Functionalized Palygorskite as a Delivery Platforms for Bioactive Asymmetric Beta-Diketone Dyes

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Abstract: Natural clay minerals are among the most versatile materials used in the biomedical field. Palygorskite has found various applications in this field, from the treatment of diarrheal diseases in the past to materials with antibacterial properties and platforms carrying bioactive compounds used in the treatment of diseases, cosmetic and healthcare products in the present. In this study, a possible delivery method of some bioactive asymmetric β-diketonic compounds is presented. Palygorskite modified with amphionic groups (P) and copper ions (PCu) was used as a platform to load bioactive curcumin derivatives (1 and 2). By varying the copper ions, the amounts of charged active compounds were monitored. Studies have shown that the hybrid materials resulting from the loading of 1 and 2 compounds on palygorskite with 30% copper ions (PCu30) can be used as delivery methods for these asymmetric curcumin derivatives, while palygorskite with 50% copper ions (PCu50) loaded with the same type of bioactive compounds has antibacterial properties.

Keywords: organomodified palygorskite; beta-diketones; antimicrobial; delivery platforms; drug release

1. Introduction

The price and availability of natural minerals are directly related to the diversity of the fields in which they have been applied [1,2]. The most common applications of natural clay minerals are as absorbents in water purification processes, for which it is important that the specific surface be as large as possible. These characteristics are obtained through top-down processes, following the modification of the surfaces through different acid, alkaline or hydrothermal treatments [3–5]. For their applications as materials for the delivery of active compounds or as an antimicrobial material in the medical field [6–9], surface modification techniques are used for bottom-up processing. The surfaces of the clay minerals in this case are adapted by grafting residues of organic molecules such as silane or surfactant groups [10,11], inorganic ions or metals nanoparticles [6,7,12] to create conditions to increase the affinity of the surfaces to the active compounds that are loaded later. These are organic–inorganic hybrid materials in which the inorganic matrix plays the role of the carrier. Palygorskite, a magnesium aluminum phyllosilicate (containing 2:1 layers where there is an octahedral sheet between two opposing tetrahedral sheets), is part of the class of one-dimensional clay minerals. Palygorskite, formed by structural tunnels with cross-sectional dimensions of 0.64 × 0.37 nm², contains exchangeable cations with zeolitic water inside them. When the zeolitic water in these tunnels is removed via heating, the sorption capacity is increased and small organic molecules or inorganic compounds can be adsorbed. The color variation of palygorskite from white, grayish
and yellowish to gray-green is closely related to the contents of metal ions in the clay mineral network. Reductions in the iron and aluminum contents are achieved by partially dissolving the salts in the presence of strong acids. At the same time, the sodium and potassium ions go into a solution while the magnesium ions can remain almost entirely in the clay, thereby leading to increases in the bleaching power and the adsorption capacity. The disaggregation of palygorskite or organically modified packages has found applications in the field of nanocomposites, with new uses in the automotive industry, in fire retardancy and packaging as a barrier, as supports for catalysts, as carriers of drugs or fertilizers and in fuel cell applications [2,13–15]. Palygorskite is an example of a clay mineral, which has been studied due to its low biotoxicity as a host matrix in drug delivery systems or diagnostic compounds [16,17]. In order to clarify the surface properties that palygorskite must have in this case, numerous studies [18–23] have been carried out regarding the parameters that influence the release of drugs. Thus, it was found that the pH is one of the important factors in terms of performance for the controlled release of drugs [18,19]. The other decisive factor, which has been evaluated through quantitative and kinetic studies and controls the release of active substances, is the architecture of the modified palygorskite matrix. The results of these studies have shown that the matrix can be modified through the use of hydrothermal methods or functionalization with various anchoring agents, including polyvinyl alcohol, 3-aminopropyltriethoxysilane (APTES), chitosan and others [18,23]. As a result, the adsorption potential of palygorskite can be increased compared to active compounds with antimicrobial (metronidazole) [20], anti-inflammatory (diclofenac acid) [21,22] and antituberculosis (isoniazid) [19] properties. The use of such carrier matrices that have the ability to release the active substance in a controlled manner results in improvements of the bioavailability of various drugs and the quality of treatments by reducing the adverse effects caused by large uncontrolled amounts of the active compound. Through surface modifications, palygorskite has been functionalized so that it can be loaded with different components, from natural extracts [24] and metal ions [25] to synthetic compounds [17] with different properties for therapeutic purposes. Following the in situ generation of metallic nanoparticles, deposits of essential oils or other cationic organic molecules, such as materials with antimicrobial properties, have been obtained on the surface of modified palygorskite [26–29]. The uses of these hybrid materials have recently been extended to numerous fields, including in targeted applications in the treatment of intestinal diseases [30], coatings for medical devices, cosmetic products [31,32] and health dressings [33]. In this context, the present study examined a new model for supporting matrices based on palygorskite surfaces modified with amphionic groups (P) and decorated with copper ions (PCu). On these newly obtained matrices, we loaded two asymmetric β-diketonic compounds (1 and 2) synthesized using an economic method in the microwave field. These compounds have a similar structure to curcumin and have been studied with regard to their bioavailability, and the results have shown that they have possible antitumor properties [34].

