

Article

Structure–Property–Transport Relationship in Hyaluronic Acid/ZnO Nanocomposite Dissolving Microneedles for Transdermal Ciprofloxacin Delivery

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Abstract

Polymeric microneedles are introduced as a promising platform for minimally invasive drug delivery and molecular transport control. In the present study, hollow dissolving nanocomposite microneedles based on a mixture of high- and low-molecular-weight hyaluronic acid (HA) in a 40:60 ratio, including zinc oxide nanoparticles (ZnO NPs), have been created and evaluated as hydrated polymer transport matrices. Surface modification of ZnO nanoparticles using citric acid was proposed to improve dispersion by reducing agglomeration of nanoparticles in the polymer matrix. ZnO nanoparticles in concentrations ranging from 1 to 10% (*w/w*) were used to study the effects of the loading level of nanoparticles on the structure, mechanical response, and controlled diffusion behavior of hydrated polymer matrices. The created nanocomposites exhibited clear hollow structures with tip radius of 18–23 μm , height of 1500 μm , and aspect ratio of 5.7. Nanoscale surface organization and particle dispersion in the polymer matrix were studied by scanning electron microscope (SEM) and atomic force microscope (AFM). Low nanoparticle concentrations were favorable for maintaining high matrix homogeneity, while high concentrations resulted in increased surface roughness and nanoparticle agglomeration. Mechanical compression testing confirmed that hydrated HA/ZnO microneedles were characterized by elastic bending behavior until fracture. Diffusion experiments performed in Franz diffusion cells showed that nanoparticle concentration significantly impacted the cumulative transport and flux of molecules through the hydrated microneedle matrix. Formulations with 5% and 7% ZnO nanoparticles were characterized by a prolonged diffusion behavior attributed to ZnO-induced tortuous transport channels in the polymer matrix. In contrast, formulations with 10% ZnO nanoparticles exhibited accelerated heterogeneous transport due to ZnO-induced changes in structure and morphology. The experimental diffusion data correlated well with the Higuchi kinetic model, and anomalous transport was detected using the Korsmeyer–Peppas model, which indicated a synergistic effect of diffusion and polymer relaxation on molecular transport. As compared to coating and tip-loaded microneedle designs, the obtained HA/ZnO nanocomposite microneedles offered a simple approach for embedding Ciprofloxacin in the hydrated polymer matrix. This was achieved due to the direct creation of microneedles containing dissolved particles.

Keywords: Hyaluronic acid; zinc oxide nanoparticles; dissolvable microneedles; transdermal drug delivery; ciprofloxacin; nanocomposite polymers



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1. Introduction

Polymer nanocomposites form a highly versatile range of materials that can be engineered to achieve microstructural systems with desired mechanical, physicochemical, and transport properties [1,2]. Polymer nanocomposites offer the possibility to precisely control structure–property relationships via interface effects, dispersion phenomena, and polymer chain dynamics [3]. These types of systems become very important for cases when mechanical stability needs to be combined with regulated mass transport capabilities for use in coatings, membranes, and biomedical devices [4–7].

Among such devices, microneedle systems present a unique example where properties on the micro- and nano-scales dictate macroscopic system behavior [8]. Microneedles are micron-scale projections that are able to penetrate the stratum corneum and form transient microchannels to enhance transdermal transport without causing considerable damage to tissues [9,10]. Various materials like metals, silicon, and ceramics were examined but polymeric microneedles attracted growing interest due to their biocompatibility, biodegradability, and mechanical properties [11].

The utilization of hyaluronic acid (HA) as a component of polymer systems is widespread owing to its hydrophilicity, viscoelastic properties, and capability of extensive hydrogen bonding [12,13]. The physical and chemical properties of HA are heavily contingent on the molecular weight of this polysaccharide, which defines chain entanglements, viscosity, and mechanical strength [14]. High-molecular weight HA (HMW-HA) usually provides structural rigidity and mechanical strength, while low-molecular weight HA (LMW-HA) increases chain mobility and diffusion rate [15–17]. Thus, combined use of HMW-HA and LMW-HA may serve as an efficient means of adjusting polymer networks and attaining superior performance [8].

However, the HA-based systems demonstrate a lack of mechanical strength and are prone to failure under compressive and insertion loads [18]. To overcome this challenge, HA-based nanocomposites can be developed using a broad range of nanofillers, such as carbon fibers, graphene, and metallic nanoparticles [19]. In particular, zinc oxide nanoparticles have gained popularity among researchers due to their high specific surface area, surface reactivity, and compatibility with hydrophilic polymers [20]. Apart from antimicrobial activity, zinc oxide nanoparticles are capable of improving the mechanical properties of the composites due to stress transfer and microstructural changes [21].

The effectiveness of nanocomposites highly depends on the dispersion pattern and interfacial interactions between polymer and nanoparticle [22]. Aggregates may create stress concentration zones and impair the mechanical properties, while proper dispersion is a prerequisite to achieving enhanced reinforcement and structural uniformity [23]. Several strategies have been developed to address these challenges, including pH-mediated stabilization and ligand-based functionalization [24]. In HA-based systems, electrostatic interactions could be applied to manipulate nanoparticle–polymer interaction without affecting the polymer structure [25].

Apart from the composition of materials used in microstructured systems, geometry plays an essential role in defining their mechanical behavior and functional properties [26]. Geometric parameters include aspect ratio, tip radius, and spatial distribution, which control stress distribution, deformation, and penetration efficiency [27]. The development of polymer nanocomposite microneedles should consider the material properties and geometric factors for achieving optimal performance [28].

Transport processes in polymeric materials are directly linked to the microstructure of the polymer and its network dynamics. Diffusion processes of drugs in hydrophilic polymers are usually determined by the interplay between Fickian diffusion and polymer relaxation, especially in cases when there is swelling or dissolution of the system. The

presence of nanoparticles may also affect transport behavior due to changes in free volume, tortuosity, and chain dynamics of the polymer system.

In this connection, the current paper addresses HA/ZnO nanocomposite systems as models of polymer networks to reveal the effect of incorporation of nanoparticles on microstructure, structural changes, and functional properties. A hybrid matrix, consisting of high- and low-molecular weight hyaluronic acids, was mixed with zinc oxide nanoparticles at different concentrations (1–10 wt%) to determine the effects of filler content on characteristics of material performance. The nanocomposite films were produced by micromolding and were analyzed in terms of morphology and nanostructure with the help of SEM and AFM. Compression and insertion tests were conducted to study material behavior under applied forces. Moreover, diffusion performance of the nanocomposites was studied using *in vitro* drug penetration experiments.

Thus, this work aims at studying the structure–properties relationship in HA/ZnO nanocomposites and their application for microstructured polymeric systems. The results obtained are expected to contribute to the development of polymer nanocomposites in terms of their structure and function. In addition to adequate mechanical properties, efficient transportation of drugs across biological tissues should be one of the key characteristics of microneedles.

However with reference to taking drugs across biological tissues, the maximum loading investigated in this study (10 wt%, corresponding to approximately 16mg ZnO per formulation) remains within concentration ranges reported in the literature to demonstrate acceptable cytocompatibility in MTT assays. Previous studies have indicated that ZnO nanoparticles toxicity is dose-dependent and is strongly influenced by nanoparticle dissolution, Zn^{2+} release, and direct cellular exposure. In composite systems where ZnO nanoparticles are immobilized within a biopolymer matrix, cytotoxic effects are generally reduced, supporting the potential suitability of the HA/ZnO composites developed herein for biomedical applications [29–31].

Furthermore, an important requirement for the efficient operation of microneedles in biological applications is their ability to release the active compounds in a fast and efficient manner. Ciprofloxacin, a broad-spectrum antibiotic that is used for the treatment of many bacterial infections, can be delivered through microneedles for increased efficiency and reduction of possible side effects. The study of drug transport through skin tissue can be performed using Franz diffusion cells together with a biological membrane, for example, porcine skin.

2. Materials and Methods

2.1. Materials

Hyaluronic acid (HA) was used as the main component of the polymer microneedle system. Two grades of HA with different molecular weights were selected: high molecular weight (HMW) HA (1300–1500 kDa) and low molecular weight (LMW) HA (30–50 kDa).

Zinc oxide (ZnO) nanoparticles, with sizes between 10 and 30 nm, were added to the microneedle system as nanofiller agents for improving their mechanical strength. The concentration of the nanoparticles was set to 1%, 3%, 5%, 7%, and 10% (*w/w*) based on the total amount of polymers.

Citric acid solution (1 M) was applied as a stabilizer of nanoparticles and an additional reagent to increase compatibility between HA and ZnO nanoparticles.

The model drug was ciprofloxacin hydrochloride for the analysis of drug transport across the biological membrane. Phosphate-buffered saline (PBS) was used as the receiving medium in drug diffusion studies. Skin samples of porcine origin were tested as biological membranes in Franz diffusion cell studies.

Hyaluronate (HA, MW~1300–1500 kDa, and 30–50 kDa), was provided by Huaxi Biotechnology Co., Ltd. (Shandong, China), Zinc Oxide Nanoparticles/Nano powder (ZnO) 99.8% purity of size 10–30 nm was prepared at the center for chemical engineering center laboratory, ITMO University. Citric acid ($C_6H_8O_7$, anhydrous, purity $\geq 99.5\%$ molecular weight = $192.12 \text{ g}\cdot\text{mol}^{-1}$) was obtained from Sigma-Aldrich (St Louis, MO, USA) and used as a chelating and stabilizing agent without further purification. Distilled water prepared in-house using the Millipore Milli-Q system with a resistivity of $18.2 \text{ m}\Omega\cdot\text{cm}$ at 25°C was used throughout the experiment and ciprofloxacin hydrochloride were provided by Merck and Tokyo Chemical Industry.

2.2. Preparation of HA/ZnO Nanocomposite Solution

The method used for preparing the HA/ZnO nanocomposite solution involved sequential dispersion aimed at ensuring an even distribution of nanoparticles in the polymer matrix. Two fractions of hyaluronic acid of different molecular weight (low-molecular-weight hyaluronic acid, LMW HA, 30–50 kDa and high-molecular-weight hyaluronic acid, HMW HA, 1300–1500 kDa) in the ratio of 60:40 (LMW:HMW) were weighed using an analytical balance before dissolving.

To prepare the solution, zinc oxide nanoparticles were first dispersed in an aqueous solution of 1M citric acid to enable surface functionalization and electrostatic stabilization. Surface functionalization enables good compatibility between ZnO nanoparticles and the highly hydrophilic HA solution, while electrostatic stabilization helps avoid any aggregation.

Subsequently, the LMW HA fraction was gradually dissolved in the ZnO dispersion while stirring. HMW HA fraction was added next to increase the viscosity of the solution. Stirring of the resulting solution continued for 4–24 h until the required level of homogeneity was achieved.

To enhance nanoparticle dispersion and destroy any formed aggregates, the solution was homogenized in a high-speed homogenizer. Prior to the microneedle manufacturing process, the solution was degassed for 12 min under vacuum at around 99 kPa.

2.3. Fabrication of HA/ZnO Composite Microneedles

A solution of the degassed HA/ZnO nanocomposite was used to prepare microneedles by using the technique of micromolding. The solution was carefully poured onto polydimethylsiloxane (PDMS) microneedle molds containing an arrangement of 140 cavities (arranged in a 14×10 pattern). To ensure that all of the cavities were completely filled and the solution of the polymer was distributed uniformly, the molds were degassed for 12 min under pressure of 99 kPa and solvent-tapped in micromolds. Once all of the molds were completely filled, they were dried to allow the evaporation of the solvent and the formation of the solid polymer microneedles.

Artificial Skin Fabrication Using Gelatin

The artificial skin model was prepared by dissolving 10% (w/v) gelatin powder in distilled water at $50\text{--}60^\circ\text{C}$ under continuous stirring at 400rpm, until a homogeneous solution was obtained. The solution was poured into Petri dishes and allowed to cool at room temperature, followed by storage at 4°C for 12 h to form a gel. The resulting gelatin layer was used as skin simulant for insertion testing.

2.4. Microneedle Geometry and Structural Design

Geometric parameters of the prepared microneedle arrays were chosen to ensure appropriate mechanical strength of microneedles and efficient penetration of the skin. Microneedles were organized in a rectangular array consisting of 140 needles (configuration

14 × 10). The needles were fabricated on the basis of the nanocomposite matrix composed of HA and ZnO.

A conical form of each microneedle was chosen to achieve efficient skin penetration during microneedling; these are basically with references to previous studies [32]. The sharp tip of the microneedle has a radius of 18–23 µm, an inter-needle spacing of 1.52 mm, and a height of 1.5 mm based on measurements carried out under a microscope. As is known, the conical shape is characterized by an increased surface pressure exerted onto a substrate (skin). Therefore, a sufficient pressure that could break down the resistance of stratum corneum and achieve efficient penetration is provided [33,34].

Micromolding was used for fabricating microneedles with the same height, base diameter, and geometrical parameters. Mechanical strength of the microneedle together with penetration capacity and controlled dissolution were taken into account when choosing parameters of the microneedles' geometry.

Moreover, the presented microneedle technology involves the fabrication of a hollow dissolvable needle with a central lumen. In particular, upon insertion into the skin, the needle is rapidly dissolved owing to hydration. Consequently, the diffusion of therapeutic agents becomes easy due to channels created in the skin.

2.5. Drug Loading of Microneedles

The ciprofloxacin hydrochloride compound was incorporated into the HA/ZnO nanocomposite solution prior to micromolding so that the resulting microneedles would contain drug delivery ability. The drug was dissolved in the polymer solution with constant stirring at 800rpm so that a uniform distribution could be achieved. The drug-containing solution went through the same process of micromolding as mentioned previously.

