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Controlled Drug Release from Gelatin-Sodium Carboxymethylcellulose Interpenetrating Polymer Networks

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ABSTRACT

Hydrogels of uncrosslinked gelatin, crosslinked gelatin (Gelx), and various compositions of semi-interpenetrating polymer network of cross-linked gelatin with uncross-linked sodium carboxymethylcellulose [Gelx-NaCMC] were investigated as potential matrices for substrate delivery. Simultaneous swelling behavior and controlled drug release under enzymatic conditions (erodible) were monitored for hydrogels of [Gelx-NaCMC] and Gelx. Results indicated a first order release indicating that the processes (rate of drug diffusion and degradation) do not follow the same kinetics.

Key Words: Hydrogels; Swelling; Drug release; Enzymatic degradation; Biodegradation; Natural polymers; Gelatin; Interpenetrating polymers networks.

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INTRODUCTION

Over the past two decades extensive research has been carried out in the design of polymeric matrices for controlled drug delivery. Currently, hydrogels are considered to be very attractive devices, because hydrogels allow control of drug diffusion upon the adjustment of several parameters (polymer concentration, crosslink density, hydrophlicity, degradability, etc.).[1–6] The most common mechanism of drug release follows the first order release kinetics (matrix type), where the rate of drug diffusion is controlled by the concentration of drug loaded.[7–11] However, once the surface concentration is depleted, the drug release slows down. We felt a bioerodible device could be the best matrix to achieve constant release. In addition, fine tuning of the matrix degradation and substrate release kinetic could ultimately lead to zero order release.

Our earlier investigations suggested that a two component semi-interpenetrating system, consisting of cross-linked gelatin (Gelx) with sodium carboxymethlcellulose (NaCMC) would be an ideal matrix.[12–14] Because, both gelatin and NaCMC are totally biocompatible, while gelatin undergoes complete enzymatic degradation[15] and NaCMC is excreted biologically. Thus, the suitability of [Gelx-NaCMC] as a biodegradable controlled release matrix, to achieve zero order release is beyond dispute. We chose bromothymol blue as the substrate for its compact structure and the ease with which it can be detected and quantified.

EXPERIMENTAL

Materials

Gelatin, sodium carboxymethylcellulose, 25% glutaraldehyde, tris- (hydroxyethyl) aminomethane and trypsin (enzyme), were procured from Loba Chemie, Bombay, India. Bromothymol blue (BTB) was obtained from British Drug Houses, England. All other reagents were of analytical grade.

Preparation of Ten Percent [Gelx-NaCMC] Hydrogels with or without Loaded Drug

Substrate free hydrogels were prepared as reported in our earlier studies.[12–14] Ten percent gels were prepared by dissolving the calculated amounts of gelatin and NaCMC in 100 ml water (w/v) at 100°C. The dissolved concentrated aqueous solution mixture was cooled and poured between the glass plates separated by Teflon gaskets and the solution was allowed to set overnight. The gelled slab of 0.5 cm in thickness was dislodged carefully, cut into circular discs of diameter 1.6 cm and soaked in 1% glutaraldehyde solution (v/v) for 6 h. Later, gels were washed with distilled water for several times to remove excess glutaraldehyde and dried the gels at room temperature (30–35°C) to constant weight. Gels were prepared with varied NaCMC composition (0–2.5), and abbreviated as A–E in Fig. 1. For preparing drug loaded gels, 90 mg of BTB was dissolved in 100 ml (w/v) of citric acid–disodium hydrogen orthophosphate buffer, of pH 7.4, followed by the addition of gelatin

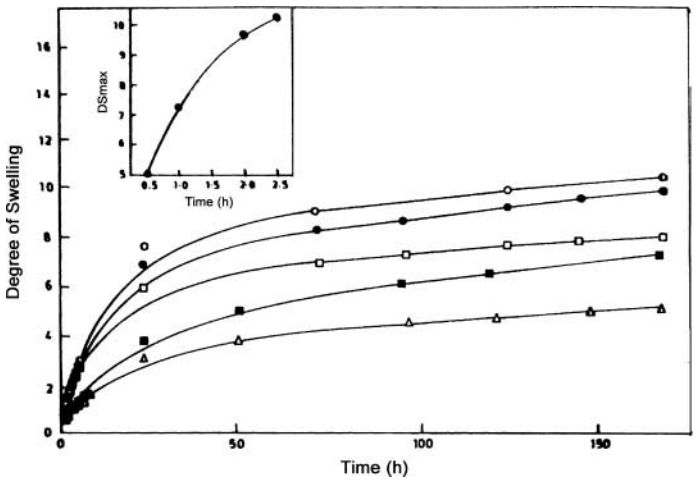

Figure 1. Dynamic swelling profiles for [Gelx-NaCMC] gels. Inset shows DS_{max} (maximum degree of swelling) for gels A, B, C, D and E keeping gelatin ratio constant to 1.0 and varying NaCMC starting from 0.05, 0.10, 0.15, 0.20 and 0.25, respectively.

