

Special Issue Reprint

Advances in Nanocomposites

Preparation, Characterization, Properties
and Applications

Edited by
Reshma B Nambiar, Emmanuel Rotimi Sadiku and Anand Babu Perumal

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Advances in Nanocomposites: Preparation, Characterization, Properties and Applications

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Guest Editors

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About the Editors

Reshma B Nambiar

Reshma B Nambiar is a Postdoctoral Researcher at Zhejiang University, China. Her research interests span food science, food biotechnology, biopolymer development, nanocomposites, nanostructured materials, sustainable food packaging, encapsulation technologies, and food preservation. Her work focuses on the development and characterization of functional and sustainable polymeric systems with improved mechanical, thermal, barrier, antimicrobial, and other functional properties for food and related applications. She is particularly interested in the development of biopolymer-based nanocomposites and advanced packaging materials using sustainable and renewable resources. Her research involves interdisciplinary approaches integrating food science, biotechnology, nanotechnology, and materials science. She has contributed to peer-reviewed scientific publications and collaborative research activities and is actively involved in developing innovative material solutions for sustainable food packaging, food preservation, and emerging food applications.

Emmanuel Rotimi Sadiku

Emmanuel Rotimi Sadiku is an Emeritus Professor at Tshwane University of Technology, Pretoria, South Africa, and has extensive research experience in polymer science, polymer nanocomposites, composites, and nanomaterials. He obtained his PhD in Polymer Science from the University of Strathclyde, United Kingdom, and subsequently undertook postdoctoral and visiting research appointments in Italy, Sweden, and Germany. His research interests include polymer physics, polymer blends and nanocomposites, X-ray diffraction, microscopy, rheology and viscoelasticity, nanomaterials, hydrogels, spectroscopy, and functional and antibacterial materials. His research has contributed to the development and characterization of advanced polymeric and nanocomposite materials for structural, environmental, biomedical, energy, and other emerging applications. He has authored and coauthored numerous peer-reviewed publications and has contributed extensively to international research collaborations and scholarly book projects. He has also served as an editor and contributor to several books on polymer nanocomposites, bionanocomposites, two-dimensional nanocomposites, and advanced functional materials, including publications with major international academic publishers. His extensive expertise in polymer nanocomposites and advanced materials provides valuable multidisciplinary perspectives for this Reprint.

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Anand Babu Perumal is an Adjunct Faculty at Saveetha Institute of Medical and Technical Sciences, Chennai, India, and a Postdoctoral Researcher at Zhejiang University, China. He received his PhD in Biotechnology through a joint doctoral program between SRM Institute of Science and Technology, India, and Tshwane University of Technology, South Africa, supported by the DST NRF Indo–South Africa bilateral research program. His research interests include sustainable polymers and polymer nanocomposites, nanomaterials, nanofibers, biodegradable and active food packaging, food preservation, probiotic encapsulation, biopolymer development, green chemistry, and agro-waste valorization. His research also focuses on developing functional and sustainable materials for food and environmental applications. He has published numerous research articles and book chapters and has contributed to several academic and research activities as an editor, guest

editor, editorial board member, and reviewer for international journals. He has also been involved in interdisciplinary research collaborations and the development of sustainable material technologies, including biodegradable polymeric films and advanced nanostructured systems.

Preface

Nanocomposites have become an important class of advanced materials, owing to their unique combination of properties and their potential to address emerging challenges across diverse scientific and technological fields. Advances in nanomaterial synthesis, polymer science, surface engineering, and characterization have facilitated the development of multifunctional nanocomposites with tailored mechanical, thermal, electrical, optical, barrier, antimicrobial, and catalytic properties.

The Reprint, *Advances in Nanocomposites: Preparation, Characterization, Properties and Applications*, brings together 13 published contributions from the Special Issue, providing a focused overview of recent research in the preparation, functionalization, characterization, properties, and applications of nanocomposite materials. The collected studies encompass polymer-based, bio-based, sustainable, hybrid, and multifunctional nanocomposite systems, with applications spanning food technology, environmental protection, energy, healthcare, sensing, coatings, and advanced engineering. The Reprint reflects the interdisciplinary nature of nanocomposite research and highlights recent approaches to material design, processing, characterization, and performance enhancement.

This Reprint is intended for researchers, academics, postgraduate students, and professionals working in nanotechnology, materials science, polymer science, chemistry, biotechnology, food science, environmental science, engineering, and related fields. It is hoped that this collection will provide useful insights into current developments in nanocomposites and encourage further research and interdisciplinary collaboration in this rapidly evolving field.

Reshma B Nambiar, Emmanuel Rotimi Sadiku, and Anand Babu Perumal

Guest Editors

Advances in Nanocomposites: Preparation, Characterization, Properties, and Applications

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Nanocomposites are a class of nanomaterials wherein one or more phases, of a nano-sized dimension (zero dimensions, one dimension, and two dimensions), are embedded in ceramic, metal, or polymer materials, etc. [1]. Nanoparticles, nanorods, nanospheres, nanofibers, nanocrystals, and carbon nanotubes (CNTs) are examples of the discrete units of nanocomposite materials [2–4]. Nanocomposites exhibit multifunctional properties, such as high surface-to-volume ratios, high mechanical strength, high electrical or thermal conductivity, good chemical resistance, desirable optical properties, decreased gas and water permeabilities, flame retardancy, redox reactivity, and catalytic activity [5–12]. Researchers have utilized nanocomposite materials for a broad range of applications [13–21]. In this Special Issue, of the nineteen manuscripts received, twelve articles were published and, among these, ten were original research articles and two were review articles, which focused on the preparation, characterization, and applications of nanocomposites, including biopolymers, thin films, etc.

AbdulRasheed-Adeleke et al. [Contribution 1] developed a thermoplastic starch (TPS) bionanocomposite films with lignin fillers from bamboo plants which have a reduced moisture absorption tendency and improved mechanical properties due to the good filler/matrix interfacial adhesion. The results show that the films produced would be suitable for application as packaging materials for food preservation. In another study, Folorunso et al. [Contribution 2] evaluated the fabrication of hybrids of polyvinyl alcohol (PVA), polypyrrole (PPy), and a reduced graphene-oxide nanocomposite. The study confirmed that the electrical conductivity of polymer composites depends greatly on the weight fraction of the fillers used. The study also indicated that the deployment of the optimal characterization/simulation tools for the prediction of the electrical conductivity of polymer composites is critical. Zhou et al. [Contribution 3] prepared biodegradable composites made up of Poly-(butylene adipate-co-terephthalate) (PBAT), thermoplastic starch, hydrophobically modified nanofibrillated cellulose (HMNC), and green surfactant (sucrose fatty acid ester) via the melt mixing and film-blowing process (PBATHMNC), which exhibited good mechanical and barrier characteristics. The *Agaricus* mushrooms preserved using these films retained their commodity value after 11 days of preservation. The developed PBAT-HMNC composites were proven to be promising nanocomposite materials for food packaging [22].

Other studies focused on the ecofriendly synthesis of bioactive glass nanofiber scaffolds, nanorod MAZ zeolite, and graphene quantum dots (GQDs). In one approach, a novel 3D hollow mesoporous bioactive glass nanofiber scaffold was synthesized with a template-assisted sol–gel method using a bacterial cellulose (BC) as a template and a nonionic triblock copolymer (P123) as a pore-directing agent, as well as ethyl orthosilicate

(TEOS), calcium nitrate tetrahydrate (CN), and triethyl phosphate (TEP) as glass precursors. The scaffold had nanofiber-like morphology and an interconnected three-dimensional network structure with a large specific surface area and a large pore volume and exhibited excellent apatite-forming ability and cellular biocompatibility, which could be used in bone tissue regeneration [Contribution 4].

Zhang et al. [Contribution 5] reported a green route for synthesizing nanorod MAZ zeolite (MAZ-N) by using a low-cost and environmentally friendly choline chloride as a template. The MAZ-N samples had good crystallinity and uniform porous structures. The study suggested the potential application of MAZ-N zeolites as a supporting catalyst compounds in industrial processes. Nancy et al. [Contribution 6] developed a one-pot, facile, and ecofriendly synthesis approach for the fabrication of GQDs via the pulsed laser irradiation of an organic solvent (toluene) without any catalyst. It was observed that the GQDs obtained displayed fluorescence features and exhibited excellent optical limiting properties, and it was expected to have potential applications in optoelectronics and light-harvesting devices.

A review paper by Ahari et al. [Contribution 7] discusses the role of AuNPs (gold nanoparticles) in improving active food packaging. There is a global concern about food loss due to reduced shelf life, hence they suggested that the administration of nanotechnology-based methods, such as metal-based nanoparticles, can reduce the unresolved issues of the shortened shelf life of food and the associated environmental concerns about packaging, especially those of green synthetic-based AuNPs in comparison to the conventional chemistry-based methods. The developed AuNPs have a large surface-to-volume ratio, and diminished biohazards, aimed at increasing stability and the induction of antimicrobial or antioxidant properties. However, it is necessary to consider the hazards, including the migration of AuNPs for food packaging purposes. Another review examines the importance of Zn for obtaining multifunctional materials with interesting properties by following certain preparation strategies, such as choosing an appropriate synthesis route, the doping and co-doping of ZnO (Zinc oxide) films to achieve conductive oxide materials with *p*- or *n*-type conductivity, and finally adding the desired polymers to the oxide systems for piezoelectricity enhancement. The authors mainly followed the results of studies from the last ten years through chemical routes, especially via the sol-gel and hydrothermal synthesis routes. They concluded that ZnO can be very useful as a seed layer, for the good adherence of the main layer to the substrate, thereby generating nucleation sites for nanowire growth, which could be useful across multiple applications [Contribution 8]. A study on ZnO was also conducted by Xiao et al. [Contribution 9], who worked on the grain boundary structures of ZnO-based ceramics that were manipulated by the incorporation of polytetrafluoroethylene (PTFE) and metal oxides, through the cold sintering process (CSP), which reduced the grain size, increased the grain boundary resistance and the Schottky barrier height.

Bilal et al. [Contribution 10] prepared novel platforms for horseradish peroxidase immobilization by testing a variety of hybrid and single nanomaterials of various origins such as chitosan, magnetic nanoparticles, and selenium ions. The physicochemical characterization of the obtained systems proved successful enzyme deposition on all the presented materials with an efficiency above 70%. Of all the tested materials, the most favorable for immobilization was the above-mentioned chitosan-based hybrid material. The selenium additive increased the immobilization yield of the enzyme and improved enzyme stability. The obtained results confirm the applicability of these nanomaterials as useful platforms for enzyme immobilization in the contemplation of the structural stability of an enzyme and the high catalytic activity of fabricated biocatalysts. In another study, Jamal et al. [Contribution 11] prepared a bilayer electrospun fibers for skin tissue engineering applications with increased cell attachment and proliferation. Different ratios of PHBV-PLLA (70:30, 80:20, and 90:10 *w/w*) blends were electrospun on previously formed electrospun PHBV membranes to produce their bilayers. The membranes had random fibers with porous morphology and the fabricated bilayer showed high mechanical strength as well as

biocompatibility with good cell proliferation and cell attachment, showing potential for skin substitute applications.

Malektaj et al. [Contribution 12] developed Ca–alginate–montmorillonite (MMT) nanocomposite gels, which were able to preserve their integrity in a wide range of pH values. The experimental data showed that the elastic moduli increase linearly with a concentration of MMT at all pH values under investigation due to the formation of physical bonds between the alginate chains and the MMT platelets. The study showed that the prepared gel could be used in wide range of applications such as tissue engineering, drug delivery, and food packaging. In conclusion, the research area of nanocomposites is growing very rapidly, and many applications in different sectors are very promising.

Author Contributions: R.B.N. and A.B.P. wrote the editorial with inputs from E.R.S. All authors have read and agreed to the published version of the manuscript.

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List of Contributions

1. AbdulRasheed-Adeleke, T.; Egwim, E.C.; Sadiku, E.R.; Ochigbo, S.S. Optimization of Lignin–Cellulose Nanofiber-Filled Thermoplastic Starch Composite Film Production for Potential Application in Food Packaging. *Molecules* **2022**, *27*, 7708. <https://doi.org/10.3390/molecules27227708>.
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Article

Development of Biocompatible Electrospun PHBV-PLLA Polymeric Bilayer Composite Membranes for Skin Tissue Engineering Applications

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Abstract: Bilayer electrospun fibers aimed to be used for skin tissue engineering applications were fabricated for enhanced cell attachment and proliferation. Different ratios of PHBV-PLLA (70:30, 80:20, and 90:10 *w/w*) blends were electrospun on previously formed electrospun PHBV membranes to produce their bilayers. The fabricated electrospun membranes were characterized with FTIR, which conformed to the characteristic peaks assigned for both PHBV and PLLA. The surface morphology was evaluated using SEM analysis that showed random fibers with porous morphology. The fiber diameter and pore size were measured in the range of $0.7 \pm 0.1 \mu\text{m}$ and $1.9 \pm 0.2 \mu\text{m}$, respectively. The tensile properties of the bilayers were determined using an electrodynamic testing system. Bilayers had higher elongation at break (44.45%) compared to the monolayers (28.41%) and improved ultimate tensile strength (7.940 MPa) compared to the PHBV monolayer (2.450 MPa). In vitro cytotoxicity of each of the scaffolds was determined via culturing MC3T3 (pre-osteoblastic cell line) on the membranes. Proliferation was evaluated using the Alamar Blue assay on days 3, 7, and 14, respectively. SEM images of cells cultured on membranes were taken in addition to bright field imaging to visually show cell attachment. Fluorescent nuclear staining performed with DAPI was imaged with an inverted fluorescent microscope. The fabricated bilayer shows high mechanical strength as well as biocompatibility with good cell proliferation and cell attachment, showing potential for skin substitute applications.

Keywords: bilayer composites; polymeric membranes; PHBV; PLLA; skin regeneration

1. Introduction

Skin covers the largest surface area of the human body and forms a barrier between the internal and external environment [1,2]. Healthy skin is significant to maintain the normal homeostasis of the human body [3]. The skin has a complex multilayer structure, which is composed of cells, fibers, capillaries, hair follicles, and additional cell networks (ECMs). Skin generally comprises three layers, an upper epidermis, a middle dermis, and the lower hypodermis. The epidermis consists of keratinocytes, the dermis is composed of fibroblasts and collagen, and the third, innermost layer, the hypodermis, possesses lipocytes and collagen [2].

Throughout the lifetime of an individual, the skin constantly heals itself and regains its integrity through a harmonized interaction between keratinocytes, fibroblasts, melanocytes, and endothelial cells in addition to the involvement of certain signaling molecules [4]. The same process is used for the repair of injured or damaged skin tissue. Modern medical interventions help wound healing and injury recovery via tissue grafting with autologous, allogenic, or xenogeneic skin structures [5,6]. However, this process involves donor site morbidity, which can be avoided by replacing autologous tissue with synthetic and natural skin substitutes [7,8]. Skin substitutes are used for the regeneration and wound healing of skin, mainly for burns, and testing of cosmetics and drugs for skin.

According to the World Health Organization (WHO), burns cause 180,000 deaths every year whereby low and middle-income countries are the major victims [9]. Over 1,000,000 people in India and 173,000 in Bangladesh are moderately or severely burnt every year. In Bangladesh, Colombia, Egypt, and Pakistan, 17% of children with burns have a temporary disability and 18% have a permanent disability [10]. Only a small percentage of patients have access to reconstructive procedures, while the rest of them remain untreated and handicapped [11,12].

Many skin healing substitutes are available in the market, each with its limitations. Some of these products act as wound dressings while others are impregnated with cells that are incapable of surviving long enough to heal the wound completely [3,12]. However, imitating natural skin with all its functions has not been achieved effectively so far. To achieve optimum mechanical properties, multilayered structures to cater for appendages and respective cellular populations, achieving innervation and vascularization, and the absence of organogenesis are major deficiencies of current models [13,14]. Other disadvantages of these models are fragility microbial contamination, lower engraftment rates, healing delay, and high cost. This prevents the substitutional skin from being fully functional, like natural skin [15].

Distinctive techniques have been utilized for the preparation of polymer nanofibers in recent years, for example, electrospinning [16,17], phase separation [18], self-assembly [19,20], drawing [21], and template synthesis [22,23]. Electrospun polymer nanofibers are used in various applications, such as biosensors, optical electronics, wound healing, nonwoven fabrics, filtration, drug delivery, and scaffolds for tissue engineering. Because of environmental concerns and sustainability problems, renewable and biocompatible polymers are getting more attention [24,25]. Bio-nanocomposites are a class of nanocomposite materials. Here, the prefix “bio” suggests that inorganic/petroleum-based constituents are replaced with bio-based, environmentally friendly alternatives [26]. In recent research, the commonly used bio-nanocomposites are HA-PLA, HA-PCL, PU-PLA, PAL-PCL, and PHBV-PLLA [27].

In this study, PHBV (Polyhydroxy butyrate-co-hydroxy valerate) was selected for its biocompatible and biodegradable nature. Furthermore, it is easy to be electrospun into nanofibrous membrane layers, resulting in high tensile strength [28]. PLLA (Poly L-lactic acid) was selected as the second polymer, which is also known for its characteristics and biocompatibility [29,30]. PLLA has been widely used for various applications ranging from pelvic floor repair material [31] to the preparation of biodegradable membranes with the combination of different polymers [32].

The novelty of this work lies in the fabrication of a nano-porous/microporous-nano-porous bilayer membrane that has a skin-like bilayer architectural structure. A thin compact

layer of PHBV was first formed through electrospinning; then, PHBV-PLLA electrospun fibers were developed on this PHBV monolayer to form a bilayer to enhance cell attachment and proliferation. The prepared bilayer mats were characterized with FTIR, the Electrodynamical Fatigue Testing system, and SEM analysis for structural, mechanical, and morphological properties, respectively. Biocompatibility was assessed by culturing the MC3T3 (pre-osteoblastic) cell line and Alamar Blue assay on days 3, 7, and 14, respectively. To gain more insight, cell attachment was observed through SEM micrograph and fluorescent nuclear staining for investigating an electrospun bilayer of the PHBV/PHBV-PLLA biodegradable polymeric matrix that can be optimized further for skin repair applications.

2. Results and Discussion

2.1. SEM for Electrospun PHBV-PLLA Bilayer Membranes

Figure 1 shows SEM images of PLLA-PHBV bilayer membranes. The surface of these membranes has a randomly uniform fibrous morphology with a 3D appearance [33]. A porous structure and uniform random fiber deposition are the main features observed in all the membranes [34]. Figure 1a–c show the bilayer membranes 90:10, 80:20, and 70:30 *w/w* PHBV-PLLA at magnifications of $5k\times$, respectively. The arrangement and distribution of fibers give a microporous/nano-porous nature to the membranes [35], which are a prerequisite for a biocompatible membrane to mimic the native extracellular matrix of the skin.

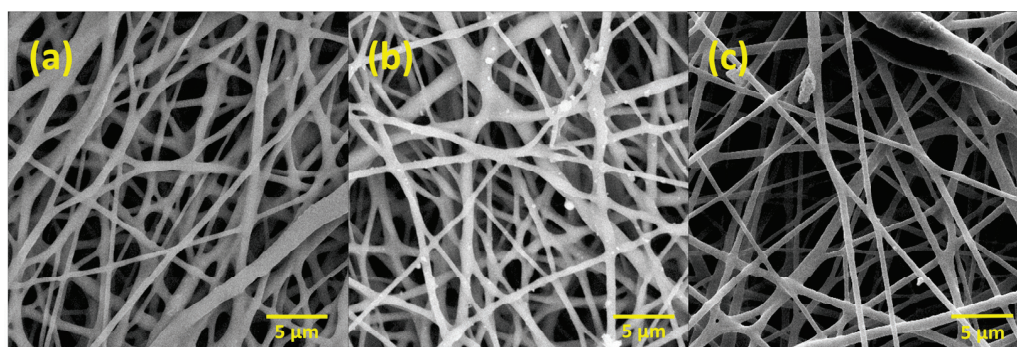


Figure 1. Electrospun bilayer PHBV-PLLA membranes at different magnifications. (a) 90:10, (b) 80:20, and (c) 70:30 (*w/w*) ratio.

Figure 2 shows the cross-sectional images of PHBV-PLLA bilayer membranes, taken at a magnification of $200\times$. In the bilayer membrane, there is a partition between the PHBV-PLLA blend fibers and the PHBV fiber layer. A dashed red line in the middle of the membrane indicates the interface of the bilayer structure of the membrane having a PHBV layer on the left and a PHBV-PLLA layer on the right (Figure 2). Interlinking fibers show a clear interlayer adherence and hence are a determinant of consistency in mechanical load distribution among the polymeric layers. The porous structure of bilayer membranes helps in cellular migration through the pores and attachment due to the higher available surface area of the fibers, which increases cell proliferation and improves biocompatibility.

2.2. Pore Diameter and Fiber Thickness Analysis

Pore diameter and fiber thickness play an important role in cell proliferation. The pore diameter and fiber thickness of the three fabricated bilayer membranes are shown in Figure 3. The pore void of bilayer 90:10 was measured as $1.8\text{ }\mu\text{m}$, that of 80:20 was $1.9\text{ }\mu\text{m}$, and 70:30 was $2.1\text{ }\mu\text{m}$. The pore diameter was higher in membranes containing higher PLLA content. Nano-pored scaffolds with pore voids less than $1\text{ }\mu\text{m}$ can be applied to improve cell–surface interaction, and larger pore size (around $1\text{--}3\text{ }\mu\text{m}$) is needed for cell-to-cell interaction on anchorage-dependent cell populations [36]. Therefore, all three membranes can allow cell-to-cell interactions for tissue engineering applications. Similarly, the fiber thickness of the 90:10 bilayer was $0.60\text{ }\mu\text{m}$, $0.65\text{ }\mu\text{m}$ for 80:20, and $0.70\text{ }\mu\text{m}$ for 70:30,

as shown in Figure 3. The difference between the means of fiber thickness of each type of bilayer is significant compared to the control. SEM analysis shows that the fibers and pore diameters were in the range of μm and provide good anchorage points for cells to the membranes with enhanced cell proliferation and migration through these interconnected pores. The fibrous architecture of these electrospun fibers mimics the collagen fibers of the extracellular matrix, providing a large surface area for cell attachment and an open porous structure for cells to migrate into the scaffolds, which is essential for good tissue integration [35], as discussed earlier.

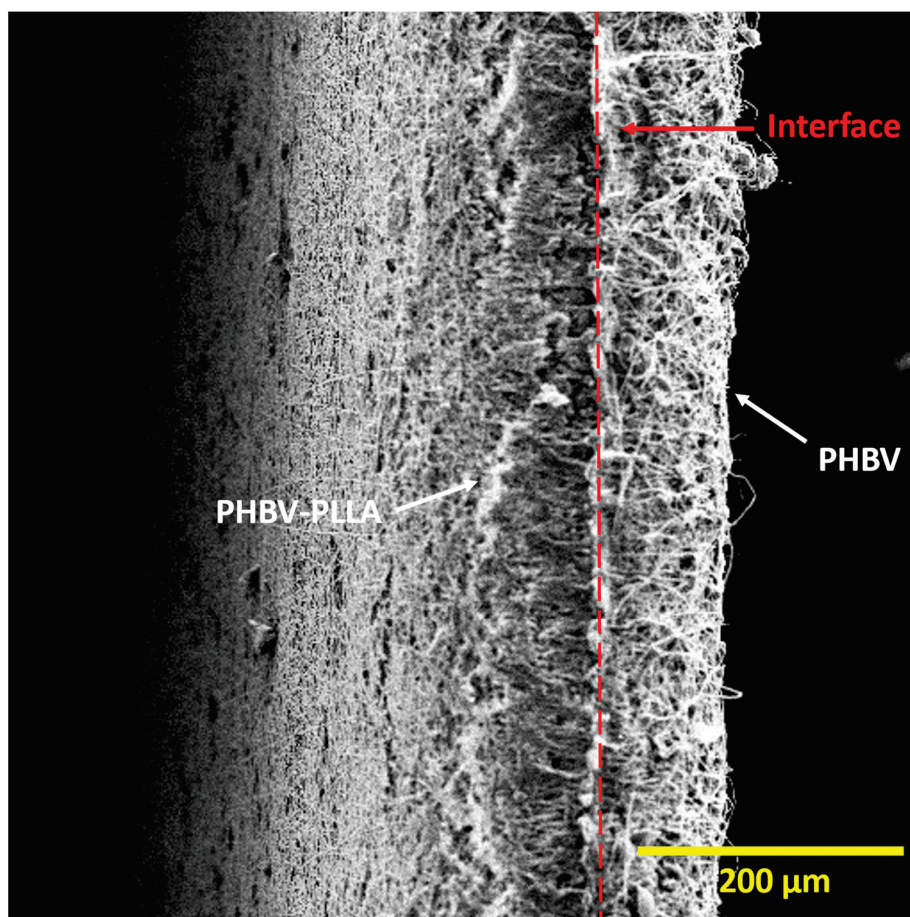


Figure 2. Cross-sectional view of the Bilayer PHBV-PLLA membranes. (White arrows: individual layers, red line: interface between two layers).

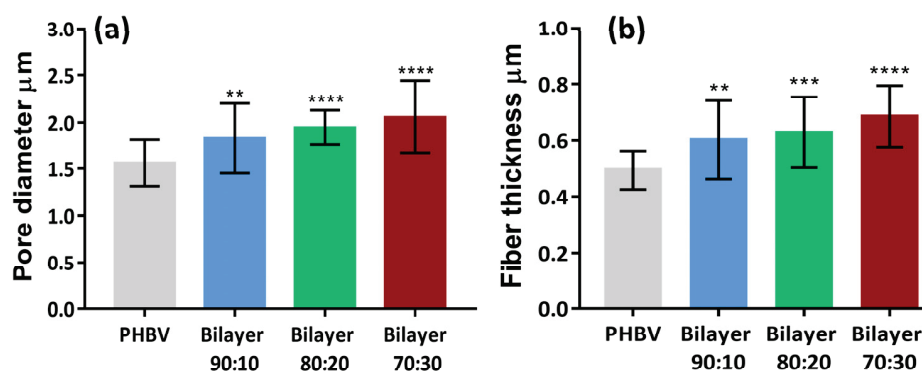


Figure 3. Comparison of (a) pore diameter and (b) fiber thickness of bilayer PHBV-PLLA membranes with monolayer PHBV. (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

2.3. Porosity Measurement

The porosity of the membranes and the apparent densities were calculated using the formulas stated in the materials and methods section and graphically represented in Figure 3. Membrane porosity ranging from 60 to 90% is preferred for the penetration of the cells [37]. For cellular integration, electrospinning creates pores of appropriate size by dispensing fibers at random orientations. The porosity of all the membranes is within the range of 63–70%, as given in Table 1. This indicates that the porosity values reached with electrospinning for this polymeric combination are acceptable for a given thickness appropriate for the epidermis [38] and possibly extendable to the dermis with more fibrous deposition.

Table 1. Morphological properties of the PHBV and PHBV-PLLA bilayer membranes.

Membranes	Bulk Density	Thickness	Porosity
PHBV	1.25 g	70 μm	65%
PHBV-PLLA bilayer	1.26 g	180 μm	67–70%

2.4. FTIR Analysis

Figure 4 shows the FTIR spectra of PHBV and PLLA membranes and stacked spectra of bilayers having PHBV-PLLA electrospun membranes with three blend compositions. The FTIR spectra of PHBV show a strong band of around 1720 accounting for C=O stretching. Symmetric -C-O-C bonding is exhibited in the range of 800–975 cm^{-1} , whereas asymmetric stretching for -C-O-C signals is seen between 1060 and 1150 cm^{-1} . In spectra obtained from PLLA, CH₃ stretching is observed in a range of 3000–2950 cm^{-1} , whereas C=O stretching is similar to that in the PHBV band. CH₂ and CH₃ bending were found at 1452 and 1279 cm^{-1} , respectively. The O-C=O stretching around 1200–1090 cm^{-1} exhibits the characteristic ester groups. The IR spectra are a combination of the IR peaks from PLLA and PHBV and were found to be 3000, 2925, and 2845 cm^{-1} for CH, CH₂, and CH₃, respectively. Other peaks include 1720 cm^{-1} for C=O stretching, 1440 and 1370 cm^{-1} for CH₂ and CH₃ for bending, and 1270–1050 cm^{-1} for C-O-C stretching. 975 cm^{-1} was found for CH₂ in-plane bending of PHBV and 869 cm^{-1} for O-C-H-CH₃, which are similar to the trend reported in HC Chang et al. [39]. As is evident from the graphs, there are no considerable peak shifts for the bilayers with compositional variations. By changing the compositions, fiber morphology and the porous structure of the membranes are changing, but no chemical change occurred, which resulted in no noticeable peak shifts. Considerable peak intensities are observed in comparison to other specimens for the bilayer samples owing to the better reflectance and absorbance by the samples due to the formation of the bilayer [40].

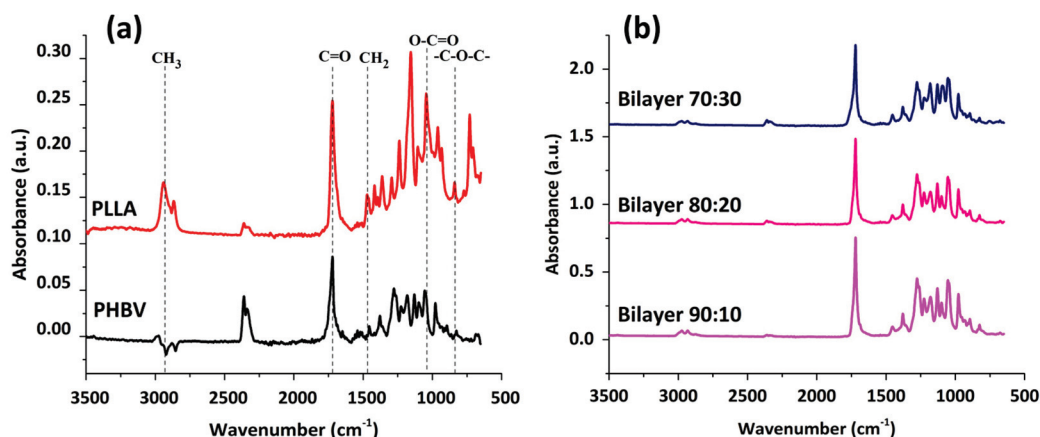


Figure 4. (a) FTIR for PHBV and PLLA membranes with characteristic peaks. (b) FTIR spectral comparison between different formulations of bilayer PHBV-PLLA membranes.

2.5. Mechanical Testing

Mechanical testing was conducted on electrospun membranes to determine the tensile strength and Young's modulus of the membranes. Figure 5 shows the stress and strain curves for all the membranes. These membranes have an overall ductile behavior under applied stress. The curves show a proportional increase in the stress and strain ratio at the beginning, where Hooke's law is being followed. All the membranes have a strain percentage in this linear portion under less than 10% [28].

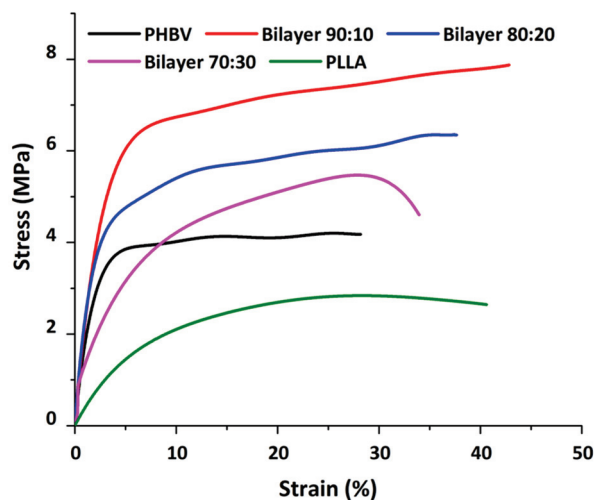


Figure 5. Stress and strain graph showing the mechanical behavior of membranes.

The stress–strain curve of the PHBV membrane in comparison to the bilayer membrane suggests the increase in the stress/strain of bilayers with the addition of PLLA to the PHBV polymer. All bilayers have higher mechanical strength as compared to the PHBV monolayer. Overall, the mechanical strength decreased from the composition of 90:10 to 80:20 and then 70:30 *w/w*, respectively. This can be attributed to the PLLA ratio in the blend; the higher the ratio, the lower the mechanical strength (Table 2), as PLLA is known to have lower mechanical strength as compared to the PHBV polymer [17]. However, the overall tensile strength was better in the bilayer compared to PHBV alone, which may be due to the PHBV bottom layer, which provides support to the blended layer and enhances the mechanical properties of the bilayer membranes.

Table 2. Mechanical properties of PHBV-PLLA membranes were calculated from stress–strain curves. (* Young Modulus rating of submandibular skin by Griffin et al. [41]).

Membrane Type	Young Modulus MPa	Ultimate Tensile Stress MPa	Elongation at Break %
Submandibular skin	1.28 MPa *	-	-
PLLA	45.65	2.450	40.05
PHBV	119.7	4.224	28.41
Bilayer 90:10	172.0	7.940	44.45
Bilayer 80:20	144.1	6.355	37.63
Bilayer 70:30	128.2	5.470	33.95

As the amount of PLLA increases from 10% to 30%, the diameter of the fiber increases, as evident from Figure 5. Hence, it is presumed that the number of fibers per unit area of the electrospun membrane decreases, resulting in a decrease in the mechanical strength. This is also apparent from the fiber thickness and void size, which increased with the addition of PLLA in the blend. It is important to note that at higher thicknesses, comparable to natural

human skin, both monolayers and bilayer membranes can show much higher modulus values with improved elongation at break (Table 2); in addition, different regions of skin yield different Young's modulus values, as measured by Griffin et al., i.e., 1.28 MPa [41]. Since these values show comparably improved mechanical properties, a bilayer scheme provides evidence as a better option to use as a substitute to mimic the skin's mechanical behavior.

2.6. Cell Culture Evaluation

In vitro cytotoxicity of each of the membranes was determined by culturing MC3T3 (pre-osteoblastic cell line) on the membranes. Cell proliferation was evaluated via Alamar Blue assay on days 3, 7, and 14. SEM images of cells cultured from membranes were taken as well as inverted microscopic imaging.

2.6.1. Alamar Blue Assay

Cell proliferation assay was performed using Alamar Blue stain. The experiment was performed for 3, 7, and 14 days via co-culturing the membranes, and the results were then compared with control. Figure 6 shows the absorbance results of the control, which shows cells seeded in the media without membrane, and bilayer 90:10, 80:20, and 70:30 *w/w* membranes for 3, 7, and 14 days, respectively [42,43].

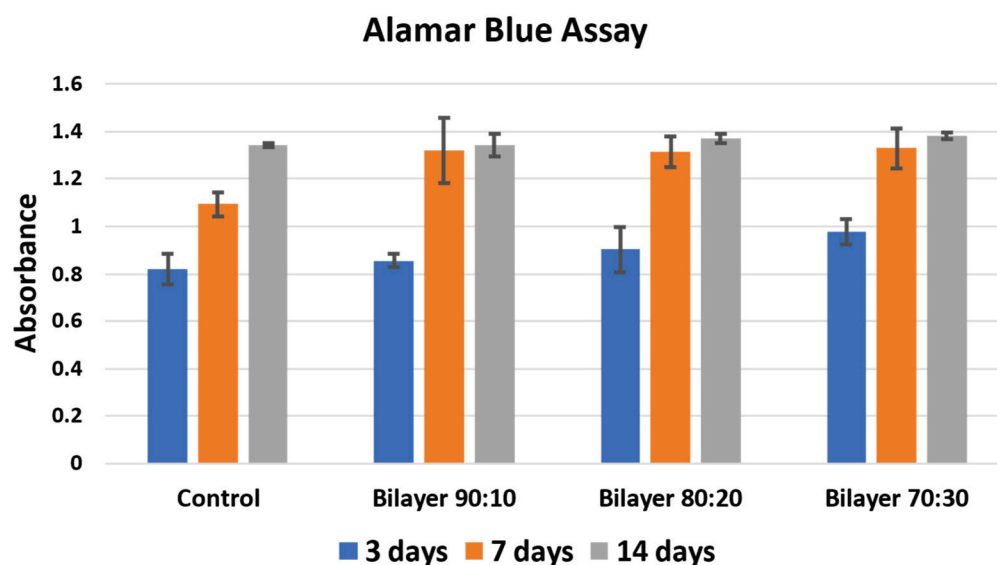


Figure 6. Cell proliferation Alamar Blue assay of bilayer PHBV-PLLA membranes. (Sidak's multiple comparison test showed no significant values for *p*).

It is evident in Figure 6 that there is an increase in cell proliferation of bilayer PHBV-PLLA membranes compared to the control. The proliferation increased with time, i.e., from 3 to 7 with visible viability increment and then up to 14 days for all the membranes and in the control. The absorbance values after incubation with the Alamar Blue stain are an indicator of proliferation. The higher the absorbance, the higher the biocompatibility and cell proliferation of the tested material [44]. The 70:30 bilayer membrane showed the highest proliferation, which may be due to higher void size for cell integration and/or due to higher participation of biocompatible PLLA. An increase in percentage proliferation as compared to control after 3 days is similar in 90:10, 80:20, and 70:30 bilayer formulations. There is less increase after 14 days of culture, which the reason might be that the cells reached a plateau stage due to the high initial number per well [45]. The 70:30 bilayer formulation showed the maximum increase in cell proliferation as compared to all other membranes after 3, 7, and 14-day culture. It seems that the pore size, fiber diameter, and mechanical strength of the 70:30 membrane also affected cell attachment and proliferation. These results also support the use of bilayer membranes as biocompatible materials for artificial skin applications.

2.6.2. Scanning Electron Micrographs of MC3T3 Cells Cultured in Electrospun Membranes

Bilayer membranes of (a) 90:10, (b) 80:20, and (c) 70:30 *w/w* formulations of PHBV-PLLA at 1k $\times$ were evaluated. SEM images show the membrane surfaces on day 14 after the initial seeding of 50,000 cells. The cells gradually increased from day 1 to 14 and, on day 14, the cells were fixated with glutaraldehyde, washed with 30, 50, 70, 90, and 100 percent alcohol, respectively, and the SEM images of the membranes were taken after drying. Adherent cells on these membranes were observed using the SEM analysis, as the attachment of cells could not be verified with the optical microscope. The cell morphology and the attachment of the cells to the membrane were found to be similar to those reported in the literature [34,35,46], confirming the proliferation of the cells onto the membranes. The confluent layer of MC3T3 cells was attached to the membranes, as shown by SEM (Figure 7). These SEM images validate the biocompatibility of the bilayer membranes by showing cell attachment and growth of the pre-osteoblast MC3T3 cells onto and through the electrospun fibrous mesh. Additionally, it can be concluded that these membranes did not show cytotoxicity to the seeded cells.

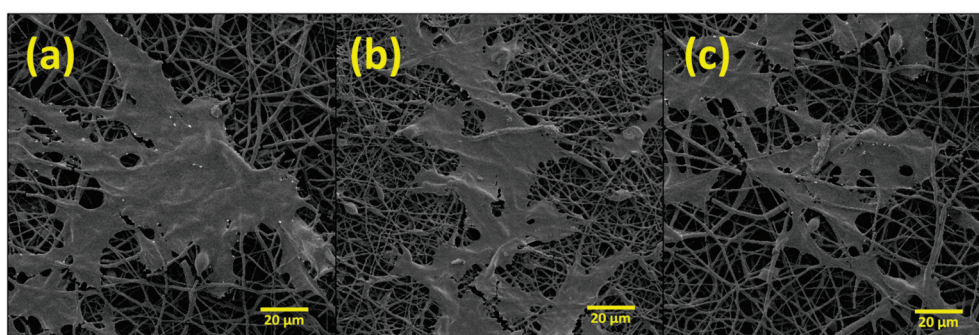


Figure 7. SEM images of PHBV-PLLA bilayer membranes taken after 14 days of culture at different 1k $\times$ magnification. (a) 90:10, (b) 80:20, and (c) 70:30 *w/w* formulations.

2.6.3. Fluorescence Imaging of MC3T3 Cells Stained with DAPI

DAPI staining and fluorescence imaging results also indicate that the membranes are not cytotoxic to the cell, as an increased number of cells on the bilayer membrane are exhibited (Figure 8b). At higher magnification, these layers were filled with cell nuclei, as shown in Figure 8a. It is concluded with this study and Alamar Blue assay that bilayer and monolayer membranes were not cytotoxic and biocompatible with cells.

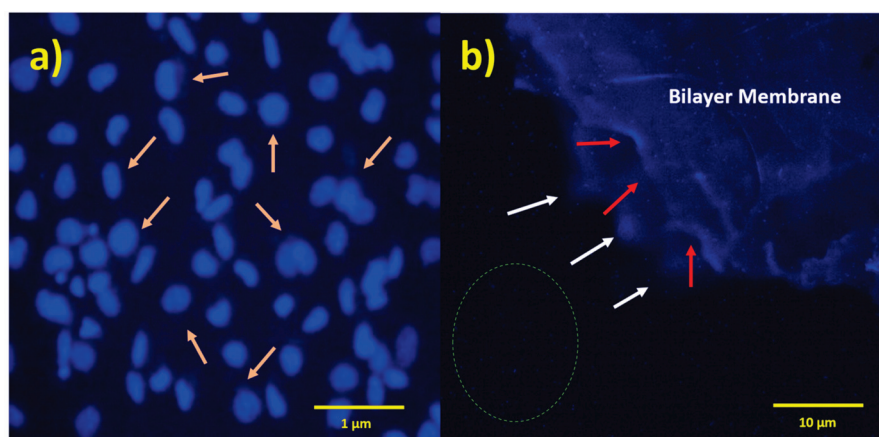


Figure 8. Fluorescent micrographs of PHBV-PLLA bilayer membranes stained with DAPI fluorescent dye. (a) Arrows indicate cell nuclei on the membrane, 40 $\times$ magnification; (b) white arrows indicate the edges of the bottom layer of the membrane; red arrows indicate the edges of the upper layer of the membrane; and dotted circle indicates one of the regions in the medium showing the cellular nuclei, 4 $\times$ magnification.

The fabrication of electrospun membranes for skin tissue applications is gaining interest in biomedical solutions, and the current study aims to highlight this point by providing a proof-of-concept material in the form of a PHBV-PLLA bilayered membrane. All the formulations can show biocompatibility and cellular proliferation in an *in vitro* mouse skin model (MC3T3 cell). Furthermore, the physical characteristics of these membranes are significant in providing enough mechanical strength as well as support to cell proliferation via its micro/nano-porous structure. Additionally, no significant differences in color or physical appearance of the fibrous mats were seen when placed at room temperature or 40 °C up to 3 months, which shows that these membranes are stable in terms of shelf life.

3. Materials and Methods

3.1. Materials

Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV; Goodfellow, Cambridge, UK), methanol (AnalaR Normapur-VWR, Lutterworth, UK), and Dichloromethane (DCM; Sigma Aldrich, London, UK). Poly-L-Lactic Acid (PLLA; Goodfellow, Cambridge, UK). Pre-osteoblast cell line (MC3T3 ATCC[®] CRL-2593[™]), alpha minimum essential medium (α -MEM; Thermo Fisher Scientific, Waltham, MA, USA), penicillin/streptomycin (Pen/strep; Sigma Aldrich, Life Sciences, St. Louise, MI, USA), fetal bovine serum (FBS; Sigma Aldrich, USA), T75 culture flask (Corning Biosystem, Flintshire, UK), Trypsin–EDTA (Sigma Aldrich, USA), Alamar Blue solution (Sigma Aldrich, UK), and phosphate-buffered saline (PBS; Gibco, Waltham, MA, USA).

3.2. Bilayer Membrane Fabrication

PHBV was dissolved in a 90:10 DCM methanol ratio followed by the addition and dissolution of Poly-L-Lactic Acid for a composite blend formation. Each bilayer formulation has been named bilayer 90:10, bilayer 80:20, and bilayer 70:30, where 90:10 means weight by weight distribution of 90 against 10 between PHBV and PLLA, respectively. The solution was prepared at room temperature on a mechanical stirrer overnight. On the following day, 3 syringes were filled with 5 mL solutions and were fitted in the multichannel needle holder. The needle gauge was 18 mm in diameter. The syringes were fixed on the (Kent Scientific, Torrington, CT, USA) syringe pump, having a flow rate ranging from 0.35 to 0.45 mL/h. The applied voltage was 15kV with a high-voltage power supply (Genvolt, Bridgnorth, UK). The fibrous scaffold was collected on a rotating drum 16 cm $\times$ 6 cm in diameter and wrapped with aluminum foil at a distance of 14 cm from the needle tips with a rotation speed of 300 rpm [34]. An amount of 15 mL of the polymer solution was dispensed to make a fibrous sheet, as described by Bye et al. [46]. Figure 9 shows the schematic fabrication of membranes.

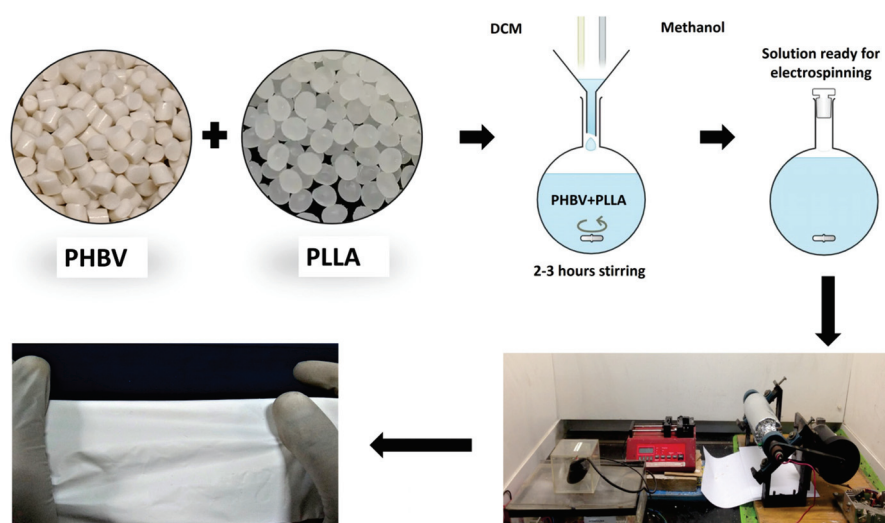


Figure 9. Fabrication of a bilayer electrospun fiber composite membrane.

3.3. Scanning Electron Microscopy

Scanning Electron Microscopy was performed using TESCAN Vega3 LMU (VEGA TC), using an ETD detector and SE mode after gold coating with a sputter coater from Quorum Technologies. SEM images were acquired using an acceleration voltage of 10 kV with a beam intensity of 4 pA.

3.4. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infra-Red Spectroscopy (Nicolet 6700, ThermoScientific, Waltham, MA, USA) was performed in Attenuated Total Reflectance (ATR) mode, where each sample was scanned 128 times at a resolution of 8 cm^{-1} in the scan range of $4000\text{--}650\text{ cm}^{-1}$.

3.5. Mechanical Testing

Mechanical or tensile testing was performed using the Walter + Bai Electrodynamic Fatigue Testing System (LFV-E), having a load of 1.5 KN. The 6 replicates were prepared for each membrane and were cut into a rectangular shape with a width of approximately 5 mm and a length of 45 mm. The thickness of these samples was measured in millimeters with the help of a Vernier caliper with the precision of 0.01 mm. From the slope of the initial linear path of the stress and strain curve, the tensile strength of Young's modulus was calculated. Strain in percentage and stress in MPa were calculated with the deformation and load values using the following equations [34].

3.6. Statistical Analysis of Membranes

With six samples from each of the experiments, all the analysis was conducted at least twice to obtain precise data. From SEM, all the data are shown as the mean. Following Tukey's post hoc test, a one-way (ANOVA) was performed; p -values < 0.05 were considered to be statistically significant. GraphPad Prism 5.0 software was used to analyze all the data.

3.7. Analysis of Fiber Thickness and Void Size

Fiber thickness was calculated by selecting at least thirty fibers, each from three frames of images taken using SEM (Version 1.50), using Image-J software (Version 1.54h) [47]. Void and pore sizes in each of the membranes were measured in the same way. The thickness of the electrospun membranes was measured using a microscope, and the apparent density and porosity of the membrane were calculated with the help of the following equations [37].

3.8. In Vitro Evaluation

MC3T3 (pre-osteoblastic cell line) cell lines were gifted from National Institute of Biotechnology and Genetic Engineering (NIBGE) Faisalabad, Pakistan. The in vitro biocompatibility test was carried out via cell culture on the membranes using pre-osteoblast cell lines. Cells were expanded in a T75 culture flask (MC3T3) that was prepared, with α -MEM with $100\text{ }\mu\text{g/mL}$ of penicillin/streptomycin and 10% FBS added, in a humidified incubator at a temperature of $37\text{ }^{\circ}\text{C}$ with 5% CO_2 . Fresh media changes were made after 2 to 3 days. The cells were expanded and grown to 90% confluence [44,48]. Afterwards, these cells were detached using trypsin-EDTA. Before seeding, the cells were counted, and 50,000 cells were seeded on each sample in the 24-well plate to check the compatibility of the MC3T3 cells with PHBV-PLLA membranes. Before seeding, the samples were sterilized with 70% ethanol for 2 h. Then, the samples were washed 3 times with PBS at 15-min intervals and the cells were cultured in tissue culture plastic plates without membranes as control.

3.9. In Vitro Characterization

3.9.1. Alamar Blue Assay

After 3, 7, and 14 days, absorbance measurements of the Alamar Blue assay were taken to check the biocompatibility of cells with the membranes and the attachment of cells onto the membranes. Based on the metabolic activity of the cells, the Alamar Blue has a redox indicator that changes from an oxidized (blue) form to a reduced (red) form as the

substrate is taken up by the cells. Cell-seeded samples were carefully washed with PBS and 0.5 mL of 1mM Alamar Blue solution was added. After that, it was incubated for 3 to 4 h at 37 °C. An absorbance plate reader (PR4100 Absorbance Microplate Reader BIO-RAD, Hemel Hempstead, UK) was used to measure the absorbance at 570 nm. Samples were fixed for DAPI staining and SEM analysis after the results were taken from the plate reader. To prepare the sample for SEM, the membranes were dehydrated and washed with the different dilutions (30, 50, 70, 90, and 100%) of ethanol and then dried.

3.9.2. Fluorescence Microscopy

Fluorescence microscopy was performed using an 89404-464 VWR microscope having 240 V with a frequency of 50–60 Hz and a Halogen lamp of 6 V and 30 W. The live cells were stained with DAPI and fluorescence images were taken after excitation with UV light with a 460 nm filter at 10× magnification.

4. Conclusions

Biocompatible PHBV-PLLA bilayer membranes containing different concentrations (70:30, 80:20, and 90:10 *w/w*) of each polymer in the blend were developed through electrospinning. SEM analysis shows the fibrous non-woven structure of the electrospun matrix with the increase in pore diameter and fiber thickness with higher PLLA content in the blends. The pore size of the 90:10 bilayer formulation was measured to be 1.8 µm and the fiber diameter 0.6 µm, while the pore size of the 70:30 bilayer was 2.1 µm and fiber diameter was 0.7 µm, respectively. The 90:10 bilayer formulation showed significantly higher tensile strength compared to the PHBV and PLLA monolayers, i.e., 7.940, 4.224, and 2.450 MPa, respectively. In addition, there was a significant increase in the tensile strength for higher concentrations of PHBV in the blended bilayer structures. In vitro biocompatibility proved that all three ratios of the bilayer membrane were highly biocompatible. Cell proliferation increased after culturing on the membranes for 3 and 7 days, with no cytotoxicity. The highest cell proliferation was observed in the 70:30 bilayer membrane, with the highest pore diameter around 2 µm. The tensile strength of bilayers with smaller pore sizes was highest, i.e., 172 MPa, but the cell proliferation rate was lowest (Figure 6), while the mechanical strength of bilayers with larger pore sizes had lower tensile strength but significantly higher cell proliferation. This research can be useful in developing biomedical materials for skin tissue scaffolds. Future work includes the seeding and culture of keratinocytes and fibroblasts.

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Article

Support Materials of Organic and Inorganic Origin as Platforms for Horseradish Peroxidase Immobilization: Comparison Study for High Stability and Activity Recovery

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Abstract: In the presented study, a variety of hybrid and single nanomaterials of various origins were tested as novel platforms for horseradish peroxidase immobilization. A thorough characterization was performed to establish the suitability of the support materials for immobilization, as well as the activity and stability retention of the biocatalysts, which were analyzed and discussed. The physicochemical characterization of the obtained systems proved successful enzyme deposition on all the presented materials. The immobilization of horseradish peroxidase on all the tested supports occurred with an efficiency above 70%. However, for multi-walled carbon nanotubes and hybrids made of chitosan, magnetic nanoparticles, and selenium ions, it reached up to 90%. For these materials, the immobilization yield exceeded 80%, resulting in high amounts of immobilized enzymes. The produced system showed the same optimal pH and temperature conditions as free enzymes; however, over a wider range of conditions, the immobilized enzymes showed activity of over 50%. Finally, a reusability study and storage stability tests showed that horseradish peroxidase immobilized on a hybrid made of chitosan, magnetic nanoparticles, and selenium ions retained around 80% of its initial activity after 10 repeated catalytic cycles and after 20 days of storage. Of all the tested materials, the most favorable for immobilization was the above-mentioned chitosan-based hybrid material. The selenium additive present in the discussed material gives it supplementary properties that increase the immobilization yield of the enzyme and improve enzyme stability. The obtained results confirm the applicability of these nanomaterials as useful platforms for enzyme immobilization in the contemplation of the structural stability of an enzyme and the high catalytic activity of fabricated biocatalysts.

Keywords: nanomaterials; support material; horseradish peroxidase; immobilization; catalytic activity; enzyme stability

1. Introduction

Science and industry have become increasingly interested in enzymatic biocatalysts due to their unique characteristics, including their robustness, selectivity, specificity, and efficient kinetics. Furthermore, enzymatic catalysts are characterized by low energy and equipment requirements and are known for their environmentally friendly characteristics [1,2]. Commercializing the use of enzymes meets several challenges, primarily in terms of economic considerations and the difficulty of reusing free enzymes. It is important to note that biocatalysts often have limited structural stability in varying reaction media and

conditions [3]. The immobilization of enzymes can improve their properties by combining them with an insoluble carrier, resulting in heterogeneous biocatalysts. Immobilization also enhances the thermal and storage stability of enzymes and enables them to maintain their activity over a wide pH range in aqueous solutions, as well as in organic solvents. Further, immobilized enzymes can be quickly and easily isolated from the reaction medium, significantly improving their reusability and reducing costs [4,5]. As a result of immobilization, efficient biocatalytic systems can be produced that combine both an enzyme's advantages as well as the physical properties of the selected carrier [6].

A prerequisite for the proper execution of the immobilization process is the selection of a suitable carrier. Substances used as carriers should be inert compounds characterized by high chemical and thermal stability [7]. Over the past years, the development of nanotechnology has provided access to diverse nanomaterials, which, due to their unique properties, have created prospects for use as nanocarriers in enzyme immobilization [8,9]. The availability of various forms of nanomaterials, from branched dendrimers and matrices through fibers and particles to tubes and dots, allows for the selection of a matrix with specific characteristics tailored to the properties of the biocatalysts, resulting in an easier immobilization protocol [10]. Compared to conventional matrices, nanocarriers feature an ease of surface modification, enhanced stability, and reusability [11,12]. Metallic nanoparticles have gained particular recognition due to their high surface-to-volume ratio, strength, reactivity, and chemical inertness [13,14]. One of the examples may be iron oxide nanoparticles, further distinguished by their superparamagnetic properties and biocompatibility, which were discussed by Mohamed et al. [15] to immobilize horseradish peroxidase (HRP). The produced system was characterized by a significant improvement in the stability of the enzyme and the ability to easily separate the product from the medium by magnetic separation, which translated into the preservation of more than 55% of its initial activity after 10 reaction cycles. Titanium dioxide nanoparticles exhibiting UV resistance and photocatalytic properties have also been successfully used in enzyme immobilization. Sahin [16], for instance, used 3-(3,4-dihydroxyphenyl) propionic acid-functionalized TiO₂ nanoparticles as matrices for HRP immobilization. Given their abundant availability and polymeric nature, the use of non-metallic nanoparticles, including biodegradable chitosan or thermally stable silica, has also gained popularity [17]. Melo et al. [18] combined chitosan nanoparticles with polyethylene glycol (PEG) for horseradish peroxidase immobilization. The steps carried out improved biocompatibility and preserved enzyme activity by up to 70%, thus representing promising biocatalytic systems for biomimetic applications. The enormous potential of nanobiocatalysts makes them applicable in the chemical, pharmaceutical, or medical industries, as well as in the production of biofuels, the degradation of harmful substances, and many others [19,20].

Horseradish peroxidase (HRP) is a widely distributed enzyme with well-researched properties and is one of the most influential enzymes in biotechnology. Due to its extensive catalytic activity and wide substrate range, it is capable of carrying out oxidation reactions of a wide variety of organic and inorganic compounds in reactions supported by hydrogen peroxide [21,22]. As a result, HRP is widely used in many applications. Due to these features, biocatalytic systems based on HRP immobilized on nanocarriers are of particular interest [23]. Essential HRP applications include the design of advanced biosensors that detect hydrogen peroxide. For example, Devaraj et al. [24] used graphene oxide–chitosan gold nanocomposites as electrode platforms for the immobilization of HRP to investigate the second generation of enzymatic biosensors. A technique for developing an enzymatic biosensor based on HRP immobilized on single-walled carbon nanotubes treated with acid has been proposed by Sahin [25]. The expansion of the global industry has resulted in a significant increase in the presence of a variety of pollutants in the environment that can negatively impact both human health and the environment. Biocatalytic systems based on HRP immobilized onto nanomaterials have remarkable potential for purifying surface waters and wastewaters of a wide range of impurities, such as dyes, pharmaceuticals, pesticides, phenols, and everyday products [26–29]. A study by Aldhari et al. [26]

demonstrated that HRP immobilized on copper oxide nanosheets is an efficient means of degrading a variety of dyes (crystal violet, methyl green, malachite green). On the other hand, Machałowski et al. [30] used silica nanopowder and 3D fibrous chitin scaffolds from the marine sponge *Aplysina fistularis* as carriers for HRP immobilization, and the implemented biosystem catalyzed almost the 100% degradation of 17α -ethinylestradiol.

The use of HRP offers a wide range of possibilities in the context of biocatalysis development. Developing new nanomaterials suitable for HRP immobilization is one of the greatest challenges that will enable the production of highly active biocatalytic systems for multipurpose applications. Nevertheless, the occurring research gaps related to the broad characterization of hybrid materials and biocatalytic systems, as well as their increased stability and activity, are not fully covered. Many studies show greater stability and the possibility of multiple applications of biocatalysts; nevertheless, the improvement in catalytic activity could be increased. There is also a lack of comprehensive studies on horseradish peroxidase immobilization on various support materials. Hence, in the presented study, it was attempted to obtain novel biocatalytic systems based on single or hybrid nanomaterials as supports for HRP immobilization to determine which material acts as a suitable platform for HRP immobilization with high activity and stability retention. The obtained systems were characterized in terms of their morphology, and their immobilization efficiency, biocatalytic activity, reusability, and storage stability were also determined. In the presented work, the broad characterization of the support materials and biocatalytic systems covers research gaps occurring in the literature, providing data about the use of novel materials with applications as immobilization platforms, as well as improving the stability and activity of the obtained biocatalytic systems.

2. Results and Discussion

2.1. Physicochemical Characterization of Materials before and after Immobilization

Due to a variety of functional groups occurring on the surface of the materials and their high surface capacity, inertness, and accessibility, zeolitic imidazole framework (ZIF-8), graphene oxide, MWCNT, MNP, and CH-M-Se materials were tested as immobilization platforms for the HRP enzyme to obtain biocatalysts with a broad spectrum of applications. It should be noted that each support material is characterized by slightly different chemical/physical phenomena, resulting in varying properties of the immobilized enzymes produced. For instance, the ZIF-8 material is known for its open 3D structure, which limits diffusional limitations and allows easy access to enzymes that might be deposited on the surface of this material into its pores. In contrast, the use of MWCNT or graphene oxide as a support results in an improvement in the transport of electrons between an enzyme and a substrate, which facilitates enzyme activity. These materials are also known for their well-developed surface area, which improves the even distribution of biomolecules. In contrast, MNPs were used due to their magnetic properties, which facilitate the separation of the biocatalysts from the reaction mixture and enhance their reusability. Finally, in the chitosan-coated magnetic nanoparticles doped with a selenium ion (Ch-M-Se) hybrid material, several features were combined to produce a synergistic effect on the enzyme's properties. Chitosan offers biocompatibility as well as hydroxyl and amine functional groups for stable enzyme binding. Magnetic nanoparticles, as mentioned above, support system recovery, whereas the addition of selenium ions improves the activity of the immobilized HRP. A comprehensive characterization of the support materials used and the materials after HRP immobilization was performed in order to clearly examine the suitability of the hybrid materials for their use in immobilization, as well as to confirm effective enzyme binding and determine changes in the support materials after enzyme deposition.

At first, Fourier-transform infrared spectroscopy was applied. The Fourier-transform infrared spectroscopy (FTIR) spectra of the ZIF-8 sample (Figure 1a) show that the broad band visible around $3500\text{--}3000\text{ cm}^{-1}$ comes from the stretching vibrations of O-H groups occurring on the sample's surface, allowing enzyme embedding. Additionally, bands from 2-methylimidazole are visible on the spectrum around 1150 cm^{-1} and 1180 cm^{-1} coming

from the vibrations of the C–N bond, and the signal around 1310 cm^{-1} and 1420 cm^{-1} occurs from the vibration of the C–H bond, which corresponds with the obtained data from Xu et al. [31]. The graphene spectrum (Figure 1b) shows multiple signs of oxygen-derived functional groups occurring in the structure. For example, the band around 1600 cm^{-1} might occur from C=O in carboxylic COOH groups. The band around 1040 cm^{-1} refers to stretching vibrations from the C–O group, and the signal at 1375 cm^{-1} is characteristic of C–OH [32,33]. The FTIR spectrum of the multi-walled carbon nanotubes (MWCNTs, Figure 1c) shows a typical band around 600 cm^{-1} , which corresponds with the bending vibrations of C–C bonds. The weak signal around 1730 cm^{-1} may be associated with the stretching vibrations of bonds from carboxylic groups, suggesting that carboxylic groups could be formed due to the oxidation of some carbon atoms on the surfaces of the MWCNTs [34].

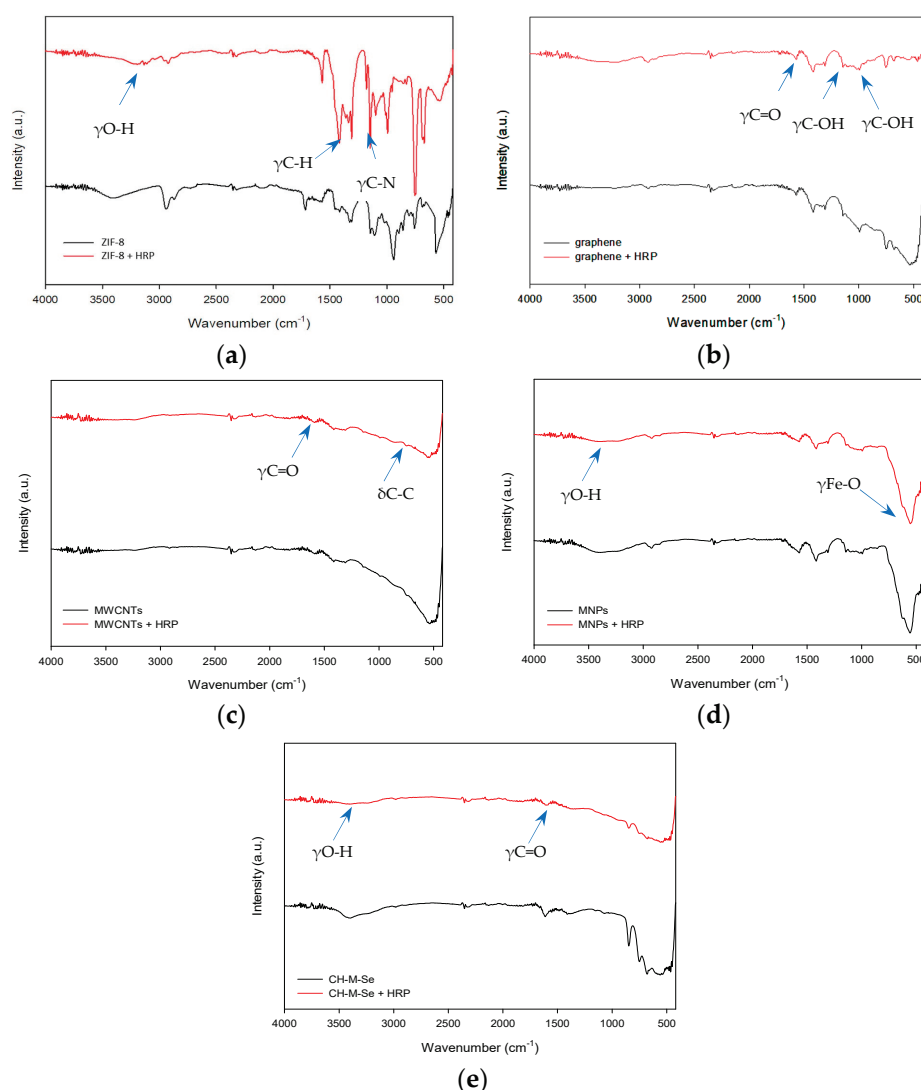


Figure 1. FTIR spectra of pristine hybrid materials and hybrid materials with immobilized enzyme: (a) ZIF-8 carrier before and after HRP immobilization; (b) graphene oxide material before and after HRP immobilization; (c) MWCNT material before and after HRP immobilization; (d) magnetic nanoparticles (MNPs) before and after HRP immobilization; and (e) CH-M-Se carrier before and after HRP immobilization.