2. Materials and Methods

2.1. Raw Materials Used for the Modification of the Palygorskite Matrix

The precursors used for the synthesis and characterization of the β-diketone derivatives were of laboratory reagent grade and were purchased from retail suppliers (Merck and Sigma-Aldrich, Darmstadt, Germany) and used without further purification. The host inorganic matrix used in this study was palygorskite, a magnesium–aluminum phyllosilicate with a fibrillar structure, in monoclinic crystalline systems (Mg, Al)₂Si₄O₁₀(OH)₄(H₂O) (PAL) obtained from SERVA (Germany, Heidelberg). The clay mineral had a cation exchange capacity (CEC) of 18.5 meq/100 g clay, determined using the Busenberg and Clemency method [35]. This involved the saturation of exchangeable cation sites with ammonium cations. The ammonium-exchanged clay was dispersed in an alkaline solution of sodium hydroxide, which released the ammonium as dissolved ammonia gas, after which it was detected with a specific-ion electrode. Next, (3-aminopropyl)triethoxysilane (99%,
2.2. Methods for Obtaining the Carrier Matrices and Loading Them with Curcumin Derivatives

Palygorskite was modified with amphionic glycine residue (C₂H₅NO₂) through a silanization process with APTES and chloroacetic acid using a method already described elsewhere [36]. The deposition of the copper ions was carried out in an aqueous medium, under magnetic stirring, using modified palygorskite (P) and Cu(NO₃)₂·3H₂O at different mass ratios for P/Cu(NO₃)₂ (1:1, 1.2, 1.3; 1:6; 1:12). The modified palygorskite was decorated with copper ions using the method of adsorption from an aqueous solution of copper nitrate at different concentrations (5–80 mg/100 mL), with a pH of approximately 3 and at 30–60 °C for 2 h. The resulting suspension was centrifuged and filtered and the solid was washed with distilled water until the pH was neutral. After being filtered and dried at 105 °C, hybrid materials loaded with copper ions were obtained at different weight percentages (n) of 10% (PCu₁₀), 20% (PCu₂₀), 30% (PCu₃₀), 48% (PCu₄₈) and 50% (PCu₅₀).

The loading percentage on the modified palygorskite was calculated by determining the content of copper ions in the aqueous solution after the sorption process. For this, a few drops of 25% ammonia solution were added to each resulting filtrate and the absorbance of the resulting Schweizer’s complex [37] was measured. The recorded results were compared with standards of known Cu²⁺ concentrations (Figure 1). The hybrid materials (PCuₙ) were subsequently used to deposit asymmetric β-diketone derivatives by adsorbing dyes from alcoholic solutions of 0.05% concentration under magnetic stirring. The products were filtered and dried at 100 °C.

The palygorskite treated with (3-aminopropyl)triethoxysilanes (Scheme 1a(i)) and after that modified with amphionic groups of glycine residues (Scheme 1a(iii)), as presented by our team in another paper [36], was subjected to a copper ion adsorption process from solutions of different concentrations (Scheme 1a(iii)). In this way, hybrid materials with different copper ion loading rates (PCu₁₀ ÷ PCu₅₀) were obtained on palygorskite. The obtained organic–inorganic composites were morphostructurally characterized and then tested as delivery systems (Scheme 1a(iv)) for curcumin derivatives synthesized at microwave frequencies [34].

![Figure 1. Variations in Schweizer’s complex absorbance rates against the concentration of copper ions in a standard aqueous solution.](image-url)
Scheme 1. The main stages in the modification of palygorskite to obtain drug delivery systems: (i) modification of palygorskite(P) surfaces with APTES in ethanol at 45 °C for 2 h; (ii) grafting of carboxylic groups with chloroacetic acid at 70 °C for 4 h; (iii) decoration of the hybrid surfaces with copper ions at 30 °C for 2 h; (iv) loading of bioactive compounds on the organic–inorganic hybrid matrices via their adsorption from alcoholic solutions (a) and structural formula of curcumin derivatives (b).

