Development of Polymeric Hydrogels for Potential Biomedical Applications:

Sol-Gel Synthesis and in Vitro Release of Mangiferin

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**Abstract**

Polymeric hydrogels consisting of polyvinyl alcohol, chitosan, and gelatin were synthesized and used as carriers for mangiferin, a natural extract from the leaves of mango, Extracts of mangiferin were characterized using FT-IR and NMR and tested for antimicrobial activity against a clear zone assay for two-component, three-component, and hybrid three-component hydrogels prepared with FT-IR and SEM-EDX. The influence of the polymer components and intermolecular hydrogen bonds in the hybrid hydrogel on swelling behavior and release of mangiferin in solutions of pH 5.5 and 10.0 was studied. Hydrogels prepared in this study showed promising potential for biomedical applications.

**Keywords** : Polyvinyl alcohol, chitosan, gelatin, mangiferin, hydrogel

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Abstract

Polymeric hydrogels based on blending of polyvinyl alcohol, chitosan and gelatin were prepared and loaded with mangiferin, a naturally occurring glucosyl xanthone extracted from leaves of the mango tree. The structure of this bioactive compound was confirmed by FT-IR and NMR and its anti-microbial property was tested by the clear zone method. The developed binary, ternary and hybrid ternary blend hydrogels were characterized by FT-IR and SEM-EDX. The effects of polymer composition and siloxane hybrid network on the swelling behavior and in vitro release of mangiferin were investigated in aqueous media of pH 5.5 and 10.0. It was found that the hybrid hydrogel systems produced in this study seem promising as potential materials for biomedical applications.

Keywords: polyvinyl alcohol, chitosan, hybrid, mangiferin, hydrogel

Introduction

Hydrogels are three-dimensional networks of hydrophilic polymers that can retain large amount of aqueous fluid in their structures. The ability of water absorption depends on nature of functional groups, backbone structure, crosslink density, morphology and others. Hydrogels have been widely used in many applications, such as tissue engineering, drug delivery device and wound healing material. Wound dressing hydrogels exhibit advantages of biocompatibility, ability of absorbing wound exudates and reducing damage from wound adherence. Many dressings, fabricated from different types of polymeric hydrogels have been developed and reported (Hu, Qiang & Wang, 2017; Majd et al., 2016; Neto et al., 2019). Polyvinyl alcohol is a hydrophilic polymer with good biodegradability, biocompatibility, processing ability, and non-cytotoxicity (Arun et al., 2017; Ghaderi et al., 2019). Among several applications, polyvinyl alcohol has been proposed as a promising hydrogel suitable for wound dressing material. The hydrogel can keep wound tissue moist, increase healing rate and allow easy removal after heating as opposed to traditional gauze and cotton wool dressing. However, polyvinyl alcohol is very sensitive towards moisture. In its swollen state, the hydrogel possesses low mechanical properties which is undesirable for applications. In order to improve the mechanical properties and stability, polyvinyl alcohol is blended with other biopolymers. Chitosan, a polysaccharide isolated from crustacean shells, has been widely studied for application in tissue engineering and biosensors due to its excellent properties as antibacterial agent, good biocompatibility and biodegradability. It has been reported that incorporation of chitosan into the polyvinyl alcohol matrix could lead to an improvement in biocompatibility and mechanical properties of the hydrogel (Ghaderi et al., 2019).

Mangiferin, a polyphenol compound, is an active natural product obtained from various plants such as Mangifera indica L. (Barreto et al., 2008). The substance has been reported to exhibit biological activities such as antioxidant, anticancer, antimicrobial, antiviral activities and others (Dar et al., 2005; Yoosook et al., 2000; Guha
et al., 1996). It was also considered as non-toxic as its oral LD$_{50}$ value tested in mice was 400mg/kg. However, a very low water solubility (0.111 mg/mL) and significantly strong inter-molecular interaction involving hydrogen bonding limit the amount of release and the use of this substance in drug delivery systems. Encapsulation of mangiferin with natural polymer blends may be a good way to increase drug loading, drug release and bioavailability (de Souza et al., 2013).