For the magnetic nanoparticles (MNPs, Figure 1d), the FTIR spectrum consists of a band around 3440 cm^{-1} from the stretching vibrations of O–H groups occurring on the surface of the material. A typical band confirming the successful material’s synthesis ap-

pears around 584 cm^{-1} , which originates from the stretching vibrations of Fe-O bonds [35]. For the last hybrid material tested, a broad band around 3500 cm^{-1} is visible, showing the presence of the stretching vibrations of O-H bonds occurring on the surface of the material. A typical band around 1740 cm^{-1} appears due to the stretching vibrations coming from the C=O bonds [36]. The functional groups of pristine materials, confirmed with FTIR spectroscopy, show the possibility of enzymes' bonding to the surface of the support. The obtained spectra for pristine materials compared with the spectra of materials with immobilized enzymes do not significantly differ; nevertheless, the changes that occurred indicate the successful deposition of the biocatalyst on the support surfaces. For the materials after the immobilization process, the characteristic bands coming from vibrations of amide I, II, and III bonds are slightly visible around 1645 , 1550 , and 1250 cm^{-1} , which are typical for the spectra of samples containing enzymes and confirm the successful immobilization [37]. Moreover, the broad band around 3500 cm^{-1} after immobilization is less intense in the analyzed samples, suggesting enzyme deposition mainly via hydroxyl groups.

In the next step, a thermogravimetric analysis was performed for all the materials (Figure 2). The obtained thermogravimetric analysis (TGA) curves of all the samples show a lack of great mass loss until $100\text{ }^{\circ}\text{C}$, which corresponds to the absence of entrapped water in pores. For the MWCNT and MNP samples, the mass loss for pristine materials and the obtained biocatalysts does not exceed 15%. This shows the great stability of the obtained materials and biocatalysts produced based on these materials. In contrast, the obtained TGA for the graphene-based biocatalysts shows a greater mass loss compared to the pristine materials due to the high enzyme loading and its degradation at high temperatures [38]. For the rest of the samples, the mass loss of the biocatalysts is comparable to that of the support materials, leading to the conclusion that the obtained biocatalysts are resistant to high temperatures and the mass of the enzyme loaded is lower; hence, the effect of the thermal degradation of the enzyme backbone on the mass of the sample is less pronounced. In comparison, the (3-aminopropyl)triethoxysilane (APTES) functionalized MNPs presented by Keshta et al. [38] display a mass loss of around 25% and 15% compared to the pristine and modified support materials, which correspond to the ones presented in the article. A greater mass loss at around $100\text{ }^{\circ}\text{C}$ may be related to the physically adsorbed water present in the material. On the other hand, Song et al. [39] used ZIF-8 nanoparticles for enzyme deposition. The free HRP's mass loss was greater at lower temperatures than that of the pristine support and the obtained biocatalyst. The results obtained confirmed the stabilizing effect of the support and its inertness at high temperatures.

Both pristine materials and the obtained biocatalytic systems after HRP immobilization were also analyzed using scanning electron microscopy (SEM, Figure 3) and by determining changes in the particle size of the support before and after immobilization (Table 1). This allowed for an evaluation of the morphological structures of the applied carriers and a partial confirmation of the immobilization process. The resulting SEM images of all the materials are shown in Figure 3. Additionally, Table 1 shows the average particle size and the particle size distribution of the initial materials and the obtained biocatalytic systems. The SEM images of the ZIF-8 material (Figure 3a) show the crystalline structure of the support, which has overlapping crystals with an average particle size of about 290 nm. It is possible to observe distinct clusters of enzymes on the surface of the support after immobilization (Figure 3b), which is confirmed by the increased average particle size of the resulting biocatalytic system (approximately 440 nm). ZIF-8 is an attractive material for immobilizing enzymes, as demonstrated in a study by Stanišić et al. [40], which showed the successful immobilization of HRP on this support mainly due to well-fitted particles. Figure 3c presents the morphology of pure MWCNTs. The presence of bundles of intertwined tubes with inhomogeneous lengths of a few micrometers is characteristic of this carrier. In the MWCNT material, its morphology allows enzymes to be efficiently immobilized since there are many open spaces where the enzymes can be localized onto and into the support's structure. It is possible to notice in Figure 3d that a thin layer of enzyme was formed on the surface of the support after HRP immobilization. A similar

distribution of the enzyme on the surface of MWCNTs is shown in studies conducted by Duarte et al. [41], confirming our assumptions on MWCNTs' suitability as supports for HRP.

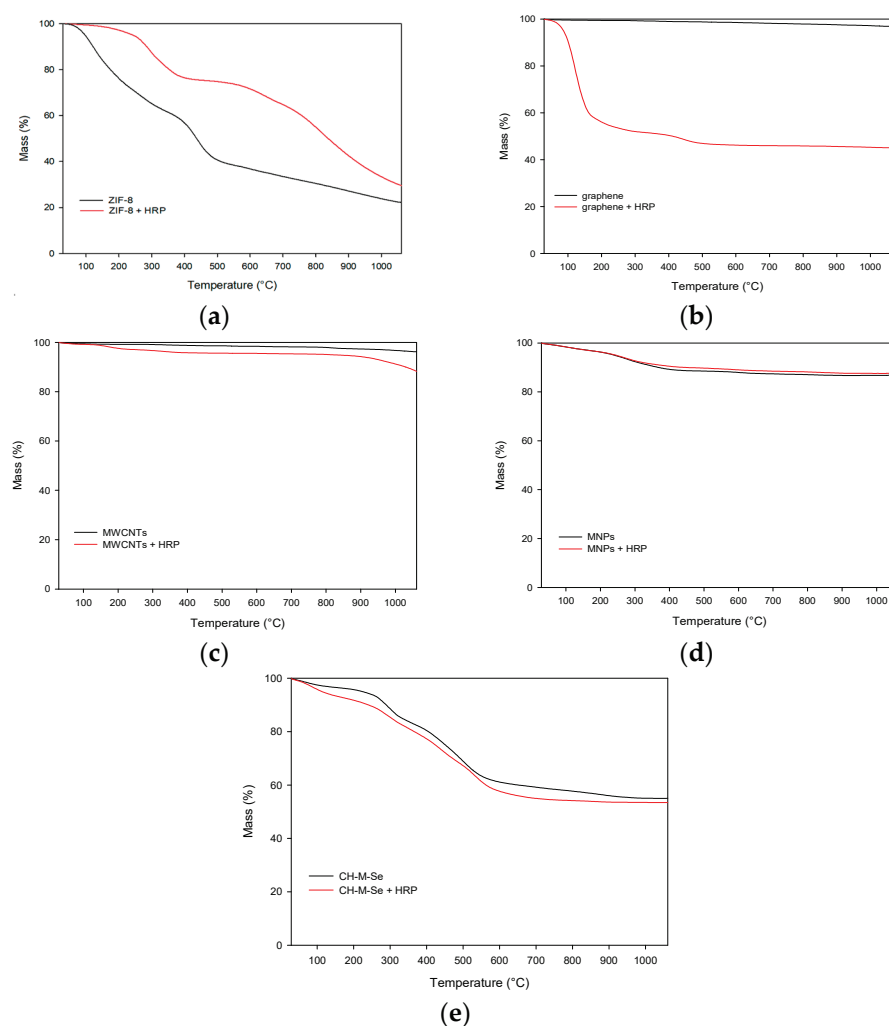


Figure 2. Results of thermogravimetric analysis of pristine hybrid materials and hybrid materials with immobilized enzyme: (a) pristine ZIF-8 carrier before and after HRP immobilization; (b) graphene oxide material before and after HRP immobilization; (c) pristine MWCNT material before and after HRP immobilization; (d) pristine MNPs before and after HRP immobilization; and (e) pristine CH-M-Se carrier before and after HRP immobilization.

Table 1. The average particle size of the support materials before and after HRP immobilization.

Type of Material	Sample	Average Particle Size (nm)	Particle Size Range (nm)
Pristine materials	ZIF-8	287	105–615
	graphene	630	458–1718
	MWCNTs	62	5.6–141
	CH-M-Se	832	190–2801
	MNPs	283	141–396
Materials after HRP immobilization	ZIF-8	443	255–825
	graphene	806	615–1281
	MWCNTs	112	17–198
	CH-M-Se	973	295–2969
	MNPs	372	37–643

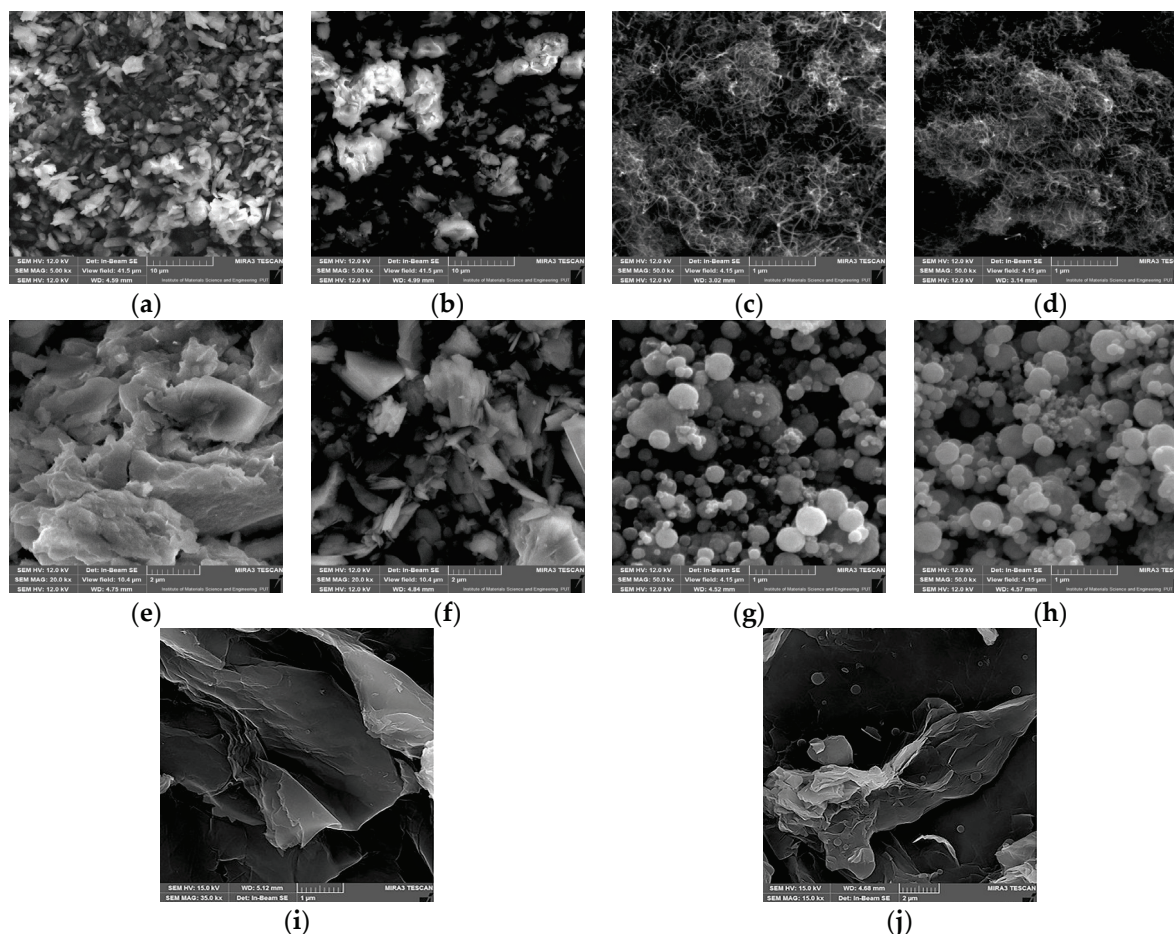


Figure 3. SEM images: (a) pristine ZIF-8 carrier; (b) ZIF-8 carrier after HRP immobilization; (c) pristine MWCNT material; (d) MWCNTs after HRP immobilization; (e) CH-M-Se carrier; (f) CH-M-Se carrier after HRP immobilization; (g) MNPs; (h) MNPs after HRP immobilization; (i) graphene oxide material; and (j) graphene oxide after HRP immobilization.

Figure 3e presents an SEM image of the pristine chitosan-coated magnetic nanoparticles doped with selenium ions. Chitosan is characterized by its smooth, non-porous structure, which is made up of crystals and microfibers of varying sizes. An analysis of the resulting biocatalytic system (Figure 3f) shows that HRP enzymes are present both as agglomerates and as single molecules. Figure 3g shows SEM images of the magnetic nanoparticles, which are characterized by a homogeneous distribution and spherical shape. The particle size of the used MNPs is quite narrow and ranges between 141 and 396 nm, while the average size is about 280 nm. As a result of dipole–dipole interactions between the Fe_3O_4 nanoparticles, this material also exhibits an agglomeration of these particles. After the immobilization of the HRP enzyme, there are no significant changes in the morphology of the support (Figure 3h), which is consistent with the observations of Gong et al. [42]. Note, however, that particles bigger than 100 nm were identified in the material upon immobilization, clearly indicating HRP deposition onto the surface of the MNPs. A morphological analysis of graphene oxide's structure (Figure 3i) shows its layered structure. The layers have irregular shapes and different sizes (particle sizes between 458 and 1718 nm). Also typical for this support is the presence of pronounced distortions, which form rough graphene oxide surfaces and are due to the presence of oxygen groups in its structure. Figure 3j provides information on the morphology of the biocatalytic system formed after the immobilization of HRP on graphene oxide. It shows the deposition of enzyme layers on the surface of graphene oxide sheets and the presence of larger agglomerates of the enzyme, supported also by the results of particle size distribution, which showed an increase in

the average particle size distribution upon immobilization. These data stay in agreement with the results presented by Besharati et al. [43] and Devaraj et al. [24]. However, HRP immobilization using graphene oxide is not widely presented in the literature.

The scanning electron microscopy analysis provides information regarding the structure and morphology of the materials, indicating that all the materials are suitable for use as enzyme carriers. In addition, the immobilization process does not affect the structure of the materials, which allows them to retain their specific characteristics even after enzyme deposition, making the produced systems stable and resistant [44,45]. However, due to enzyme binding, in most cases, an increase in the particle size of the support materials occurred, which confirms efficient HRP immobilization.

The energy-dispersive X-ray microanalysis (EDS) provided valuable information on the elemental composition of the obtained materials as well as the resulting biocatalytic systems after HRP immobilization (Table 2). For example, the EDS results can be used to verify the chemical composition of the MNPs, whose high iron content (above 60%) confirms the correct synthesis process. The presence of selenium in the CH-M-Se material also confirms the incorporation of selenium nanoparticles and the formation of the desired hybrid material. Furthermore, the immobilization of the enzymes increases the oxygen content of all the resulting biocatalytic systems due to the presence of many oxygen groups in the enzyme structure. In materials with immobilized enzymes, there is also an increase in nitrogen content. The work of Işık [46] noted a significant increase in oxygen and nitrogen content after immobilizing chitosan beads. Similarly, Zdart et al. [47] observed that enzyme deposition led to an increase in carbon and oxygen content, as well as the appearance of nitrogen due to the presence of an enzyme immobilized on the carrier.

Table 2. Elemental composition of carriers before and after immobilization.

Samples	Content (%)							
	C	N	O	S	Mn	Fe	Zn	Se
ZIF-8	42.04	6.49	30.27	-	-	0.01	21.16	-
ZIF-8 + HRP	42.19	10.78	30.78	-	0.02	0.03	16.18	-
graphene	97.38	-	2.17	0.25	0.01	0.19	0.01	-
graphene + HRP	82.32	-	17.19	0.21	0.03	0.22	0.02	-
MWCNTs	91.83	-	7.92	0.02	-	0.23	-	-
MWCNTs + HRP	85.11	-	14.73	0.01	-	0.15	-	-
CH-M-Se	15.99	0.13	32.45	0.10	26.87	2.37	0.01	22.08
CH-M-Se + HRP	12.72	0.15	33.25	0.05	29.75	3.09	-	20.98
MNPs	10.24	-	29.21	-	0.03	60.51	-	-
MNPs + HRP	24.79	-	32.12	0.02	0.03	43.02	0.03	-

ZIF-8 is characterized by its easy preparation and biocompatibility and has been previously demonstrated to be an efficient carrier for enzyme immobilization. Within the performed experiments, small particles with visible crystal structures of the ZIF-8 material were visualized using the atomic force microscopy (AFM) technique (Figure 4a). This method enables a non-destructive analysis of the carrier surface topography and the characterization of immobilized enzyme molecules on the carriers' surfaces [48]. According to the photographs of the ZIF-8 surface after HRP immobilization, this process resulted in a rounding of the carrier particle shape and a smoothing of their edges. Visible changes in the particle morphology and the increased size of the carrier particle agglomerates prove the sufficient enzyme immobilization on the tested material. It is worth noting that the ZIF-8 material has been tested for the efficient immobilization of various enzymes, e.g., pepsin, as performed by Yang et al. [49]. The next tested material, multi-walled carbon nanotubes, is an important class of new materials with numerous useful properties, including suitability for enzyme immobilization. The performed experiments resulted in the deposition of the enzyme molecules on the surface of the nanotubes as well as inside the tubes, which was compared to the pure carrier (Figure 4b,d).

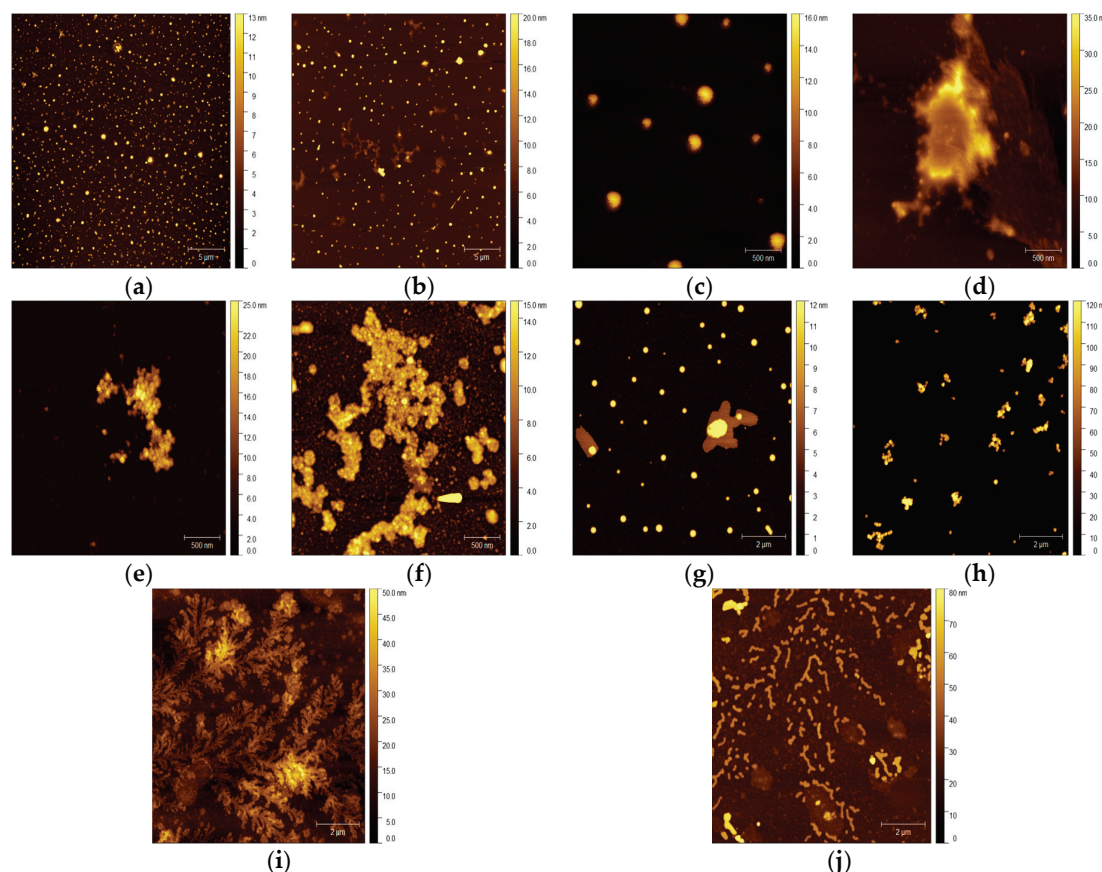


Figure 4. AFM images: (a) pristine ZIF-8 carrier; (b) ZIF-8 carrier after HRP immobilization; (c) pristine MWCNT material; (d) MWCNTs after HRP immobilization; (e) CH-M-Se carrier; (f) CH-M-Se carrier after HRP immobilization; (g) MNPs; (h) MNPs after HRP immobilization; (i) graphene oxide material; and (j) graphene oxide after HRP immobilization.

The observed increases in the nanotube size align with the findings of Rastan et al. [50], who employed MWCNTs for lipase immobilization. They also applied MWCNT surface functionalization for better incorporation of the enzyme, an approach that was tested in our study in the case of the selenium-functionalized material Ch-M-Se. The presence of these ions increases the stability of the biocatalyst, resulting in better properties for the created system. Comparing the pictures of the pure and enzyme-loaded Ch-M-Se carriers (Figure 4e,f), one can see changes in the carrier surface topography after immobilization, as well as a higher tendency to form aggregates. This was also confirmed by the particle size investigation of the support materials before and after HRP immobilization. Another class of materials with possible applications in enzyme immobilization are magnetic nanoparticles, which, due to their magnetic properties, enable the easy separation of the carrier from the reaction mixture [51]. This feature makes them a promising tool for industrial bioprocess applications. Our study confirmed changes in the MNPs' behavior after the immobilization (Figure 4h), showing a higher preference for self-association. The increase in the size of the tested MNPs is in line with reports by Mendes et al. [52] and Arredondo et al. [53], who tested various magnetic carriers in tyrosinase immobilization. Finally, graphene oxide (GO), a cheap and easy-to-process material that can be viewed as a single atomic layer of graphite with various oxygen-containing functionalities, was applied to HRP immobilization. Comparing the topography of the native and HRP-immobilized graphene oxide surfaces, a significant increase in the height of the surface can be seen, which confirms the effective deposition of the enzyme on the surface of the material. Regarding the analysis presented by Zhang et al. [54], the determinants of the conformation of the immobilized enzyme are the interactions between the functional groups of GO and HRP. The presented results

confirm that AFM is a powerful tool for analyzing material topography modifications after enzyme immobilization. In this case, this technique allows for the confirmation of effective HRP immobilization on matrices with various properties and applications, supporting results from other experiments.

2.2. Biocatalytic Characterization of the Produced Systems

The immobilization process was carried out under the most favorable conditions, and to confirm the effectiveness of the produced biocatalytic systems, the process yield, the amount of immobilized enzyme, and the retained catalytic activity were determined (Table 3). The process of HRP immobilization on all the tested supports occurred with an efficiency of at least 69%, regardless of the process parameters used, with the maximum at around 85–90%. The highest efficiencies were recorded for the processes using MWCNTs and CH-M-Se materials, which were 89.2% and 84.9%, respectively. Further, the amount of immobilized enzyme was examined to determine its loading and possible distribution within the support structures. The process occurs most efficiently at the initial stage, when the availability of active sites on the surface of the carrier is the highest. The MWCNTs and CH-M-Se also turned out to be matrices that allowed for the attachment of as much as 44.6 mg and 42.5 mg per 1 g of carrier, respectively. These nanomaterials exhibit a unique combination of stiffness and strength compared to other fibrous materials and offer ideal properties such as resistance to weight transfer and large surface areas. However, the amount of immobilized enzyme is not everything, as it is crucial for the production of effective biocatalytic systems that the immobilized protein maintains high activity. Analyzing the data in Table 3, it can be concluded that the produced systems retain high activity, which is about 80%. The exception is graphene, which was characterized by the lowest activity of immobilized HRP (62%). In contrast, CH-M-Se distinguished itself from the other materials by retaining catalytic activity exceeding 90%. Selenium's favorable properties, such as high biological activity, non-toxicity, and good bioavailability, have made the element increasingly popular. Moreover, the combination of chitosan and magnetic nanoparticles in the structure of this hybrid support facilitates easy substrate/product flow and reduces possible diffusional limitations. For example, Nonsuwan et al. [55] used selenium to develop organic–inorganic hybrid pullulan/SeNPs microflowers, which exhibited an excellent enzyme adsorption of 142.2 mg/g. Taking all parameters into account, the process of HRP immobilization on the proposed materials can be considered efficient and allows for the immobilization of significant amounts of biocatalyst on the surface of the carrier, supported by high activity recovery. CH-M-Se is the carrier with the most favorable properties for binding the HRP enzyme. Chitosan-based nanofibers are characterized by increased porosity, excellent mechanical properties, and a high surface-to-volume ratio, and the addition of selenium gives these carriers unique properties that can improve enzyme immobilization efficiency and enzyme stability.

Table 3. Results of immobilization yield, amount of immobilized enzyme, and activity recovery.

Sample	Immobilization Yield (%)	Amount of Immobilized Enzyme (mg/g)	Activity Recovery (%)
ZIF-8	69.8	34.9	81.2
graphene	84.6	42.3	62.7
MWCNTs	89.2	44.6	87.3
CH-M-Se	84.9	42.5	91.2
MNPs	74.3	37.1	83.8

An undoubtedly distinguishing feature of the immobilized enzymes is the improvement in their stability and potential for reuse. To determine the reusability of the produced biosystems, 10 consecutive reaction cycles were carried out, the results of which are summarized in Figure 5a. Analyzing the data presented, it can be seen that 100% of activity

was retained only after the first cycle, and this gradually decreased with each subsequent cycle. All the materials were good matrices for HRP immobilization, as they retained their activity after 10 cycles, but clear percentage differences were noted. The lowest activity was retained by graphene and the MNPs, at levels of 39% and 46%, respectively, while the highest potential was shown by CH-M-Se, retaining its activity at 73%. Such a high percentage result may indicate the formation of stable bonds between the enzyme and the surface of the carrier, which effectively prevents the leaching of HRP. The addition of selenium further increases the stability of the enzyme and makes the system more resistant to varying process conditions and repeated use, as the selenium ions show a positive effect on HRP activity. Finally, the addition of magnetic nanoparticles as a part of the hybrid support strongly enhances the operability of the immobilized system because the simple separation of the system from the post-reaction mixture is possible, which reduces the number of operational stages negatively affecting the enzyme stability. The storage stability of the biocatalytic systems and the free enzyme was also investigated. The results of these tests are shown in Figure 5b. A decrease in the activity of the free HRP was observed from the first days of testing, and this eventually led to the preservation of only about 46% of the activity after 30 days. The loss of activity over one month for the immobilized enzyme was significantly less. All the support materials tested significantly improved the retained activity, and these results confirm the benefits of immobilization in increasing enzyme stability. The best results were achieved for the CH-M-Se and ZIF-8 materials, as the HRP immobilized on their surfaces retained activity at 84% and 80%, respectively, after 30 days of storage. Similar results were also observed by Xie et al. [56], who observed that after 60 days of storage, the activity of HRP immobilized using the multi-arm magnetic composite $\text{Fe}_3\text{O}_4\text{@PAA-6-arm-PEG-NH}_2$ was 71% higher than that of the free enzyme, which also confirms the protective properties of the carrier for HRP. All the examples cited confirm the validity of using carriers as scaffolds that stabilize the structure of enzymes and exhibit protective functions against catalytic proteins, thus limiting conformational changes of proteins during storage. Table 4 presents a comparison of results from previous studies with the results presented in this study to highlight the importance of the obtained results. It should be added that, compared to traditional enzyme carriers, the use of Ch-M-Se and MWCNT materials characterized by unique properties translates into effective enzyme immobilization and improved stability. The use of these matrices ensures the preservation of high enzymatic activity and provides the possibility of reusable systems, which affects the cost-effectiveness of the process. The structure of MWCNTs additionally provides a large enzyme contact area, which increases the efficiency of enzymatic reactions, while Ch-M-Se materials exhibit excellent biocompatibility, which promotes the long-term stability of biocatalytic systems. Despite the higher cost of Ch-M-Se material synthesis as compared to other simple and commercially available support materials the significant benefits of using it as a carrier for enzyme immobilization make the resulting biocatalytic systems economically viable solutions at the laboratory scale. Nevertheless, deeper studies on the cost-effectiveness of the entire biocatalytic process supported by the immobilized HRP are required, which will facilitate the translation of the proposed system onto a larger scale.

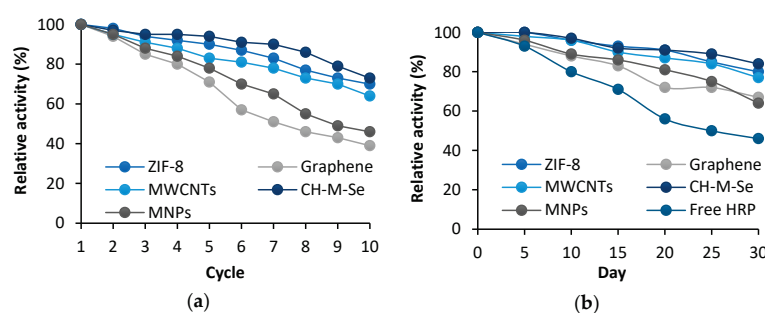
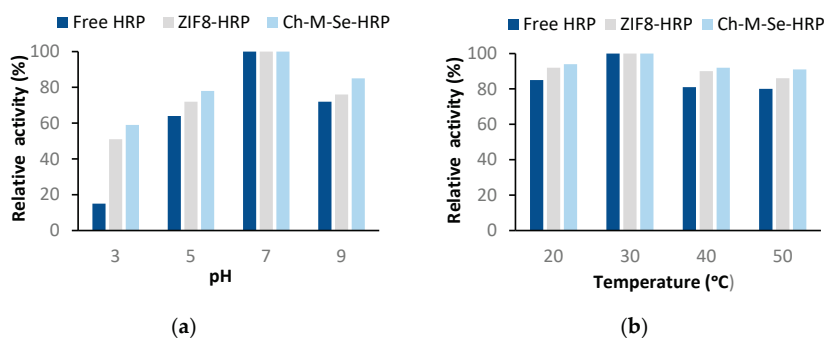


Figure 5. Reusability (a) and storage stability (b) of HRP immobilized using tested support materials. The results are the mean values of three repeated experiments with an error value of less than 5%.

Table 4. Comparison of the HRP immobilization results obtained with those obtained from previous studies.

Carrier	Immobilization Method	Immobilization Efficiency (%)	Activity Recovery (%)	Reusability	Optimum pH and Temperature	Reference
MNPs	adsorption	7	100	55% after 10 cycles	7.5 50 °C	[15]
MNPs	adsorption	74.3	83	46% after 10 cycles	7 30 °C	present study
graphene	covalent bonding	30	90	70% after 10 cycles	7 50 °C	[43]
graphene	adsorption	84.6	61	39% after 10 cycles	7 30 °C	present study
ZIF-8	encapsulation	98.4	89	>80% after 6 cycles	6.5 70 °C	[57]
ZIF-8	adsorption	69.8	81	70% after 10 cycles	7 30 °C	present study
MWCNTs	covalent bonding	81	80	not available	7 30 °C	[58]
MWCNTs	adsorption	89.2	87	70% after 10 cycles	7 30 °C	present study
CH-M-Se	adsorption	84.9	91	73% after 10 cycles	7 30 °C	present study

After the evaluating the stability potential of the immobilized HRP and characterizing the resulting biocatalytic systems, it is also important to determine its activity changes under different process conditions. In this section, we decided to present only systems obtained using the chitosan-based hybrid material and ZIF-8 materials, because when using these supports, the highest activity recovery and stability were attained. Analyzing the effect of pH and temperature on the retained catalytic activity of the systems (Figure 6), one can see the greater tolerance of the immobilized enzyme to changing conditions. The only exception where identical profiles and the highest activity were obtained for the free and immobilized HRP is at 30 °C and a pH of 7, hence, at the optimal process conditions. Outside these conditions, the free HRP showed lower activity and was found to be more prone to inactivation. More favorable results for the immobilized enzyme were achieved when using the Ch-M-Se material as a support. This system showed over 80% activity over a pH range of 5 to 9 and over the whole analyzed temperature range, clearly showing the most stabilizing and protective effect of this support on the HRP structure. This is related to the fact that chitosan contains amine and hydroxyl groups in its structure that support stable enzyme binding, even including multipoint attachment, which makes the enzyme structure more rigid and stable under harsh process conditions.

**Figure 6.** Effect of pH (a) and temperature (b) on the relative activity of free and immobilized HRP on support materials. The results are the mean values of three repeated experiments with an error value of less than 5%.

3. Materials and Methods

3.1. Materials and Reagents

Graphene, multi-walled carbon nanotubes, zeolitic imidazole framework, magnetic nanoparticles, and chitosan-magnetic nanoparticles doped with selenium ions hybrid material were synthesized by the members of the Division of Chemical Technology at the Faculty of Chemical Technology, Poznan University of Technology and were used as delivered, without any further purification. Horseradish peroxidase (EC 1.11.1.7) (HRP), hydrogen peroxide solution (30%), 50 mM phosphate buffer at pH 7, Bradford reagent, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were delivered by Sigma-Aldrich (St. Louis, MO, USA).

3.2. HRP Immobilization

The immobilization of horseradish peroxidase (HRP) using various materials was carried out as follows. Precisely, 100 mg of the selected carrier material was weighed, and an HRP solution with a concentration of 0.5 mg/mL (10 mL) was added. The process lasted for 24 h at room temperature and pH 7, and the samples with the carrier and enzyme solution were mixed at a speed of 300 rpm in a thermomixer. After immobilization, the samples were centrifuged. The resulting precipitate was allowed to dry at room temperature, while the solution above the sediment was analyzed to determine the content of the unbound enzyme and calculate the process efficiency.

3.3. Physicochemical Characterization of the Produced Systems

The morphology of the used materials before and after immobilization was determined based on SEM images (EVO40, Zeiss, Oberkochen, Germany) after being coated by gold (Balzers PV205P, Balzers, Switzerland). An energy-dispersive X-ray microanalysis (EDS) was carried out using the EDS analyzer contained in the scanning electron microscope (Tescan machine (Tescan, Switzerland) with Gamma-Tec tooling from Princeton Inc. (Princeton, NJ, USA). Before the analysis, the samples were attached to the base with a carbon paste or tape. The FTIR spectra of the samples were made using a Vertex 70 spectrometer (Bruker, Bremen, Germany). The samples were analyzed in the form of KBr pellets in the wavenumber range 4000–400 cm^{-1} at a resolution of 0.5 cm^{-1} with 64 scans per sample. An AFM (Park NX10, Park Systems Corp., Suwon, Korea) was implemented in the experiments to determine changes in the topography of the support surface before and after immobilization. Topography measurements were performed in non-contact mode with an All-in-One cantilever, type D (BudgetSensors, Sofia, Bulgaria), with a nominal resonance frequency of 350 kHz and a nominal force constant of 40 N/m. The scan size was set to $10 \times 10 \mu\text{m}^2$ with a sampling of 512 lines for each image and a scan rate of 0.4–0.5 Hz. For each sample, at least three measurements were performed at different positions. The measurements were performed in air at room temperature. The results of the imaging were further investigated using Gwyddion v 3.05 version open-source software. Particle size distributions were determined using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern UK), enabling measurements of particle diameters in the range 0.6–6000 nm based on a noninvasive backscattering technique, NIBS. The measurement involves transmitting a red laser beam with a wavelength of 663 nm through the material. A thermogravimetric analysis (TGA) of the materials before and after immobilization was performed using a Jupiter STA 449F3 apparatus (Netzsch, Selb, Germany). Measurements were carried out over the temperature range of 25–1000 °C under nitrogen flow (10 mL/min) at a heating rate of 10 °C/min, with an initial 10 mg mass of the sample.

3.4. Biocatalytic Characterization of the Produced Systems

The activity and stability of the free and immobilized HRP were examined based on a model reaction using ABTS as a substrate by measuring the rate of formation of ABTS*. Briefly, 10 mg of free enzyme or an appropriate amount of the biocatalytic system containing 10 mg of immobilized HRP was added to the solution containing 15 mL of

100 mM ABTS solution in 50 mM phosphate buffer at pH 7.0 and 1 mL of 5 mM hydrogen peroxide. The process was carried out for 10 min at 30 °C, and the progress of the oxidation reaction was measured with a Jasco V-750 (Tokyo, Japan) spectrophotometer at $\lambda = 420$ nm. One unit of free and immobilized HRP activity was defined as the amount of enzyme that produces 1 mM of ABTS* per minute under assay conditions.

The amount of immobilized enzymes on tested support materials was determined according to the Bradford method by considering the mass of the immobilized enzyme and the mass of the support material. After immobilization, the solutions were mixed with Bradford reagent in a 0.5 mL:0.5 mL ratio. Following 10 min of incubation, the absorbance was measured at 595 nm, and the HRP concentration was determined using a standard curve of BSA. The immobilization yield and activity recovery were calculated according to Equations (1) and (2):

$$\text{Immobilization yield (\%)} = \frac{A_i - A_f}{A_i} \times 100\% \quad (1)$$

$$\text{Activity recovery (\%)} = \frac{A_t}{A_i} \times 100\% \quad (2)$$

where A_i denotes the initial activity of the HRP used for immobilization, A_f denotes the total activity of the enzyme in the supernatant, and A_t denotes the activity of the immobilized HRP. The activities of the supernatant and reference samples were determined under the standard conditions based on the above-presented reaction.

The effect of pH on the activity of the immobilized HRP was examined according to the above-mentioned ABTS reaction at 30 °C over a pH range of 3 to 9, whereas the effect of temperature was examined over a temperature range of 20 to 50 °C at pH 7.

The reusability of the produced system was examined for over 10 consecutive catalytic cycles using ABTS as a model substrate. The ABTS relative conversion efficiency was calculated based on the final concentration of ABTS free radicals after the process. After each cycle, the system tested was washed with 50 mM of phosphate buffer at pH 7.0 and placed in a fresh substrate solution. The storage stability of the free and immobilized HRP stored at 4 °C in phosphate buffer (pH 7.0) was tested for over 30 days. The relative activity was determined over time based on the ABTS model oxidation reaction. For analyzing the storage stability and reusability, the initial activity was defined as 100% activity. All measurements were conducted in triplicate, and the results are presented as mean value $\pm$ standard deviation.

4. Conclusions

This study synthesized and tested novel biocatalytic systems based on various hybrid and single materials as successful platforms for enzyme deposition. The morphological analysis, stability, and activity tests were performed to fully characterize the successful biocatalysts' deposition and their catalytic activity. Based on the morphological analysis of the obtained biocatalytic samples, such as the FTIR, TGA, and AFM images, the synthesis of the materials and immobilization were proven. A further indication of the success of the immobilization process was the increase in particle size following the immobilization of the enzyme. The analyses carried out using UV-Vis spectroscopy confirmed the ability to apply significant amounts of the enzyme to the surface of the carriers. The immobilization of HRP on all the tested supports occurred with an efficiency of at least 69%, and for the MWCNT and CH-M-Se materials, the efficiency reached up to 90%. The obtained results showed that the retained activity was high at around 80%, reaching over 90% for CH-M-Se. Considering the results of all the above analyses, it was concluded that CH-M-Se was the carrier with the most favorable properties for binding the HRP enzyme. The addition of selenium gives these nanomaterials unique properties that translate into efficient enzyme immobilization. Also, the storage stability tests and analyses of the reusability of the biocatalytic systems showed the most favorable results for the enzyme immobilized on CH-M-Se, which clearly

confirms the suitability of using these nanomaterials as scaffolds that stabilize the structure of HRP and show protective functions against catalytic proteins. The presented studies demonstrate the applicability of the proposed hybrid and single materials as platforms for the successful synthesis of biocatalytic systems. The developed systems exhibit a broad spectrum of potential applications due to their high catalytic activity and reusability, hence promoting the growth of the green and sustainable technologies industry.

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Abbreviations

ABTS	(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))
AFM	Atomic force microscopy
APTES	(3-Aminopropyl)triethoxysilane
Ch-M-Se	Chitosan-magnetic nanoparticles doped with selenium ions hybrid material
EDS	Energy-dispersive X-ray microanalysis
FTIR	Fourier-transform infrared spectroscopy
GO	Graphene oxide
HRP	Horseradish peroxidase
MNPs	Magnetic nanoparticles
MWCNTs	Multi-walled carbon nanotubes
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
TiO ₂	Titanium dioxide
ZIF-8	Zeolitic imidazole framework

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Article

The Effect of pH on the Viscoelastic Response of Alginate–Montmorillonite Nanocomposite Hydrogels

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Abstract: Ionically cross-linked alginate hydrogels are used in a wide range of applications, such as drug delivery, tissue engineering, and food packaging. A shortcoming of these gels is that they lose their strength and degrade at low pH values. To develop gels able to preserve their integrity in a wide range of pH values, Ca-alginate–montmorillonite nanocomposite gels are prepared, and their chemical structure, morphology, and mechanical response are analyzed. As the uniformity of nanocomposite gels is strongly affected by concentrations of MMT and CaCl₂, it is revealed that homogeneous gels can be prepared with 4 wt.% MMT and 0.5 M CaCl₂ at the highest. The viscoelastic behavior of nanocomposite gels in aqueous solutions with pH = 7 and pH = 2 is investigated by means of small-amplitude compressive oscillatory tests. It is shown that Ca-alginate–MMT nanocomposite gels preserve their integrity while being swollen at pH = 2. The experimental data are fitted by a model with only two material parameters, which shows that the elastic moduli increase linearly with a concentration of MMT at all pH values under investigation due to formation of physical bonds between alginate chains and MMT platelets. The presence of these bonds is confirmed by ATR-FTIR spectroscopy. The morphology of nanocomposite gels is studied by means of wide-angle X-ray diffraction, which reveals that intercalation of polymer chains between clay platelets increases the interlayer gallery spacing.

Keywords: alginate; hydrogel; montmorillonite; nanocomposite; mechanical properties

1. Introduction

Applications of biopolymeric hydrogels in controlled drug delivery have recently received significant attention [1–4]. The pH sensitivity of gels is crucial for the design of drug release systems to treat gastric diseases, prevent infections, or deliver cancer therapy in the acidic environment of tumors [5–8]. The shortcomings of some biopolymeric hydrogels are their decreased stability, degradation, and erosion in aqueous solutions with low pH values. These phenomena affect their ability to retain drugs and reduce their effectiveness as drug release systems.

Ca-alginate hydrogels are commonly used materials for drug delivery with versatile applications [9–11]. However, the range of their applications is limited to weak acidic conditions with pH > 5.5 only [12,13]. When Ca-alginate gels are exposed to strong acidic environments, calcium ions that cross-link alginate chains in the gel matrix are replaced with protons, which leads to disintegration of the gel structure [14]. This hinders applications of Ca-alginate gels in pH below the pK_a of alginate chains (the value of pK_a belongs to the interval between 3.6 for mannuronic acid and 3.8 for guluronic acid [15]).

Montmorillonite (MMT) is a natural clay mineral known for its high surface area and its capacity for cation exchange. These properties make it valuable to modulate the release of pharmaceuticals [16–18]. The incorporation of MMT into biopolymer gels

improves the moisture barrier properties [19], enhances the pervaporation dehydration performance [20,21] and the entrapment efficiency of drugs [22], and enhances the mechanical properties of alginate gels [19,23,24]. The biocompatibility of MMT and alginate makes their nanocomposites suitable for biomedical applications [11,25]. Chemical modification of MMT clay before mixing with biopolymers enhances their biocompatibility, the mechanical strength of the composites, and their structural integrity [20,21].

The objective of this study is twofold. Our first purpose is to find suitable ranges of MMT and CaCl_2 concentrations that allow homogeneous alginate–MMT nanocomposite gels to be prepared with repeatable mechanical responses. The uniformity of the gels is important because enhancement of the mechanical properties of MMT biopolymer gels depends strongly on the uniform dispersion of clay [20]. When an alginate gel contains relatively high amounts of MMT and CaCl_2 (which both serve as cross-linkers in its polymer networks), the gel becomes inhomogeneous [26]. Nanocomposite biopolymer gels were prepared in previous studies [27–29] with concentrations of MMT up to 5 wt.%.

Secondly, cross-linking of alginate with MMT and CaCl_2 leads to the formation of two different types of physical bonds: between alginate chains and MMT platelets, and between alginate chains bridged by Ca^{2+} ions [30]. Our objective is to investigate the difference between these bonds and their influence on the mechanical response characterized by dynamic mechanical analysis (DMA). For this purpose, alginate–MMT hydrogels were prepared with different MMT concentrations at $\text{pH} = 7$. Afterwards, the gels were immersed in aqueous solutions with $\text{pH} = 2$. It is known that bonds between alginate chains and Ca^{2+} ions are partially broken upon immersion of the gels in acidic environments with $\text{pH} = 2$ [14].

To analyze the effect of pH of aqueous solutions on the viscoelastic response of nanocomposite hydrogels, we focus on solutions with $\text{pH} = 2$ and $\text{pH} = 7$ (below and above the pK_a of alginate chains). $\text{pH} = 2$ was chosen because this pH value is typical for biomedical applications related to gastric diseases. Experimental data for the storage and loss moduli in small-amplitude oscillatory tests (the frequency-sweep mode) are matched simultaneously by a simple model in linear viscoelasticity with two material parameters. Fitting shows that the elastic modulus of the polymer network increases linearly with concentration of MMT platelets at both pH values under investigation, whereas a measure of inhomogeneity of the network remains practically independent of the concentration of nanofiller. An increase in the elastic modulus of the network with mass fraction of MMT at normal $\text{pH} = 7$ is explained by electrostatic interactions between negatively charged COO^- groups of alginate chains and positively charged edges of clay platelets. The growth in the elastic modulus with concentration of MMT at $\text{pH} = 2$, when all the carboxyl groups are protonated and electrostatic interactions disappear, is ascribed to formation of hydrogen bonds between neutral COOH groups of alginate chains and OH groups attached to the surfaces of MMT platelets.

The novelty of this study consists of the following: (i) limits are determined on concentrations of MMT clay and Ca^{2+} ions, within which nanocomposite gels remain homogeneous on the one hand and preserve their integrity at low pH values on the other; (ii) it is demonstrated that the elastic modulus of nanocomposite gels increases with mass fraction of MMT clay in aqueous solutions with all pH values under consideration; (iii) to explain this growth, a model is proposed that presumes replacement of electrostatic interactions between negatively charged carboxyl groups of alginate chains and positively charged edges of platelets at high pH values with hydrogen bonds between neutral carboxyl groups and OH groups attached to the surfaces of MMT platelets at low pH values.

2. Results

2.1. Preparation of Uniform Nanocomposite Gels

To choose proper concentrations of MMT and CaCl_2 that ensure uniformity of nanocomposite gels, several series of gels were prepared with concentrations of MMT clay ranging from 1 to 4.5 wt.% and concentrations of CaCl_2 ranging from 0.5 to 1.5 M. Figure 1A shows

good repeatability of data in the DMA test on two samples prepared with 4 wt.% MMT and 0.5 M CaCl_2 . When the content of MMT and CaCl_2 went beyond 4 wt.% and 0.5 M, respectively, poor repeatability of observations in the DMA test was observed in Figure 1B, which shows the storage modulus E' and the loss modulus E'' of two samples prepared with 4.5 wt.% MMT and 1.5 M CaCl_2 . Relatively large deviations between the data in Figure 1B can be attributed to the fact that the homogeneity of samples decreases with an increase in MMT and CaCl_2 concentration [31,32]. The inhomogeneity is caused by the disk-like morphology of MMT clay platelets, which serve as barriers that block the gel and prevent diffusion of the CaCl_2 [26]. Moreover, at concentrations of MMT above 4 wt.%, clay platelets and their stacks interact with each other, which results in a substantial reduction in the stiffness and strength of nanocomposites [33].

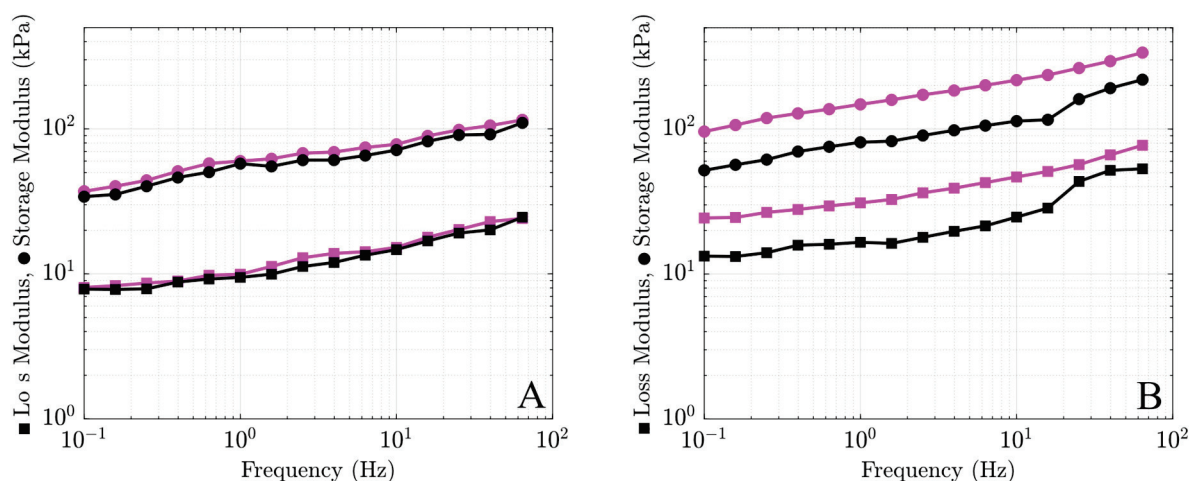


Figure 1. Storage modulus E' (circles) and loss modulus E'' (squares) versus frequency f for alginate–MMT hydrogels cross-linked with (A) 4 wt.% of MMT and 0.5 M CaCl_2 and (B) 4.5 wt.% of MMT and 1.5 M CaCl_2 in aqueous solutions with pH = 7. Each figure shows observations on two samples (black and purple lines) with identical preparation conditions.

In what follows, the nanocomposite gels prepared with concentrations of 1, 3, and 4 wt.% MMT and 0.5 M CaCl_2 are analyzed.

2.2. ATR-FTIR Spectroscopy

To evaluate interactions between the MMT platelets and the alginate chains, attenuated total reflection-Fourier-transform infrared spectroscopy (FTIR) analysis was performed. The FTIR spectra of alginate, MMT, and alginate–MMT nanocomposite hydrogels with 1, 3, and 4 wt.% MMT are presented in Figure 2.

The absorption bands of MMT are assigned as follows: 3624 cm^{-1} corresponds to OH stretching vibration in Si–OH and Al–OH bonds. The band at 1637 cm^{-1} corresponds to the H–O–H bending vibration of adsorbed water, and the peak at 1025 cm^{-1} corresponds to Si–O stretching [34,35].

Sodium alginate is characterized by asymmetric and symmetric stretching vibrations at 1595 cm^{-1} and 1405 cm^{-1} due to carboxylic acid and at 1019 cm^{-1} for oxygen stretching in a cyclic ether bridge. The band at 3268 cm^{-1} corresponds to OH stretching vibration [34].

The FTIR spectrum of the nanocomposite hydrogels revealed that the chemical structures of the nanocomposite hydrogels are similar to alginate, which is the major fraction in the nanocomposite [34]. Comparing the alginate–MMT gels and the pristine MMT, it can be concluded that the Si–OH stretching of MMT at 3624 cm^{-1} disappears in the spectra of the nanocomposite gels, confirming that active sites of the polymer matrix interact with MMT. This phenomenon is explained in Refs. [20,21] by hydrogen bonding between the silanol hydroxyl groups and alginate carboxyl and hydroxyl groups.

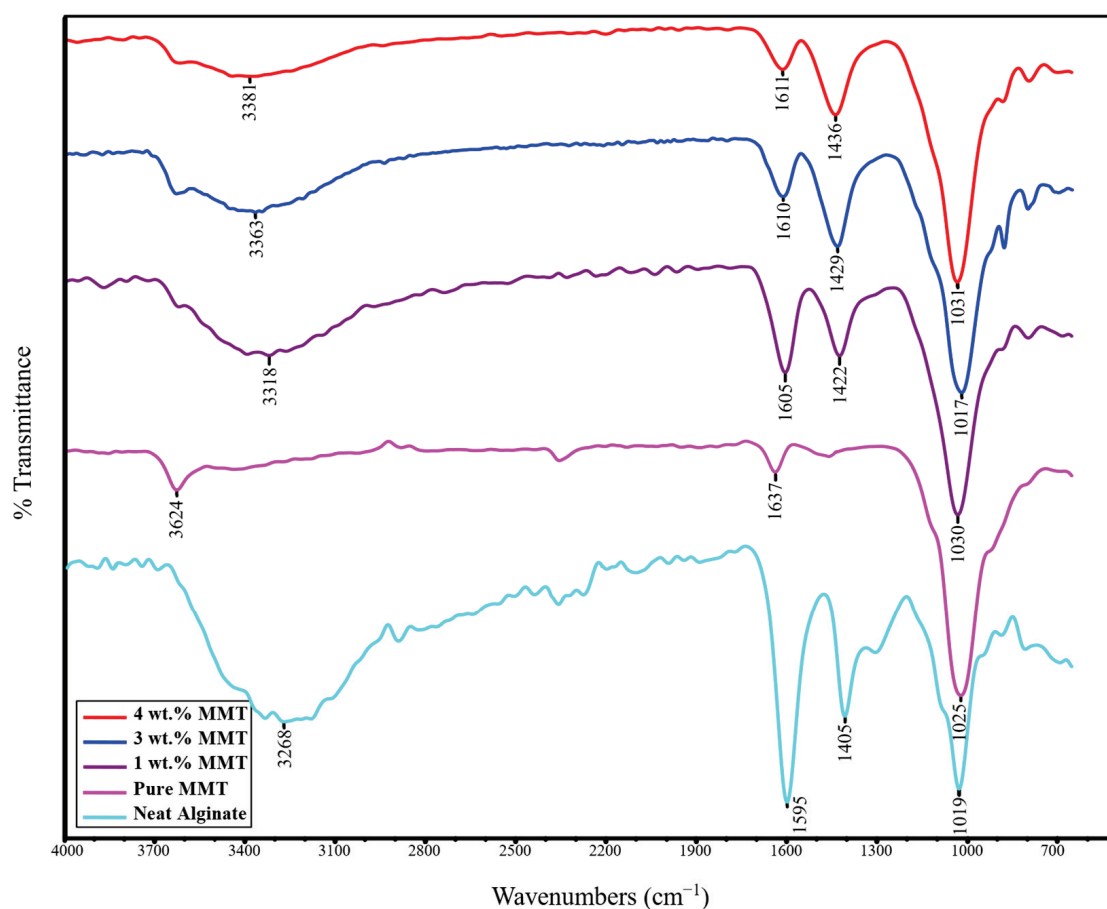


Figure 2. FTIR spectra of alginate, MMT, and Ca-alginate/MMT hydrogels with 1, 3, and 4 wt.% MMT.

The other result showing interaction of MMT with alginate chains is that the COOH alginate stretching bands at 1595 cm^{-1} and 1405 cm^{-1} shifted to higher values (see Table 1) in the nanocomposite gels. This shift is caused by interactions between OH groups attached to MMT platelets and carboxyl groups bound to alginate chains [36].

Table 1. FTIR peaks and corresponding functional groups in MMT, alginate, and alginate–MMT nanocomposite.

	Asymmetric Stretching Vibrations of Carboxylate Groups	Symmetric Stretching Vibrations of Carboxylate Groups	Oxygen Stretching	Stretching Vibration of Hydroxyl Groups	Bending Vibration of H–O–H	Stretching Vibration of Si–O–Si Bonds
MMT	-	-	-	3624 cm^{-1}	1637 cm^{-1}	1025 cm^{-1}
Alginate	1595 cm^{-1}	1405 cm^{-1}	1019 cm^{-1}	3268 cm^{-1}	-	-
1 wt.% MMT	1605 cm^{-1}	1422 cm^{-1}	1030 cm^{-1}	3318 cm^{-1}	-	-
3 wt.% MMT	1610 cm^{-1}	1429 cm^{-1}	1017 cm^{-1}	3363 cm^{-1}	-	-
4 wt.% MMT	1611 cm^{-1}	1436 cm^{-1}	1031 cm^{-1}	3381 cm^{-1}	-	-

2.3. X-ray Diffraction

The degree of intercalation of MMT clay platelets in alginate–MMT nanocomposite hydrogel is investigated by means of wide-angle X-ray diffraction (XRD). The results are presented in Figure 3. It appears from the XRD pattern of pure MMT that the diffraction characteristic peak of the MMT plane [001] is at $2\theta = 7.34^\circ$ ($d = 12.03\text{ \AA}$), which coincides with the value reported in Ref. [29]. It is known that intercalation of polymer chains into the

clay stacks increases the interlayer spacing of the clays, leading to a shift in the diffraction peak towards lower angle values. Figure 3 shows that the characteristic peaks of MMT 2 θ changed from 7.34° to 5.91° ($d = 14.92 \text{ \AA}$) for nanocomposite hydrogels. The expanded gallery distance in modified MMT makes polymer molecules enter into the MMT gallery more easily [37]. The decrease in the intensity of the diffraction characteristic peak of the MMT plane [001] most likely indicates the disordered intercalated structure [37,38].

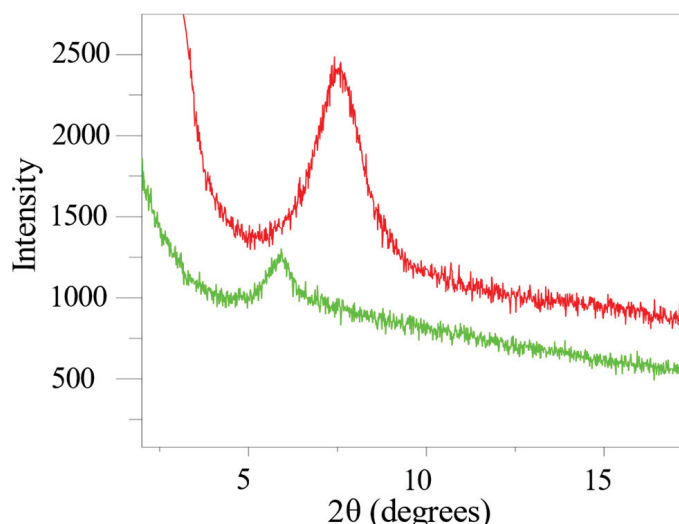


Figure 3. XRD patterns of pure MMT (red) and Ca-alginate/MMT nanocomposite hydrogel (green).

2.4. Mechanical Properties of Alginate–MMT Hydrogels

The storage modulus (E') and loss modulus (E'') of alginate–MMT hydrogels (with 0, 1, 3, and 4 wt.% MMT concentrations), immersed in solutions of pH = 7 or pH = 2, were determined by means of small-amplitude compressive oscillatory tests in the frequency-sweep mode. The influence of frequency f on these moduli is demonstrated in Figure 4.

The storage E' and loss E'' moduli of the alginate–MMT nanocomposite hydrogels at pH = 7 (Figure 4B–D) are higher than those of the neat alginate hydrogel (Figure 4A) in the entire frequency range due to formation of physical bonds between alginate chains and MMT platelets. The storage modulus E' and loss modulus E'' of the nanocomposite gels increase with the concentration of MMT. The storage modulus E' and loss modulus E'' of all samples in aqueous solutions with pH = 2 are lower than those in water with pH = 7.

Alginate chains in Ca-alginate–MMT nanocomposite gels are cross-linked by two types of bonds: between Ca^{2+} and alginate chains, and between MMT platelets and polymer chains. To understand which bonds are broken at pH = 2, the storage modulus E' and the loss modulus E'' measured at frequency $f = 1 \text{ Hz}$ are plotted versus MMT concentration in aqueous solutions with pH = 7 and pH = 2. The results are depicted in Figure 5, where the experimental data are approximated by the linear functions. This figure shows that the slopes of both dependencies (for the storage modulus E' and the loss modulus E'') are relatively close at pH = 2 and pH = 7.

The storage and loss moduli of alginate–MMT nanocomposite hydrogels characterize the structure of their polymer networks because they are proportional to concentrations of physical bonds between chains. These bonds are formed when alginate chains are cross-linked by Ca^{2+} ions and MMT platelets (negatively charged ionized carboxyl groups of alginate chains form electrostatic bonds with positively charged edges of clay platelets) under preparation of the gels at pH = 7 (see Figure 6). Reduction in pH from pH = 7 to pH = 2 causes notable changes in the structure of the polymer network. Under strongly acidic conditions, the concentration of bonds formed by Ca^{2+} ions decays due to release of these ions, whereas all the carboxyl groups of alginate chains become protonated. As a result, electrostatic interactions between chains and clay platelets are replaced by hydrogen

bonds formed between neutral COOH groups of alginate chains and OH groups attached to the surfaces of MMT platelets (Figure 6).

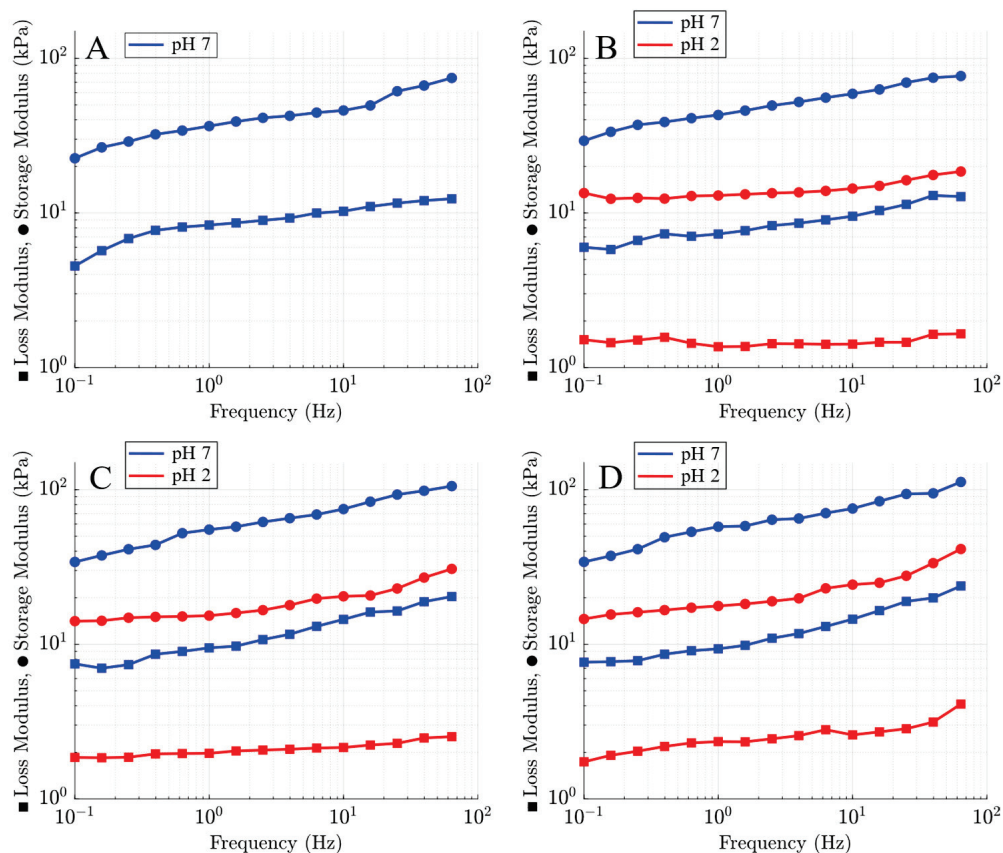


Figure 4. Storage modulus E' (circles) and loss modulus E'' (squares) versus frequency f for alginate–MMT gels with (A) 0, (B) 1, (C) 3, and (D) 4 wt.% of MMT in aqueous solutions with pH = 7 and pH = 2.