In order to evaluate the adsorption capacity of the host matrix against the two asymmetric β-diketonic compounds (Scheme 1b), the absorbance of the alcoholic solutions of the compounds at known concentrations was measured. Subsequently, in the range of values in which the absorbance follows the Lambert–Beer law (Figure 2a), we measured the curcumin derivative concentrations in the residual solutions after the adsorption on the supporting material. The results show that the adsorption of β-diketonic derivatives on the support takes place differently (Figure 2b). This process is influenced by the stereochemistry of the curcumin derivatives and the types of bonds established between the auxochromes of the dyes and the host matrix. In this regard, it was established that 2 absorbs better on the PCu50 hybrid material compared to 1.

Figure 2. Calibration scale formed by measuring the absorbances of the dye solutions at known concentrations (a) and the absorbances of the residual filtrates after the dye adsorption process on the hybrid materials (b).
2.3. Structural Characterization Methods

UV–VIS absorption spectra were acquired for the solutions in ethanol at a $1 \times 10^{-5}$ molar concentration (for the residual filtrates of the adsorption processes) and 25 ± 0.5 °C in rectangular quartz cuvettes with an optical path length of 10 mm, using an UV–VIS–NIR Jasco V-570 spectrometer (Jasco Int. Co., Ltd., Tokyo, Japan). The fluorescence spectra were recorded with a JASCO FP 6500 spectrofluorimeter (Jasco Int. Co., Ltd., Tokyo, Japan) at 25 °C, using 10 mm path-length quartz cuvettes for liquids or the support for solid samples, at an excitation wavelength of 420 nm. The UV–VIS absorption and fluorescence spectra were processed with Spectra Manager I (Jasco Int. Co., Ltd., Tokyo, Japan). The IR spectra were recorded in solid samples on a Jasco FTIR 6300 spectrometer (Jasco Int. Co., Ltd., Tokyo, Japan) equipped with a Specac ATR Golden Gate system (Specac Ltd., Orpington, UK), in the range of 400 to 4000 cm$^{-1}$ (32 accumulations at a resolution of 4 cm$^{-1}$). The spectra were processed with Spectra Manager II (Jasco Int. Co. Ltd., Tokyo, Japan).

The presence of copper ions on the palygorskite hybrid material was analyzed in the elemental analysis of the obtained composites. A Vanta C series portable XRF (Olympus, Waltham, MA, USA) equipped with a 40 kV X-ray tube with a rhodium anode and a silicon drift detector (acquisition time of 60 s for each beam) in the precalibrated GeoChem mode was used to evaluate the samples. By recording XRD diffractograms in the range of 20, 2–90° with a RigakuSmartLab system (Rigaku Corporation, Tokyo, Japan), the hybrid materials and active compounds derived from curcumin were characterized and the diffractograms were interpreted using PDXL software (ver. 2.7.2.0, Rigaku Corporation, Tokyo, Japan), followed by a comparison with the ICDD (International Centre for Diffraction Data) database entries. The particle morphology and elemental analyses were performed with an SEM (TM4000Plus; HITACHI, Tokyo, Japan; accelerating voltage: 15 kV) equipped with an EDS (X-stream-2; Oxford instruments, Oxford, UK). AZtecOne software (ver.1.0, Oxford instruments, Oxford, UK) was used for the EDS analysis. The weight concentrations of copper and all detected elements of organic compounds were quantified and expressed as weight percentages (wt%) in the analyzed area. The characterization of the delivery matrices and hybrid materials loaded with curcuminic derivatives by surface area, pore volume and size was performed using a Nova 2200e Quantachrome (Quantachrome Instruments Corporate Drive Boynton Beach, FL, USA) automated gas adsorption system. The samples were analyzed after being gassed at 150 °C for 4 h, then after N$_2$ adsorption–desorption the isotherms were measured at −196 °C. The specific surface areas ($S_{BET}$) and total pore volumes ($V_{total}$) of the samples were determined using heBET (Brunauer–Emmett–Teller) and BJH (Barrett–Joyner–Halenda) methods, respectively.

The tests for the evaluation of the antimicrobial activity were carried out in Petri dishes on a specific agar medium and Muller–Hinton medium inoculated in the canvas with the bacterial strains $S. aureus$ (ATCC 25923) and $E. coli$ (ATCC 25922), respectively, along with Sabouraud medium in the case of the $C. albicans$ strain (ATCC 10231) from the microbial collection of ICECHIM. The working inoculum was represented by a suspension in A.F.S. made from a fresh 18–24h culture (3–4 isolated colonies), developed on a solid medium, with a density of $1–3 \times 10^5$ UFC/mL, which was adjusted nephelometrically (McFarland standard $0.5 = 1.5 \times 10^8$ UFC/mL) and spectrophotometrically. Then, 10mg/mL of each composite was dispersed in DMSO (dimethylsulfoxide) and the samples were inoculated on the disk in 25 or 30 µL of the medium seeded in the cloth with the tested microorganisms. The plates were incubated for 24 h at 37 °C for bacteria and 28 °C for fungi. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone, the clear zone (halo) that appears around the inoculation.