In the present work, mangiferin loaded binary blend, ternary blend and hybrid ternary blend of polyvinyl alcohol/chitosan/gelatin (PVOH/CHI/GEL) delivery systems were fabricated by solvent casting approach. Reasons of choosing gelatin as the third component in ternary blend were its non-toxicity, miscibility and less preferential affinity of mangiferin to gelatin than to polyvinyl alcohol and chitosan, therefore better profile of drug release was expected. The efficiency of different matrices for mangiferin release was investigated in pH 5.5 and 10.0 buffer solutions. This work may provide a new potential application for PVOH/CHI/G bio-hydrogels not only in biomedical application, such as drug delivery and wound healing, but also in other different areas where biological activities of mangiferin are required.

Methods

Materials

Mangiferin, a natural bioactive agent, was extracted from Mangifera indica L. and purified in our laboratory. Gelatin powder (GEL), CAS 9000-70-8, was obtained from Merck. A medium molecular weight chitosan (CHI), No.448877: 75-85% deacetylated degree, polyvinyl alcohol (PVOH), 87-90% hydrolyzed: average molecular weight of 30,000-70,000, tetraethoxy silane (TEOS), and other chemicals were purchased from Sigma-Aldrich.

Preparation of mangiferin loaded hydrogels

A solution of polymers was prepared by dissolving 2 g of polymer mixture (Table 1) in 20 mL of aqueous glacial acetic acid (2% v/v) under constant stirring at 50°C for 1 h. In the case of preparing hybrid hydrogel, a desired volume of TEOS (crosslinker) was gradually dropped into the reaction mixture and stirred for 24 h. The viscous mixture was carefully poured into a mold and kept in a hot air oven at 50°C for another 24 h. The cast film samples were then stored in a desiccator until further use. The mangiferin loaded hydrogels were prepared by the same procedure. In addition, mangiferin powder was weighed and dissolved into polymer solution at the first step.
Table 1 Formulations for hydrogel preparation

<table>
<thead>
<tr>
<th>Sample</th>
<th>PVOH (g)</th>
<th>CHI (g)</th>
<th>GEL (g)</th>
<th>TEOS (µL)</th>
<th>Mangiferin (g)</th>
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<tr>
<td>M95PV/5CH</td>
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<td>-</td>
<td>-</td>
<td>0.05</td>
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<tr>
<td>90PV/5CH/5GE</td>
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<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90PV/5CH/5GE-T1</td>
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<td>0.1</td>
<td>0.1</td>
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<tr>
<td>90PV/5CHI/5GEL-T2</td>
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<td>0.1</td>
<td>0.1</td>
<td>60</td>
<td>-</td>
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<tr>
<td>M90PV/5CH/5GE</td>
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<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>M90PV/5CHI/5GEL</td>
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<tr>
<td>M90PV/5CHI/5GEL-T2</td>
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<td>0.1</td>
<td>60</td>
<td>0.05</td>
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</table>

Study of antimicrobial activity

Antimicrobial activity of mangiferin against Micrococcus luteus 9341 and Pseudomonas aeruginosa 27853 was tested using an agar disc diffusion method. 20 µL suspensions of organism equivalent to 0.5 and 2 McFarland were spread on nutrient agar plates. 20 µL of mangiferin solution was loaded on each sterile filter disk (diameter 6 mm). The disks were placed on the prepared agar plates. After 24 h incubation at 37°C, the diameter of inhibition zone was measured. The solvent, DMSO, was used for reference.

ATR-FTIR spectroscopy

The spectra of samples were recorded with an ATR-FTIR spectrophotometer (PerkinElmer-Frontier) in the wave number range of 4000-400 cm⁻¹ and a resolution of 4 cm⁻¹.

Fluid uptake study

The fluid uptake behavior of the hydrogel was evaluated in pH 5.5 and 10.0 buffer solutions for 8 hours. After an immersion of the sample in a solution for different periods of time, the amount of fluid uptake was measured by removing of sample from the medium and wiping off excess fluid with a filter paper. The %swelling ratio associated with fluid uptake was measured by the following equation:

\[
\text{Swelling ratio (\%)} = \frac{(W_s - W_d)}{W_d} \times 100 \quad \text{(Eq. 1)}
\]

where \(W_s\) and \(W_d\) are the swollen weight and the dried weight of the hydrogel sample.

