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Characterization of Genipin-Crosslinked Gelatin Hydrogel Loaded with Curcumin

Masrina Mohd Nadzir, Lau Sin Mun and Chan Pei Juan
School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus,
14300 Nibong Tebal, Penang, Malaysia

Abstract: Gelatin hydrogels are attractive for use in biomedical applications due to its well defined structural, physical and chemical properties. This study aimed to evaluate the physicochemical properties of crosslinked gelatin hydrogel incorporated with curcumin and the release characteristic of curcumin from the hydrogel matrix. The addition of naturally occurring crosslinking agent, genipin has improved the mechanical strength of the gelatin hydrogel. Low concentration of genipin (0.1% w/w) added to gelatin solution prior to solidification resulted in average pore size more than 1000% larger than the average pore size of un-crosslinked gelatin hydrogel. However, average pore size was reduced with further increased in genipin concentration. This is accompanied with decreased in swelling capacity of hydrogel. Curcumin entrapment and release analysis confirmed that the alteration of pore size through crosslinking with varying genipin concentration enabled the controlled release of curcumin from hydrogel matrix.

Key words: Gelatin hydrogel, genipin, curcumin, crosslinking, biomedical applications

INTRODUCTION

Turmeric, derived from the rhizome of perennial herb Curcuma Longa L. has been used for centuries as spices with no known harmful or lethal side effects and also been used to treat a wide variety of ailments (Gupta *et al*., 2013a). Studies have proven that the therapeutic activities associated with turmeric are owing to curcumin (Gupta *et al*., 2013b). Curcumin has been shown to exhibit antioxidant, anticancer, antiviral, antibacterial and antifungal activities, so much so that a number of studies have been conducted on the incorporation of curcumin in developing therapeutic and antimicrobial products (Liu *et al*., 2016; Ravindra *et al*., 2012; Kasoju and Bora, 2012).

Gelatin as a base material offers advantages such as well documented structural, physical and chemical properties. Furthermore, gelatin is abundant in nature, biodegradable and low cost (Mariod *et al*., 2013). However, the main disadvantage of gelatin based products is its poor mechanical and water resistance (Hanani *et al*., 2012) which limits its possible applications. An alternative way to overcome this problem is by modifying it using crosslinking techniques, especially by naturally-occurring crosslinking agents.

Genipin, a naturally occurring crosslinking agent can be used to improve the strength of gelatin based product and to protect the activity of curcumin. Genipin is derived from the fruits Genipa Americana and Gardenia jasminoides Ellis (Ramos *et al*., 2016). It has the ability to crosslink with amino acids or proteins and has been reported to be 5000-10000 times less cytotoxic than conventional crosslinking agent such as glutaraldehyde (Wang *et al*., 2013).

In the present study, gelatin hydrogel incorporated with curcumin and crosslinked with genipin were prepared and characterized. We also evaluate the curcumin loading and release characteristics for the hydrogel system.

MATERIALS AND METHODS

Preparation of genipin crosslinked gelatin hydrogel loaded with curcumin: Gelatin hydrogels were prepared with type B, bovine skin gelatin (Sigma-Aldrich, USA) with concentration of gelatin 6% w/v and genipin (Challenge Bioproducts, Taiwan) concentrations at 0.1, 0.3, 0.5 and 0.7% w/w. Gelatin powder was initially dissolved in distilled water at 50°C. Subsequently, the appropriate amount of 25% w/v genipin in 60% v/v ethanol solution was added to gelatin solution and stirred for 3 min at 50°C. The hot solution was then poured into a plastic mould and allows to cures for 24 h at room temperature and then frozen at -80°C for 1 h followed by lyophilization. Curcumin was loaded into the hydrogel by submerging the sample in 0.1% w/v curcumin in 60% v/v ethanol solution for 24 h at room temperature in the dark. Swelled sample was taken out from curcumin solution and washed with distilled water to remove the excess of curcumin present on the surface. Finally, sample was air dried in the dark for 48 h.