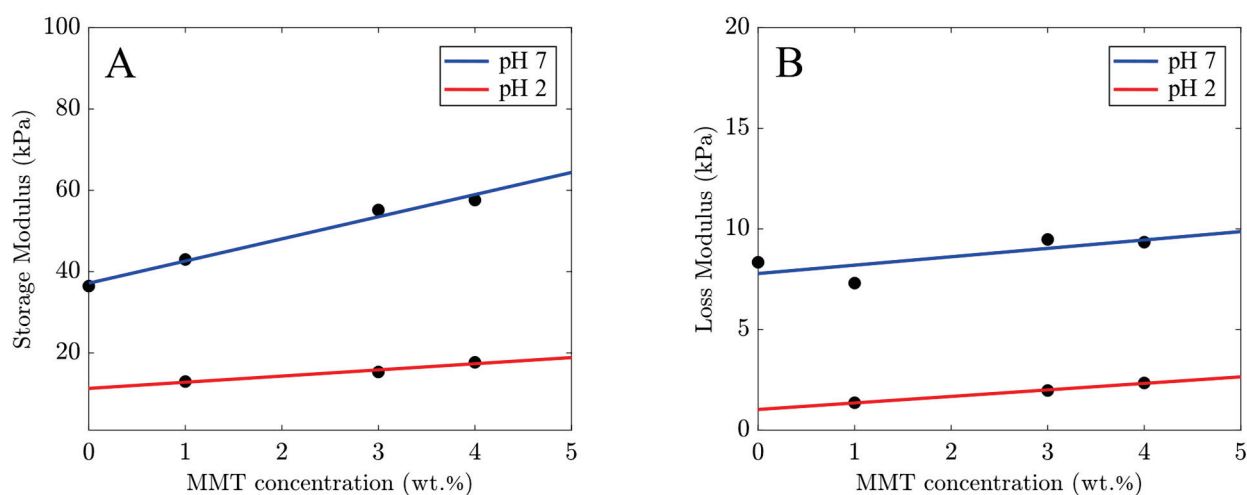


Figure 5. (A) Storage modulus E' and (B) loss modulus E'' at frequency $f = 1$ Hz versus concentrations of MMT in aqueous solutions with pH = 7 and pH = 2. Circles: experimental data. Solid line: their approximation by a linear function.

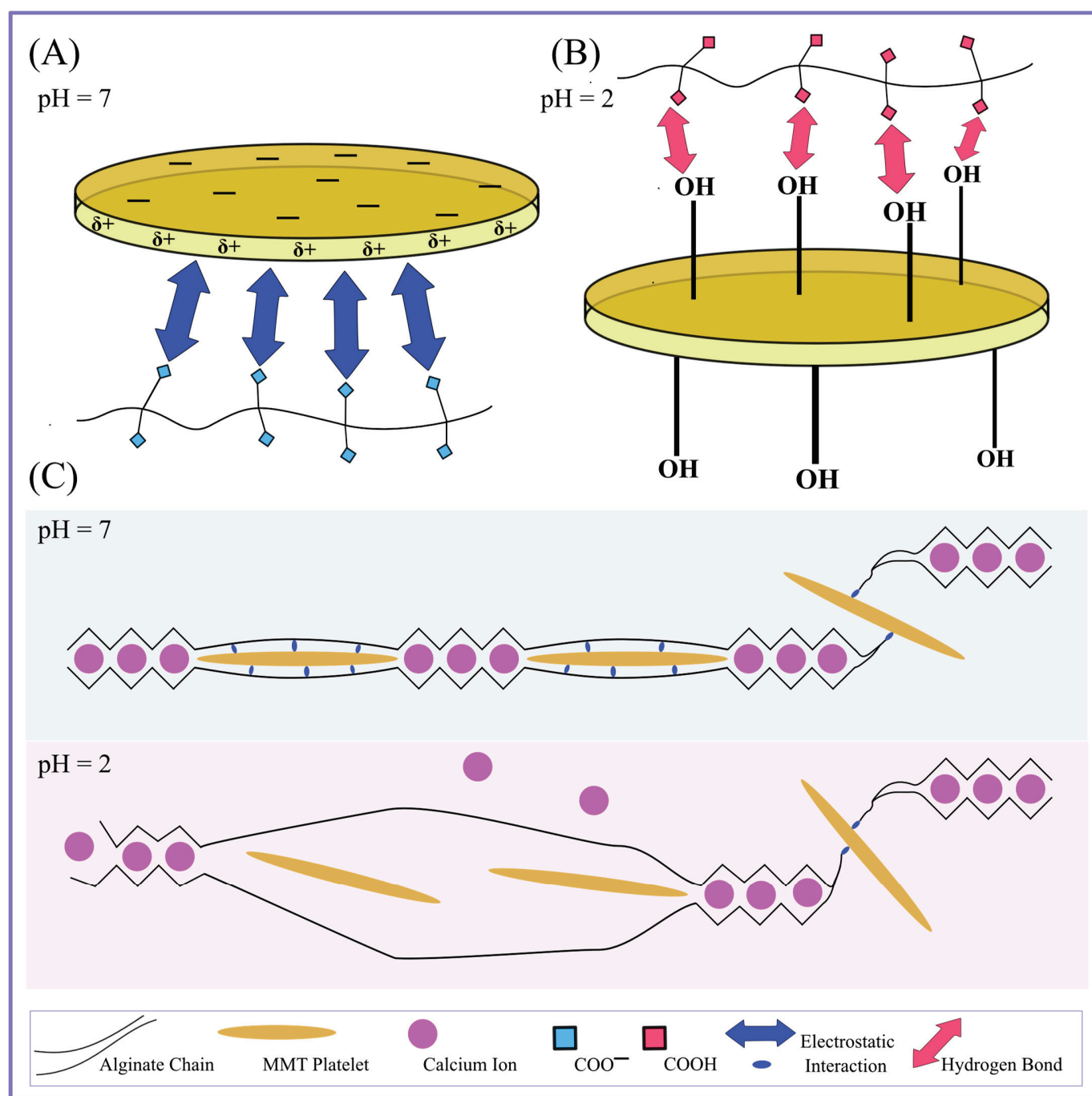


Figure 6. (A) Schematic presentation of the electrostatic links between the positively charged edge of MMT and the negatively charged alginate chains at pH = 7, and (B) hydrogen bonds between the OH groups of MMT and protonated COOH groups of alginate chains at pH = 2. (C) By lowering the pH from 7 to 2, the weak “egg-box” structure is broken; consequently, the electrostatic connection between MMT and alginate chains is broken.

To assess how this phase transition affects the mechanical properties of nanocomposite gels, the experimental data depicted in Figure 4 were fitted by means of the model in linear viscoelasticity proposed in Ref. [39]. The model treats a nanocomposite gel as a transient network of flexible polymer chains linked by permanent and temporary (reversible) bonds. Temporary bonds break and reform at random instants. Rearrangement of these bonds is driven by thermal fluctuations. Each temporary bond is characterized by its dimensionless (normalized by the thermal energy) activation energy for breakage v . The system of

temporary bonds between chains is presumed to consist of bonds with activation energies v that vary from zero to infinity [40]. The ratio of the number of temporary bonds with activation energy v belonging to the interval $[v, v + dv]$ to the total number of temporary bonds in the network reads $f(v)dv$, where $f(v)$ stands for the probability density to find a reversible bond with activation energy v . The following quasi-Gaussian expression is adopted for the probability density:

$$f(v) = f_0 \cdot \exp\left(-\frac{v^2}{2\Sigma^2}\right) \quad (1)$$

An advantage of Equation (1) is that it involves the only parameter Σ that is treated as a measure of inhomogeneity of a polymer network, while the coefficient f_0 is determined from the normalization condition.

The rate of breakage $\Gamma(v)$ for a temporary bond with activation energy v is determined by the Eyring equation

$$\Gamma(v) = \Gamma_0 \cdot \exp(-v) \quad (2)$$

where the pre-factor Γ_0 stands for the attempt rate for breakage of temporary bonds.

The storage modulus $E'(\omega)$ and the loss modulus $E''(\omega)$ are determined by the formulas

$$E'(\omega) = E \int_0^\infty f(v) \frac{(1-\kappa) \Gamma^2(v) + \omega^2}{\Gamma^2(v) + \omega^2} dv \quad (3)$$

$$E''(\omega) = E \int_0^\infty f(v) \frac{\kappa \Gamma(v) \omega}{\Gamma^2(v) + \omega^2} dv \quad (4)$$

where E denotes the elastic modulus (which is proportional to the total number of permanent and temporary bonds between chains), and κ stands for the ratio of the number of temporary bonds to the total number of bonds between polymer chains. A detailed derivation of Equations (3) and (4) is provided in Ref. [39].

Given an angular frequency $\omega = 2\pi f$, Equations (1)–(4) involve four adjustable parameters: (i) E stands for the elastic modulus of a nanocomposite gel, (ii) κ is the ratio of the number of temporary bonds in the network to the total number of bonds, (iii) Σ characterizes the distribution of temporary bonds with various activation energies, and (iv) Γ_0 is the attempt rate for breakage of temporary bonds. These coefficients are found by fitting the experimental data reported in Figure 4.

To simplify the analysis, we presume Γ_0 to be independent of pH and mass fraction of MMT clay. Γ_0 is set to 15.000 s^{-1} in numerical simulation. In the matching procedure, we use the values $\Sigma = 6.9$ and 13.5 for $\text{pH} = 7$ and $\text{pH} = 2$, respectively. These values are determined from the condition of the best fit of the experimental data on samples with 1 wt.% of MMT. The strong decay in Σ with pH confirms that the inhomogeneity of nanocomposite gels increases at $\text{pH} = 2$ due to the breakage of bonds between chains and replacement of electrostatic interactions between MMT platelets and alginate chains with hydrogen bonds. The remaining two parameters, E and κ , are determined by approximating each set of data in Figure 4 separately.

Figures 7 and 8 demonstrate an acceptable agreement between the data and the results of the numerical simulation.

The effect of mass fraction of MMT c on the parameters E and κ is illustrated in Figure 9, where the data are fitted by the linear functions

$$E = E_0 + E_1 c, \quad \kappa = \kappa_0 + \kappa_1 c \quad (5)$$

with the coefficients determined by the least squares method.

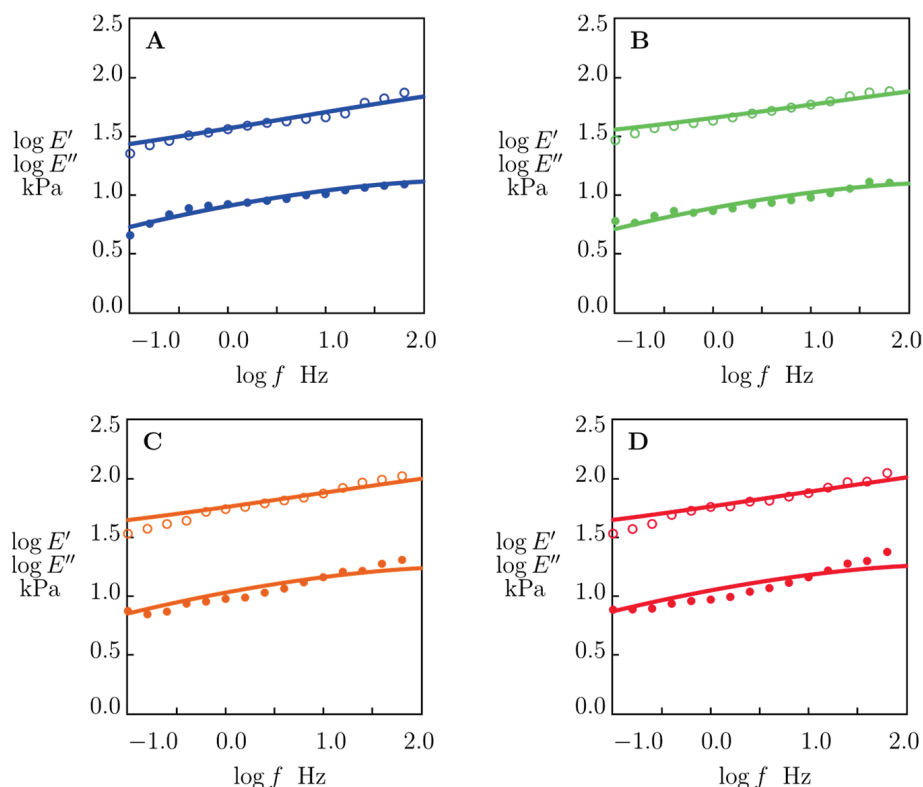


Figure 7. Storage modulus E' ($\circ$) and loss modulus E'' ($\bullet$) versus frequency f . Symbols: experimental data in small-amplitude oscillatory tests at room temperature on alginate–MMT nanocomposite gels with various concentrations of MMT swollen in water with pH = 7. (A) 0, (B) 1.0, (C) 3.0, (D) 4.0 wt.% MMT. Solid lines: results of numerical analysis.

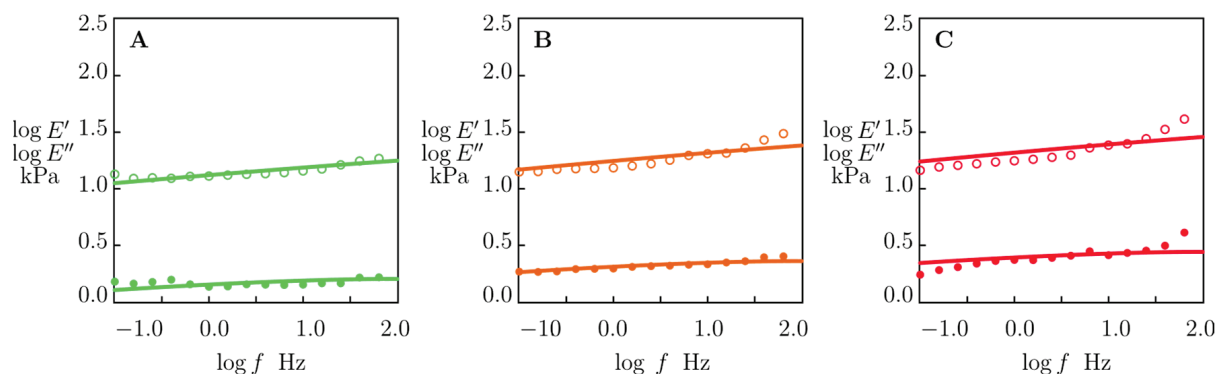


Figure 8. Storage modulus E' ($\circ$) and loss modulus E'' ($\bullet$) versus frequency f . Symbols: experimental data in small-amplitude oscillatory tests at room temperature on alginate–MMT nanocomposite gels with various concentrations of MMT swollen in water with pH = 2. (A) 1.0, (B) 3.0, (C) 4.0 wt.% MMT. Solid lines: results of numerical analysis.

The slopes of the graphs depicted in Figure 9A equal 12.61 at pH = 7 and 4.46 at pH = 2, which means that the effect of MMT on the elastic modulus of nanocomposite gels is substantially (by about three times) stronger at pH = 7 than at pH = 2. Figure 9B shows that the coefficient κ (the ratio of the concentration of transient bonds to the total concentration of bonds between chains) is practically independent of the concentration of MMT, and its value is close to 0.8. Weak deviations of κ from the constant value can be explained by inconsistencies in measurements.

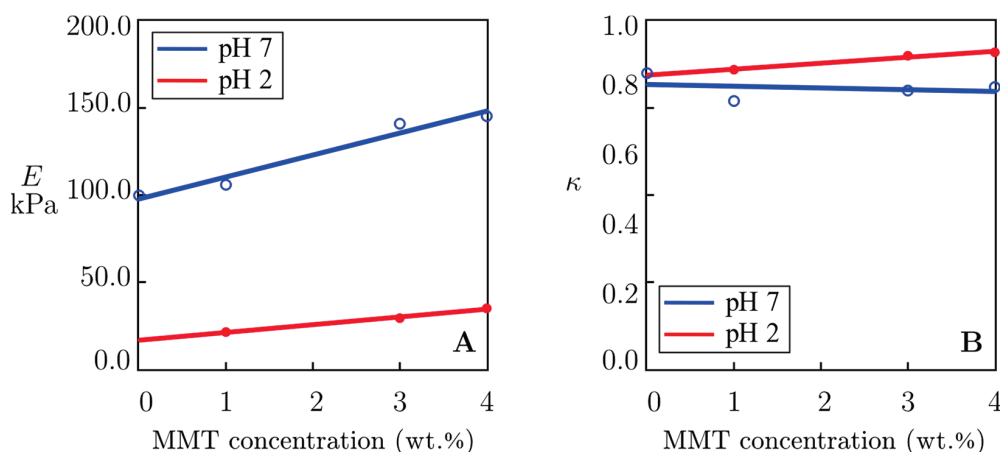


Figure 9. (A) Elastic modulus E and (B) coefficient κ versus MMT concentration. Symbols: treatment of experimental data at room temperature on alginate–MMT nanocomposite gels swollen in water with pH = 7 and pH = 2. Solid lines: results of numerical simulation.

3. Discussion

The alginate–MMT nanocomposite hydrogels were prepared with various concentrations of MMT clay. By using the DMA technique, the interactions between MMT platelets and alginate chains in the gels swollen at pH = 7 and pH = 2 were investigated.

The frequency-sweep mode of small-amplitude oscillatory tests revealed a monotonical rise in both storage modulus E' and loss modulus E'' with concentration of MMT at both pH values under investigation (Figure 4), indicating an increase in the elasticity and viscoelasticity of the nanocomposite gels.

The fact that the storage modulus E' and the loss modulus E'' of the alginate–MMT nanocomposite gels exceed those of the neat alginate hydrogel (Figure 5) confirms the formation of cross-links between alginate chains and MMT platelets at pH = 2 and pH = 7 [28] (Figure 9A). A decrease in pH from pH = 7 to pH = 2 leads to a strong decrease in the storage and loss moduli (Figure 5), which can be explained by the breakage of weak bonds between chains at pH = 2 [41].

A model with two adjustable parameters was used to fit the experimental data obtained from small-amplitude oscillatory tests (Figures 7 and 8). The effects of pH and concentration of MMT platelets on these quantities are illustrated in Figure 9.

Figure 9A shows that the elastic modulus E increases noticeably with concentration of MMT at pH = 7, but this growth becomes less pronounced at pH = 2. Presuming E to be proportional to the total number of bonds between chains, it can be concluded that the concentration of cross-links in the polymer network is reduced when the nanocomposite gel is swollen at pH = 2. In our previous study [14], it was shown that some egg-box bonds between alginate chains formed by Ca^{2+} ions were broken at low pH.

To explain why the effect of concentration of MMT on the elastic modulus becomes less pronounced at pH = 2, it is supposed that the structure of a polymer network in alginate–MMT nanocomposite gels changes dramatically when pH decreases from pH = 7 (which is strongly above the pK_a of alginate chains) to pH = 2 (which is below pK_a).

At pH = 7, all the carboxyl groups attached to alginate chains are ionized, while all the MMT platelets in the gel have positive charges at their edges and negative charges at their surfaces [42]. Physical cross-links between the chains and the platelets are formed due to electrostatic interactions between negatively charged COO^- groups of chains and positively charged edges of the platelets.

At pH = 2, all the carboxyl groups become neutral. Physical bonds between the alginate chains and the clay platelets are formed due to hydrogen bonds between COOH groups attached to the chains and hydroxyl groups OH at the surfaces of clay platelets [43] (see

Figure 6, where the interactions between MMT platelets and alginate chains are represented schematically).

The decrease in pH from pH = 7 to pH = 2 causes not only unzipping of some egg-box bonds between alginate chains but also phase transition in the bonds that link alginate chains with MMT platelets (Figure 6C). Both effects increase inhomogeneity in the nanocomposite gels, which is observed as an increase in the measure of inhomogeneity from $\Sigma = 6.9$ at pH = 7 to $\Sigma = 13.5$ at pH = 2. Replacement of “strong” electrostatic interactions between the chains and platelets at pH = 7 with “weak” hydrogen bonds results in a decay in the influence of concentration of MMT platelets on the elastic modulus E in accordance with the observations reported in Figure 9A.

According to Figure 9B, the coefficient κ (the ratio of the concentration of transient bonds to the total concentration of bonds between chains) is practically independent of the concentration of MMT. This result is rather unexpected, and several possible explanations for it can be provided. One of them is that the strength of bonds formed by egg-boxes between alginate chains is close to the strength of bonds formed between alginate chains and MMT platelets. In other words, when a gel is immersed into water with pH = 2, the number of unzipped egg-box bonds and the number of broken bonds between alginate chains and clay platelets decrease proportionally to each other (Figure 6C).

4. Materials and Methods

4.1. Materials

The sodium alginate from brown algae was purchased from Acros Organics (Geel, Belgium). Cloisite Na⁺, a natural and commercial montmorillonite (MMT) enriched with sodium, was acquired from Southern Clay Products (Gonzales, TX, USA). Calcium chloride (CaCl₂) was provided by Merck (Burlington, MA, USA). Hydrochloric acid (HCl), 37% (v/v), was supplied by VWR International (Rosny-sous-Bois, France). Deionized water was used in the preparation of hydrogels and in all measurements.

4.2. Modification of MMT

MMT was modified in order to increase the gallery spacing between the platelets and to allow for a more effective mixing of the polymer with the MMT clay. First, 1 g of MMT powder was slowly added to 50 mL pre-heated deionized water at 80 °C under magnetic stirring at a speed of 500 rpm. After 4 h of stirring, the resulting MMT suspension was left to settle for 24 h. Then, the MMT suspension was ultrasonicated for 15 min, followed by centrifugation for 15 min at 4000 rpm. The resulting sediment was washed with deionized water and centrifuged again for 15 min at 4000 rpm. Washing operations were repeated until the supernatant became clear. The washed sediment was dried at 40 °C for 3 days, and then ground by hand into a fine powder for further processing [38,44].

4.3. Preparation of Hydrogels

A series of ionically cross-linked alginate–MMT hydrogels were prepared with various concentrations of MMT and a constant amount of CaCl₂ by a method described previously in Ref. [14]. First, the pH of a 1 wt.% alginate solution (molecular weight of 216.12 kDa, G:M ratio 30:70) was reduced to 3.5 using HCl. This results in a decrease in the ionization degree of carboxyl groups along the alginate backbone. Then, 1, 3, and 4 wt.% of modified MMT powder was slowly added to the alginate solution and stirred until a homogeneous mixture was obtained. The alginate–MMT mixture was poured into a mold, and then a CaCl₂ (0.5 M) solution was added. The mixture was left for 3 days to finalize the cross-linking procedure. Thereafter, the remaining unreacted moieties were removed by immersing hydrogels overnight in water with pH = 7.

DMA tests were conducted on swollen hydrogels in aqueous pH = 7 or pH = 2 (see Section 4.6). For XRD test, the gel with 2 wt.% MMT and, for FTIR test, the gel with 1, 3, and 4 wt.% MMT was dried at a temperature $T = 30$ °C for two days.

4.4. ATR-FTIR

ATR-FTIR measurements were conducted to assess the interactions between MMT platelets and alginate chains using a Spectrum One spectrometer from Perkin Elmer (Waltham, MA, USA). The analysis was performed in transmittance mode, employing a zinc selenide crystal with a resolution of 16 cm^{-1} and conducting 128 scans per measurement across the $500\text{ to }4000\text{ cm}^{-1}$ range.

4.5. X-ray Diffraction

X-ray diffraction using an Empyrean diffractometer from PANalytical (Almelo, The Netherlands) with Cu K α radiation at 45 kV and 40 mA was employed to explore the effect of ionic gelation with CaCl_2 on the extent of MMT intercalation/exfoliation. The measurement range was between 2 and 17° .

4.6. Mechanical Tests

Small-amplitude compression oscillatory tests were performed to measure the storage modulus E' and the loss modulus E'' by means of DMA Q800 V20.9 (TA Instruments, New Castle, DE, USA). The strain amplitude was 0.5% and frequency f was between 0.1 and 60 Hz at room temperature ($T = 22^\circ\text{C}$).

The DMA Q800 V20.9 (TA Instruments, New Castle, DE, USA) was employed to evaluate the storage modulus (E') and loss modulus (E'') through small-amplitude compression oscillatory tests. These tests involved a strain amplitude of 0.5% and a frequency (f) ranging from 0.1 to 60 Hz, conducted at room temperature (22°C).

First, the gels immersed in an aqueous solution with $\text{pH} = 7$ were assessed by measuring their storage modulus (E') and loss modulus (E''). Then, the samples were immersed into an aqueous solution with $\text{pH} = 2$ for 2 days (to reach equilibrium, Figure S1) [14], and the DMA tests were repeated.

4.7. Statistical Analysis

In the small-amplitude compression oscillatory tests, three repetitions were conducted on disk-shaped samples with a diameter of 8.5 mm and a thickness of 4.5 mm, and their mean values were reported. The standard deviations of the data were less than 5% of their mean values.

5. Conclusions

A series of alginate–MMT nanocomposite hydrogels have been prepared with various concentrations of MMT platelets and CaCl_2 as cross-linkers. Homogeneous nanocomposite gels are manufactured with MMT and CaCl_2 up to 4 wt.% and 0.5 M, respectively. XRD analysis reveals an increase in the MMT gallery spacing from 12.03 to 14.92 Å. FTIR analysis demonstrates interactions of MMT platelets with alginate chains. The mechanical properties of nanocomposite gels were studied in aqueous solutions with $\text{pH} = 7$ and $\text{pH} = 2$ by means of DMA. It is shown that the storage modulus E' and the loss modulus E'' increase with concentration of MMT platelets and decrease with pH . To explain these findings, the experimental data have been fitted by a model with only two adjustable parameters. It is demonstrated that the elastic modulus E grows linearly with MMT concentration at both pH values under investigation, but the increase in E is weaker at $\text{pH} = 2$. This finding is explained by replacement of electrostatic interactions at $\text{pH} = 7$ with hydrogen bonds at $\text{pH} = 2$ between MMT platelets and alginate chains. This phase transition is accompanied by a pronounced growth in the inhomogeneity of the polymer network (characterized by the measure of inhomogeneity Σ). It is shown that alginate–MMT nanocomposite gels preserve their integrity while being swollen in water with $\text{pH} = 2$, which implies that these hydrogels can be used as a drug carrier in environments with low pH .

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29010244/s1>, Figure S1: Degree of swelling Q versus time t for alginate hydrogels in aqueous solutions with pH = 7.

Author Contributions: Conceptualization, H.M., A.D.D. and E.F.; Data curation, H.M. and A.D.D.; Formal analysis, H.M.; Funding acquisition, J.d.C.C.; Investigation, H.M.; Methodology, H.M., A.D.D. and E.F.; Supervision, A.D.D., E.F. and J.d.C.C.; Validation, A.D.D.; Writing—original draft, H.M.; Writing—review and editing, H.M., A.D.D., E.F. and J.d.C.C. All authors have read and agreed to the published version of the manuscript.

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Article

Cold-Sintered ZnO Ceramic Composites Co-Doped with Polytetrafluoroethylene and Oxides

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Abstract: Grain boundaries play a significant role in determining the performance of ceramic-based materials. The modulation of interfacial structures provides a promising approach to improve the physicochemical and electrical properties of ceramic materials. In this work, the grain boundary structures of ZnO-based ceramics were manipulated by incorporating polytetrafluoroethylene (PTFE) and metal oxides through the cold sintering process (CSP). It was found that the grain size of ZnO-based ceramics can be effectively reduced from 525.93 nm to 338.08 nm with an addition of PTFE and metal oxides of CoO and Mn₂O₃. Microstructural results show that most of the PTFE phase and metal oxides were distributed along the grain boundaries, which may lead to the increased grain boundary resistance from 1.59×10^6 ohm of pure ZnO to 6.21×10^{10} ohm of ZnO-based ceramics doped with PTFE and metal oxides, and enhanced Schottky barrier height from 0.32 eV to 0.59 eV. As a result, the breakdown field and nonlinear coefficient of the ZnO-based ceramics were improved to 3555.56 V/mm and 13.55, respectively. Therefore, this work indicates that CSP presents a feasible approach to design functional ceramic composites through the integration of polymer and metal oxides.

Keywords: ceramic composite; cold sintering process; grain boundaries; ZnO

1. Introduction

Grain boundaries act as interfaces separating two homogeneous crystalline phases within polycrystalline systems such as ceramics, metals, or organic crystals [1–3]. The chemical properties and physical structures of the grain boundaries have significant impacts on the performances of both structural and functional materials [4,5]. Consequently, the manipulation of grain boundary structures was widely used to design new ceramic materials or improve their electrical and mechanical properties [6,7]. Specifically, for varistor ceramics with double Schottky barriers at the grain boundaries, the meticulous modulation and optimization of grain boundary structures significantly contributes to its electrical and physicochemical properties [8–10]. At present, a wide range of varistor materials such as ZnO, TiO₂, SrTiO₃, CaCu₃Ti₄O₁₂, and SnO₂ [11–15] have been extensively developed and studied. Among these materials, ZnO-based varistors are the most commonly employed as surge absorbers or lightning arrester in electronic circuits and power systems due to their excellent non-ohmic electrical behavior. The external lightning or internal switching of the circuit system may lead to transient overvoltage, which results in an abnormal current and damages the components used in the power system [16]. ZnO varistors, utilizing their non-ohmic current–voltage (*I*–*V*) characteristics, swiftly convert to a low-resistance state when the voltage exceeds the predesign threshold voltage, which can redirect overvoltage

to the ground, preventing damage to the equipment and ensuring the stable operation of the circuit or system [16].

ZnO is a typical n-type semiconducting material with a wideband gap of 3.37 eV [17]. Generally, the stable ZnO presents a hexagonal crystal structure with a space group of $C_{6v}^4 = P63mc$ [18], where O atoms are arranged in the hexagonal close-packed lattice. All octahedral voids and half of the tetrahedral voids are unoccupied by atoms. Therefore, the crystal structure of ZnO is in a relatively open state, making it easy for additive doping. The inherent ease for dopants introduced into ZnO makes it widely applied across various fields. Priyanka Jood et al. found that the incorporation of Ti and Al into ZnO can enhance the thermoelectric conversion capabilities of ZnO materials [19,20]. Dietl et al. reported that the introduction of magnetic ions into ZnO induces outstanding ferromagnetic properties [21]. Furthermore, as III-group elements such as Ga and In were doped into ZnO, a substantial increase in electrical conductivity can be achieved, broadening its applications in conductive oxides [22].

ZnO-based varistors were generally fabricated through the high-temperature sintering process of ZnO powder with various metal oxides such as Bi_2O_3 , Co_2O_3 , MnO_2 , Sb_2O_3 , SiO_2 , etc. Bi_2O_3 , with a low melting point of 825 °C, is particularly important since it provides the medium for liquid-phase sintering, enhances the growth of ZnO grains, and affects the stability of the non-ohmic current–voltage characteristics of the material. The metal oxides were mainly distributed at the grain boundaries during the sintering and build a high Schottky barrier, which provides the basis for the non-ohmic behavior of ZnO-based ceramics [23]. The conventional sintering temperature of ZnO varistors is typically as high as around 1200 °C, with a long soaking time, spanning several hours to several days [24–26]. The high sintering temperature can induce an overgrowth of grains in ZnO varistor ceramics, resulting in a low-breakdown electrical field (E_b) [27,28]. In order to mitigate the sintering temperature, many researchers have made significant efforts to develop novel low-temperature sintering techniques over the past decades such as liquid-phase sintering [29], microwave sintering [30], spark plasma sintering (SPS) [31], etc. Nevertheless, sintering temperatures of more than 800 °C are still required to complete the densification of ceramic materials. As a result, additives used for the grain boundary design of ZnO ceramics are limited to inorganic fillers only, since organic polymers can hardly resist temperatures above 400 °C. Traditionally, ceramic powders were homogeneously dispersed into a polymer matrix to produce a ceramic-polymer composites with an improved structure or electrical properties [32]. Regarding ceramic-based composites, ceramic powders are usually sintered first to form a ceramic skeleton, followed by the infiltration of polymers [33]. The differences in the processing temperature gap of ceramic and polymer materials make it difficult to fabricate ceramic-based composites without damaging the polymers in a single step.

In 2016, C. A. Randall et al. introduced the concept of the cold sintering process (CSP) to fabricate ceramic-based materials at a remarkably low temperature (≤ 300 °C) [34,35]. The cold sintering process covers several different mechanisms, and the critical enabling parameter is the transient chemical phase that activates the dissolution and precipitation process, together with a moderate temperature and pressure [36]. The densification process with CSP occurs under limited exposure to water vapor and/or aqueous solutions to provide a transient liquid phase that becomes evaporated under a uniaxial pressure with a controlled heating above 100 °C, in a die that is not completely closed. The CSP involves a series of interconnected nonequilibrium processes that collectively enable an irreversible densification of ceramic powders. First, the liquid phase aids particle rearrangement under the applied external pressure; the heating, pressure, and acidity accelerate chemical dissolution from powders to the aqueous liquid phase. With continued heating, this liquid evaporates, driving a high concentration of the solute, and eventually supersaturates the liquid and drives the precipitation and subsequent pseudo hydrothermal epitaxial crystal growth process that completes the final stages of sintering. The innovative sintering technology offers a solution to challenges that cannot be solved by conventional sintering

processes and exhibits its potential applications in bridging the sintering gap of ceramics and polymers [37,38], reducing energy consumption and cost [39], and tailoring the grain size and grain boundaries [40] in ceramic materials and ceramic-based composites. Recently, ceramic–polymer composites were successfully produced via the CSP route. Zhao et al. [41] reported that ZnO–PTFE (polytetrafluoroethylene) composites could be designed based on CSP and densified at a low temperature of 285 °C. Furthermore, PTFE was also used to modulate the microstructure and electrical properties of other ceramics such as SiO₂ [42] and BaTiO₃ [43], based on CSP. Recently, Si et al. successfully integrated the thermoplastic polymer of poly-ether-ether-ketone (PEEK) with some nanoscale metal oxides into ZnO ceramic using CSP [5]. The ZnO–PEEK–oxide composite presents a switch-like effect which refers to high resistance state under a low electric field, and low resistance state under a high electric field.

Inspired by all these recent works, we prepared a new ZnO–PTFE–oxide (CoO, Mn₂O₃) composite employing CSP. Of the theoretical density of the ZnO-based composite, ~97% was achieved after an isothermal dwell at 300 °C for 1 h with an assisted pressure of 300 MPa. The effects of PTFE and metal oxides on the microstructural evolution of ZnO ceramics were observed via SEM. The breakdown electric field and non-linear coefficient of the ceramic composites were significantly improved. The roles of incorporating PTFE and metal oxides on the grain boundaries of the ceramic composites was discussed.

2. Results and Discussion

2.1. Density and Crystal Structure

Sintering parameters such as external pressure, sintering temperature, and soaking time are very crucial factors for ceramic densification during CSP. In 2017, S. Funahashi et al. [34] first prepared high-dense ZnO ceramics under 300 MPa at 305 °C using CSP. In this work, ZnO powder with particle size of ~200 nm was adopted, and its densification process was studied. As shown in Figures S1–S3 (Supplementary Materials), the various densities of ZnO ceramics fabricated under uniaxial pressure, from 50 MPa to 300 MPa, sintering temperature from room temperature (RT) to 300 °C, and soaking time from 0 to 9 h were studied using CSP. It was found that ZnO ceramics can be well densified when cold-sintered at 300 °C under a uniaxial pressure of 300 MPa for 1 h. These CSP parameters were also selected to fabricate the ZnO–PTFE composites. Figure 1 shows that all the samples present a high density of more than 97%. However, it is noteworthy that there is a slight downward trend in the relative density from samples S1 to S4, which can be associated with the increased presence of doping agents, mildly retarding the densification process.

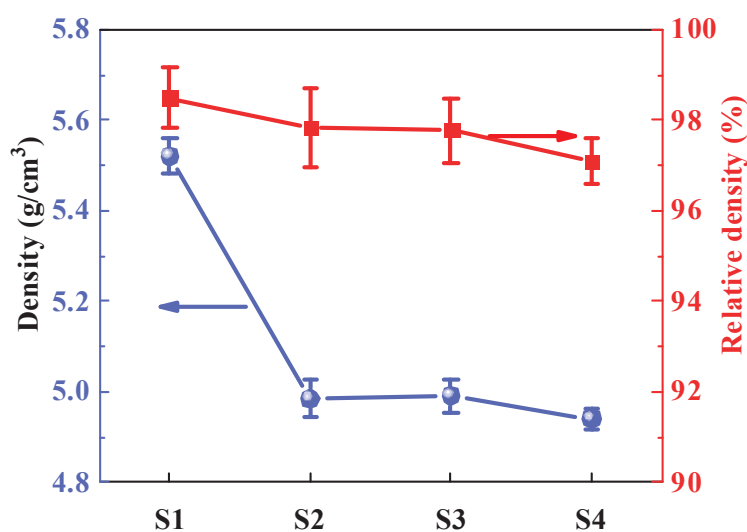


Figure 1. The density of ZnO-based composite ceramics.

Figure 2 demonstrates the X-ray diffraction (XRD) patterns of PTFE and cold-sintered ZnO samples S1, S2, S3, and S4. As a semi-crystalline polymer, PTFE has a combination of crystalline and amorphous structures and presents obvious X-ray peaks at around 18.0° . The major XRD peaks of S2, S3, and S4 are indexed with their Miller indexes based on the PDF#99-0111. Sample S1 presents the wurtzite structure with the space group of P63mc, and there are no obvious impurity X-ray peaks detected in ZnO ceramics, while for samples S2, S3, and S4, another very low peak at around 18.0° that can be attributed to the PTFE polymer can be found. However, the X-ray peaks of CoO and Mn₂O₃ doped in S3 and S4 were absent, which could have resulted from their low doping concentration or retention in the amorphous state during low-temperature CSP.

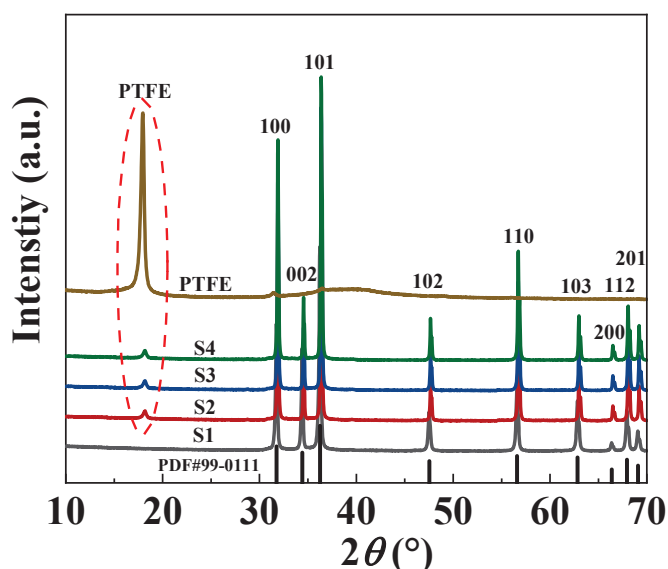


Figure 2. XRD patterns of ZnO-based composite ceramics.

2.2. Microstructure and Dielectric Properties

Figure 3a–d depicts the microstructural evolution of ZnO-based ceramic and composites with various additives. The mean grain size was calculated based on more than 100 grains using the linear intercepts method [44,45], as shown in the inset of Figure 3a–d. Figure 3a shows that the grain size of a pure ZnO ceramic increased from 200 nm of the initial powder to 525.93 nm after CSP. In Figure 3b, the grain size of the cold-sintered ZnO–PTFE composite was only 338.08 nm, suggesting that PTFE effectively inhibits excessive growth of the ZnO grain. Moreover, energy-dispersive spectroscopy (EDS) was employed to check the element distribution in sample S2, and two specific regions (Spectrum 1 and 2) were selected for point-scanning. Spectrum 1 was selected for ZnO grains, while Spectrum 2 was selected for the filamentous structures, as shown in Figure 3b. The elemental analysis spectra revealed that spectrum 1 exhibited a high content of Zn and O, whereas spectrum 2 displayed an obvious peak of the F element, corresponding to the polymer PTFE.

Figure 3c,d demonstrates the microstructure of samples S3 and S4, respectively. It can be observed that the grain size of the two samples decreased to 290.15 nm and 258.73 nm as CoO and Mn₂O₃ were introduced, indicating that the incorporation of metal oxides can further restrain grain growth. In Figure 3d, some segregated small particles can be observed at the grain boundaries and were proved to be Co and Mn elements according to the EDS results shown in Figure 3f. It can be inferred that CoO and Mn₂O₃ may not react with ZnO under CSP but preferentially segregate at the grain boundaries, thereby further suppressing grain growth.

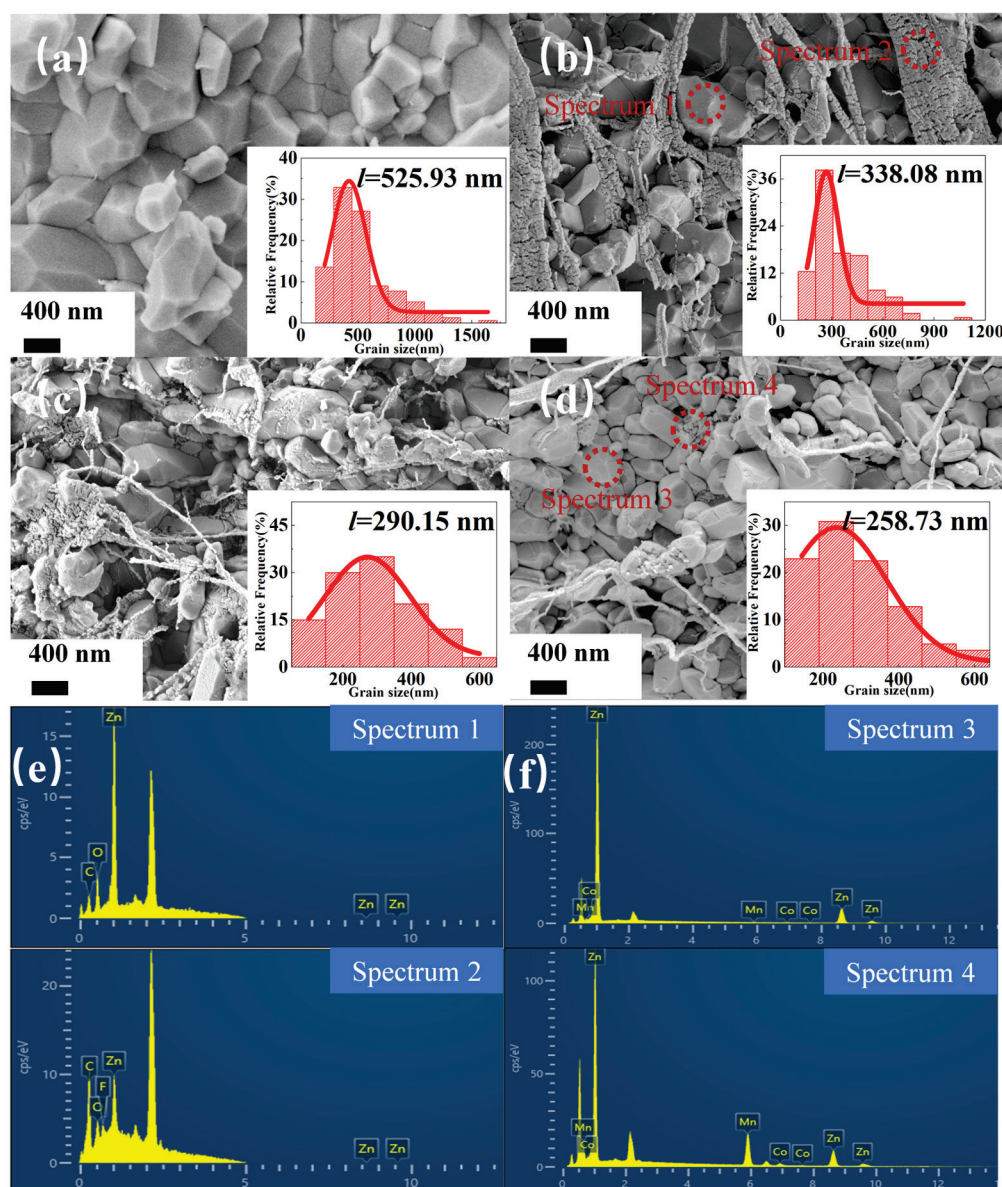


Figure 3. The microstructures of the fracture surfaces of samples sintered via CSP. The SEM images (a–d) for samples S1–S4 and the EDS elemental analysis spectra (e,f) for samples S2 and S4.

Figure 4 shows the impedance spectroscopy of samples S1, S2, S3, and S4 in the Nyquist plots at room temperature. The intercept at a low frequency corresponds to the resistance of the grain boundaries [46], which increases from 1.59×10^6 ohm of sample S1 to 5.16×10^8 , 3.43×10^{10} , and 6.21×10^{10} ohm with doping PTFE and metal oxides for sample S2, S3, and S4, respectively, while the high-frequency intercepts related to ZnO grain resistance remain nearly unchanged. The doped PTFE and metal oxides showed synergism in controlling the ZnO grain growth, meanwhile creating a high-resistance grain boundary phase between the smaller ZnO grains. Therefore, the incorporation of the PTFE and metal oxides CoO and Mn_2O_3 can dramatically increase the grain boundary resistance of ZnO ceramic composites, and improve the breakdown electric field of ZnO-based ceramic composites.

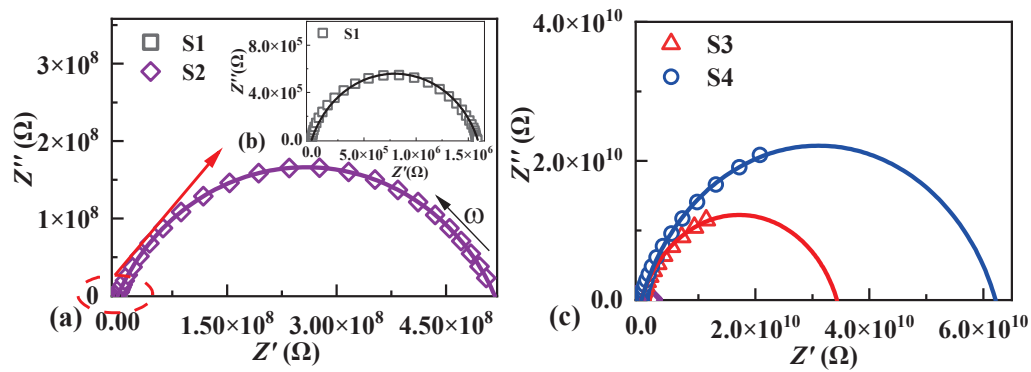


Figure 4. Nyquist plots of impedance for all samples at room temperature, (a) sample S2, (b) sample S1, (c) sample S3 and S4.

Figure 5a shows the frequency-dependent permittivity of ZnO-based ceramic composites at room temperature. It can be seen that the permittivity decreases with increasing frequency in the range from 10 Hz to 10^6 Hz. Due to the low permittivity of PTFE ($\epsilon_r = 2.1$) [47], the permittivity of ZnO-based composites with PTFE is lower than that of pure ZnO ceramics. Furthermore, with the introduction of metal oxides CoO and Mn_2O_3 , the permittivity of ZnO-based ceramic composites further decreases. In Figure 6b, the frequency-dependent variation in the dielectric loss tangent ($\tan\delta = \epsilon''/\epsilon'$) for ZnO-based ceramic composites were tested at room temperature. The relationship between dielectric loss $\tan\delta$ and conductivity can be expressed as follows [48]:

$$g = \frac{\epsilon_0(\epsilon_s - \epsilon_\infty)\omega^2\tau}{1 + \omega^2\tau^2} \quad (1)$$

$$\tan\delta = \frac{\gamma + g}{\omega\epsilon_0\epsilon_r} \quad (2)$$

where γ is the dc conductivity of the dielectric, g is the equivalent conductivity of relaxation polarization, τ is relaxation time, ω is the angular frequency, ϵ_s is the static relative permittivity, ϵ_∞ is the optical frequency relative permittivity, ϵ_0 is permittivity of the vacuum, and ϵ_r is the relative permittivity of the dielectric. Equation (2) can be transformed into Equation (3) when g is much smaller than γ in the low-frequency range, and can be neglected as follows:

$$\tan\delta = \frac{\gamma}{\omega\epsilon_0\epsilon_r} \quad (3)$$

where the predominant factor influencing the value of $\tan\delta$ is the low-frequency dc conductivity.

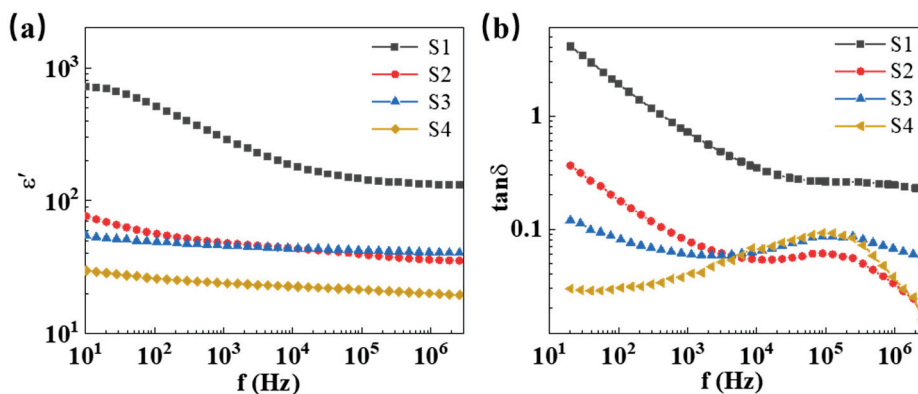


Figure 5. The permittivity (a) and dielectric losses (b) of samples at room temperature.

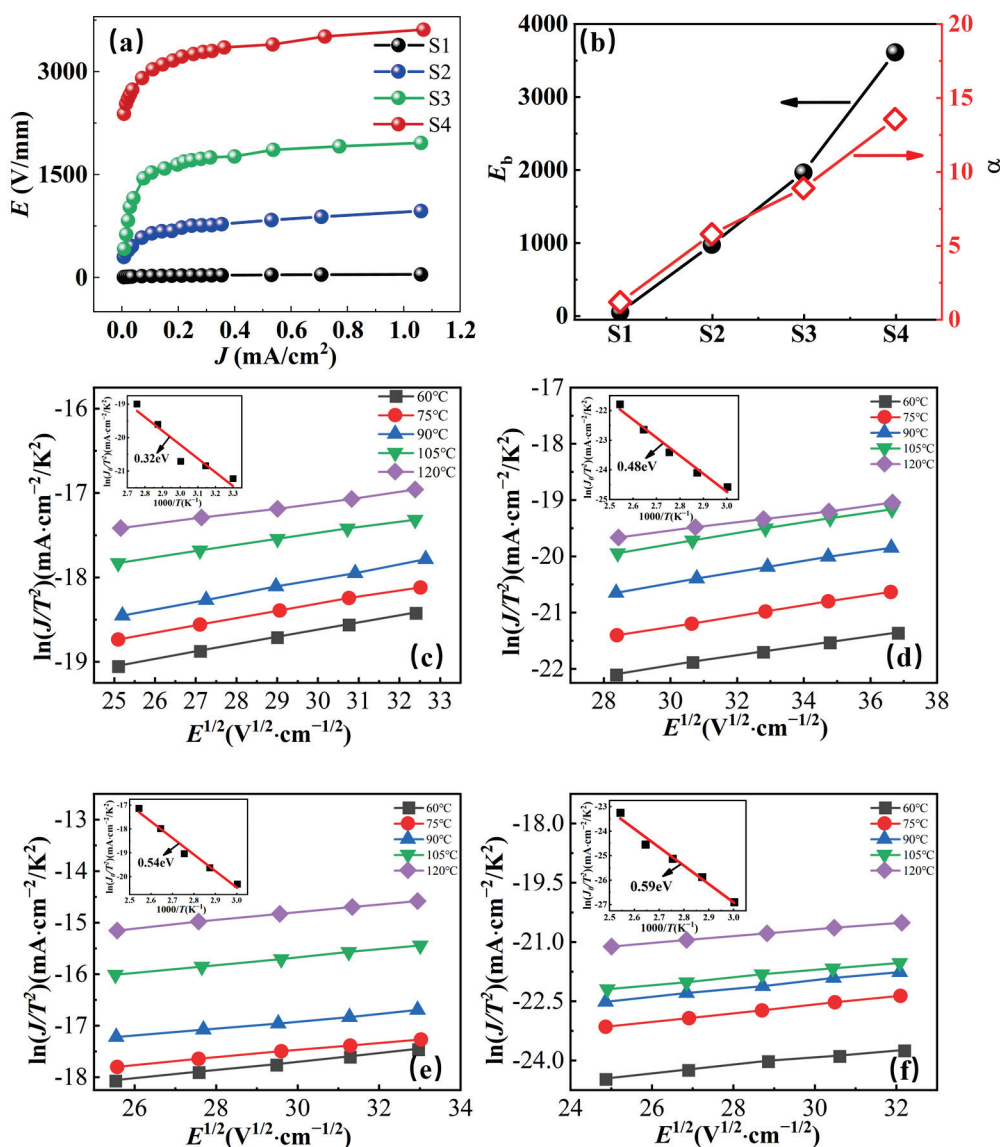


Figure 6. (a) Current-Voltage dependence of the ZnO-based varistor ceramics at room temperature. (b) The breakdown electric field (E_b) and nonlinear coefficient (α) of ZnO-based varistor ceramics. Current-voltage-temperature data for ZnO-based varistor samples S1 (c), S2 (d), S3 (e), and S4 (f) in the temperature range of 60–120 °C. The Schottky barrier height is shown in the inset.

As shown in Figure 5b, dielectric loss $\tan\delta$ is inversely proportional to the frequency below 1000 Hz, indicating that dc conductance mainly contributes to dielectric loss in the low frequency range. Typically, at the power frequency of 50 Hz, sample S1 exhibits a very high dielectric loss tangent of 2.437, whereas the value of $\tan\delta$ for sample S4 was significantly reduced to 0.028 because the contribution of dc conductivity to dielectric loss was well-suppressed with the introduction of PTFE and metal oxides. At high frequencies, a relaxation peak is observed in samples S2, S3, and S4, which can be attributed to the electronic relaxation processes of an intrinsic oxygen vacancy defect [49]. Thus, it is believed that incorporation of PTFE, Co, and Mn can optimize the grain boundary characteristics of ZnO-based ceramic composites, thereby enhancing their dielectric performance.

2.3. Nonlinear Ohmic Characteristics and Schottky Barriers

The I - V characteristics and nonlinear coefficient α of ZnO-based ceramic composites at room temperature are presented in Figure 6a,b, respectively. It can be found that the pure ZnO ceramic (S1) exhibits a quasi-ohmic behavior. As PTFE was introduced, E_b and α

increased from 43 V/mm, 1.13 of sample S1 to 967 V/mm and 5.74 of sample S2. Moreover, with the introduction of metal oxides CoO and Mn₂O₃, E_b and α were further enhanced to 1960 V/mm, 8.86 of sample S3, and 3611 V/mm, 13.55 of sample S4, respectively.

In order to analyze the electrical conduction mechanism of cold-sintered ZnO-based ceramic composites, the current density–electric field (J – E) relationship at temperatures from 60 °C to 120 °C is given in Figure 6c,f. The J – E characteristics of ZnO-based ceramic composites follow the Schottky emission process and can be described by Equation (4) [41]:

$$J = AT^2 \exp[(\beta E^{1/2} - q\phi_B)/kT] \quad (4)$$

where J is the current density, T is the absolute temperature, A is the Richardson constant, E is the applied electric field, β is a constant, k is the Boltzmann constant, and ϕ is the Schottky barrier height. Under low electric fields, higher temperatures and electric field strength result in increased energy acquired by electrons. Consequently, electrons are more likely to overcome the barrier, leading to a higher current density. As shown in Figure 6c–f, the current density was apparently enhanced with the thermal excitation electric field E as the test temperature increased from 60 °C to 120 °C. On the other hand, a linear relationship between $E^{1/2}$ and $\ln J$ was achieved, which indicates that the grain–grain boundary structures in ZnO-based ceramic composites are formed and consequently the thermionic emission current flows across the Schottky barrier in the grain boundary region as described by Equation (4). The Schottky barrier height of ZnO-based composite ceramics was determined using Arrhenius fitting ($1/T$ and $\ln J/T^2$), as shown in the insets of Figure 6c–f. The barrier height gradually increased from 0.32 eV of sample S1 to 0.59 eV of sample S4.

From the I – V results, it is found that the introduction of polymer PTFE to ZnO ceramic induces a Schottky barrier of 0.48 eV at the grain boundary and results in the fundamental non-ohmic behavior of ZnO-PTFE composites [41]. The incorporation of CoO and Mn₂O₃ impedes grain growth and raises the Schottky barrier to 0.59 eV, enhancing the breakdown field and nonlinearity. It is believed that co-doping of PTFE and metal oxides in ZnO plays a synergistic effect in improving the I – V characteristics of ZnO-based ceramic composites.

3. Experimental Procedure

3.1. Sample Preparation

The initial ZnO powder with an approximate particle size of ~200 nm was purchased from Acros Organics (Morris Plains, NJ, USA). The PTFE suspension (60 wt%) dispersed in H₂O was obtained from Sigma-Aldrich (Burlington, MA, USA), featuring an average particle size of ~0.5 microns. Mn₂O₃ powder was prepared by calcining Mn(OOCH₃)₂ (Alfa Aesar, Ward Hill, MA, USA) at 600 °C for 5 h. The CoO powder was acquired from Alfa Aesar with a purity of 99.9%.

As shown in Figure 7, ZnO powder was mixed with a PTFE suspension and two metal oxides (CoO and Mn₂O₃) based on the formulations presented in Table 1, and the initial composite powders denoted as P1, P2, P3, and P4 were prepared, respectively. The ball milling equipment with a model of XQM-2 was purchased from Changsha Tianchuang Powder Technology Co., Ltd. (Changyuan, China). The rotation speed during the ball milling process was set to 380 revolutions per minute. Then, the resulting mixtures were dried in an oven at 70 °C for 12 h. Afterward, a small quantity of an acetic acid solution (2 mol/L, approximately 20 wt%) was introduced to the mixture and manually ground in a mortar for a few minutes. Then, the moistened powders were poured into a steel die with a diameter of 12.7 mm, and pressed using a manual press (model YLJ-24T) under a pressure of 300 MPa, first at room temperature for 10 min, and then the steel die was heated to 300 °C with a ramp rate of 15 °C per minute. The temperature was isothermally kept at 300 °C for 1 h, and then cooled down to room temperature before taking out the sample. The ZnO ceramic composites prepared from the four different initial powders (P1, P2, P3, and P4) were labeled as S1, S2, S3, and S4, respectively.

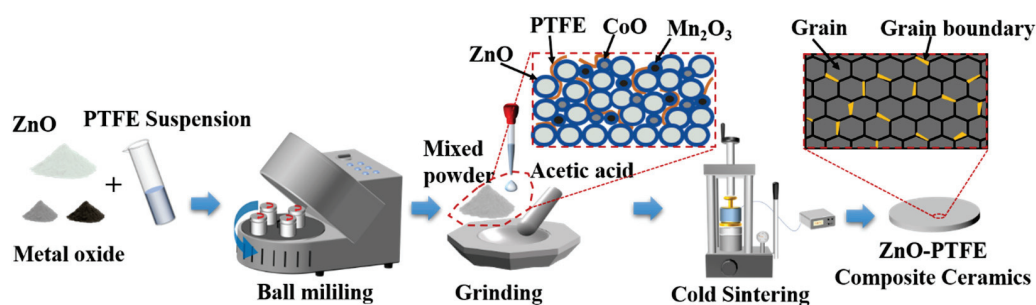


Figure 7. The schematic of the preparation of ZnO-based composites with PTFE and metal oxides using CSP.

Table 1. Various composite powders (mol%) of ZnO–PTFE–oxides.

Composite Powder	ZnO	PTFE	CoO	Mn ₂ O ₃
P1	100	0	0	0
P2	85	15	0	0
P3	84	15	1	0
P4	83.5	15	1	0.5

3.2. Characterization

The relative densities of the cold sintered samples were measured using a geometric and an Archimedes method in ethanol. In the experimental procedure, the samples underwent polishing and ultrasonic cleaning in anhydrous ethanol to eliminate surface impurities. Subsequently, a constant-temperature drying oven (DHG-9013A) was employed to remove any residual liquid from the samples. The completely dried samples were then weighed on a precision analytical balance, resulting in a recorded mass denoted as m_1 (g). Subsequently, the samples were immersed in anhydrous ethanol and placed on a filter, with the measured mass recorded as m_2 (g). After the removal of anhydrous ethanol and surface drying, the saturated weight, m_3 (g), was determined. Using the density of anhydrous ethanol (ρ_w), the real density of the ceramic samples (ρ) was calculated using Equation (5). The relative density was then calculated based on the theoretical density (ρ'), as shown in Equation (6).

$$\rho = \frac{m_1}{m_3 - m_2} \times \rho_w \quad (5)$$

$$\rho_r = \frac{\rho}{\rho'} \times 100\% \quad (6)$$

X-ray diffraction analysis (PANalytical Empyrean, Stanford, CA, USA) was employed for a comprehensive assessment of the phase composition of the samples, utilizing Cu-K α radiation with a wavelength (λ) of 0.15416 nm. The scanning range of 2-theta angles spanned 10° to 70°, with a step size of 0.026°, enabling the acquisition of detailed crystalline information. Microstructural observations of the cross-sections of the ZnO-based composite ceramic samples were conducted using a field emission scanning electron microscope (SEM, JEOL JSM-6390A, Tokyo, Japan) to characterize the morphology and investigate the influence of additive doping on the internal grain boundary structure, with magnifications ranging from 20,000 to 50,000 times. The structural features and distribution of dopants were determined through point scanning tests using energy-dispersive spectroscopy (EDS). For the electrical measurement, both sides of the samples were polished and sputtered with Au as electrodes by a sputter coater (Quorum Q150T). The diameters of the electrodes on both sides of the samples were 8 mm and 6 mm, respectively, which were both smaller than the diameter (12.7 mm) of the samples to prevent the surface–flashover breakdown during the current density–electric field property test.

The current density–electric field characteristics were measured using a pA meter (HP4140B, Hewlett Packard, Palo Alto, CA, USA) and the temperature was controlled by

a Delta oven (Delta 9023, Cohu Inc., Poway, CA, USA) in the range of room temperature to 120 °C. The breakdown field, E_b , was determined using Equation (7) and the nonlinear coefficient α was obtained via Equation (8), where d is the thickness of the sample, and U_{1mA} and $U_{0.1mA}$ are the voltage measured at the current of 1.0 mA and 0.1 mA, respectively. For the testing and analysis of the barrier heights, it was necessary to measure the J – E characteristics at different temperatures. The samples fixed in an electrode system were placed in a Delta oven for the temperature-variable J – E test. Impedance spectra and dielectric loss were tested via broadband dielectric spectroscopy (Novocontrol Concept 80, Montabaur, Germany) using a 1 V peak-to-peak ac small-signal voltage in the range from 10 Hz to 10^6 Hz.

$$E_b = \frac{U_{1mA}}{d} \quad (7)$$

$$\alpha = \frac{\log_{10}(I_{1mA}/I_{0.1mA})}{\log_{10}(U_{1mA}/U_{0.1mA})} = \frac{1}{\log_{10}(U_{1mA}/U_{0.1mA})} \quad (8)$$

4. Conclusions

In summary, a new type of ZnO–PTFE–oxide ceramic composite was introduced, which presented an extremely high-breakdown electrical field and improved nonlinear coefficient. The ZnO-based ceramic composites were fabricated using CSP at 300 °C under a uniaxial pressure of 300 MPa, and achieved a large relative density of $\geq 97\%$. The microstructural results show that the grain sizes of ZnO were severely restricted to hundreds of nanometers, and filamentous PTFE and segregated metal oxides could be found at the grain boundaries. The introduction of PTFE and metal oxide fillers to the ZnO ceramic during CSP synergistically produced a high-resistance grain boundary phase, which induced the non-ohmic electrical behavior and enhanced the breakdown field. Therefore, the varistor-like J – E behavior of the ZnO–PTFE–oxide ceramic composite was observed due to the Schottky barrier at the grain boundaries. The impedance spectroscopy analysis indicated that the contribution of dc conductivity to dielectric loss can be restrained because the incorporation of the PTFE phase and metal oxides increased the Schottky barrier height at the grain boundaries. This work provides a promising processing route for grain boundary design with polymer and metal oxides in ceramic composites through the cold sintering process.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29010129/s1>, Figure S1. The density of ZnO ceramic samples at different sintering pressures. Figure S2. The density of ZnO ceramic samples at different sintering temperatures. Figure S3. The density of ZnO ceramic samples at different sintering time.

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Article

Biodegradable Nanofibrillated Cellulose/Poly-(butylene adipate-co-terephthalate) Composite Film with Enhanced Barrier Properties for Food Packaging

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Abstract: Biodegradable composites consisting of Poly-(butylene adipate-co-terephthalate) (PBAT), thermoplastic starch, hydrophobically modified nanofibrillated cellulose (HMNC), and green surfactant (sucrose fatty acid ester) were prepared via the melt-mixing and film-blowing process (PBAT-HMNC). The composites were characterized using the Fourier transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), and thermogravimetric analyzer (TGA). The mechanical and barrier properties were systematically studied. The results indicated that PBAT-HMNC composites exhibited excellent mechanical and barrier properties. The tensile strength reached the maximum value (over 13 MPa) when the HMNC content was 0.6% and the thermal decomposition temperature decreased by 1 to 2 °C. The lowest values of the water vapor transmission rate (WVTR) and the oxygen transmission rate (OTR) were obtained from the composite with 0.6 wt% HMNC, prepared via the film-blowing process with the values of 389 g/(m²·day) and 782 cc/(m²·day), which decreased by 51.3% and 42.1%, respectively. The *Agaricus* mushrooms still had a commodity value after 11 days of preservation using the film with 0.6 wt% HMNC. PBAT-HMNC composites have been proven to be promising nanocomposite materials for packaging.

