INTRODUCTION

The use of natural polymers (e.g., gelatin, chitosan, and silk proteins) for the development of hydrogels is gaining in importance owing to their inherent biocompatibility.\(^1\) Hydrogels are high-water content materials prepared from crosslinked polymers that can provide sustained, local delivery of a variety of therapeutic agents. Use of the natural polymer chitosan as a scaffold material for hydrogels has been intensively investigated because of the polymer’s biocompatibility, low toxicity, and biodegradability. Therefore, the advanced development of chitosan hydrogels has led to new drug delivery systems that release their payloads in response to varying environmental stimuli.\(^2,3\)

Chitosan (see Figure 1), the product of the \(N\)-deacetylation of chitin, has also received particular attention because it is produced as a waste product during crustacean (shrimp and crab) processing.\(^4\) In addition, it offers other advantages; chitosan can not only be used to control the release of active agents but also be prepared without the use of hazardous organic solvents because it is soluble in aqueous acidic solutions. Given the above-mentioned properties, chitosan is extensively used for the development of drug delivery systems.

![Chitosan structure](Image)

**Figure 1.** Chitosan structure (red: oxygen, white: hydrogen, blue: carbon and purple: nitrogen atoms)

Chitosan has been effectively used in drug delivery applications as hydrogel systems, drug conjugates, biodegradable release systems, and other components.\(^5,6\) Chitosan-based systems are used for the delivery of proteins/peptides, growth factors, anti-inflammatory drugs, and antibiotics, as well as in gene therapy and bioimaging applications.\(^9,11,12\)

A hydrogel comprised of chitosan crosslinked using the low-toxicity crosslinker genipin was prepared, and the absorption of glibenclamide by the hydrogel was investigated. Optimized structures and their molecular electrostatic potentials were calculated using the AM1 method, and the results were used to evaluate the molecular interactions between the three compounds. The quantitative structure-property relationship model was also used to estimate the activity of the chemicals on the basis of their molecular structures. In addition, theoretical Fourier transform infrared spectra were calculated to analyze the intermolecular interactions in the proposed system. Finally, the hydrophilicity of the hydrogel and its influence on the absorption process were also estimated.

**Keywords:** chitosan; genipin; glibenclamide; FTIR; QSPR modeling.

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**Artigo**

**INTERACTIONS OF CHITOSAN/GENIPIN HYDROGELS DURING DRUG DELIVERY: A QSPR APPROACH**

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![Genipin structure](Image)

**Figure 2.** Genipin structure (red: oxygen, white: hydrogen and blue: carbon atoms)

Glibenclamide (see Figure 3), also known as glyburide, is an antidiabetic drug in the class of medications known as sulfonylureas, which are closely related to sulfa drugs. Sulfonylureas bind to ATP-dependent \(K^+\) channels in beta cells of the pancreas, depolarizing them and stimulating the release of \(Ca^{++}\), which in turn stimulates insulin production.\(^22,23\)

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In this study, Fourier transform infrared (FTIR) spectroscopy was used to analyze the intermolecular interactions in the proposed system. As one of the ways to verify the obtained results, we apply the theoretical calculations by means of the computational chemistry tools. Using the quantitative structure-property relationship (QSPR) model/method, we estimate the activity of the chemicals on the basis of only their molecular structure. Sorption of organic chemicals in soils or sediments is usually described by sorption coefficients.

In addition, the log P value of the hydrogel was determined as a measure of its hydrophobicity. The log P value of a compound, which is the logarithm of its partition coefficient between n-octanol and water (c_{octanol}/c_{water}), is a well-established measure of the compound’s hydrophobicity. Low hydrophilicities, and therefore high log P values, cause poor absorption or permeation. It has been shown that compounds must have a log P value less than 5 in order to have a reasonable probability of being well absorbed. Typically, the log P value of a substance at pH 7.4 is considered as an index of the compound behavior in plasma.

Finally, the molecular electrostatic potential (MESP) was investigated using the Austin Method 1 (AM1) calculations. This method provides information about the regions in which intermolecular interactions occur between compounds. The electrostatic potential is the energy of the interaction of a point positive charge (an electrophile) with the nuclei and electrons of a molecule. Negative electrostatic potentials indicate areas that are prone to electrophilic attack. The electrostatic potential can be mapped onto the electron density by using color to represent the value of the potential.