Corresponding Author: Masrina Mohd Nadzir, School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, 14300 Nibong Tebal, Penang, Malaysia

Characterization of gelatin hydrogel: The morphology of gelatin hydrogels was evaluated using scanning electron microscope (Quanta FEG 450, Netherlands). The bloom strength of sample was measured using a TA.XT-Plus texture analyzer (Stable Micro System, Surrey, UK) at a rate of 0.5 mm/sec. The maximum force (N) was recorded when the specified penetration distance was reached.

Swelling study: This test determined the maximum hydration capacity of the gelatin hydrogel. The percentage swelling index I_s (%) of gelatin hydrogels was determined by initially weighing dried samples (W_d) before immersing in Phosphate Buffer Saline (PBS, Gibco®, Life Technologies, NY, USA) at room temperature. The weight of wet samples (W_w) was determined every 15 min for up to 120 min. The water absorption of sample was calculated using the following Equation:

$$I_S = \frac{W_w - W_d}{W_d} \times 100 \quad (1)$$

Curcumin entrapment efficiency: The Entrapment Efficiency, EE (%) of curcumin was estimated by soaking the gelatin hydrogel for 72 h in PBS. After removal of hydrogel, the solution was analyzed by UV-Vis spectrophotometer (Thermo Scientific Genesys 20, USA) at 485 nm. The amount of entrapped curcumin was calculated using the following Equation:

$$EE(\%) = \frac{\text{Actual curcumin content in hydrogel}}{\text{Total curcumin}} \times 100 \quad (2)$$

In vitro drug release study: Curcumin loaded gelatin hydrogel (10 mg) was immersed in 2 ml of PBS in centrifuge tube attached to a shaker (Infors HT, Switzerland) set at 300 rpm and 37°C. Periodically, the solution containing loaded hydrogel was centrifuged at 5,000 rpm for 7 min to separate the released curcumin from the hydrogel. The supernatant containing curcumin was dissolved in 3 mL ethanol and then analyzed by UV-Vis spectrophotometer at 485 nm. The concentration of released curcumin was calculated using a standard curve of curcumin in ethanol. The percentage of released curcumin was determined from the following equation:

$$\text{Drug release}(\%) = \frac{\text{Concentration of curcumin}}{\text{Total concentration of loaded curcumin}} \times 100 \quad (3)$$

Drug release kinetics: The release profile data was fitted into the following mathematical models to analyze curcumin release kinetics and mechanism. Here, M_t/M_∞ is the fraction of drug released at time t and k_1, k_H and k represent First order release constant, Higuci constant and Korsmeyer-Peppas constant, respectively (Kasoju, and Bora, 2012; Mariod and Adam 2013; Hanani *et al*., 2012; Ramos *et al*., 2016; Wang *et al*., 2013; Dash *et al*., 2010). In Korsmeyer-Peppas Model, n is the release exponent and is indicative of drug release mechanism. The values of k_1, k_H, k and n were determined by fitting the release data into respective equation:

First order Model:

$$\frac{M_t}{M_\infty} = 1 - \exp(k_1 t) \quad (4)$$

Higuchi Model:

$$\frac{M_t}{M_\infty} = k_H t^{1/2} \quad (5)$$

Korsmeyer-Peppas Model:

$$\frac{M_t}{M_\infty} = kt^n \quad (6)$$

RESULTS AND DISCUSSION

Morphology and mechanical characteristics of gelatin hydrogels: The fabricated gelatin hydrogels were found to have a three-dimensional microporous structure (Fig. 1A-C). Crosslinking plays a crucial role in regulating the pore dimension. The average pore size for gelatin hydrogel when crosslinked with 0.1% w/w genipin was 51.86±13.33 μm which was >1000% larger than the average pore size of un-crosslinked gelatin hydrogel (3.86±1.02 μm). The average pore size decreased to 37.00±9.71 μm when the concentration of genipin was increased to 0.5% w/w due to increased crosslinking density. Loading of curcumin to the gelatin hydrogel did not significantly alter the morphology of the hydrogel (Fig. 1 D and E). Table 1 shows the differences in hardness (resistance to compressive deformation) values of gelatin hydrogel crosslinked with varying concentration of genipin. The data suggest that the