Keywords: nanofibrillated cellulose; green surfactant; biocomposites; packaging

1. Introduction

Environmentally friendly materials are research hotspots and a focus due to the “white pollution” issue. The excessive utilization of conventional plastic products, such as shopping bags, packaging films, bottles, courier bags, etc., has seriously threatened the environment and social development. Compared with metal, stone, and wood, plastic products have the advantages of low-cost and strong plasticity, which are widely used in many fields. However, the drawback of traditional plastic products is that they are non-biodegradable, resulting in a serious environmental issue. As reported, a plastic waste mountain was found in the ocean, and the area is increasing yearly [1,2]. Generally, plastic products such as shopping bags, packaging boxes, and agricultural mulch film are only used for a few minutes, but it takes hundreds of years or even longer for these to decompose in nature. Researchers focused on finding the ideal candidates to replace traditional plastic products. In this case, the development of green-based materials such as natural polymers (cellulose, chitosan, starch, alginate, etc.), man-made biodegradable polymers (Poly-(butylene adipate-co-terephthalate), poly (lactic acid), Polypropylene carbonate, Polyhydroxyalkanoates, Polybutylene succinate, etc.) are the focus of researchers in recent years, which will be conducive to the overall development of the economy and society, and the harmonious coexistence of human and nature [3–5].

Among those conventional packaging materials, plastic products are widely used due to the low cost and light-weight properties. In addition, such plastic products usually maintain functional properties, such as grease resistance and gas barrier properties. In recent years, green-based materials (cellulose and regenerated cellulose) or biodegradable plastics (PLA, PBAT, PHB, and PHBV) have become research hotspots in the field of packaging. Herein, Poly-(butylene adipate-co-terephthalate) (PBAT) is considered a completely biodegradable aliphatic aromatic polyester. PBAT is a biodegradable material with exceptional mechanical and film-forming properties. Despite exhibiting low water vapor resistance, PBAT displays excellent oxygen barrier properties, even more than that of low-density polyethylene (LDPE). Such qualities have earned PBAT polymers the reputation of being one of the more cost-effective alternatives to commodity petrochemical-based materials. The nanocomposite films were prepared with a different source of fillers and biodegradable polymers. The as-prepared composite films were systematically characterized to verify the effects of the different types of fillers on the morphological, water vapor barrier, thermal stability, color, optical, and antimicrobial properties. The results indicated that the biodegradable composite films showed improved properties (optical and mechanical). The composite films exhibited strong antibacterial activity [6]. PBAT-based biodegradable films incorporated with nano particles were prepared to improve the mechanical and water vapor barrier properties of PBAT film. The as-prepared nanocomposite films exhibited strong antibacterial activity against *Escherichia coli* and *Listeria monocytogenes*. The developed films have a high potential for being used as UV-screening and antimicrobial packaging films [7]. Other work also reported the development of antifungal bioplastic films based on poly-(lactic acid) (PLA) and poly-(butylene adipate-co-terephthalate) (PBAT) blends with incorporated trans-cinnamaldehyde using cast extrusion. The synthesized films reduced bacterial and fungal growth in bread, extending shelf-life for more than twenty days. Despite the advantages of PBAT, the relatively high-cost and low water vapor barrier property of PBAT in comparison to fossil-based polymers has impeded its extensive use in areas such as packaging. Therefore, the PBAT/PLA composite incorporated with the nano-polyhedral oligomeric silsesquioxane as a reactive compatibilizer was prepared using melt processing. The as-prepared biodegradable films exhibited improved water vapor, CO₂ and O₂ permeability, which offered a method for preparing high-performance biodegradable plastic packaging films [8]. In addition, nanocomposite technology has the potential to improve polymer properties and expand the applications of PBAT. Shankar and Rhim [9] prepared three different types of zinc oxide nanoparticles (ZnONP), and the PLA/PBAT nanocomposite films were then prepared with the reinforcement of ZnONP. The nanocomposites films showed improved optical and mechanical properties and exhibited strong antibacterial activity. The PBAT/silver nanoparticles (AgNPs) composite films were synthesized; the results indicated that the incorporation of AgNPs improved the mechanical and water vapor barrier properties. The developed films have a high potential for being used as UV-screening and antimicrobial packaging films [10]. In lieu of this, the effects of the fillers such as single-layered MMT nanosheets [11] and nanocellulose [12] on PBAT composites have also been examined. Nanofillers, such as MMT and nanocellulose, improve the barrier properties of the biodegradable substrate dramatically while being well-dispersed. In the reported work [11], the addition of the MMT, the biodegradable polymer, and the implemented orientation process resulted in a decrease in the viscosity of the composites and an increase in the crystallinity of PLA (up to 25%), and the wettability tests confirmed the synergic behavior of the selected polymer blend. However, the dispersion ability of the nanocellulose will dramatically affect the barrier property of the polymer film, this key factor has seldom improved in the above literatures.

Generally, the dispersion of the nanofillers in substrates is the key factor affecting the barrier property of the polymer matrices. Depending on the interaction between nanofillers and polymers, there are usually four different types of composite morphology that might be obtained: exfoliated; flocculated; intercalated; and aggregated. For the above types, the exfoliated one is the most ideal morphology to obtain dominant barrier properties for H_2O , O_2 , CO_2 , etc., involving immense polymer penetration with the individual delaminated platelets and dispersed in the polymers randomly. Materials with excellent barrier properties are especially noticed by researchers. The use of renewable materials in packaging has earned many accolades [13]. Cellulose fiber is the most abundant biopolymer on earth and is utilized in numerous fields due to the advantages of being sustainable, biodegradable, and environmentally friendly. In recent years, many cellulose derivatives, including cellulose nanomaterials (cellulose nanocrystals (CNC), nanofibrillated cellulose (NFC) or cellulose nanofiber (CNF), and bacterial nanocellulose (BNC)), have been commercially produced with excellent properties for film-forming. These derivatives are relatively low-cost and have demonstrated excellent gas barrier properties. In addition, nanocellulose materials have great potential to improve the mechanical and barrier properties while being used as nanofillers in packaging film [14]. The activated hydroxyl groups are the principal structural components in cell walls, which can be easily modified with other functional groups via various derivation reactions including esterification, halogenation, oxidation, and etherification. Such modifications improve the properties of cellulose substantially, particularly the dispersibility. In this case, instead of using inorganic filler, hydrophobic-modified cellulose derivatives are usually used for enhancing the barrier property; hydrophobic-modified cellulose derivatives extend the water vapor transmission path, and thus do not permit the penetration of water through the molecular system [15,16]. However, most of the reported work carried out the chemical modification with many organic solvents, resulting in environmental pollution issues. Our previous work successfully prepared a novel composite consisting of dissolved cellulose fiber and microfibrillated cellulose for dye removal. The combination of microfibrillated cellulose with substrate significantly increased the adsorption capacity and mechanical properties of the biocomposite. The as-prepared cellulose-based composites were environmentally friendly and renewable bioadsorbents, which were not only applied in the field of environmental pollution control, but might also be promising for synthesizing the chemically modified cellulose derivatives products [17].

As a typical commercial biodegradable polymer, PBAT has the advantages of excellent processing performance and good barrier performance. However, the high-cost limits its application in the field of packaging. TPS is made using starch and plasticizer, which is biocompatible and low-cost. Filling TPS into PBAT will not only decrease the cost of the composite film, but also increase the biocompatibility of the final product. In this work, green-based biocomposites consisting of the biodegradable polymer (PBAT), thermoplastic starch, hydrophobically modified nanofibrillated cellulose (HMNC), and green surfactant (sucrose fatty acid ester) were prepared via the melt-mixing and film-blowing process without using any solvents, which has seldom been reported in other literature previously. A comparison of the properties of those biocomposites prepared using the solution-casting method was also systematically investigated. The morphology, thermal stability, and functional groups of the as-prepared biocomposites were systematically characterized. The mechanical properties and the influence factors by adding HMNC were systematically studied; the water vapor permeability (WVP) and oxygen transmittance rate (OTR) were also tested to provide a guide for maximizing the potential of the as-prepared biocomposite films for a variety of applications.

2. Results and Discussion

2.1. FT-IR Spectroscopy of PBAT-HMNC

FT-IR was applied to verify the changes in the functional groups on PBAT, PBAT-TPS, and composites with different HMNC content (PBAT-HMNC-0.3, PBAT-HMNC-0.5, and PBAT-HMNC-0.6); the obtained results are indicated in Figure 1. The characteristic peaks of PBAT were identified (2953 cm^{-1} , 2869 cm^{-1} , 1712 cm^{-1} , $1460\text{--}1450\text{ cm}^{-1}$, 1101 cm^{-1} , 1276 cm^{-1} , and 729 cm^{-1}); [8] the peaks at 1101 cm^{-1} and 1276 cm^{-1} are attributed to the vibration adsorption of the C-O-C in the glucose ring from PBAT. The bending vibration absorption peak of the C-H on the para-disubstituted benzene ring is at 729 cm^{-1} , and the absorption peak at $700\text{--}900\text{ cm}^{-1}$ further proves the existence of the benzene ring. The peaks at $1460\text{--}1450\text{ cm}^{-1}$ are moderate bending vibrations caused by -CH_2 , and the peaks at 2953 cm^{-1} and 2869 cm^{-1} are the stretching vibration absorption peaks of -CH_2 . The above peaks show the characteristic functional groups of PBAT [18]. In addition, the FTIR spectrum of all samples except PBAT gives the peaks at around 3400 cm^{-1} (the adsorption peak at 3340 cm^{-1}), which might be due to the -OH group from the TPS and HMNC. The peak at 1712 cm^{-1} corresponds to the stretching vibration of -C=O group from PBAT and HMNC [19]. The stretching vibration absorption peaks of -CH_2 (the peaks at 2953 cm^{-1} and 2869 cm^{-1}) are deeper from the spectrum of PBAT-TPS, HMNC-0.3, HMNC-0.5, and HMNC-0.6, also corresponding to the addition of TPS and HMNC.

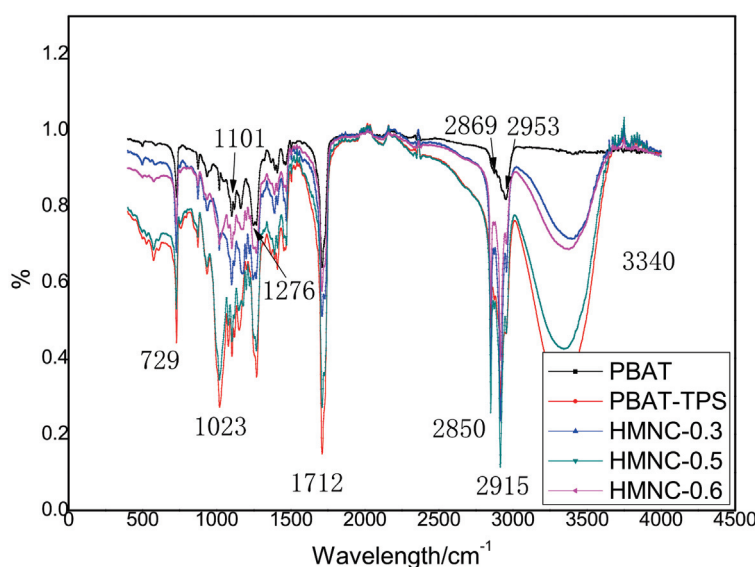


Figure 1. The FT-IR spectrum of PBAT-HMNC.

2.2. SEM Images of PBAT-HMNC

The SEM can be applied to identify the morphology of the composite (PBAT-HMNC); the morphology of the composites (PBAT-TPS) and the composites with HMNC (PBAT-HMNC-0.3 and PBAT-HMNC-0.6) are compared to show whether the HMNC has been dispersed well in the substrate, and the obtained images are indicated in Figure 2. The compatibility between PBAT and TPS is excellent due to the smooth morphology obtained in Figure 2a,b. The TPS granules are dispersed well in the PBAT and the homogenous phase can be observed [20]. In addition, the HMNC also disperses ideally in the substrate (PBAT-TPS) via the application of extrusion and lab-scaled film blowing (from Figure 2c,d). As the content of HMNC increased, more HMNC fibers can be observed in the images (from Figure 2e,f). However, the ideal compatibility between HMNC and the substrate PBAT-TPS can also be observed; the fibers disperse well in the composites [21].

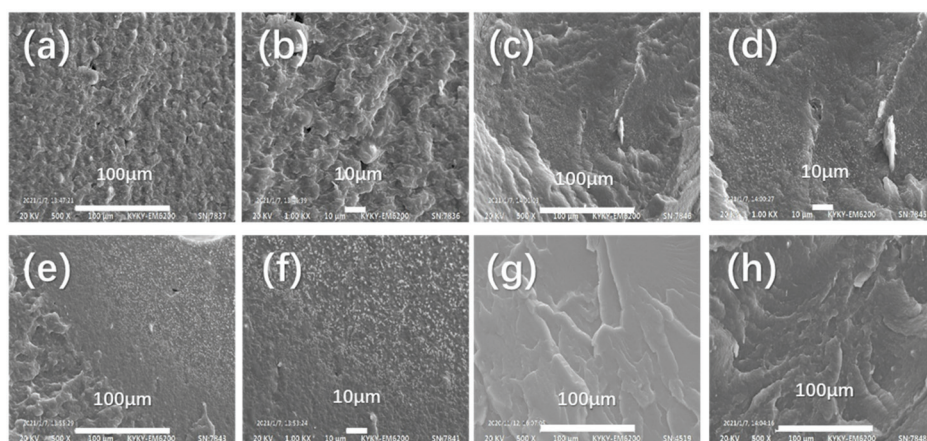


Figure 2. The SEM images of PBAT-HMNC composites: (a,b) PBAT-TPS composite; (c,d) HMNC-0.3; (e,f) HMNC-0.6; (g) PBAT; and (h) TPS.

2.3. The Thermal Stability of the Composites (TG and DTG)

The material will undergo a sudden change in weight at a specific temperature, and TGA measurement can be used to determine the thermal degradation performance of the samples [22]. In this work, the thermal degradation performance of PBAT and PBAT-HMNC composites (PBAT-HMNC-0.3, PBAT-HMNC-0.5, and PBAT-HMNC-0.6) were tested using TGA and the corresponding results, including the TG and DTG curves, are indicated in Figure 3. Generally, PBAT shows a single thermal decomposition process at a certain temperature. From Figure 3, PBAT enters an obvious thermal degradation at 402 °C from Figure 3b (the thermal decomposition stage theoretically appears when the temperature is higher than 400 °C); during the process, some unstable components are decomposed into small molecule gases and condensable macromolecules. The residual of PBAT at 600 °C is less than 10%. In terms of the thermal decomposition stages of PBAT-HMNC composites, there are main three stages, which depend on the different contents of the matrix. Compared with the curves of TG and DTG, the first stage, which happens at 128 °C, is related to the evaporation of water from the composites (the temperature is higher than the boiling point of water); the mass ratio of the composites decreases no more than 10%. The second stage, obtained at 299 °C, corresponds to the thermal decomposition of TPS from the composites; the mass ratio of the composites decreases at around 30%, which matches the content of the TPS from the composite films. The third stage occurs at 402 °C with 54% mass loss of the composites, presumably due to the PBAT content. In a comparison of the DTG curves between PBAT, HMNC-0.3, HMNC-0.5, and HMNC-0.6, the thermal decomposition temperature of the third stage decreases by 1 or 2 °C, which might be due to the fillers (TPS and HMNC) changing the continuous structure of PBAT, thus destroying the continuous phase of PBAT and lowering thermal stability [23].

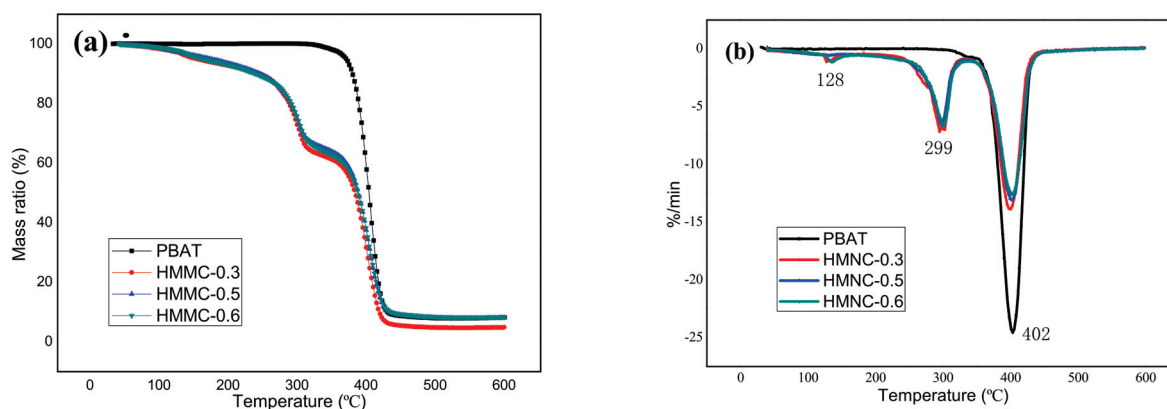


Figure 3. The DG and DTG curves of PBAT-HMNC: (a) the TG curves; (b) The DTG curves.

2.4. The Mechanical Properties of PBAT-HMNC Composites

Tensile strength can be measured using the tensile testing machine. In the tensile test, the tensile strength is shown by the maximum tensile stress of the sample until fracture. The elongation at break is expressed as a percentage (%) and usually refers to the ratio of the displacement of the sample at break to the original length. Tensile strength and elongation at break are extremely important factors for composite films; the corresponding results are revealed in Figure 4. The tensile strength and elongation at break exhibit the contrast trend with the addition of HMNC, the tensile strength increases and the elongation at break decreases with the increase in HMNC content. The HMNC is evenly dispersed in the PBAT matrix, and a well-dispersed structure can be observed (see the SEM images), resulting in increased tensile strength [24]. The elongation at break decreases with the increase in HMNC content, which might be due to the continuous phase of the PBAT molecule chains being destroyed, and defect points appear during the test. The sample breaks faster when the testing equipment pulls the samples.

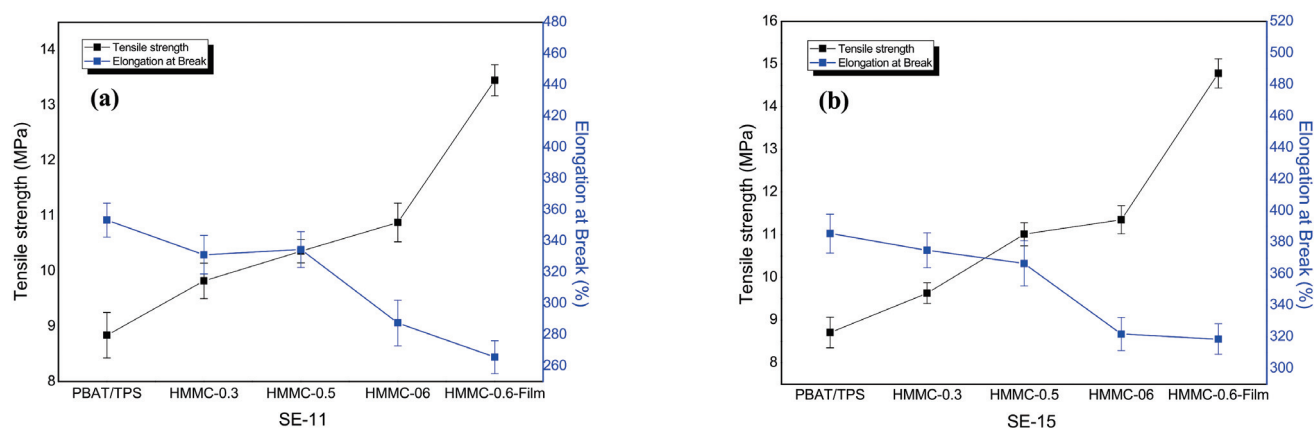


Figure 4. The tensile strength and elongation at break of PBAT-HMNC composites: (a) SE-11 and (b) SE-15.

In addition, the tensile strength reaches the maximum value when the HMNC content is 0.6% for the composite with both SE-11 and SE-15. The HMNC-0.6 film, produced by a film-blowing process, reveals the maximum tensile strength compared to the films with the HMNC contents of 0.3% and 0.5%. During the film-blowing process, the film forms with a vertical force, resulting in the formation of an oriented network where the HMNC and TPS reach an ideal dispersion state. The force from tensile testing equipment, therefore, will be transferred from the samples to HMNC and TPS while stretching, resulting in improved tensile strength of the composite films. This phenomenon is also demonstrated with the report that biaxial tensile processing can improve the film properties.

2.5. The Barrier Properties of PBAT-HMNC Composites

The barrier performance is an evaluation of the permeability of the packaging materials with respect to a certain penetration object. It is an important indicator for packaging materials, and also a basic function that the packaging materials must have, especially for food and medicine. The barrier properties of the composites have been tested and the corresponding results, including the WVTR and OTR, are indicated in Figure 5. The influence of the HMNC content on the PBAT/TPS film is also shown. As indicated, the PBAT/TPS composite has a relatively lower barrier property towards water vapor and oxygen, with the WVTR and OTR of around 800 g/(m²·day) and 1350 cc/(m²·day), respectively. The WVTR and OTR decrease with the increase in HMNC content. The lowest values of WVTR and OTR are 389 g/(m²·day) and 782 cc/(m²·day) for PBAT/TPS film with 0.6 wt% HMNC produced via a film-blowing process, which is 51.3% and 42.1% lower than that of the PBAT/TPS composite without HMNC. Generally, the HMNC can improve

the gas barrier property, thus reducing gas dissolution on the surface of the composite film and reducing the gas diffusion rate. The improved gas barrier property is mainly due to the “multi-path” effect; in addition, the molecular chain of the HMNC is flexible and easy to wind, which forms a dense three-dimensional network. Meanwhile, the film-blowing process makes the HMNC easier to disperse evenly and form an oriented structure, resulting in a higher gas barrier property of the composite film [25].

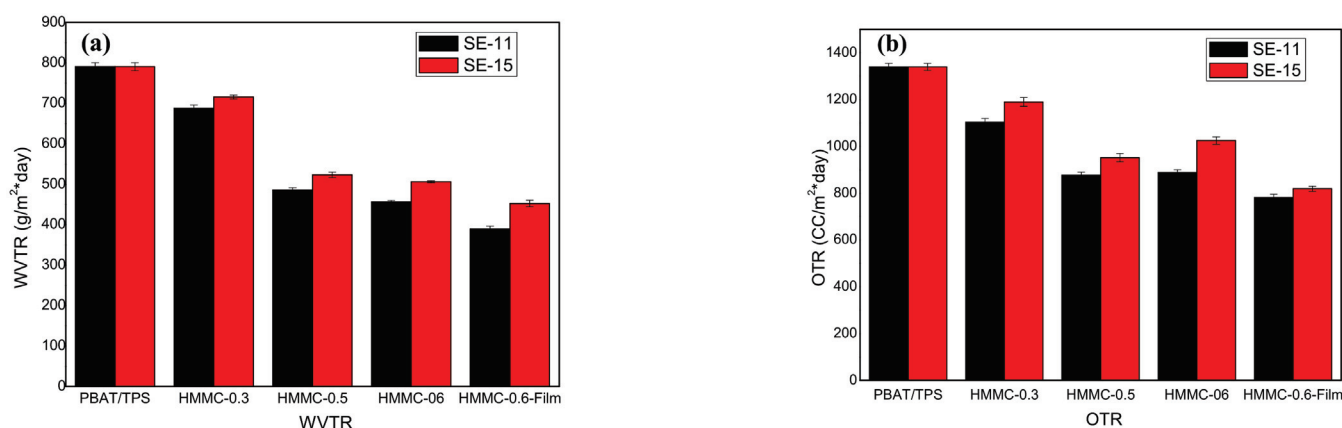


Figure 5. The WVTR (a) and OTR (b) of PBAT-HMMC composites (SE-11 and SE-15 added).

2.6. The Freshness Preservation Evaluation

As a fresh-keeping packaging material, better barrier performance is a critical parameter. The research shows that film with a certain barrier performance and certain permeability has the best fresh-keeping effect for fruits and vegetables. The freshness preservation evaluation of the high-valued mushroom (*Agaricus* mushroom) was carried out using PBAT/TPS film with 0.6 wt% HMNC and commercial polyethylene punch film as the packing films at the conditions of 5 °C and normal humidity. The weightlessness rate was calculated and the morphology was recorded by a digital camera to verify the changes in the quality of the products; the obtained morphology results are indicated in Figure 6. The *Agaricus* mushrooms still had a commodity value after 11 days of preservation using the film prepared in this work, and the weightlessness rate was 18.51%. In addition, the mushrooms packed in the PE film lost 49.5% of the total weight after 11 days of preservation. Most of the mushrooms were dry and lost their commercial value. The reason might be due to the fact that fungi, fruits, and vegetables also breathe in the packaging films and this requires a certain amount of oxygen in the film (the film needs a certain oxygen permeability). In addition, the water vapor generated by respiration also needs to be removed in time, which can easily lead to the deterioration of the mushrooms, fruits, and vegetables. In a reported work, the CNC has been successfully filled in biodegradable film and applied to the fresh-keeping of mango, which proved that biodegradable composite films were ideal candidates for food packaging [26]. In this work, all the raw materials are food contact materials and the as-prepared packaging films can also be used to extend the freshness period of common fruits and vegetables. The PBAT/TPS biodegradable film was proved to be the most proper candidate to pack edible fungi such as *Agaricus* mushrooms, oyster mushrooms, matsutake mushrooms, etc.



Figure 6. The freshness preservation evaluation of *Agaricus* mushrooms using commercial PE film and PBAT-HMNC (PBAT-HMNC-0.6).

3. Materials and Methods

3.1. Materials

Poly-(butylene adipate-co-terephthalate) (PBAT, C1200, density 1.25–1.27 g/cm³, melt volume flow (MVR) (190 °C/2.16 kg) 2.5–4.5 cm³/10 min) was purchased from BASF, Ludwigshafen, Germany; nanofibrillated cellulose (NFC, solid content 4.5% ± 0.5%) was obtained from Guilin Qihong Technology Co., Ltd. (Guilin, China). Corn starch, glycerol, palm wax, sucrose fatty acid ester (SE-11 and SE-15, hydrophile lipophile balance, hydrophilic and lipophilic equilibrium values (HLB values) are 11 and 15, ethylene, and other reagents were used as received from Macklin reagent Co. (Shanghai, China)

3.2. Preparation of the Palm Wax Emulsion

First, 100 g palm wax was added to a beaker and heated up to about 85 °C, the wax was melted and then a total volume of 400 mL surfactant (SE-11 and SE-15) (1 wt% based on the weight of palm wax) aqueous solution with a temperature of 90 °C was added to the melted palm wax. After 3 min, emulsification took place using the high-speed homogenizer (BRS-200, Anhui Bunkin Chemical Machinery Co., Ltd., Hefei, China); the palm wax emulsion was obtained and quickly transferred to the ice water bath. Finally, the emulsion was obtained after being cooled down to room temperature and the resulting emulsion was labeled as PWE (white color).

3.3. Preparation of the Hydrophobically Modified Nanofibrillated Cellulose (HMNC)

The HMNC was prepared via an emulsion modification with the addition of PWE. First, the solution with 5 wt% of the nanofibrillated cellulose (NFC) was prepared in three-necked flasks, the PWE was then added with a fixed weight ratio (5% towards the content of NFC). The matrix solution was then placed on a stirrer after being fully dispersed. The chains of the cellulose were loaded with the PWE; the hydrophilicity of NFC was changed

by the reaction that occurred between -OH on cellulose chains and PWE for a one-hour modification process. The water was then removed via filtration and the modified NFC was then obtained (HMNC) [27]. The resulting HMNC was then dried at 80 °C in a vacuum oven for 24 h, ground and stored in a dry cabinet for future utilization.

3.4. Preparation of PBAT- HMNC Composite Films

The PBAT-HMNC with four different HMNC concentrations (0, 0.3, 0.5, and 0.6 wt%), PBAT, and TPS were prepared using a melt extrusion in a twin-screw extruder (H1GD 20, Guangzhou Hartek Technologies Co., Ltd., Guangzhou, China 150 °C, 100 rpm, and residence time of 5 min; the TPS was prepared by mixing the 74 wt% of corn starch with the 26 wt% of glycerol under the conditions of 140 °C, 100 rpm, and residence time of 5 min). The PBAT was dried for at least 1 h at 80 °C before use. The masterbatch of PBAT-HMNC (marked as PBAT-HMNC-0.3, PBAT-HMNC-0.5, PBAT-HMNC-0.6) was obtained from the cutter, and the composite films were produced via lab-scaled film-blowing equipment (XH-432-25, Dongguan Xihua Testing Machine Co., Ltd., Dongguan, China). The films were stored properly for further testing and application. Subsequently, the masterbatch of PBAT-HMNC was hot pressed (XH-406BWP-30-300, Dongguan Xihua Testing Machine Co., Ltd., Dongguan, China). Plates with dimensions of 25 mm × 1 mm were obtained for tensile testing.

Meanwhile, the non-solvent process was carried out by adding the materials (PBAT, TPS, NFC, surfactants, and wax) directly into the twin-screw extruder, followed by the film-blowing and hot-pressing processes for preparing the corresponding films and plates for further characterization.

3.5. Characterization

The surface morphology of PBAT-HMNC was tested via a Scanning Electron Microscope (SEM, FEI Phenom Prox, 5 kV, Phenom-world B.V., Eindhoven, The Netherlands). Samples were prepared by cutting before being sprayed with a thin gold layer. The functional groups of the synthesized samples were measured using FTIR spectra (Invenio S, Bruker Corporation, Billerica, MA, USA) and the samples were tested directly with an ATR spectrophotometer. The data were collected 32 times at a resolution of 1 cm⁻¹. The thermal stability of the composite films was examined using thermogravimetric analysis (TG 209 F3 Tarsus, Tarsus GmbH, Berlin, Germany); the test was carried out from 35 °C to 700 °C at a heating rate of 20 °C/min under nitrogen condition (flow rate of 20 cm³/min). The tensile tests were performed in a Testing Machine (Model E43, MTS Industrial System (China) Co., Ltd., Shanghai, China) according to GB/T 1040.3 Standard. The dog bone specimens were prepared before testing. Five specimens were analyzed for each composition. The tensile strength and elongation at break were obtained from the tests.

3.6. The Measurement of the Oxygen and Water Vapor Barrier Properties

The gas barrier properties (OTR and WVTR) were characterized using Oxygen Permeation Analyzer (OX-TRAN, Ametek Mocon, Minneapolis, MN, USA) and Water Vapor Permeability (Permatran-W 1/50G, Ametek Mocon, Minneapolis, MN, USA). During the WVTR test, the sample is installed in the cavity, and a certain humidity difference is formed on both sides of the sample. The water vapor will penetrate through the sample from the high-humidity side to the low-humidity side under the action of the humidity difference. Through the measurement of the water vapor penetration, the WVTR of the samples can be obtained. The OTR test is carried out with similar mechanism via Oxygen Permeability (OP) Analyzer.

4. Conclusions

Biodegradable composites consisting of HMMC and PBAT/TPS were successfully prepared via the extrusion and film-blowing processes, followed by the systematic characterization of the SEM, FTIR, mechanical, and barrier properties. The resulting PBAT-HMMC

composites exhibited excellent mechanical and barrier properties. The lowest values of WVTR and OTR were obtained from the composite with 0.6 wt% HMMC, prepared via the film-blowing process. Overall, PBAT-HMMC composites have been proven to be easy to prepare, environmentally friendly, and promising gas barrier nanocomposite materials for packaging, showing a great economic value and the application can be readily extended to other fields, such as agricultural mulch, medicine capsules, etc.

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Sample Availability: Not applicable.

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Article

Fluorescence and Nonlinear Optical Response of Graphene Quantum Dots Produced by Pulsed Laser Irradiation in Toluene

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Abstract: Graphene quantum dots (GQDs), the zero dimensional (0D) single nanostructures, have many exciting technological applications in diversified fields such as sensors, light emitting devices, bio imaging probes, solar cells, etc. They are emerging as a functional tool to modulate light by means of molecular engineering due to its merits, including relatively low extend of loss, large outstretch of spatial confinement and control via doping, size and shape. In this article, we present a one pot, facile and ecofriendly synthesis approach for fabricating GQDs via pulsed laser irradiation of an organic solvent (toluene) without any catalyst. It is a promising synthesis choice to prepare GQDs due to its fast production, lack of byproducts and further purification, as well as the control over the product by accurate tuning of laser parameters. In this work, the second (532 nm) and third harmonic (355 nm) wavelengths of a pulsed nanosecond Nd:YAG laser have been employed for the synthesis. It has been found that the obtained GQDs display fluorescence and is expected to have potential applications in optoelectronics and light-harvesting devices. In addition, nonlinear optical absorption of the prepared GQDs was measured using the open aperture z-scan technique (in the nanosecond regime). These GQDs exhibit excellent optical limiting properties, especially those synthesized at 532 nm wavelength.

Keywords: pulsed laser irradiation; graphene quantum dots; fluorescence; optical limiting

1. Introduction

Graphene quantum dots (GQDs) are a distinguished zero-dimensional group of graphitic nanomaterials having various topologies. They are minute splinters of carbonaceous materials with particle size below 10 nm and an sp²-carbon structure core, revealing exciton confinement and quantum size effect [1,2]. Generally, the fabrication of broadband modulators, solar cells, photodetectors, etc., were based on the materials having broadband absorption (BBA) and emission that covers the UV-Visible, near-IR regions of the electromagnetic spectrum [3–8]. The materials with BBA also exhibit non-linearity and hence they can have potential applications in the fields of high harmonic generation, optical parametric oscillation, multiphoton imaging and Kerr effect [9–12]. Hence, inventing novel materials with both optical nonlinearity and BBA characteristics are of great interest nowadays. Quantum dots belong to the groups IV to VI, namely, CdS, CdSe, PbSe, ZnSe, HgTe, etc., have already been applied in variety of applications, such as bio imaging, light emitting diodes and solar cells, due to their tunable absorption and desired optical nonlinear characteristics [13–17]. Materials generated from high bandwidth semiconductors, such as GaN, ZnSe, ZnS and AlN, exhibit UV optical activity, and materials generated from rare

earth doped GaN and CdS possess near IR activity [18,19]. Even though the size tuning of quantum dots can alter the active optical range, it cannot give the entire range completely, i.e., simultaneous UV-Vis and IR activity. To achieve this, GQDs and modified GQDs are promising candidates for such active optical activity [20–22]. Hence, GQDs are a better substitute for these toxic groups IV–VI quantum dots due to their biocompatibility, superior photo stability and cost-effective preparation methods.

Regardless of the synthetic route, GQDs display photoluminescence (PL) features. The PL emission is heavily dependent on the morphology and micro structure instigated during the synthesis. Despite the enormous effort paid to this field of research, the exact origin of photoluminescence (PL) and the fate of the excitation energy are still under debate. In the past couple of years, GQDs were subjected to extensive study and found that the photoluminescence (PL) emission may be aroused from electron-hole recombination, doping, quantum size effect, surface defects in the functional groups of GQDs, etc. [23]. In addition to PL, GQDs may exhibit interesting nonlinear optical properties. Recently, lots of theoretical and experimental research has been conducted on the BBA of GQDs of different sizes, shapes and functionalities, and great progress has been registered in elucidating the shape and size dependent nonlinear activity of GQDs [24–29]. In particular Ma et al., using z-scan techniques with femtosecond laser at 532 nm, investigated the third-order nonlinear optical responses of carbon dots and found that both samples exhibited positive nonlinear refractivity and negative nonlinear absorption coefficient [30].

Concerning the routes to produce GQDs, various methods, such as solvothermal, hydrothermal, electrochemical synthesis, ultrasonic, pyrolysis of organic compounds etc., have been incorporated to synthesize GQDs with control in microstructure and morphology [31–36]. However, many of these methods were complicated, time consuming and required pre-treatment and heavy purification processes. Later, the researchers focused on tuning the optical properties, chemical and surface characters of the GQDs through doping with heteroatoms, namely, Si, N, B and S [36–39]. Almost all these methods required costly precursors and long reaction time. Patil et al. introduced pulsed laser ablation in liquid (PLAL) as a novel, comparatively easier and single step to synthesize nanoparticles in 1987 [40,41]. In PLAL, a solid target is submerged in a liquid medium, and ablated with a beam of high energy Laser. During this process, the interaction between intense ($\sim 10^6$ to 10^{14} W/cm²) and short ($\sim 10^{-13}$ to 10^{-8} s) pulses of laser and the interface between the surface of the target material and liquid would generate bubbles with high temperature and pressure. This results in vaporization and the subsequent emission of surface materials brings nucleation and nanoparticle generation [42,43]. A couple of studies were reported on pulsed laser ablation of solid carbon targets in liquid media to produce carbon dots and GQDs. Furthermore, carbon nanoparticles generated by PLAL show solvent dependent optical properties since the surface can be functionalized or modified by the reaction with the solvent [44–47]. Pegylated GQDs were generated in situ through PLAL of a graphite target in polyethylene glycol solution as in-vivo fluorescent image agent [48]. It is also reported that N-doped GQDs can be fabricated through PLAL synthesis by using aqueous solution of graphene oxide as carbon source and diethylene triamine as N source [49]. These reports revealed the possibility of surface passivation and doping with heteroatoms, which can augment the quality of GQDs. Recently, pulsed laser assisted sythethis has been demonstrated as a clean and efficient one-step method to fabricate GQDs and carbon nanostructures from a bottom-up approach using precursors such as toluene [50], chlorobenzene and o-dichlorobenzene [51].

Using a similar bottom-up approach, the motivation of this work is to generate GQDs in toluene, by pulsed laser irradiation, without using many reagents and catalysts. A variety of GQDs are produced by using both the second (532 nm) and third harmonic (355 nm) wavelengths of an Nd:YAG laser. The GQDs exhibit unique optical limiting properties, especially those synthesized at 532 nm wavelength. This study highlights the impact given by nanosecond laser radiation upon the size controlled of synthesis of GQDs from in an organic liquid with exceptionally small reaction time. Apart from the

previous report by Yu et al. [50], here we explored the GQDs synthesis with second (532 nm) and third harmonic (355 nm) wavelengths of Nd:YAG laser other than the fundamental wavelength (1064 nm) without using any reaction protective gases such as Argon. The whole experiment was conducted in room temperature and atmospheric pressure. We found that, at fluence, $F \geq 3.1 \text{ Jcm}^{-2}$, the ablation mechanism is apparently explosive boiling and does not lead to QDs formation and we fabricated less than 10 nm GQDs at lowest laser fluences in these particular experimental conditions, apart from the study reported by Yu et al. [50]. Even though the direct photodissociation of toluene molecules to carbon dots under pulsed laser irradiation in the absence of surfactants or catalysts was also reported by Zhu et al. [52], we had generated GQDs in nanosecond regime and established the size control by varying the laser wavelength and established the effect of the same to its NLO properties for the first time.

2. Experimental Details

2.1. Synthesis

An Nd:YAG Laser beam (Litron LPY 674G-10, repetition rate-10 Hz, pulse width-8 ns) was focused into a glass cuvette containing 30 mL of toluene and ablated that carbon source for 120 s, corresponding to different laser energy densities (starting from 1 Jcm^{-2} to 3 Jcm^{-2}), which immediately results in the generation of graphene quantum dots (see Figure 1). The experiments have been performed at second (532 nm) and third (355 nm) harmonic laser wavelengths. Here, the bottom-up synthesis approach for generating highly pure colloidal GQDs has been employed. Laser irradiation creates high non-equilibrium conditions within liquids with extremely high pressure and temperature where anomalous processes and the fast growth of the fragmented nano-sized species can occur. A change in color of the solution to transparent yellow after the irradiation has been observed (Figure 1), which infers the formation of GQDs. Further, the samples were centrifuged at 6000 rpm for 20 min and then the GQDs will remain in the supernatant liquid.

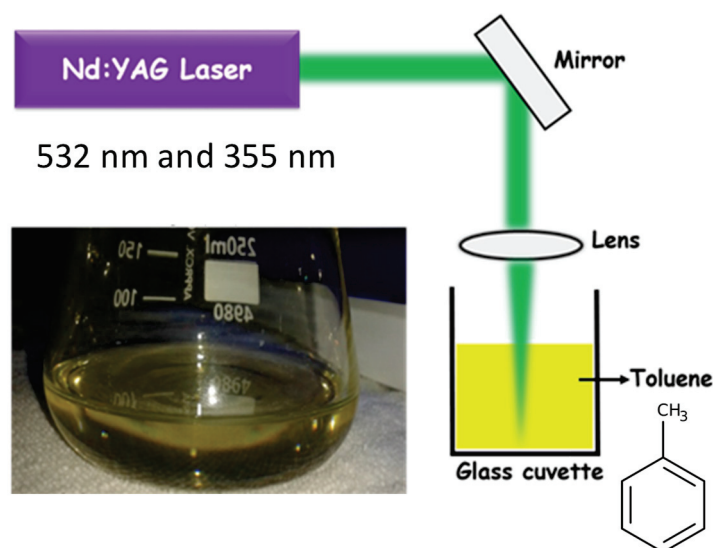


Figure 1. Experimental layout of production of GQD via laser irradiation in toluene and the yellowish GQD solution just after irradiation.

2.2. Characterization

The FTIR spectra of the samples were recorded using ATR-FTIR (Shimadzu, IR Prestige 21) spectrometer in the range of $4000\text{--}450 \text{ cm}^{-1}$. The Raman spectra were recorded with Confocal Raman Microscope with AFM WITec Germany, Alpha 300RA. TEM measurements were performed on JEOL JEM 2100 electron microscope at operating voltage of 200 kV. Fluorescence spectra were recorded on a fluorescence spectrophotometer (HORIBA

Fluoromax-4 spectrofluorometer). Prompt decay is the instrumental response function, the response of the instrument to a zero-lifetime sample. This curve is typically collected using a dilute scattering solution such as colloidal silica (Ludox) and no emission filter. This decay represents the shortest time profile that can be measured by the instrument. The width of the IRF is due to both the characteristics of the detector and the timing electronics. Shimadzu (model 2450) UV-Vis spectrophotometer was used for the absorption measurements and the spectra were typically measured in the range of 85–800 nm. X-ray photoelectron spectroscopy (XPS) was carried out using Kratos Analytical, Axis ultra 1486 eV spectrometer with monochrome Al K α as the excitation source. The nonlinear optical properties (NLO) of the GQDs have been examined by the z-scan technique as described in reference [53].

3. Results and Discussion

The GQDs via pulsed laser irradiation of toluene have been prepared within a short span of time. When the laser beam impinges on the surface of the toluene, the electrons are under the influence of the electric field of the laser pulses and are accelerated to produce plasma. This laser produced plasma enhances the breakdown of toluene to form GQDs. The experiments have been performed at second (532 nm) and third (355 nm) harmonic laser wavelengths with laser fluence ranging from 1 to 3 Jcm⁻². At large fluences ($F \geq 3.1$ Jcm⁻²), the irradiation mechanism becomes explosive and that does not lead to GQD formation. The preparation requires utilization of very small reaction time (2 min) and less laser energy compared to the previous reported literatures [6,7]. The physical, chemical and morphological characteristics were studied by FTIR and Raman spectroscopy, X-ray photoelectron spectroscopy and Transmission Electron Microscopy. The optical characteristics were studied by UV-Visible spectroscopy and photoluminescence spectroscopy. The nonlinear optical response of the GQDs has been examined by z-scan technique.

3.1. Morphological Analysis

The structure and morphology of the as-prepared GQDs were investigated by transmission electron microscopy (TEM). Transmission Electron Microscopy (TEM) images were recorded using JEOL JEM 2100 electron microscope. Figure 2 illustrates the TEM images of GQDs, in which GQD@355 nm displays particle size in the range of 3 ± 2 nm, and GQD@532 nm in the range 5 ± 3 nm. It has been noted that there is a slight increase in the particles size of GQDs with the long wavelength laser ablation. Almost all the GQDs show uniformity in size and shape. The particle size distribution curve is included in the inset. The HRTEM images reveal clear details about the inter planar distance of 0.212 nm for the GQD which is tie in with the (100) plane of graphene. The selected area electron diffraction (SAED) of the as-prepared GQDs exhibited diffraction rings due to the presence of nanocrystals randomly oriented and most of them are associated with the graphitic structure. The TEM analysis had demonstrated the presence of a graphitic core, unlike an amorphous core in ordinary carbon dots.

3.2. XPS, FT-IR and Raman Analyses

XPS measurements were carried out to probe the composition of GQDs. As seen in Figure 3a, the XPS show a dominant graphitic C1s peak at 284.8 eV and O1s peak at 532 eV for GQDs. The measured spectrum of C1s is nicely de-convoluted into four surface components, which correspond to sp² C (C=C, C–C) at a binding energy of 284.6 eV, sp³ C (C–OH) at 285.9 eV, C–O–C at 287.3 eV and C=O at 289.6 eV [54]. These results suggest that functionalization of the graphene sheet with oxygen containing functional groups took place during the course of the reaction. The presence of sp³ C peak in the XPS spectra suggests that planarity in GQD structure has reduced severely, and defects appeared in the prepared GQDs. The XPS spectrum of O1s is resolved into peaks at 532.7 eV corresponding to C=O and these results suggest that the prepared GQDs are rich in oxygen containing functional groups.

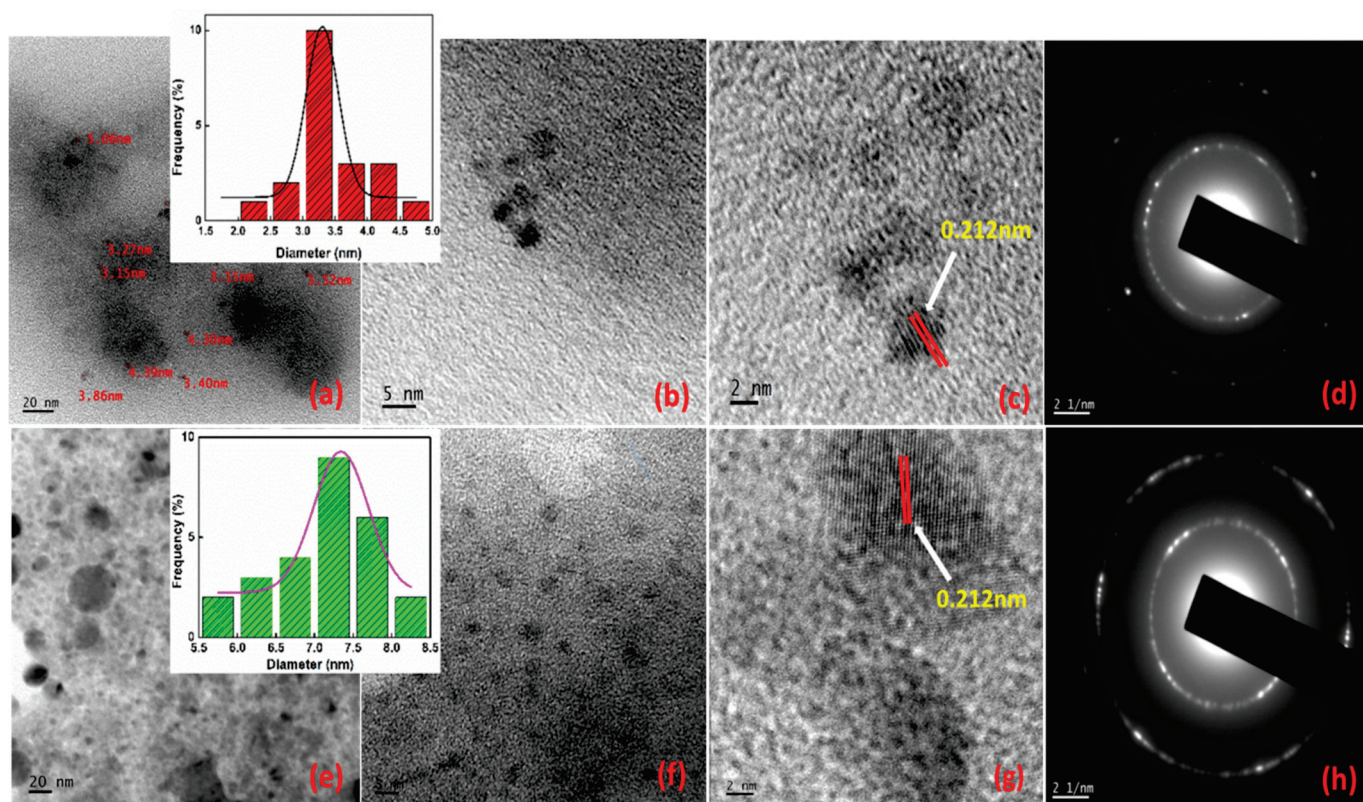


Figure 2. TEM and HRTEM images of (a–d) GQD@355 nm and (e–h) GQD@532 nm.

The Raman spectra of GQDs are shown in Figure 3d, where peaks around 1329 cm^{-1} and 1582 cm^{-1} represent the D-band and G-band, respectively. The D and G peaks appeared in the spectrum attributed to the aromatic domains of graphene quantum dots. The peak at 1329 cm^{-1} is associated to the disordered band due to structural defects, dangling and edge sp^3 carbon bonds that break the symmetry and the peak at 1582 cm^{-1} correspond to the in plane stretching vibration of sp^2 carbon atoms. The intensity of the G band is related to the amount of sp^2 domains whereas the D band to the sp^3 domains. The intensity ratio between the D and G bands (I_D/I_G) can be employed to examine the sp^3 structure of the GQDs. There is no appreciable difference in the I_D/I_G ratio (0.94) for both GQD@532 nm and GQD@355 nm. This implies that the synthesized GQDs have more disordered structure and this could be due to the presence of more oxygen-containing functional groups in the prepared GQDs. Consequently, the intensity of Raman scattering was determined by the concentration of particles confirming that the scattered Raman intensity has increased in the case of GQD@532 nm. The functional groups of GQDs were identified by FTIR spectroscopy by analyzing their vibrational spectra. Existence of C=O, O–H and C–O groups in GQDs are substantiated by FTIR spectroscopy. Figure 3e shows the FTIR spectra of GQDs synthesized at two different laser wavelengths. The FTIR spectra of GQDs (in solution form) show highly resolved peaks corresponding to stretching frequency of O–H, C=O and C–O groups. The bands at 2981 cm^{-1} and 1375 cm^{-1} are attributed to the stretching and bending vibrations of C–H groups, respectively. The broad band at 3423 cm^{-1} is assigned to the stretching frequency of O–H groups. The bands at 2981 cm^{-1} and 1375 cm^{-1} , respectively, are attributed to the stretching and bending vibrations of C–H groups. The bands around 1728 cm^{-1} and 1225 cm^{-1} are due to the stretching of C=O and C–O groups, respectively. The peak at 1625 cm^{-1} indicates the existence of aromatic C=C groups. It can be seen that the C–H bending and stretching occurs at 1468 cm^{-1} and 2930 cm^{-1} respectively and the C=C bonds shows the presence of aromatic carbon which is situated at around 1735 cm^{-1} [55]. Of note, after being laser irradiated, the FTIR spectra

of the GQDs suspension showed the same characteristics as pure toluene (compare spectra in Figure 1e,f), confirming that the solvent after laser irradiation was still toluene.

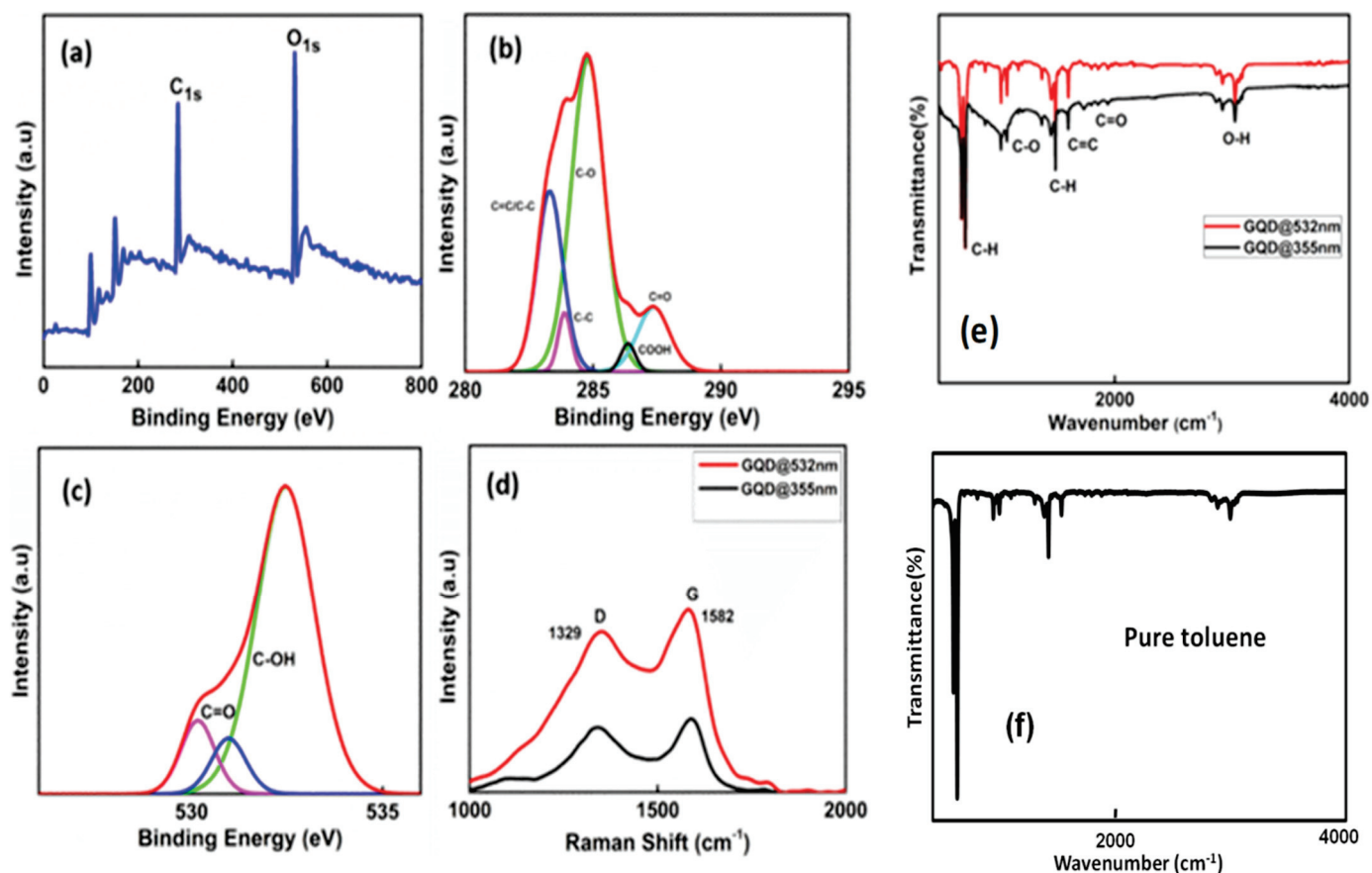


Figure 3. X-ray photoelectron spectroscopy (XPS) and Raman spectra of the laser synthesized GQDs. (a) Wide scan spectra. (b) C_{1s} spectrum. (c) O_{1s} spectrum. (d) Raman spectra. (e,f) FT-IR spectra.

3.3. UV-Visible Absorption Spectroscopy

To obtain the optical properties, UV-Vis analysis of GQDs were carried out. Figure 4a) shows the UV Visible absorption spectra of GQD@532 nm and GQD@355 nm synthesized at 2.6 J cm^{-2} laser fluence. The absorption spectra of both the prepared samples are almost identical and at 287 nm with a small shoulder peak at around 340 nm that corresponds to the $n\text{-}\pi^*$ transitions of the carbon oxygen bond (C=O), which is most obviously seen in graphene [56]. Here, it can also be seen that the absorption intensity increases with increase in the laser wavelength and fluence which indicates the increase in particle size and concentration at higher wavelength ablated species (Figure 4b). Since the absorption cross section is proportional to square diameter of absorbing particles, much smaller absorbance occurred in the case of 355 nm indicating smaller density of absorbing particles. Irradiation with a 355 nm wavelength resulted in a surprisingly small production rate compared to longer wavelengths, such as 532 nm, due to absorption of the laser radiation on a short path.

3.4. Photoluminescence Spectra

The photoluminescence properties of the samples are shown in the Figure 5a. The PL emission of GQDs synthesized at 2.6 J cm^{-2} at second and third harmonics of laser wavelengths and the PL emission is observed at 406 nm at an excitation wavelength 360 nm. From this it could be inferred that the GQDs exhibits blue fluorescence, which is the contribution of emission from LUMO to HUMO [35]. It has also been observed that there is a small luminescence quenching takes place with a little reduction in intensity in the case of GQD@355 nm. Moreover, the PL intensity enhances with the increase in laser wavelength

and the strong photoluminescence activity of the prepared GQDs in the ultraviolet-visible region can be explored in various sensing applications.

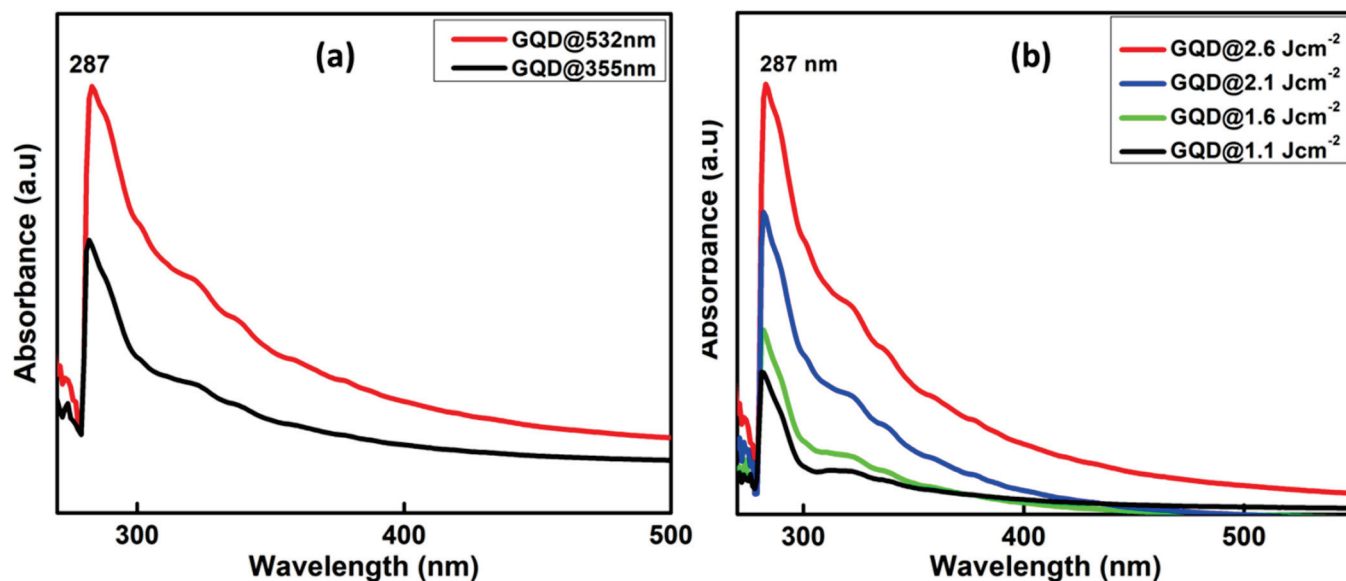


Figure 4. (a) UV-Visible absorption spectra of GQDs synthesized at different laser wavelengths and (b) at different laser fluences.

The fluorescence lifetime of the GQDs (Figure 5b) was investigated using time-correlated single photon counting (TCSPC) equipped with the HORIBA Fluoromax-4 spectrofluorometer. The measurement of the fluorescence lifetime is one of the absolute methods to distinguish between static and dynamic fluorescence quenching. The fluorescence decay curves of the colloidal solution of GQDs were fitted to multi-exponential functions. Here, GOD requires three time constants for satisfactory fits and they are $T_1 = 3.135083 \times 10^{-9}$ s, $T_2 = 1.170301 \times 10^{-9}$ s, $T_3 = 1.05686 \times 10^{-8}$ s respectively. The mean life time value can be calculated using Equation (1) [57].

$$T_m = a_1 T_1 + a_2 T_2 + a_3 T_3 \quad (1)$$

where, a_1 , a_2 , a_3 , T_1 , T_2 and T_3 are the relative amplitudes and decay times of the multi exponential components of the fluorescence decay. The mean life time of the prepared GQD obtained from the time resolved measurement is 3.7 ns and the quenching effect was identified as static fluorescence quenching. The prepared GQDs display very low fluorescence quantum yields (estimated to $\sim 10^{-4}$ to 10^{-3} using the reference method and rhodamine B as reference dye), mainly due to a very strong absorbance in the UV range and absence of a comparable level of absorbance at the wavelengths of maxima of the excitation spectra.

Commission International d'Eclairage (CIE) 1931 system has been used to evaluate the color luminescence emission intensities and to identify the dominant emission wavelength of the fabricated GQDs for evaluating the material performance which is shown in Figure 5c. CIE chromaticity studies can be useful in this context to give more idea about the possible application in LED lighting devices. The color of the phosphor can be frequently expressed by (CIE) coordinates. To reflect the true color of GQD luminescence, CIE 1931 (x, y) coordinates are calculated [58,59]. The CIE coordinates calculated are (0.175, 0.178). Hence, the prepared GQDs while excited with commercially available blue LED's can be used as potential candidates for blue emitting light sources.

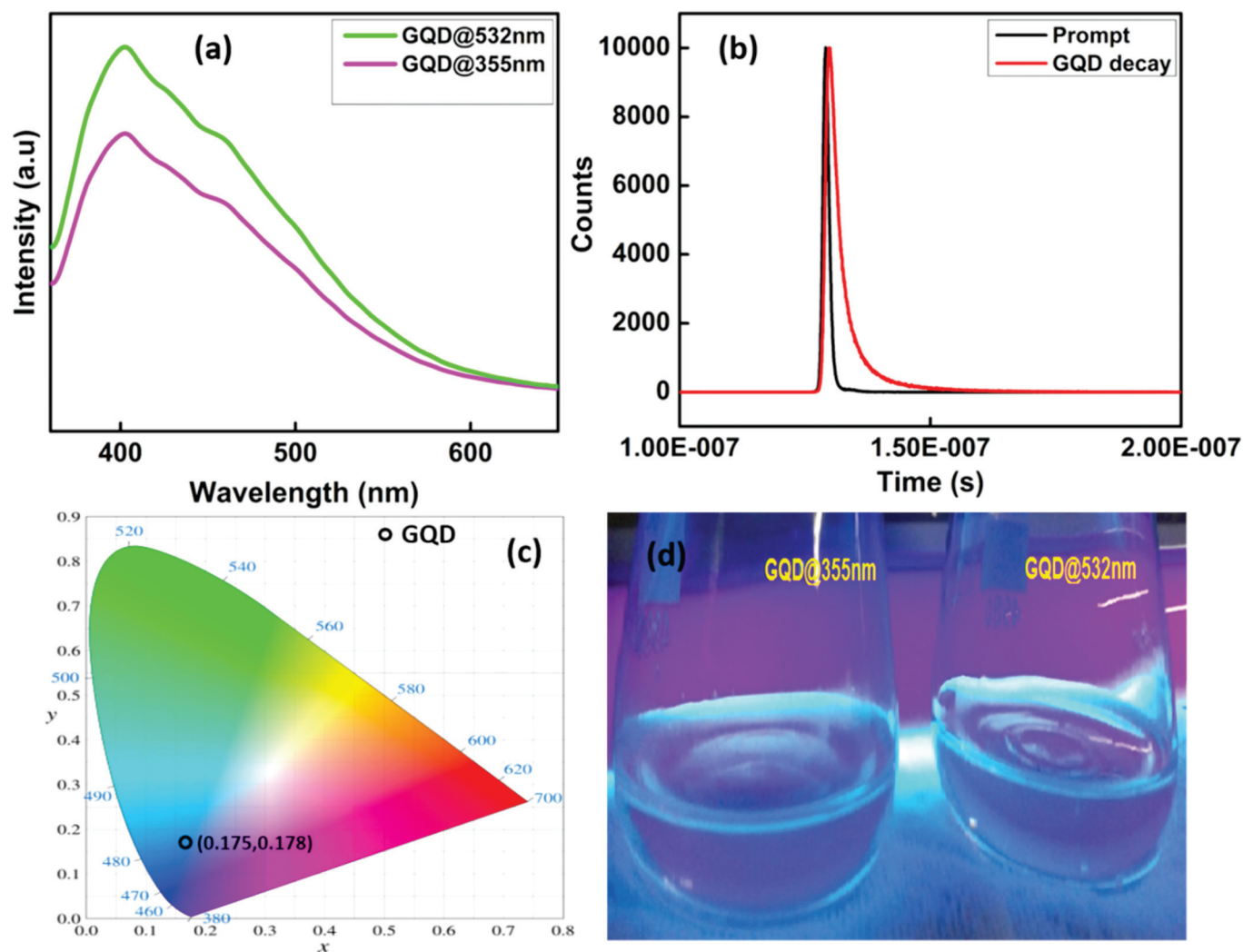


Figure 5. (a) Photoluminescence spectra of GQDs at an excitation wavelength 380 nm. (b) Fluorescence decay curve. (c) CIE chromaticity color coordinate diagram fluorescence to naked eye shown by the samples when subjected to UV radiation (d).

