Transport of curcumin from cross-linked chitosan matrices: A comparative study

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Abstract. Chitosan hydrogels are widely studied as drug delivery vehicles. The present work involves investigating the effects of cross-linking on morphology of chitosan gels and their impact on the curcumin release from cross-linked gel matrices. Chitosan usually suffers from poor mechanical strength so to improve the mechanical and thermal stability the gels are often chemically cross-linked. In this study, chitosan hydrogels are prepared and then the gels are cross-linked with glutaraldehyde (synthetic cross-linker) and genipin (a naturally occurring cross-linker). Cross-linked gels are subjected to a number of tests, such as compression test, swelling study, and scanning electron microscopy analysis. Release study was performed by forming drug loaded gels by incorporating curcumin into the chitosan matrices. Morphological analysis for the cross-linked gels revealed compact network structure. Chitosan gel cross-linked with genipin, indicated higher compressive strength ($0.1539 \pm 0.03$) MPa than gel cross-linked with glutaraldehyde ($0.032 \pm 0.01$) MPa. Gels cross-linked with glutaraldehyde showed higher swelling and also higher release of curcumin ~90.67% whereas, genipin cross-linked gels having more compact network shows better release profile of curcumin ~79.26% over 24 hours ($P<0.05$). Release behaviour indicates swelling dependent release.

1. Introduction
The biomedical field has witnessed an immense increase in the use of hydrogels as efficient drug delivery systems and also in many other versatile applications [1]. Water molecules can be retained within the three dimensional network of hydrogel, and also, often find use as vehicles in drug delivery owing to their biocompatibility and biodegradability. Besides, hydrogel facilitate controlled release of drugs as they can disperse the drug within their network due to presence of porous structure [2]. The release system and the biodegradability of hydrogels can be modulated by varying the parameters such as temperature, pH, electric and magnetic fields etc. [2]. Chitosan, gelatin, alginate, dextran, silk etc., are the commonly used biopolymers used for the fabrication of hydrogel. Crustacean exoskeletons are usually a rich source of chitin which can be deacetylated to obtain the natural polymer chitosan [3]. Chitosan is a strong biopolymer candidate for the application of drug delivery as it possesses the desirable characteristics of biocompatibility, biodegradability and anti-bacterial properties [4]. Biopolymer based hydrogels usually suffer with poor mechanical and thermal stability so often that they require an enhancement of mechanical strength. Both synthetic and natural cross-linking agents are employed to give mechanical stability to proteins and biomaterials. However, natural cross-linkers such as genipin, starch etc. are usually preferred over synthetic cross-linkers owing to lesser or negligible toxicity. Proteins, gelatin, chitosan, are effectively cross-linked by genipin which is obtained from geniposide [5]. Porosity of matrix and enhanced rigidity which are essential in cartilage tissue engineering and drug delivery application, are effectively met by genipin cross-linked chitosan–gelatin composite hydrogels [5]. The herb Curcuma longa naturally yields curcumin, which is a weak base. Curcumin is a known antioxidant, and antibacterial agent having antitumor and anti-inflammatory properties, which enables it to find its application in drug delivery [6]. The therapeutic application of curcumin can be increased by extending its release behaviour.
The present work aims at designing suitable glutaraldehyde and genipin cross-linked stable hydrogels which will behave as a carrier and regulate the release of the biomolecules.

2. Experimental

2.1. Materials

Chitosan (90% deacetylation) was procured from Marine Chemicals (Kerala, India). Glutaraldehyde and Lactic Acid (99%) were purchased from HiMedia (Mumbai, India). Genipin was procured from Challenge Bioproducts (Taipei, Taiwan). Deionized water was used for all experiments.

2.2. Preparation of curcumin loaded cross-linked chitosan hydrogel

First, 1% (w/v) chitosan solution was prepared in lactic acid (2% v/v) and magnetically stirred for 15 minutes to dissolve the chitosan completely in lactic acid solution. 25 mg of curcumin was added into the solution and stirred and gently heated for complete dissolution of the curcumin. Then the solutions were kept in the freezer at about -40°C. After keeping it for 15-20 minutes a gel was formed. The gel formed had very low mechanical strength. The gels were cross-linked with glutaraldehyde and genipin to fabricate curcumin loaded cross-linked gels. 15% (w/v) glutaraldehyde solution was incorporated to 1% (w/v) chitosan solution, and was kept in a magnetic stirrer at 40°C for about 10 minutes. Chitosan was cross-linked with glutaraldehyde to form a hydrogel. Similarly, the chitosan hydrogel was also cross-linked by incorporating 1% (w/v) genipin solution into it and kept for 24 hours at room temperature to facilitate complete dispersion.

2.3. Characterization

2.3.1. Swelling Test: Hydrogel’s absorption capacity was determined by swelling test. 0.5 g of samples were dipped in 10 ml of water for 24 hours at room temperature. After removing the samples from water at different intervals of time, blotting paper was used to remove excess water followed by recording their respective weights. Then the hydrogels’ swelling index was determined according to standard protocol as described (7).

\[ SI = \frac{(W_s - W_d)}{W_d} \times 100 \]  

2.3.2. Compression test: Compression test is a technique employed to analyze the mechanical properties of hydrogels. Hydrogel matrices were compressed at 60mm min^-1 compression rate (EZ test, Shimadzu Corporation, Japan). The hydrogel matrices were 35mm in diameter. This was followed by the determining compressive modulus using curve fitting in the linear region of the stress-strain curve. Results were performed in triplicates for each hydrogels and were expressed as mean ± standard deviation.