3. Results and Discussion

3.1. Obtaining Organic–Inorganic Hybrid Materials Loaded with Asymmetric Curcumin Analogs

3-Aminopropyltriethoxysilane (APTES) is one of the most effective modifiers for inorganic phases with hydroxyl groups, through the hydrolysis reaction of siloxane groups with hydroxyl groups on the surfaces of mineral clays. The remaining available amine groups
were used together with the carboxyl groups grafted by the reaction with chloroacetic acid to create an amphiphilic surface on the palygorskite. In this way, a versatile surface was obtained with increased compatibility with active compounds and with various structural configurations. Furthermore, the architecture of the supporting matrix was decorated with copper ions through which β-diketonic derivatives of the inorganic phase could be attached and quantified. The role of copper ions is to form structural complexes with diketonic compounds in order to increase their bioavailability. The hybrid materials, namely palygorskite samples modified with organic residues and decorated with copper ions, were used as supports for the delivery of asymmetric curcumin derivatives. For this study, two asymmetric β-diketonic compounds obtained at microwave frequencies following an equimolecular reaction between acetylacetone and vanillin (1) or 4-acetamido-benzaldehyde (2) were chosen (Scheme 1b).

Knowing that the structure of palygorskite is fibrillary, we considered it interesting to explore it as a supporting material and to analyze the extent to which the deposited asymmetric β-diketone dye would be exposed on the surface of the hybrid material. At the same time, an attempt was made to quantify the dye deposited in relation to the copper ions on the support (Figure 3), based on the fluorescence quenching properties that these ions have on the curcumin derivatives [38–40]. Considering the above, it can be observed that the presence of copper ions on the modified palygorskite with glycine residues has weak effects on reducing the fluorescence intensity of the support in Figure 3a. However, the presence of these ions leads to a severe reduction in the fluorescence intensity of the host material loaded with β-diketonic compounds in Figure 3b. These findings led us to further study the fluorescence quenching ability depending on the curcumin derivative to Cu$^{2+}$ ratio (Figure 4). Thus, the fluorescence intensity (Figure 4a) levels of solution 1 at the beginning of the adsorption process and with known 1/Cu$^{2+}$ molar ratios (1:0–1) were measured. These were compared with the fluorescent intensity levels of the residual solutions after the adsorption process (Figure 4b). The results obtained for the decrease in fluorescence intensity of the residual solutions of 1 after adsorption on the hybrid matrix decorated with copper in different proportions were almost half of the value measured for the carrier matrix without copper. On the other hand, the fluorescence intensity of the residual solutions decreased from PCu$_{50}$ to PCu$_{30}$, consistent with the increase in fluorescence intensity measured on the hybrid materials from PCu$_{50}$-1 to PCu$_{30}$-1 (Figure 3a). At the same time, comparing the fluorescence intensity levels of the residual solutions (PCu$_{30}$ and PCu$_{48}$) to the fluorescence of the solutions with known curcumin derivative/Cu$^{2+}$ molar ratios, it was observed that the concentration of copper ions to which the fluorescence quenching corresponded was greater than the calculated 2.5·10$^{-7}$ mol/L for an equimolecular ratio of 1:1. We assumed that these values were due to the additional adsorption of the dyes on the inorganic substrate, in addition to the complexation of the asymmetric β-diketonic derivative with copper ions. This adsorption process was limited to PCu$_{50}$, probably due to the higher density of copper ions on the surface of the hybrid material and the steric hindrance of 1. A decrease in fluorescence intensity that was greater than expected for the concentration of copper ions calculated for a 1:1 equimolecular ratio was also recorded for PCu$_{50}$-2 (Figure 4c,d). In these cases, we supposed that the decrease in fluorescence intensity was due to the formation of the copper complex with the β-diketonic derivative, although the fluorescence quenching paths from these interactions were not sufficient to totally extinguish the fluorescence emissions. These were supplemented by the fluorescence processes that took place due to the physical interactions established between the uncomplexed asymmetric curcumin derivative compounds and the support matrix. The residual solution of PCu$_{50}$-1 had the lowest quenching rate of the fluorescence emissions, probably due to the low curcumin derivative/Cu$^{2+}$ molar ratio, as corroborated by it having the lowest adsorption rate onto the supporting matrix compared to the other asymmetric β-diketonic derivative.
3.2. Structural Characterization of Hybrid Materials as Curcumin Compound Delivery Systems