**EXPERIMENTAL**

The AM1 method was initially used to optimize the structures of the compounds investigated in the present study because it generates lower-energy structures, even when the initial structures are far away from the minimum structures. The Polak-Ribiere algorithm was used for mapping the energy barriers of the conformational transitions. For each structure, 5715 iterations, a level convergence of 0.001 kcal/mol/Å, and a line search of 0.1 were performed.

**Structural parameters**

The optimized structural parameters were used for the vibrational wavenumber calculations with AM1 method in order to characterize all the stationary points as minima. The structural parameters were calculated by selecting the Constrain bond and length options from the Build menu for the two methods of analysis.

**FTIR**

An infrared spectrum is commonly obtained by passing infrared electromagnetic radiation through a sample that possesses a permanent or induced dipole moment and determining what fractions of the incident radiation are absorbed at particular energies. The energy of each peak in an absorption spectrum corresponds to the frequency of the vibration of part of the molecule, thus allowing qualitative identification of certain bond types in the sample.

The FTIR spectra were obtained by first selecting from the Compute menu, vibrational and rotational spectrum options; after this step, using the vibrational spectrum option, FTIR spectrum pattern is obtained for the two methods of analysis. The results of the analyses for the optimized structures for chitosan, genipin, and glibenclamide obtained using the AM1 method are listed in Tables 1–3, respectively.

**Table 1. FTIR results of chitosan**

| Assignment            | Experimental (Frequencies cm\(^{-1}\))\(^{27,28}\) | AM1 (Frequencies cm\(^{-1}\)) |
|-----------------------|---------------------------------------------------|-------------------------------|
| CH (CH\(_2\)-OH) stretching | --------                                 | 4374                          |
| OH and CH (CH\(_2\)-OH) | 3550                                             | 3676                          |
| CH (CH\(_2\)-OH) stretching | --------                                 | 3512                          |
| NH                                | 3314                                             | --------                       |
| OH (CH\(_3\)-OH)                  | --------                                 | 3300                          |
| C-N                               | 1360-1080                                       | 2041                          |
| C-C                               | --------                                 | 1909                          |
| NH\(_2\)                           | 1580                                             | --------                       |
| C-O (chitosan ring)               | 1150                                             | 1489                          |

**Table 2. FTIR results of genipin**

| Assignment        | Experimental (Frequencies cm\(^{-1}\))\(^{30-32}\) | AM1 (Frequencies cm\(^{-1}\)) |
|-------------------|-----------------------------------------------------|-------------------------------|
| OH stretching     | --------                                             | 6052                          |
| CH\(_3\) asymmetric stretching                | --------                                             | 4694, 4530                    |
| CH stretching (ring)                                  | 3745                                             | 4091, 3885                    |
| C=C                                | 3398, 3245                                       | 3354                          |
| CH\(_3\) (scissoring)                         | 3100                                             | 3039                          |
| C-C, C-O                                  | 2520                                             | 2680, 2571                    |
| C–C, C–O, CH                                    | 1681, 1622,1105                                  | 1663, 1038                    |
| CH\(_3\), CH\(_2\)                                        | 1443                                             | 1405                          |
| C–O–C                                          | 830, 835                                        | 818                            |
| C–O–C (out of plane)                          | 773                                              | 755                            |

**Table 3. FTIR results of glibenclamide**

| Assignment        | Experimental (Frequencies cm\(^{-1}\))\(^{35-39}\) | AM1 (Frequencies cm\(^{-1}\)) |
|-------------------|-----------------------------------------------------|-------------------------------|
| NH asymmetric stretching | --------                                             | 5583                          |
| CH asymmetric stretching                | --------                                             | 5007                          |
| CH\(_3\) stretching                  | --------                                             | 4511                          |
| CH\(_3\), (O-CH\(_3\))                | 3591                                             | 4028                          |
| NH (amide)                          | 3367, 3311, 1713                                   | 3314                          |
| CH=CH                          | 3035                                             | 3529                          |
| C=C (ring)                           | 1591                                             | 2830                          |
| C=C, S=O                           | 2412                                             | 2474                          |
| C=O                             | 1652, 1618                                       | 1720, 1715                    |
| S=O\(_2\)                        | 1341-1332, 1158                                   | 1316                          |
| C–C, C–N, C–O                          | 1995, 1334, 1154, 1090, 1018, 924, 793           | 1332, 1124, 1028, 569         |
Electrostatic potential

After obtaining the Gibbs free energy for the optimized geometries using the AM1 method, two-dimensional contour diagrams of the electrostatic potentials surrounding the three molecules, their total electron densities and spin densities, their molecular orbitals, and the electron densities of the individual orbitals were plotted.