2.3.3. SEM analysis: The surface morphology of the cross-linked constructs was studied using a scanning electron microscope (SEM) (EVOMA18 with Oxford EDS-X-act). Images were acquired after gold sputter coating the samples. The cross-linked constructs were freeze dried for 48h using a freeze dryer prior to SEM analysis.

2.3.4. In-vitro drug release: Diffusion cell having a barrier of semi permeable membrane was used to measure the hydrogels’ drug release profile. Diffusion study using crosslinked hydrogels (genipin and glutaraldehyde both) 2g of curcumin loaded with 2 gm of curcumin was performed at room temperature in for 24 hours in deionized water. 5ml of samples were taken out at regular time intervals and the collected samples were analyzed spectrophotometrically at 265nm.

2.3.5. Statistical Analysis: All quantitative experiments were performed in triplicates followed by expressing the results in means ± standard deviation (SD) for n = 3. Statistical analyses of the data for all experiments were performed by Graph Pad Prism 7 software ((La Jolla, California USA), p ≤ 0.05 were deemed statistically significant.
3. Results and discussion

Chitosan based hydrogels, when cross-linked with different cross-linkers appear in different colours. When cross-linked with glutaraldehyde the gel had a mustard yellow colour whereas, when cross-linked with genipin it had a dark blue colour [7]. The change of colour provides the proof for the cross-linking process (figure 1).

![Figure 1](image)

Figure 1. (A) Chitosan gel without cross-linking, (B) glutaraldehyde cross-linked (C) genipin cross-linked.

The swelling study is essential because it is a key regulating factor to study the release of drug from the hydrogel. Release of drug from the gel occurs because of the ingress of water leading to its swelling. Figure 2 demonstrates the swelling index of both gels studied for 24 hours. It was observed that after 8 hours the uncross-linked gel has a swelling of over >90% swelling resulting in the disintegration of gel. Crosslinking hydrogels with glutaraldehyde and genipin enhanced their stability and a swelling of 92.6 ± 0.04% for glutaraldehyde cross-linked gel and 79.27±0.07 for genipin cross-linked gel, were recorded for 24 hours (P<0.05) (figure 2).

![Figure 2](image)

Figure 2. % Swelling Index of the cross-linked chitosan gels

| Table 1: Compression test values of chitosan based hydrogels. |
|-------------------------------------------------------------|
| Sample composition | Compressive modulus (Mean + SD) MPa     |
| Chitosan + glutaraldehyde                    | 0.032 ± 0.01               |
| Chitosan + genipin                           | 0.1539 ± 0.03              |

From the above table (Table 1), it can be seen that the compression values for the chitosan based hydrogels cross-linked with genipin is greater than hydrogels cross-linked with glutaraldehyde. This may be due to the fact that genipin provides better stability and it forms stronger bonds with the hydrogel matrices.
Figure 3. SEM micrographs for (a) chitosan cross-linked with glutaraldehyde (b) chitosan cross-linked with genipin at a magnification of 500X, 1000X and 2000X

From figure 3, it can be understood that the cross-linking between the chitosan-genipin hydrogels seemed to be more defined and the bonding between the structures are more prominent when compared with chitosan-glutaraldehyde hydrogels. Surface morphology of hydrogels cross-linked with glutaraldehyde is different from the hydrogels that are cross-linked with genipin. Thus, by using different cross-linkers surface morphology of hydrogel changes. This demonstrates that genipin is more potent than glutaraldehyde, therefore, genipin based hydrogels have better cross-linking properties than glutaraldehyde [7].

Figure 4. % Cumulative release of curcumin from glutaraldehyde and genipin cross-linked gel matrices.

Figure 4 demonstrates the release profile of curcumin from glutaraldehyde cross-linked and genipin cross-linked composite matrices. The uncross-linked gel has low stability causing it to show a burst release followed by its disintegration in less than 8 hours (data not shown in graph). When the hydrogel is immersed in the solvent, the pore size increases due to increase of interstitial spaces between the polymer chains leading to diffusion of entrapped drug molecule out into the solution. The burst effect is produced by the release of loosely bound drug molecules on the gel surface, into the dissolution medium. However, controlled release profile of curcumin is better for the matrix cross-linked with genipin (~79.26%) than the gel cross-linked with glutaraldehyde (~90.67%) for 24 hours (P<0.05). The polymer network of the hydrogel becomes more compact as its stability is enhanced by crosslinking [8]. The polymer networks’ enhanced rigidity after crosslinking leads to reduced swelling.
rate. Entrapped molecules are released from the matrix by sustained diffusion as solvent ingress causes controlled relaxation of the polymer network. This occurs because the transport of drug indicates swelling dependent release profile. The results are in line with the compression test data.

4. Conclusion
Biolopolymers of natural origin are the current interest of researchers all around the world in the biomedical field and chitosan has indicated promising results in multiple applications. To improve the mechanical strength of hydrogels, crosslinking as a strategy has proved to be beneficial. Also, as demonstrated by the results of this study, a sustained release cannot be obtained from un-cross-linked gels due to the absence of a compact nature and stability. This study also aided in improving the pharmacokinetics of curcumin and enhancing its stability by crosslinking. It has also been observed that, owing to genipin’s natural origin, the biocompatibility of genipin is better as compared to synthetic cross-linkers. Hence, paving a way for future investigations, as it is a safe and effective cross-linker. Besides, the studies also indicated that the fabricated hydrogel cross-linked with genipin exhibited a better transport of curcumin than the glutaraldehyde cross-linked gel. Thus, the results attribute that transdermal and site specific drug delivery can be targeted by a suitable polymeric carrier using hydrogels cross-linked with genipin. Various other biomedical application can also be explored using this concept in both acidic and basic buffer with other model drugs.

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