and NaCMC of composition 1.0:0.1, respectively at 100°C. Gels were crosslinked with 15% glutaraldehyde solution (v/v) for 1 h, so as to increase the rate of crosslinking and to minimize the drug diffusion. The total amount of BTB present in the disc was estimated by grounding the disc to fine powder, weighed and transformed into cellulose thimble and subjected soxhlet extraction with 200 ml of water for 48 h. The extract was filtered and made up to 250 ml. The BTB concentration was determined by correlating the absorbance of the solution at 616 nm to the calibrating curve.

Controlled Release Experiments

[Gelx-NaCMC] being bioerodible,[15] we first established the bioerodibility of Gelx, and [Gelx-NaCMC] hydrogels by enzymatic degradation studies and then studied the release of BTB from the respective hydrogels under non-erodible (non-enzymatic) and erodible (enzymatic) conditions according to the reported literature.[16–17] All experiments were conducted in duplicates with [Gelx-NaCMC] hydrogels of Gelx to NACMC ratio at 1.0:0.1.

Enzymatic Degradation Studies

The enzymatic degradation studies were done for gels of gelatin, crosslinked gelatin (Gelx) and [Gelx-NaCMC], respectively using trypsin as an enzyme based on the reported literature.[16] The experiments were carried out in Perkins Elmer UV/Visible spectrophotometer Lambda 2 with provision for heating the samples.

Swollen gels of gelatin (0.012 g), Gelx (0.0115 g) and [Gelx-NaCMC] (0.0094 g) were transferred into three separate UV Quartz cells of capacity 3 ml. Each cell contained 0.04% of trypsin in 2.5 ml (w/v) of 1 M tris(hydroxymethyl)amino methane buffer of pH 8.6. The UV cells were maintained at 44°C and the amount of protein degraded was recorded at regular intervals by monitoring absorption at 280 nm. The percent of protein degraded was calculated from calibration curve. For the calibration curve, 1 g of gelatin were dissolved in 100 ml of distilled water (w/v). From the stock solution, a series of gelatin solutions of concentrations in the range 0.1 to1.0% (w/v) were prepared and recorded optical density at 280 nm.

Estimation of BTB Release from Matrix

BTB release under enzymatic[17] and non-enzymatic conditions for Gelx and [Gelx-NaCMC] were monitored using a Perkins Elmer UV/Visible spectrophotometer model Lambda 2. The exact amount of BTB release from the matrix was estimated using a BTB calibration curve.

BTB release studies were done at 37°C by suspending the BTB loaded disc in 10 ml of citric acid-disodium hydrogen orthophosphate buffer of pH 7.4. At definite intervals, the disc was taken out and the amount of dye released into the buffer was determined by absorption at 616 nm. The disc was transferred into a 10 ml fresh buffer solution for the next measurement.

Dye release studies for Gelx and [Gelx-NaCMC] were also conducted in the presence of an enzyme trypsin at 44°C in tris(hydroxymethyl)amino methane of pH 8.6. These studies were done in 20 ml of buffer solution, with varying amounts of trypsin, 10 mg, 20 mg and 50 mg per 100 mg disc (w/w). The amount of enzyme had to be increased because its specific activity was very low. The recordings of the optical density were done as described earlier, at each interval the disc was transferred into a 20 ml fresh buffer for the next measurement.

Swelling Cycles

The ability of hydrogel samples to repeated swelling and drying were performed so as to understand the robustness of the swelling phenomenon. Gels of [Gelx-NaCMC] of set B (1.0:0.1), were dried at room temperature (35°C) to constant weight, and allowed to swell to equilibrium weight. The swollen discs were again allowed to dry at room temperature (35°C) to attain constant weight. This process of swelling and drying were continued through several cycles.