2.6. Microneedle Insertion Test

The ability of insertion and mechanical strength of the produced microneedles were tested using an artificial skin prepared from gelatin. The microneedle arrays were kept vertical on the top of the gelatin layer, where a compressive load of 70 g was applied for mimicking the application pressure. After the insertion process, the microneedles were examined by a digital microscope 500X. Based on their orientation in the skin, the needles were considered either upright, bent or broken to determine the mechanical strength of the microneedles.

2.7. Diffusion Transport Studies

Diffusion-controlled transport behavior was evaluated using a Franz diffusion cell system with a receptor volume of 12 mL and an effective diffusion area of 1.77 cm². Porcine skin membranes were mounted between the donor and receptor compartments. At predetermined intervals (30–300 min), receptor solution aliquots of 1mL were withdrawn and immediately replaced with equal volume of fresh PBS maintained at the same temperature as physiological condition of 37 °C and analyzed using UV–Vis spectroscopy at 271 nm. The concentration of the initial solution containing diffused ciprofloxacin decreases as a 1 mL aliquot in the donor compartment is removed and replaced with fresh PBS. Each aliquot was diluted to make up the required mark of 4 mL concentration in a quartz cuvette of 1 cm optical path length for recommended UV measurement (190–400 nm). The drug concentration was quantified using UV-VIS spectroscopy (UV-1900i Plus, Shimadzu Corporation, Kyoto, Japan) in absorbance mode at 271 nm, corresponding to the characteristic absorption maximum (max) of ciprofloxacin hydrochloride reported in previous studies [35].

2.7.1. UV-Visible Spectrophotometric Calibration of Ciprofloxacin

A calibration curve of ciprofloxacin was prepared using UV–visible spectrophotometry to quantify the drug release. A stock solution (100 µg/mL) was prepared by dissolving 10 mg of ciprofloxacin in 100 mL of phosphate-buffered saline (PBS, pH 7.5). Working standard solutions in the range 1–6 µg/mL were prepared by serial dilution of the stock solution with PBS as the diluent. The absorbance of each standard solution was measured at 271 nm against a blank solution of PBS. The calibration curve was plotted as blank-corrected absorbance against ciprofloxacin concentration. Linear regression analysis showed a high correlation between absorbance and concentration. The calibration equation was $A = 0.161C - 0.047$ with a coefficient of determination (R^2) of 0.989. This equation was used to determine the ciprofloxacin concentrations in release samples obtained during the in vitro drug release study.

Therefore, drug concentration was determined using the calibration equation

$C = \frac{A}{0.161}$, where A = absorbance, C = concentration (µg/mL).

The dilution-corrected concentration was calculated according to $C_{\text{real}} = C \times 4$.

Cumulative transport behavior was calculated using

$$Q_n = C_n V + \sum (C_p V_g)$$

Q_n = cumulative transport, V = receptor volume (12 mL), V_g = withdrawn sample volume (1 mL).

Steady state flux was determined according to $J = Q/AT$ where

J = flux (µg cm⁻² h⁻¹)

- Q = cumulative permeated drug
- A = diffusion Area
- T = diffusion time

Steady state flux permeation flux (J) was calculated from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot according to the formula stated above.

2.7.2. Kinetic Modeling

Transport kinetics were evaluated using the Higuchi model and Korsmeyer–Peppas Model to investigate diffusion-controlled transport mechanisms within the HA/ZnO nanocomposite system.

Higuchi model

- $Q = k_H \sqrt{t}$

Korsmeyer–Peppas model

- $Mt/M_\infty = kt^n$

Linearized form

$\log (Mt/M_\infty) = \log k + n \log t$, where Mt/M_∞ = fractional transport, n = transport exponent and k = kinetic constant.

2.8. Microneedle Morphology Characterization

Assessment of the morphology and structure of the synthesized HA/ZnO composite microneedles was conducted by optical microscopy and scanning electron microscopy (SEM). This evaluation considered the microneedle dimensions, their shape and sharpness, and also the uniformity of arrays.

Where N represents the total number of needles in the array (140 needles). The insertion pressure at the microneedle tip was calculated using: $P = F/A$

Where P is the pressure, F is the force per needle, and A is the contact area of the microneedle tip. The tip contact area was estimated using $A = \pi r^2$; where r represents the microneedle tip radius.

2.9. Statistical Analysis

All the data collected from characterization and performance evaluation of the HA/ZnO composite systems were statistically analyzed in order to provide reliable and reproducible results. All measurements were done in triplicate ($n = 3$) unless otherwise stated, and results are presented in terms of mean $\pm$ SD.

When needed, regression analysis was applied to estimate relations between concentration of ZnO and such properties of composites as surface roughness, mechanical strength, and insertion force, which allowed for quantitative analysis of these structure–property relationships.

3. Results

3.1. Microneedle Morphology and Geometry

In the current study, hyaluronic acid and zinc oxide nanocomposite microneedles have been developed using a micromolding method with Polydimethylsiloxane (PDMS) molds through vacuum-filling of the molds. As a result, it was possible to obtain uniform microneedles with identical geometrical shapes in terms of filling the microcavities thoroughly. Every microneedle array contained 140 microneedles distributed on 14×10 matrices as shown under SEM in Figures 1–6. These are controlled microneedles of HA only, HA/ZnO 1%, HA/ZnO 5%, HA/ZnO 7%, and HA/ZnO 10% respectively. Each microneedle had a mean length of 1500 μm and radius of tips that ranged from 18 to 23 μm and internal structure is hollow, with dimension for lumen diameter of approximately 23–30 μm and a wall thickness of 4–8 μm . Microneedles also had varying widths at the base that ranged from 253 to 272 μm , and an inter-needle distance from 1.49 to 1.54 mm at a considerable average value of 1.52 mm. Microneedles with aspect ratios greater than approximately 3–5 are generally considered high-aspect ratio structures suitable for skin penetration. The fabricated microneedles exhibited an aspect ratio of approximately 5.7 [36].

These dimensions are consistent with reported hollow microneedle architectures, where lumen diameters generally range from 5 to 70 μm depending on the fabrication method and intended applications [37].

Sharp tip radius and high aspect ratio are crucial elements that determine the effectiveness of penetration into the stratum corneum and minimize insertion force. These geometrical properties are regarded as suitable for a microneedle system since they contribute to enhanced skin permeation and structural integrity upon application.

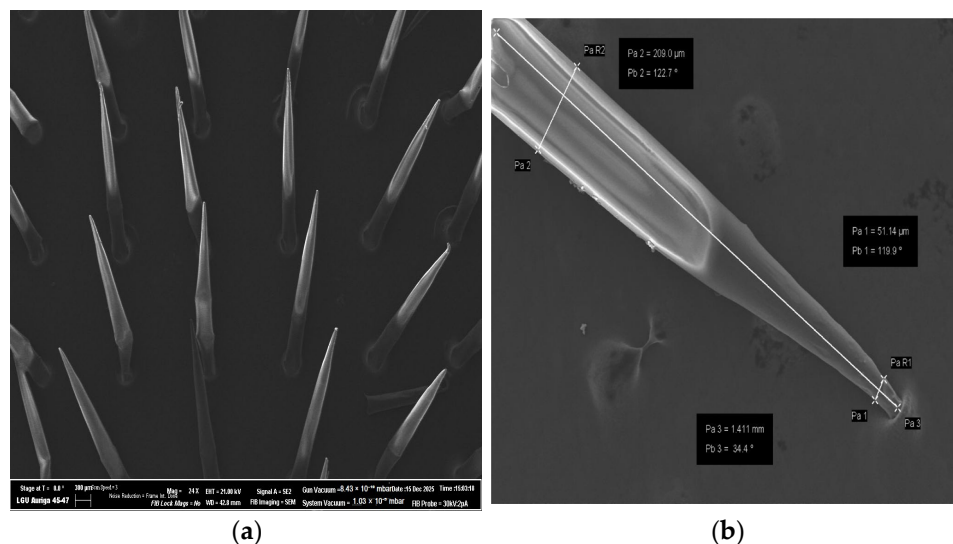


Figure 1. (a) Microneedle arrays from HA-based microneedles without zinc oxide and (b) single needle geometry of the same microneedle without zinc oxide incorporation. A representative SEM image illustrating the morphology of HA microneedles reproduced from our previous work [8] under the Creative Commons Attribution (CC BY) license.

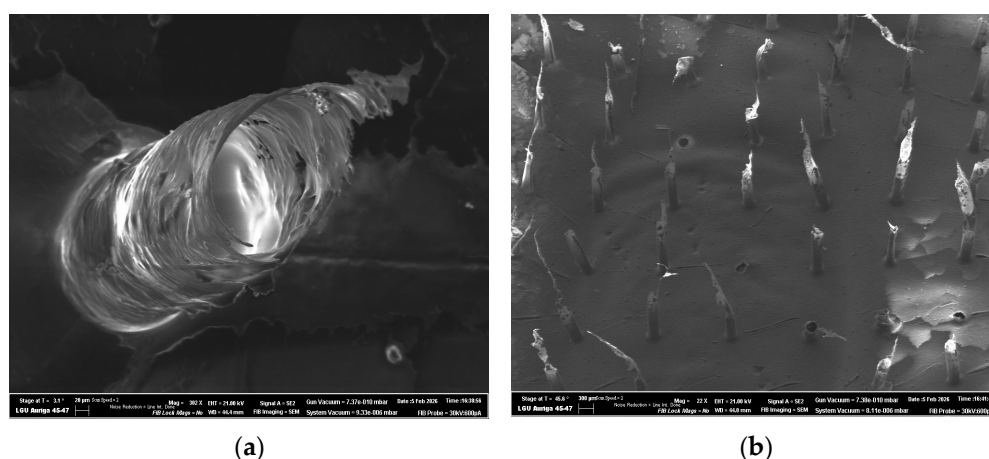


Figure 2. Shows HA = ZnO 1% under SEM observation: (a) cross-section of internal nucleation, (b) wide view of needle arrays.

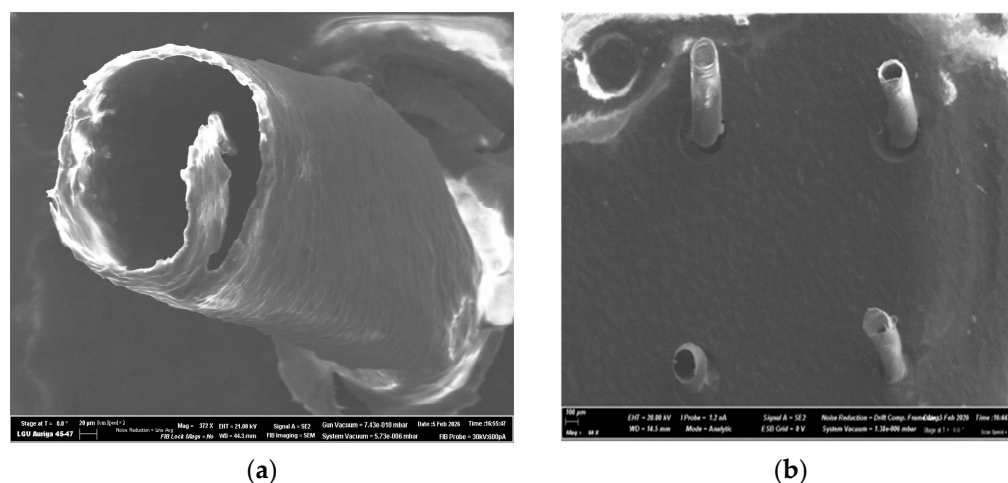


Figure 3. Shows HA = ZnO 3% under SEM observation: (a) cross-section of internal nucleation, (b) wide view of needle arrays.

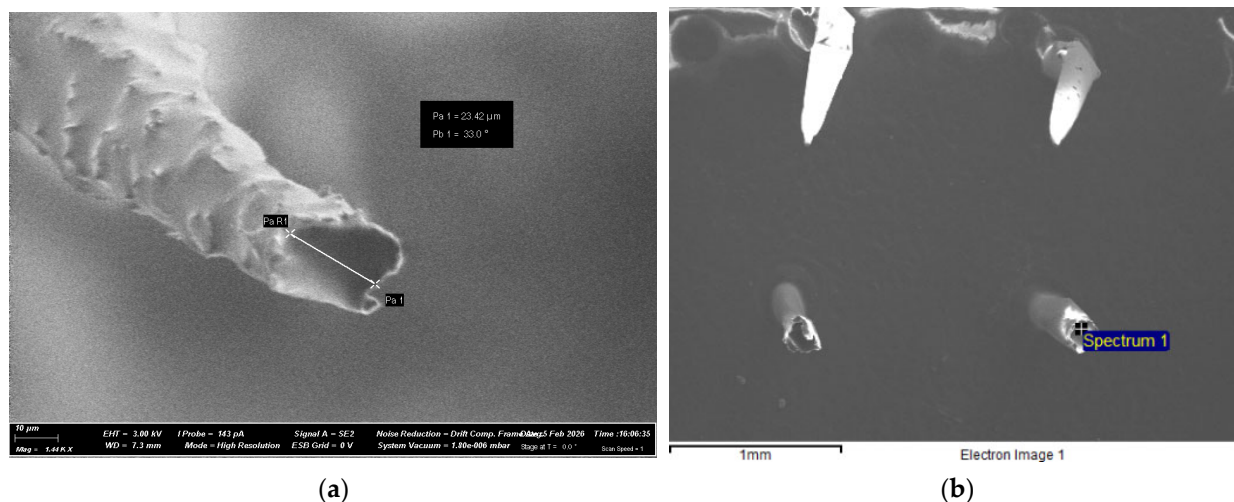


Figure 4. Illustrates the microneedle array for HA + ZnO 5%: (a) cross section, (b) tip radius measurement.