The presence of the elements characteristic of the hybrid materials, namely palygorskite samples modified with organic residues and decorated with copper ions, was confirmed by XRF analysis (Table 1). A modified natural clay matrix was used as the support for the delivery of asymmetric curcumin derivatives. The crystalline structure of the finished composite was characterized using X-ray diffractograms and compared with those of the loaded active compound to confirm its multicomponent structure. Figure 5 shows the diffractograms recorded for the palygorskite modified with organic residues and decorated with copper ions (PCu) used as a carrier matrix for the compounds derived from curcumin, namely 1 and 2, respectively, as well as those of the final multilayer products (PCu-1 and PCu-2). After the deposition of copper ions on the modified palygorskite substrate, it was observed by comparison with the literature data (ICCD01-088-1950) [36] that the resulting structure had an amorphous character (Figure 5). 

Figure 3. Fluorescence intensity levels of hybrid materials with different concentrations of Cu\(^{2+}\) and loaded with \(1\) (a) and of PCu50 hybrid materials loaded with \(\beta\)-diketone derivatives (b).

Figure 4. Fluorescence intensity measurements depending on the \(1/\text{Cu}^{2+}\) molar ratio (a) and dye/\(\text{Cu}^{2+}\) molar ratio (c) and the fluorescence spectra of the residual filtrates (f) after the dye loading processes on the hybrid materials (b,d).
recorded diffractograms for the final hybrid materials with peaks characteristic of the deposited layers at $2\theta = 13.74^\circ, 16.46^\circ, 19.86^\circ, 30.82^\circ$ and $45.8^\circ$ (Figure 5).

Table 1. The elemental analysis of the raw palygorskite and palygorskite decorated with copper ions.

<table>
<thead>
<tr>
<th>Element</th>
<th>Si (wt %)</th>
<th>Al (wt %)</th>
<th>Fe (wt %)</th>
<th>Mg (wt %)</th>
<th>K (wt %)</th>
<th>Ca (wt %)</th>
<th>Ti (wt %)</th>
<th>Cu (wt %)</th>
<th>L.E. (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>25.79</td>
<td>3.65</td>
<td>4.19</td>
<td>3.70</td>
<td>0.48</td>
<td>4.02</td>
<td>0.63</td>
<td></td>
<td>56.74</td>
</tr>
<tr>
<td></td>
<td>(±0.48)</td>
<td>(±0.21)</td>
<td>(±1.2)</td>
<td>(±0.02)</td>
<td>(±0.07)</td>
<td>(±0.05)</td>
<td></td>
<td></td>
<td>(±0.9)</td>
</tr>
<tr>
<td>PCu50</td>
<td>25.68</td>
<td>3.20</td>
<td>4.45</td>
<td>2.97</td>
<td>0.50</td>
<td>1.80</td>
<td>0.65</td>
<td>2.33</td>
<td>58.42</td>
</tr>
<tr>
<td></td>
<td>(±0.50)</td>
<td>(±0.10)</td>
<td>(±0.05)</td>
<td>(±0.01)</td>
<td>(±0.04)</td>
<td>(±0.01)</td>
<td>(±0.06)</td>
<td></td>
<td>(±0.7)</td>
</tr>
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</table>

L.E.—light elements (not detectable by XRF).

Figure 5. XRD patterns of the hybrid materials decorated with copper (PCu) and loaded with curcumin derivatives 1 (a) and 2 (b).

Following the textural analysis of the surfaces decorated with copper (Figure 6a) and comparison with the previous ones modified only with glycine residues [36], it was observed that the surfaces of the new hybrid materials increased considerably. This result was attributed to the formation by the copper ions of some clusters on the modified surfaces of the palygorskite. This resulted in an increase in the specific surface of approximately four times compared to the initial one, without a significant change in the volume of the pores but with a decrease in their diameter (Table 2). These results are also supported by the X-ray diffractogram of PCu in Figure 5b, which confirms the amorphous structure of the modified palygorskite matrix. Through this process carried out from the bottom up, carrier matrices were obtained on which the active compounds derived from curcumin were then loaded. Depending on their molecular structure and especially the auxochromes on the aromatic ring, the diameter of the pores decreased (Figure 6b,c). From the data presented in Table 2, it can be seen that for the auxochromes with smaller volumes of 1, the average diameter of their pores increased less than for those loaded with 2 and their overall volume decreased, probably due to the increased affinity of copper ions to oxygen atoms, which facilitates better adsorption in the pores. In comparison with the same type of substrate modified with copper after loading with active compounds, it can be observed that the specific surface of the hybrid material loaded with 1 is smaller than that of the hybrid material loaded with 2, which shows us that the adsorption of 2 is better on the surface of the carrier matrix. These assumptions are supported by the X-ray diffractograms that are illustrated in Figure 5a for composite materials with a crystalline structure, while in Figure 5b it can be seen that for PCu-2 the XRD pattern suggests a mixture of phases.
Figure 6. The nitrogen adsorption–desorption isotherms (a) and poresize distributions of the hybrid materials (PCu<sub>30</sub> and PCu<sub>50</sub>) and drug delivery systems (b,c).