Measurement of in vitro mangiferin release

The hydrogel films loaded with mangiferin were placed in 30 mL of buffer solutions (pH 5.5 and 10.0) for 8 h. Aliquots of 1 mL were drawn in intervals of 30 min for the first hour, and 60 min for the last 7 h and analyzed by
a UV-Vis spectrophotometer. The UV absorbance of mangiferin in release solutions was measured at 257 nm (pH 5.5) and 390 nm (pH 10.0) and then converted to the %cumulative release according to a calibration curve.

Results

Characterization of mangiferin

The bioactive agent, mangiferin powder, prepared in our laboratory was purified and characterized before use. \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)) and ATR-FTIR spectra of mangiferin are displayed in Figure 1. The activity of mangiferin against microorganism expressed in terms of inhibition zones at different concentrations of mangiferin is presented in Figure 2 and Table 2.

Figure 1  Chemical structure and characterization of mangiferin a) \(^1\)H NMR spectrum and b) ATR-FTIR spectrum.

Figure 2  Antibacterial effect of mangiferin at different concentrations (25, 50, 75 and 100 mg/mL), tested against:

a) M. luteus  and  b) P. aeruginosa
Table 2  Antimicrobial effect of mangiferin at different concentrations

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Inhibition Zone (mm) measured from different concentrations of mangiferin</th>
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<tr>
<td></td>
<td>25 mg/mL</td>
</tr>
<tr>
<td>M. luteus 9341</td>
<td></td>
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<tr>
<td>P. aeruginosa 27853</td>
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</table>

Investigation on the binary and ternary blend hydrogels

- **ATR-FTIR**

  ATR-FTIR spectroscopy was used to confirm functionality of the hydrogels. Figure 3 shows the ATR-FTIR spectra of the fabricated 90PV/5CH/5GE and its homopolymers.

- **In vitro mangiferin release from the binary and ternary blend hydrogels**

  It is generally known that the pH of the healthy skin surface is between 4.2 and 5.6 and an increase in pH value indicates a sign of local infection. It was reported that the pH value measured from wound exudates of second degree burns could reach the maximum value of 10.0 (Ono et al., 2015). In order to investigate the effects of hydrogel component and pH on the release behavior, we have measured the %cumulative release of different hydrogels in both pH 5.5 and 10.0 buffer solutions and results are shown in Figure 4.

![Figure 3: ATR-FTIR spectra of (a) gelatin, (b) chitosan, (c) polyvinyl alcohol and (d) ternary blend of polyvinyl alcohol/chitosan/gelatin.](image-url)
Figure 4 The cumulative release of mangiferin from different hydrogels in a) pH 5.5 and b) pH 10.0 buffer solutions.

Investigation on the hybrid ternary blend hydrogels

- **SEM-EDX and ATR-FTIR**

The elemental analysis and ATR-FTIR spectroscopy were used to examine the hybrid ternary blend hydrogels and the results are shown in Figure 5 and Figure 6, respectively.

Figure 5 SEM-EDX analysis of a) 90PV/5CHI/5GEL, b) 90PV/5CHI/5GEL-T1 and c) 90PV/5CHI/5GEL-T2 hydrogels.
**Fluid uptake**

It is generally known that the entrapment of wound exudate by the dressing hydrogel is one of the important considerations, as exudate in wound can cause the growth of bacteria and microbial infection. It also prevents the wound from maceration. In this work, the fluid uptake of the fabricated ternary blend hydrogels was investigated in pH 5.5 and 10.0 buffer solutions and the results are displayed in Figure 7.