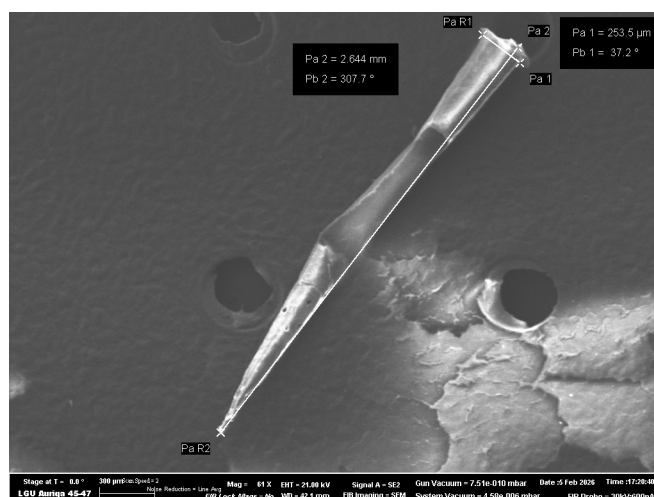


Figure 5. Shows the single microneedle dimension for HA + ZnO 7%.

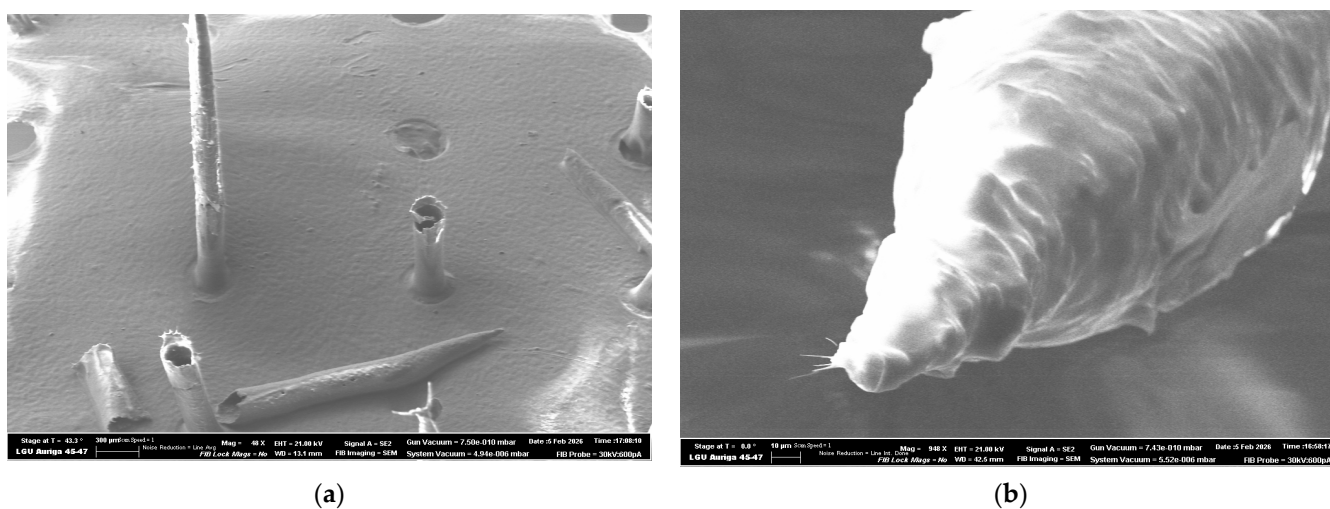


Figure 6. Illustration of HA + ZnO 10%: (a) surface area, (b) microneedle tip closer view.

Morphology analysis through scanning electron microscopy showed that the microneedles retained the same conical shape, with a smooth surface and sharp tips. Addition

of ZnO nanoparticles into HA-based material did not significantly alter the geometrical shape of the microneedles, which implies that the micromolding technique successfully transferred the geometries from the mold despite varying nanoparticle concentrations.

Surface Topography Analysis

The effects of nanoscale surface properties of the HA/ZnO composite structures have been studied using atomic force microscopy (AFM), as seen in Figure 7a–c below. Surface roughness is an important parameter for nanoparticle dispersion in a polymer matrix and material properties evaluation.

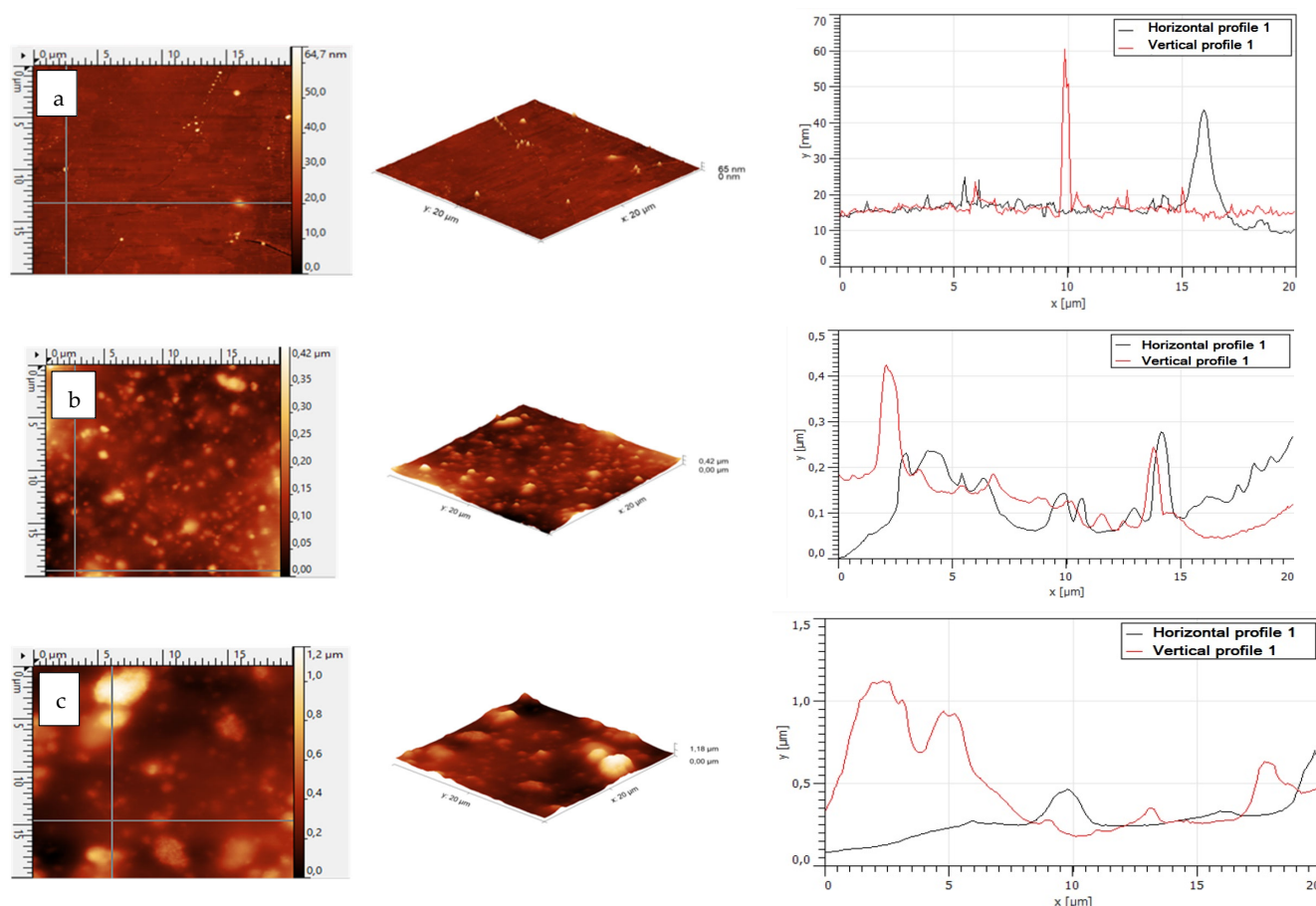


Figure 7. AFM images and height profiles of three different sample surfaces labeled (a) HA, (b) HA/ZnO5%, and (c) HA/ZnO10% composite films. Each figure includes a 2D topography image (**top**), a 3D surface rendering (**middle**), and line scan height profiles (**bottom**) with distance in micrometers (μm) on the x-axis and height in micrometers on the y-axis. Each sample was scanned over a 30 × 30 μm area. In the figure above the commas represents decimal points

The measurements of average surface roughness (R_a) have been performed for HA/ZnO composites with different ratios between nanoparticles and the matrix, as illustrated in Table 1 below. The changes in surface roughness of the samples have been observed depending on the amount of incorporated nanoparticles. Strategically the morphology was studied for HA only and HA/ZnO for 5 and 10% respectively. Zinc oxide nanoparticles modified with citric acid add nano-structuring elements to the polymer surface without causing nanoparticle agglomeration. It improves nanoparticle distribution and affects the microstructure of composites.

Table 1. AFM surface roughness parameters of HA and HA/ZnO films.

Sample	Mean Ra (nm)	SD (nm)	SEM (nm)	Coefficient of Variation (%)
HA (0.8% <i>w/v</i>)	1.92	1.21	0.61	63.1
HA/ZnO (5% <i>w/w</i>)	55.89	41.00	20.50	73.4
HA/ZnO (10% <i>w/w</i>)	122.10	97.81	48.90	80.1

Modifications of the surface roughness may affect the interfacial properties and the behavior of a composite with drugs incorporated. They can also have a certain effect on mechanical properties of microneedles.

Therefore, AFM data help to evaluate the insight into HA/ZnO composite structures and the effects of nanoparticle introduction to the polymer matrix.

AFM results show a significant increase in surface roughness with ZnO concentration (1.92 → 122.10 nm), confirming successful nanoparticle integration into the HA matrix. This increase is not merely morphological but reflects:

- Enhanced interfacial area
- Increased polymer–particle interactions
- Formation of heterogeneous microdomains

Citric acid stabilization plays a key role by modifying ZnO surface charge, improving dispersion and reducing agglomeration. At moderate loading (≈ 5 wt%), nanoparticles are likely well-dispersed within the polymer network, contributing to uniform stress distribution. At higher concentrations (≥ 7 wt%), the excessive particle content likely leads to:

- Localized stiffness heterogeneity
- Polymer chain disruption
- Reduced effective load transfer

3.2. Compression and Fracture Behavior of Microneedle Arrays

The mechanical performance of the microneedle arrays was evaluated using a progressive compression test under incremental loading from 5 g to 50 g, as seen in Figure 8a,b. The number of upright, bent and fractured needles was recorded after each load application ($n = 3$) and the after load deformation is shown in Figure 9a–e, Table 2 and the plotted stacked bar reflects the mechanical distribution in Figure 10.

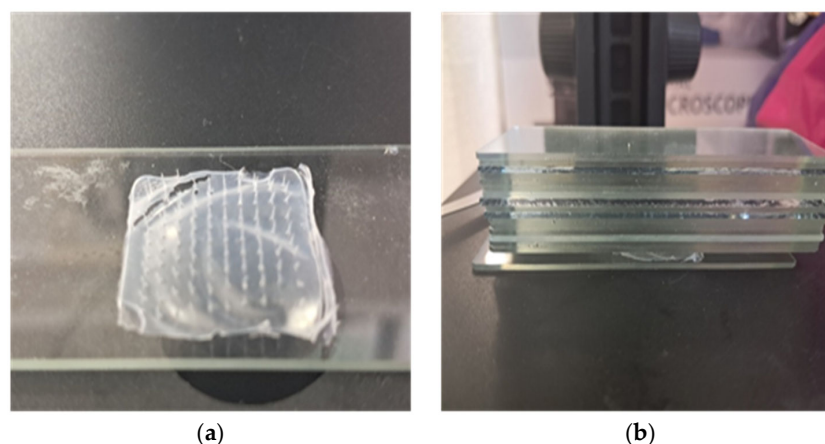


Figure 8. Shows (a) microneedle before axial loading for compression measurement and (b) manual loading of mass on the microneedle array.

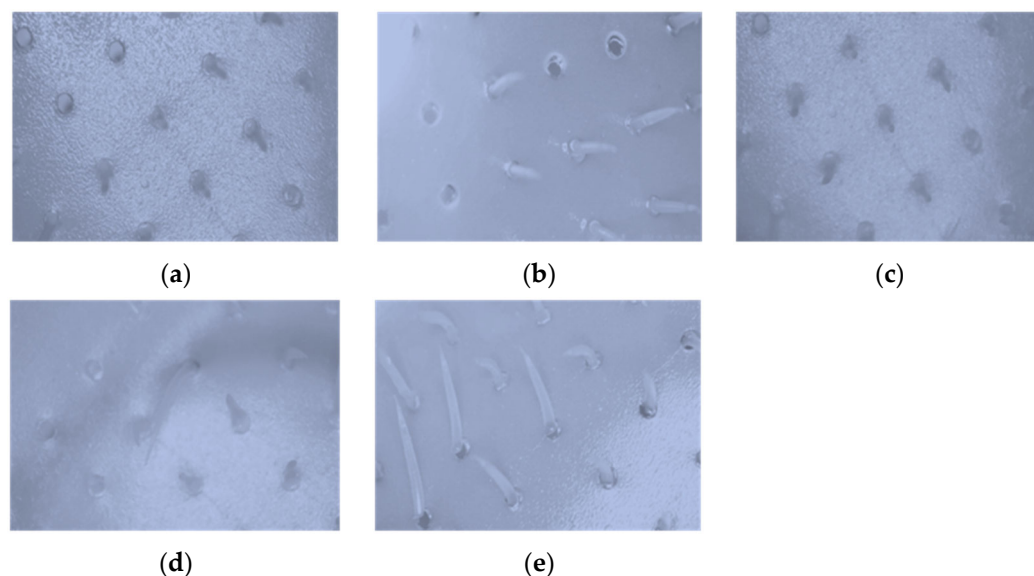


Figure 9. Shows after-effect of compression force application on each microneedle: (a) ZnO1%, (b) ZnO3%, (c) ZnO5%, (d) ZnO7% and (e) ZnO10% by digital microscope 500 $\times$.