Table 1: Mechanical characteristics of gelatin hydrogels crosslinked at varying concentration of genipin

Variable (% w/w)	Resistance to compression (N)
Control	3.32
0.1	7.31
0.3	20.52
0.5	45.10
0.7	47.65

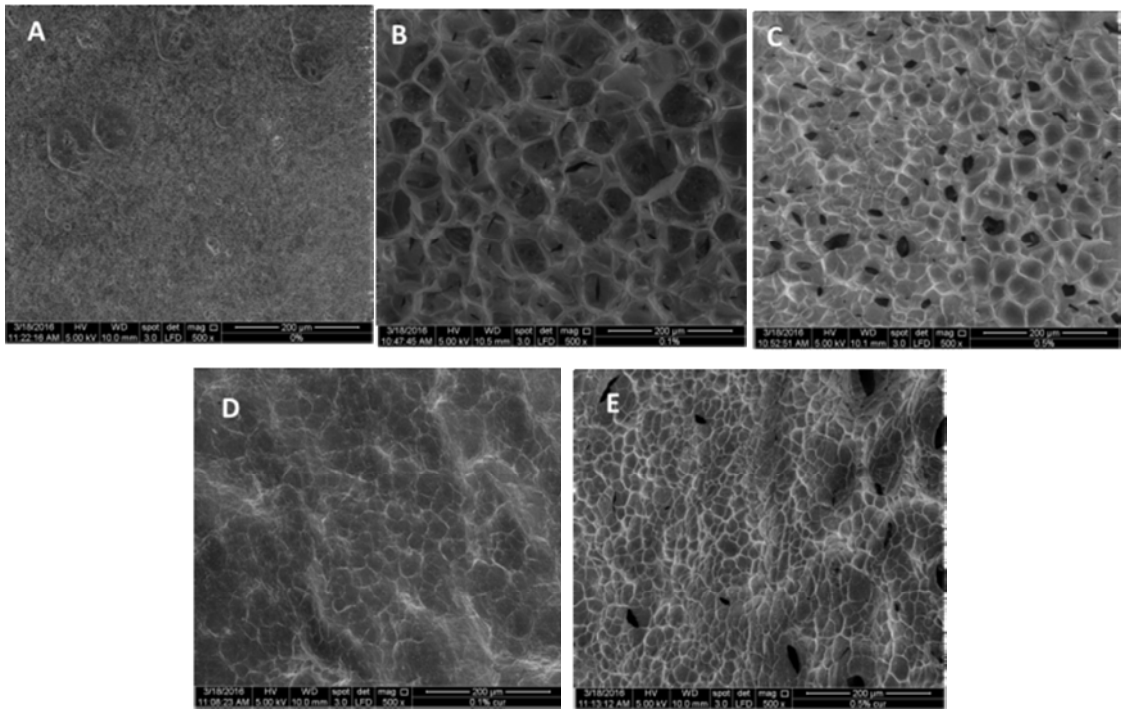

Fig. 1: A) SEM images showing the structure of unloaded gelatin hydrogel without crosslinking; B) Crosslinked with 0.1% w/w genipin; C) Crosslinked with 0.5% w/w genipin; D) Curcumin loaded gelatin hydrogel which was initially crosslinked with 0.1% w/w genipin and E) 0.5% w/w genipin

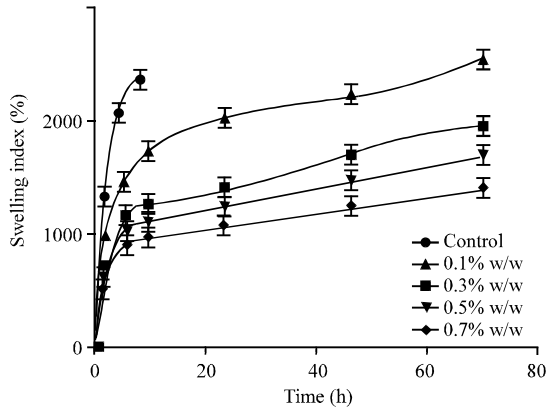

Fig. 2: The water uptake of gelatin hydrogels crosslinked via different genipin concentration

increase of genipin concentration resulted in the increase in hardness and hence decreased the flexibility of the hydrogel which could affect swelling.