**In vitro release of mangiferin from the hybrid hydrogels**

The vitro release of mangiferin from the hybrid hydrogels was examined in pH 5.5 and 10.0 media and shown in Figure 8.
Discussion

**Characterization of mangiferin**

Figure 1a shows the $^1$H-NMR spectrum (400 MHz, DMSO-$d_6$) of mangiferin sample and the data are as follows: $\delta$ 13.76 (1H, s, 1-OH), 10.55 (2H, brs, 6-OH, 7-OH), 7.38 (1H, s, H-8), 6.86 (1H, s, H-5), 6.37 (1H, s, H-4), 4.84 (2H, brs, OH-3′, OH-4′), 4.60 (1H, d, $J = 9.6$ Hz, H-1′), 4.58 (1H, brs, 6′-OH), 4.45 (1H, brs, 2′-OH), 4.05 (1H, t, $J = 8.8$ Hz, H-2′), 3.69 (1H, brd, $J = 11.2$ Hz, H-6′), 3.40 (1H, dd, $J = 11.2$, 6.4 Hz, H-6′a), 3.17 (3H, m, H-3′, 4′, 5′). The ATR-FTIR spectrum (Figure 1b) of mangiferin shows major absorption bands at 3500-3250, 1619, 1490, 1250, 1190, 1095 and 828 cm$^{-1}$ indicating main characteristic of the compound. Similar finding was also reported by de Souza and co-workers (de Souza et al., 2009).

It was observed from Figure 2 and Table 2 that mangiferin showed inhibition effect against *M. luteus* and *P. aeruginosa* at the concentrations of 50 and 75 mg/mL, respectively and the inhibition zones increased with increasing concentration of the compound. The solvent for mangiferin dissolution, used as a reference, did not show antimicrobial activity. The obtained results confirmed a characteristic of this natural compound as a product with antimicrobial effect which is in correspondence with other works (Stoilova et al., 2005).

**Investigation on the binary and ternary blend hydrogels**

It has been reported that polyvinyl alcohol, chitosan and gelatin are self-associating components and capable of interacting by intra- and inter-molecular hydrogen bonding. The blends of these homopolymers have been reported to have good mechanical properties under investigation for the biomedical application (Yuvaraja et al., 2017). The superior properties of the materials have been attributed to their strong molecular interactions (Yanga et al., 2016; Garnica-Palafax & Sanchez-Arevalo, 2016). The bioactive mangiferin, polyphenol compound, used in this work is also capable of hydrogen bonding with the amide and hydroxyl pendent groups in the structure of the homopolymer matrices. The proposed schematic for the interactions between the homopolymers and mangiferin through inter- and intra-molecular bindings is shown in Figure 9.
• **ATR-FTIR**

ATR-FTIR spectroscopy was used to confirm functionality of the hydrogels. Figure 3 shows the ATR-FTIR spectra of 90PV/5CH/5GE and its homopolymers. The spectra of gelatin and chitosan show some similar absorption bands, as their repeating units include a number of identical functional groups. The major characteristic peaks around 3300 and 2920 cm\(^{-1}\) are due to N-H stretching and C-H stretching vibrations, respectively. The absorption peaks at 1658 and 1520 cm\(^{-1}\) correspond to the amide I and amide II groups. The sharp bands around 1050 and 1110 cm\(^{-1}\) indicate the C-O stretching vibration. For the spectrum of pure polyvinyl alcohol, the broad band around 3300 cm\(^{-1}\) is a result of the O-H stretching. The strong peaks around 2950 and 2975 cm\(^{-1}\) could be assigned to the C-H stretching of alkyl groups. The stretching band around 1740 cm\(^{-1}\) refers to the C=O from the remaining acetate group of vinyl acetate unit. From the spectrum of 90PV/5CH/5GE, the ternary blend with a high proportion (90%) of polyvinyl alcohol, it can be noted that the bands at 3300, 2975, 2950, 1740 cm\(^{-1}\) are the main characteristic peaks of polyvinyl alcohol as it was the main component in the blend. The amide I and amide II absorption bands shown in the spectrum are due to the minor components of chitosan and gelatin.

![Schematic diagram](image)

**Figure 9** The proposed schematic of inter-molecular and intra-molecular hydrogen bonding in the mangiferin loaded hydrogels: a) binary blend and b) ternary blend.
• **In vitro release of mangiferin from the binary and ternary blend hydrogels**