Table 2. Specific surface area, pore volume and average pore diameter values for organic–inorganic hybrids and drug delivery systems.

<table>
<thead>
<tr>
<th>Sample</th>
<th>S&lt;sub&gt;BET&lt;/sub&gt; (m&lt;sup&gt;2&lt;/sup&gt;/g)</th>
<th>V&lt;sub&gt;total&lt;/sub&gt; (cm&lt;sup&gt;3&lt;/sup&gt;/g)</th>
<th>D&lt;sub&gt;pore&lt;/sub&gt; (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL</td>
<td>117</td>
<td>0.327</td>
<td>13</td>
</tr>
<tr>
<td>P</td>
<td>36</td>
<td>0.319</td>
<td>15</td>
</tr>
<tr>
<td>PCu&lt;sub&gt;30&lt;/sub&gt;</td>
<td>132</td>
<td>0.312</td>
<td>9</td>
</tr>
<tr>
<td>PCu&lt;sub&gt;30&lt;/sub&gt;-1</td>
<td>79</td>
<td>0.225</td>
<td>11</td>
</tr>
<tr>
<td>PCu&lt;sub&gt;30&lt;/sub&gt;-2</td>
<td>91</td>
<td>0.310</td>
<td>14</td>
</tr>
<tr>
<td>PCu&lt;sub&gt;50&lt;/sub&gt;</td>
<td>158</td>
<td>0.340</td>
<td>9</td>
</tr>
<tr>
<td>PCu&lt;sub&gt;50&lt;/sub&gt;-1</td>
<td>76</td>
<td>0.220</td>
<td>12</td>
</tr>
<tr>
<td>PCu&lt;sub&gt;50&lt;/sub&gt;-2</td>
<td>85</td>
<td>0.228</td>
<td>16</td>
</tr>
</tbody>
</table>

The hybrid materials loaded with dyes were characterized using FTIR spectroscopy (Figure 7). By analyzing the FTIR spectra, characteristic peaks of the inorganic support were identified at 3615 and 3545 cm<sup>-1</sup> corresponding to the stretching vibrations of the OH groups belonging to Al, Mg or Si atoms of the supporting matrix. In the range of 1500–1300 cm<sup>-1</sup>, signals characteristic of organic structures were found. Thus, the bands at 1517–1470 cm<sup>-1</sup> is characteristic of aromatic C-H stretching vibrations and the one around 1400 cm<sup>-1</sup> to the stretching vibration of the –NH<sub>2</sub> and C-N bonds of APTES, respectively. The band at 1320 cm<sup>-1</sup> was attributed to the stretching vibrations characteristic of amides in the structure of compound 2. The broad band at 1100–900 cm<sup>-1</sup> is due to the overlap of the stretching vibrations of the C-O bonds in the organic compounds with those of the Si-O bonds in the host matrix.

The SEM images enable direct observations of the surface microstructures of the host matrices. The images show that progressive changes occurred on the surface of palygorskite modified with amphionic groups (Figure 8a) after it was decorated with copper ions (Figure 8b). The scanning electron microscopy images of the hybrid palygorskite–curcumin derivatives (Figure 8c) clearly indicate the absence of the shiny, bulky particles that can be observed on the structure of the host matrix. The morphology of the product changed once the β-diketonic derivative was deposited on the substrate to show agglomerates formed by rod- and plate-like structures.
The element mapping using scanning electron microscopy and the corresponding EDX spectra of the surface-modified palygorskite authenticated the presence of carbon and copper with the four main constituents oxygen, magnesium, aluminum and silicon in PCu$_{50}$ due to the surface modification of the palygorskite by aminosilane and decoration with copper ions. The EDX measurements were recorded for a qualitative analysis of the elemental composition of the palygorskite modified with the glycine residue after being decorated with copper ions and then loaded with curcumin derivatives. The EDX spectra in Figure 9a,b confirm that the copper ions were sorbed onto the modified palygorskite. All data are shown in Table 3 and provide direct evidence of the adsorption of copper onto the inorganic matrix and the loading with β-diketone derivatives. Thus, it can be observed that the percentages of chemical elements such as oxygen and carbon, which are found in the basic structures of organic compounds, increase at the expense of the calcium ions, as shown in the elemental analysis of the hybrid materials loaded with β-diketone derivatives.