Table 2. Compression response of HA/ZnO microneedles (mean values).

Array	ZnO (%)	Upright	Bent	Fractured	Load (g)
14 $\times$ 10	1	48 $\pm$ 2	49 $\pm$ 3	43 $\pm$ 2	50
14 $\times$ 10	3	51 $\pm$ 3	33 $\pm$ 2	56 $\pm$ 3	50
14 $\times$ 10	5	66 $\pm$ 2	38 $\pm$ 3	36 $\pm$ 2	50
14 $\times$ 10	7	41 $\pm$ 3	40 $\pm$ 2	46 $\pm$ 3	50
14 $\times$ 10	10	42 $\pm$ 2	56 $\pm$ 3	43 $\pm$ 2	50

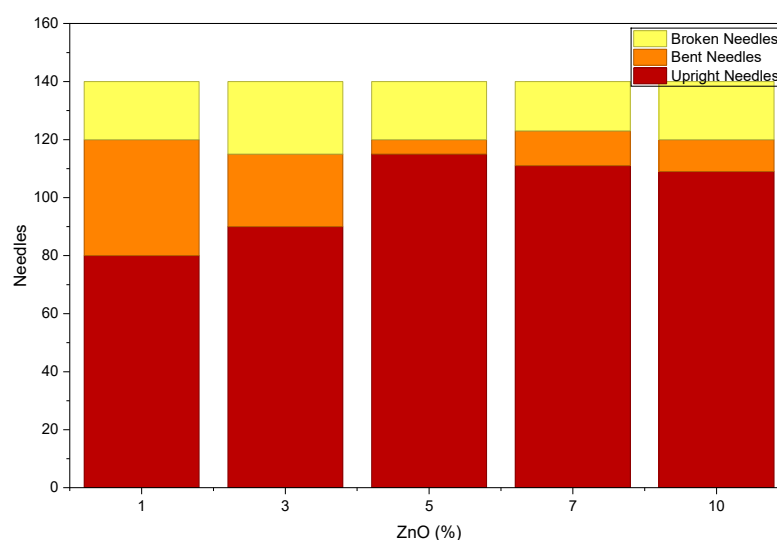


Figure 10. Mechanical failure distribution of HA/ZnO microneedle arrays under 50 g axial load; data are represented as mean $\pm$ SD ($n = 3$).

The tabulated compression of Table 2: response of the HA/ZnO microneedle shows the mean values in response to axial load application, which resulted in resistance, bending and fracture. The deformation was counted for each microneedle array and placed in the three specified categories, as the present study simply focused on categorized sections

within each array. Spatial mapping of failure locations, either at the edge or within the arrays, was not performed. This is recommended for future research.

Based on the findings, the microneedles deformed mainly by bending instead of sudden breakage, which suggests that the composite structure of HA/ZnO has the ability to absorb stresses through elastic deformation. To examine the structural robustness of HA/ZnO microneedle array structures, the samples were subjected to mechanical loads up to 50 g. The data have been categorized into three groups: upright needles, bent needles, and fractured needles, as shown in Figure 6 in the form of a stacked bar graph. In view of these findings, no further statistical analysis was performed for this dataset.

3.2.1. Mechanical Integrity and Fracture Ratio Analysis

To quantitatively assess mechanical performance, mechanical integrity and fracture ratio were calculated as follows and summarized in Table 3, and visual interpretation in Figure 11 below.

$$\text{Mechanical Integrity (\%)} = \frac{\text{Number of Upright Needles}}{\text{Total Number of Needles}} \times 100$$

$$\text{Fracture Ratio (\%)} = \frac{\text{Number of Fractured Needles}}{\text{Total Number of Needles}} \times 100$$

where $N_{\text{total}} = N_{\text{upright}} + N_{\text{bent}} + N_{\text{fractured}}$; where N_{upright} is the number of upright microneedles after compression testing, $N_{\text{fractured}}$ is the number of fractured microneedles, and N_{total} is the total number of microneedles in the array (140 needles) [38].

Table 3. Mechanical integrity and fracture ratio (mean $\pm$ SD, $n = 3$).

ZnO (%)	Mechanical Integrity (%)	Fracture Ratio (%)
1	34 $\pm$ 1.4	30.95 $\pm$ 1.2
3	36.43 $\pm$ 1.6	40.00 $\pm$ 1.5
5	47.38 $\pm$ 1.2	25.48 $\pm$ 1.3
7	29.05 $\pm$ 1.5	32.62 $\pm$ 1.4
10	29.76 $\pm$ 1.3	30.48 $\pm$ 1.2

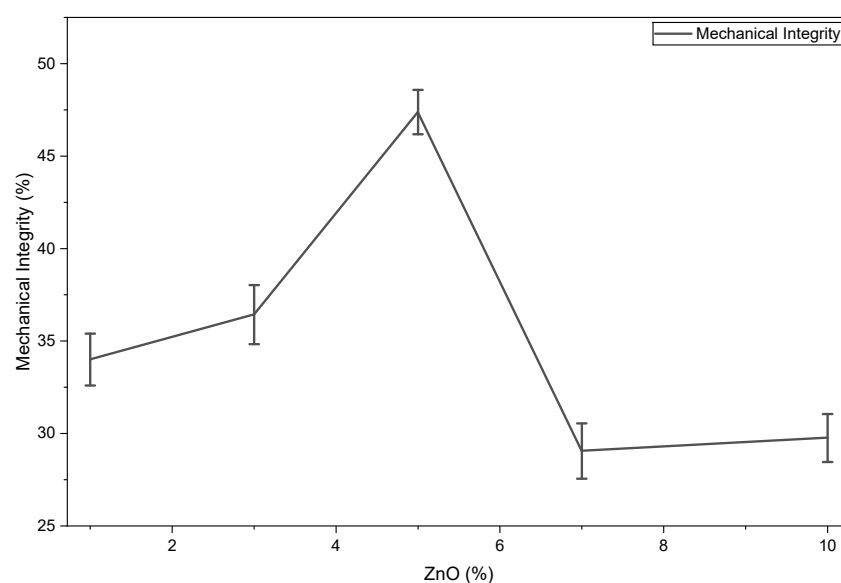


Figure 11. Effect of ZnO concentration on mechanical integrity of HA microneedles.

There was an increase in mechanical strength of the microneedles with the inclusion of 5% of zinc oxide nanoparticles, thus implying that the HA matrix had been effectively strengthened by the nanoparticles. However, beyond this quantity, there was a marked decline in mechanical strength. It was also not observed through scanning electron microscopy images that there had been any significant agglomeration of the nanoparticles, and therefore it is reasonable to assume that the reason for such a drop in strength is related to the breakdown of the HA polymer structure due to excessive nanoparticle content.

3.2.2. Coefficient of Variation and Data Reliability

The reliability of the mechanical data was assessed using the coefficient of variation (CV) as shown in Table 4

$$CV (\%) = \frac{SD}{Mean} \times 100$$

Table 4. Coefficient of variation (CV%) of mechanical parameters.

ZnO (%)	Mechanical Integrity CV (%)	Fracture Ratio CV (%)
1	4.08	3.87
3	4.39	3.75
5	2.53	5.10
7	5.16	4.29
10	4.37	3.94

All CV values remained below 5–6%, confirming good reproducibility of the compression testing and reliability of the dataset.

3.2.3. Reinforcement Effect of ZnO Nanoparticles

To evaluate the reinforcing effect of ZnO, a mechanical reinforcement factor (MFR) was calculated using the 1% ZnO formulation as a reference, as summarized in Table 5 and the bar chart in Figure 12.

$$MFR = \frac{\text{Mechanical Integrity}}{\text{Mechanical integrity 1\%}}$$

Table 5. Reinforcement factor of ZnO nanoparticles.

ZnO%	Reinforcement Factor
1	1.00
3	1.06
5	1.38
7	0.85
10	0.87

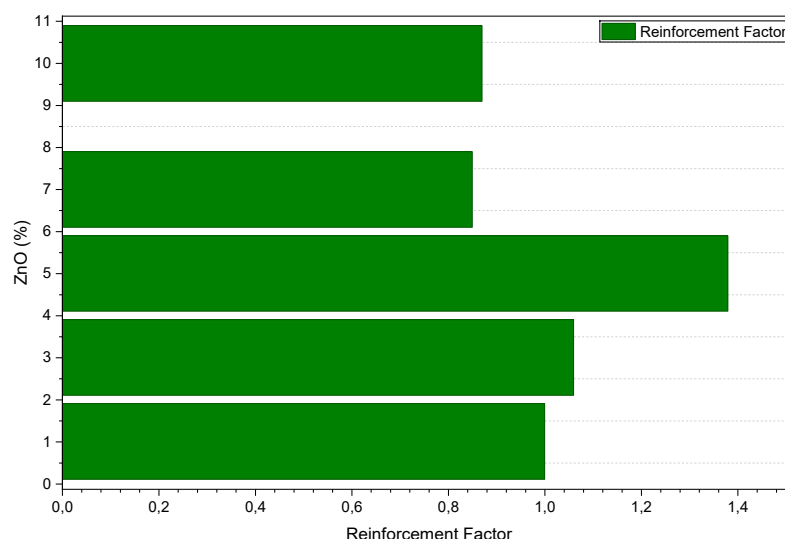


Figure 12. ZnO nanoparticles reinforcement effect on HA microneedles.

The maximum reinforcement was observed at 5% ZnO (MFR = 1.38), indicating a 38% improvement in mechanical integrity compared to the baseline formulation. This enhancement is attributed to improved ZnO-HA interaction and uniform nanoparticle dispersion, which strengthens the polymer matrix and enhances load distribution.

3.3. Microneedle Insertion Test and Mechanical Safety Factor

3.3.1. Insertion Performance on Artificial Skin

The insertion ability and mechanical stability of HA/ZnO microneedles were evaluated using a gelatin artificial skin model. Typically, gelatin substrates are employed in artificial skin studies due to their similar mechanical properties compared to human skin stratum corneum and dermis. Load of 70 g was uniformly applied to the microneedle array at incremental bases from 5 g for 12 s per load as described in Figure 13, to simulate the pressure applied manually. Force calculation is based on Newton's second law.

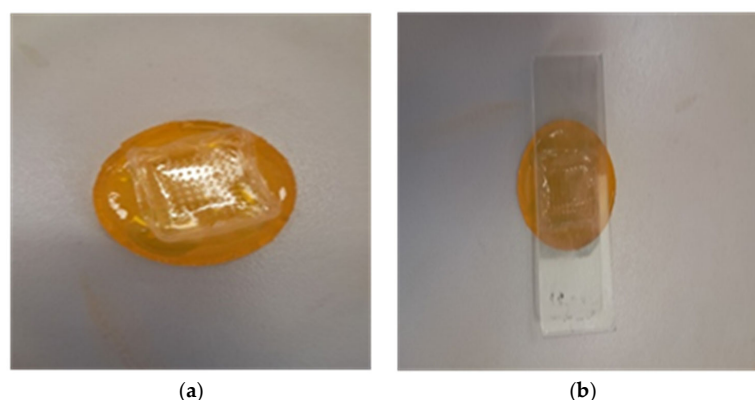


Figure 13. Describes the insertion test using gelatin skin model: (a) insertion in gelatin, (b) axial load placed on the microneedle to force deep insertion.

$F = m \times g$; where F = applied force (N); m = mass (g); g = gravitational acceleration (9.81 m/s^2) $F = 0.07 \times 9.81 = 0.6867 \text{ N}$.

Thus, the total compressive force applied to the microneedle array was approximately 0.687 N. Since the array contained 140 microneedles, the estimated force distributed per needle was: $F_{\text{needle}} = 0.6867/140$; $F_{\text{needle}} \approx 0.0049 \text{ N}$ (4.9 mN). After insertion, the microneedles were examined under an optical microscope and categorized as upright (successfully

inserted), bent, or fractured to evaluate structural integrity, as seen in Figure 14a–e. The after-effect of the insertion test under a digital microscope that shows the fractured and bent microneedles is illustrated in Figure 15a–e. The summary of the mechanical integrity after compression can be seen as tabulated in Table 6.

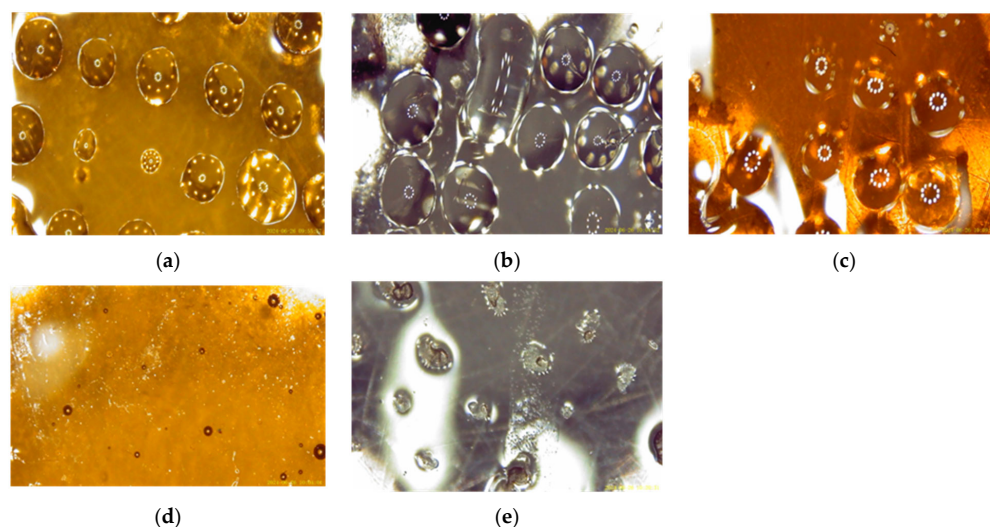


Figure 14. Morphology of HA-ZnO microneedles during gelatin insertion test for 12 s. With axial load of 70g for each microneedle: (a) HA/ZnO 1%, (b) HA/ZnO 3%, (c) HA/ZnO 5%, (d) HA/ZnO 7%, and (e) HA/ZnO 10% by digital microscope 500 $\times$.