The cumulative release profiles of mangiferin (Figure 4) from binary and ternary blends indicated that the release profiles depended upon the hydrogel composition as well as the pH. It was observed that the %cumulative release of drug from all hydrogels in pH 5.5 was higher than in pH 10.0 medium and a major fraction of the bioactive drug was retained in the hydrogel after 8 hours of the test. The lowest release rate and content of mangiferin were measured from the 90PV/10CH hydrogel. At the end of the test, only 52 wt% of mangiferin was released in pH 10.0 (Figure 4b). In this case, the retained bioactive compound in this hydrogel was unfavorable, as a large fraction of the costly drug was not efficiently used for the required treatment. In the case of 95PV/5CH hydrogel fabricated with lower composition of chitosan (5 wt%), a more effective release profile was observed comparing to the 90PV/10CH in terms of release rate and amount. This could be due to the strong inter-molecular hydrogen bonding between the numerous hydroxyl groups of polyphenolic mangiferin and the amide and hydroxyl pendent groups of chitosan (Neelakandan & Kyu, 2009). From our preliminary study, we found that mangiferin showed significantly preferential affinity to chitosan than to PVOH and the release of mangiferin from pure chitosan hydrogel was extremely low especially in basic medium. In order to reduce the confined and inefficient use of mangiferin in the hydrogels, a ternary blend of 90PV/5CH/5GE was prepared and its release profile was examined. We found that a more desirable release profile and higher efficient use of mangiferin was achieved by the ternary blend hydrogel. This could be due to the hydrophilic nature and the structure of gelatin that consists of a multitude of branching and heterocyclic bulk groups in its backbone. An incorporation of this polymer in the hydrogel could diminish the intensity of hydrogen bonding and also increase a free volume between polymeric chains in their swollen states. Therefore, the ternary blend seems promising to overcome the confinement problem and ineffective use of mangiferin (Pulat & Ugurlu, 2016). It should be noted that, the cumulative release of 90PV/5CH/5GE in pH 10.0 medium representing an infected wound condition was 75% after 8 h and should be optimized. Here, we proposed a sol-gel synthesis for the preparation of hybrid organic-inorganic hydrogels with siloxane linkage. The release behavior of the prepared materials is further discussed in next section.

**Investigation on the hybrid ternary blend hydrogels**

Two reactions used to describe the sol gel process of hybrid hydrogels are hydrolysis (Eq. 2) and condensation (Eq. 3-4) (Pipattanawarothai *et al.*, 2017). The elemental analysis obtained from SEM-EDX (Figure 5) confirms the appearance of siloxane linkage by the Si and O compositions in the hybrid 90PV/5CHI/5GEL-T1 and 90PV/5CHI/5GEL-T2 hydrogels. The mangiferin loaded hybrid hydrogels were fabricated and characterized by ATR-FTIR spectroscopy (Figure 6). The strong band around 1095 cm\(^{-1}\) was due to the characteristic of mangiferin. The structure of bioactive loaded hybrid terpolymer comprised three dimensional network of siloxane is proposed and shown Figure 10.
Hydrolysis: \[ \text{Si-(OR)}_4 + \text{H}_2\text{O} \rightarrow \text{HO-Si-(OR)}_3 + \text{ROH}, \quad \text{R= ethyl group} \] (Eq. 2)

Polycondensation: \[
\begin{align*}
\text{(RO)}_3\text{-Si-OH} + \text{HO-Si-(OR)}_3 & \rightarrow \text{(RO)}_3\text{-Si-O-Si-(OR)}_3 + \text{H}_2\text{O} \\
\text{(RO)}_3\text{-Si-OH} + \text{RO-Si-(OR)}_3 & \rightarrow \text{(RO)}_3\text{-Si-O-Si-(OR)}_3 + \text{ROH}
\end{align*}
\] (Eq. 3) (Eq. 4)

- **Fluid uptake**

  From Figure 7, it was found that these hydrogels could quickly absorb a high volume of fluid with %swelling ratio in the range of 220-350 and 580-880 in pH 10.0 and 5.5 buffer solutions, respectively. The swelling of all samples reached their equilibrium states within approximately 60 min in both solutions. Higher swelling ratio in pH 5.5 environment was due to the protonation of amine functionality giving cationic groups (NH\(_3^+\)) and causing a repulsion between chains and a hydrated structure. It is interesting to note that swelling behavior of the hydrogels was complicated, as it was governed by several factors such as the nature of the three parent polymers, polyvinyl alcohol, chitosan and gelatin, and the density of siloxane hybridization. We found that the terpolymer hydrogels without the siloxane hybridized network could swell to a large extent in pH 5.5 medium. The reason for this was due to the interlocking of molecular chain after hybridization that restricted the repulsion between protonated polymeric chains. On the other hand, the hybridization promoted an increasing in the swelling degree in pH 10.0 medium. A high ionic strength of buffer solution and a hydrophilicity of siloxane hybridized network could contribute for this behavior.