3.5. Nonlinear Optical Studies

The nonlinear optical transmission measurements were carried out using the open aperture z-scan technique, which is actually a measurement of the optical transmission of the material as a function of input light intensity. The z-scan set up is comprised of a pulsed laser beam having known energy, a converging lens to focus the laser beam, and laser energy detectors. The propagation direction of the laser beam is assumed to be in the z-axis, such that the fluence is maximum at the focal point $z = 0$ which decreases towards either direction on the z-axis. The sample is moved along the z-axis, towards or away from the focal point to vary the light fluence falling on it. The position versus transmission curve (z-scan curve) can be drawn by measuring the transmitted energy for different sample positions using a detector. Optical nonlinearity coefficients can then be calculated numerically by fitting the measured z-scan curve to standard nonlinear optical transmission equations [60–62].

The laser sources used in the current setup is a Q-switched Nd:YAG laser (Minilite, continuum) emitting at the second harmonic wavelength of 532 nm. The Gaussian beam is focused through a plano-convex lens of focal length 10.75 cm. The laser pulse widths (FWHM) are approximately 5 ns. The samples are taken in a 1 mm cuvette. The cuvette is mounted on a stepper motor controlled linear translational stage and is translated along the z-axis through the focal region. The sample sees different laser fluencies at each z positions

and the corresponding transmittance is measured using a pyroelectric energy probe (Rj7620, Laser probe Inc., Utica, NY, USA) placed behind the sample. In all measurements, the lasers were running in a single shot mode by externally triggering the laser output. Figure 6 shows the open aperture z-scan curves and the subsequent fluence dependent nonlinear transmissions acquired for GQDs for 532 nm and 355 nm, 5 ns laser excitation at input laser pulse energy of 25 μ J. The optical limiting measurements are quantified using the limiting threshold value, which is defined as the input fluence at which the sample's normalized transmittance drops to 50% of its linear transmittance. Materials having lower limiting thresholds exhibit maximum optical limiting performance. The limiting threshold value of GQDs is better than that of standard materials such as C60 in toluene and carbon black in water [63]. Mostly, the optical limiting property of a material can obtain contributions from phenomena such as excited state absorption, (ESA) including free-carrier absorption, excited singlet or triplet absorption, etc., and two or three-photon absorption, which strongly depend on the material, excitation pulse width and wavelength. It has been shown from the previous reports that ESA is much stronger for nanosecond pulse excitations in graphene-based materials [64].

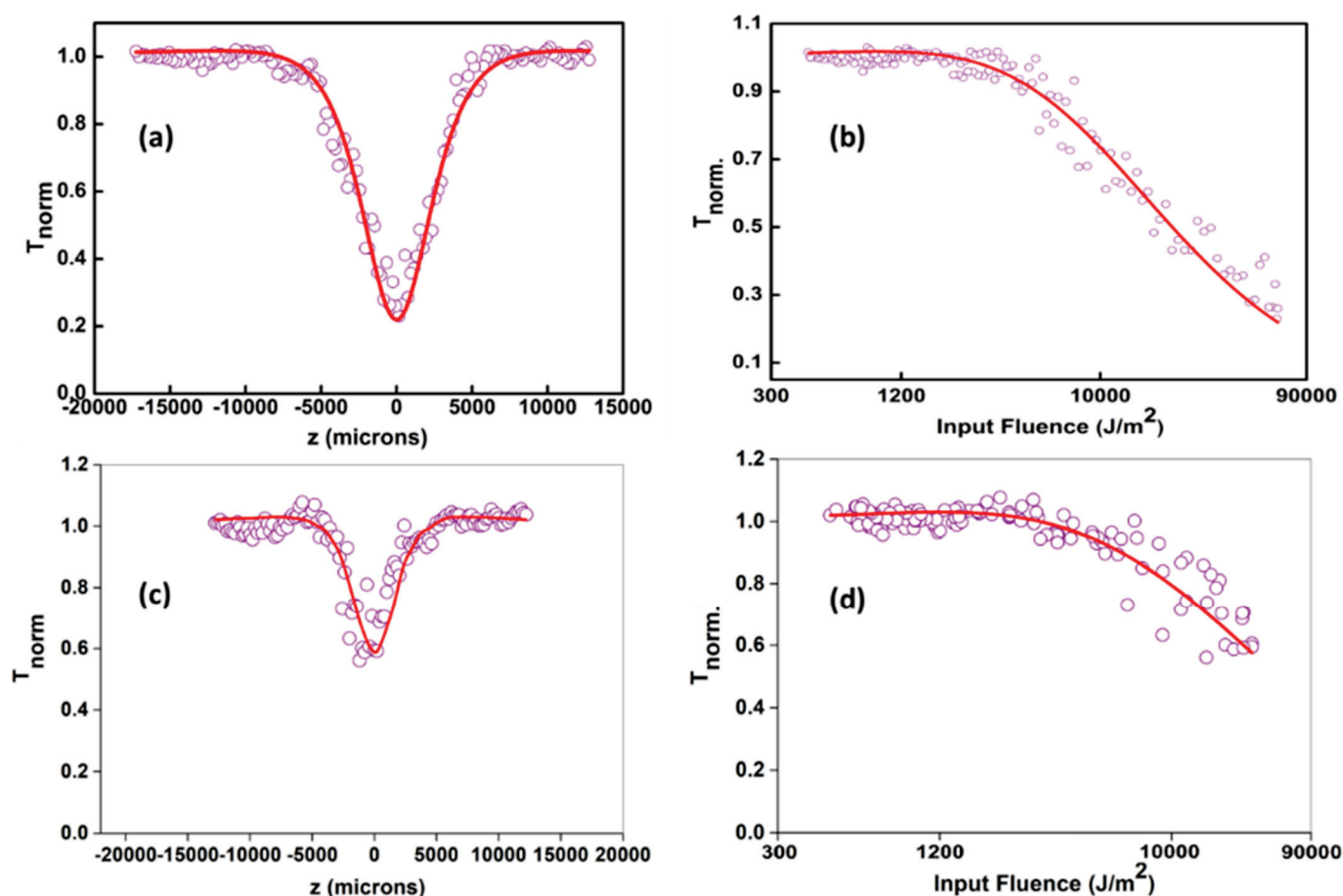


Figure 6. (a,b) Open aperture z-scan of GQDs@532 nm, 5 ns laser pulse excitation at laser pulse energy of 25 μ J. (c,d) Open aperture z-scan of GQDs@355 nm, 5 ns laser pulse excitation at laser pulse energy of 25 μ J. Open circles: data points, solid lines: numerical fits to the data points.

The intensity dependent effective nonlinear absorption co-efficient $\alpha(I)$ is given by the equation [65]

$$\alpha(I) = \alpha_0 / (1 + (I/I_{sat})) + \beta_{eff} I \quad (2)$$

where I and I_{sat} represent the laser input and saturation intensities, respectively, while α_0 and β_{eff} represent the linear absorption and effective two-photon absorption coefficients respectively.

Optical limiters are devices which are active at high intensity as opaque materials, whereas at low intensity, they act like transparent materials. Therefore, these devices are indispensable in optical sensors and eye protective devices. Figure 6 depicts input fluence plotted against normalized transmittance using open aperture z-scan data. GQDs exhibit optical limiting nature as the normalized transmittance decreased with the input fluence. Results reveal that the optical limiting efficiency of GQD@532 nm sample was significantly higher than that of GQD@355 nm, which may be explained by the increase in particle size and concentration at higher wavelength ablated species.

In this work, two photon absorption (TPA) became the effective nonlinear absorption mechanism when using the ns laser pulses. TPA is an instantaneous nonlinearity that involves the simultaneous absorption of two laser photons with assistance of the intermediate state. TPA behavior was strongly affected by the pulse duration and the field intensity. The values of the TPA cross-section increased with the broadening of the incident laser pulse and increasing intensity. Therefore, the TPA effect became much weaker when irradiated with a short laser pulse that is faster than the intersystem transition. Under the laser irradiation with low energy, the available ground state carriers were depleted, while the excited states became almost occupied, and, thus, the Pauli Exclusion Principle came into effect [66]. Moreover, it has exhibited the reverse saturable absorption (RSA) nature of GQDs. The optical limiting threshold is found to be 0.45 Jcm^{-2} for the laser synthesized GQDs@532 nm. The obtained nonlinear parameters for nanosecond laser pulse excitations are given in Table 1.

Table 1. Nonlinear absorption coefficient (β_{eff}) and saturation intensity (I_{sat}) for ns excitations, obtained by numerical fits to the data points.

Sample	Energy (μJ)	β_{eff} ($\times 10^{-10} \text{ mW}^{-1}$)	I_{sat} ($\times 10^{10} \text{ Wm}^{-2}$)	Optical Limiting Threshold (Jcm^{-2})
GQDs@532 nm	25	4.3	57.7	0.45
GQD@355 nm	25	0.48	139.9	6.4

4. Conclusions

The optical properties of GQDs synthesized using nanosecond laser pulses with a carbon source followed by a fast and one-step process have been studied critically. Absorption and photoluminescence spectrum gives direct evidence of the formation of GQDs. Imaging using TEM is sufficient to prove the existence of spherical GQDs having homogenous size less than 10 nm. The as-prepared GQDs exhibit outstanding photoluminescence and chemical stability and the preparation requires utilization of very small reaction time (2 m) and less laser energy. The fluorescent nature of GQDs is trustworthy for biological and optoelectronic applications. It may also be noted that by controlling the laser parameters such as laser fluence, laser wavelength and repetition rate, one can fine-tune different properties such as concentration, particle size, etc., as per the application requirements. Moreover, the third-order-like NLO properties of these GQDs is investigated using open aperture z-scan technique which revealed the RSA nature of GQD so that it can be used as excellent optical limiter especially GQDs synthesized at 532 nm wavelength. These GQDs can be used as an efficient two-photon probe for bio-imaging applications due to their strong TPA. Conclusively, the laser ablation technique, thus, is a successful method for preparing GQDs within a very short span of time, while the GQDs synthesized at longer wavelengths and laser fluences have improved optical properties.

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Article

Preparation and In Vitro Bioactivity Study of a Novel Hollow Mesoporous Bioactive Glass Nanofiber Scaffold

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Abstract: In this study, a novel three-dimensional hollow mesoporous bioactive glass nanofiber scaffold has been synthesized with a template-assisted sol-gel method using bacterial cellulose (BC) as a template and nonionic triblock copolymer (P123) as a pore-directing agent, ethyl orthosilicate (TEOS), calcium nitrate tetrahydrate (CN), and triethyl phosphate (TEP) as glass precursors. Scanning and transmission electron microscopies, X-ray diffraction, nitrogen adsorption-desorption, and nuclear magnetic resonance method were applied to characterize the morphology, crystal structure, and chemical structure of the mesoporous bioactive glass nanofiber scaffold. Furthermore, the in vitro bioactivity and biocompatibility were also explored. The obtained scaffold depicted nanofiber-like morphology and interconnected three-dimensional network structure that replicated the BC template. The scaffold showed a large specific surface area ($230.0 \text{ cm}^2 \text{ g}^{-1}$) and pore volume ($0.2 \text{ m}^3 \text{ g}^{-1}$). More importantly, the scaffold exhibited excellent apatite-forming ability and cellular biocompatibility. We believe that the hollow mesoporous bioactive glass nanofiber scaffold has great potential application in bone tissue regeneration.

Keywords: sol-gel method; three-dimensional; hollow; mesoporous bioactive glass nanofiber scaffold; bioactivity

1. Introduction

Mesoporous bioactive glasses (MBGs) have attracted extensive attention in clinical applications due to their excellent in vitro bioactivity [1–6], bone conductivity [7], and bone inducibility [8]. Compared to traditional bioactive glasses (BGs), MBGs exhibit large specific surface area and pore volume due to their well-ordered mesoporous structure. Yan et al. prepared MBGs and observed the MBGs possessed excellent bone-forming bioactivity compared with conventional BGs [9]. Li et al. fabricated multifunctional mesoporous silica nanoparticles and the multifunctional theranostic nano-platform for simultaneous photoluminescence imaging [10]. Kim et al. synthesized mesoporous bioactive glass nanoparticles to deliver therapeutic molecules, and the in vitro bioactivity test indicated their excellent apatite-forming ability [11]. However, the current research mainly focuses on MBG nanoparticles [12]. Bioactive glass nanofiber scaffolds have been recently reported, but their fiber diameter is on the micron scale, whereas the collagen fibers in a natural extracellular matrix (ECM) are between 50 and 500 nm in diameter [13]. Consequently, the structural similarity between glass fibers and the ECM is low.

Generally, an MBG nanofiber scaffold with higher similarity to the ECM is commonly prepared by the electrospinning technique, but electrospinning is complex, and the diameters of the obtained fibers are a few hundred nanometers [14,15]. Besides, as the fiber

thickness is also on the micron scale, creating real-life biomimetic ECM structures is extremely challenging [14,16–19]. As a result, MBG hollow fibers with nanoscale diameters are almost never reported.

Bacterial cellulose (BC) has been widely applied as the scaffold in bone tissue engineering as its three-dimensional (3D) spatial network structure closely mimics the ECM structure [20–22]. Moreover, BC is increasingly used as the template in the preparation of inorganic materials [23,24]. However, the synthesis of MBG nanofiber scaffolds (especially those with hollow structures) on BC templates is rarely reported.

Motivated by these considerations, the present authors prepared a novel 3D hollow MBG nanofiber scaffold composed of hollow ultrafine MBG nanofiber scaffold (ca. 36 nm) via sol–gel assisted heat treatment technology, which was the smallest among all currently reported MBG fibers [25,26]. The sol–gel process is a low-temperature process employed to prepare MBG. Hydrolysis of the alkoxysilane groups is followed by condensation to form the glass network. A hybrid material can be obtained by introducing a polymer into the sol–gel process. The silica network develops in the dissolved polymer, thus allowing the silica network and the polymer to interact at the molecular level. In this preparation, the template was BC, and the pore-foaming agent was nonionic three-block copolymer P123 (polyethylene oxide–polypropylene oxide–polyethylene oxide). The morphology and structure of the 3D hollow MBG nanofiber scaffold were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), N₂ adsorption–desorption isotherm analysis, and nuclear magnetic resonance (NMR). The *in vitro* bioactivity and cellular biocompatibility of the MBG nanofiber scaffold were also investigated.

2. Results

2.1. Synthesis of Hollow Mesoporous MBG Nanofiber Scaffold

The synthetic procedure is shown in Figure 1. A hollow MBGs nanofiber scaffold was synthesized using BC as a template. Firstly, the BC template was immersed into P123/anhydrous ethanol solution for 24 h and then introduced to H₂O/anhydrous ethanol solution for 24 h to complete hydrolysis and polycondensation. Finally, the 3D hollow mesoporous bioactive glass nanofiber scaffold was obtained using the sol–gel method and heat treatment technology.

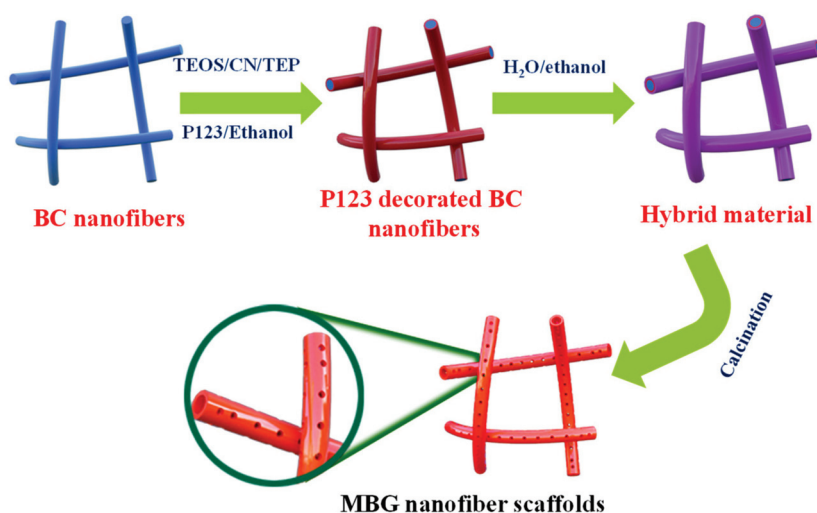


Figure 1. Schematic illustration of the synthesis of MBG nanofiber scaffold.

2.2. Physicochemical Properties of the MBG Nanofiber Scaffold

SEM and TEM images of the MBG scaffold are shown in Figure 2. The MBG scaffold is composed of nanofibers forming an obvious 3D spatial network with large interconnected pores (Figure 2a). This structure well replicates the structural characteristics of the

BC template, so it is expected to promote cell proliferation, nutrient delivery, and bone growth [27,28]. Based on the SEM image, the diameter of the nanofiber scaffold is measured, and the average fiber diameter of the scaffold is approximately 36 ± 10 nm. The structural characteristics of the nanofibers were further analyzed by TEM. The MBG scaffold presents a large number of nanotube structures with uniform wall thickness (Figure 2b), which are advantageous for loading growth factors or drugs. The selected area electron diffraction (SAED) pattern (Figure 2b, inset) is a large spot, indicating an amorphous structure of the MBG scaffold. This phenomenon indicates that the inorganic components coated on the surface of the BC nanofibers were not crystallized after holding at 600°C for 5 h and maintained their amorphous structure.

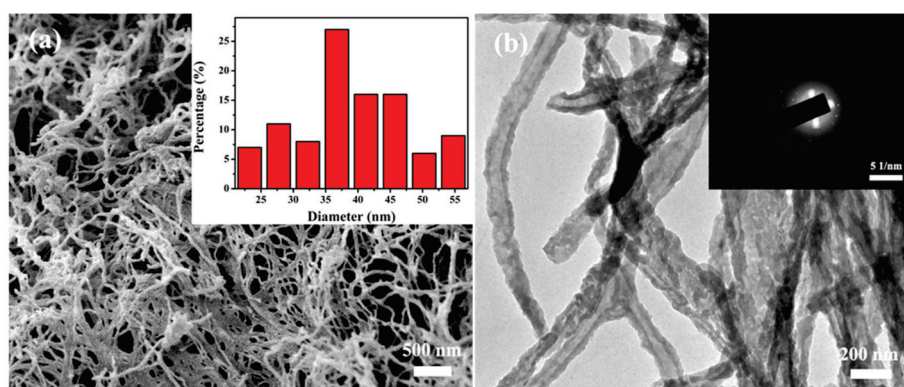


Figure 2. (a) SEM image and TEM image of MBG nanofiber scaffold (Inset shows the diameter distribution (a) and SAED pattern (b) of MBG nanofiber scaffold).

The crystal structure of the MBG scaffold was determined from the XRD patterns shown in Figure 3a. The wide-angle diffraction peaks of the MBG scaffold are broad, with no obvious crystallization peaks, again indicating the amorphous nature of the MBG scaffold after high-temperature calcination. This result consolidates the SAED result (Figure 2b, inset). The wide SiO_2 peak at $2\theta \approx 23^\circ$ can be attributed to the amorphous silica. In the FT-IR spectrum of P123, BC, and hybrid material (Figure 3b), the major characteristic peaks of BC (3345 and 1493 cm^{-1} to O–H, 1164 and 1060 cm^{-1} to C–O) and P123 (2980 – 2850 cm^{-1} to C–H, 3000 cm^{-1} to O–H) is depicted in the FT-IR spectra of hybrid material. This clearly indicated the formation of P123 decorated BC nanofibers, hybrid materials, and calcination. For the MBG scaffold, the absorption peak at 1493 cm^{-1} is attributed to the hydroxyl group (–OH), and the characteristic peaks at 805 and 1040 cm^{-1} can be assigned to stretching and bending vibrations of the Si–O–Si bond, respectively [29,30].

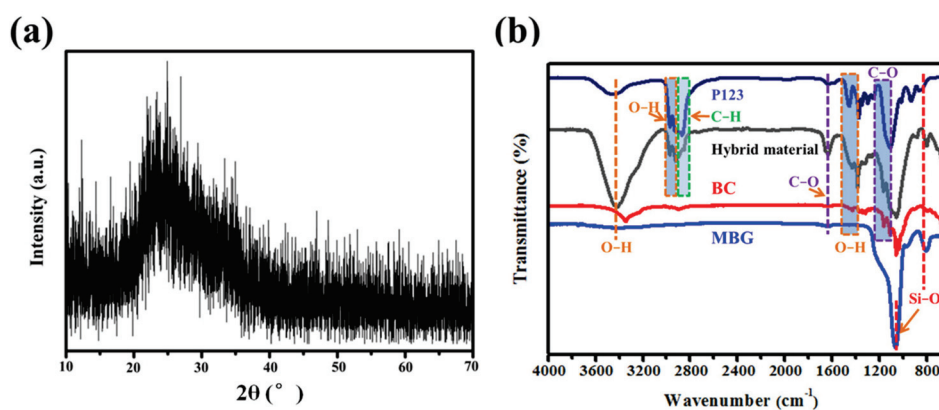


Figure 3. (a) XRD patterns and (b) FT-IR spectra of P123, BC, hybrid material, and MBG nanofiber scaffold.

Figure 4 displays the N₂ adsorption–desorption isotherm and pore size distribution curve of the MBG scaffold. The sorption isotherm (Figure 4a) is a type IV isotherm with a B-type hysteresis loop in the 0.6–1.0 range of relative pressures caused by capillary agglutination. According to this result, the MBG scaffold is a typical mesoporous material [31,32]. The mesopore distribution curve of the MBG scaffold (Figure 4b) exhibits a high cusp distribution concentrated around 22.0 nm. The specific surface area, pore size distribution, and pore volume of the MBG scaffold, calculated using the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda methods, are shown in Table 1. The MBG scaffold has a large specific surface area (230.0 m² g^{−1}) and a total pore volume of 0.2 cm³ g^{−1}, implying a porous structure of the MBG scaffold. Such high porosity and large specific surface area will promote the loading and slow release of growth factors or drugs.

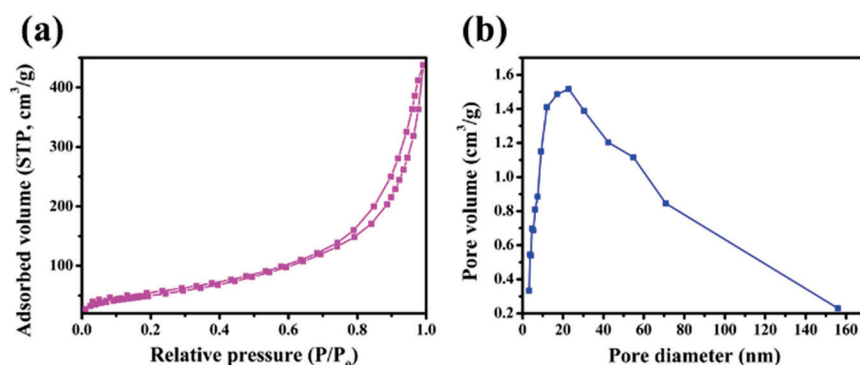


Figure 4. (a) N₂ adsorption-desorption isotherms and (b) pore size distribution curves of MBG nanofiber scaffold.

Table 1. Specific surface area, pore volume, and nanopore size of MBG scaffold.

Samples	Specific Surface Area (m ² g ^{−1})	Pore Volume (cm ³ g ^{−1})	Pore Size (nm)
MBG	230.0	0.2	22.0

Figure 5 shows the ²⁹Si–NMR spectrum of the MBG scaffold. The obvious absorption peaks around chemical shifts of 83 and 90 ppm correspond to Si(OSi)₂(OH)₂ (Q²) and Si(OSi)₃(OH) (Q³) in the silicon-oxygen network structure, respectively. Specifically, silicon contains two and one non-bridging oxygen bond at chemical shifts of 83 and 90 ppm, respectively. The additional weak peaks near chemical shifts of 76 and 99 ppm can be attributed to Si(OSi)(OH)₃ (Q¹) and Si(OSi)₄ (Q⁴) in the silicon-oxygen network structure, respectively, which contain three and one non-bridging oxygen bonds, respectively. The above results reveal numerous non-bridging oxygen bonds in the MBG scaffold, which are generated by the breakage of the Si–O–Si network after introducing the basic metal ions [33]. The glass network structure also contains many non-bridging oxygen bonds, which can improve ion dissolution in the MBG scaffold, the degradation performance of the MBG scaffold, and the capacity of in vitro apatite formation. These results imply excellent in vitro biological activity of the MBG scaffold.

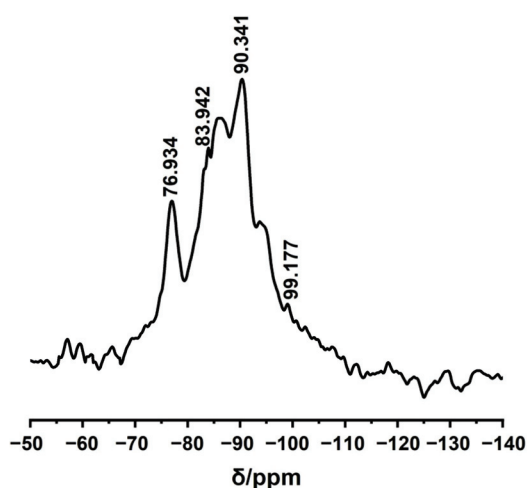
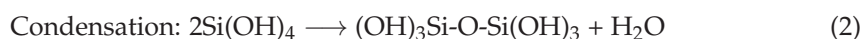


Figure 5. ^{29}Si NMR spectra of MBG scaffold.

3. Discussion

3.1. Formation Mechanism of Hollow MBG Nanofiber Scaffold

Based on previous studies and reports [34], the authors suggest the following formation mechanism of the hollow MBG nanofiber scaffold. First, the Si, Ca, and P precursor solution and P123 were evenly dispersed in the ethanol solution. As no hydrolysis reactions occurred between the precursors and water during stirring in the sealed state, SiO_2 nanoparticles could not form in the solution of precursors in ethanol. Dissolution in ethanol reduced the concentrations of TEOS, CN, and TEP before the BC was soaked in the mixture. As BC, with its 3D network structure, has excellent hydrophilicity, the precursor solution rapidly permeated the internal and external surfaces of the BC template and reacted with the $-\text{OH}$ groups on the nanofiber surfaces, forming Si–O bonds via the in situ silicification reaction of the TEOS precursor (as shown in (1)). This reaction produced uniform $(\text{OR})_3\equiv\text{Si}-$ deposits on the BC nanofibers. The strong polarity of the symmetrically structured $(\text{OR})_3\equiv\text{Si}-$ promoted the adsorption of the other precursors, which underwent partial hydrolysis reactions to ethanol. However, the preceding hydrolysis reactions were impeded in the current experiment since the solvent was ethanol. The majority of the precursors just adsorbed on the BC nanofiber surfaces. Adsorption lowered the precursor concentrations in the BC nanofiber network structure, causing the precursor solution to diffuse continuously around the BC nanofiber into the 3D network structure. Finally, the precursor concentrations reached dynamic equilibrium on the BC nanofiber surfaces.



When the precursor-absorbed BC aerogel was immersed in the mixture of water and anhydrous ethanol, the $(\text{OR})_3\equiv\text{Si}-$ groups and absorbed precursors underwent progressive hydrolysis and polycondensation (as shown in (2)). Similar to the synthesis of silica nanotubes by Hanagata [35], hydrolysis and polycondensation of the precursors produced a large number of colloid particles in the sol system. The BC nanofibers provided sites for the nucleation and growth of sol-gel-derived precursor oligomers, which were deposited on the BC nanofiber surface to a certain thickness along the axial direction. Eventually, a layer of amorphous precursor mixture was formed on the BC nanofibers. Over a prolonged soaking time, the concentration of the P123 surfactant exceeded the critical micelle concentration, inducing self-assembly in the Si, Ca, P, P123, and BC systems. At this time, mesoporous structures were formed on the inner and outer surfaces of the BC nanofibers. After sufficient hydrolysis, the BC nanofibers and P123 template were completely calcined, affording the MBG nanofiber scaffold with an ordered mesoporous structure and nanotube morphology.

3.2. In Vitro Bioactivity of the MBG Nanofiber Scaffold

Figure 6 displays the SEM images of the MBG scaffold mineralized in SBF at different times. Mineralization obviously changed the morphology of the MBG scaffold from that of the pre-mineralized sample (Figure 2a). After mineralization, a flaky mineral compound was deposited on the MBG scaffold's surface, and the fibers were then attached. The mineral layer thickened as the soaking time was extended, and after 72 h of mineralization, the nanofibers were no longer discernible. The MBG scaffold's huge surface area increased the SBF solution's contact area and is anticipated to bestow superior in vitro biological activity. In particular, the large surface area enhances the dissolution velocity of the ions through the MBG scaffold and promotes the formation of silicon –OH groups on the scaffold surface, which act as nucleation sites for Ca and P ions.

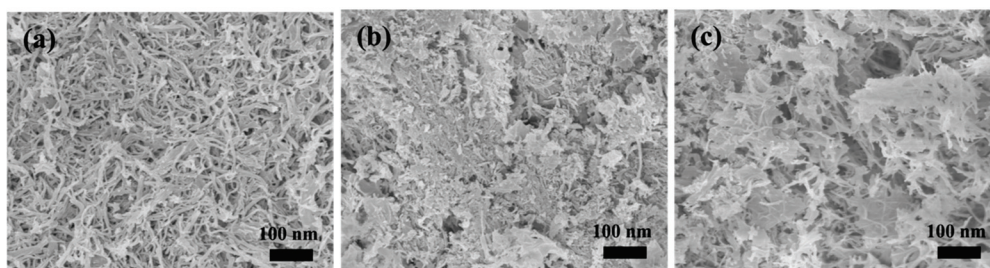


Figure 6. SEM images of MBG scaffold after immersion in SBF for (a) 12 h, (b) 24 h, and (c) 72 h.

Figure 7 displays the XRD patterns of the MBG scaffold mineralized in SBF at different times. After 12 h of mineralization, the XRD pattern of the scaffold showed not only amorphous peaks (Figure 3a) but also weak diffraction peaks (e.g., at $2\theta \approx 32^\circ$). Lengthening the mineralization time gradually increased the intensity of the peaks attributed to mineral formations on the MBG scaffold surface, and the crystalline state gained prominence. The peaks emerging at $2\theta = 29^\circ, 33^\circ, 37^\circ, 43^\circ, 49.5^\circ$, and 53° were assigned to the (002), (211), (310), (222), (213), and (004) crystal planes of hydroxyapatite [36], respectively. These results affirm that the MBG scaffold can rapidly deposit hydroxyapatite in SBF and hence exhibits excellent in vitro biological activity. These results were consistent with those of the SEM and ^{29}Si -NMR.

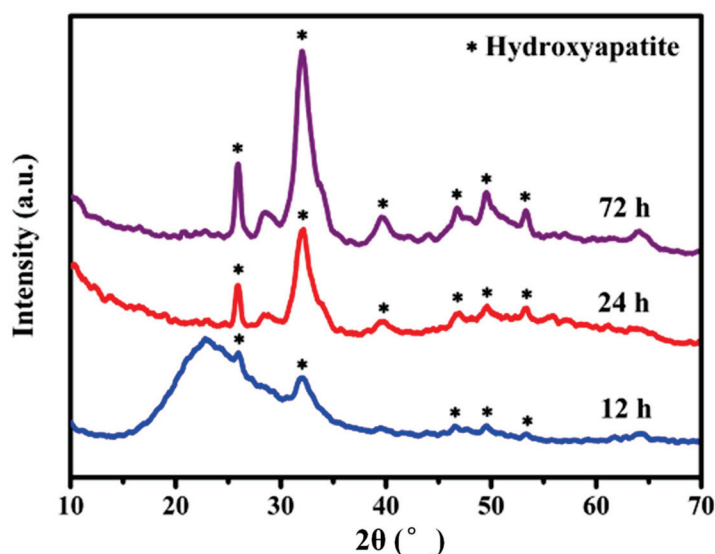


Figure 7. XRD patterns of MBG scaffold after immersion in SBF for different times.

Figure 8 shows the release-concentration curves of ions (Ca^{2+} , PO_4^{3-} and SiO_4^{4-}) from the MBG scaffold at 4, 8, 16, 24, and 72 h. The Ca^{2+} concentration reached a maximum

of 127.9 ppm after the first four hours and then gradually reduced until mineralization was complete. After 24 h of mineralization, the Ca^{2+} concentration reduced to 99.7 ppm. According to literature reports, the Ca^{2+} concentration change in SBF solution is related both to the dissolution of Ca^{2+} in the MBG scaffold and the formation of hydroxyapatite on the surface [37,38]. When the sample was immersed in SBF, the Ca^{2+} rapidly dissolved via hydrogen ion (H^+) exchange in the solution. During the whole soaking process, the Ca^{2+} concentration in SBF would continuously decrease if the consumption rate of Ca^{2+} by hydroxyapatite exceeded the dissolution rate of Ca^{2+} ; otherwise, it would gradually increase. In the initial stage, the amount of Ca^{2+} released from the MBG scaffold exceeded the amount consumed by hydroxyapatite, so the Ca^{2+} concentration increased. At later times, the Ca^{2+} concentration reduced because ions were released from the MBG scaffold and many silica-rich gel layers formed on the surface of the MBG scaffold via silicon $-\text{OH}$ groups. The gel layers provided nucleation sites for the Ca and P ions in the solution; consequently, the ions were deposited and crystallized on the gel surfaces. The consumption rate of Ca^{2+} then increased, and the Ca^{2+} concentration reduced. Meanwhile, the PO_4^{3-} concentration trended downward in the SBF solution and decreased to 12.0 ppm after 24 h, mainly because PO_4^{3-} was consumed by hydroxyapatite formation. In contrast, the SiO_4^{4-} concentration trended upward because Si was released from the MBG scaffold.

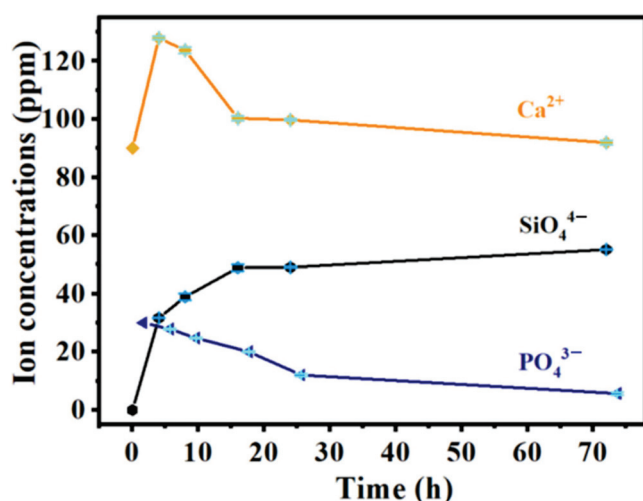


Figure 8. The curves of ion concentration released from MBG nanofiber scaffold for different time periods.

3.3. Cellular Responses to MBG Scaffold

The attachment and morphology of hBMSCs on the MBG scaffold were examined by SEM (Figure 9). Many hBMSCs were observed to attach and spread well on the surface of the nanofiber scaffold after 1, 4, and 7 days, respectively. Besides, numerous filopodia were noted, demonstrating close cell contact with the scaffold. The result indicated the good biocompatibility of the MBG nanofiber scaffold.

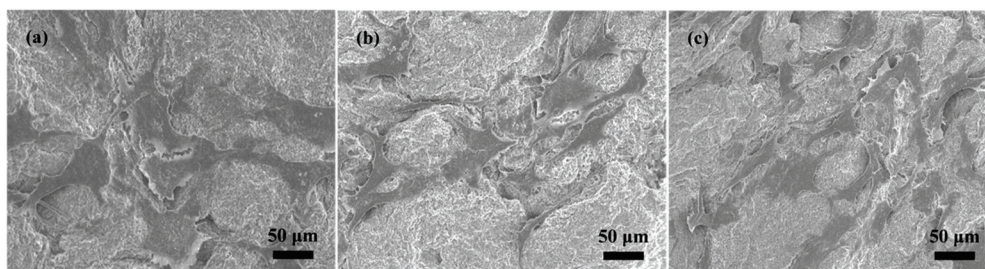


Figure 9. Morphology of the hBMSCs after culture on nanofiber scaffold for (a) 1, (b) 4, and (c) 7 days.

4. Materials and Methods

4.1. Materials and Chemical

Pure ethyl alcohol, triethyl phosphate (TEP, 99.8%), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 99%), and tertiary butanol were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Tetraethyl orthosilicate (TEOS, 98%) was purchased from Beijing Innochem Science & Technology Co., Ltd. (Beijing, China). Nonionic block copolymer $\text{EO}_{20}\text{PO}_{70}\text{EO}_{20}$ (P123, Mw = 5800 g/mol) was purchased from Sahn Chemical Technology Co., Ltd. (Shanghai, China).

4.2. Preparation of MBG Nanofiber Scaffold

- (1) First, 4 g of P123 was added to 50 mL of anhydrous ethanol and stirred until completely dissolved. To this solution, we successively added 9 mL of tetraethyl orthosilicate (TEOS), 1.75 g of calcium nitrate (CN), and 1 mL of triethyl phosphate (TEP). The obtained mixed solution was thoroughly stirred until it became uniform. Next, 25 mg of BC aerogel was soaked in this solution for 24 h. Once removed, the BC aerogel was washed twice quickly with ethanol and stored for later use.
- (2) Second, 5 mL of H_2O and 45 mL of anhydrous ethanol were combined and stirred to form an evenly mixed solution. The aerogel sample prepared in Step (1) was immersed in this solution for 24 h to complete hydrolysis and polycondensation. The obtained sample was freeze-dried to afford a $\text{SiO}_2/\text{CaO}/\text{P}_2\text{O}_5/\text{BC}$ hybrid material, which was then placed in a tubular furnace and heated from room temperature to 300 °C at 1 °C/min. After 30 min holding at 300 °C, the sample was heated to 600 °C and held at that temperature for 5 h, affording the targeted 3D hollow MBG nanofiber scaffold.

4.3. Characterization of MBG Nanofiber Scaffold

The microstructure of the scaffold material was observed using field-emission SEM with an acceleration voltage of 5 kV. The internal microstructure of the scaffold material was analyzed using field-emission TEM with an acceleration voltage of 200 kV. The wide-angle X-ray diffraction pattern of the sample was recorded by X-ray diffraction (XRD, Rigaku D/Max 2500 v/pc) with Cu Ka radiation. The chemical structure of the scaffold surface was measured with a Fourier transform infrared (FT-IR) spectrometer, and the surface area and pore size distribution of the scaffold were determined from the N_2 adsorption–desorption isotherms. The ^{29}Si NMR spectrum of the sample was recorded on an NMR spectrometer.

4.4. In Vitro Bioactivity Experiment of the MBG Nanofiber Scaffold

The scaffold was immersed in an appropriate amount of simulated body fluid (SBF). The mass: volume ratio of the material to the SBF was 1 mg mL^{−1}. The SBF containing the scaffold was placed in an incubator rotating at 90 r min^{−1} at 36.5 °C for 12, 24, or 72 h. The SBF was refreshed every 24 h. The samples collected at different times were rinsed with deionized water, freeze-dried, and stored in sample bags for later use. Their surface morphologies and crystal structures before and after mineralization were characterized by SEM and XRD.

4.5. Cell Study

The hBMSCs used in this study were provided by Cyagen (China). The hBMSCs were then transplanted into culture flasks supplemented with human bone marrow stromal cell basal media (Cyagen, Taicang, China) and incubated at 37 °C in a humidified 5% CO_2 incubator. Once the hBMSCs were passaged to about 80% confluence, the attached hBMSCs were further expanded. The medium was refreshed twice a week until the hBMSCs confluence was achieved. Only early passage cells were used in the next experiment.

To analyze the effect of the MBG scaffold on the cell morphology of hBMSCs, the cell/scaffold constructs were collected after being cultured for 1, 4, and 7 days in the growth medium, rinsed in PBS, and then fixed with 2.5% glutaraldehyde in PBS for 1 h. The construct was then removed by washing in PBS buffer containing 4% (*w/v*) sucrose

and post-fixed in 1% osmium tetroxide in PBS. Then the constructs were dehydrated in a graded series of ethanol (50%, 60%, 70%, 80%, 90%, and 100% (v/v)) for 15 min. Finally, the scaffold was coated by spraying with Pt for 120 s. The morphologies of the attached hBMSCs were observed using SEM.

5. Conclusions

The repair of large bone defects is a challenging clinical problem. Limitations associated with existing treatments, such as autologous bone grafts and allografts, have increased the need for synthetic bone graft substitutes. This study aimed to investigate the in vitro bioactivity and cellular biocompatibility of novel 3D hollow MBG nanofiber scaffold as synthetic bone graft substitutes, which was successfully prepared from a BC template via the sol-gel-assisted heat treatment technology with P123 as the pore-forming agent. The obtained MBG scaffold revealed a 3D spatial network structure with an ultrafine tubular (around 36 nm) structure and possessed a large specific surface area of $230.0 \text{ m}^2 \text{ g}^{-1}$ and a total pore volume of $0.2 \text{ cm}^3 \text{ g}^{-1}$, implying a porous structure of the MBG scaffold. Additionally, the 3D MBG nanofiber scaffold showed good in vitro bioactivity, which was attributed to its large specific surface area. Cell study indicated the good biocompatibility of the MBG nanofiber scaffold, ascribed to the 3D spatial network structure. Therefore, this investigation demonstrated that the MBG nanofiber scaffold with excellent bioactivity and cellular biocompatibility would be a promising scaffold in bone tissue regeneration.

Author Contributions: J.X. (Jian Xiao) and Q.W. contributed equally to this work. Conceptualization, J.X. (Jian Xiao) and F.Z.; methodology, J.X. (Jian Xiao) and Q.W.; validation, J.X. (Jian Xiao), Q.W. and Z.Y.; formal analysis, J.X. (Jian Xiao), Z.D. and J.X. (Jinhong Xue); resources, J.X. (Jian Xiao) and Z.D.; data curation, J.X. (Jian Xiao) and Q.W.; writing—original draft preparation, J.X. (Jian Xiao) and Q.W.; writing—review and editing, J.X. (Jian Xiao) and F.Z.; visualization, J.X. (Jian Xiao), J.X. (Jinhong Xue) and Q.W.; supervision, F.Z.; project administration, J.X. (Jian Xiao); funding acquisition, J.X. (Jian Xiao) and F.Z. All authors have read and agreed to the published version of the manuscript.

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Article

Low-Cost and Environmental-Friendly Route for Synthesizing Nano-Rod Aluminosilicate MAZ Zeolite

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Abstract: Preparation of nano-rod aluminosilicate Mazzit (MAZ) zeolite under low-cost and environmental-friendly route is attractive, but still challenging. Herein, we report a green route for synthesizing nano-rod MAZ zeolite (MAZ-N) using low-cost and environmental-friendly choline chloride as template. Various characterizations including powder X-ray diffraction (XRD), scanning electron microscope (SEM), N₂ sorption, and thermogravimetry-differential thermal analysis (TG-DTA) show that MAZ-N samples have good crystallinity and uniform porous structures. Furthermore, the crystallization process and impact of synthesis conditions of MAZ-N samples have been investigated in detail. These results suggest the potential applications of MAZ-N zeolites as supporting catalyst compounds in industrial processes.

Keywords: zeolites; low-cost; environmental-friendly; aluminosilicate MAZ; nano-rod

1. Introduction

As one of the most important microporous materials, zeolites have been widely applied in industrial processes for the production of chemicals and fuels due to their large internal surface area, high hydrothermal stability, unique channel structure, and superior solid acidity [1–12]. The chemical composition and pore structure of zeolites are significant, but also the control of their morphology has great effect on their performance in catalytic applications [13–20]. Additionally, the improved mass diffusion ability of nano-rod zeolites would provide plenty of potential opportunities for the further application in catalysis, adsorption and environmental protection.

MAZ zeolite consists of gmelinite cages that are linked in columns parallel to the *c*-axis to build main pores with 12-membered rings [21]. Due to this unique structure, MAZ zeolites show excellent catalytic performance in the methane to methanol [22,23], alkylation of aromatics [24,25], oil cracking [26], hydrocracking [27], and in the isomerization of paraffin and aromatics [28]. There are several MAZ samples that have been successfully synthesized with costly organic template. In the conventional synthesis of zeolite omega, the tetramethylammonium cation (TMA⁺) is used as the organic structure direct agent (OSDA). Both Martins group and Figueras group fabricated MAZ samples using tetramethylammonium hydroxide, which is a classical organic template of MAZ synthesis [29,30]. TMA⁺ may be occluded in the gmelinite cages during omega crystal growth process, which is supposed to be powerful OSDA during the zeolite synthesis [21]. Zones group prepared MAZ zeolites in the presence of dioxane as an organic template [31]. Furthermore, Dong group obtained MAZ zeolites using piperazine at 150 °C for 4 d [32]. However, the above organic templates are expensive, toxic and environmentally unfriendly, which would hinder the further application of MAZ zeolites in petrochemical processes. Thus, the development of a low-cost and environmental-friendly route for synthesizing a nano-rod aluminosilicate MAZ zeolite is still imperative and challenging.

Here, we focus on choline, a low-cost, nontoxic and biodegradable template. Choline is a water-soluble vitamin-like essential nutrient which refers to quaternary ammonium

salt containing N,N,N-trimethylethanolammonium cation [33]. Moreover, choline has been successfully applied in synthesizing several zeolite crystals as a structure-directing agent [34,35]. In this study, we reported a green route for synthesizing nano-rod MAZ zeolite using low-cost and environmental-friendly choline chloride as template. The zeolite product obtained had good crystallinity and uniform nano-rod morphology. Moreover, we systematically investigated the crystallization of MAZ zeolite on various synthesis conditions and enhanced the comprehension of crystallization process of the nano-rod such as MAZ zeolite. The combination of low cost and high mass transfer ability presents good opportunities for industrial applications of MAZ zeolite in the future.

2. Results and Discussion

Figure 1A shows the XRD pattern of the as-synthesized MAZ-N zeolite, which provides a series of characteristic peaks associated with that of the MAZ-C zeolite (Figure S1). These peaks basically remain after calcination, showing good thermal stability of the MAZ-N zeolite. Figure 1B displays the SEM image of the as-synthesized MAZ-N zeolite, showing the uniform assembled nano-rod morphology, which is very different from that of the MAZ-C zeolite (Figure S2). These nanorods are uniform with widths of ca. 200 nm and lengths in the range of ca. 3–5 μm . This result suggests the high quality of MAZ-N zeolite with nano-rod morphology could be successfully obtained, in good agreement with the result from XRD pattern in Figure 1A.

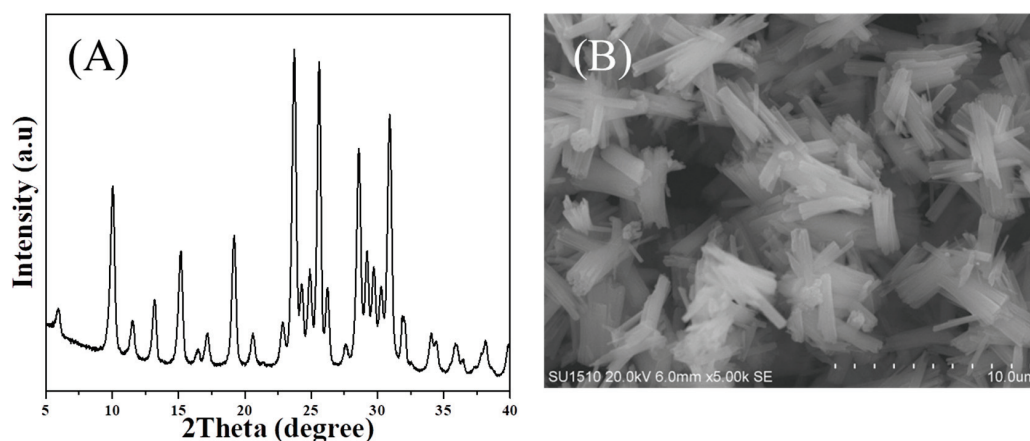


Figure 1. (A) XRD pattern and (B) SEM image of the as-synthesized MAZ-N sample, respectively.

Figure 2 shows the N_2 sorption isotherms of H-MAZ-N zeolite, indicating steep increases in the adsorbed volumes at a relative pressure ($10^{-6} < P/P_0 < 0.01$), which is due to the filling of micropores. In addition, the hysteresis loop appeared to be at the relative pressure range of 0.5–0.95. The BET surface area and micropore volume of the MAZ-N zeolite are measured at about $173 \text{ m}^2/\text{g}$ and $0.05 \text{ cm}^3/\text{g}$, which are similar to those of the conventional MAZ zeolite reported in the bibliography [36].

Figure 3 displays the TG-DTA curves of the as-synthesized MAZ-N zeolite in the temperature range from 25 to 800°C . The weight loss associated with the adsorbed water appears to be below 250°C , while the weight loss assigned to the combustion of organic template shows that the temperature ranged from 400 to 630°C . After 700°C , no obvious weight loss was observed. The DTA curve exhibits a main exothermic peak within the temperature range of 400 – 630°C accompanied by the weight loss at approximately 3.55% assigned to the combustion of organic template in the micropores of MAZ-N zeolite. A brief summary of these characterizations confirms that MAZ-N has a good crystallinity and nano-rod morphology. The low-cost and environmental-friendly template could be promising for large batch production of the high-effective MAZ zeolite in industrial processes.

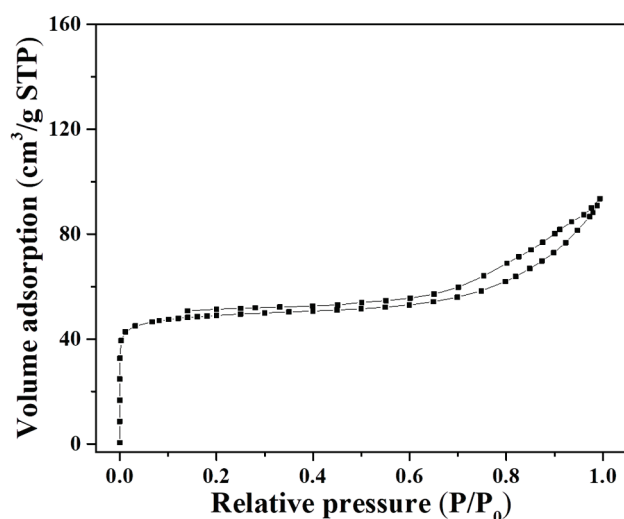


Figure 2. N_2 sorption isotherm of the H-MAZ-N sample.

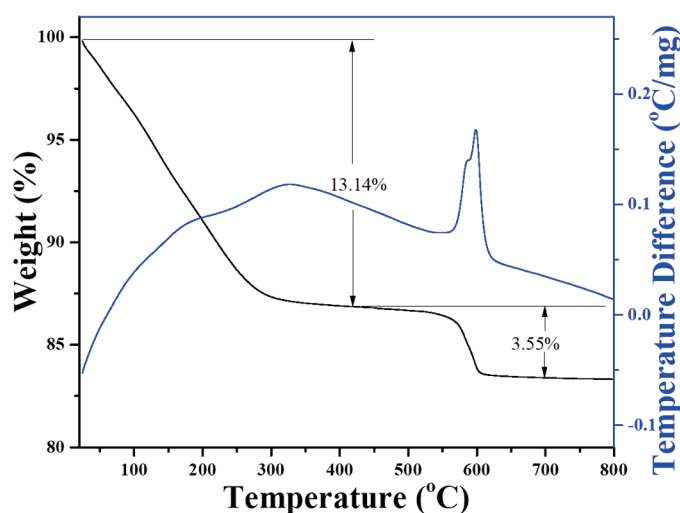


Figure 3. Thermogravimetry–differential thermal analysis (TG-DTA) curves of the as-synthesized MAZ-N sample.

As shown in Table 1 and Figures 4–6, we systematically investigated the crystallization of MAZ zeolite at 150 °C in the presence of low-cost and environmental-friendly choline chloride on various synthesis conditions. When the Na_2O/SiO_2 molar ratio in the starting gel is adjusted from 0.187 to 0.250, products with amorphous phases, pure MAZ, a mixture of MAZ with the analcine (ANA) structure are elicited (Run 1–5, Table 1, Figure 4). The Na_2O/SiO_2 molar ratio for synthesizing a pure MAZ structure ranges from 0.203 to 0.219 (Run 2–3, Table 1).

Furthermore, when the SiO_2/Al_2O_3 molar ratio in the starting gel is distributed in the range of 10–18, all of the products are pure MAZ zeolites (Run 6–8, Table 1, Figure 5). When the SiO_2/Al_2O_3 molar ratio in the starting gel is higher than 18, the products contain MAZ zeolite and ANA structure.

In addition, the template/ SiO_2 molar ratio in the starting gel is vital and carefully studied. We found that the relatively low template/ SiO_2 molar ratios (below 0.382) in the starting gel would result in a mixture of MAZ with the amorphous phase (Run 9, Table 1, Figure 6). When the template/ SiO_2 molar ratio comes up to 0.446, a pure MAZ zeolite with good crystallinity is obtained (Run 9, Table 1, Figure 6). The relatively high template/ SiO_2 molar ratio has no effect in the crystallization of MAZ zeolite (Run 10–12, Table 1, Figure 6).

After the systematic synthesis, aluminosilicate gel for crystallization of nano-rod MAZ zeolites at 150 °C for 1 day with molar ratio of 3.06 Na₂O/Al₂O₃/14 SiO₂/6.25 Choline Chloride/164 H₂O is appropriate.

Table 1. Impact of synthesis conditions on the crystallization of aluminosilicate MAZ-N zeolites (H₂O/SiO₂ = 11.7, at 150 °C for 1 d).

Run	Na ₂ O/SiO ₂	SiO ₂ /Al ₂ O ₃	R/SiO ₂ ^a	Products ^b	Crystallinity ^c (%)
1	0.187	14	0.446	Amor.	/
2	0.203	14	0.446	MAZ	90
3	0.219	14	0.446	MAZ	100
4	0.234	14	0.446	MAZ + ANA	/
5	0.250	14	0.446	MAZ + ANA	/
6	0.219	10	0.446	MAZ	90
7	0.219	12	0.446	MAZ	100
8	0.219	18	0.446	MAZ	125
9	0.219	14	0.382	MAZ + Amor.	/
10	0.219	14	0.511	MAZ	85
11	0.219	14	0.575	MAZ	80
12	0.219	14	0.639	MAZ	76

^a R stands for choline chloride as template. ^b The phase appearing first is dominant. ^c Run 3 was designated as 100% crystallinity.

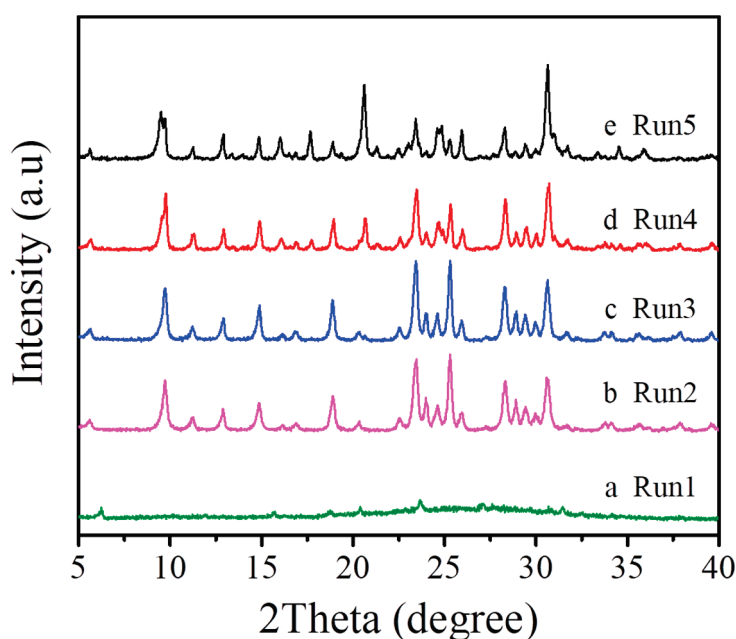


Figure 4. XRD patterns of the samples synthesized with different Na₂O/SiO₂ molar ratios ((a) Na₂O/SiO₂ = 0.187, (b) Na₂O/SiO₂ = 0.203, (c) Na₂O/SiO₂ = 0.219, (d) Na₂O/SiO₂ = 0.234, and (e) Na₂O/SiO₂ = 0.250).

Figure 7 displays the crystallization process of the MAZ-N zeolite detected by XRD and SEM techniques. Before crystallization, the XRD pattern shows amorphous phase in the starting aluminosilicate gel (Figure 7a), which is associated with the basically amorphous morphology in the SEM image (Figure 8a). Prolonging the crystallization time from 12 to 15 h causes the peak intensities assigned to MAZ zeolite crystals to become higher (Figure 7b–d). At the same time, several zeolite crystals could be found in the corresponding SEM images (Figure 8b). Further prolonging the crystallization time from 16.5 to 22.5 h causes the peak intensities to continue improving (Figure 7e–g). Correspondingly, much more MAZ zeolite crystals with nano-rod morphology could be observed in the SEM images (Figure 8c–f). When the crystallization time was longer than 24 h, there was no

obvious change on peak intensities tested by XRD techniques. In addition, the SEM image (Figure 1B) presents the perfect MAZ zeolite crystals with nano-rod morphology after crystallized for 24 h, suggesting the complete crystallization of the MAZ zeolite. Figure 9 shows the dependence of the MAZ zeolite crystallinity on crystallization time, which is consistent with the S-shaped curve of crystal growth.

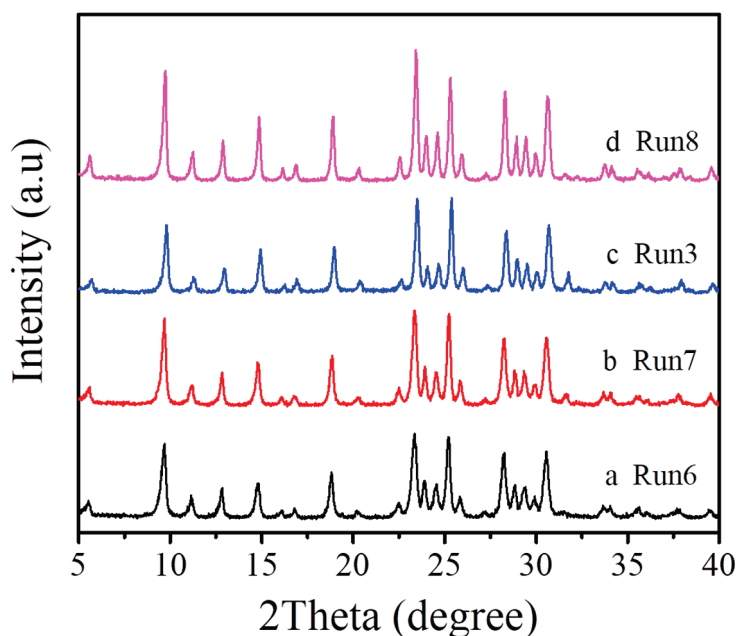


Figure 5. XRD patterns of the samples synthesized with different $\text{SiO}_2/\text{Al}_2\text{O}_3$ molar ratios ((a) $\text{SiO}_2/\text{Al}_2\text{O}_3 = 10$, (b) $\text{SiO}_2/\text{Al}_2\text{O}_3 = 12$, (c) $\text{SiO}_2/\text{Al}_2\text{O}_3 = 14$, and (d) $\text{SiO}_2/\text{Al}_2\text{O}_3 = 18$).

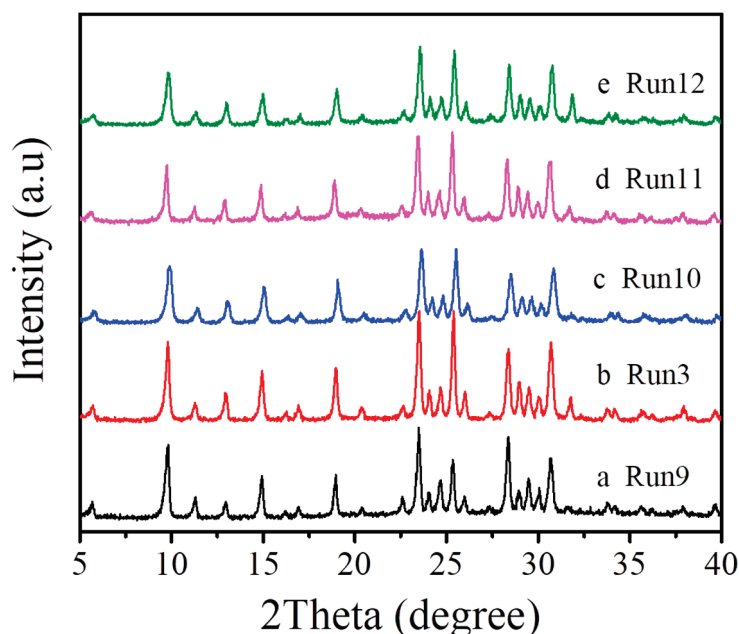


Figure 6. XRD patterns of the samples synthesized with different R/SiO_2 molar ratios ((a) $\text{R}/\text{SiO}_2 = 0.382$, (b) $\text{R}/\text{SiO}_2 = 0.446$, (c) $\text{R}/\text{SiO}_2 = 0.511$, (d) $\text{R}/\text{SiO}_2 = 0.575$, and (e) $\text{R}/\text{SiO}_2 = 0.639$).

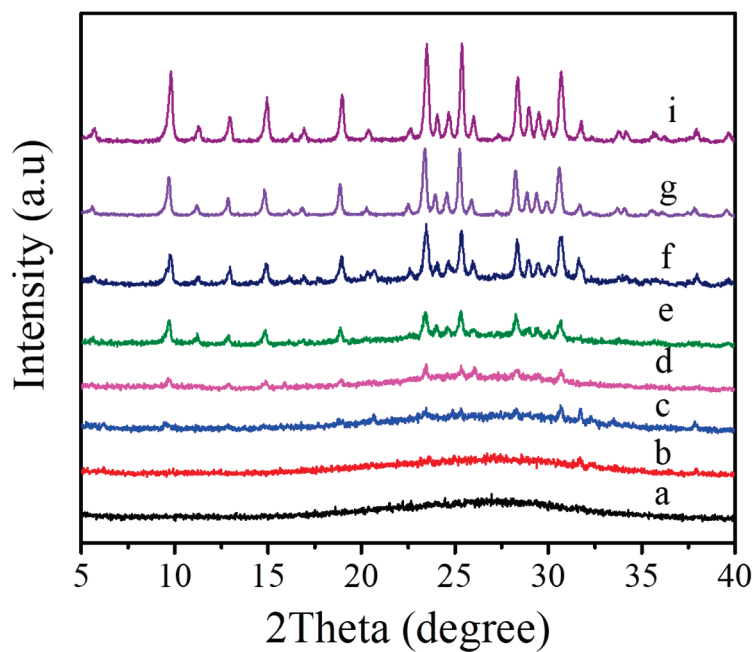


Figure 7. XRD patterns of the as-synthesized MAZ-N samples crystallized at (a) 0, (b) 12, (c) 13.5, (d) 15, (e) 16.5, (f) 18, (g) 22.5 and (i) 24 h, respectively.

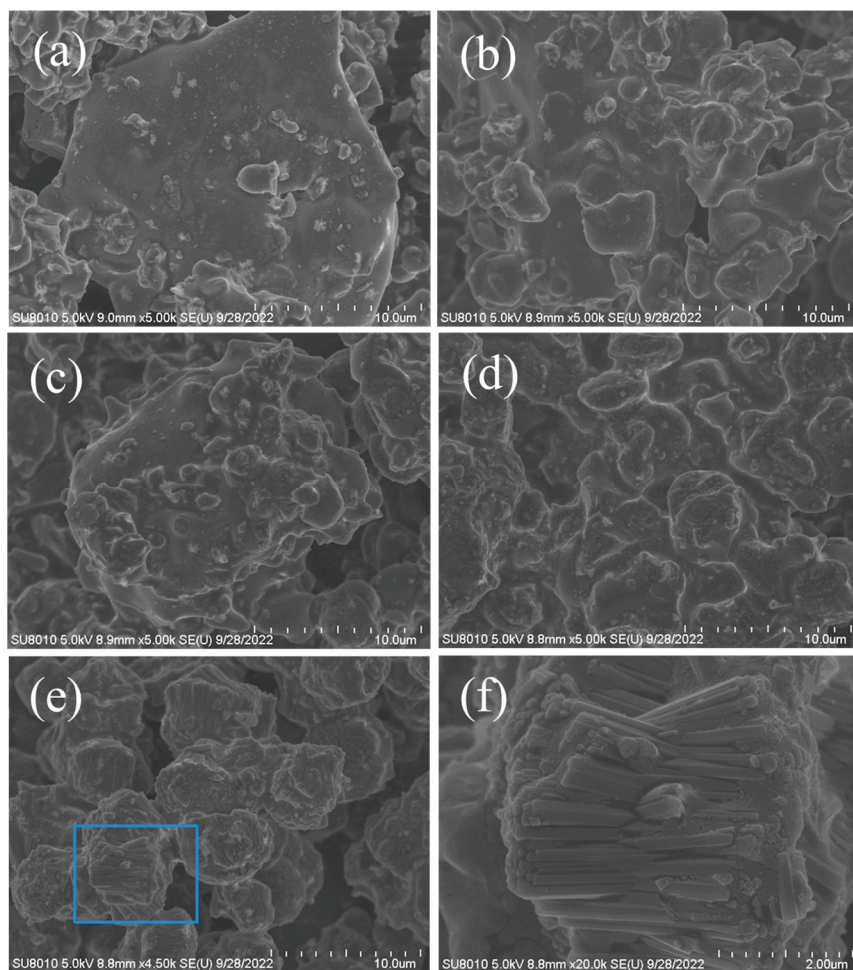


Figure 8. SEM images of the as-synthesized MAZ-N samples crystallized at (a) 0, (b) 15, (c) 16.5, (d) 18, (e,f) 22.5 h, respectively.