Swelling studies: We could find that the swelling capacity of gelatin hydrogels decreased by increasing the genipin concentration (Fig. 2). The water uptake of the hydrogel strongly depends on the microstructure and the hydrophilic nature of the hydrogel. The ability to retain the sample porous structure and the reduction of the hydrophilic groups which are consumed during the crosslinking reaction seems to be the main explanation for the differences observed in the swelling. The range of I_s of the gelatin hydrogels can be generally divided into two groups. In the first group, the I_s of the un-crosslinked sample and sample crosslinked with 0.1% w/w genipin were in the range of 1300-2000% for the first 8 h. Although, swelling capacity of un-crosslinked gelatin hydrogel was the highest, poor mechanical properties of the hydrogel led to the collapse of the porous structure in PBS after 8 h (data not shown). In comparison, when the genipin concentration exceeded 0.1% w/w, the I_s was in the range of 800-1000%. Attenuated swelling tendency of crosslinked hydrogels was observed when the soaking period exceeded 12 h, implying that the hydrogels were nearly saturated with water. Upon submerging the gelatin hydrogel in PBS, the absorbed water and swelling action stretches the pores within and results in wider pore size distribution. The concentration of water inside the crosslinked hydrogel is dependent on the diffusivity of the solution, resulting in a concentration dependent diffusivity. It is obvious that hydrogel with more extensive crosslinking possesses a tighter structure. The increased degree of crosslinking in the hydrogel network results in restrained capacity of gelatin chains to undergo relaxation which brings about a fall in the amount of water

Table 2. The release constants and corresponding regression coefficient values

Genipin concentration (% w/w)	Mathematical models for drug release kinetics		
	First order	Higuchi	Korsmeyer-Peppas
0.1	$k_1 = 5.3\times10^{-5}$ $R^2 = 0.979$	$k_H = 0.047$ $R^2 = 0.961$	$k = 1.63$ $R^2 = 0.927$ $n = 0.628$
0.3	$k_1 = 6.9\times10^{-5}$ $R^2 = 0.987$	$k_H = 0.057$ $R^2 = 0.888$	$k = 1.97$ $R^2 = 0.850$ $n = 0.811$
0.5	$k_1 = 7.1\times10^{-5}$ $R^2 = 0.979$	$k_H = 0.059$ $R^2 = 0.858$	$k = 2.10$ $R^2 = 0.914$ $n = 0.893$
0.7	$k_1 = 6.9\times10^{-5}$ $R^2 = 0.947$	$k_H = 0.057$ $R^2 = 0.812$	$k = 1.82$ $R^2 = 0.868$ $n = 0.712$