*Figure 10* The proposed schematic of intermolecular and intramolecular hydrogen bonding in mangiferin loaded hybrid-ternary blend hydrogel.
In vitro release of mangiferin from the hybrid hydrogels

Figure 8 shows the in vitro cumulative release of mangiferin in pH 5.5 and 10.0 media. The effect of sol gel hybridization on the cumulative release was observed. Referring to the swelling data, we found that the release path could be a swelling controlled mechanism followed by diffusion of mangiferin, as the release profiles of all samples corresponded to the swelling behavior. Interestingly, the slower release of mangiferin in pH 5.5 was observed from the M90PV/5CH/5GE-T2 prepared with higher siloxane hybridization. This hydrogel was more suitable for the release in basic environment (pH 10.0) representing the simulated infection condition. After 8 h, the cumulative releases of M90PV/5CH/5GE-T2 were 87.9 and 91.2% in pH 5.5 and 10.0 solutions, respectively. The release kinetics was also investigated by introducing power law equation (Bhowmik et al., 2012):

\[
\frac{M_t}{M_\infty} = kt^n
\]

where \(M_t\) is the amount of released drug at time \(t\) and \(M_\infty\) is the amount of released drug at equilibrium. \(k\) is a characteristic constant related to the structure of the hydrogel network. \(n\) is a diffusional constant that relates to the release transport mechanism. By plotting the \(\ln(\frac{M_t}{M_\infty})\) against \(\ln t\), the constants can be calculated from the intercept and slope of the plot. Three classes of release mechanisms are classified. Case I or Fickian diffusion occurs when the rate of diffusion is much less than that of relaxation, in which the \(n\) values \(\leq 0.5\). For Case II, relaxation-balanced diffusion, where \(n \geq 1.0\), the diffusion is very fast relative to the relaxation rate. In the case of Non-Fickian diffusion, where \(0.5 < n < 1\), the rates of diffusion and polymer relaxation are comparable. It was found that the release of mangiferin is between the limiting of Case I and Case II. Using the fractional release at time, the \(n\) and \(k\) for the three samples are given in Table 3. The calculated \(n\) values are below 0.5, indicating a Fickian swelling transport mechanism diffusion. Similar values of \(n\) were reported (Pulat & Ugurlu, 2016).

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Values of “k”</th>
<th>Value of “n”</th>
<th>Correlation coefficient, (R^2)</th>
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<tr>
<td></td>
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Conclusions

Polyphenol mangiferin, a natural compound extracted from leaves of mango trees, possesses many bioactivities such as pharmacological, antimicrobial and antioxidant effects. The use of this natural component in controlled delivery system is limited due to its low solubility and capability of strong intermolecular interaction through hydrogen bonding. As a result, the transportation of mangiferin from its loaded matrix is not efficient for therapeutic treatment. To overcome this problem, different types of mangiferin loaded PVOH based hydrogels have been prepared including PVOH/CHI binary, PVOH/CHI/GEL ternary and hybridized PVOH/CHI/GEL ternary blends. Because of the strong intermolecular interaction between mangiferin and the homopolymers, the cumulative released of drug from 90PVOH/10CHI binary blends was low in pH 10.0 medium which was not beneficial for the medical treatment. To enhance the release and the competent use of bioactive compound, the PVOH/CHI/GEL ternary blend and its hybridized hydrogels were fabricated. These samples were test for potential mangiferin delivery matrices. The preliminary results from swelling behavior and in vitro release study presented the hybridized M90PV/5CHI/5GEL-T2 hydrogel as the most suitable matrix for controlled release of mangiferin. Further studies, the cytotoxicity and in vivo release, needed for the use of this fabricated hydrogel in wound dressing and biomedical applications are under investigation.

Acknowledgements

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