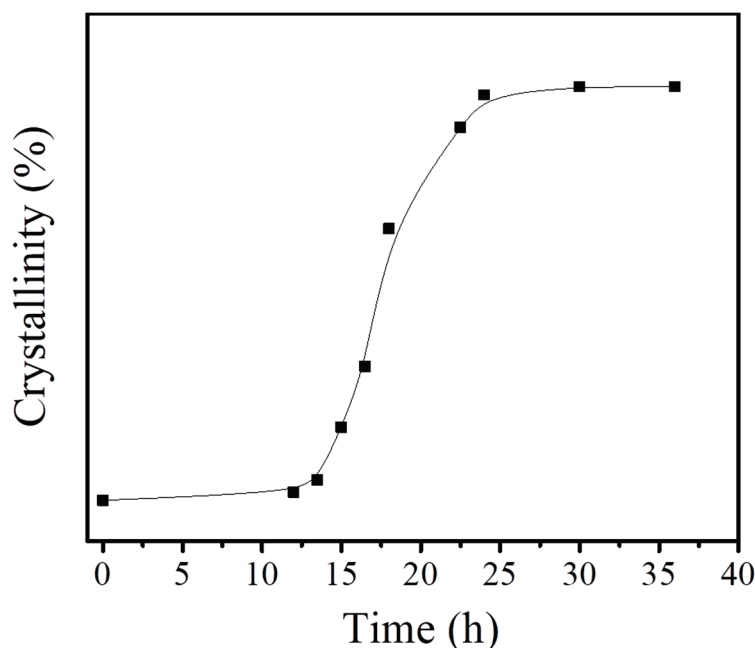


Figure 9. The dependence of the MAZ-N zeolite crystallinity on crystallization time.

3. Materials and Methods

3.1. Materials

The following chemicals were utilized: sodium hydroxide (NaOH, AR, 96%, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), sodium aluminate (NaAlO_2 , 36.6% Na_2O , and 43.3% Al_2O_3 , Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), silica sol (LUDOX HS-40, 40% SiO_2 in water, Sigma-Aldrich, Shanghai, China), choline chloride ($\text{C}_5\text{H}_{14}\text{ClNO}$, AR, 98%, Aladdin, Shanghai, China), tetramethylammonium hydroxide (TMAOH, 25% in water, Aladdin, Shanghai, China), and ammonium nitrate (NH_4NO_3 , AR, 99%, Beijing Chemical Reagent Co., Ltd., Beijing, China) were employed without further purification.

3.1.1. Synthesis of MAZ Zeolite

MAZ zeolite with nano-rod morphology was hydrothermally synthesized at the temperature of 150 °C for 1 day with 3.06 $\text{Na}_2\text{O}/\text{Al}_2\text{O}_3/14\text{SiO}_2/6.25$ Choline Chloride/164 H_2O molar ratios of initial synthesis gels in the presence of choline chloride. As a typical run, 0.53 g sodium hydroxide was added to 6.8 g deionized H_2O , followed by introducing 0.94 g sodium aluminate. After stirring for 1 h, 6.0 g silica sol was added to the mixture dropwise, followed by the last addition of 3.5 g choline chloride when the mixture turned into clear solution. After being stirred at room temperature for 2 h, the final mixture was transformed into an autoclave to crystallize at 150 °C for 1 d. After filtration, wash with deionized H_2O , air drying, and calcined to remove the organic template, the MAZ zeolite product was collected. The H-form of MAZ sample was prepared by triple ion-exchange with a solution of 1 mol/L NH_4NO_3 at room temperature for 2 h and calcination at 500 °C for 4 h, designed as H-MAZ-N zeolite.

3.1.2. Synthesis of Conventional MAZ Zeolite

Conventional MAZ zeolite was hydrothermally synthesized at the temperature of 100 °C for 7 days with 2.4 $\text{Na}_2\text{O}/\text{Al}_2\text{O}_3/10\text{SiO}_2/0.24$ TMAOH/1100 H_2O molar ratios of initial synthesis gels according to the previously reported procedure. The product was collected after filtration, washed with deionized H_2O , air drying and calcined to remove the organic template. The H-form of the sample was prepared by triple ion-exchange with a solution of 1 mol/L NH_4NO_3 at room temperature for 2 h and calcination at 500 °C for 4 h, designed as H-MAZ-C zeolite.

3.2. Characterization

Powder X-ray diffraction (XRD) (Rigaku, Tokyo, Japan) data were measured at room temperature with a Rigaku Ultimate VI X-ray diffractometer (40 kV, 40 mA) using CuK α 1 radiation ($\lambda = 1.5406 \text{ \AA}$). Scanning electron microscopy (SEM) (Hitachi, Tokyo, Japan) experiments were acquired with Hitachi SU-1510 and SU-8010 electron microscope. Nitrogen sorption experiments were performed on a Micromeritics ASAP 2460 M (Micromeritics Instrument Corporation, Atlanta, GA, USA) and Tristar system apparatus at -196°C . The micropore volume and specific surface area were calculated using the t-plot and BET methods, respectively.

4. Conclusions

In summary, we developed a low-cost and environmental-friendly route for preparing nano-rod aluminosilicate MAZ zeolite. The obtained MAZ zeolites have advantages of high crystallinity, uniform morphology of a nano-rod, high BET surface area, and good mass transfer ability. Moreover, the crystallization process of MAZ-N zeolites has been investigated in detail, which is consistent with the rule of crystal growth. We hold the opinion that this sustainable green route might offer good opportunities for practical applications of MAZ zeolites as high-efficient catalysts in the future.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules27227930/s1>, Figure S1: XRD pattern of the as-synthesized MAZ-C zeolite; Figure S2: SEM image of the as-synthesized MAZ-C zeolite.

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Article

Optimization of Lignin–Cellulose Nanofiber-Filled Thermoplastic Starch Composite Film Production for Potential Application in Food Packaging

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Abstract: The optimization of the production of thermoplastic starch (TPS) bionanocomposite films for their potential application in food packaging was carried out, according to the Box–Wilson Central Composite Design (CCD) with one center point, using Response Surface Methodology (RSM) and fillers based on lignin and nanofiber, which were derived from bamboo plant. The effects of the fillers on the moisture absorption (MAB), tensile strength (TS), percent elongation (PE) and Young's modulus (YM) of the produced films were statistically examined. The obtained results showed that the nanocomposite films were best fitted by a quadratic regression model with a high coefficient of determination (R^2) value. The film identified to be optimum has a desirability of 76.80%, which is close to the objective function, and contained 4.81 wt. % lignin and 5.00 wt. % nanofiber. The MAB, TS, YM and PE of the identified film were 17.80%, 21.51 MPa, 25.76 MPa and 48.81%, respectively. The addition of lignin and cellulose nanofiber to starch composite was found to have reduced the moisture-absorption tendency significantly and increased the mechanical properties of the films due to the good filler/matrix interfacial adhesion. Overall, the results suggested that the produced films would be suitable for application as packaging materials for food preservation.

Keywords: lignin; nanocomposite; cellulose nanofiber; optimization; percent elongation; starch; tensile strength; Young's modulus

1. Introduction

Synthetic plastics have dominated every field of human activity, particularly the packaging industries [1]. Despite their many merits, synthetic plastics have been a major environmental concern for some time. Since they are non-biodegradable and also dependent upon a non-renewable petroleum resource, the blooming usage of these plastics has caused grave energy crises, as well as environmental pollution associated with their disposal, including damage to the eco-system, water supplies and sewer systems, as well as rivers and streams. As a result, great attention is being drawn to natural polymers, e.g., starch, due to its potential as a resource for making environmentally friendly useable products to replace those derived from petroleum. The reason for starch being favored for this purpose is because it is inherently biodegradable, renewable, cost effective and available from many plants. In order to make it suitable for industrial application, such as packaging, starch is usually processed in the presence of a plasticizer to obtain a product called thermoplastic (TPS). However, TPSs alone cannot be employed for packaging because they have low mechanical strength and high water sensitivity [2], which falls below the requirements

of a packaging material. Thus, in order to overcome these drawbacks, there is the need for the incorporation of reinforcing fillers, such as cellulosic fibers, whiskers, nanofibers, etc., to produce new and inexpensive starch biocomposites with improved properties [3–8]. Lignin and nanofibers are potential fillers which are currently in great demand due to their natural abundance and susceptibility to biodegradability, being derived from plants. The term “lignin”, from the Latin word *lignum*, meaning “wood”, was first used by Swiss botanist Candolle [9]. Lignin, a by-product that is mainly obtained from pulp and the paper industry, is the largest aromatic polymer in nature. Although its exact molecular structure is still subject to controversy, lignin is believed to result from the dehydrogenative polymerization of three monomer species, namely p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The amounts and proportions of the main functional reactive groups such as hydroxyl, methoxyl, carboxyl and carbonyl groups in lignin vary according to the plant species and extraction processes applied. Together with cellulose and hemicellulose, lignin forms the structural components of trees and various plants, and constitutes the most common natural polymers from plants. When lignin and cellulosic fibers are used as fillers in natural polymers, the resulting products are eco-friendly and have improved physical properties. Organic membranes prepared from cellulose derivatives usually have low mechanical strength as well as poor resistance to oxidation. In order to overcome these limitations, cellulose-based films make use of lignin [10]. The use of lignin and/or cellulosic fibers to improve the properties of TPS intended to be processed as packaging materials has been reported by a number of researchers. For example, in a study carried out by Kaushik et al. [11], cellulose nanofibrils were extracted from wheat straw using steam explosion, acidic treatment and high-shear mechanical treatment. These nanofibrils were dispersed in thermoplastic starch (TPS) using a Fluko high-shear mixer in varying proportions, and films were casted out of these nanocomposites. The results showed that the mechanical properties increased with the nanofiber concentration. The barrier properties also improved with the addition of nanofillers up to 10%, but further addition deteriorated the properties due to possible fiber agglomeration. In order to improve the mechanical properties and the resistance to water absorption of thermoplastic starch (TPS), Kaewtatip and Thongmee [12] employed kraft lignin (KL) and esterified lignin (EL) as fillers for the TPS matrix. EL was produced via esterification of the KL hydroxyl groups. The TPS/lignin composites were prepared using compression molding. The amount of each of the lignins used in the composites was 5 wt. % (dry starch basis). The TPS and composites were investigated using Fourier-transform infrared spectroscopy (FTIR), water absorption and tensile testing. The FTIR spectra showed that the interaction between the TPS and each lignin caused the peak of the OH stretching shift to lower the wavenumber. This result indicated that both the TPS/KL and the TPS/EL composites had improved mechanical properties over TPS. The tensile strength of the TPS/KL and TPS/EL composites was higher than for the TPS by about 17% and 32%, respectively. In addition, the presence of lignins in the TPS matrix significantly decreased the water-absorption properties. The combination of lignin and cellulosic fiber in the production of a new TPS composite was created by Narchamnan and Sakdaronnarong [13], using a laccase-mediator system to enhance the binding efficiency of natural fibers and lignin compounds into a cassava starch composite matrix. In this work, violuric acid (VA) was tested for its effect as a mediator for laccase treatment. The influence of different fiber, lignin and water contents of the biocomposite was statistically investigated. The results showed that adding 15% (*w/w*) fibers into biocomposite at 44% (*w/w*) water content increased the flexural strength and modulus by four times compared with the control. A combination of fibers + VA gave the greatest enhancement of the modulus at 1140% and flexural strength at 375.8%, as much as neat starch biocomposite. The presence of fibers, lignin and VA as mediators for laccase treatment substantially enhanced the water resistance of starch biocomposite, which was detected by a change in the water drop contact angle on the biocomposite surface.

So far, studies based on the simultaneous incorporation of lignin and cellulose nanofibers into thermoplastic starch composite film, besides the one referenced above, have not been

reported in the literature. Moreover, by changing the sources of the lignin and fibers used in TPS, new biocomposites with unique properties can result. In this work, the lignin and nanofibers were obtained from bamboo plant (*Bambusa vulgaris*). Further to this, work on the optimization of the experimental variables for the development of flexible films from starch for food packaging is limited, hence, the justification for the present study. Therefore, the objective of this research is to produce a lignin–cellulose nanofiber-filled thermoplastic starch composite film, with the optimization of key process variables, for potential application in food packaging.

2. Results and Discussion

2.1. Film Morphologies

The SEM micrographs of the pure starch (representing the negative control sample) and that of lignin–cellulose nanofiber-filled thermoplastic starch composite film (the target sample) are shown in Figure 1 and Figure 2, respectively. The SEM micrograph of the pure starch, as seen (Figure 1), showed the characteristic near-spherical morphology of the cassava starch granules with varying sizes [14]. The micrograph showed that the starch granules were generally in the order of sizes that ranged between 2 and 7 μm . This morphology is, however, destructured during the starch gelatinization process, subsequent to the incorporation of the fillers, in order to pave the way for good mixing in the composite formation stage. In Figure 2, the SEM micrograph shows that the fillers (lignin and cellulose nanofiber) were homogeneously distributed within the matrix of the starch nanocomposite film, a situation that usually results in improved mechanical properties. The white dots with varying sizes on the composite are considered as the fillers embedded in the starch matrix. The sizes of the cellulose nanofibers and lignin were evaluated using transmission electron microscopy (TEM), and their respective micrographs were as shown in Figure 3a,b. It was observed that the sizes of the cellulose nanofibers varied from 20 to 100 nm, while those of lignin fell between 20 and 200 nm. The determined characteristics of the used cellulose nanofiber are hereby shown in Table 1.

Table 1. The characteristics of the used cellulose nanofiber prepared from bamboo (*Bambusa vulgaris* schrad).

TGA Data			
$T_{\text{onset}}/^{\circ}\text{C}$	Peak Temperature/ $^{\circ}\text{C}$	End Degradation Temperature/ $^{\circ}\text{C}$	Residue/wt. %
200.05	328.00	500.58	12.20
Size range/nm			
TEM and particle size distribution		20–100	

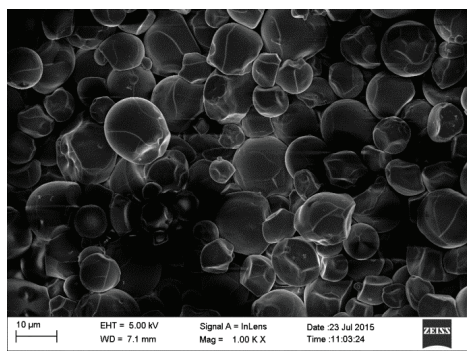


Figure 1. SEM micrograph of pure cassava starch film.

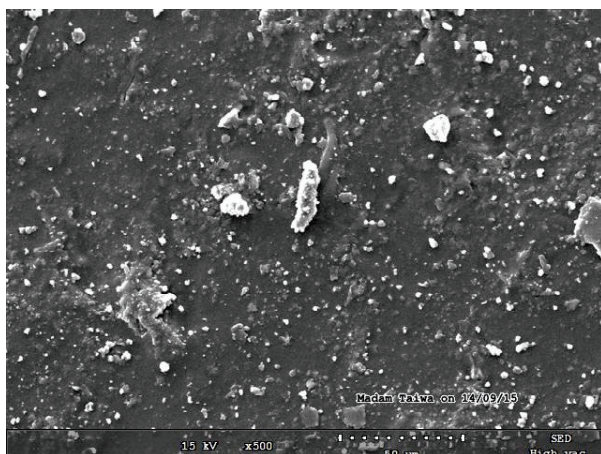


Figure 2. SEM micrograph of lignin-cellulose nanofiber-filled thermoplastic starch composite film.

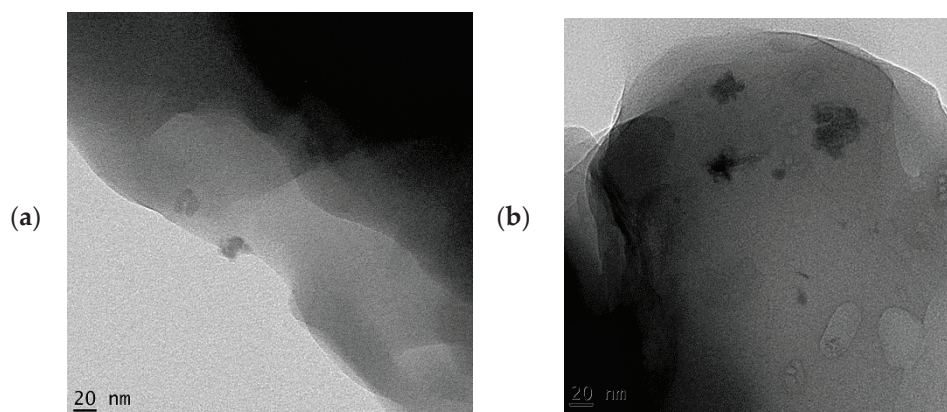


Figure 3. TEM micrographs of (a) cellulose nanofiber; (b) lignin fillers.

2.2. Development of Regression Model Equations by Central Composite Design (CCD) for Lignin-Cellulose Nanofiber-Filled Thermoplastic Starch Composite Film

The quadratic models, in terms of the actual factors used in achieving the desired optimum or ideal films, were obtained from an analysis of variance (ANOVA) (Tables 2–5), and are given in Equations (1)–(4). The positive signs in the models indicate synergetic effects, whereas the negative signs, antagonistic effects. The model for predicting the moisture-absorption capacity of the lignin-cellulose nanofiber-filled thermoplastic starch composite film in terms of actual factors is given in Equation (1).

$$MAB = 35.53 + 0.837l - 0.321n - 0.755ln \quad (1)$$

$$\left\langle R^2 = 91.02\% \mid R_{adj}^2 = 85.63\% \mid R_{pred}^2 = 64.30\% \right\rangle$$

where: MAB = moisture-absorption capacity, l = lignin (wt. %), n = nanofiber (wt. %).

The model was significant at a p -value of 0.0048, and the coefficient of determination (R^2) of 0.9102 was obtained. This indicates that the statistical model could explain 91.02% of the variability, while the remaining 8.98 wt. % could not be accounted for by the independent variables [15]. The predicted R^2 of 64.30% reasonably agrees with the adjusted R^2 (85.63%), as should normally be the case for model adequacy due to the very small blocking effect of the data used. The precision of the model is high since the adequate precision value obtained was ~11.156 [16].

The model for predicting the percentage elongation of the lignin–cellulose nanofiber-filled thermoplastic starch composite film in terms of actual factors is given in Equation (2).

$$PE = 50.35 + 0.10l - 1.09n + 0.077ln - 0.067l^2 + 0.124n^2 \quad (2)$$

$$\langle R^2 = 84.49\% | R_{adj}^2 = 58.65\% | R_{pred}^2 = -82.39\% \rangle$$

where: PE = elongation (wt. %); l and n retained their usual meaning.

The model was significant at a p -value of 0.1792, and the coefficient of determination (R^2) of 0.8449 was obtained. This indicates that the model could describe 84.49 wt. % variability, while 15.51 wt. % was inexplicable by the independent variables [15]. In addition, the negative predicted R^2 value of -82.39 wt. % implied that the fitted model, though precise (with adequate precision value of 5.37), is not a suitable predictor of the percentage elongation of the starch composite film.

The model for predicting the tensile strength of the lignin–cellulose nanofiber-filled thermoplastic starch composite film with respect to the actual factors is given in Equation (3).

$$TS = 17.32 + 0.37l + 0.48n \quad (3)$$

$$\langle R^2 = 83.46\% | R_{adj}^2 = 77.94\% | R_{pred}^2 = 61.72\% \rangle$$

where: TS = tensile strength (MPa); l and n retained their usual meaning.

The model was significant at a p -value of 0.0045, and the coefficient of determination (R^2) of 0.8346 was obtained. This indicates that the statistical model could explain the 83.46% value of the variability, while a 16.54% value was inexplicable by the independent variables [15]. In addition, the predicted R^2 of 61.72 wt. % agrees with the R^2 (adjusted) of 77.94%, as should normally be the case for adequate model. The model is precise in its capacity to predict the tensile strength of the starch mixture film, since an adequate precision value of 10.91 was recorded [16].

The model for predicting the Young's modulus of the lignin–cellulose nanofiber-filled thermoplastic starch composite film in terms of actual factors is given in Equation (4).

$$YM = 24.5 + 0.09l + 0.165n \quad (4)$$

$$\langle R^2 = 95.01\% | R_{adj}^2 = 93.34\% | R_{pred}^2 = 91.81\% \rangle$$

where: YM = Young's modulus (MPa); l and n retained their usual meaning.

Table 2. ANOVA for response surface quadratic model for *MAB* of lignin–cellulose nanofiber-filled thermoplastic starch composite film.

Source	Sum of Squares	df	Mean Square	F Value	p -Value Prob > F	
Model	245.9	3	81.97	16.89	0.0048	Significant
A-lignin	48.91	1	48.91	10.08	0.0247	
B-cellulose nanofiber	160.52	1	160.52	33.08	0.0022	
AB	36.47	1	36.47	7.52	0.0407	
Residual	24.26	5	4.85			
Cor total	270.17	8				
Std. dev.	2.2		R-squared	0.9102		
Mean	28.28		Adj R-squared	0.8563		
C.V. wt. %	7.79		Pred R-squared	0.643		
PRESS	96.44		Adeq precision	11.156		

Table 3. ANOVA for response surface quadratic model for TS of lignin–cellulose nanofiber-filled thermoplastic starch composite film.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -Value Prob > F	
Model	8.86	2	4.43	15.14	0.0045	Significant
A-lignin	3.27	1	3.27	11.18	0.0155	
B-cellulose nanofiber	5.59	1	5.59	19.09	0.0047	
Residual	1.76	6	0.29			
Cor total	10.61	8				
Std. dev.	0.54		R-squared	0.8346		
Mean	19.88		Adj R-squared	0.7794		
C.V. wt. %	2.72		Pred R-squared	0.6172		
PRESS	4.06		Adeq precision	10.908		

Table 4. ANOVA for response surface quadratic model for YM lignin–cellulose nanofiber-filled thermoplastic starch composite film.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -Value Prob > F	
Model	0.85	2	0.42	57.09	0.0001	Significant
A-lignin	0.2	1	0.2	26.37	0.0021	
B-cellulose nanofiber	0.65	1	0.65	87.81	<0.0001	
Residual	0.045	6	0.00744			
Cor total	0.89	8				
Std. dev.	0.086		R-squared	0.9501		
Mean	25.26		Adj R-squared	0.9334		
C.V. wt. %	0.34		Pred R-squared	0.9181		
PRESS	0.073		Adeq precision	20.514		

Table 5. ANOVA for response surface quadratic model for PE of lignin–cellulose nanofiber-filled thermoplastic starch composite film.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -Value Prob > F	
Model	1.43	5	0.29	3.27	0.1792	Not significant
A-lignin	0.11	1	0.11	1.22	0.3502	
B-cellulose nanofiber	0.32	1	0.32	3.6	0.154	
AB	0.38	1	0.38	4.29	0.1302	
A ²	0.14	1	0.14	1.62	0.2922	
B ²	0.49	1	0.49	5.62	0.0985	
Residual	0.26	3	0.088			
Cor total	1.69	8				
Std. dev.	0.3		R-squared	0.8449		
Mean	48.76		Adj R-squared	0.5865		
C.V. wt. %	0.61		Pred R-squared	−0.8239		
PRESS	3.09		Adeq precision	5.373		

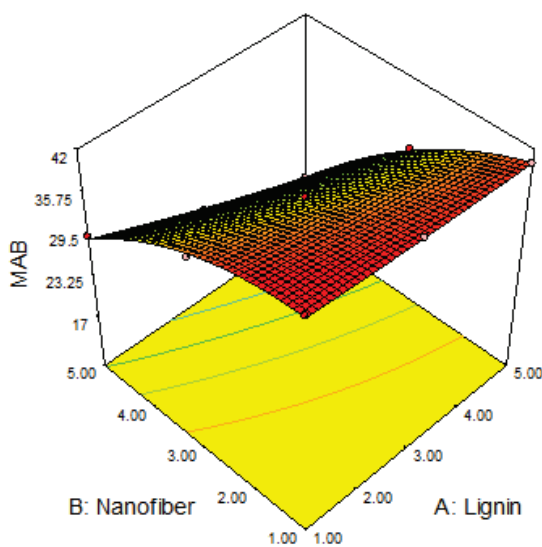
The model was found to be significant at a 0.0001 *p*-value, and the coefficient of determination (R^2) of 0.9501 was obtained. This indicates that the model could explain 95.01% of the variability, while 4.99% was inexplicable by the independent variables [15]. The predicted R^2 value of 91.81% reasonably agrees with the R^2 adjusted value of 93.34% for the adequate model, and this was caused by the small block effect of the data used in generating the model. In addition, the model is precise in its capability to forecast the lignin–cellulose nanofiber-filled thermoplastic starch composite film's Young's modulus, since the adequate precision recorded value was 20.51 [16].

2.3. Analyses of Response Surfaces

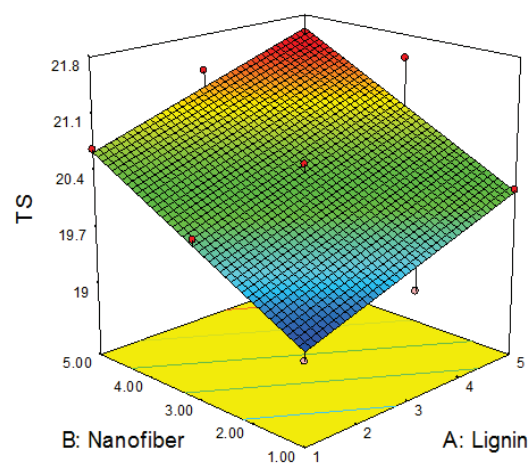
The 3D response surface plots of the joint effects of the independent variables (lignin and cellulose nanofiber) on the *MAB*, *TS*, *YM* and *PE* of lignin–cellulose nanofiber-filled thermoplastic starch composite film, as performed according to the working conditions stated in Table 6, are presented in Figure 4. The 3D response surfaces were plotted in order to investigate the probable interactions among the variables, and to determine the optimum conditions of each factor for the minimum moisture absorption and maximum strength of the starch nanocomposite films.

Table 6. RSM factorial design matrix for lignin–cellulose nanofiber-filled thermoplastic starch composite film.

Run	Factors		Responses			
	Lignin (wt. %)	Cellulose Nanofiber (wt. %)	<i>MAB</i> (%)	<i>TS</i> (MPa)	<i>YM</i>	<i>PE</i> (%)
1	3.0	1.0	32.558	18.370	24.960	49.300
2	1.0	3.0	31.818	19.250	25.050	48.250
3	1.0	1.0	32.787	18.070	24.800	49.650
4	5.0	3.0	28.571	21.510	25.520	48.525
5	3.0	5.0	22.034	20.510	25.675	49.000
6	5.0	5.0	15.590	20.980	25.740	48.575
7	5.0	1.0	31.884	19.750	25.100	48.500
8	1.0	5.0	28.571	20.490	25.425	48.500
9	3.0	3.0	30.693	19.970	25.100	48.500



(A)



(B)

Figure 4. Cont.

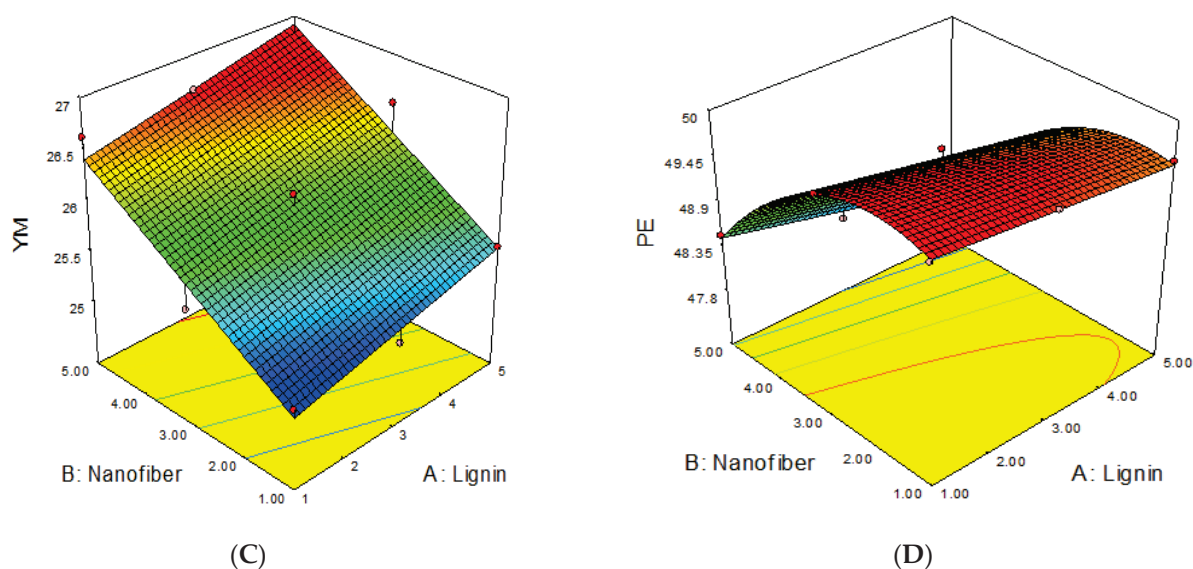


Figure 4. The influence of filler contents on (A) *MAB*, (B) *TS*, (C) *YM* and (D) *PE* of the lignin–cellulose nanofiber-filled thermoplastic starch composite film.

The response surface plots for the influence of lignin and cellulose nanofiber contents on the *MAB*, *TS*, *YM* and *PE* of the lignin–cellulose nanofiber-filled thermoplastic starch composite film are presented in Figure 4.

The results revealed that the inclusion of cellulose nanofiber content had the most significant effect on the moisture absorption and mechanical properties of the lignin–cellulose nanofiber-filled thermoplastic starch composite film, followed by lignin inclusion content. The *MAB* reduced rapidly as the cellulose nanofiber content increased when compared to that of lignin content. The lignin and cellulose nanofiber contents interacted negatively. This shows that the moisture absorption reduced as the lignin and cellulose nanofiber contents increased. The decrease in *MAB* with the increase in the lignin and cellulose nanofiber contents was because of the strong filler–matrix interaction, which reduced the molecular mobility and diffusivity in the matrix material, thus, limiting the degree of moisture uptake [17]. This result is in accordance with the findings of Khan et al. [18], Mitchell [19], and Taghizadeh and Sabouri [20], who reported low moisture-absorption rates for cocoyam, tapioca and modified corn starch films, respectively, with an increase in filler loading.

The results also showed that the *TS* and *YM* of the starch nanocomposite film increased as the lignin and cellulose nanofiber contents increased, while *PE* decreased with the increase in lignin and cellulose nanofiber contents. The significant increase in the *TS* and *YM* of the lignin–cellulose nanofiber-filled thermoplastic starch composite film with the increase in filler contents, particularly nanofiber, was due to the small sizes (nano-range), smooth surface and large surface area of these fibers, which produced a good fiber/matrix interaction, thus, improving the composite film’s strength [21]. It might also be that lignin improved the compatibility between the starch and cellulose nanofiber [22]. Moreover, Suarez et al. [23] also reported the fact that a good interfacial region increases stress transfer efficiency from the matrix to the fillers, thereby increasing the composite’s strength.

This result corroborated with that of Wang et al. [22], who confirmed a rise in the *TS* and *YM*, but a decrease in the elongation-at-break of a PLA nanocomposite reinforced with lignin–cellulose nanofiber (L-CNF), as the L-CNF content rose from 25 to 35 wt. %. Patpen et al. [24] also reported the effect of cellulose addition on the *TS* of polylactic acid biocomposite; they observed an increase in this parameter as cellulose loading increased. Tawakkal et al. [25] also reported how kenaf-derived cellulose (KDC)-loaded polylactic acid affected the material’s tensile properties and documented an improvement in both *TS* and tensile modulus, as KDC loading increased from 30 to 60 wt. %. On the contrary,

Sawpan et al. [26] studied the mechanical properties of hemp fiber-reinforced PLA biocomposites and established a non-linear relationship between *TS* and fiber content, indicating that the strength of the biocomposite somewhat decreased as the fiber content increased from 0 to 40 wt. %.

2.4. Optimization of Lignin–Cellulose Nanofiber-Filled Thermoplastic Starch Composite Film Production

The result of the optimization of the experimental variables (lignin and cellulose nanofiber contents), showing the desirability function, with respect to the films prepared, is shown in Figure 5. The goal for both the water barrier and mechanical optimization was to minimize the moisture absorption and improve the mechanical property; thus, the target value of the responses, as obtained from the obtained experimental results, was at the lowest and uppermost values, respectively. The result from the optimization showed that increases in the lignin and cellulose nanofiber contents, from 1 to 5 wt. %, significantly affected the desirability of the films. Therefore, it is obvious from the optimization result that the selected film with a desirability closer to the goal is the one that was produced with the blend of 4.81 wt. % lignin and 5.00 wt. % nanofiber at 76.80% desirability. The corresponding *MAB*, *TS*, *YM* and *PE* of the selected film, apparently the optimum sample, are 17.80%, 21.51 MPa, 25.76 MPa and 48.81%, respectively. This finding is similar to that of Wang et al. [22], who documented optimum values of 21.6 MPa and 21.6% for the *TS* and elongation, respectively, for PLA composites reinforced with L-CNF. Akbar et al. [27], however, obtained optimum values of 50 MPa for the *TS*, 2.15 MPa for the *YM* and 141.07% for elongation, during the optimization of polyvinyl alcohol nanocomposite films. Patpen et al. [24] also reported an optimum value of 46.207 MPa for the optimization of PLA-based biocomposites that were reinforced with cellulose obtained from durian peel.

Design-Expert® Software

Desirability



X1 = A: Lignin
X2 = B: Nanofiber

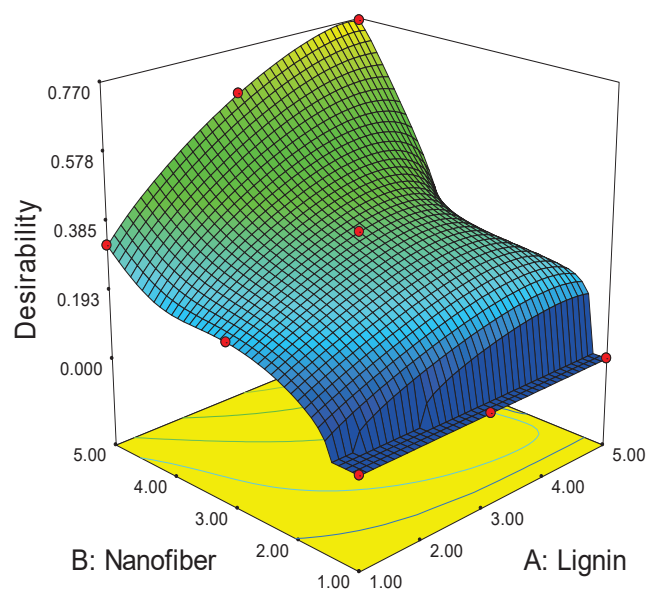


Figure 5. Desirability function of selecting the ideal film prepared.

This study thus affirms that lignin and cellulose nanofiber addition to starch composite minimized *MAB* and improved the mechanical properties of the produced films because of the good filler/matrix interfacial adhesion, as evident by the SEM micrograph. Hence, the produced films can be applied to package food.

3. Materials and Methods

3.1. Plant Materials

Cassava (*Manihot esculenta* crantz) was purchased from a farm in Minna, Nigeria, whereas bamboo (*Bambusa vulgaris* schrad) was collected from the river banks of Gurara in Izom, Niger State. They were identified and authenticated by a botanist at the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja. Specimen voucher numbers (NIPRD/H/6792 and NIPRD/H/6793), respectively, were placed at their herbarium for references in the future.

3.2. Chemicals

Analytical grade chemicals were employed for the study and included ethanol (BDH chemicals, London, UK); acetic anhydride (Sigma Aldrich, Burlington, MA); sodium hydroxide (Kermel, China); citric acid (BDH chemicals, UK); hydrochloric acid (Griffin and George, UK); sulfuric acid (BDH chemicals, UK); hydrogen peroxide (BDH chemicals, UK); sodium sulphate (BDH chemicals, UK); and ammonium hydroxide (Griffin and George, London, UK). Glycerol (BDH chemicals, UK) was used as the plasticizer for the study.

3.3. Preparation Lignin and Cellulose Nanofiber from Bamboo (*Bambusa vulgaris* Schrad)

Lignin was extracted from bamboo according to standard procedure, as reported by Yong et al. [28], Alemdar and Sain [29] and Ming-Fei et al. [30]. The bamboo stalks obtained were first sun-dried and then chipped into small pieces. The sun-dried pieces of bamboo were ground and screened to obtain a 40–60 µm mesh fraction. The ground bamboo stalk (20 g) was first soaked in NaOH (4% *w/w*) at room temperature for 24 h. It was then filtered and washed with distilled water (1 L) until it was free of alkali. The residue was re-dispersed in 1 L of distilled water, filtered again and treated with 10% (*w/w*) NaOH at 121 °C in an autoclave for 4 h. Furthermore, the residue was again washed in distilled water to free it of residual alkali (1 L) and filtered. Lignin was precipitated from the filtrate by acidifying it to pH 2 with H₂SO₄. The precipitates were separated from the mixture by filtration. The separated lignin was washed with water several times and then oven-dried at 40 °C. In order to obtain bamboo fiber, the supernatant liquid left after the alkali treatment was bleached in 8% (*v/v*) H₂O₂ at room temperature for 24 h. Finally, the material was again washed and filtered as before to obtain bamboo fiber. The bamboo fiber was then converted to nanofiber using acid hydrolysis [31]. The bamboo fibers obtained after lignin removal were steeped in HCl (10% *w/w*) with ultrasonic agitation at 60 °C for 2 h using an Ultrasonicator (SB25-12DT, Scientz, Ningbo, China). The material was given a final wash and then placed in a high shear homogenizer (Heidolph DIAX 900, Burladingen, Germany) for 15 min to produce bamboo nanofibers.

3.4. Experimental Design and Optimization of Starch Nanocomposite Film Production

Design Expert software (version 7.0, Start-Ease Inc., Minneapolis, MN, USA) was employed for the experimental design, while Response Surface Methodology (RSM) was used for optimizing the conditions required for preparing the starch nanocomposite films. As a result, 1 center-point Box–Wilson Central Composite Design (CCD) was utilized. After the designed experiment was performed, linear regression was used to obtain the results. The design consisted of 9 experimental runs. The RSM considered the effect of two variables: lignin content (wt. %) and cellulose nanofiber content (wt. %) used as fillers in the nanocomposite preparation with 5 levels each. The response functions measured were moisture absorption (MAB), tensile strength (TS), percent elongation (PE) and Young's modulus (YM). An analysis of variance (ANOVA) was employed to analyze the obtained data, in order to determine the interactions that exist between the process variables and the responses. Accurate and proper models were picked at $p < 0.05$ and had a significant correlation. The fitting model's quality was expressed by the coefficient of determination R^2 and adjusted R^2 . The factors' level with their codings are shown in Table 7.

Table 7. Experimental variables and their coded levels of variables levels for CCD.

Variables	Units	Coded Levels		
		−1	0	+1
Lignin	wt. %	1.0	3.0	5.0
Cellulose nanofiber	wt. %	1.0	3.0	5.0

In the optimization selection, two factors (lignin and cellulose nanofiber contents) were considered in order to build desirability indices. The objective was to reduce the *MAB* while improving *TS*, *PE* and *YM*; therefore, the target value of the responses was lowest for *MAB* and highest for *TS*, *PE* and *YM* from the experimental results obtained.

3.5. Preparation of Lignin–Cellulose Nanofiber-Filled Thermoplastic Starch Composite Film

Cassava starch (2 g) granules were dispersed in 50 mL of distilled water and heat was applied at 70 °C for 20 min under constant stirring over a magnetic stirrer. Glycerol (50 wt. % based on dry cassava starch content) was added to the dispersion while the heating at 70 °C was continued under constant stirring speed for the next 2 min. Next, the lignin and cellulose nanofiber (varied wt. %, with respect to the dry cassava starch content, and based on the statistical formulation of the Central Composite Design adopted) were added to the dispersion under the same conditions for another 2 min. Before being introduced into the plasticized starch mixture, the cellulose nanofibers were sonicated for 10 min by using a 60 W rated Sonicator. The mixture of dispersion was then cast into a mold and oven-dried at 50 °C using a still-air oven for 18 h, in order to obtain dry lignin–cellulose nanofiber-filled thermoplastic starch composite films [32,33], whose average thickness was found to be 0.12 mm. The control (TPS) sample was also prepared using the same process mentioned above, except that there were no fillers added to it. All films were conditioned at 55 ± 5% RH and 25 ± 2 °C before testing their permeability and mechanical properties, as described by the ASTM standard D882-09 [34] and Detduangchan et al. [35]. The conditioning was performed by inserting the films into desiccators containing a saturated solution of Mg (NO₃)₂•6H₂O for 72 h. Part of the films were tested for water absorption, *TS*, *PE* and *YM*, and the rest, which were for other tests, were kept in plastic bags and inserted into desiccators.

3.6. Characterization of Lignin–Cellulose Nanofiber-Filled Thermoplastic Starch Composite Film Water-Absorption Test

The film pieces (20 mm × 20 mm) were pre-conditioned by drying in the oven at 50 °C for 24 h and then weighed to determine the dry weight. They were then immersed in a bath containing distilled water at room temperature. The film samples were removed from distilled water after intervals of 1, 3, 5, 7, 9 and 11 h and, after wiping off the excess water on their surfaces with tissue, their weights were determined. The water-absorption capability (WAC) was, thus, calculated using Equation (5) [32,36]:

$$\text{WAC wt. \%} = \frac{(W_{\text{wet}} - W_{\text{dry}})}{W_{\text{dry}}} \times 100 \quad (5)$$

where: W_{wet} = Wet specimen weight and W_{dry} represents the dry specimen weight.

3.7. Mechanical Properties

The *TS*, *PE* and the *YM* values were determined with a universal testing machine (DBBMTCL-2500kg Testometric, Rochdale, UK), according to the ASTM D882 Standard [34]. Prior to testing, each sample was conditioned at a temperature of 25 °C and a relative humidity (RH) of 55% for 24 h. The average thickness of the samples was about 0.12 mm. The tensile test was carried out using 1.3 mm/min crosshead speed. Each of the determinations was obtained from triplicate specimens.

3.8. Scanning Electron Microscopy (SEM)

The morphological structures of the lignin–cellulose nanofiber-filled thermoplastic starch composite films were determined using SEM (Zeiss Auriga HRSEM) at an accelerating voltage of 15 kV. The as-prepared samples were, respectively, placed on a stub with a double-sided adhesive tape, after which they were coated with a thin layer of gold. The micrographs were captured using a magnification of 350 times the original specimen size [33,37].

3.9. Statistical Analysis

Design Expert Software (version 7.0) was employed to analyze the obtained data. The determination of interaction effects between the factors and a quadratic surface plot was generated using an analysis of variance (ANOVA). The model adequacy was examined using the ANOVA, a normal probability plot and a residual plot, according to the method described in the literature [38]. An F-Test was also employed to determine the model's statistical significance and the regression coefficients' significance.

4. Conclusions

In conclusion, the film with a desirability of ~76.80%—which is closest to the objective function—and containing 4.81 wt. % lignin and 5.00 wt. % cellulose nanofiber, was selected as the optimum sample. The *MAB*, *TS*, *YM* and *PE* of the selected film were 17.80%, 21.51 MPa, 25.76 MPa and 48.81%, respectively. This film presented the maximum mechanical potency and minimal moisture-absorption capacity. The addition of lignin and cellulose nanofiber concurrent to the TPS matrix evidently caused a decrease in moisture absorption, while at the same time an improvement in the mechanical properties of the films. Consequently, the prepared films have the potential to be employed as films for packaging foods.

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Article

Fabrication and Model Characterization of the Electrical Conductivity of PVA/PPy/rGO Nanocomposite

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Abstract: Owing to the numerous advantages of graphene-based polymer nanocomposite, this study is focused on the fabrication of the hybrid of polyvinyl alcohol (PVA), polypyrrole (PPy), and reduced graphene-oxide. The study primarily carried out the experimentation and the mathematical analysis of the electrical conductivity of PVA/PPy/rGO nanocomposite. The preparation method involves solvent/drying blending method. Scanning electron microscopy was used to observe the morphology of the nanocomposite. The electrical conductivity of the fabricated PVA/PPy/rGO nanocomposite was investigated by varying the content of PPy/rGO on PVA. From the result obtained, it was observed that at about 0.4 (wt%) of the filler content, the nanocomposite experienced continuous conduction. In addition, Ondracek, Dalmas s-shape, dose–response, and Gaussian fitting models were engaged for the analysis of the electrical transport property of the nanocomposite. The models were validated by comparing their predictions with the experimental measurements. The results obtained showed consistency with the experimental data. Moreover, this study confirmed that the electrical conductivity of polymer-composite largely depends on the weight fraction of fillers. By considering the flexibility, simplicity, and versatility of the studied models, this study suggests their deployment for the optimal characterization/simulation tools for the prediction of the electrical conductivity of polymer-composites.

Keywords: polyvinyl alcohol; polypyrrole; graphene; electrical conductivity; energy storage; models; percolation threshold

1. Introduction

The ability to control the properties of polymers by the inclusion of nano-fillers gives rise to the fabrication of devices for energy storage, medical applications, computer systems, civil construction, automobile parts, and industrial equipment. For instance, Al-Zu'bi et al. [1] stated that in the process of retrofitting, high-performing polymer-composite can be achieved when carbonaceous fillers are composited with polymers and cement. The excellent electrochemical activity, electrical conductivity, large surface area, and ion transition path are some of the classified properties of conducting polymer for energy storage [2]. The properties of polythiophene doped Iron (III) chloride and polyamide 6 (PA6) make the composite suitable for the manufacturing of solar cells [3].

The composites of polylactic acid, polydopamine, and polyurethane with some compatible inorganic materials are often used for antibacterial infections [4]. The electric double-layer capacitor performances of PVA-doped-potassium-iodide- and -glycerol, has been reported by Aziz et al. [5]. As a biocompatible polymer, PVA is environmentally benign with relatively low cost, and it is suitable for the manufacturing of electrodes for supercapacitors and batteries [6–9].

The invaluable need for energy storage in the present and future generation due to the dilemma caused by the use of fossil fuel fosters the urgency for the development of a plethora of types of electrodes for electrochemical energy storage. Amongst others, polymer-composites for supercapacitors and batteries are envisaged to ravage the problems confronting the present-day electrodes. Conducting polymers such as polypyrrole and polyaniline possess high electrical conductivity and good redox reaction, but poor cyclability sets them at bay. However, the hybrid or composite of conducting polymers with two-dimensional materials, such as graphene and its derivative, produces materials having excellent electrochemical, thermal, and mechanical properties for energy storage electrodes [10–12]. Graphene is electrically conductive, as well as chemically, thermally, and mechanically stable.

The electrical conductivity of polymer-composites largely depends on the preparation methods and the intrinsic conductivity of the individual materials [13,14]. Of further concern is the percolation trend of polymer-composites' electrical conductivity. The description of the electrical conductivity of polymer-composites cannot be singly achieved by experimentation technique, hence the need for analytical and descriptive models. While envisaging polymer-composites for energy storage, models must be meaningfully engaged in order to produce optimized and less costly electrodes. In our previous study, the electrical conductivity models for different polymer-composites were discussed [15]. The network usually formed by the mixture of filler and matrix can be easily analyzed and characterized by some set of well-formulated mathematical equations. In a study by Clingerman [16], the performances of statistical percolation, thermodynamic, structure-oriented, and geometry percolation models for the prediction of the electrical conductivity of polymer-composite were reported. The Mamunya model [17], which is an example of a thermodynamic model, was described by Clingerman as most suitable for the prediction of the electrical conductivity of polyacrylonitrile filled carbon fibers. Several authors [18–21] have enunciated the dependency of polymer-composites' conductivity on some parameters. Of course, previous models are viable and useful for the prediction of the electrical conductivity of polymer-composite. However, further study on most suitable models cannot be overstated due to the many parameters on which the properties of polymer-composites depend.

The purpose of this study is to experimentally investigate the electrical property of the nanocomposite of polyvinyl alcohol (PVA), polypyrrole (PPy), and reduced graphene oxide (rGO)—PVA/PPy/rGO nanocomposite. Secondly, the characterization of the electrical conductivity of PVA/PPy/rGO nanocomposite by using various models as discussed in Sections 1.1–1.4 was conducted. The models were validated by using the experimental measurements, and their results were presented accordingly. The results showed that the models are versatile for the prediction of the electrical conductivity of polymer-composites. However, further modification may be required in order to achieve high accuracy in subsequent studies.

1.1. Ondracek Model

The model developed by Ondracek [22], considered four (4) parameters, which are: shape factor, orientation, volume fraction, and conductivity. The model is given by Equation (1).

$$1 - C_D = \frac{\phi_D - \phi_c}{\phi_D - \phi_m} \left(\frac{\phi_m}{\phi_c} \right)^h \left(\frac{\phi_c + g\phi_D}{\phi_m + g\phi_D} \right)^k \quad (1)$$

where $h, k, g = f(F_D, \cos^2 \alpha_D)$, C_D ($0 \leq C_D \leq 1$) is the volume fraction of filler, ϕ_D and ϕ_m are the filler and matrix conductivity phase, F_D ($0 \leq F_D \leq 0.5$) is the filler shape factor, and $\cos^2 \alpha_D$ ($0 \leq \cos^2 \alpha_D \leq 1$) is the orientation factor. The Ondracek model is not often considered an effective and appropriate model for the prediction of polymer-composites' electrical conductivity due to its inability to predict composite electrical conductivity when the filler phase conductivity is of higher magnitude than the matrix counterpart. However, the model can be linearly re-parameterized to give a better prediction accuracy. By expanding Equation (1) while ignoring the effects of exponential parameters h and k , Equation (2) is as given.

$$\frac{g\phi_D^2}{\phi_m} - \phi_m - C_D\phi_D - \frac{gC_D\phi_D^2}{\phi_m} + C_D\phi_m + gC_D\phi_D = \frac{g\phi_D^2}{\phi_c} - \phi_c \quad (2)$$

Assuming: $g\phi_D^2 \ll \phi_c^2$, Equation (3), is as presented:

$$\phi_c = C_D\phi_D(1 - g) + \phi_m(1 - C_D) - \frac{g\phi_D^2}{\phi_m}(1 - C_D) \quad (3)$$

Equation (3) is equivalent to Equation (4).

$$\sigma = v_f\sigma_f(1 - g) + \sigma_p(1 - v_f) - \frac{g\sigma_f^2}{\sigma_p}(1 - v_f) \quad (4)$$

where v_f, σ_f, σ_p , and σ , are the filler volume fraction, filler, polymer, and composite conductivities; and $g = 1 - \frac{1}{2F_D + (1-3F_D)\cos^2 \alpha_f}$. Equation (4) clearly shows the importance of filler volume fraction in the determination of the electrical conductivity of the nanocomposite. That is, the composite conductivity equals to the conductivity of filler when the volume fraction of the filler equals zero. The linearized form of Equation (1) is given by Equation (4). On a logarithm scale, Equation (4) can be used to measure and predict the electrical conductivity of polymer-composites to a certain degree of accuracy.

1.2. Dalmas s-Shape Model

In its original form, the s-shape function proposed by Dalmas [23], for polymer-composites electrical conductivity, is as given in Equation (5).

$$S(\psi) = \sigma_s + \frac{\sigma_{cE} - \sigma_s}{1 + e^{-a\psi+b}} \quad (5)$$

where $\sigma_{cE}, \sigma_s, S(\psi)$, are the contact, surfactant, composite conductivities; ψ, a , and b are the volume fraction and fitting parameters. Dalmas provided the performances of Equation (5) by using it to describe the experimental data of MWNT-polymer nanocomposites. Equation (6) is equivalent to Equation (5).

$$\sigma = \sigma_1 + \frac{\sigma_2}{1 + e^{-av_f+b}} \quad (6)$$

where σ_1 and σ_2 are the initial and maximum electrical conductivities of the polymer-composites.

1.3. Dose-Response Model

A dose-response model is a sigmoidal model that has been extensively used in pharmaceutical and clinical industries to measure the cause and effect of drug, illness, death, and diseases [24]. For instance, Park et al. [25] studied the treatment of tumor via radiotherapy exposure by using dose-response theory. In the study, Park et al. discovered that a high dose of radiation gives a better treatment response for hepato-cellular carcinoma. Furthermore, Li et al. [26], carried out a dose-response investigation on fat distribution and

incidental liver cancer. The result of the investigation showed that liver disease increases the chance of atypical obesity. In mathematical terms, dose is an independent parameter (cause), while response is the dependent parameter (effect). The dose–response theory can be used to determine the properties of polymer-composites by assuming that the various determining factors of the properties are associated to dose parameters, while the response is the resulting property. Rahaman et al. [27] previously studied the applicability of sigmoidal equations to polymer-composites’ electrical conductivity determination.

According to Ritz et al. [28], the four-parameter-log-logistic model, presented in this study as Equation (7), is the most frequently used dose–response equations.

$$\sigma(v_f, (\sigma_1, \sigma_2, c, d)) = \sigma_1 + \frac{\sigma_2 - \sigma_1}{1 + e^{(c(\log(v_f) - \log(d)))}} \quad (7)$$

Herein, c is the slope or steep factor and d is the effective dose parameter. From the experimental data presented in this study, the performance of the dose–response model of Equation (7) was slightly modified for better accuracy. The modified form of Equation (7) is as presented in Equation (8). Another possible way to achieve a better accuracy from the prediction of Equation (7) is to critically set the initial values of the dependent parameters from the simulation code.

$$\sigma(v_f, (\sigma_1, \sigma_2, c, d)) = \sigma_1 + \frac{\sigma_2 - \sigma_1}{1 + e^{-c \log(v_f)} e^{-c \log(d)}} \quad (8)$$

1.4. Gaussian Fitting Model

The Gaussian model is another important predictive model considered in this study. The versatility of the Gaussian model has been proved by many researchers [29–32]. It is, therefore, important to test how useful Gaussian fitting model can be in predicting the property of polymer-composite, viz.: electrical conductivity. Amongst others, filler orientation, synthesis method, initial conductivity of matrix/filler, weight fraction, and shape are factors that contribute to the electrical property of polymer nanocomposites. The Gaussian equation presented by Equation (9), is a three-parameter equation.

$$\sigma = \sum_n^N \sigma_n e^{-\left(\frac{x - k_n}{z_n}\right)^2} \quad (9)$$

where z_n and k_n are factors relating to the percolation threshold and saturating weight fraction; σ_n is the electrical conductivity of filler/matrix.

2. Results

A typical micrograph of PVA/PPy/rGO nanocomposite is shown in Figure 1. The micrograph of polymer-composite is very important in order to observe the interaction between the fillers and matrices. As shown in Figure 1, the ternary materials are homogeneously mixed; however, the interaction was by van der Waals and $\pi - \pi$ interactions [33].

In the preparation and electrical conductivity measurement of polymer-composites, the most important parameter of interest is the percolation threshold. A polymer-composite with a high percolation threshold suggests a low electrically conductive composite; such a composite will require a high-volume fraction of filler, which is synonymous with a high cost of production. On the other hand, a low percolation threshold gives rise to an increase in the electrical conductivity, reduces filler weight and improves mechanical and chemical properties of the polymer-composites. One of the determinants of polymer-composites’ percolation threshold is the preparation method. The interface between filler/filler and filler/matrix, are functions of the morphology of the composite, a factor which can be controlled by the hybridization and synthesis methods.

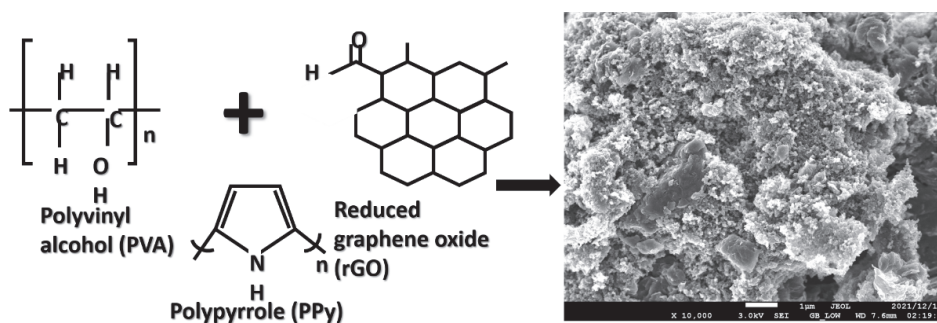


Figure 1. The structures and the morphology of PVA/PPy/rGO nanocomposite (0.4 (wt%)).

The electrical conductivity measurement of the fabricated PVA/PPy/rGO nanocomposite is as shown in Figure 2. As it can be seen from the figure, the electrical conductivity of the nanocomposite gained a continuous conductive path at about 0.4 (wt%) of the hybrid filler. With respect to the recorded low percolation threshold, it can be ascertained that the electrical property of the materials has been improved. The low percolation threshold also suggests that the filler possesses a high aspect ratio [34].

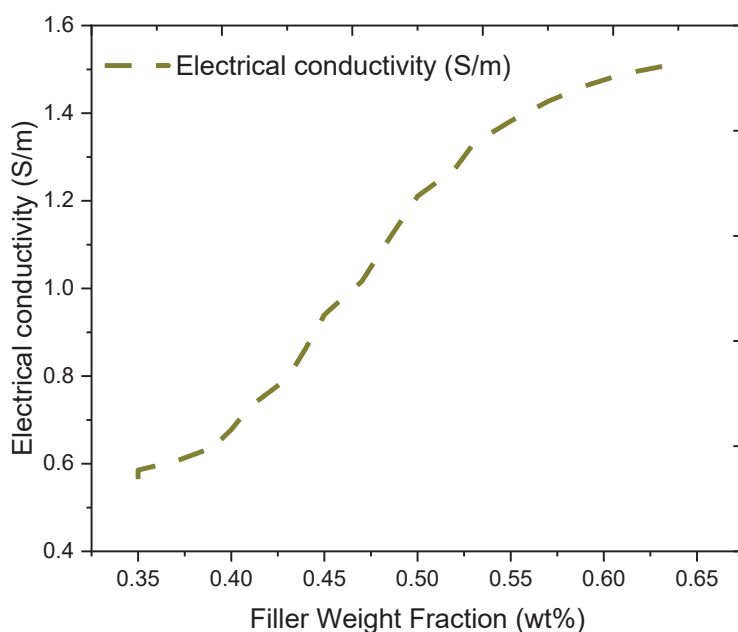


Figure 2. Electrical conductivity measurement of PVA/PPy/rGO nanocomposites.

Experimental Data and Modeling Analysis

The models previously mentioned in Sections 1.1–1.4, were used to describe the electrical conductivity data of the fabricated PVA/PPy/rGO nanocomposite. The description of the experimental data is important in order to theoretically formulate and propose models for the prediction of the electrical conductivity of polymer-composites. It is also necessary to mention that the models can save the time and cost of preparing polymer-composite.

Figure 3 shows the comparison and the description of PVA/PPy/rGO nanocomposite conductivity data by the linearized Ondracek model. The Ondracek model is concerned with orientation of filler, shape, weight fraction, and conductivities of the filler and matrix. From the linearized Ondracek equation, the effects of the aforementioned factors are presented by χ_1 and χ_2 , as shown in Table 1. The re-parameterized equation of Equation (4) is as shown by Equation (10); where x is the volume fraction. From the results, it can be observed that the effect of g -factor in the equation, which represents the shape and orientation factor, can be positive or negative. Subsequent to the fact that the model is

linear, it is difficult to truly observe how the g -factor affects the percolation threshold of the nanocomposite. A high percolation threshold is usually associated with anisotropic orientation of fillers; otherwise, a low percolation shows that the orientation is isotropic [35]. In addition, further observation from the figure is that the model can predict the conducting region of the composite before saturation. Table 1 displays the accuracy of the model. The deviation of the model from the experimentation data is 0.03 (0.038 adjusted). The per unit errors for the calculated parameters concisely give the performance of the model. Therefore, the model is proposed for re-modification in order to give better accuracy by producing agreeable simulation data with experimental data.

$$\sigma = \log(\chi_1 x + \chi_2(1 - x)) \quad (10)$$

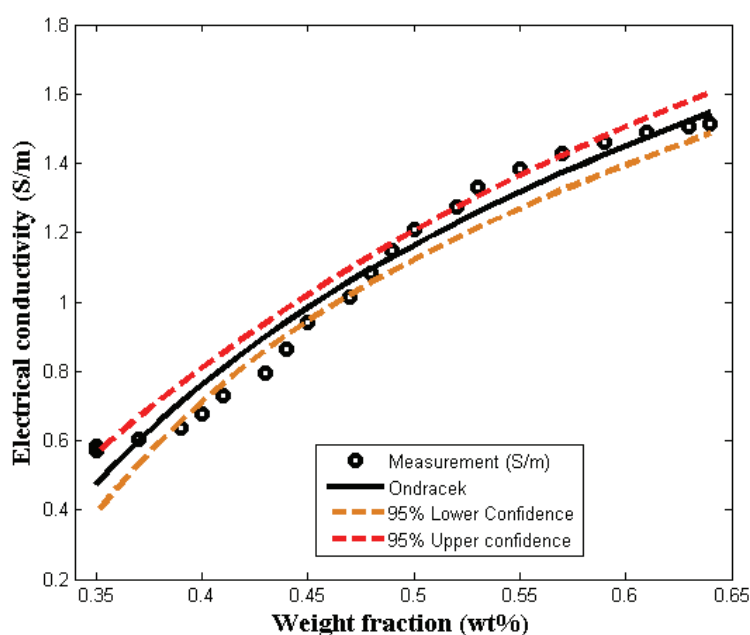


Figure 3. Comparing the linearized Ondracek model (LOM) with experimental measurement.

Table 1. Linearized Ondracek model performance data.

Model	Parameters	Parameter Values	Standard Error	Per-Unit Standard Error	R ²	R ² -adj
Ondracek	χ_1	8.51	0.27	0.03	0.967	0.962
	χ_2	−2.11	0.21	0.09		

The electrical conductivity of polymer-composite usually depicts an s -like shape, which can be segmented into before and after percolation threshold, and saturation regions. These regions often form s -shapes like graphs for most polymer-composite electrical conductivity. As shown in Figure 4, the comparison of PVA/PPy/rGO nanocomposite electrical conductivity data with the Dalmas s -shape model, is elucidated. In Equation (6), the Dalmas s -shape equation having σ_1 as the matrix conductivity and σ_2 as the saturation conductivity of the composite is presented. According to the literature, the fitting parameters, a and b , account for the effects of aspect ratio and shape factor. The aspect ratio and shape factor determine the point at which the polymer-composite changes from linear conductivity to the point of saturation [36]. As shown in Figure 4, the Dalmas s -shape model fits the experimental data well, and it was able to trace all the conductivity regions of the composite. The accuracy of the prediction can be found in Table 2. From the results presented in Table 2, it can be asserted that the model is in good agreement with the experimental data. Provided by Equation (11) is the Dalmas s -shape equivalent electrical

conductivity predictive model for PVA/PPy/rGO nanocomposite. This model can be generalized to predict or determine the electrical conductivity of polymer-composites other than PVA/PPy/rGO nanocomposite. The model is simple, unique, and versatile, and it can be deployed into computer system application for the prediction of the electrical conductivity of polymer-composites.

$$\sigma = 0.5 + \frac{1.03}{1 - e^{-22.37x+10.46}} \quad (11)$$

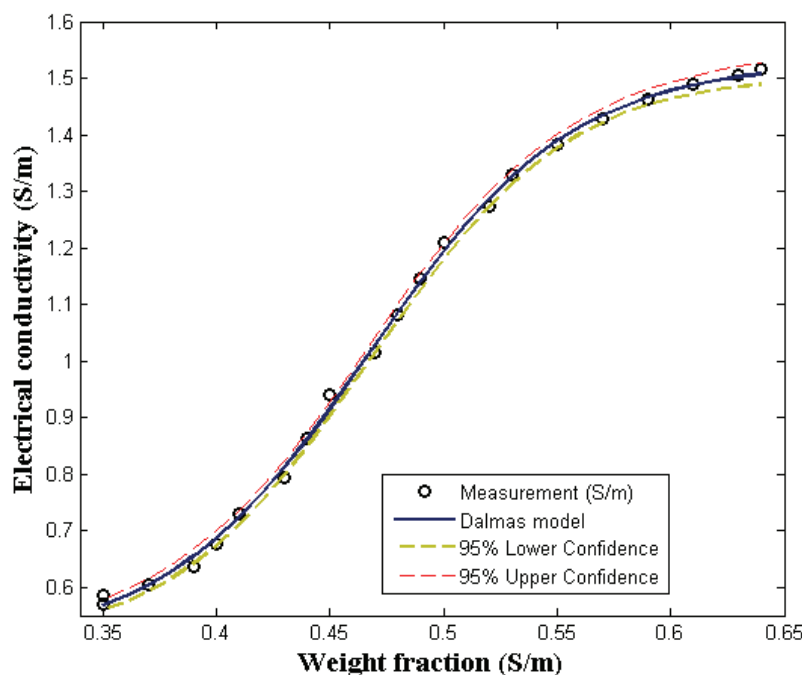


Figure 4. Comparing the Dalmas s-shape model (DsM) with experimental measurement.