HyperChem software displays the electrostatic potential as a contour plot when the appropriate option in the Contour Plot dialog box is selected. Atomic charges indicate where large negative values (sites for electrophilic attack) are likely to occur. However, the largest negative value for the electrostatic potential is not necessarily adjacent to the atom with the largest negative charge.

RESULTS AND DISCUSSION

Structural parameters

The thermodynamic data obtained are listed in Table 4. The Gibbs free energy value (−947.998 kcal/mol) indicates that the crosslinking reaction involves crosslinking of two free amino groups in chitosan with one molecule of genipin (see Figure 4). Nucleophilic attack by the primary amine occurs at the carbon atom in genipin, while the secondary amine attacks the aldehyde group.

Figure 4. Structure of chitosan/genipin using computational chemistry

Table 4. QSPR properties results

| Property     | Chitosan/Genipin | Chitosan/Genipin-Glibenclamide |
|--------------|------------------|--------------------------------|
| ΔG (kcal/mol)| -947.998         | -243.08                        |
| Mass (amu)   | 1455.43          | 2442.44                        |
| Surface area (Å²) | 1798.11       | 2910.84                        |
| Volume (Å³)  | 3507.66          | 5860.58                        |
| Log P        | -22.15           | -46.33                         |

Swelling and diffusion in hydrogels are also influenced by the hydrophilicity of the crosslinked polymer. Therefore, the Log P value for chitosan/genipin was calculated and found to be −22.15. This value represents the hydrophilic property of the substance and is considered to be an indicator of the ability of the hydrogel to absorb molecules. In hydrogels formed by chitosan crosslinked with itself, release is largely controlled by the crosslinking density; consequently, the higher the crosslinking density, the lower the release rate.

However, other system parameters, such as drug concentration, often play a major role. To our knowledge, there are no examples of hydrogels formed by chitosan crosslinked with itself that exhibit pH-sensitive swelling.

Indeed, the numerous inter-chain interactions formed by crosslinking inhibit swelling because most of the amino groups of chitosan react with the crosslinker. Such systems do not present a release profile that can be further modulated after administration; for example, drug release cannot be targeted in the gastro-intestinal tract, which limits their range of application.

Table 4 shows the thermodynamic results of glibenclamide absorption process; here the Gibbs free energy value −243.08 kcal/mol is spontaneous because of the formation of hydrogen bonds between the carbonyl groups of glibenclamide and NH groups of chitosan. Nucleophilic attack of the amino group of chitosan at the carbonyl of the genipin leads to formation of a stable bond (amide), and an oxygen atom in genipin is replaced by a nitrogen atom from chitosan (see Figure 5).

Figure 5 shows that the title system (chitosan-genipin/glibenclamide) is not planar, and based on observations, deformation of the ring structure depends on the nature of the substituents (OH, NH₂, and CH₃–CH₂). Figure 5 shows the results of the computational analysis to determine the optimized geometries for chitosan/genipin and chitosan (genipin)/glibenclamide. Their molecular structures as well as the numbering of their atoms are also shown in the figure.

Figure 5. Structure of (chitosan (genipin)/glibenclamide using computational chemistry

The optimized structural parameters for chitosan, chitosan crosslinked with genipin, and the chitosan/genipin hydrogel with absorbed glibenclamide are listed in Tables 5–7, respectively, in accordance with the atom numbering scheme given in Figures 4 and 5. The title system (chitosan-genipin/glibenclamide) is not planar, and based on observations, deformation of the ring structure depends on the nature of the substituents (OH, NH₂, and CH₃–CH₂). Therefore, we could compare the calculation results given in Tables 5–7 with experimental data. As discussed in the previous literature, several authors have explained the changes in frequency or bond length of the C–H bond upon substitution due to a change in the charge distribution on the carbon atom of the ring. The substituents may either be electron withdrawing (Cl, Br, F) or electron donating (CH₃, C₂H₅).

The carbon atoms are bonded to hydrogen atoms via σ bonds, and substitution of a hydrogen atom for a halogen atom on the benzene
ring reduces the electronic density at the carbon atom owing to an induction effect. The ring carbon atoms exhibit a larger attraction for the valence electron cloud of the remaining hydrogen atoms, resulting in an increase in the C–H force constant and a decrease in the corresponding bond lengths. The reverse holds true for substitution with electron donating groups.