Figure 7. FTIR spectra of the hybrid materials loaded with asymmetric curcumin analogs.

Figure 8. SEM images of palygorskite modified with amphionic groups (a), palygorskite modified with amphionic groups and decorated with copper ions (PCu$_{50}$) (b) and the curcumin-derivative-loaded hybrid material (PCu$_{50}$-2) (c).
Table 3. Elemental compositions of samples of P, PCu and PCu–curcumin derivative.

<table>
<thead>
<tr>
<th>Element</th>
<th>P Weight %</th>
<th>ACu20 Weight %</th>
<th>ACu30 Weight %</th>
<th>PCu30 Weight %</th>
<th>PCu30-1 Weight %</th>
<th>PCu30-2 Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>49.0 (±0.4)</td>
<td>36.4 (±0.3)</td>
<td>41.2 (±0.3)</td>
<td>46.1 (±0.7)</td>
<td>68.3 (±0.3)</td>
<td>64.6 (±0.5)</td>
</tr>
<tr>
<td>Mg</td>
<td>4.6 (±0.1)</td>
<td>2.1 (±0.0)</td>
<td>2.5 (±0.0)</td>
<td>3.8 (±0.3)</td>
<td>1.4 (±0.0)</td>
<td>2.5 (±0.0)</td>
</tr>
<tr>
<td>Si</td>
<td>23.7 (±0.2)</td>
<td>7.8 (±0.1)</td>
<td>10.3 (±0.1)</td>
<td>14.9 (±0.7)</td>
<td>6.0 (±0.1)</td>
<td>10.8 (±0.1)</td>
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<tr>
<td>C</td>
<td>17.0 (±0.6)</td>
<td>49.7 (±0.4)</td>
<td>7.8 (±0.1)</td>
<td>28.9 (±0.7)</td>
<td>22.3 (±0.3)</td>
<td>18.1 (±0.4)</td>
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<td>Al</td>
<td>3.4 (±0.1)</td>
<td>1.6 (±0.0)</td>
<td>1.9 (±0.0)</td>
<td>2.7 (±0.2)</td>
<td>1.0 (±0.0)</td>
<td>1.9 (±0.0)</td>
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<tr>
<td>Fe</td>
<td>1.3 (±0.1)</td>
<td>0.7 (±0.0)</td>
<td>0.7 (±0.1)</td>
<td>1.6 (±0.3)</td>
<td>0.4 (±0.0)</td>
<td>0.7 (±0.0)</td>
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<tr>
<td>Ca</td>
<td>0.5 (±0.0)</td>
<td>0.7 (±0.0)</td>
<td>0.3 (±0.0)</td>
<td>0.6 (±0.1)</td>
<td>0.1 (±0.0)</td>
<td>0.3 (±0.0)</td>
</tr>
<tr>
<td>Na</td>
<td>1.3 (±0.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>K</td>
<td>0.2 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>0.1 (±0.0)</td>
</tr>
<tr>
<td>Cl</td>
<td>0.6 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>0.1 (±0.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>-</td>
<td>0.7 (±0.1)</td>
<td>0.9 (±0.1)</td>
<td>1.2 (±0.3)</td>
<td>0.4 (±0.1)</td>
<td>0.8 (±0.1)</td>
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3.3. Release Study of β-Diketone Derivatives at Variable pH Levels

The release profiles of the β-diketonic derivatives loaded on inorganic support (PCu30-1 and PCu30-2) were studied for three different pH values at 27 °C. With these pH values, we aimed to replicate the environments in which the hybrid materials can cross through the body. The results of the studies showed that in slightly acidic environments (Figure 10), the release processes of the curcumin derivatives take place at a low speed and the organic structures superficially adsorbed on the surface of the inorganic support were probably the first to be released. These desorption processes are a little more intense as the pH decreases and are influenced by the cleavage of London or van der Waals physical bonds established between the palygorskite support and the asymmetric β-diketonic compounds. Thus, it can be observed that depending on the auxochrome groups, which can be found grafted on the aromatic residues, the release processes decrease in the order of 2 < 1. However, the significant desorption processes of β-diketonic derivatives from the inorganic substrate take place in a slightly alkaline medium, specific to the small intestine in the human digestive tract, at which point the physical bonds between the host inorganic matrix and the biologically active organic compound are probably broken. In this case, the release of the curcumin derivatives is influenced by the polarity of the medium, which leads to the modification of the molecular structure, probably by shifting the tautomeric
equilibrium of the β-diketonic compounds to the keto-enolic form, leading to the disruption of intermolecular association processes [41].