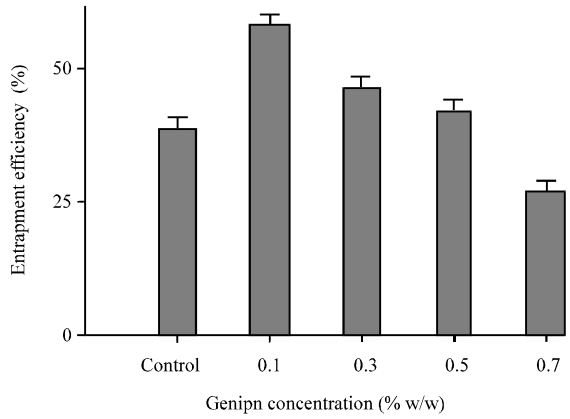

Fig. 3: The drug entrapment efficiency for gelatin hydrogels

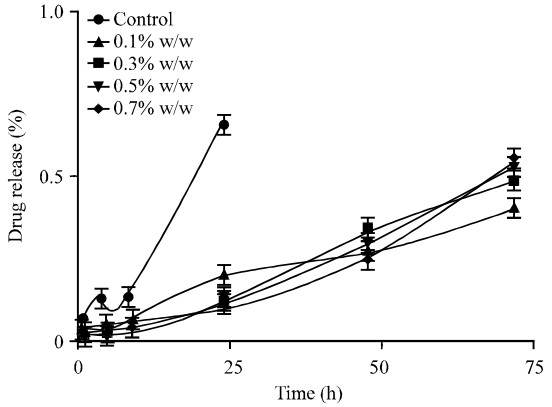

Fig. 4: The release of curcumin from gelatin hydrogels

molecules and swelling. This change in swelling with genipin concentration correlates with the hardness of the hydrogels where samples crosslinked with 0.7% w/w genipin has the highest hardness and the lowest swelling capacity.

Drug entrapment: It is shown in Fig. 3 that the highest drug entrapment efficiency (58.39%) was achieved when gelatin hydrogel was crosslinked with 0.1% w/w genipin. Curcumin was loaded into the hydrogel by means of diffusion, thus pore size plays a dominant role in determining how much curcumin could be entrapped inside the hydrogel. In this context, the 0.1% w/w genipin crosslinked hydrogel gave the highest entrapment efficiency because it having the largest pore size (Fig. 1B).

Gelatin hydrogel crosslinked with 0.7 % w/w genipin has an entrapment efficiency of 27.07% which was the lowest among all the samples. This might be due to higher extent of crosslinking which leads to smaller pore diameter as well as denser hydrogel. Thus, the void space present inside the hydrogel is significantly reduced and eventually less curcumin can be loaded into the hydrogel.

Drug release analysis: The results of the in vitro release behavior of curcumin from gelatin hydrogel are shown in Fig. 4. The release (%) profile showed a slow and sustained release over a prolonged period.

The prolonged release of curcumin suggested the release of curcumin from the hydrophobic domain of the gelatin hydrogel is largely dependent on the interaction between the drug and the domain. A slow and sustained released of curcumin from the hydrogel suggested the non-covalent interactions between curcumin and the hydrophobic domain of the gelatin hydrogel. To find out the mechanism of curcumin released from crosslinked gelatin hydrogel, we have analyzed the experimental drug release data with three different kinetic models. Based on the regression analysis, we found that the First Order Model was best fitted with drug release kinetics data (Table 2) and hence found that curcumin was released from gelatin hydrogel predominantly by means of diffusion. Since, curcumin exist in gelatin hydrogel as dispersed crystals, the release can be attributed to the expansion of pore size of hydrogel due to swelling in PBS. In this view, diffusion does not initiate from the hydrogel phase rather the penetrant moves into the hydrogel system and causes the relaxation of the polymer chains. This allows the drug to diffuse out of the swollen area of the polymer.

Controlled release of curcumin from gelatin matrix could be achieved by changing the pore size by varying the genipin concentration. Here with increase of genipin concentration the release of curcumin from crosslinked hydrogel was delayed. As for un-crosslinked gelatin, significantly fast released of curcumin was observed after several hours in PBS. However, the structure of the un-crosslinked hydrogel collapsed after 8 h, releasing the remaining curcumin from hydrogel. On the other hand, genipin crosslinked hydrogel still maintained its structure

for up to 72 h. Therefore, the addition of crosslinking agent is proven to provide a stronger mechanical structure for gelatin hydrogel so that hydrogel can last for longer period.

CONCLUSION

In this reaearch, we investigated crosslinked gelatin hydrogel incorporated with curcumin. Among all of the samples studied, the hydrogel crosslinked with 0.1% w/w genipin has the best curcumin entrapment efficiency. The large pores allow more curcumin to diffuse inside the hydrogel. Although, the hardness of this sample was the lowest for crosslinked hydrogels, the porous structure of the sample was retained for a long period. It is concluded that low concentration of genipin is suitable to attain long lasting hydrogel which entrapped high amount of curcumin loaded by means of diffusion. Our future interest will be toward exploring the potential of the synthesized gelatin hydrogel as antimicrobial wound dressing because the slow and sustained released of curcumin from hydrogel over a prolonged period makes it attractive for this application.

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