Table 2. Dalmas s-shape performance data.

Model	Parameters	Parameter Values	Standard Error	Per-Unit Standard Error	R ²	R ² -adj
Dalmas s-shape	σ_2	1.02	0.008	0.007	0.9989	0.9988
	a	22.37	0.462	0.021		
	b	10.46	0.203	0.019		

Figure 5 compares the dose–response model with the experimental data. The initial/maximum conductivity, orientation factor, and aspect ratio are the parameters considered in the model. In the determination of the electrical conductivity of polymer-composite, the effect of the aspect ratio and orientation is inverse proportionality to percolation threshold. As it is shown in Figure 5, the calculated value for the percolation threshold is the same with the experimental value. The model agrees with the experimental data. This is evidence that a dose–response model can be effectively applied to predict and characterize the electrical behavior of polymer-composite [27]. In addition, Table 3 provides the performance and accuracy of the dose–response model considered in this study. In order to further simplify the model for the prediction of the electrical conductivity of PVA/PPy/rGO nanocomposite, Equation (12) is as presented. The correlation between the experimental data and the dose–response model as applied to the present study is

an indication that the model can be generalized for the prediction of polymer-composite electrical conductivity of any type.

$$\sigma = 0.5 + \frac{1.07}{1 + 7.40e^{9.79 \log x}} \quad (12)$$

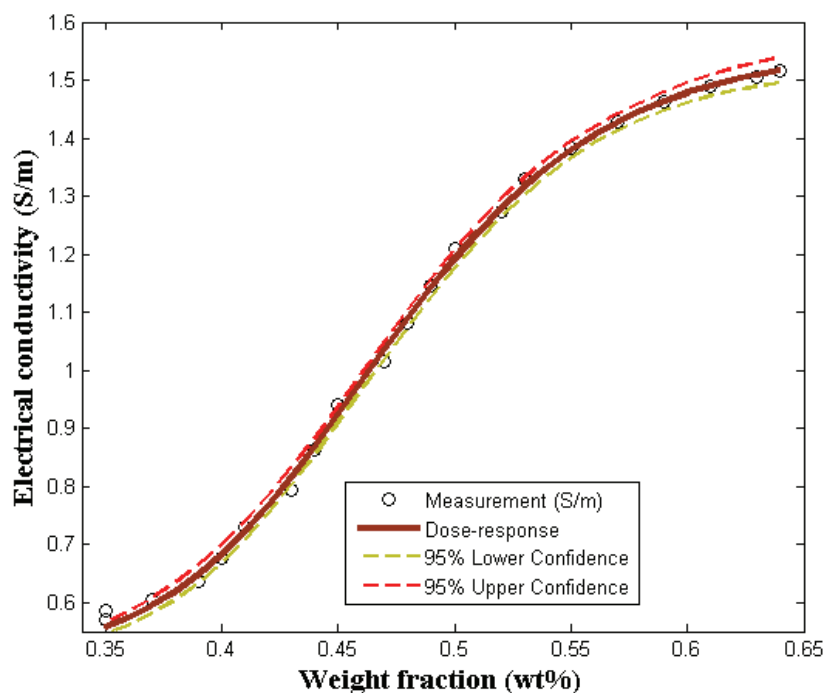


Figure 5. Comparing the dose-response model with experimental measurement.

Table 3. Dose-response performance data.

Model	Parameters	Parameter Values	Standard Error	Per-Unit Standard Error	R ²	R ² -adj
Dose-response	$\sigma_2 - \sigma_1$	1.07	0.01	0.01	0.9986	0.9984
	c	−9.79	0.24	0.03		
	$e^{-c \log(d)}$	7.40	0.21	0.03		

Moreover, according to the experimental and the Gaussian model results shown in Figure 6, respectively, the electrical conductivity of polymer-composites depends largely on the quantity of fillers in the matrix [34]. The Gaussian model is a fascinating and simple model, which is suitable for the prediction and classification of random data. At micro-scale, polymer-composites are heterogeneous; therefore, their data can be classified as random data [37]. From the results shown in Figure 6, it can be seen that the Gaussian model is appropriate for the prediction of the electrical conductivity of polymer-composites. However, for $n = 1$ (Figure 6a), the model was moderately adequate to predict the percolation threshold of PVA/PPy/rGO nanocomposite. Figure 6b clearly showed that at $n = 2$, the model accurately predicts the nanocomposite conductivity. Moreover, Tables 4 and 5 give the performance and accuracy of the model. Equations (12) and (13) are the Gaussian predictive models developed for the study experimental data.

$$\sigma = 1.52e^{-\left(\frac{x-0.64}{0.28}\right)^2} \quad n = 1 \quad (13)$$

$$\sigma = 0.138e^{-\left(\frac{x-0.346}{0.032}\right)^2} + 1.505e^{-\left(\frac{x-0.62}{-0.244}\right)^2} \quad n = 2 \quad (14)$$

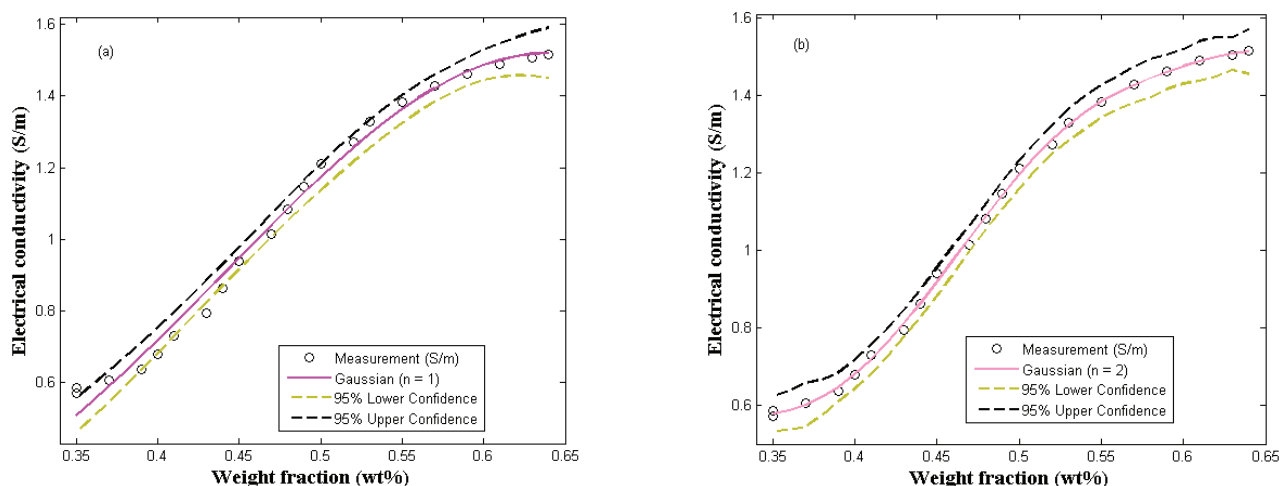


Figure 6. Comparing the Gaussian model with experimental measurement (a) $n = 1$ (b) $n = 2$.

Table 4. Gaussian fitting performance data ($n = 1$).

Model	Parameters	Parameter Values	Standard Error	Per-Unit Standard Error	R ²	R ² -adj
Gaussian	σ_1	1.52	0.024	0.02	0.9989	0.9902
	k_1	0.64	0.011	0.02		
	z_1	0.28	0.012	0.04		

Table 5. Gaussian fitting performance data ($n = 2$).

Model	Parameters	Parameter Values	Standard Error	Per-Unit Standard Error	R ²	R ² -adj
Gaussian	σ_1	0.138	0.033	0.236	0.9983	0.9975
	k_1	0.346	0.019	0.056		
	z_1	0.032	0.019	0.578		
	σ_2	1.505	0.008	0.005		
	k_2	0.620	0.004	0.007		
	z_2	−0.244	0.007	0.027		

3. Materials and Methods

The graphene (rGO) used was obtained from the CeNam-CSIR, Pretoria, South Africa. The polypyrrole with CAS No: 30604-81-0; solid, 20 wt% carbon content loading and >300 °C melting point, was purchased from Sigma Aldrich, Gauteng, South Africa. Polyvinyl alcohol with CAS No: 9002-89-5 was purchased from Sigma Aldrich, South Africa. A $\overline{M}_w$ of between 146,000–186,000, 99+% hydrolyzed, hydrophilic, and crystalline power, are the properties of the polyvinyl alcohol. Moreover, acetone was purchased from Sigma Aldrich and deionized water was sourced from the CSIR, Pretoria laboratory. Refer to [19] for the electrical conductivity measurement method.

Fabrication Method

The fabrication of PVA/PPy/rGO nanocomposite was achieved by solvent blending/drying method. The first sample was produced by the following procedures. A total of 300 mg of PVA was measured into a beaker containing 20 mL of deionize water, and it was magnetically stirred for 2 h. Secondly, a 100 mg of rGO was dispersed in 300 mL of acetone/deionized water and ultra-sonicated for 1/3 h. In addition, 100 mg of PPy was added to the rGO solution and further ultra-sonicated for 1/3 hr. The ultra-sonicated mixture of PPy/rGO was loaded onto the dissolved PVA (300 mg) and rigorously stirred for more than 12 h. The resultant solution of the nanocomposite was washed by using

acetone and deionize water. Moreover, the nanocomposite was vacuum-dried at 60 °C for 12 h. Moreover, the preparation of the other samples followed the same process; the PVA content was kept constant, while PPy and rGO contents were varied.

4. Conclusions

Herein, the electrical conductivity of PVA/PPy/rGO nanocomposite was experimentally and mathematically studied. The concept adopted in this research can usher in new insights into the development of highly efficient energy storage electrodes. The mathematical equations employed for the conductivity characterization of PVA/PPy/rGO nanocomposite showed results that correlate with the experimental measurements. The Gaussian, Dalmas s-shape, and dose-response models showed results, which are closer to the experimental measurement. However, the limitation of the models is that initialization of values for the dependent parameters is very difficult. Further studies would endeavor to provide more insights into how these models can be effectively used to characterize the electrical properties of polymer-composites, more accurately.

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Various Applications of ZnO Thin Films Obtained by Chemical Routes in the Last Decade

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Abstract: This review addresses the importance of Zn for obtaining multifunctional materials with interesting properties by following certain preparation strategies: choosing the appropriate synthesis route, doping and co-doping of ZnO films to achieve conductive oxide materials with p- or n-type conductivity, and finally adding polymers in the oxide systems for piezoelectricity enhancement. We mainly followed the results of studies of the last ten years through chemical routes, especially by sol-gel and hydrothermal synthesis. Zinc is an essential element that has a special importance for developing multifunctional materials with various applications. ZnO can be used for the deposition of thin films or for obtaining mixed layers by combining ZnO with other oxides (ZnO-SnO₂, ZnO-CuO). Also, composite films can be achieved by mixing ZnO with polymers. It can be doped with metals (Li, Na, Mg, Al) or non-metals (B, N, P). Zn is easily incorporated in a matrix and therefore it can be used as a dopant for other oxidic materials, such as: ITO, CuO, BiFeO₃, and NiO. ZnO can be very useful as a seed layer, for good adherence of the main layer to the substrate, generating nucleation sites for nanowires growth. Thanks to its interesting properties, ZnO is a material with multiple applications in various fields: sensing technology, piezoelectric devices, transparent conductive oxides, solar cells, and photoluminescence applications. Its versatility is the main message of this review.

Keywords: ZnO thin films; sol-gel; seed layer; doping; nanostructures; composite materials

1. Introduction

Zinc oxide is a versatile material with a wide range of applications in different fields such as chemistry, materials science, biology and nanotechnology due to its simple and environmentally friendly synthesis, biocompatibility, and high chemical stability [1]. Over time, as a result of their impressive properties, involving a wide band gap of 3.37 eV, exceptional electron mobility (1 to 200 cm²/Vs), and an exciton binding energy of 60 meV [2,3], ZnO has been comprehensively explored in different forms for certain applications [4]. Thus, depending on the synthesis methods, ZnO can be obtained as: bulk [5,6], nanostructures [7,8], thin films, or hybrid materials [9,10]. The properties of ZnO-based materials can be tailored and enhanced by controlling and optimizing several parameters (solution concentration [11], dopant level [12,13], synthesis [14–17], and annealing temperature [18] or pH [19]). In the last decade, the development of nanostructures with various morphologies [20–27] such as: nanowires, nanorods (NR), nanoflowers, nanosheets, nanobelts, nanoneedles, nanoplates, has gained tremendous attention, being used in biological applications [8,28–34]: bioimaging, biosensing, antibacterial and drug delivery agents. In particular, ZnO thin films have optical (photoluminescence) [33,35–42], electrical (thermoelectric [43–46], piezoelectric [47–53]) and biological (antimicrobial [54–56], antibacterial [57–59]) characteristics, which make them excellent candidates for the development of optoelectronic [60–65], piezoelectric [66–68], transparent conductive oxides (TCO) devices [69–71], ultraviolet (UV) photodetectors [13,72,73], solar cells [74–78], photocatalysts [79,80], gas sensors [81–90] or biosensors [91–93]. The previous papers have discussed ZnO films with varying morphologies (e.g., nano-particles or nanorods) that were grown on a wide range of substrates, using

different chemical methods for different applications. In 2022 alone, at least nine reviews were published on ZnO, suggesting the topicality of a review about materials with Zn in various combinations and forms.

To illustrate the continuous progress and growing research interest in ZnO, a graph depicting the annual number of publications on ZnO and ZnO thin films from 1980 to 2023 (Figure 1) was included, obtained with SCOPUS. This graph highlights a significant growth in the publication numbers between the years 2000 and 2015. The inset in Figure 1 shows a constant interest in this topic, for the last decade.

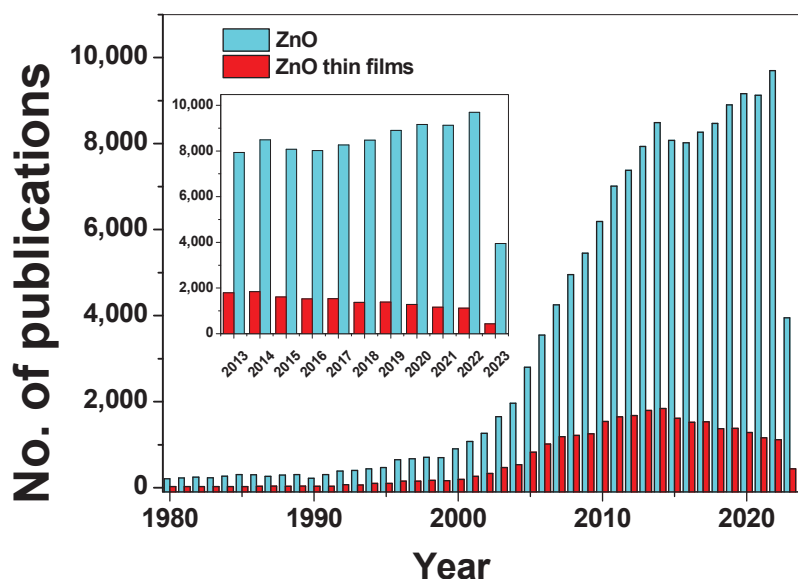


Figure 1. Evolution of the number of scientific papers related to the search of “ZnO” and “ZnO thin films” phrases published between 1980–2023. Inset: illustration of the last decade. Source: Scopus (accessed on 12 May 2023).

The novel viewpoint of this review is that it starts from ZnO nanoparticles (NPs), continuing with mono- and mixed ZnO layers, covering doped and codoped ZnO, and finally, considering Zn as a dopant by itself.

2. Preparation Methods of ZnO Films

Over the years, various physical and chemical methods have been used to prepare ZnO films. In this chapter, we focus on the chemical methods and highlight some unique aspects based on recent literature findings. Some of the best-known chemical methods for ZnO film preparation include sol-gel (SG) (using dip-coating and spin-coating for deposition), hydrothermal (HT), chemical bath deposition (CBD), and successive ionic layer adsorption and reaction (SILAR).

2.1. Sol-Gel Synthesis

The SG technique is one of the most popular deposition methods, extensively used in the last years to prepare inexpensive ZnO thin films. A graphic illustration (Figure 2) of the SG solution preparation, the spin-coating deposition of a seed-layer, and the HT growth of ZnO nanorods is presented in Ref. [94].

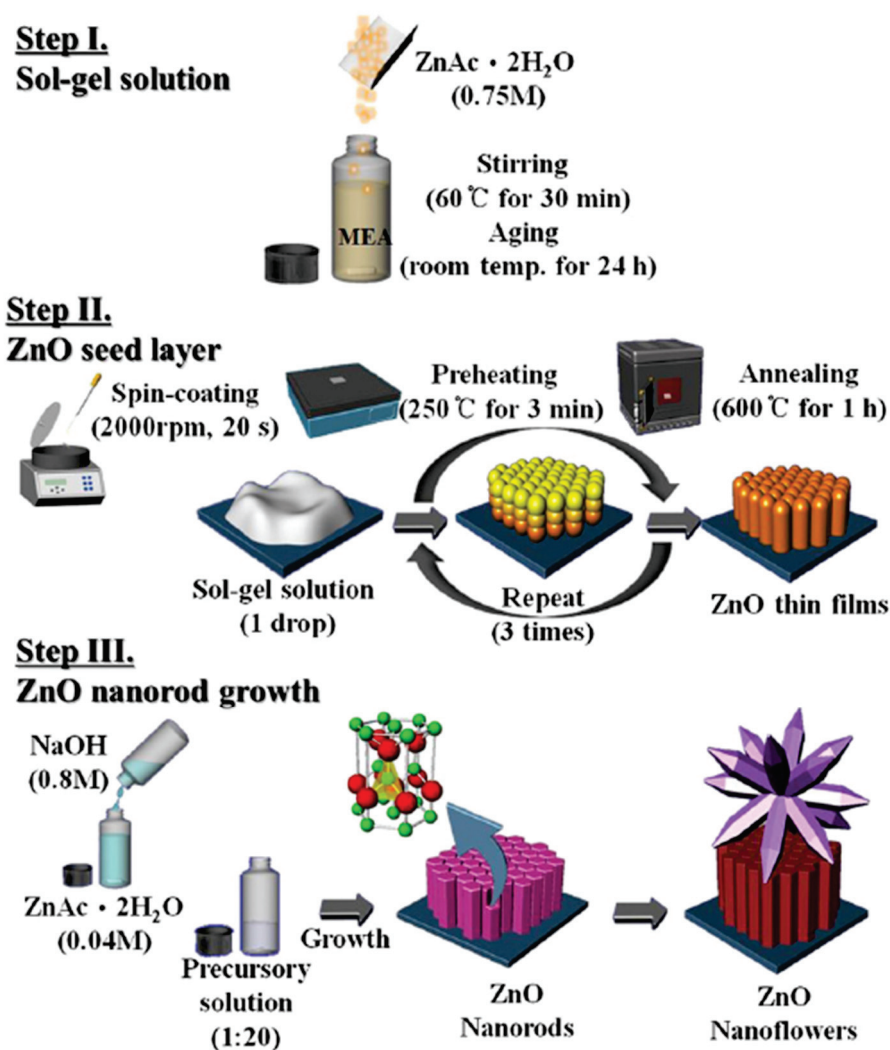


Figure 2. Graphic illustration of sol-gel solution preparation, ZnO seed layer deposition and ZnO nanorod growth. Reprinted from [94] with permission from Elsevier.

The synthesis conditions of the SG method can modify the film surface and at the same time, they can improve the catalytic and chemical sensing properties of the film. The variation of precursor concentrations is among the best-known procedures to obtain the desired properties.

Other factors that may influence the final properties of the films are the microwave treatment of the sol [95] or the polymer modifiers [96–99]. The application of microwave heating in the SG method is a good way to reduce the preparation time and to obtain nanostructured films at lower temperatures [96]. On the other hand, hydroxypropyl cellulose (HPC) or ethylcellulose (EC) added into the sol-gel precursor solution led to high photocatalytic activity, high chemical sensitivity [96] and the control of the particle size [97] and film porosity [98].

Shankar [99] showed that adding the monomer of the polymer poly(vinyl alcohol) (PVA) along with dehydrated zinc acetate led to an enhanced carrier concentration in ZnO nanorods and in turn increased the sensitivity to ethanol detection at room temperature.

Jang [100] proved that the modification of ZnO with siloxane polymers increased the sensitivity of ethanol detection at room temperature.

Significantly enhanced charge transport characteristics necessary in sensor applications were obtained using conjugated polymer (CP) semiconductors [101], contributing to the next generation of high-performance organic field-effect transistor (OFET) sensors.

2.2. Hydrothermal Method

The hydrothermal method is usually performed to grow ZnO NRs in an autoclave, from a water-based solution at specific conditions of high pressure and temperature. A previously deposited SL on the substrate induces the formation and the growth of NRs with a specific morphology [94,102–104].

The growth characteristics of nanorods and nanowires are as many as their use in specific applications [105–108], with requirements regarding the density, length, and thickness of the nanowires. These requirements can be met by varying the temperature (higher temperatures generally lead to faster growth rates), the pressure (the use of a low-pressure can result in the growth of long and thin nanowires), the growth time (affecting the length of the resulting nanorods or nanowires), the number of deposition cycles, etc.

2.3. Chemical Bath Deposition

Chemical Bath Deposition is a process used to deposit thin films onto a substrate which involves the use of a chemical solution that contains precursors for the desired material [109–112]. The substrate is immersed in the solution and a reaction occurs at the surface of the substrate, resulting in the deposition of a thin film. Figure 3 [113] illustrates the step-by-step process of ZnO thin film formation using the CBD technique.

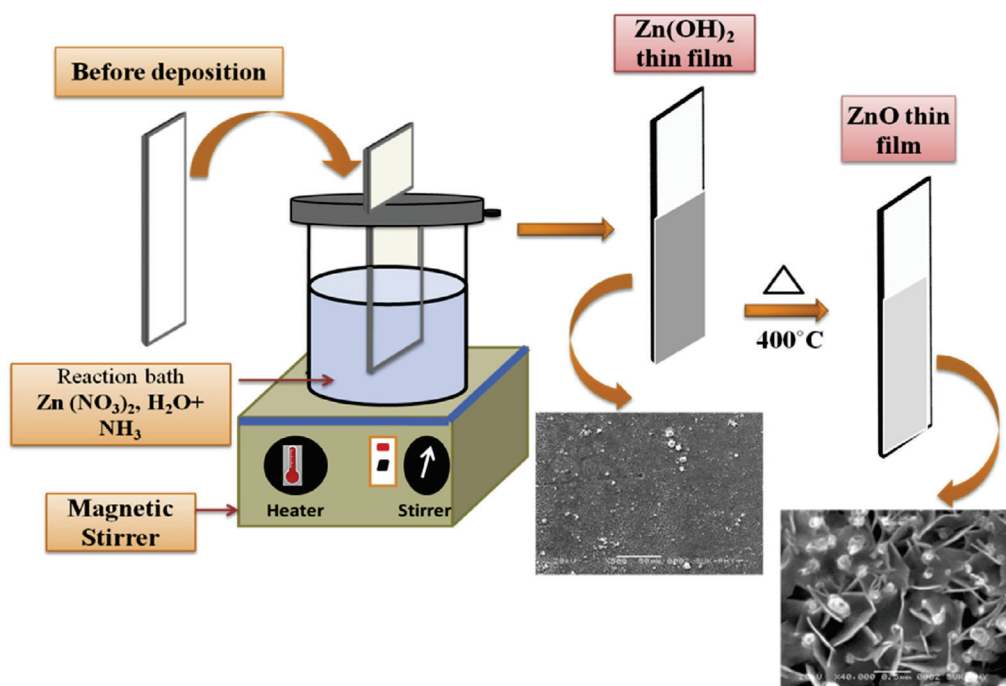


Figure 3. Schematic diagram of CBD technique used to obtain ZnO thin films. Reprinted from [113] with permission from Elsevier.

The CBD process offers several advantages, including low cost, ease of use, and the ability to deposit films on a wide range of substrates, including plastics and glass. The process can also be used to deposit films with controllable thickness and composition, making it useful for a variety of applications, including solar cells, sensors, and optoelectronics.

2.4. Successive Ionic Layer Adsorption and Reaction

The successive ionic layer adsorption and reaction deposition method was first described by Nicolau [114] and Ristov et al. [115]. It is an ion-by-ion deposition at room temperature, which is conducted by alternately immersing the substrate, first in the cation solution and then in the anion solution of a given compound, each step being followed by the rinsing of the excess solution with deionized water. A schematic diagram of this process

is presented in Figure 4 [116]. The thickness and the morphology are better controlled than in other related methods, such as CBD [117]. The thickness is monitored using the number of successive immersions, as well as the concentration and the type of the reactant precursors, tuning the thickness from the nm to μm range, depending on their intended use.

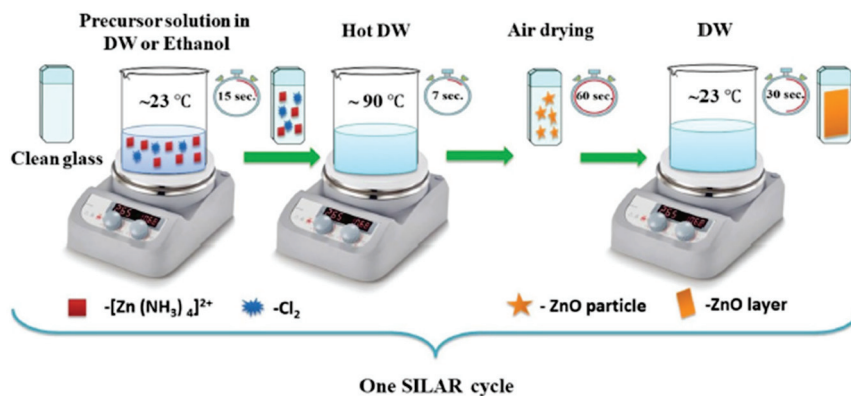


Figure 4. Graphical representation of SILAR method used to obtain ZnO thin films. Reprinted with permission from [116]. Copyright 2022, Yergaliuly et al.

Among other precursors, zinc chloride is proven to be the most suitable precursor for Al-doped ZnO thin films [118], with good morphological, optical, and electrical properties, for applications in optoelectronic devices.

The number of SILAR cycles plays a major role in obtaining a texture suitable for photoelectrochemical applications [119], with a preferential orientation along the (002) plane. With the increase of the deposition cycles from 60 to 120, a more compact growth of the nanorods was observed, which can be explained by the formation of more nucleation centers on the substrate. A second increase from 120 to 180 cycles leads to an agglomerated growth with a deviation from vertical shape, with a negative impact on the photoelectrochemical performance.

2.5. Other Chemical Methods: Spray Pyrolysis, Inkjet Printing, Chemical Vapor Deposition (CVD)

Another chemical method is spray pyrolysis [120–122], popular for its low-cost results, in which the precursor solution is deposited on a heated substrate using a high-velocity gas carrier. The film thickness and properties can be controlled by adjusting the concentration of the precursor solution, the spray rate, and the substrate temperature.

Spray pyrolysis was used to obtain ZnO thin films. The observed increase of the bandgap energy is a result of the ZnO SL presence, which also induces changes in the morphology from flower-like ($\sim 6 \mu\text{m}$ diameter) for samples without SL to horizontal nanorods ($\sim 0.8 \mu\text{m}$ length) in the presence of the SL [123]. This method was also used to obtain undoped and Mg-doped ZnO films on indium tin oxide (ITO) SL [124]. The choice of a different material for the SL had, as a result, the selective nucleation and further growth of ZnO grains which exhibited a good H_2 sensing response, even more, improved by doping ZnO with Mg.

This method is a relatively simple and cost-effective process used to deposit a wide range of materials, such as oxides, nitrides, and metals, for various applications, including sensors, photovoltaics, and catalysis.

A variation of the spray pyrolysis method is the Inkjet printing, where using jets with different pressures, the material is deposited on previously designed shapes [125–128]. The inkjet printing technique offers several advantages for ZnO thin film deposition. It allows for precise control of the thickness and morphology of the film, as well as the ability to pattern the film with high spatial resolution. It is also a low-cost method that can be used to obtain ZnO thin films on a variety of substrates, including flexible ones. Inkjet printing

is a promising technique for the fabrication of ZnO thin films for different applications, such as solar cells, sensors, and electronic devices.

The CVD method is used to grow different films on a substrate using a chemical reaction between vapor phase reactants [129,130]. The CVD method allows for the deposition of high-purity films with exceptional uniformity over large areas and enables the growth of complex structures (nanowires, thin films, multilayers, etc.), with precise control over composition, thickness, and orientation [131–133].

In summary, there are several effective methods for preparing ZnO films, including SG, CVD, spray pyrolysis, etc., each with its advantages and disadvantages depending on the desired film properties and application requirements.

3. ZnO as Seed Layer (SL)

In this chapter, a brief overview of the importance of SL is emphasized, together with some applications in which the SL is essential in tuning the final material properties.

An SL is typically defined in the literature as a very thin layer deposited on different substrates to further assist the growth of specific nanostructures, such as nanorods, nanowires, nanosheets, etc. The ZnO SL quality is critical, because it plays a significant role in inducing a preferential orientation of the ZnO films grown on its surface, and at the same time it influences their morphology, diameter, and crystallinity.

The deposition of a SL is also important in obtaining a good adherence of a subsequent layer to the substrate. In various applications, such as piezoelectric materials, for example, the nucleation sites of a SL offer the possibility to grow nanowires of different dimensions and directions, depending on the SL thickness.

Especially in the hydrothermal method (HT), a SL is necessary before growing 2D structures. This technique was successfully used to obtain columnar ZnO grains of 1300–1700 nm thickness and 80–160 nm in diameter, grown from a ZnO SL deposited by the SG method [47]. Figure 5 shows (a) the bright-field (BF) transmission electron microscopy image and (b) the high-angle annular dark-field (HAADF) image of ZnO NWs film grown on Au/Ti/SiO₂/Si substrate by HT method [47].

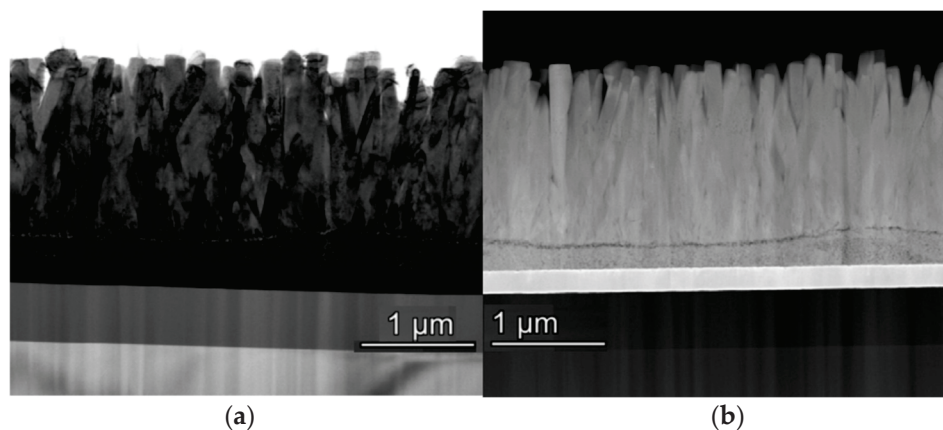


Figure 5. BF TEM (a) and HAADF images from ZnO NWs (b) on Au substrate. Reprinted from [47] with permission from Elsevier.

Good quality ZnO SL is also used to reduce the lattice mismatch between a substrate and the layers to be grown on it, developing vertically aligned ZnO nanorods with a good density and crystallinity control [134,135].

Many works have investigated the effect of deposition parameters on the initial SL in obtaining vertically oriented nanorods. The SL quality is highly influenced by the preheating temperature and annealing conditions. The preheating stage is the thermal treatment performed after each deposition on a multi-layer SG film and its temperature varies depending on the different precursor solutions used in the deposition process.

As shown in [136], a preheating temperature of 400 °C is necessary to decompose the organics by-products and to obtain vertically aligned ZnO nanorods, while in [137] it is stated that the preheating process is providing the necessary energy for the nucleation and growth kinetics of the ZnO film, directly affecting the crystalline quality of the final film. The influence of different preheating temperatures on the growth of vertical nanorods is studied in [135,138] and in this case, the best nanorods alignment along the c-axis was found in the samples with a SL obtained at 350 °C preheating temperature.

In other words, the effect of ZnO SL annealing temperature on the growth of ZnO nanorods has been studied. A higher temperature (500 °C) led to an increase in the diameter and length of ZnO NR, due to the larger size of the SL grains [139]. UV sensors were produced based on SLs obtained at different annealing temperatures (100 ÷ 500 °C) [140] with the highest response for the sample annealed at 500 °C.

An increase from one to five successively deposited ZnO layers results in a thicker and denser SL on the substrate, when using the SG spin coating technique [141]. In the case of the CBD method, the increase of the number of adsorbed atoms provides a larger growth area for ZnO nanowires, and the highest density and rod-like structure of ZnO nanowires is obtained for the sample with five depositions of ZnO SL [141]. When ZnO nanowires were grown on the thinner SL, they had a flower-like structure [141].

Even though most thermal treatments are performed to improve the final properties of the samples, the annealing of the ZnO SL was reported in some cases to have a negative impact on the device performances, in applications such as NO sensors [142]. The annealing process enabled a larger diameter of the nanorods and lower porosity and, consequently a few percent decrease in gas sensing response in comparison with the as-deposited ZnO nanorods.

Banari et al. [108] have studied the UV photodetection properties of ZnO nanorods grown by two-step (spin coating and hydrothermal) method on SLs, with thickness varying in the range of 50–125 nm. Depending on the successive number of spin-coating depositions (3, 5, 7, and 9), increasing the SL thickness first leads to an increase in the carrier concentration from three to five layers, followed by a decrease in the last two cases. The high value of the carrier concentration was assigned to the surface defects and oxygen vacancies in the ZnO films.

In summary, the SL is a critical factor as it affects the nucleation, diameter, length, and uniformity of the resulting nanostructures.

4. ZnO in Composite Thin Films

The possible growth directions and the morphologies of ZnO (1D, 2D and 3D) are illustrated in Figure 6 [143]. In this chapter, we focus on different double-layer composites, with respect to the overall improvement of the composite thin films' properties.

There are different combinations of double layers that contain ZnO and are efficient in the development of many applications. Such examples are presented in Table 1 for sensor applications.

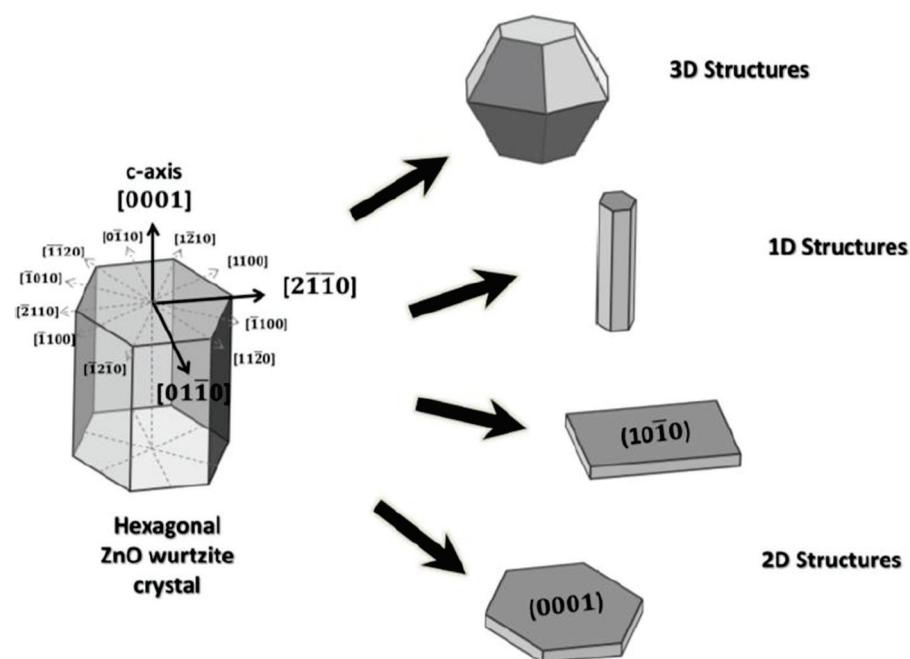


Figure 6. The growth directions of ZnO wurtzite crystal and possible morphologies. Reprinted from [143]. Copyright 2017, Leonardi, S.

Table 1. ZnO in composite layers deposited by chemical methods for sensor applications.

No.	Hetero-Structures	Method	Year	Sensor Application	Main Results	Ref.
1	ZnO-NiO	HT	2019	H ₂ S	The performance of the gas sensor toward H ₂ S was significantly improved after the formation of NiO/ZnO heterostructures.	[144]
2		HT	2020	VOC	Selective VOCs sensors based on NiO/ZnO p-n heterojunction diode for 2-propanol, toluene, and formaldehyde vapors detection can be attained by controlling the applied voltage. An advantage of this diode is the ability to modify the forward bias voltage, tailoring the number of carriers implied in the sensing process. A higher forward voltage leads to the increase of the O ⁻ adsorbates that exist on the ZnO surface.	[145]
3		HT	2020	NH ₃	The improvement of gas sensing properties could be assigned to the hierarchical structure which leads to a better adsorption of gas molecules and also the formation of n-ZnO/p-NiO heterojunction.	[146]
4		HT	2022	H ₂ CO	The detection of formaldehyde at low temperatures was improved by the formation of ZnO/NiO heterostructures with high porosity which promotes the adsorption of gas molecules on the surface.	[147]
5	CdO-ZnO	HT	2021	H ₂ CO	The gas sensing measurements highlighted an improved response of CdO-ZnO nanorices structures towards formaldehyde gas sensing, compared to the ZnO nanoflowers.	[148]

Table 1. Cont.

No.	Hetero-Structures	Method	Year	Sensor Application	Main Results	Ref.
6	ZnO-CuO	SG	2018	H ₂ CO	The gas sensing properties of the ZnO sensor can be enhanced through CuO addition to creating a CuO/ZnO heterojunction. The experimental results proved that the CuO/ZnO-based sensor exhibits exceptional selectivity and sensitivity for room temperature formaldehyde detection.	[149]
7	ZnO-graphene	SG	2018	NO ₂	G-ZnO composite thin films act as selective sensors for NO ₂ detection at low temperature, the superior capabilities being due to the concomitant adsorption of NO ₂ gas and molecular oxygen on the graphene and ZnO surfaces.	[150]
8		SG	2021	NO ₂	The hybrid materials based on ZnO/graphene heterostructures improve gas detection sensitivity at low temperatures due to the combination between the specific properties of ZnO and graphene.	[151]
9		Reflux method	2018	CO	The rGO-ZnO composites enhance the sensor performance, in terms of reducing the working temperatures for CO gas detection.	[152]
10		SG	2016	CO	The ZnO-SnO ₂ composite materials with different content of SnO ₂ selectively detect the CO gas.	[153]
11	ZnO-SnO ₂	HT	2019	C ₂ H ₅ OH	The SnO ₂ /ZnO heterostructures show a higher gas sensing response in contrast with the ZnO nanorods. The formation of SnO ₂ /ZnO heterojunction may be responsible for the improved performance of the sensors.	[154]
12		Spray pyrolysis	2019		The Zn:Sn molar ratio has an important role in the morphology of the nanostructures, the best gas sensing results being obtained in the case of a higher content of ZnO nanorods. Thus, a better sensitivity was found in the films with higher amounts of ZnO, due to their higher crystallinity.	[155]
13	ZnO-Cr ₂ O ₃	Two-step chemical route	2018	H ₂ CO	The gas sensing measurement showed that the Cr ₂ O ₃ -ZnO heterostructures exhibit excellent gas sensing properties for formaldehyde, which can be assigned to the formation/presence of hierarchical structures.	[156]

ZnO-graphene is an interesting and promising combination. Graphene and its derivatives present nonlinear optical properties (NLO), with high absorption and dispersion properties, with the ultrafast optical response, which makes them suitable for the application of the mode-locked lasers [157]. ZnO has strong second and third-order nonlinear optical features [158,159]. Sreeja et al. [160] showed that adding reduced graphene oxide (rGO) to the AZO layers leads to an increase in the absorption and decrease of the optical band gap, an effect that intensifies with the increase of the rGO concentration. The increase in the percentage of rGO from 2 to 6 wt.% leads to an enhanced formation of sheet-like structures in rGO, which subsequently merge with agglomerated ZnO particles, as could be seen in Figure 7 [160].

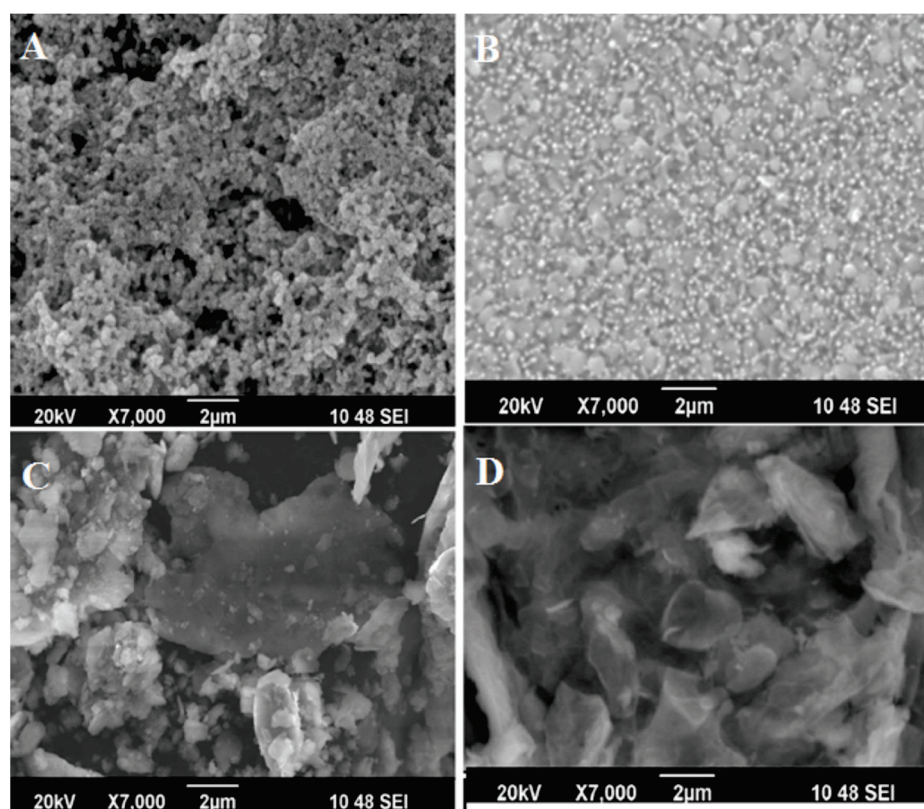


Figure 7. SEM images of (A) AZO, (B) AZO:rGO (2 wt.%), (C) AZO:rGO (4 wt.%), and (D) AZO:rGO (6 wt.%) composite thin films. Reprinted from [160] with permission from Elsevier.

ZnO-graphene is also used in gas sensors [161,162] because it improves their selectivity, shortens the recovery time, and operates at lower temperatures [162]. At the same time, *this combination* managed to mitigate electromagnetic radiation (with around 30 dBs) in the domain of 10–20 GHz [163], the attenuation being a function of the ZnO/graphene nanoplatelets ratio and of the frequency employed.

ZnO-polymer can form very efficient piezoelectric coatings on flexible metallic substrates. Chelu et al. [47] showed that a vertical ZnO nanowires (ZnO NWs) array grown on flexible Ti substrate by the hydrothermal method at low temperature and covered with a layer of poly(methyl methacrylate) (PMMA) leads to high values, above 120 pC/N, of the piezoelectric coefficient d_{33} . With such values, it is possible to foresee that PMMA/(ZnO NWs)/Ti nanostructures open the way towards integration in wireless or defense technologies and in wearable or implantable biomedical systems as efficient harvesters.

Gen-Wen Hsieh et al. [164] demonstrated that the composite dielectric film of poly(dimethylsiloxane)-PDMS-elastomeric silicone and zinc oxide tetrapod display remarkable sensing performance in the capacitance change and a pressure sensitivity of 2.55 kPa^{-1} over that of pristine polymer sensors, enabling a minimum detectable pressure of only 1.0 Pa. At the same time the composite has a rapid response and reliable sensing stability for over 1000 cycles. By introducing stress-sensitive additives of zinc oxide nanostructures, PDMS-ZnO composites may provide the basis for potential applications in touch sensing, electronic skin and sensitive wearable healthcare devices.

Another example of the role of **ZnO-polymer** composites is their use in medicine in sutures, dermal fillers, or stents applications [165]. Venkatesh et al. [166] used polylactic acid (PLA) and polypropylene (PP) polymers in urinary stent applications. Their combination with ZnO increased the antibacterial properties and polymer degradation.

The combination of **ZnO-Poly**(butylene adipate-co-terephthalate) (PBAT) is very useful in mechanical, thermal, and biological activity for food packaging [167] showing superior antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. The tensile

strength in the nanocomposite film with 10 wt.% ZnO enhanced to 45.0 MPa compared to 37.9 MPa of pure PBAT film, as well as increased thermal stability due to the good dispersion of ZnO nanoparticles in the PBAT matrix.

Another application was recently presented by B.C. Kang [162], in which **ZnO NW, polymers, and carbon** were used together in Wearable Pressure/Touch Sensors Based on Hybrid Dielectric Composites of Zinc Oxide Nanowires (NWs)/Poly(dimethylsiloxane) (PDMS) and Flexible Electrodes of Immobilized Carbon Nanotube (CNT) Random Networks. The incorporation of ZnO NW into PDMS increased the sensitivity of the composite in low-pressure regions, from $1.32 \times 10^{-4} \text{ Pa}^{-1}$ to $8.77 \times 10^{-4} \text{ Pa}^{-1}$. This effect appears due to the enhancement of piezoelectricity induced by ZnO NW on flexible CNT electrodes.

Combining the high charge carrier mobility of ZnO with the good film-forming properties of the polymer, the ZnO-polyethyleneimine (PEI) composite layer served as a cathode buffer layer for organic and perovskite solar cells [161]. Power conversion efficiency of the composite is improved compared to that of each individual ingredient and the performance of the perovskite as a solar cell increases from 10.05% to 11.76%. Chen et al. [168] also used PEI for doping ZnO for an efficient electron transport layer (ETL) in solar cell applications. At 7 wt.% PEI, they obtained vertical transport and the power conversion efficiency improved to 4.6% from a value of 3.7% of the corresponding device with pristine ZnO.

Another category of examples is the class of multi-component films based on ZnO, such as p-CuS-ZnS/n-ZnO heterostructures, prepared by SG [169] which can produce non-toxic, stable UV photodiode with an excellent rectifying behavior and very fast response.

5. Zn as Dopant

Zn can be interesting not only as a basic element in the ZnO films but also as a dopant introduced in other oxidic films. In Table 2 the role of Zn as a dopant is exemplified for SG films, as pointed out by recent works.

Table 2. Effect of Zn doping on different oxides prepared by SG.

Year	Dopant Ions	Doped Oxide	Doping Effect	Ref.
2022	Co^{2+} , Cu^{2+} , Zn^{2+}	NiO	Changes in the NiO film color	[170]
2022	Zn^{2+}	MgO	Biosensors-detection of glucose level	[171]
2022	Zn^{2+}	ITO	Improved sensor response to CO_2 and TCO characteristics for solar cell	[172]
2022	Zn^{2+}	SrTiO_3	Good effect on the dielectric response	[173]
2022	Zn^{2+}	ITO	Optimized electrical conductivity and carrier density	[174]
2021	Zn^{2+}	NiO	Refractive index increase with the Zn concentration (1–5%)	[175]
2018	Zn^{2+}	BiFeO_3	Significant decrease of the leakage current of BiFeO_3 film at low electric fields.	[176]
2018	Zn^{2+}	CuO	Increasing band gap with Zn concentration	[177]
2017	Cu^{2+} , Zn^{2+} , Mn^{2+}	BiFeO_3	Considerably lower leakage currents in doped films compared with pure BFO film	[178]

The examples above can be expanded with films prepared by other methods than SG, such as: *Spray pyrolysis* [179–183]; *Chemical bath deposition* [184–186]; *thermally vacuum evaporation* [187], and others [188–190]. When oxide layers are doped with Zn, their concentration plays an important role in improving parameters in various applications. The optoelectronic devices and solar cells have a better performance due to the enhancement of

refractive index with the Zn concentration increase (1–5%) [175] and with the increase of smoothness of the surface morphology as the Zn wt.% grows to 10 wt.% [180].

In some cases, ZnO may have a positive effect only when it is in a small amount, while when its concentration is slightly higher, the effect is negative. Such a case is presented by Sheikh et al. [191] for Polyether block amide (PEBA) nanocomposites doped with ZnO. The effect of low ZnO ($\leq 0.5\%$) concentration on the thermal and mechanical properties of prepared PEBA/ZnO nanocomposite thin films is very good but with increasing the concentration to 1%, it weakens due to agglomeration of the nanoparticles.

6. Doped and Codoped ZnO Films; p-Type Conductivity

Through the SG method, it is relatively easy to prepare stable n-type ZnO, but it is very difficult to obtain a p-type material due to the generation of donor-type defects which compensate for the charge of acceptor dopants (self-compensation effect) and the low solubility of the acceptor dopant ions [192]. According to Li et al. [193], p-type ZnO is characterized by a low concentration and mobility of holes, making it unstable over time. This instability leads to the tendency of the p-type ZnO to revert to n-type at room temperature within a specific time interval.

Thus, undoped ZnO shows n-type conductivity due to intrinsic defects such as interstitial Zn and oxygen vacancies. By doping and co-doping, p-type ZnO can be obtained through three fundamental approaches: (a) replacing Zn with the group I and IB elements (Li, Na, K, Ag, Cu), (b) by doping with group V elements (N, P, As, Sb) which replace oxygen in the lattice or (c) co-doping with donors and acceptors (Li-Ni, In-N, Al-N, F-Ag) [193–195]. In recent years, group I elements have been reported to possess better dopant behavior than group V or group III elements in terms of acceptor, and donor level, respectively [196,197].

An extensive discussion on the ZnO band structure, the partial density of states (PDOS), and the lattice parameters are presented in [198] and illustrated in Figure 8a,b.

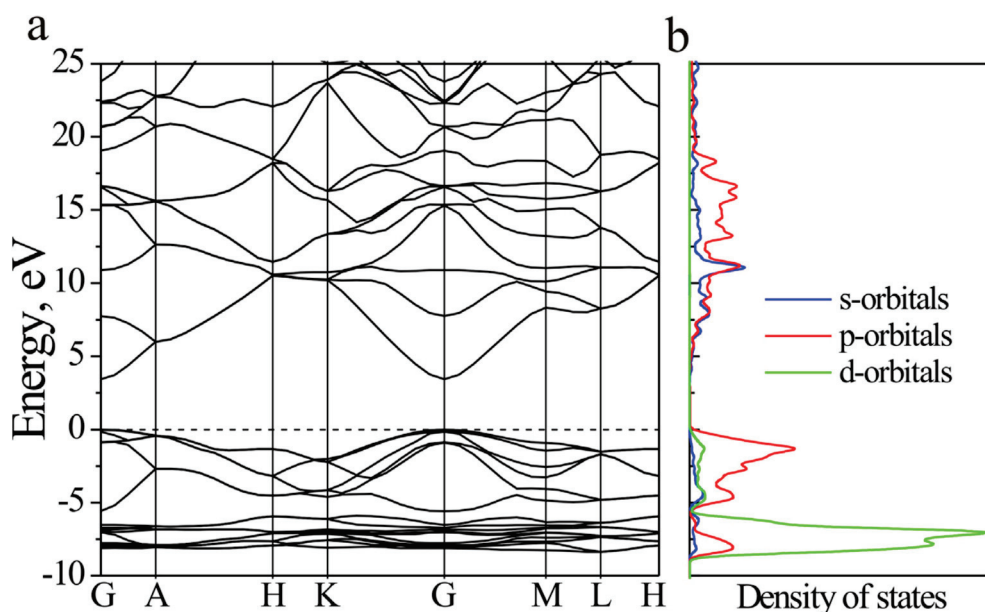


Figure 8. Band structure (a) and partial density of states (b) of ZnO. Reprinted from [198] with permission from Elsevier.

Li is considered the most suitable element from group I (Li, Na, K) to produce p-type ZnO modifying the strain effects and energy levels through the replacement of Zn with Li [199,200]. The reason for this is the small ionic radius of Li of 0.68 Å, which is very close to the ionic radius of Zn (0.74 Å). As a result, Li can occupy the Zn vacancy (V_{Zn}) and induce the desired effects [201–203].

According to [204,205], another option is to dope ZnO with Na ions (0.95 Å), which replace Zn ions (Na_{Zn}) and create a shallow acceptor state [205].

Also, it has been reported that apart from the ionic radius that can affect doping, the substrate material also plays a role in the doping of ZnO. For example, p-type behavior was thus obtained for Li-doped ZnO deposited on a silicon substrate, and n-type behavior was obtained for ZnO deposited on a glass substrate [199,203].

Maksimov [206] has reported that obtaining p-type ZnO materials via anion substitution, by replacing oxygen with other group VI elements (S, Se, Te) is used for the fabrication of photovoltaic devices [206,207].

ZnO has been doped with transition metals (such as Mn, Ni) to obtain dilute magnetic semiconductors (DMS) for applications in spintronic devices [199] and also has been doped with Ni or F elements to fabricate high-quality humidity sensors [208,209].

Co-doping of ZnO is reported to be feasible due to the strong attractive acceptor-donor interaction, which overcomes the repulsive interactions between the acceptors and leads to the formation of acceptor-donor-acceptor complexes [197].

In summary, based on the literature and the structural, morphological, elemental, optical, and electrical analysis, the p-type conductivity of ZnO (doped/co-doped) is attributed to the formation of an impurity band above the maximum of the valance band, resulting in a reduction of the band gap and a decrease in the energy of ionization of the acceptor (Li, Na, P, N) [194,199–201,205] respectively of the donor (Al, In, Ni) [196,199,207] highlighted in the spectroscopic analysis through the red shift in the UV emission [197,202].

The Raman spectra showed an increase in the intensity of phonon mode E1(LO) [197,200] which is associated with impurities and the formation of defects such as oxygen vacancies, demonstrating that the doping occurred.

A widening of the band-gap is usually observed for ZnO doped with donors, while a reduction of the band gap was noticed by doping with acceptors [197,199,205,207,210].

The change of the lattice parameters indicates that the dopant (in the form of ions) replaces the Zn ions and was incorporated into the ZnO lattice (confirmed by XRD, XRF, XPS measurements) [192,194,199,204,206,211].

The Hall, Seebeck or the current–voltage (I–V) measurements indicated that, usually, the undoped ZnO film exhibited n-type conductivity, while the doped ZnO films generally exhibited p-type conductivity with low carrier concentration [192–194,196,199,201,205,209–213].

Repeated measurements after a period of time (4–12 months) demonstrated that the conduction type is stable over time [199,211,212]. The p-type conductivity in thin films is generated by free O^{2-} vacancies and interstitial Zn atoms or substitution sites of Zn^{2+} ions.

In addition, the SG method [192,199,211,214] has proved to be an attractive technique for obtaining p-type ZnO films among others due to the low deposition costs compared to other more expensive methods such as RF magnetron sputtering [210,212] pulsed laser deposition [215] or spray pyrolysis [196,213].

The ionic character of elements used for doping ZnO is presented below in Table 3.

Table 3. The ionic character of some elements used for doping ZnO.

Ionic Character			
Acceptor	Donor		Acceptor-Donor
Li [197,199–203]	F [204,209,214]	Te [206,207]	Li-Ni [199]
Na [192,197,204,205]	Cl [214]	Ga [214,215]	Ga-N [213]
K [197]	Al [216,217]	Ni [199,208,217]	In-N [203]
N [193,194,212]	In [196]	Mn [218]	Al-N [210]
P [194]	S [206]		F-Ag [195]
Sn [215]	Se [206]		

7. Applications

In the present section, a special attention is given to the applications of ZnO thin films prepared by sol-gel and hydrothermal methods.

The versatility of the material is demonstrated in Figure 9.

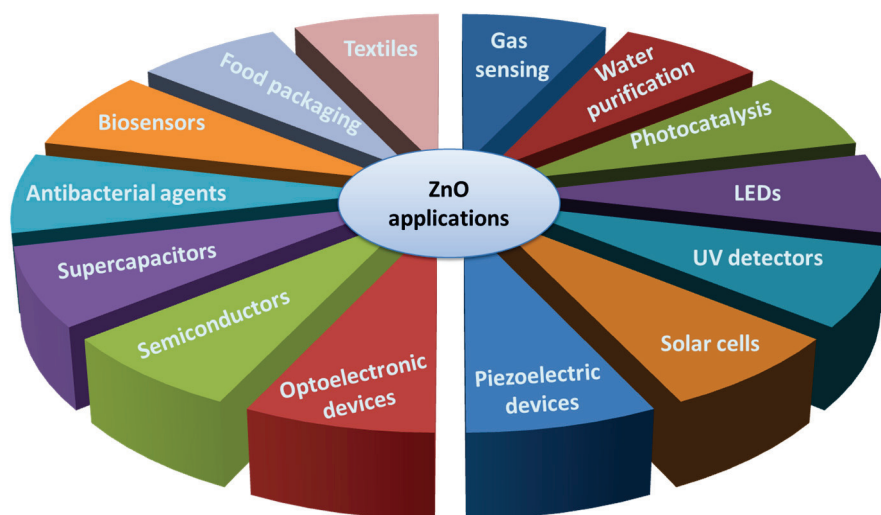


Figure 9. Applications of ZnO in different fields.

An overview of the SG-ZnO applications in the literature of the last ten years is presented in Table 4.

Table 4. An overview of the SG-ZnO applications in the last ten years.

Year	Title	Application	Review Content	Ref.
2023	Controlled Growth of Semiconducting ZnO Nanorods for Piezoelectric Energy Harvesting-Based Nanogenerators	Piezoelectric Nanogenerator; Energy harvesting	ZnO nanorods; Piezoelectric properties; Piezoelectric devices;	[219]
2023	Recent Advances in Integrating 1D Nanomaterials into Chemiresistive Gas Sensor Devices	Gas sensors	1D Nanomaterials; Electrical properties; Gas sensing	[220]
2022	92 years of zinc oxide: has been studied by the scientific community since the 1930s- An overview	Rubber industry; Biosensors; Textile industry; Agriculture (nano-fertilizers)	Vulcanization properties; Biological properties; UV blocking property; photo-catalytic self-cleaning; Electrical conductivity; Photoluminescence (PL) properties; Anti-fungal properties	[1]
2022	A review of flexible lead-free piezoelectric energy harvester	Piezoelectric Nanogenerator; Energy harvesting; Flexible Nanogenerator	ZnO NWs; Electrical properties; Piezoelectric behavior	[52]
2022	Morphological evolution-driven semiconducting nanostructures for emerging solar, biological, and nanogenerator applications	Solar cells; Nanogenerator; Biological applications	ZnO nanostructures; Antimicrobial properties; Antitumor activity; Anticancer activity; Piezoelectric properties	[21]
2022	ZnO Transducers for Photoluminescence-Based Biosensors	Biosensors	PL Properties	[33]
2022	A Review of the Impact of Zinc Oxide Nanostructure Morphology on Perovskite Solar Cell Performance	Solar Cell	Zinc Oxide Nanostructure; Electron mobility	[22]

Table 4. Cont.

Year	Title	Application	Review Content	Ref.
2022	Immobilization of zinc oxide-based photocatalysts for organic pollutant degradation: A review	Photocatalysis	Photocatalytic activity	[221]
2021	Economic Friendly ZnO-Based UV Sensors Using Hydrothermal Growth: A Review	UV sensors	Piezo-phototronics and piezotronics; conductivity; photoresistivity	[7]
2021	Review of ZnO-based nanomaterials in gas sensors	Sensors	ZnO nanomaterials; ZnO nanocomposite; Gas sensing properties; Electronic properties	[83]
2020	Photoluminescence of ZnO Nanowires: A Review	Photoluminescence applications	ZnO Nws; Optoelectronic properties; PL properties	[24]
2020	A review on ZnO: Fundamental properties and applications	Field effect transistors (FET); Gas sensing; LED devices; Environmental applications	ZnO; Optical, magnetic, and PL properties	[222]
2020	Advances in doped ZnO nanostructures for gas sensor	Gas sensors	ZnO nanostructures; Metal doping; Hetero atomic doping	[223]
2019	ZnO as a Functional Material	Biomarkers; Gas sensors	ZnO p-type; PL	[4]
2019	Enhanced sensing performance of ZnO nanostructures-based gas sensors	Sensors; Gas sensors	ZnO nanostructures; Nanocomposites; Gas sensing properties; Metal doping; UV activation; heterojunction	[224]
2018	Synthesis, properties, and applications of ZnO nanomaterials with oxygen vacancies: A review	Photocatalyst; Photoelectrochemical water oxidation; Antibacterial agents; Gas sensors; Supercapacitors; Electronic devices	ZnO nanomaterials; PL; Electrical properties; Ferromagnetism; Antibacterial activity; Gas sensing properties	[225]
2018	Fabrications and Applications of ZnO Nanomaterials in Flexible Functional Devices-A Review	Solar cell; Supercapacitors; Flexible piezoelectric NGs; UV photodetectors (PDs); Photodiodes; Flexible and porous 3-D ceramics; Functional surface coating; Biosensors; Gas sensors	ZnO nanomaterials; Thin films; Optical and electrical properties	[226]
2017	ZnO Nanowire Application in Chemoresistive Sensing: A Review	Gas sensors; Biosensors	ZnO NWs; ZnO Nanowire Sensors; Sensing, photoresponse, and semiconductor properties	[227]
2017	Zinc oxide nanostructure-based dye-sensitized solar cells	DSSCs	ZnO nanomaterials; Photosensitizer dyes; Photoconversion efficiency	[228]
2016	Optical biosensors based on ZnO nanostructures: advantages and perspectives. A review	Optical biosensors	ZnO nanostructures; Functionalization of ZnO surface	[229]
2015	ZnO nanostructured thin films: Depositions, properties, and applications—A review	Gas Sensors; SAW Devices Thin Film Transistors (TFT); LED; Solar Cells	ZnO thin films; Optical and electrical properties	[230]
2014	Zinc Oxide Nanomaterials for Biomedical Fluorescence Detection	Biomedical	Optical and electronic properties ZnO NR	[231]
2013	p-Type ZnO materials: Theory, growth, properties, and devices	LED; Photodetector; Field-effect transistor (FET); Sensors; Piezoelectric NG	Homo- and heterojunctions p-doping of ZnO films; Emission properties	[232]

In the previous sections, we mentioned the applications every time we considered the different types of layers and their preparation methods. This is obvious in Table 1, which is dedicated to sensor applications of composite layers containing Zn. Nevertheless, additional remarks are in order, regarding fluorescence because it is one of the most widely used detection mechanisms in many fields such as biology, biophysics, biochemistry, genomics, proteomics, drug discovery, disease diagnostics, and environmental analysis. A few recent examples are presented below:

7.1. Medical Field

In the reference [231], the status of 1D ZnO in vitro as bio-detection supports is summarized, as well as the challenges and future outlook concerning their application as enhanced *biomedical detection* platforms are presented.

Al-doped ZnO (AZO) thin films, annealed at different temperatures (250 °C, 450 °C, and 650 °C for 1 h in air) are used for the detection of glucose based on *fluorescence quenching* [30]. From the AZO450 PL spectra (Figure 10) in the presence of glucose at different concentrations and immobilized with glucose oxidase (GOx), a systematic decrease in the PL intensity is observed with the increase in concentration from 20 μ M to 20 mM.

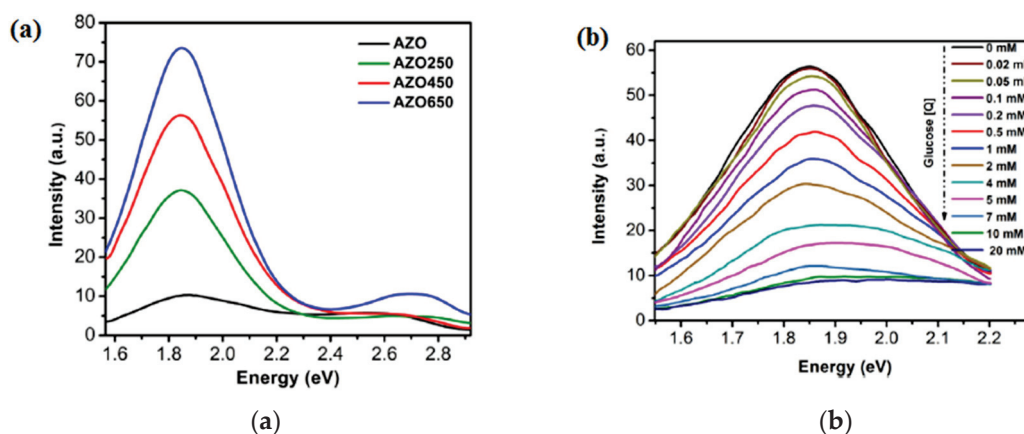


Figure 10. (a) PL spectra for as-grown and annealed AZO samples, (b) AZO450 PL spectra before and after glucose exposure at different concentrations in the presence of GOx. Reprinted from [30] with permission from Elsevier.