The actual change in the C–H bond lengths is influenced by the combined effects of the inductive–mesmeric interaction and the electric dipole field of the polar substituent. The calculated geometric parameters can be used as the foundation for calculating other parameters for the compound.\textsuperscript{36}

### FTIR

The calculated FTIR results for chitosan/genipin and chitosan (crosslinked with genipin)/glibenclamide are presented in Table 8. The second column indicates the absorption bands at 2320 and 1097 cm\(^{-1}\), which clearly appeared after crosslinking with genipin. The peak at 1097 cm\(^{-1}\) represents the C–N stretching vibration for the secondary amine and formation of a double bond at the carbon associated with the heterocyclic ring of the product. Moreover, the adsorption band that appeared at 1236 cm\(^{-1}\) (amide III) represents a mixed CO–N/N–H vibration mode, and the peak at 813 cm\(^{-1}\) is a characteristic absorption band for the C–H stretching vibration associated with the heterocyclic ring of the product.

An amine group in the chitosan macromolecule undergoes nucleophilic attack at the C–OH group of genipin, resulting in the formation of a new covalent bond between the aldehyde group and the secondary amine and formation of a double bond at the carbon in the ortho-position.\textsuperscript{36,17,36-38} One of the most important changes is evident in the reduction of the carbonyl group of the genipin, which reacts with the primary amine in chitosan to form an amide, which

### Table 5. Structural parameters calculated for chitosan crosslinking with genipin using AM1 method

| Bond | Bond Length (Å) | Bond | Bond Length (Å) | Bond | Bond Length (Å) |
|------|-----------------|------|-----------------|------|-----------------|
| C1-C3 | 1.5904 | C4-C5 | 1.6290 | O26-C27 | 1.4199 |
| C1-O2 | 1.4392 | C6-C23 | 1.4606 | C27-C28 | 1.7307 |
| O2-C6 | 1.4106 | O23-C24 | 1.4979 | C28-C29 | 1.7857 |
| C6-C5 | 1.5326 | C24-C25 | 1.7343 | C29-C30 | 1.7105 |
| C5-C4 | 1.5599 | C10-N12 | 1.3435 | C31-C32 | 1.6139 |
| O43-C45 | 1.5737 | C14-C15 | 1.6129 | C31-C32 | 1.6139 |
| C45-C46 | 1.6548 | N12-C14 | 1.4551 | C7-C15 | 1.5556 |
| C46-C47 | 1.5737 | C14-C15 | 1.6129 | C31-C32 | 1.6139 |
| O47-C48 | 1.5008 | C15-C16 | 1.5797 | C32-C33 | 1.5749 |
| C48-C49 | 1.5816 | C16-C17 | 1.5147 | C33-C34 | 1.6242 |
| C49-C50 | 1.7053 | C17=C21 | 1.3415 | C34-C29 | 1.7610 |
| C50-C45 | 1.6717 | C21-C20 | 1.4668 | C26-C27 | 1.2279 |
| O1-C3 | 1.5116 | C20-C19 | 1.3411 | S22-O25 | 1.5798 |
| C3-C4 | 1.6016 | C19-C18 | 1.4664 | S22-O24 | 1.5794 |
| C4-C5 | 1.6762 | C18=C16 | 1.3563 | C10-O11 | 1.2316 |
| C5-C6 | 1.6404 | C20-S22 | 1.7650 | N28-C29 | 1.4657 |
| C6-C2 | 1.4415 | S22-N25 | 1.7177 | C29-C30 | 1.5879 |
| C2-O1 | 1.4579 | N25-C26 | 1.3478 | C30-C31 | 1.6051 |
| C4-C7 | 1.7066 | C26-N28 | 1.3436 | C9-C5 | 1.5969 |
| C7=C8 | 1.4762 | C8-C9 | 1.6465 | |