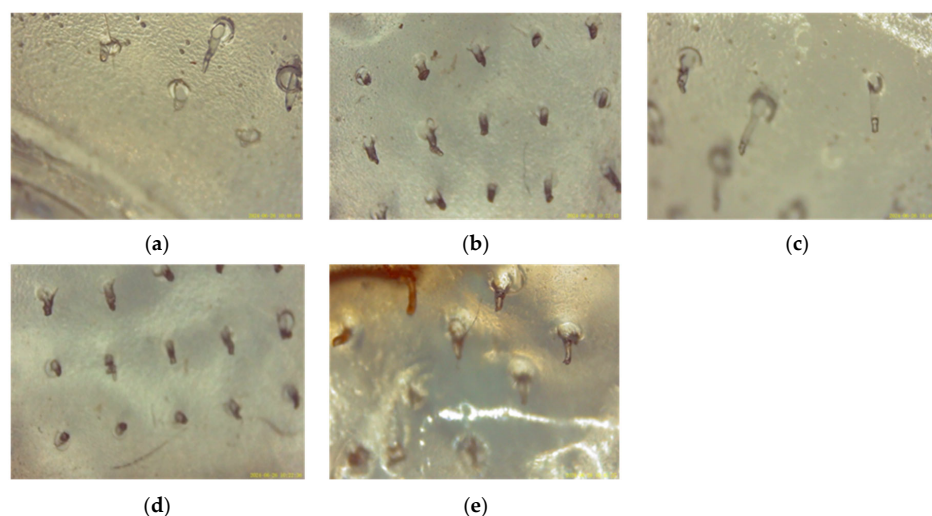


Figure 15. Morphology of HA-ZnO microneedles after insertion test for 12 s with axial load of 70 g: (a) HA/ZnO 1%, (b) HA/ZnO 3%, (c) HA/ZnO 5%, (d) HA/ZnO 7%, and (e) HA/ZnO 10% by digital microscope 500 $\times$.

Table 6. Mechanical integrity results.

ZnO %	Upright	Bent	Fractured	Upright (%)
1	80	40	20	57.14
3	90	25	25	64.29
5	109	11	20	77.86
7	111	12	17	79.29
10	115	5	20	82.14

It is important to note that penetration behavior is influenced not only by microneedle geometry and mechanical strength but also by mechanical characteristics of the substrate, including gelatin concentration, stiffness, and viscoelastic properties. Furthermore, the loading rate (application velocity) can affect the measured insertion force, as higher insertion velocities may alter the deformation and fracture behavior of the gelatin layer, potentially reducing the force required for penetration. In the present study, the insertion tests were conducted under constant loading conditions; however, the influence of different application velocities was not systematically investigated.

Theoretical consideration of HA/ZnO hollow microneedles (AFM–mechanical correlation):

The observed insertion behavior can be further interpreted in terms of the effective mechanical response of the HA/ZnO composite system, which is influenced by the nanoscale dispersion and interfacial interactions identified in the AFM analysis. The resistance of hollow microneedles to elastic instability under compressive insertion loading can be approximated using Euler buckling theory:

$$P_{cr} = \pi^2 E_{eff} I / (kL)^2$$

where E_{eff} represents the effective modulus of the HA/ZnO composite governed by nanoparticle dispersion and interfacial adhesion, I is the second moment of area (reduced due to the hollow architecture), L is the effective needle length, and K is the column effective length factor reflecting boundary conditions during insertion.

From the AFM-derived microstructural observations, improved ZnO dispersion and enhanced interfacial compatibility are expected to increase E_{eff} , thereby improving structural rigidity of the microneedle matrix. This is consistent with the experimentally observed reduction in bending fraction at higher ZnO loadings, indicating improved resistance to elastic deformation during insertion. However, the system remains sensitive to off-axis loading due to its high aspect ratio geometry, which may still promote localized bending prior to failure.

Therefore, the reported insertion force is interpreted within the context of the specific experimental conditions employed as seen in Figure 16. Future studies should evaluate the effect of insertion velocity and substrate mechanical properties to better replicate in vivo skin insertion behavior.

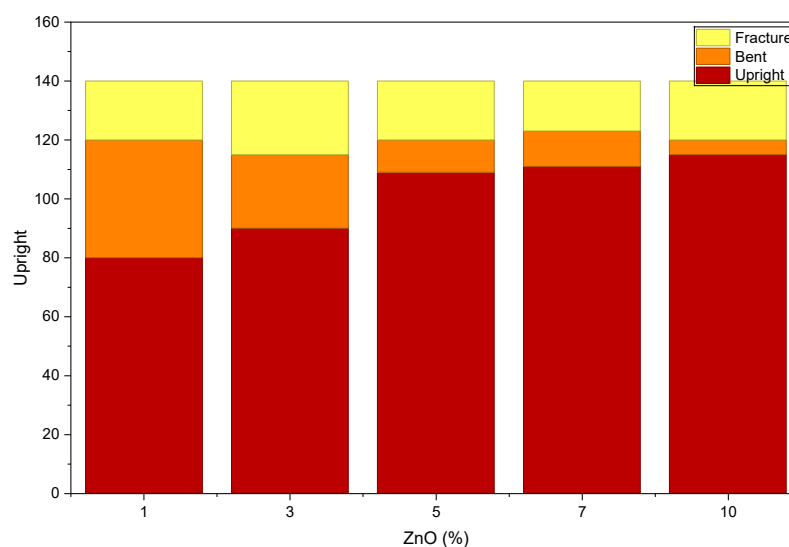


Figure 16. Mechanical integrity of HA/ZnO microneedle arrays after insertion testing under a 70 g compressive load; data are represented as mean $\pm$ SD ($n = 3$).

3.3.2. Factor of Safety Analysis

The mechanical reliability of microneedles is often assessed using factor of safety (FoS) = $F_{\text{fracture}}/F_{\text{insertion}}$.

The insertion forces required for polymeric microneedles to pierce through the skin fall in the range of 0.058–0.1 N/needle. Moreover, the high number of upright microneedles post-insertion supports the assertion that the applied force was enough for piercing the gelatin artificial skin.

3.3.3. Microneedle Insertion Pressure Analysis

For the purpose of evaluating the ability of the fabricated HA/ZnO composite microneedles to penetrate the skin, the pressure exerted at the tip of the microneedles was evaluated. Penetration into the skin depends on both the force that is applied and the pressure at the tip of the needle. In the process of insertion, the microneedle array was subjected to a vertical force of 70 g, which acted on the microneedles attached to the artificial skin made from gelatin. Therefore the inserted pressure at the microneedle tip was calculated using $P = F/A$: where P represents the insertion pressure (pa), F is the force applied per needle (N), and A is the microneedle tip contact area (m^2). The microneedles fabricated in this study exhibited a tip radius ranging from 18 to 23 μm . For the minimum tip radius $A = \pi r^2$; for tip radius of 18 μm , the contact area is $1.02 \times 10^{-9} \text{ m}^2$; and for 23 μm , the contact area is $1.66 \times 10^{-9} \text{ m}^2$. Hence, $P = 3.0\text{--}4.8 \text{ Mpa}$.

Therefore, despite the force distributed per needle, the small tip radius of the microneedle enabled the generation of sufficient localized stress to puncture the artificial skin substrate. This is shown as a bar chart in Figure 17 [32,39].

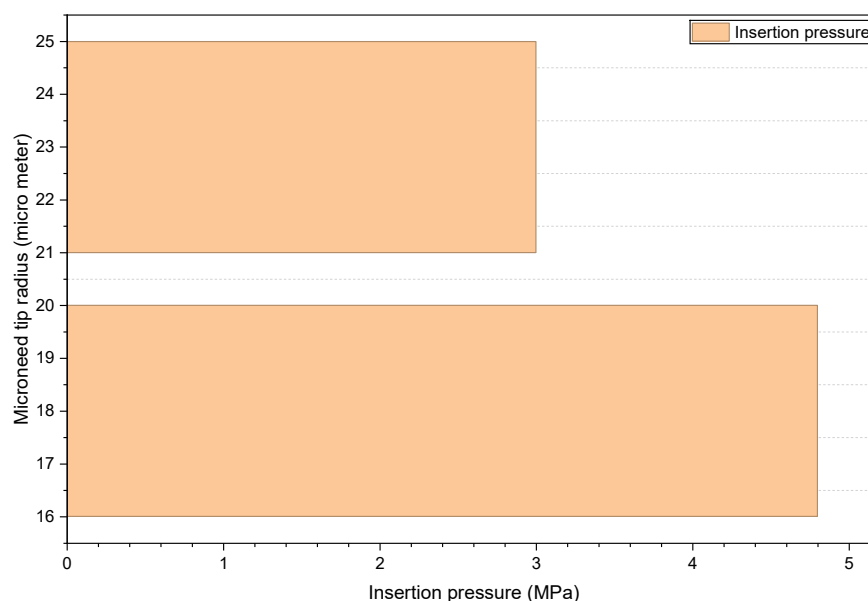


Figure 17. Estimated insertion pressure at the microneedle tip based on experimentally measured tip radius of 18 μm and 23 μm .

3.3.4. Structure–Mechanical Performance Relationship

A clear relationship between structure and mechanical performance was observed

- ZnO concentration governs polymer reinforcement and nanoparticle dispersion
- Needle density governs load distribution
- Mechanical performance is maximized at 5% ZnO with optimized geometry

3.4. Diffusion-Controlled Transport Behavior

3.4.1. Calibration Curve of Ciprofloxacin

The UV–Vis absorption spectra of ciprofloxacin exhibited a prominent absorption maximum at 271 nm (Figure 18), which was utilized for subsequent quantitative analyses. The calibration curve for ciprofloxacin demonstrated a linear correlation between concentration and absorbance within the examined range of 1–6 µg/mL (Figure 19). The linear regression analysis yielded the equation $A = 0.161C - 0.047$, with a coefficient of determination ($R^2 = 0.989$), signifying exceptional linearity and suitability of the analytical approach for the quantitative assessment of ciprofloxacin. The enhanced coefficient of determination verifies that the variations in absorbance were directly proportional to the alterations in drug concentration within the designated range. Consequently, the calibration model was deemed enough reliable for the following determination of ciprofloxacin concentrations during in vitro release assays of the HA/ZnO microneedle formulations. The UV–Vis absorption spectra of ciprofloxacin exhibited a prominent absorption maximum at 271 nm (Figure 18), which was utilized for subsequent quantitative analyses. The calibration curve for ciprofloxacin demonstrated a linear correlation between concentration and absorbance within the examined range of 1–6 µg/mL (Figure 15). The linear regression analysis yielded the equation $A = 0.161C - 0.047$, with a coefficient of determination ($R^2 = 0.989$), signifying exceptional linearity and suitability of the analytical approach for the quantitative assessment of ciprofloxacin. The elevated coefficient of determination verifies that the variations in absorbance were directly proportional to the alterations in drug concentration within the designated range. Consequently, the calibration model was deemed enough reliable for the following determination of ciprofloxacin concentrations during in vitro release assays of the HA/ZnO microneedle formulations.

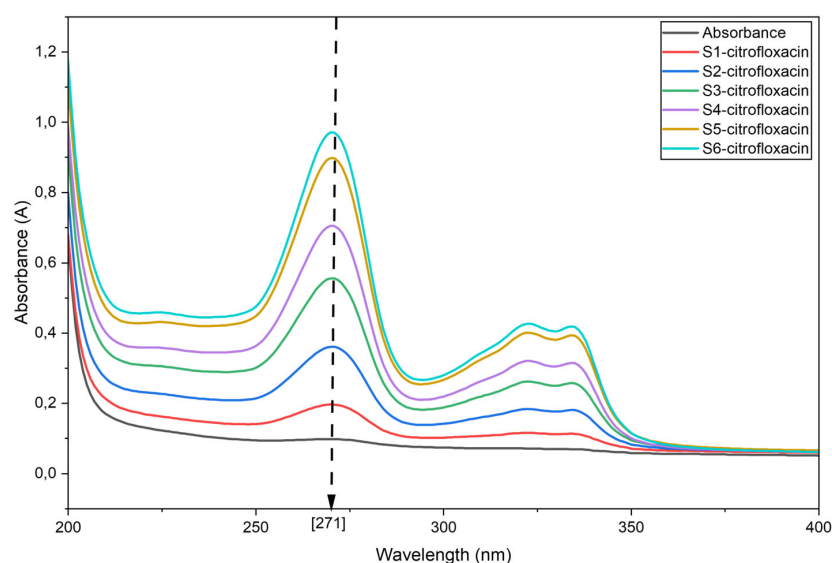


Figure 18. UV-Vis absorption spectrum of ciprofloxacin in PBS (pH 7.5) showing a characteristic absorption maximum ($\lambda_{\max}$) at 271 nm, which was selected for quantitative analysis during drug release studies.