In 2014, ZnO nanoparticles (NPs) have been employed for fluorescence lifetime imaging in human skin [233] and in the same year, Wolska et al. [234] have shown that rare earth (RE) elements can activate ZnO NPs to work as biomarkers, for medical visualization. ZnO NPs possess biocompatibility with the living organism and if they are doped with RE element, their route inside the organism can be monitored by the luminescence of RE atoms.

7.2. Antibacterial Field

The *antibacterial activity* of SG ZnO films presented by Kaviyarasu et al. [59] with different concentrations of ZnO particles (100–600 μ g/mL) was successfully used against Gram-positive and Gram-negative bacteria (*S. pneumonia*, *S. aureus*, *E. coli* and *E. hermannii*). At the same time, the photocatalytic activity of ZnO under sunlight increases the degradation rate of Rhodamine-B (RhB), which is one of the common water pollutants emitted by textile and paper industries.

This *biological application* became more attractive due to their *duality in toxicity: benefits in drug delivery and the antibacterial effects*, as underlined in [20].

8. Summary, Conclusions, and Future Prospects

This review covers the major recent results on materials based on Zn, highlighting low-cost preparation methods (like chemical ones, especially SG). It focuses on the versatility of Zn in different combinations: as thin films (doped, undoped) for SL or main layer, as a thin film in a multilayer stack, as a component in a mixed thin film, or as a dopant in other films. A material with improved properties can be achieved by controlling and tailoring its morphology, crystallinity, and porosity.

The importance of the SL and its properties for obtaining thin films have been discussed, its presence being imperative for the growth of nanostructures with different orientations. Some strategies for the improvement of ZnO properties were discussed such

as: doping and co-doping of ZnO films and the addition of polymers, graphene, or other oxide materials in the ZnO matrix. Each approach is discussed in connection with the intended application. In order to prepare sensing (gas or biomarker) or transparent conductive materials the incorporation of one or more dopants is necessary, to induce p- or n-type conductivity, while the piezoelectricity is improved by growing 2D structures or polymer coatings.

The future major challenges regarding the development of sensitive materials consist of a better understanding of the sensing mechanism (gas sensor: adsorption reactions; biosensor: host–guest interactions), improving the sensitivity, selectivity, and stability of the samples as well as reducing the operating temperature to room temperature. An expanded analysis concerning the increase of gas performance of ZnO-based materials can be conducted through (a) control of morphology (optimization of synthesis parameters), (b) defects generation (finding the suitable dopant concentration), (c) investigation of photophysical (photons generation under excitation) and photochemical properties (generation of electrons after excitation) and (d) development of new composite materials (creating of surface defects which can lead to a better adsorption by electron transfer).

In the case of biological applications, additional studies are needed to elucidate the interaction mechanism between the sample and analyzed species and the study of biocompatibility of ZnO composite materials (implants or stents). From a biological point of view, an important challenge is the immobilization of the biomolecules on the surface of the sensor, while the extinction of the fluorescence would be a major problem for the photoluminescent sensors. The piezoelectricity of ZnO-based systems remains a promising topic for medical applications. An interesting approach to achieving piezoelectric properties involves the growth of nanostructures with preferential orientations and good uniformity coated with polymer layers, the number of layers having an important influence on the piezo–response of the final materials. As mentioned above, there are still many aspects that need to be further investigated in order to improve the performance of the studied materials.

In conclusion, due to its versatility, ZnO has gained a great interest in the scientific community since its discovery and will be studied in the future for a wide range of applications.

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Abbreviations

List of acronyms (in alphabetical order)

AZO	Al-doped ZnO
BF	Bright field
CBD	Chemical Bath Deposition

CNFs	Ceramic nanofibers
CNT	Carbon nanotubes
CP	Conjugated polymer
CVD	Chemical Vapor Deposition
DMS	Dilute Magnetic Semiconductors
DSSCs	Dye-sensitized solar cells
EC	Ethylcellulose
ETL	Efficient electron transport layer
FET	Field effect transistors
HT	Hydrothermal synthesis
HPC	Hydroxypropyl cellulose
ITO	Indium Tin Oxide
LED	Light emitting diodes
NLO	Nonlinear optical properties
NG	Nanogenerator
NPs	Nanoparticles
NR	Nanorods
NWs	Nanowires
OFET	Organic field-effect transistor
PBTA	Poly(butylene adipate-co-terephthalate)
PDMS	Poly(dimethylsiloxane)
PDOS	Partial density of states
PEBA	Polyether block amide
PEI	Polyethyleneimine
PL	Photoluminescence
PLA	Poly(lactic acid)
PP	Polypropylene
PVA	Poly(vinyl alcohol)
POPs	Persistent organic pollutants
RE	Rare earth
ROS	Reactive oxygen species
rGO	Reduced graphene
RhB	Rhodamine-B
SEM	Scanning Electron Microscopy
SG	Sol-Gel
SILAR	Successive Ionic Layer Adsorption and Reaction
SL	Seed Layer
TCO	Transparent Conductive Oxide
TEM	Transmission electron microscopy
TFT	Thin Film Transistors
UV	Ultraviolet
VOC	Volatile organic compound

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Review

Role of AuNPs in Active Food Packaging Improvement: A Review

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Abstract: There is a worldwide concern about food loss due to reduced shelf life among food science researchers. Hence, it seems that any techniques contributing to improved food packaging are most welcome in the food sector. It has been demonstrated that the administration of nanotechnology-based techniques such as metal-based nanoparticles can fade away the unresolved obstacles in shortened shelf life and environmental concerns. Along with substantial signs of progress in nanoscience, there is a great interest in the usage of green synthesis-based methods for gold nanoparticles as the most advantageous metals, when compared to conventional chemistry-based methods. Interestingly, those aforementioned methods have significant potential to simplify targeted administration of gold nanoparticles due to a large surface-volume ratio, and diminished biohazards, aimed at increasing stability, and induction of anti-microbial or antioxidant properties. However, it is necessary to consider the hazards of gold nanoparticles including migration for food packaging purposes.

Keywords: antimicrobial effects; food packaging; gold nanoparticles; nanotechnology; shelf life

1. Introduction

It has been demonstrated that there is a worldwide concern about the growing rate of food loss related to perishable food [1,2]. Results acquired from those surveys depict that expiring on the shelves of food stores makes problematic challenges leading to food loss [3]. In other words, food spoilage is accelerated by several microbial activities, and enzymatic activation of oxygen-driving processes [4]. Accordingly, these issues are addressed in imposing a financial burden on the economy, health system, environment, and even on recycling industries [5]. Thereafter, it seems legal to move forward to the establishment of any technological approaches aimed at the improvement of food quality.

Proper food packaging (FP), which is traced back to the 18th century, can play an indispensable role in the prolongation of shelf life of food products [6], and also preserves nutritional values during various stages such as transport, distribution, and storage [3]. Additionally, reduction of microbial contamination especially during harvesting, processing, slaughtering, and packaging steps in developing countries, is of high practical and industrial importance [1,7,8].

As mentioned earlier, there is an imperative need for signs of progress toward more efficient biotechnology-based applications in FP. A wide range of technologies including biomaterials and nanotechnology are utilized in FP [9]. Many researchers have focused on the employment of nanotechnology for food packaging purposes (Figure 1) [10].

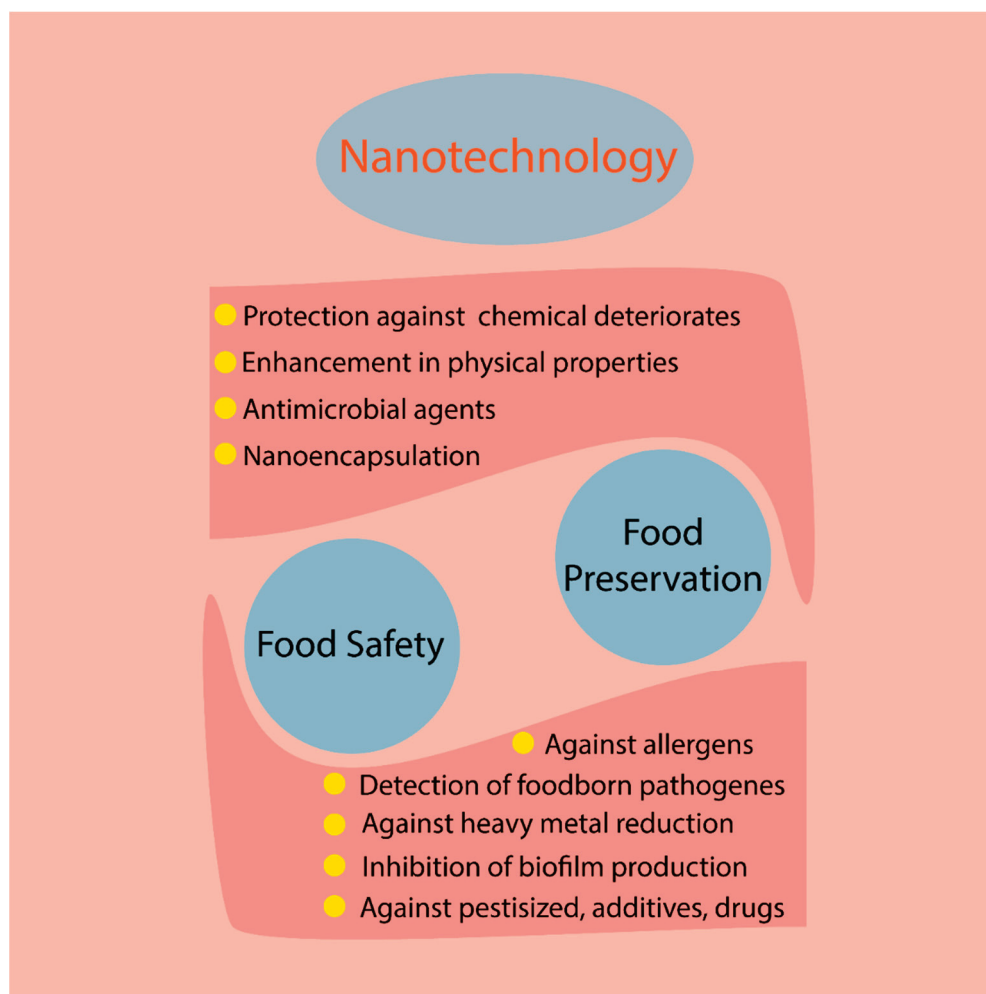


Figure 1. A schematic presentation of nanotechnology applications in daily life.

Nanotechnology in FP has a wide array of advantages, including making a barrier between the content of the food and exterior atmosphere [11], and assuring prevention of possible post-contamination of food, aimed at potential production and processing of healthier, safer, and high-quality foods [12]. There is a wide range of nanoparticles that are extensively employed in food packaging systems with distinct purposes including antibacterial [13], gas scavenging [14], antioxidant [15], and barrier properties [16] while considering food quality detectors as smart food packaging systems [17]. Metal nanoparticles (e.g., Ag, Au, Cu, CuO, Zn, ZnO, TiO₂), nano clay [18], nanoemulsions (e.g., essential oil of plants such as rosemary, ginger) metal-organic frameworks (MOF) [19] are known as the most employed nanoparticles in FP systems [13].

Amongst the existing nanoparticles, metal-based nanoparticles have attracted the attention of scientists to incorporate with the biomaterials to makes suitable packaging. Considering metal-based nanoparticles, gold nanoparticle (Au) has shown noticeable potential in improving food packaging systems from the viewpoint of antibacterial activity, improving barrier properties, using as biosensors, scavenging and so on. The incorporation of AuNPs into polymeric composites plays a crucial role in the active packaging of food. Recently, incorporation of AuNPs and grapheme oxide separately on PVA (polyvinyl alcohol) composite films on banana demonstrated antimicrobial activity against *Escherichia coli*. Banana shelf life was qualitatively enhanced with PVA-glyoxal-AuNPs nanocomposite for preserving food, validating its application in food packaging [18]. In another study, AuNPs conjugated with gallic acid (GA-AuNPs) were examined as an effective and promising strategy for reducing oxidative damage and physiological degradation of food products.

The potential capacity to scavenge free radicals and green catalytic activity against microorganisms are features of the compound. Results showed that GA-AuNPs is the safest and most effective food packaging liner in the food business [13].

In this paper, the main aim is to provide a comprehensive review of the role of gold nanoparticles (AuNPs) in food packaging systems. In this regard, first, a brief discussion was presented considering the way nanoparticles are employed in FP systems; then, the synthesis of AuNPs, and their potential in FP systems are discussed.

2. Search Method

Data of this review study were collected through an online literature review (in the English language) in Scopus, PubMed, Cochrane, Science Direct, and Google Scholar databases/search engines by 8 keywords (including 5 main keywords: (Gold nanoparticle, Au nanoparticle, AND Food Packaging, AND Food Safety), and 3 complimentary keywords (Anti-bacterial, Anti-oxidant, Shelf life)) commencing from January 2011 until June 2021 time intervals.

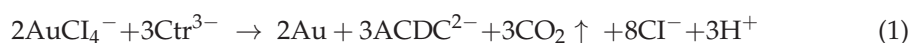
3. Synthesis of AuNPs

The modern era of AuNPs synthesis began over 150 years ago with the work of Michael Faraday, who was possibly the first to observe that colloidal gold solutions have properties that differ from bulk gold. Among the several forms of NPS created by biological systems, the gold ones are recognized as being the most compatible and non-toxic to the human body. Based on their particular characteristics and distinct surface functions, AuNPs have been used in food packaging. The simplicity of AuNP operation and functionalization results in a multi-platform in food packaging systems for antibacterial, gas scavenging, preventing biofilm formation, inhibiting gas permeability, and other purposes [19].

Along with tremendous development in the usage of AuNPs-based approaches in a variety of FP systems, there are growing concerns about the commercialization of conventional synthesis methods due to environmental or clinical hazards [20]. Over the past few decades, a wide range of solution-based approaches is employed to control size [21], shape [22], and surface functionality [23].

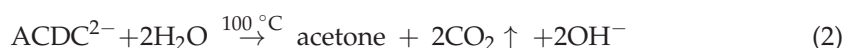
However, so far there is no available report regarding the nano-toxicity of biologically produced gold NPs (AuNPs) in vitro and in vivo. There are several reports regarding the synthesis of AuNPs via different chemical agents. For instance, citrate and gold salt are two famous components to produce gold nanoparticles (Equation (1)) [24]. Scientists showed that environmental condition including temperature affects the AuNPs dimension and their life cycle. It was reported that citrate and oxidation by-products (e.g., ACDC^{2−} (acetone dicarboxylate)) promote gathering and bridging Au(III) ions around the AuNPs seeds in the initial growth step [24].

Equation (1)



ACDC^{2−} can be converted to acetone at 100 °C according to Equation (2). However, past studies approved that the products from the Equation (2) can react with auric chloride and cause complete conversion to produce Au⁰ (Equation (3)) [24,25].

Equation (2)



Equation (3)



Summing up Equations (1)–(3), the following equation is obtained in which the effect of temperature ACDC^{2−} degradation is not considered:

Equation (4)



In another study, Equation (5) has been speculated at room temperature [26]:

Equation (5)



In another research, it was shown that the polarity of the solvent affects the AuNPs production and their size [26,27]. It was shown that the smaller size of AuNPs was synthesized in the high polarity index of the ethanol/water solvent as the reaction medium, while the bigger size of AuNPs was synthesized in the low polarity index. Polarity can be calculated from Equation (6) [28].

$$P' = \sum p'_i \phi_i \quad (6)$$

In which:

p'_i : the polarity index of solvent i in the mixture;

ϕ_i : the volume fraction of solvent i in the mixture.

It is mandatory to diminish concerns about by-products as toxic chemicals and increase the stability of AuNPs. It has been demonstrated that it is necessary to consider safe and cost-effective methods and pave the path for the brisk emergence of green synthesis of AuNPs [29–31]. There is a relationship between the functions of AuNPs and life cycle evaluation on their environmentally friendly nature. It seems that employing green synthesis of AuNPs is of great prominence due to sustainable and renewable suppliers of chemical energy [32]. There are several benefits in green biosynthesis procedures to synthesize AuNPs, when compared with classical chemistry methods. Those aforesaid biological resources act as nano-factories with less reaction time, size-controlled capability, and high acceptability [33]. Among the types of NP production techniques, the biological method is widely accepted because the use of the living organisms in the production pathway is safer than other methods. In the rest, two techniques (using microbial strains and using leaf extract) for the green synthesis of AuNPs are discussed.

The aqueous reduction of gold salts with sodium citrate is one of the most typical techniques for the production of AuNPs [34], compared to the shape-controlled synthesis of AuNPs [35]. It has been demonstrated that reagents acting as reducing and stabilizing ones such as chitosan [36] or alginate can serve as the most efficient reagents for the fabrication of AuNPs in situ [37,38].

As a prime study conducted by Irshad A. Wani et al., they tried to evaluate the size and shape-dependent anti-fungal activity of various AuNPs [39].

Those recruited AuNPs were different in their reducing agents affecting particle size, morphology, and properties of nanoparticles under similar ultrasonic frequency circumstances and without using any stabilizer [40]. Accordingly, two types of AuNPs, including gold nanodiscs of the average diameter of 25 nm (tin chloride as reducing agent), and polyhedral (nanocrystal) structure of the average size of 30 nm (sodium borohydride as reducing agent), were used in this study. Gold nano-discs reveal a much higher surface area and stronger fungicidal activity through inhibition of H⁺-ATPase against *Candida albicans* (*C. albicans*) [41].

3.1. Biosynthesis of AuNPs Using Microbial Strains

Researchers have a strong interest in the use of microbial strains, such as bacterial, due to their manageability and cost-effectiveness [41], actinomycete [42,43], fungal [44], algal [45], and yeast strains [46], for development of AuNPs for food packaging purposes.

As it was pointed out before, in addition to the existence of phytochemicals in plants, there are other co-chemical substances synergized with phytochemicals agents such as

citrate or cetyltrimethyl ammonium bromide (CTAB), being affected by photomutagenicity processes in AuNPs [47].

Results from the microbial study show that biosynthesis of AuNPs using microbial strains (such as *Shewanella oneidensis*) can be considered as highly efficient bio-reduction-based fabrication approaches [48].

It was reported that *Bacillus subtilis* could reduce Au^{3+} ions to AuNPs. The AuNPs size was reported between 5 and 25 nm inside the cell walls [49]. In an experimental study, Baker Syed et al. [50] investigated the synthesis of AuNPs from endophytic *Pseudomonas fluorescence* 417 inhabiting *Coffea arabica* L., and the assessment of their biological (anti-microbial) activities against clinically significant pathogens. AuNPs were confirmed by hyphenated techniques such as ultra violet (UV)-visible spectrophotometry (changing color of reaction from yellow to ruby red within 5 min of incubation), XRD, TEM, and FTIR (majorly in a spherical shape) [50]. Those AuNPs showed the most antibacterial properties against *P. aeruginosa* (MTCC 7903), *E. coli* (MTCC 7410), *S. aureus* (MTCC7443), *B. subtilis* (MTCC 121), and *Klebsiella pneumoniae* (*K. pneumoniae*) (MTCC 7407), respectively (results confirmed by colony forming unit (CFU) method and antibiogram tests). According to the results acquired from this study, the authors introduced such methods as efficient alternatives for the treatment of MDR strains [50].

In another study, Pourali and her colleagues employed two microorganisms (*Bacillus cereus* and *Fusarium oxysporum*) for the biosynthesis of AuNPs and compare their nanotoxicity under in vitro assays [51]. In this study, the authors added 100 microliters of HAuCl_4 to the 100 mL of the separated microbial supernatant and incubated shaker incubator for 24 h at 37 °C and 200 rpm. Both strains showed success in AuNP synthesis. Results revealed that the AuNPs produced by both strains were hexagonal, spherical, and octagonal with sizes of around 20–50 nm and irregular contours.

Singh and his colleagues reported AuNPs production using *Rhodopseudomonas capsulata* and *Pseudomonas aeruginosa* bacteria [52] under pH values from 4 to 7. They reported that pH value was the important parameter to control the shape and size of AuNPs. The produced AuNPs had a spherical shape and a diameter between 20 and 80 nm at pH 6.5. They also reported that the spherical AuNPs with the size of 10–20 nm were produced at pH = 7 while many nanoplates were detected at pH = 4. *Shewanella* algae were found to reduce Au^{3+} ions forming 10–20 nm AuNPs extracellularly with the assistance of hydrogen gas [52,53]. On the other hand, the AuNPs have been prepared on the surface of bacteria as a result of incubation of the cells with Au^{3+} ions [54].

Fungi were capable of reducing the metal ions such as gold salt into their corresponding nanometals i.e., nanogold either in the intracellular or extracellular state depending on the position of the reduction enzymes. In other words, the nanoparticles were formed extra-cellular when the cell wall's reduction enzymes were responsible for metal ions reduction and the reduction enzymes are secreted extra-cellular [55]. Sastry and coworkers have reported the extracellular synthesis of AuNPs by fungus *Fusarium oxysporum* and actinomycete *Thermomonospora* sp., respectively [56].

3.2. Biosynthesis of AuNPs Using Leaf Extract

According to the mentioned advantages for green biosynthesis of AuNPs, there is a growing trend in the usage of eco-friendly, cost-effective, non-hazardous, and sustainable methods for the synthesis of AuNPs [57]. As far as a wide arrow of studies is being devoted to green biosynthesis of nanoparticles, some of them believe that usage of several biopolymers as starch, chitosan, cyclodextrins, or even bacterial biomass [58], or environmental-based material such as olive-mill wastewater can be incorporated for reduction of AuNPs by phenolic compounds [59]. There are different industrial and laboratory approaches for the synthesis of leaf (or other parts of plants such as bark, root, or fruit) extract aimed at the usage for the biosynthesis of AuNPs [40,60]. They include (a) solvent-based extraction, (b) microwave-assisted extraction, (c) maceration extraction, and (d) ultrasound-assisted extraction [40]. In this part, several recently conducted studies aimed at the investigation on

the green biosynthesis of AuNPs by leaf extract or marine algae, are summarized: Rajathi et al., aimed at investigation of anti-bacterial effects of AuNPs produced by *Stoechospermum marginatum* (kützing) as a brown alga in their experimental study [61]. Their results from transmission electron microscopy (TEM) indicated the presence of majorly spherical shapes in the nanoparticles with (photoluminescent features) and 45.92% elemental gold. Interestingly, hydroxyl groups were recognized as reduction agents (present in the diterpenoids of the brown seaweed), effectively acting against bacterial pathogens [61].

In another study by Chidambaram Jayaseelan et al. [62], anti-fungal properties of AuNPs produced by seed aqueous extract of *Abelmoschus esculentus* (green biosynthesis of AuNPs with crystalline nature), was investigated. *Puccinia graminis critic*, *A. flavus*, *A. niger*, and *C. albicans* were the fungal strains that were tested. The authors reported that all of the nanoparticles were spherical with a narrow size range of 45–75 nm. Anti-fungal activities were observed among all of the fungal strains, especially *C. albicans*, and *Puccinia graminis* with the most inhibition zone (18, and 17 mm, respectively.). The authors concluded that as this approach is a low-cost, and user-friendly approach (done at room temperature), it also leads to better control over their nanostructures. This approach could be significantly considered in nanomedicine, and food packaging industries (as nano-preservative agents).

Marjan Shariari et al. [63], designed their study aimed at biosynthesis, and characterization of anti-AuNPs produced by *Allium noeanum* Reut. ex Regel leaves. The authors declared that antioxidant compounds in fresh leaves can be responsible for reducing agents (and forming monodisperse particles), confirmed by Fourier-transform infrared spectroscopy (FTIR) techniques. Based on the results, not only synthesized AuNPs depicted excellent antioxidant potential against 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and improved solubility, but they also revealed significant non-toxicity properties against human umbilical vein endothelial cells.

Additionally, it is of high prominence to mention that those synthesized AuNPs indicated antibacterial activities against a wide array of Gram-negative or Gram-positive pathogens (*Streptococcus pneumoniae* (*S. pneumoniae*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus saprophyticus* (*S. saprophyticus*), *Salmonella typhimurium* (*S. typhimurium*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella flexneri*, and *E. coli* O157:H7). They introduced that leaves extract incubated with aqueous gold ions can be considered as the most reproducible and biocompatible methods [63].

Jayanta Kumar Patra et al. [64] in their experimental study aimed at the synthesis of AuNPs from aqueous extract of dried outer onion peel (onion peel as food waste materials), and assessment of their biological activities. It was reported that various water-soluble phenolic compounds and cysteine derivatives were responsible for the reduction of nanoparticles (45.42 nm in diameter). Promisingly, synergistic anti-bacterial (against five different foodborne pathogenic bacteria), anti-oxidant, and proteasome inhibitory potentials were reported. They declared that this aforesaid approach can be an eco-friendly, and nontoxic procedure being able to be conducted in one step.

Annamalai et al. [65] in their experimental study, aimed at the synthesis of AuNPs from *Euphorbia hirta* L. leaf extract, and assessment of their biological (anti-microbial) activities. They recruited surface plasmon resonance (SPR) for characterization and confirmation of synthesized AuNPs through changing the color of the extract from pale yellow to purple (nanoparticles with the size varying from 6 nm to 71 nm). Promisingly, anti-bacterial properties of those synthesized nanoparticles against several bacterial strains of *E. coli*, *P. aeruginosa*, and *K. pneumoniae* were reported at very low concentrations (tested concentrations varying from 1.25 to 200 g/mL) of nanopowders used for nanoparticles. Interestingly, complete inhibition on bacterial growth was reported at the highest concentration, and the most inhibited bacterial growth was reported for *K. pneumoniae* (94%). They declared that this aforesaid approach (especially with the usage of combinative aqueous and methanolic extracts of *Euphorbia hirta* L. leaf) can be an eco-friendly, a rapidly conducted, and efficient procedure (due to synergized agents), being potentiated for usage in a wide range of industries or clinical applications [65].

Several researchs are dedicated to the improvement of the antimicrobial effects of metal-based nanoparticles, as well as considerable indicators of success in the case of the combinational use of AuNPs with others. Exemplary examples of these studies are collected. One comparative experimental study conducted by Priyanka Singh et al. [66] was aimed at the evaluation of anti-inflammatory properties of spherical silver nanoparticles, and monodisperse hexagonal AuNPs. Those nanoparticles were produced by green biosynthesis from fruit extract of *Prunus serrulata* at 80 °C. In their study, nanoparticles were confirmed and characterized through visual observation (with the appearance of color change in reaction for silver, and AuNPs, including from ruby red to brown within 50 min, and from light ruby red to deep purple color within 30 s, respectively), UV-VIS FETEM, EDX, elemental mapping, FTIR, XRD, and DLS. Criteria for anti-inflammatory effects of both nanoparticles were inhibition of downstream NF- κ B activation in macrophages [66]. Results of MTT assay from this study reported a significantly attenuated expression of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PEG2), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and LPS-induced activation of NF- κ B signaling pathway in LPS-induced RAW264.7 cells line as in vitro model. According to the results acquired from this study, the authors introduce the mentioned approach as an eco-friendly synthesis with biocompatible nature, being used as novel therapeutics for the prevention and cure of inflammation [66].

In another comparative experimental study done by R Geethalakshmi et al. [67], synthesis of gold and silver nanoparticles from *Trianthema decandra* L. root extract, and assessment of their biological (anti-microbial) activities were investigated. The shape of AuNPs was confirmed through FESEM (with the size for gold, and silver nanoparticles varying from 33 to 65 nm, and 36–74 nm, respectively), and the presence of metallic gold and silver in the nanoparticles was approved by EDX and FTIR. Phytochemical constituents were responsible for reducing agents. Both nanoparticles showed significant anti-bacterial/anti-fungal activities against *S. aureus* (MTCC 29213), *Streptococcus faecalis* (*S. faecalis*) (MTCC 0459), *Enterococcus faecalis* (*E. faecalis*) (MTCC 2729), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1035), *Proteus vulgaris* (*P. vulgaris*) (MTCC 1771), *B. subtilis* (MTCC 121), *Yersinia enterocolitica* (*Y. enterocolitica*) (MTCC 840), and *C. albicans* (MTCC 183), especially in Gram-negative bacteria. Comparatively, the anti-bacterial properties of silver nanoparticles were more than AuNPs. But both of those fabricated nanoparticles showed notable anti-bacterial effects on *Y. enterocolitica*, *P. vulgaris*, *E. coli*, *S. aureus*, and *S. faecalis*. They declared that due to the smaller size of AuNPs (varying from 37.7 nm to 79.9 nm) compared to bacteria, adherence to the bacterial cell wall is simplified and accelerated, leading to bacterial cell death [67].

In another study conducted by S. Lokina et al. [68], promising results from an investigation on biological (anti-microbial) properties of AuNPs produced from *Punica granatum* fruit (pomegranate) extract, were reported. For characterization and confirmation of AuNPs (with the size ranging from ranges from 5 nm to 17 nm), UV-Vis, fluorescence, high-resolution AuNPs (HRTEM), XRD, FTIR, selected area electron diffraction (SAED), and thermogravimetric analysis (TGA), were used. Through SPR, it was found that auric chloride (chloroauric acid or AuCl₄) is the responsible agent for the bio-reduction of AuNPs (through observing the color change from yellow to black in the reaction mixture) [68]. According to the minimum inhibitory concentration (MIC) assay, they reported an excellent anti-microbial activity of synthesized AuNPs against *C. albicans* (ATCC 90028), *A. flavus* (ATCC 10124), *S. aureus* (ATCC 25175), *Salmonella typhi* (ATCC 14028), and *Vibrio cholerae* (ATCC 14033). Additionally, according to the MTT assay, they reported excellent cytotoxic activity of synthesized AuNPs against HeLa cancer cell lines at different concentrations. Interestingly, the percentage of cancerous cell death was 98.9% in the presence of the highest dose of AuNPs (1000 μ g/mL). They introduced the aforementioned green synthesis method as low cost, non-toxic, and eco-friendly [68]. Table 1 shows a wide range of studies, aimed at the investigation of green synthesis of AuNPs from plant sources.

Table 1. Several recent studies evaluating on the usage AuNPs from plant sources.

Plant Source	Reducing Agents	Biological Effects	Microorganisms	Wavelength (nm)	Shape	NP Size (nm)	Year (Ref)
prunus cerasifera pissardii nigra leaf	extract	Anti-microbial and anti-fungal properties	<i>E. coli</i> , <i>S. aureus</i> , <i>B. subtilis</i> , <i>P. aeruginosa</i> , and <i>C. albicans</i> .	535	Spherical (20 nm)	20	2021 [69]
licorice root	extract	Antimicrobial and anticancer	<i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , and <i>Salmonella typhi</i>	517	Spherical	53.7	2021 [70]
<i>Turnera diffusa</i> Willd	oplopanone, γ -eudesmol, hydroquinone- β -d-glucoside (arbutin) and inositol	antimicrobial properties and immune response	<i>Vibrio parahaemolyticus</i> and <i>Aeromonas hydrophila</i> <i>Longfin yellowtail</i>	540	multiple shapes, mostly spherical	24	2021 [71]
banana pith extract	Alkaloids, Flavonoids	antibacterial activity and catalytic reduction	<i>Bacillus subtilis</i> , <i>E. coli</i> , <i>Pseudomonas aeruginosa</i>	530–560	spherical	470	2021 [72]
<i>Ricinus communis</i> L.	alkaloids, terpenoids, steroids	antibacterial activity	<i>Bacillus cereus</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>B. cereus</i>	550	spherical	100	2021 [73]
<i>Mentha Longifolia</i> leaf	-	anti-human breast carcinoma	breast carcinoma (Hs 578Bst)	512	spherical shape particles	36	2021 [74]
curcumin	Curcuma pseudomontana isolated curcumin	antimicrobial, antioxidant, and anti-inflammatory activities, antioxidant and radical scavenging activities.	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> and <i>Escherichia coli</i>	542	spherical shape particles	20	2021 [75]
sing Pimenta dioica Leaves	extract	Photocatalyst, antioxidant, and antibacterial	<i>S. aureus</i> and <i>E. coli</i>	517	spherical shape particles	11	2021 [76]
Garcinia kola Pulp	extract	Antibacterial activity	<i>Staphylococcus epidermidis</i> , <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i>	564	spherical shape particles	18–38	2021 [77]
Kaempferia parviflora rhizome	extract	antimicrobial, antioxidant, and catalytic degradation agent	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i>	540	spherical structure with high crystal in nature	20–60	2021 [78]
Clerodendrum inerme	extract	Antimicrobial, and antioxidant activities	<i>B. subtilis</i> , <i>S. aureus</i> , <i>Klebsiella</i> , and <i>E. coli</i> , <i>A. niger</i> , <i>T. harzianum</i> , and <i>A. flavus</i>	520	spherical	5	2020 [79]
<i>Nigella arvensis</i> leaf extract	flavonoids, alkaloids, and proteins	antibacterial, antioxidant, cytotoxicity against H1299 and MCF-7 cancer cell lines, and catalytic activities	<i>S. epidermidis</i> , <i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>Serratia marcescens</i> , and <i>P. aeruginosa</i>	546	spherical shape mostly spherical in shape and less triangle, pentagon and hexagon shapes	3–37	2017 [80]
Aqueous Extract of Garcinia mangostana Fruit Peels	phenols, flavonoids, benzophenones, and anthocyanins	-	-	540–550 (UV-vis)	spherical shape particles	32	2016 [81]
<i>Mimosa tenuiflora</i> Bark Extract	deprotonation of hydroxyl groups present in polyphenolic molecules of extract	a moderate cytotoxic effect at 24 and 48 h was found Cytotoxicity on HUVEC cells using MTT, Cellular uptake, and catalysis 12.5 mg/L of Mt extract, we obtained a 50% inhibition (L50),		maximum in 280 nm and broad of 50 nm	multiple shapes	20–200	2019 [82]

Table 1. Cont.

Plant Source	Reducing Agents	Biological Effects	Microorganisms	Wavelength (nm)	Shape	NP Size (nm)	Year (Ref)
Chenopodium formosanum shell extract	phenolic groups	antibacterial properties	<i>E. coli</i> , and <i>S. aureus</i> .	533	Most of the resultant Au NPs were spherical	8	2018 [83]
Leaf Extract of Ziziphus zizyphus	antioxidants, enzymes, and phenolic moieties	Antimicrobial and antifungal activity	<i>E. coli</i> , <i>S. marcescens</i> or <i>C. albicans</i>	peak in the range of 525–540 nm with a peak maximum in the range of approximately 527–535 nm	Majorly spherical and monodisperse	30–50	2018 [84]
Brazilian red propolis	hexane, dichloromethane and ethyl acetate, prenylated benzophenones	Antimicrobial, antifungal, and anticancer activities (dose-dependent cytotoxicity activities in bladder and prostate cancer cells)	<i>S. aureus</i> , <i>E. coli</i> , <i>S. mutans</i> , <i>C. albicans</i>	prominent peak at a range of 523–541 nm	mostly spherical shapes	8–15	2021 [85]
fresh peel (aqueous) extracts of Benincasa hispida	reducing enzymes as well as capping agents such as secondary metabolites	In vitro toxicity (antibacterial and anticancer)	Different Gram-positive and Gram-negative bacteria. Furthermore, the biosynthesized GNPs exerted remarkable in vitro cytotoxicity against human cervical cancer cell line, while sparing normal human primary osteoblast cells	a sharp absorption peak at 520 nm,	spherical in shape	22	2021 [86]
Platycodon grandiflorum leaf extract (Balloon flower plant)	flavonoids, saponins, alkaloids, amino acids, proteins, and carbohydrates	antipathogenic activity under optimal conditions	<i>E. coli</i> and <i>B. subtilis</i>	absorption at 545 nm	spherical in shape	15	2020 [87]
Hibiscus rosa-sinensis extract	alkaloids and flavonoids	-	-	520	spherical sized nanoparticles	16–30	2014 [88]
Galaxaura elongata (powder or free ethanolic) based extract		Anti-microbial properties	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , and Methicillin-Resistant <i>S. aureus</i>	520	spherical with a few rods, triangular, truncated triangular, and hexagonal AuNPs	3–77	2017 [89]
of Memecylon umbellatum leaf extract	Saponins, alkaloids, phytosterols, phlobatannins, phenolic compounds, phytosterols, and quinones	biocompatibility and anti-microbial activities	<i>B. subtilis</i> , <i>S. pneumoniae</i> , <i>S. aureus</i> , <i>S. typhimurium</i> , <i>Klebsiella aerogenes</i> , and <i>E. coli</i>	540	spherical-shaped nanoparticles	15–20	2018 [90]
Solanum nigrum leaf extract	phenolic compounds	strong DPPH radical and hydroxyl radical scavengers and antibacterial activity	<i>S. saprophyticus</i> , <i>B. subtilis</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	537	spherical-shaped nanoparticles and crystalline	50	2014 [91]

4. Potential Application of AuNPs in Food Packaging

Although the emergence of nano-biotechnology approaches is not a newly introduced concept in the food or agriculture sectors, there is a recent development in this area with novel aspects [92,93] Growing competitions and considerations for health concerns lead to increasing demand for sustainable and cost-effective production of food [94], and the development of more novel approaches for food packaging, coating, and processing to

meet consumer's demands [93,95]. In case of the emergence of nanotechnology in food packaging, as mentioned earlier, it should not be underestimated that targeted delivery of bioactive compound with reduced side-effects, stability of delivery systems [96,97], easiness in fabrication, regulation of food processed with nanomaterials, and public acceptance with affordable prices are highly encouraged to meet consumer's demands, as well [98].

Manufactured nanomaterials such as thin films, nanotubes, and nanoparticles (made in one, two, and three-dimensional structures, respectively), have been extensively used in food sectors [97]. Usage of those aforementioned structures in food industries are categorized into food supplementations, food ingredients, food additives, food nutrients, and delivery systems (in the forms of nanoemulsions, nanoparticles, surfactant micelles, emulsion bilayers, and reverse micelles aimed at improvement in texture, color, and flavor). These structures are aimed at improving the taste or color of the food, and masking unpleasant tastes.

4.1. Antibacterial Activities

In addition to the general properties of AuNPs [92,99], significant chemical properties of gold as a metal with the less corrosive one, and non-toxic effects [100,101], has attracted the focus of researches toward the usage of AuNPs in material based food industries (Figure 2). In a recent study, the function of 3-aminopropyltrimethoxysilane (APTMS) in the chitosan gold nanoparticles system was investigated. The addition of APTMS to chitosan resulted in a significant reduction in apparent viscosity, which can contribute to the achievement of higher miscibility in the structure. Additionally, the antimicrobial properties of a film composed of chitosan, APTMS, and AuNPs was evaluated against the *Salmonella* bacterium. The combined antibacterial activity of the three components was enhanced by their synergistic impact [102]. Anti-cancerous, anti-oxidant, and anti-microbial [103–105] properties of gold-based compounds, including anti-bacterial (especially against gram-negative bacteria when compared to Gram-positive bacteria [106]), anti-cancerous [41,107], anti-biofilm [108], anti-fungal (especially against *Aspergillus niger* (*A. niger*) and *Fusarium oxysporum*) [35,109–111], (majorly square-planar complexes of AuNPs [61]), anti-malaria, anti-amoebic, anti-leishmanial, and anti-trypanosomal features play an indispensable role in achieving success in food packaging, and prolongation of shelf life [16,103,110,112]. Mycelial extracts of the edible wild fungus *Cantharellus* sp. were used to manufacture AuNPs. On a variety of microorganisms, the antibacterial and antifungal capabilities of these NPs were evaluated. Significant antibacterial and antifungal properties were shown by the NPs [113]. Some studies tend to evaluate the effective ingredients responsible or in synergistic relations for induction of anti-bacterial effects in AuNPs against some bacterial strains such as: *E. coli*, *S. epidermidis*, and *Pseudomonas aeruginosa* [114]. Whereas, other studies recommend administration of other metals recruited as nanoparticles, including alloying colloidal silver-based nanoparticles with AuNPs.

Results from one experimental study carried out by Yongwen Zhang et al. [115] aimed at the investigation of anti-bacterial effects of colloid AuNPs, indicated promising findings. Successful preparation of AuNPs, high dispersion stability, and excellent antimicrobial efficiency was carried out through an in situ reduction and stabilization of hyperbranched poly (amidoamine) with terminal dimethylamine groups (HPAMAM-N(CH₃)₂) in water, and an adjusted molar ratio of AuNPs (from 10 to 30) in feed [7,115]. HPAMAM-N(CH₃)₂ was used as an effective reducing and stabilizing agent. Anti-microbial effects of several strains, including *E. coli* (ATCC 8739), *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 21332), and *Klebsiella mobilis* (*K. mobilis*) (ATCC 13048), *A. niger* (ATCC 16404), and *Penicillium citrinum* (*P. citrinum*) (ATCC 10499), were assessed by administration of 10⁸ CFU/mL from each bacterial cell suspension. Smaller particle size and narrower distribution were due to increased molar ratio and exacerbated anti-microbial effects (increased inhibition ratio up to ca. 98% at the low content of 2.8 µg/mL for AuNPs [115,116]).

To be more precise, AuNPs show less cytotoxic effects controversially compared with other metal-based nanoparticles such as silver [100,117]. They, as soluble complexes in

organic solvents, have a strong binding affinity to other biological agents, which makes them more potentiated in releasing ions, and anti-microbial activities, as well [47,118].

Results from a multidisciplinary study conducted by Enrique Lima et al. [119] aimed at investigating the anti-microbial properties of AuNPs as biocide material against *E. coli* and *Salmonella typhi* after assessment of size, dispersion, and roughness of AuNPs. Those properties were supported by zeolite. Interestingly, they reported that there were anti-bacterial features by elimination of the aforementioned bacteria by Au-faujasite dispersed nanoparticles (particles sized 5 nm at the surface) [119].

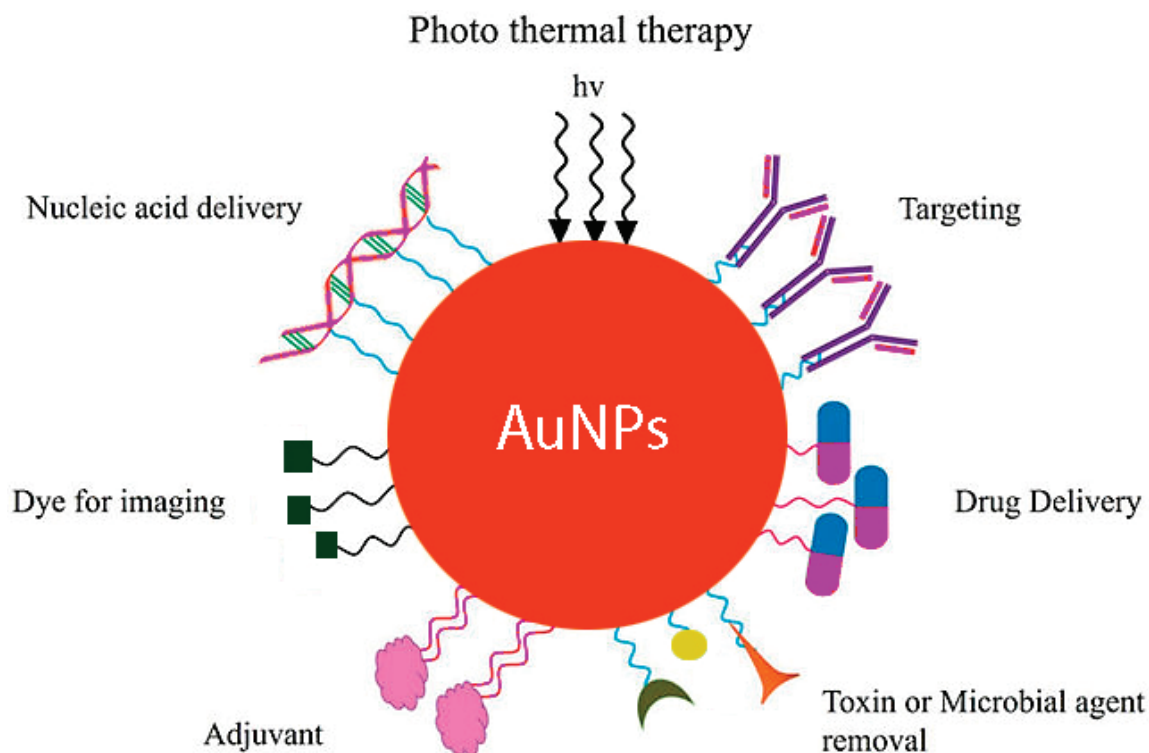


Figure 2. Functional properties of AuNPs.

An experimental study conducted by Saima Hameed et al. [120] was aimed at investigating the shape and concentration-dependent anti-microbial properties in AuNPs (with the size varying from 300 to 800 nm) against *E. coli*, *P. aeruginosa*, and *S. aureus*. Considered shapes for AuNPs were as following: nanospheres, nanostars, and nanocubes [120]. UV-vis spectroscopy, XRD, and TEM were used for the characterization and confirmation of AuNPs. AuNPs in the form of nanocubes depicted full ability in the inactivation of foodborne bacteria (zero survival percentage rate against all bacterial strains). Several cellular procedures, including cell loss, loosening of the cell wall, loss of flagella, releasing nucleic acid (or nucleic acid leakage), and loss of cellular matrix were reported after usage of those AuNPs through visual analysis (at lower concentrations). In this study, they concluded that despite similar surface areas in those AuNPs, anti-microbial interactions of gold nanoparticles with the Gram-negative or Gram-positive bacteria at lower concentrations will be shape-dependent [120,121].

Results from a preliminary interdisciplinary study carried out by Thirumurugan, A. et al. [122] demonstrated constructive effects of AuNPs exacerbated by incorporated food bio-preservatives (such as peptide of bacteriocin produced by *Lactobacillus plantarum* strain ATM11, and nisin) against reduction of microbial spoilage (*Micrococcus luteus*, *B. cereus*, *E. coli*, and *S. aureus*), that makes them proper to be used aimed at the prolongation of shelf life [122]. The most significant anti-bacterial activities were observed in the combinative usage of AuNPs/nisin, and AuNPs/bacteriocin against *E. coli*. They concluded that

peptide-producing organisms presented more anti-microbial abilities against mentioned food-borne pathogens. Additionally, the combination of bacteriocins with AuNPs showed more anti-bacterial properties when compared to AuNPs/nisin [122].

Carlos H. Pagno and his colleagues [123], in their study, aimed at investigating anti-microbial properties of active biofilms of quinoa (*Chenopodium quinoa* W.) starch-containing AuNPs against *E. coli* (ATCC 25972), and *S. aureus* (ATCC 1901) [123]. Anionic silsesquioxane containing the 1,4-diazoniabicyclo, octane chloride group (a silica-based hybrid polymer) was used for stabilizing AuNPs [123]. The final product was in the form of AuNPs dispersed to biofilms of quinoa starch with enhanced tensile strength. The significant anti-microbial activity was reported as inhibition percentages of 99% against *E. coli*, and 98% against *S. aureus*. In addition to the significant anti-bacterial properties, and increased UV radiation absorption and a decreased solubility make them more proper for food packaging applications [123].

Sujan Chowdhury et al. [124] assessed the effects of poly(vinyl) alcohol cross-linked film containing AuNPs on prolongation in the shelf life of bananas. They compared acquired results to the incorporation of graphene oxide into poly(vinyl) alcohol cross-linked film. They reported that improved mechanical and physical properties (such as tensile strength, Young's modulus value, water vapor transmission rate, and water solubility) are beholden to the dispersion of AuNPs and graphene oxide in the PVA cross-linked composites [124]. In case of advantages for anti-microbial efficacies, graphene oxide incorporated into poly(vinyl) alcohol showed less inhibition zone on disk diffusion agar tests against food-borne pathogens. They concluded that poly(vinyl) alcohol cross-linked film containing AuNPs was more efficient in increasing shelf life for bananas, and this efficacy in AuNPs was to a greater extent when glyoxal had been used as a cross-linker agent in poly(vinyl) alcohol cross-linked composite films. Mechanism of action for AuNPs against *E. coli* was cell lysis, which is accelerated by ethanol present in the poly(vinyl) alcohol film, electronic effects due to the changes in local electronic structures of the surfaces and their smaller sizes (less aggregated form and larger exposed expose a larger available surface area), as well [124].

Siying Tang et al. [125] designated their comparative study aimed at investigation of photocatalytic, and anti-microbial properties of gold-TiO₂/Sodium alginate nanocomposite films (as a degradable film and adsorber for visible light), which were produced by hydrothermal and casting method. Mechanical properties of produced film containing AuNPs, conferred them good shape stability. In the case of assessed anti-bacterial properties, there was an improved antibacterial activity against *S. aureus* and *E. coli*. This activity is accelerated by synergistic effects SPR of AuNPs, and increased production of reactive oxygen species [125,126].

Biologically, those useful properties are highly dependent on ligands. Furthermore, migration of AuNPs due to high surface to volume ratio of AuNPs [51], production of radical oxygen (scavenging free radical species via catalytic reaction), hydrophobicity [52], ability to interact with both gram-negative and positive bacteria with adjusted size and morphology (even optimized concentration) [127–129] have been considered as a merit for their reaction with food-borne pathogens, making an impressive decrease in the incidence of bacterial pathogenesis in food, and subsequently in consumers. Figure 3, indicates that how the chemical synthesis of AuNPs affects their potential applications.

To be more specific, there are several strategies for enhancing the antimicrobial characteristics of AuNPs. These methods may be roughly classified into three groups, including chemical reduction, green synthesizing [11], and incorporation of AuNPs with other nanoparticles. Multiple research suggest that these properties are dependent on chemical agents bound to the AuNPs surface. Therefore, maximizing the antimicrobial activity of synthesized AuNPs is a controversial subject.

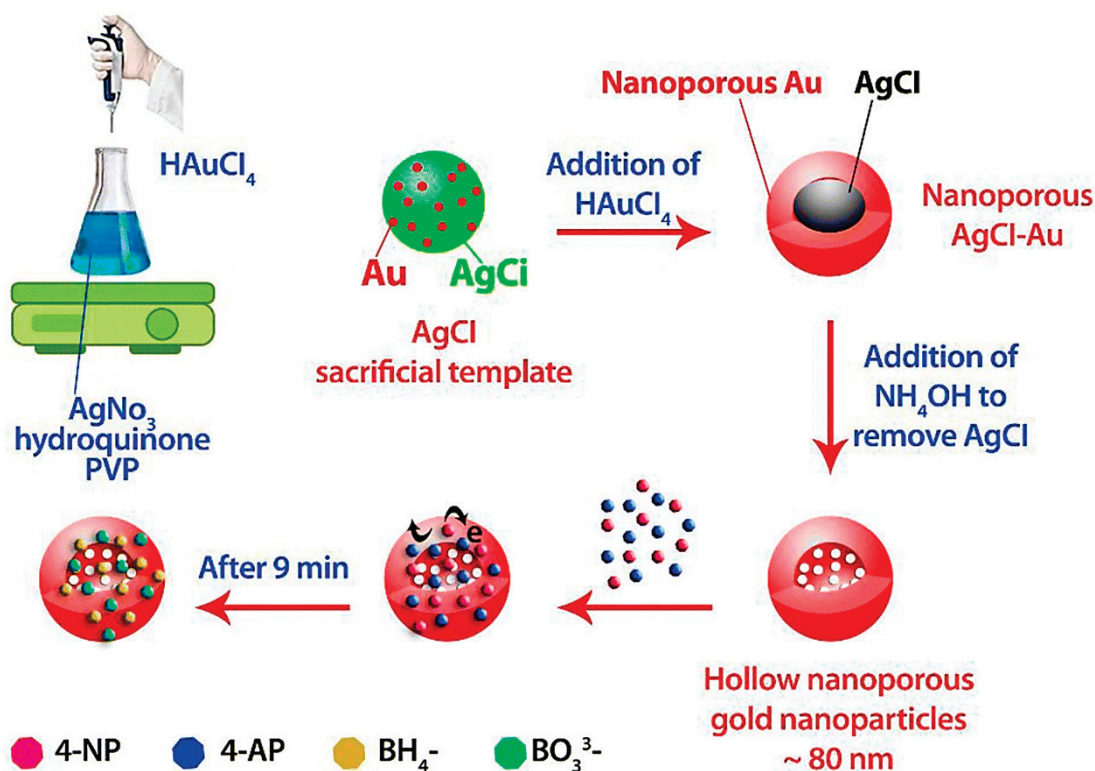


Figure 3. Chemical synthesis of AuNPs loading with different agents.

4.2. Barrier Properties

Permeability of water vapor, gases (e.g., CO_2 , O_2 , and N_2), aroma chemicals, and light are barrier properties. The presence of moisture in food promotes microbial development and rapid food deterioration. Therefore, packaged food goods may have a much longer shelf life if they are wrapped in a film with enhanced barrier qualities (WVTR) [130]. Not only anti-microbial properties of AuNPs are of food packaging systems, but gas barrier properties also make them a proper choice. In general, there are not enough studies discussing the effect of AuNPs on barrier properties. However, similar to other metal nanoparticles, AuNPs depicted a notable improvement in barrier properties.

In a research by Pagno et al., it was reported that the presence of AuNPs does not negatively affect the water vapor permeability of quinoa starch film ($p > 0.05$). They also reported the improvement of permeability of oxygen and carbon dioxide after AuNPs were embedded within the biofilm ($p < 0.01$). The presence of AuNPs in the biofilms can significantly reduce the permeability of gases ($p < 0.01$), so that AuNPs, like other metal nanoparticles, are considered an additional barrier to the penetration of gases and consequently, due to the elongation and complication of the path within the film matrix, the penetration of gases leads to a delay in oxygen transfer. They also reported that increasing the AuNPs concentration from 2.5% to 5% did not change the permeability significantly ($p > 0.05$). In another study, AuNPs were embedded with a poly(vinyl) alcohol-based film [124]. It was reported that the addition of AuNPs enhanced the molecular structure tortuosity ($p < 0.05$), thus the water molecules are unable to pass through [45].

Barrier properties are reduced when a high concentration of AuNPs are employed which the reason turns back to the agglomeration of AuNPs at higher concentration. It was shown that AuNPs can own distinct sizes (Table 2) and shapes which shows different behavior in agglomeration [131]. So, it is important to optimize the AuNPs concentration within the film matrix to reach the best barrier properties. Furthermore, the shape of AuNPs may affect the diffusion pathway which may result in better or worst barrier properties.

Table 2. AuNP dimension, weights, and volume.

AuNPs	Average Size (nm) (Diameter)	m_{AuNP} (10^{-18} g)	Volume (10^{-25} m ³)
Citrate AuNS	12.5	19.7	10.2
Tryptophan AuNS	8.4	5.9	3.1
Tyrosine AuNS	9.9	9.8	5.1
CTAB/citrate AuNS	8.9	7.1	3.7
AuNS (CTAB)	77.9	4800	2475.2
AuNR (CTAB)	58.8 (length) 15.3 width	270	137.6
AuNPr (CTAB)	94.7 (side length)	1900	970.8
AuNC (CTAB)	47.5 (side length)	2100	1071.7

Cetyltrimethylammonium bromide (CTAB), spherical AuNP (AuNS), cubic AuNPs (AuNC), rod-shaped (AuNR), prismatic AuNPs (AuNPr).

4.3. Antioxidant Properties

Metal and metal oxide NPs, carbon nanotubes (CNT), and different types of polymeric antioxidant NPs loaded with antioxidants demonstrated notable antioxidant properties. AuNPs as well as other common metal NPs own antioxidant potential. AuNPs are capable of interacting with both stable and unstable free radicals [132]. However, it was reported that AuNP's potential as an antioxidant agent can be varied in a dose-dependent manner [133].

In a study, J. Beurton et al. [112], aimed to investigate the pressing of controlled antioxidant properties (DPPH% and 2,2-azino-di(3-ethylbenzothiazolin-6-sulfonate) (ABTS^{•+}) in the well-individualized immobilized AuNPs on glass support (conducted through electrostatic interactions). As there was not any other peak except a localized SPR at 514 nm, no aggregation was reported. Promising results indicated a long-lasting antioxidant capacity characterized by a different kinetic (than colloidal AuNPs), and with the same efficiency. They designated the ability for prevention from the degradation of molecules (containing a thiol function) as a criterion for adaptable support of immobilized AuNPs. They represented that a ten-fold increase in the half-life of *N*-acetylcysteine, proved the efficacy of immobilized AuNPs for food packaging purposes with an adoptable covered surface, and the number of deposited AuNPs [112].

It has been reported that the high surface area to volume ratio of nanoparticles plays a vital role in their ability to scavenge free radicals [134]. Functionalized NPs attracted scientist's attention in food packaging systems because of benefits such as unique and localization interactions of NPs with the bio components from the foods. In research carried out by Patra and his colleagues [64], AuNPs were synthesized by green synthesis using onion peel extract and named OP-AuNPs. Antioxidant activity was one of the main parameters under study. Based on the results, they reported that OP-AuNPs showed a notable DPPH scavenging capacity of 14.44%/100 µg/mL in comparison with 36.54%/100 µg/mL by butylated hydroxytoluene as a commercial antioxidant. OP-AuNPs also demonstrated higher radical scavenging compared to butylated hydroxytoluene. It was reported that the main reason turns back to the secondary metabolites, aromatic phenolic, and flavone groups in the onion peel extract and high surface area to volume ratio of nanoparticles [126,135].

4.4. Biosensing

One of the main concerns of customers is food quality and safety. Therefore, exploring fast, accurate, and efficient techniques to detect food quality is significantly important for human health. Biosensors depict a cutting-edge frontier in food packaging management [19], and nanomaterial such as AuNPs have been employed in the food contamination detection field to monitor, assess, and control food quality [136].

The intrinsic properties of AuNPs such as high stability, easy to control size and shape of the NPs, large surface-to-volume ratio, and excellent biocompatibility, offer themselves various benefits as sensors to make smart food packaging. For instance, a

high surface/volume ratio provides high sensitivity and rapid responses. AuNPs also can be considered as multiple detection platforms [137]. Colorimetric sensors are one of the most common AuNPs-based sensors. AuNPs have shown their detecting potential in food contamination monitoring [137]. However, there are a few reports about employing AuNPs in food packaging systems.

M.R. Bindhu et al. [138] aimed to assess biosensory capabilities of gold and silver nanoparticles by a green approach (using *Solanum lycopersicum* extract). Carboxylic acid groups in the mentioned extract were recognized as reducing agents. UV-vis, FTIR, TEM, and energy dispersive spectroscopy (EDS) techniques were used for the characterization and confirmation of metal-based nanoparticles. Results of this study for monodispersed spherical gold, and silver nanoparticles showed significant SPR peaks at 546 nm and 445 nm, average size of 14 nm, and 12 nm, respectively. In addition to significant anti-microbial activity against *S. aureus* and *P. aeruginosa* existed in water samples, they were able to sense ions of heavy metals such as Fe^{3+} and Cu^{4+} in water by an SPR optical sensor, being efficient in water purification [131]. Results of this study were in agreement with other studies carried out by Govindhan Maduraiveeran et al. [132] indicating successes in the usage of AuNPs for sensitive detection of multi-target analytes with food safety and environmental monitoring purposes.

One innovative experimental study done by Cheuk-Fai Chow [139], was aimed at the investigation of correlations between the sensitivity of a colorimetric probe based on AuNPs (as a nano detector) to detection of microbial spoilage in meat products (stored at 4 °C), and volatile biogenic markers (such as dimethyl sulfide and histamine,). The ability of detection in the colorimetric probe is beholden to the ability in the transformation of the non-aggregated form of AuNPs to its aggregated form, after binding to aforesaid biomarkers. Of note, 0.5 and 0.035 µg/mL of AuNPs could detect dimethyl sulfide, and histamine biomarkers, respectively [139]. Additionally, they demonstrated that the constitution of AuNPs in the 2:1 H₂O/DMSO mixture confers them to high stability to show the selective colorimetric response for mentioned biomarkers. In the case of microbial assessments, sensing signals of AuNPs for detection of mentioned volatile biogenic markers on 12 different raw meat samples were estimated. CFUs of 10⁷ were recognized as an alarm for the level of spoilage. For raw meat, preserved raw meat, raw fish, and raw crustaceans, UV-vis signals (at 520 nm) of 0.15, 0.1, 0.45, and 0.35 were reported alarm values, indicating harmful levels of food spoilage (CFUs of 10⁷).

The development of nanotechnology offers several industries, particularly the food packaging industry, significant prospects. As functional additives, several nanoparticles (such as nanoclay and metallic nanoparticles) have recently been incorporated to food packaging. Their beneficial impacts on modern packaging materials have been widely reported. According to the findings, the introduction of nanoscale fillers into the polymer matrix will alleviate packaging material difficulties while also enhancing functional properties [140]. These polymers incorporating nanocomposites have enhanced barrier characteristics, thermal properties such as melting temperature and glass transition, and altered functions such as surface properties and hydrophobicity. Inorganic nanoparticles have the ability to inhibit bacterial development inside packaging. By adding nanoscale components into biopolymer-based packaging composites, packaging-related waste may be decreased.

The migration behavior of nanomaterials from packaging may vary from that of conventional materials, and migrating nanoparticles may be more reactive and have a distinct toxicological profile. Thus, certain nanomaterials are prohibited in the EU due to insufficient toxicity data [141].

Multiple factors, such as temperature, time, the concentration of nanoparticles in polymer matrix, polymer characteristics, placement of the nanoparticles in the packaging material, interaction between the nanoparticles and the materials, sample selection type of material, contact type of material, and the nature of the food/food simulants, have been shown to influence the migration of nanomaterials from food packaging substance into

food or food simulants. In general, nanoparticles have the ability to migrate into food, particularly when exposed to more acidic chemicals [142].

5. Hazard

Allergies and the release of heavy metals as the migration phenomenon are the two main safety concerns of nanoparticles. AuNPs depict a good safety profile. Considering AuNPs, as well as other metal nanoparticles have the potential of toxicity. AuNPs are capable of migration from packaging to food matrix and finally, they will be released in the human body after food consumption which is a toxin for different cells and tissues [138].

Currently, AuNPs are employed in active and smart food packaging systems at a relatively faster rate without desired knowledge and regulations, which can affect health and the environment. There is a likelihood of interacting between AuNPs and food molecules which leads to unwanted and toxic products. This issue is also important in smart food packaging systems which may result in false-positive detection [143].

Nevertheless, experimental use of AuNPs presented possible medical hazards as the surface to volume ratio causes catalytic properties and can make particles very reactive. Furthermore, nanoparticles easily pass cell membranes and can interact with intracellular metabolism [139]. Environmentally, AuNPs can be released into the ecosystem along with the nanocomposite. However, they are benign to the ecosystem if no accumulation happens in the environment. For instance, there is a report considering the non-toxicity of AuNPs to zebrafish. As at nano-scale gold-particles may exhibit size-related properties that differ significantly from the known properties of non-nano-scaled gold-particles, one cannot predict reliably the nature of AuNPs and a biologic system and interactions between AuNPs and living cells. Moreover, AuNPs showed different shapes with different charge and surface-chemistry and each one may have distinct behavior [144].

6. Future Prospective

To improve nano-packaging systems via AuNPs, it is necessary to consider all aspects including hazards. AuNPs as well as other common NPs, can migrate from packaging into the food matrix. However, AuNPs due to having various shapes can be a good candidate to modify a film structure with desired features.

Surface modification of the inner surface of the polymeric films (the surface which touches the food) can decrease the NPs diffusion by limiting the micro-channels. Creating a bilayered film, in which the inner layer is responsible for controlling NPs migration, can be a good solution. By the way, it is dictated that increasing the layers may consume more materials which can be considered as a hazard to the environment, so modifying the thickness of the layers is recommended.

As another recommendation, NPs modification can be another recommendation to decrease the rate of migration. Chemically, connection of NPs to the inner structure of the films can lower the migration phenomenon. It is also recommended that this connection, based on the various factors, can be permanent or temporary. Considering the previous point, AuNPs are known as good carriers. When AuNPs are loaded with an antibacterial agent, it is predicted to improve both barrier properties and migration rate.

As the final recommendation, AuNPs and ionic gold can show different activities against a range of microorganisms. It can be recommended for future studies to evaluate the level of improvement by employing both types of gold.

7. Conclusions

Food packaging is one of the most important challenges which has a direct effect on human health. In this regard, it is mandatory to engineer a packaging system that not only can increase foods shelf life but also can provide online information about food quality. Nanotechnology plays a vital role in this area of science. Metal NPs showed their potential in improving food packaging systems.

AuNPs with their unique potential demonstrated that can be integrated with other technologies in food packaging to achieve an active and smart food packaging system with suitable antibacterial, antioxidant, and scavenging properties while providing appropriate information about food quality. AuNPs have the potential to migrate from the film or coating packaging to the food matrix. Thereby, the way of polymeric film manufacturing, the concentration of AuNPs, employed agents, used biomaterial, type of food, and storage condition can affect AuNPs activity as antibacterial/antioxidant/biosensor agents.

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