### Table 6. Bond length calculated for chitosan (crosslinking with genipin)/glibenclamide using AM1 method

| Bond | Bond Length (Å) |
|------|-----------------|
| C1-C3 | 1.5904 |
| C1-O2 | 1.4392 |
| O2-C6 | 1.4106 |
| C6-C5 | 1.5326 |
| C5-C4 | 1.5599 |
| O43-C45 | 1.5737 |
| O47-C48 | 1.5008 |
| C48-C49 | 1.5816 |
| C49-C50 | 1.7053 |
| C50-C45 | 1.6717 |
| O1-C3 | 1.5116 |
| C3-C4 | 1.6016 |
| C4-C5 | 1.6762 |
| C5-C6 | 1.6404 |
| C6-C2 | 1.4415 |
| C2-O1 | 1.4579 |
| C4-C7 | 1.7066 |
| C7=C8 | 1.4762 |
Table 7. Angle calculated for chitosan (crosslinking with genipin)/glibenclamide using AM1 method

| Bond              | Angle (°) | Bond              | Angle (°) | Bond              | Angle (°) |
|-------------------|-----------|-------------------|-----------|-------------------|-----------|
| C1-C3-C4          | 127.626   | C45-O47-C46       | 106.193   | C6-C10-O12        | 136.273   |
| C5-C3-C4          | 113.831   | C45-C51-C46       | 135.376   | C10-O12-C13       | 146.014   |
| C6-C5-C4          | 115.859   | C45-O47-C48       | 143.381   | C9-C5-C6          | 124.608   |
| O2-C5-C6          | 123.559   | C49-O47-C48       | 109.344   | C1=C2-C6          | 119.726   |
| C1-O2-C6          | 132.357   | C49-C50-C48       | 121.732   | C2=C6-C5          | 119.633   |
| C1-C3-O2          | 106.768   | C49-C50-C45       | 117.545   | C6=C5-C4          | 121.828   |
| C1-C3-C7          | 121.067   | C46-C50-C45       | 121.806   | C5=C4-C3          | 118.351   |
| O12-C3-C4         | 109.22    | C50-C49-N61       | 106.1840  | C4=C3-C1          | 119.103   |
| O21-C3-C4         | 114.184   | O1=C3-C4          | 107.2720  | C1-C3-O8          | 121.394   |
| C5-C6-N16         | 147.176   | C3-C4-C5          | 116.783   | C3-O8-C9          | 133.702   |
| C6-O2-O23         | 84.6643   | C4-C5-C6          | 116.972   | C5-C4-C10         | 117.572   |
| C24-O23-C25       | 109.381   | C5-C6-C2          | 127.341   | C3-C1-C2          | 121.358   |
| C24-C25-O26       | 74.1901   | C6=C2-O1          | 104.610   | C4-C10-N12        | 120.165   |
| C25-C26-C27       | 154.839   | C2-O1-C3          | 147.022   | C4-C10=O1         | 120.411   |
| C28-C26-C27       | 84.0271   | O1=C3-O14         | 132.731   | O11=C10-N12       | 119.425   |
| C27-C28-C29       | 117.165   | C4-C5-C7          | 97.6437   | C10-N12-C14       | 124.203   |
| C24-C28-C29       | 111.158   | C4=C7-C8          | 109.065   | N12-C14-C15       | 137.288   |
| C24-C29-O37       | 102.782   | C7=C8-C9          | 118.035   | C14-C15-C16       | 145.455   |
| C27-C28-N40       | 140.123   | C8=C9-C5          | 96.8362   | C15-C16-C18       | 118.951   |
| C27-C28-O43       | 84.0492   | C9-C5-C4          | 118.42    | C15-C16-C17       | 127.566   |
| C25-C30-O26       | 96.3597   | C4=C7-C15         | 133.485   | C16-C17-C21       | 123.344   |
| C27-O43-C45       | 124.622   | C7=C15-O16        | 126.308   | C17=C21-C20       | 120.522   |
| C45-O43-C46       | 132.147   | C5-C6-C10         | 125.969   | C21-C20-C19       | 117.485   |
| C20-C19-C18       | 121.978   | C18=C16-C17       | 113.483   | C33-C34-C29       | 126.013   |
| C19-C18=C16       | 123.188   | C28=C29-C30       | 121.280   | N28-C26-O27       | 122.574   |
| C20-S22-N25       | 128.431   | C29-C30-C31       | 131.325   | N25-C26-O27       | 124.259   |
| S22-N25-C26       | 132.168   | C30-C31-C32       | 122.219   | O24=S22-N25       | 97.0564   |
| N25-C26-N28       | 113.167   | C31-C32-C33       | 113.669   | O23=S22=O24       | 40.1588   |
| C26-N28-C29       | 130.906   | C32-C33-C34       | 122.978   | C20-S22-O23       | 94.3538   |
| C19=C20-S22       | 117.351   |                    |           |                   |           |

is indicated by the growth of the absorption band at 1630 cm⁻¹ in the FTIR spectrum of the hydrogel.