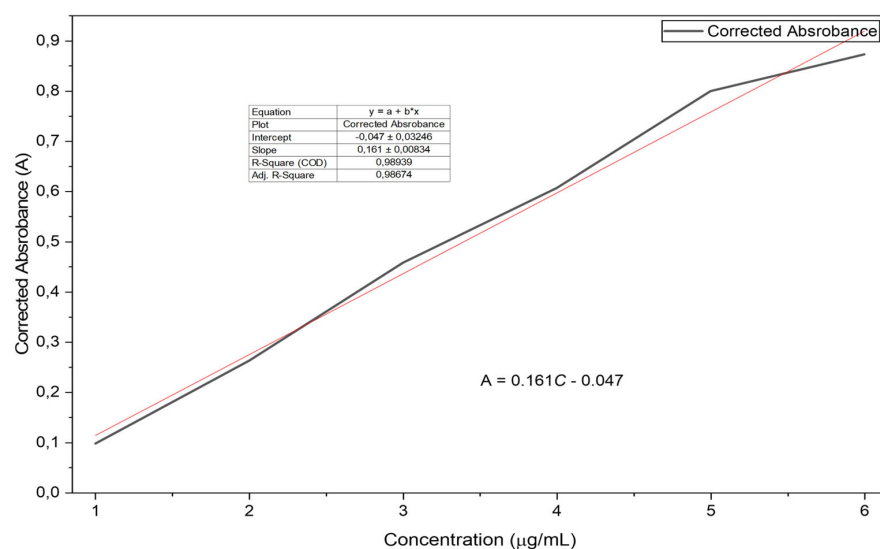


Figure 19. Calibration curve of ciprofloxacin in PBS (pH 7.5) at 271 nm showing the relationship between corrected absorbance and drug concentration (1–6 µg/mL). The linear regression analysis yielded the equation $A = 0.161C - 0.047$ with $R^2 = 0.989$.

3.4.2. In Vitro Release Profiles

The in vitro release behavior of ciprofloxacin from HA/ZnO composite microneedles was studied in phosphate-buffered saline (PBS, pH 7.5) for 300 min as described in Figure 20a–e. The cumulative release profiles showed the significant effect of the concentration of ZnO nanoparticles on the diffusion and transport of ciprofloxacin through the hydrated HA matrix. All formulations showed a time-dependent increase in the cumulative drug release, indicating the progressive diffusion of ciprofloxacin from the network of microneedles into the receptor medium. The HA/ZnO-3% formulation showed the second highest cumulative release (859.4 µg), followed by the HA/ZnO-1% formulation (689.6 µg). On the other hand, HA/ZnO-5% and HA/ZnO-7% formulations showed a relatively lower cumulative release of 587.9 µg and 582.8 µg respectively as shown in Table 7. The release profiles showed an initial burst release followed by a slower release stage, which is a typical characteristic of diffusion-controlled transport in hydrophilic polymer systems. The HA matrix hydrated and swelled when immersed in PBS, which enabled the diffusion of ciprofloxacin into the surrounding medium. Variations in the internal microstructure of the composite network with different ZnO concentrations affected the diffusional resistance and drug transport pathways. The increased ZnO loading was observed to enhance the overall drug release, while intermediate concentrations of ZnO showed a more limited release, implying a higher resistance to molecular diffusion within the matrix. The data presented here suggest that the concentration of ZnO nanoparticles can be efficiently used to control the transport of ciprofloxacin from the HA-based microneedles. The concentration-dependent release behavior observed indicates the potential of HA/ZnO composite microneedles as tunable transdermal drug delivery systems for delivery of controlled and sustained antibiotic release.

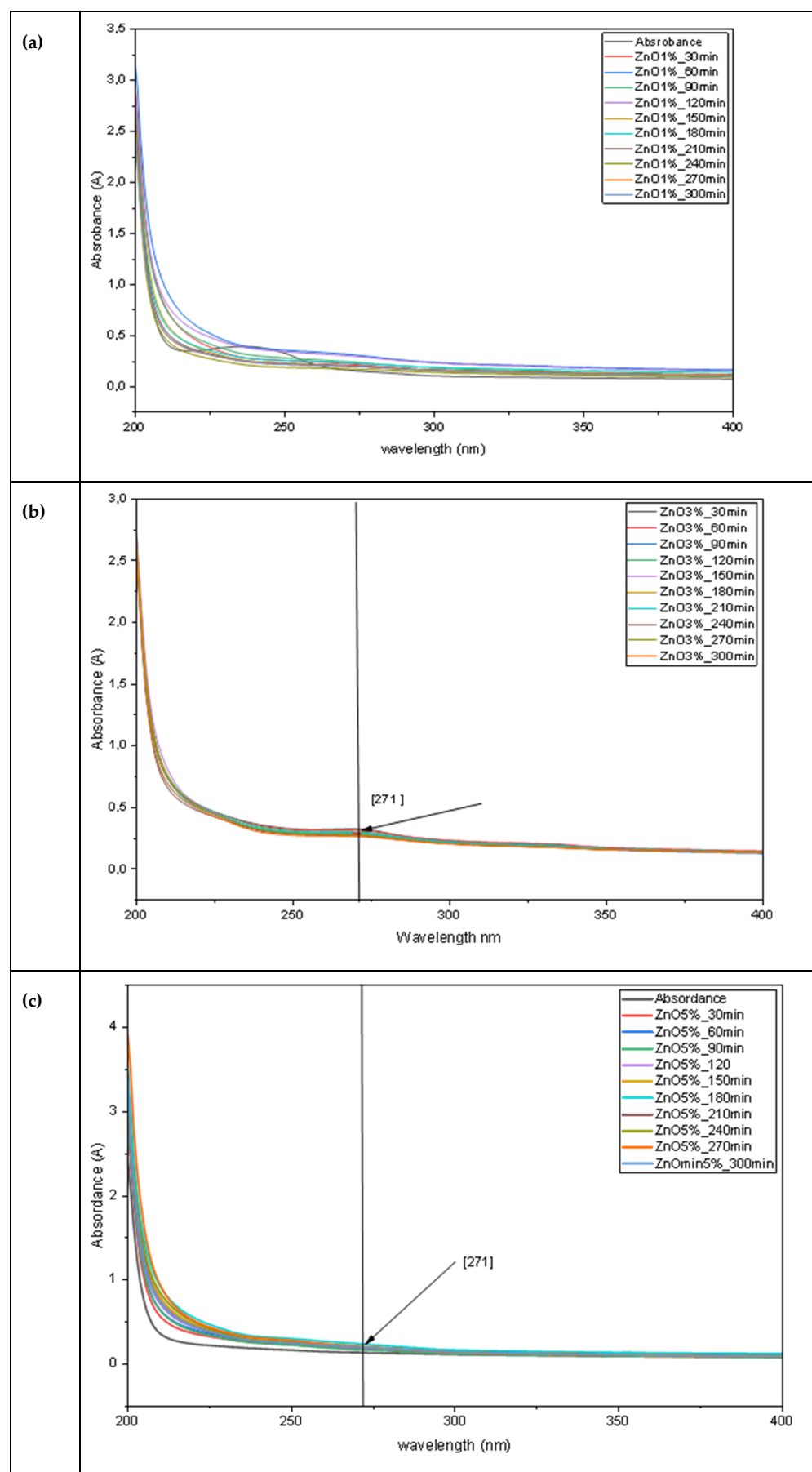


Figure 20. Cont.

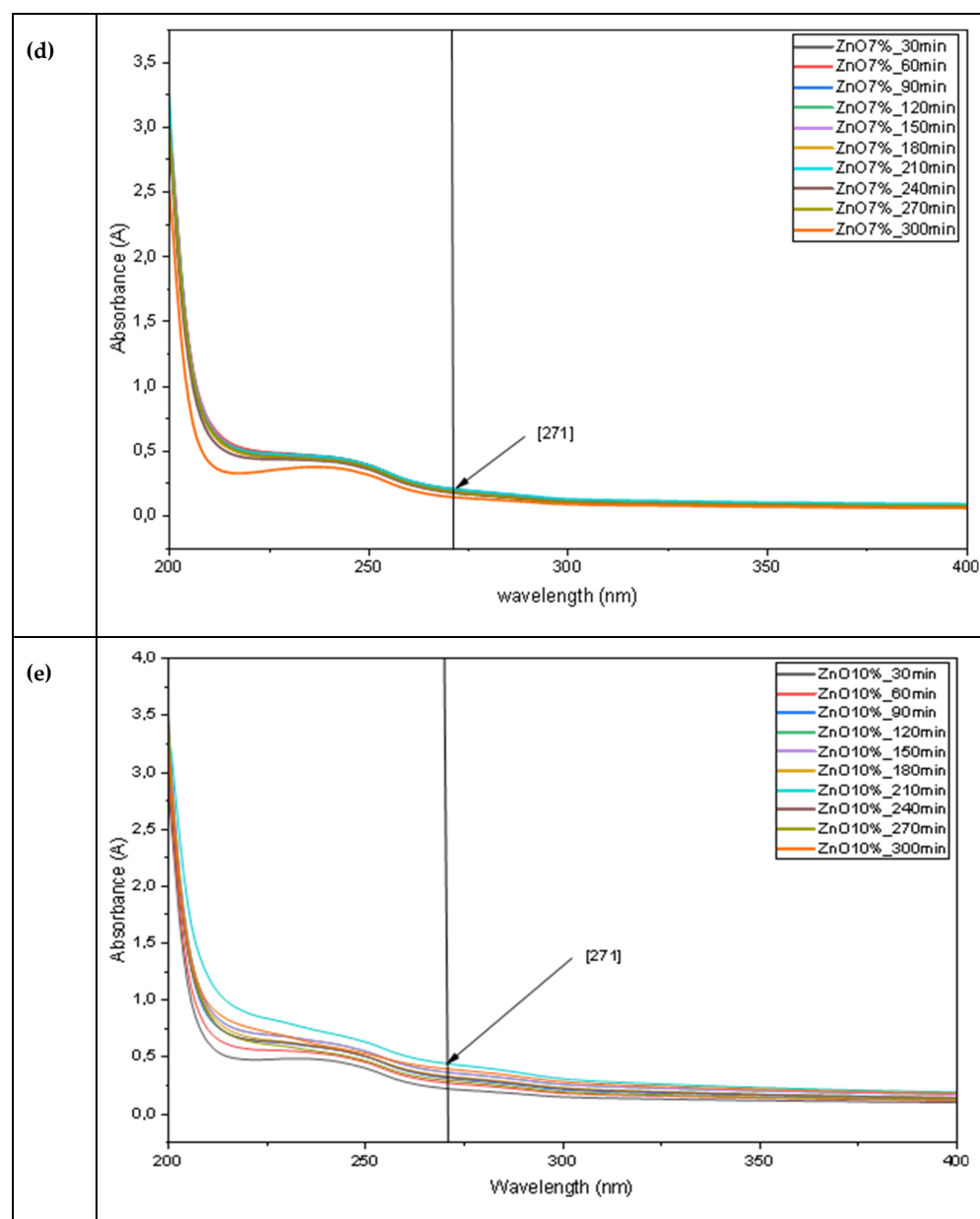


Figure 20. (a) In vitro ciprofloxacin release profile of HA/ZnO-1% composite microneedles in PBS (Ph 7.5) at 37 °C over 300 min, (b) Wavelength In vitro ciprofloxacin release profile of HA/ZnO-3% composite microneedles in PBS (Ph 7.5) at 37 °C over 300 min, (c) In vitro ciprofloxacin release profile of HA/ZnO-5% composite microneedles in PBS (Ph 7.5) at 37 °C over 300 min, (d) In vitro ciprofloxacin release profile of HA/ZnO-7% composite microneedles in PBS (Ph 7.5) at 37 °C over 300 min, and (e) In vitro ciprofloxacin release profile of HA/ZnO-10% composite microneedles in PBS (Ph 7.5) at 37 °C over 300 min.

Table 7. Cumulative ciprofloxacin release from HA/ZnO composite microneedle after 300 min.

Formulation	ZnO Concentration (%)	Cumulative Release (µg)
HA/ZnO-1%	1	689.6
HA/ZnO-3%	3	859.4
HA/ZnO-5%	5	587.9
HA/ZnO-7%	7	582.8
HA/ZnO-10%	10	971.4

3.4.3. Effect of ZnO Concentration on Drug Transport

Transport studies performed using the Franz diffusion cell with porcine skin as seen in Figure 21a,b revealed that the concentration of ZnO had a pronounced influence on diffusion behavior in hydrated HA/ZnO nanocomposite microneedles. Specifically, formulations of 1% and 3% ZnO exhibited controlled and regulated transport behaviors, indicating that a lower concentration of nanoparticles in such systems promoted higher matrix homogeneity and continuous diffusion pathways via hydration effects, thus reducing transport irregularities caused by nanoparticle agglomeration.

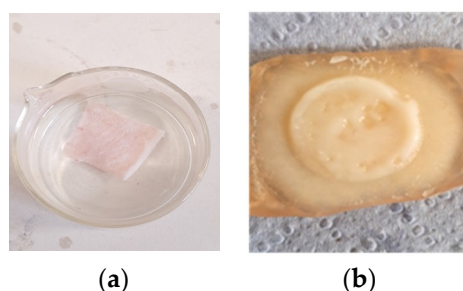


Figure 21. (a) Shows the porcine skin of equal size and thickness 1–1.5 mm in soaked PBS before and (b) after insertion in Franz diffusion cell.