**Figure 10.** The release behaviors of asymmetric curcumin analogs 1 (a) and 2 (b) in saline solution (9 mg/mL) with different pH values (pH = 2.5, 5) and buffer media (pH = 7.4).

3.4. Evaluation of the Antimicrobial Activity of Composite Materials

The two types of hybrid materials (PCu30 and PCu50) loaded with active compounds were tested to evaluate their antimicrobial activity. The PCu30 matrices loaded with asymmetric curcumin derivatives did not show antimicrobial activity for any of the fungal or bacterial cultures. This result was due to the insufficient amount of active compound released in the test medium. The antioxidant properties of β-diketonic derivatives are confirmed in the specialized literature [42,43]. The antimicrobial activity is most often attributed to the oxidative processes of these active compounds on cell membranes, thereby causing damage to the cell structure. Accordingly, in this case it can be observed that compound 1 with the hydroxy group present in the structure (PCu50-1) exerted better antimicrobial activity compared to PCu50-2 against all three strains (S. aureus Figure 11a,d, E. coli Figure 11b,e and C. albicans Figure 11c,f), showing inhibition zone diameters of 14 and 11 mm, respectively (30 µL inoculum). These results are in agreement with the results of the active compound release tests, and the antimicrobial activity can be explained by the presence of a larger amount of 1 in the biological environment. The S. aureus strain was the most sensitive to the inhibitory action of the tested samples.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>E. coli</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
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<td><img src="image2.png" alt="Image" /></td>
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</tr>
<tr>
<td></td>
<td>(a)</td>
<td>(b)</td>
<td>(c)</td>
</tr>
</tbody>
</table>

**PCu-1**

**Figure 11. Cont.**
In this study, we considered it important to find a method for conditioning certain active compounds, namely β-diketone derivatives. In this sense, we tried to obtain a support on which to load asymmetric curcumin derivatives, so as to obtain hybrid materials to facilitate the access of the active compounds to hard-to-reach places in the human body, such as the intestinal tract. For this, materials (palygorskite, silane derivatives) already known to be friendly to the human body and used as drug carriers [44–46] were considered. Copper is a micronutrient that is essential for energy utilization, brain function (neurotransmitter regulation), soft tissue and bone formation (collagen synthesis), nutrient metabolism (especially iron) and antioxidant defense against free radicals [47]. Moreover, palygorskite–copper hybrid materials have been tested on mice in vitro and in vivo. The test results suggested that these composite materials can be an alternative to antibiotic therapies against diarrhea caused by Salmonella [25]. In this study, the connection between the copper ions and the inorganic matrix was achieved through the use of glycine residues [48] (the smallest non-essential, neutral and metabolically inert amino acid) grafted on palygorskite by means of silane chains (APTES). All of these changes on the surface of the inorganic matrix were made with the aim of loading the carrier matrix as evenly as possible with β-diketonic compounds by complexing them with copper ions. The loading of curcuminic derivatives was successful, being confirmed by X-ray, FTIR, SEM and EDX analyses of the obtained hybrid materials. The loading of asymmetric β-diketonic compounds on the carrier matrix took place via the complexation of copper ions to the ketone groups of the curcumin derivatives, a process that resulted in a drastic reduction in the fluorescence intensity of the hybrid materials. The decrease in intensity but not the total extinction of the fluorescence of the composite materials was probably caused by the physical interactions of the bioactive compound with the host matrix, established by asymmetric β-diketonic derivatives directly adsorbed on the modified palygorskite. Such hybrid materials must be studied further from a structural point of view, both by varying the trace elements deposited on the inorganic matrix and by improving the deposition methods or behavior under various influences such as pH or temperature. Next, the bioactive properties of the asymmetric β-diketonic compounds must be tested in vivo and at the same time the composite materials must be studied to confirm the preservation of the properties of the loaded bioactive compounds.

4. Conclusions

In this study, a method for loading bioactive compounds on an inorganic palygorskite-type matrix modified on the surface with glycine residues and copper ions was tested. These composite materials are proposed as delivery platforms for asymmetric β-diketonic compounds in hard-to-reach environments in the human body, such as the intestinal tract. By changing the percentage of copper ions deposited on the palygorskite, it was possible to change the dose of active compounds loaded on the carrier matrix. In this case, with an increase in the amount of charged curcumin derivative, the final composite material exhibited antibacterial properties.
Author Contributions: Conceptualization, F.M.R. and V.R.; methodology, A.R.; validation, V.R. and F.M.R.; formal analysis, M.G., M.C. and I.R.; investigation, A.R.; writing—original draft preparation, F.M.R.; writing—review and editing, F.M.R. and R.C.F.; visualization, R.C.F.; supervision, V.R.; project administration, V.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare they have no conflicts of interest.

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