As a consequence of these two main reactions, the intensity of the absorption band for the amino groups at 3597 cm⁻¹ is drastically reduced in the spectrum of the hydrogel.⁶⁰⁻⁶⁰

The third column in Table 8 lists the absorption bands that appeared after the absorption of glibenclamide by the chitosan/genipin crosslinked hydrogel. These peaks include a characteristic amide band at 3407 cm⁻¹ and peaks for SO and SO₂ stretching vibrations at 2683 and 1369 cm⁻¹, respectively. The site of interaction on glibenclamide is likely the C=O group, which would also affect the NH vibration.⁶⁸

The absorption peaks observed at 2412 and 793 cm⁻¹ are associated with C–C, CH, C–O, and OH bending vibrations, while the absorption bands at 1555, 1334, 1090, and 924 cm⁻¹ are due to the C–C and C–O bonds in the chitosan crosslinked with genipin.⁴¹ The new broad peak that appeared near 1415 cm⁻¹ after crosslinking with genipin is associated with the ring-stretching vibrations of the heterocyclic amine. In addition, the C–N stretching of amide III at 1233 cm⁻¹ shifted to 1260 cm⁻¹ after crosslinking with genipin, while the peak at 1740 cm⁻¹, which corresponds to the carboxylic groups in the electrosynthetic chitosan fibers, disappeared in the genipin-crosslinked chitosan.⁹

### Electrostatic potential

The MESP values (Figure 6) ranged from −0.075 to 0.607 eV and 0.065 to 0.718 eV for the crosslinking of chitosan with genipin and glibenclamide absorption by the crosslinked hydrogel, respectively.

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**Table 8. FTIR results using computational chemistry**

| Assignments / Wavenumber (cm⁻¹) | Chitosan/Genipin | Chitosan(Genipin)-Glibenclamide |
|--------------------------------|-----------------|--------------------------------|
| N–H and CH stretching          | 3859            | 4412                           |
| OH stretching                  |                 | 4412                           |
| CH₃ scissoring (genipin)        | 3597            | 3460                           |
| NH (glibenclamide)             |                 | 3407                           |
| CH₃ scissoring (glibenclamide)  |                 | 3298                           |
| C=O (glibenclamide)            |                 | 2884                           |
| CH=CH=CH₂O, S=O (glibenclamide)|                 | 2683                           |
| NH₃ asym stretching and OH     | 3458, 3295      |                                 |
| stretching                     |                 |                                |
| C–C, CH, C–O and OH (absorption of glibenclamide) | 3089, 1441, 721 | 2320                           |
| C–O, C–C, C–H (genipin)        | 2884, 2683, 1952| 2320                           |
| C–C, C–O, C–N (absorption of glibenclamide) | 3089, 1441, 721 | 2320                           |
| S=O₂                         |                 |                                |
| C=O (Genipin-amide group)      | 1630            | 1715                           |
| CO–N, N–H                    | 1236            | 1235                           |
| C–O, C–N, C–C (chitosan and genipin) | 813            | 724                            |
The negative regions appeared near the OH groups (C–OH bonds) in the crosslinked chitosan.

The absorption of glibenclamide by the chitosan crosslinked with genipin mainly involved the formation of hydrogen bonds between the CH₂OH and CH groups. Figure 7 shows the structure of the genipin-crosslinked chitosan with absorbed glibenclamide.

CONCLUSIONS

Genipin, a natural crosslinking agent, reacts with compounds containing primary amine groups, such as chitosan, to form covalently crosslinked networks. In the case of chitosan, genipin reacts with the free amino groups present in the glucosamine units. The results of this study support the relevance of genipin as a valuable natural, nontoxic, crosslinking agent for controlled drug release in drug delivery systems based on chitosan.

The absorption of glibenclamide by the chitosan crosslinked with genipin mainly involved the formation of hydrogen bonds between the NH/C–OH and C=O bonds, respectively. FTIR results revealed that secondary amide linkages are formed via the reaction of genipin ester groups with chitosan amino groups, yielding a polymeric network structure. The MESP values indicated that the nucleophilic and electrophilic regions mainly involved the NH/C–OH and C=O bonds, respectively.

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