It should be emphasized that diffusion transport in nanocomposites at intermediate levels of ZnO concentration (5–7%) was characterized by a slower rate due to the formation of tortuous diffusion pathways as a result of incorporation of ZnO nanoparticles into hydrated HA matrices, see Figure 22. Nanoparticle domains can be viewed as intermediary barriers preventing rapid molecular diffusion and promoting diffusion-controlled rather than dissolution-mediated drug release.

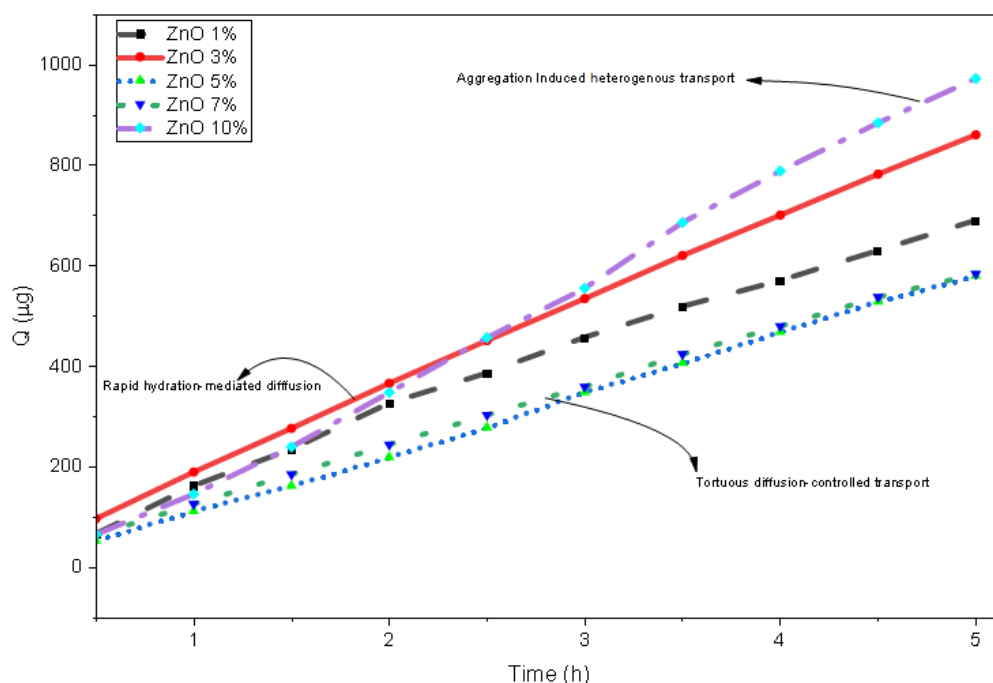


Figure 22. Cumulative diffusion transport profiles of HA/ZnO nanocomposite microneedles. Lower ZnO concentrations (1–3%) exhibited rapid hydration-mediated diffusion, while intermediate concentrations (5–7%) demonstrated sustained transport associated with tortuous diffusion pathways. The 10% ZnO formulation exhibited aggregation-induced heterogeneous transport characterized by accelerated permeation behavior.

However, it is worth noting that increased concentration of nanoparticles (10%), despite being expected to contribute to diffusion tortuosity, resulted in accelerated transport behavior with increased permeation fluxes in the system, see Figure 23 and Table 8 respectively. The observed effect can be associated with an excess nanoparticle content exceeding the ability of the matrix to sustain stable nanoparticle dispersion, thereby causing localized agglomerates disrupting the continuous polymer structure.

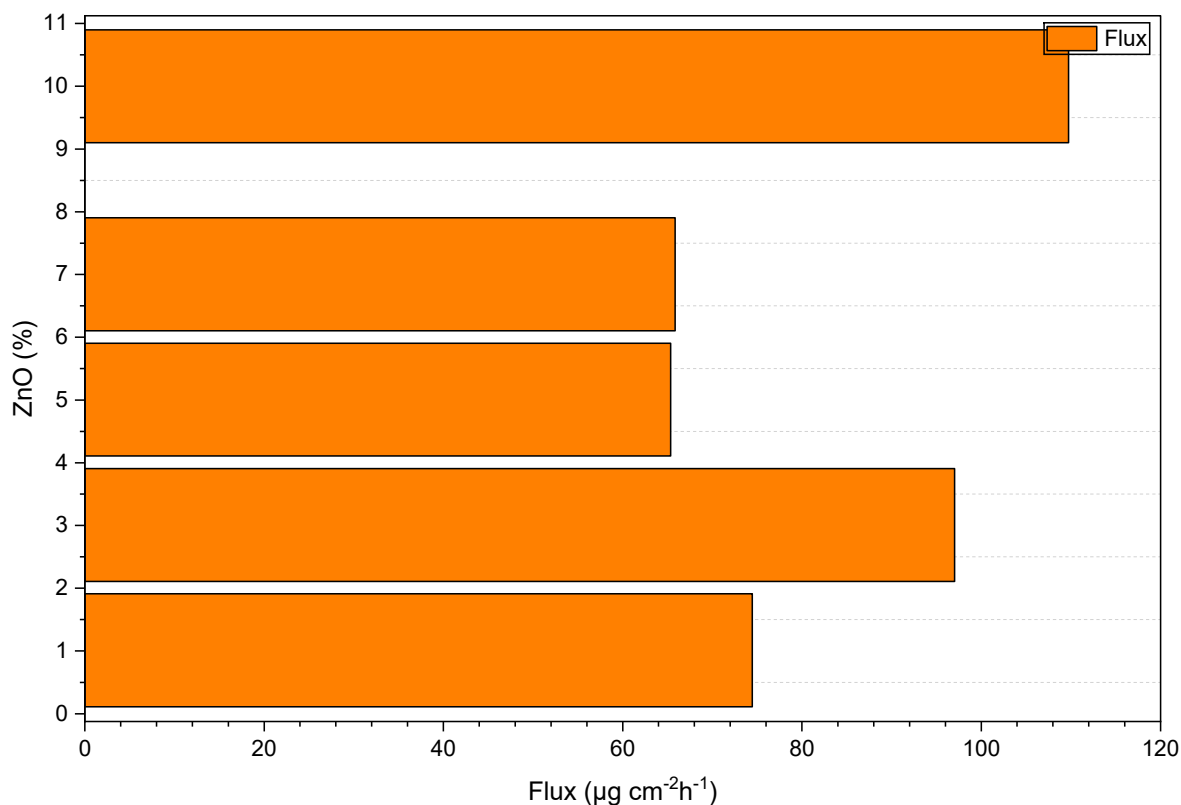


Figure 23. Comparison of permeation flux values of HA/ZnO nanocomposite microneedles as a function of ZnO concentration. Intermediate ZnO concentrations (5–7%) exhibited reduced flux associated with tortuous diffusion-controlled transport, while the 10% ZnO formulation demonstrated accelerated permeation behavior due to aggregation-induced heterogeneous transport pathways.

Table 8. Cumulative transport and flux parameters of HA/ZnO nanocomposites.

Formulation	Final Cumulative Transport Q (μg)	Flux J ($\mu\text{g cm}^{-2}\text{h}^{-1}$)	Transport Behavior
HA/ZnO 1%	661.5	74.7	Moderate diffusion transport
HA/ZnO 3%	859.4	97.1	Stable and controlled transport
HA/ZnO 5%	579.2	65.4	Sustained diffusion-controlled transport
HA/ZnO 7%	582.8	65.9	Sustained transport with increased tortuosity
HA/ZnO 10%	971.4	109.8	Highest transport but heterogeneous diffusion

Thus, the formation of heterogeneous diffusion regions accompanied by the emergence of void-like pathways can provide conditions favorable for rapid diffusion through matrix domains. As a result, the current nanocomposite formulation with the highest nanoparticle

concentration can be viewed as a shift from controlled diffusion transport to heterogeneous transport facilitated by nanoparticle agglomeration.

At the same time, microneedles containing intermediate concentration of nanoparticles provided the optimal balance between the nanoparticle dispersion level in the polymer matrix and diffusion pathway organization, enabling diffusion-controlled transport.

Comparing the current approach of loading the therapeutic agent in microneedles with previously described methods of drug delivery utilizing surface coating, dipping, dropping, or tip-loading microneedle architectures, the presented HA/ZnO nanocomposite hollow dissolving microneedles provide some unique advantages in terms of fabrication, transport behavior, and drug distribution.

In general, microneedles used in previous microneedle drug-delivery strategies were designed via coating the therapeutic agent onto needle surfaces and thus involved post-fabrication steps relying heavily on surface interactions and interfacial compatibility. For example, in previous microneedle coating studies involving the use of capillary interactions for transferring drug solutions to the needle surfaces, the drug localization in such systems was strictly defined by the capillary filling processes and drug coating uniformity [40].

Similarly, tip-loaded dissolving microneedles fabricated through multilayer casting methods localized the therapeutic agent mainly within the microneedle tip region in order to reduce drug loss within the backing layer [41].

However, the current HA/ZnO nanocomposites included the drug molecule inside the hydrated polymeric microneedles, resulting in homogeneous molecular distribution inside the hollow dissolving structures. The inclusion of the molecules directly inside the microneedles avoids post-fabrication steps and minimizes variation due to the application of surface-deposition methods. Furthermore, the formation of diffusion pathways facilitated by ZnO particles resulted in a diffusional transport mechanism compared to a simple dissolution-driven transport mechanism.

Therefore, the utilization of embedded molecular loading, fabrication of hollow structures, and nanoparticle-controlled transport in this case study could be considered as an effective method for producing more reproducible devices for controlled transdermal delivery of therapeutic drugs. In addition, it should also be noted that the casting-based fabrication method used in the current work can prove to be advantageous when compared to multiple-layer or surface deposition approaches in terms of manufacturability.

Overall, these findings demonstrate that diffusion transport within HA/ZnO nanocomposite systems is strongly governed by nanoparticle-mediated microstructural organization, interfacial compatibility, and diffusion pathway tortuosity within the hydrated polymer network, while simultaneously providing advantages in sustained transport behavior, fabrication simplicity, and structural integration of the loaded therapeutic agent.

3.5. Transport Kinetic Modeling

3.5.1. Higuchi Model Analysis

Kinetic analysis of transport through the Higuchi model showed excellent linearity between the amount of transport versus the square root of time, suggesting that the transport was diffusional in nature within the hydrated polymeric matrix, as shown in Figure 24.

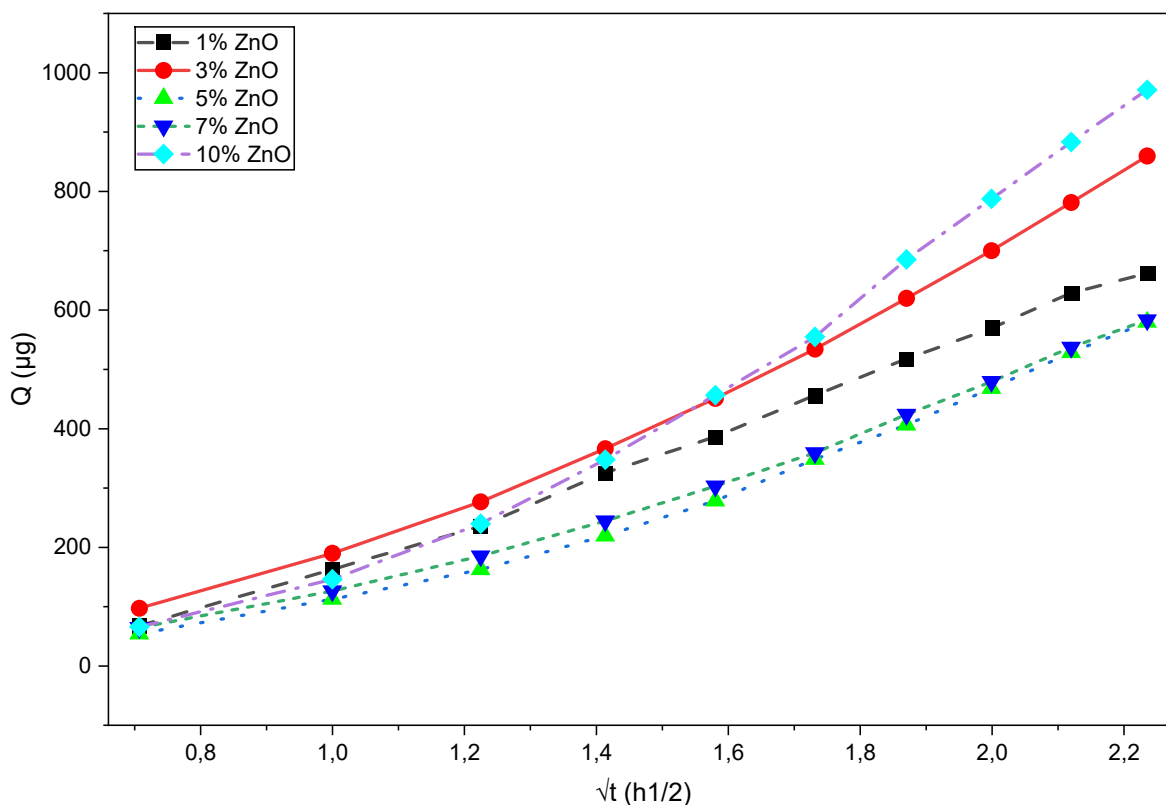


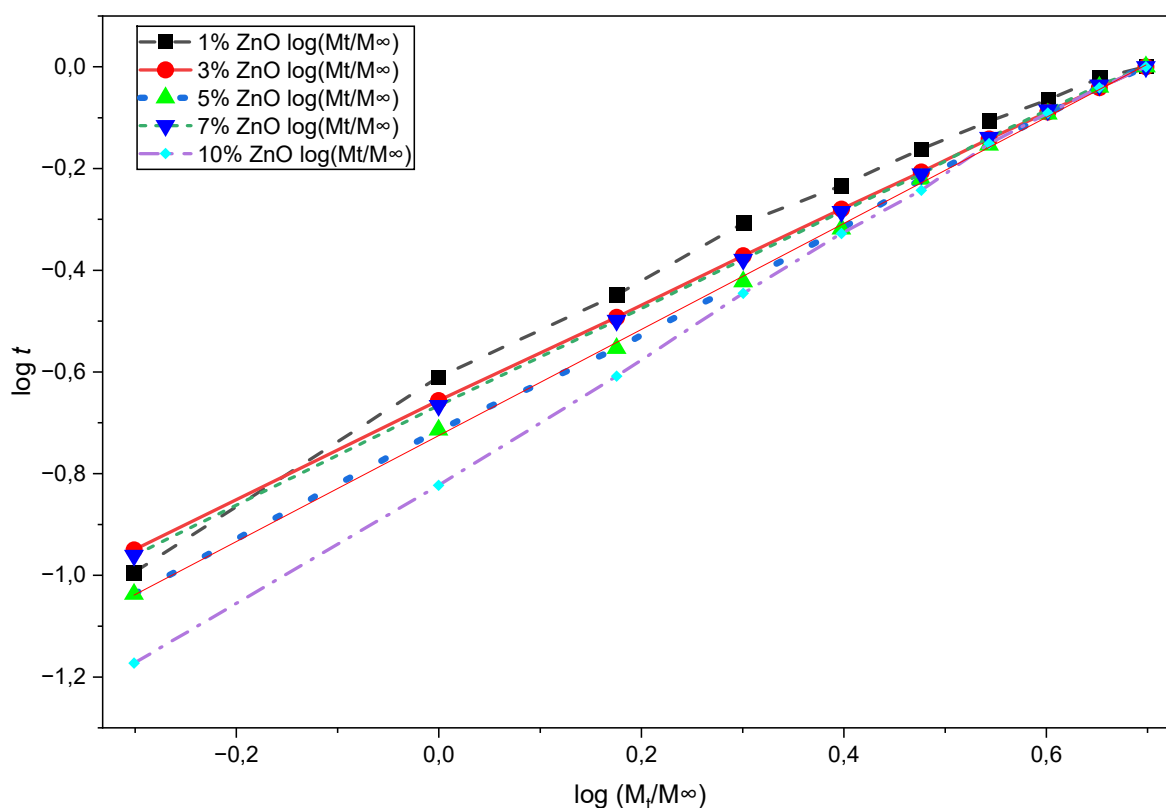
Figure 24. Higuchi transport kinetic plots of HA/ZnO nanocomposite systems.

3.5.2. Korsmeyer–Peppas Analysis

The Korsmeyer–Peppas model was used to better understand the mechanism of ciprofloxacin transport from HA/ZnO nanocomposite microneedles. The model was found to fit very well to the experimental release data with coefficients of determination (R^2) in the range of 0.990–1.000, indicating that the model was well suited to describe the release behaviour of all formulations. The release exponent (n) ranged from 0.950 to 1.193, indicating that drug transport was not only controlled by Fickian diffusion. As can be seen in Table 9, the n values of the HA/ZnO-1%, HA/ZnO-3%, and HA/ZnO-7% formulations were between 0.95 and 0.98, indicating anomalous (non-Fickian) transport, where both diffusion and polymer relaxation contributed to ciprofloxacin release. The HA/ZnO-5% formulation showed an exponent of $n = 1.043$, which indicated a more prolonged and tortuous transport process associated with an increase in the resistance to molecular diffusion within the polymer–nanoparticle network. In contrast, the HA/ZnO-10% formulation showed the highest release exponent ($n = 1.193$), which indicated a super case-II transport mechanism, where polymer relaxation and structural reorganization played a more prominent role in drug release than simple diffusion. The progressive increase of the release exponent with increasing ZnO concentration suggested that nanoparticle incorporation changed the internal architecture of the HA matrix and influenced the balance between diffusion and polymer relaxation mechanisms. These results suggest that ZnO loading not only influences the extent of ciprofloxacin release but also modulates the fundamental transport mechanism governing drug delivery from the microneedle system. More details are graphically expressed in Figure 25.

Table 9. Transport kinetic parameters of HA/ZnO nanocomposite microneedles.

Formulation	Higuchi Constant k_H	Higuchi Equation	Higuchi R^2	Peppas Exponent n	Peppas Equation	Peppas R^2	Dominant Transport Mechanism
HA/ZnO 1%	402.27	$Y = 402.27x - 257.8$	0.997	0.980	$Y = 0.980x - 0.633$	0.990	Super case II Transport
HA/ZnO 3%	507.96	$Y = 507.96x - 322.6$	0.984	0.950	$Y = 0.950x - 0.657$	1.000	Super case II Transport
HA/ZnO 5%	354.65	$Y = 354.65x - 204.4$	0.973	1.043	$Y = 1.043x - 0.715$	0.999	Super case II Transport
HA/ZnO 7%	349.27	$Y = 349.27x - 225.7$	0.982	0.967	$Y = 0.967x - 0.668$	1.000	Super case II Transport
HA/ZnO 10%	620.49	$Y = 620.49x - 505.8$	0.971	1.193	$Y = 1.193x - 0.829$	0.999	Super case II Transport

**Figure 25.** Shows the Korsmeyer–Peppas transport kinetics plots of HA/ZnO nanocomposite microneedles showing anomalous diffusion behaviour governed by combined polymer relaxation and diffusion-controlled transport mechanisms.

Higuchi Model Equation

$Q = K_H t^{1/2}$ where Q = cumulative transport (μg), $t^{1/2}$ = square root of time and K_H = Higuchi transport constant.

3.6. Implications for Functional Polymer Systems

The results demonstrate that HA/ZnO nanocomposites can be engineered to simultaneously optimize mechanical strength and transport properties through controlled nanoparticle incorporation and polymer architecture design.

This dual functionality highlights the potential of such nanocomposite systems as tunable polymer platforms, where both structural and functional performance can be modulated through compositional control. The findings emphasize the importance of

nanoparticle dispersion and polymer–nanoparticle interactions in governing both mechanical and transport behavior in hydrated polymer systems.

3.6.1. Mechanism of Drug Transport

The process of transport of drugs in the HA/ZnO microneedle device could be described by the combination of structural changes and physicochemical mechanisms. First of all, insertion of microneedles results in disruption of the stratum corneum layer and formation of microchannels, which significantly decreases the resistance of the skin barrier. Subsequent interaction of microneedles with interstitial fluid leads to fast hydration of the HA matrix, thus facilitating dissolution of the drug matrix and its release. At the same time, relaxation of polymer chains due to hydration of the matrix increases the drug transport rate and contributes to the anomalous nature of the transport mechanism. Finally, the addition of ZnO nanoparticles not only provides mechanical support but slightly modifies the drug transport pathways through the polymer matrix. This is described schematically in Figure 26.

Mechanism of Drug Transport from HA/ZnO Microneedle Device

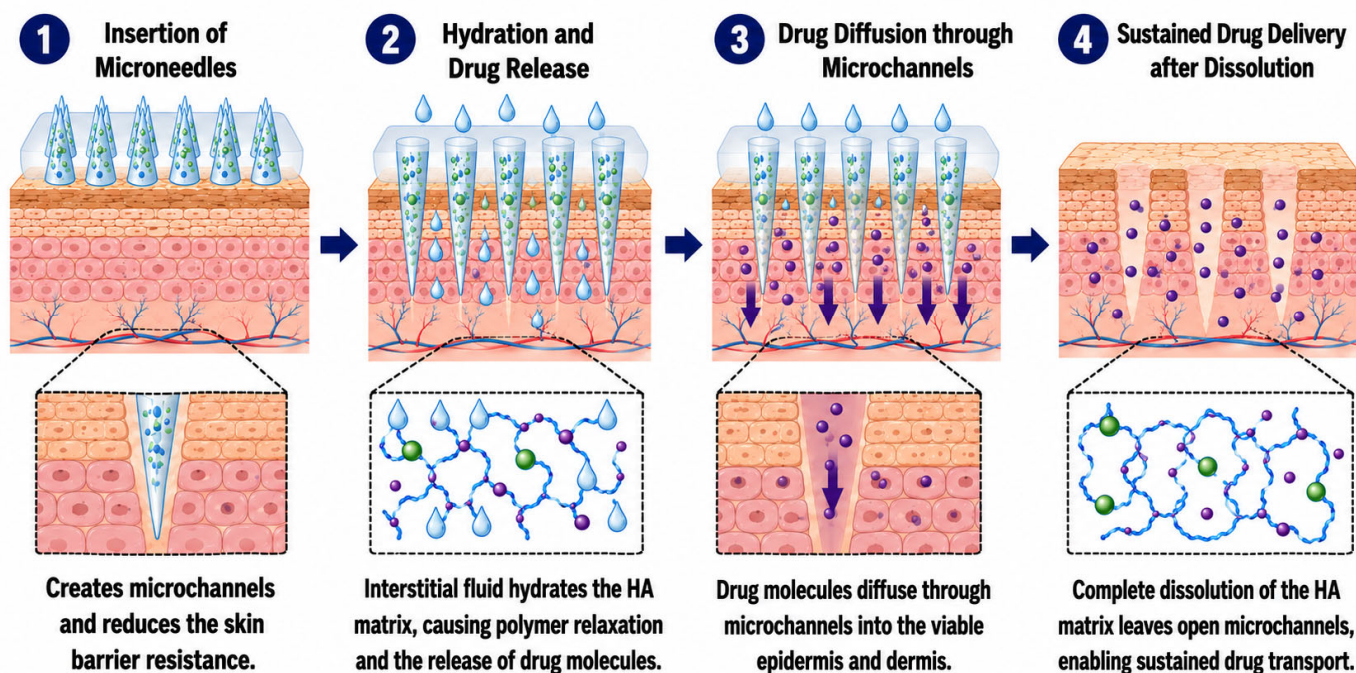


Figure 26. Illustration of the mechanism of drug transport from HA/ZnO dissolvable microneedle.

3.6.2. Structure–Transport Relationship

A clear relationship between polymer structure and transport behavior was observed. The diffusion characteristics are governed by:

- Polymer chain mobility (controlled by HA molecular weight distribution)
- Nanoparticle dispersion and interfacial interaction
- Microstructural organization and surface roughness

The increase in surface roughness with higher ZnO content (as observed from AFM analysis) reflects increased nanoscale heterogeneity, which contributes to altered diffusion pathways. The optimized formulation (5 wt% ZnO) provides a balance between structural integrity and controlled transport.

4. Conclusions

Hollow dissolving hyaluronic acid/zinc oxide nanocomposite microneedles using a combination of high and low molecular weight HA (40:60) were successfully prepared and studied as hydrated polymer nanocomposites. Citric acid stabilization played an important role in ZnO nanoparticle dispersion and matrix organization, mechanical response, and diffusional processes.

Surface analysis using SEM and AFM studies confirmed the presence of hollow microneedles and their characteristic surface morphology depending on ZnO concentration. Lower ZnO concentrations resulted in more homogeneous dispersion and matrix structure, whereas higher concentrations led to increased surface roughness. Mechanical tests showed that the microneedles possessed predominantly elastic bending properties, confirming their structural integrity and viscoelasticity.

Studies of the diffusive processes showed that ZnO concentration played a significant role in controlling transport behavior and fluxes. The 3% ZnO microneedles showed the most stable transport profile; however, 5 and 7% ZnO formulations had sustained transport due to the presence of tortuous transport paths mediated by ZnO nanoparticles inside the hydrated HA polymer matrix. Contrary to that, the 10% ZnO microneedles provided the highest cumulative amount transported along with maximum flux; however, the transport process was heterogeneous.

The presented HA/ZnO microneedles showed direct incorporation of ciprofloxacin into the hydrated polymer matrix, providing integrated drug loading capacity along with diffusion-regulated transport compared with other coating and tip-loaded microneedles. The presented fabrication technique based on casting is relatively simple.

Kinetic modeling studies showed predominance of the Higuchi model, while modeling with the use of the Korsmeyer–Peppas equation revealed an anomalous transport mechanism including diffusion and polymer relaxation mechanisms. Overall, the results demonstrate the potential of citric acid-stabilized HA/ZnO hollow microneedles as hydrated polymer nanocomposite systems for controlled transdermal drug delivery applications.

Nevertheless, several aspects require further investigation. Future studies should evaluate ZnO concentrations below and above 10 wt% to better understand the concentration-dependent transition observed in transport behavior and to identify the optimum nanoparticle loading. Additional work is also required to optimize citric acid-mediated stabilization and further elucidate nanoparticle dispersion within the hydrated polymer matrix using advanced microstructural characterization techniques. Furthermore, comprehensive cytocompatibility and biocompatibility studies are needed to assess the biological safety of HA/ZnO microneedles, particularly at higher nanoparticle concentrations. Finally, although gelatin-based substrates are widely used as a reproducible skin simulant for microneedle insertion studies, future research should include detailed mechanical and viscoelastic characterization of the gelatin model and comparison with human skin properties. Such investigations would improve the understanding of insertion mechanics, penetration, efficiency, and microneedle deformation behavior, thereby strengthening the predictive value of in vitro insertion models and supporting future clinical translation of HA/ZnO nanocomposite microneedles.

Author Contributions: K.S.D.: Writing—review and editing, Writing—original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization; R.O.O.: Conceptualization, Visualization, Validation, Supervision. F.F.Z.: Methodology; V.D.K.: Methodology; O.N.P.: Methodology. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The primary data required to support the findings of this study are included within the article. However, the underlying raw datasets generated during the current study are not publicly available due to ongoing research and commercialization restrictions related to a pending patent application.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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