RESULTS AND DISCUSSION

[Gelx-NaCMC] hydrogels of various compositions were prepared by keeping gelatin weight constant and increasing NaCMC as shown in Fig. 1. From the analysis of swelling studies on [Gelx-NaCMC] hydrogels of various compositions,[12–14] the hydrogels of gelatin to NaCMC ratio at 1.0:0.1, i.e., set B was selected for our further studies on

enzymatic degradation and controlled drug release. Figure 1, shows the dynamic swelling profile for [Gelx-NaCMC] gels of various compositions developed. We observed that as the NaCMC content is increased, the degree of swelling increases, decreasing the gel strength due to the reasons as reported earlier.[12–13]

Enzymatic Degradation Studies

To monitor the bioerodibility of the [Gelx-NaCMC] hydrogels, several samples were subjected to enzymatic degradation. As controls both gelatin and cross-linked gelatin (Gelx) were included. Figure 2, shows the enzymatic degradation of gelatin, Gelx and [Gelx-NaCMC] hydrogels. Uncrosslinked gelatin gel registered 73% of protein degradation within 2 h; whereas [Gelx-NaCMC] and Gelx registered only 24% and 19% of protein degradation respectively even after 5 h. The [Gelx-NaCMC] being more hydrophilic than Gelx, the extent of degradation is more.

Entrapment and Release of BTB from [Gelx-NaCMC] Hydrogels

There are several methods for incorporating a substrate into a polymer device and monitoring drug release. For incorporation the choice is invariably based on the chemistry of the substrate and the polymer matrix. A method that causes non-or least alteration to the characteristics of the loaded drug is always chosen. From this perspective, hydrogels have definite advantages over other polymeric materials. Hydrogels are generally very compatible with most of the drugs because the system is mostly water. The extensive

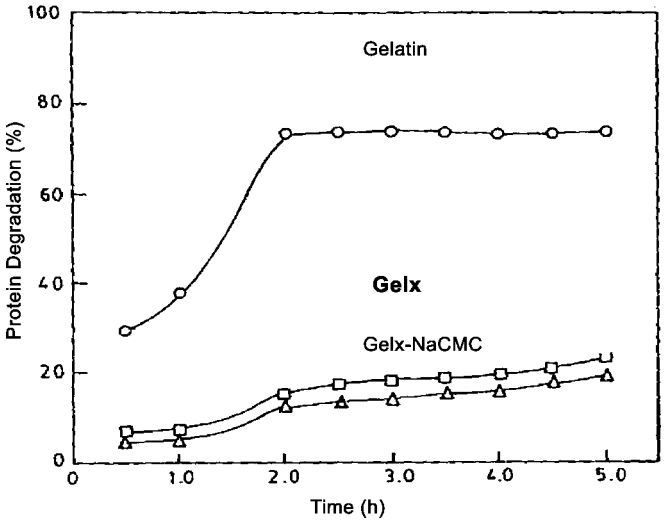

Figure 2. Degradation of ten percent gels of gelatin, Gelx and [Gelx-NaCMC] of set B.

swelling makes drug loading easier. The density of the polymer matrix can be easily adjusted by changing the polymer content or the cross-link density.

In our studies, though BTB could be loaded into the dry pre-made gel by soaking into a BTB solution of appropriate concentration, we chose to incorporate BTB at the gel making stage itself by dissolving it along with the monomer, because BTB does not contain any susceptible functional groups to interact with the polymer matrix.

The process of drug release from a polymer matrix in the surrounding medium (buffer) can be described in most cases by Fickian diffusion. In modeling reservoir or matrix systems, the equations describing Fickian diffusion are solved with appropriate initial and boundary conditions.

In monolithic dosage forms, the drug dissolved in a solid block of polymer or embedded in the matrix, diffuses to the surface of the matrix. The total amount of drug released from a film or a thin slab is proportional to $\sqrt{\text{time}}$,[18] and the rate of release gradually declines. The kinetics of drug release is governed by the physical properties, the geometry of the device and the total amount of drug incorporated. Alternatively the dosage form can be designed with large reservoir of drug bounded by a rate of controlling diffusion barrier. Changing the surface area, density of the matrix, and the solubility of the drug or its diffusion coefficient can alter the diffusion rate.

Usually the release of substrate entrapped in a polymeric device obeys a general expression:

$$M_t/M_\infty = kt^n$$

Where M_t and M_∞ are the quantities of the drug released at time t and ∞, k is the constant release and n is the index of release.

BTB Release from Non-eroding Disc

Figures 3 and 4 depict the BTB release (M_t/M_∞) from Gelx and [Gelx-NaCMC] hydrogels, respectively. The corresponding increase in the disc weight (in grams) as the disc swells is also shown in the figure. The rate of release of dye form both Gelx and [Gelx-NaCMC] are comparable, about 3–4% in 60 min. The extent of swelling is also comparable. The swelling of Gelx disc was 750% where as [Gelx-NaCMC] registered 880%. At such high swelling degrees, a small molecule like BTB can easily diffuse out of the system. However, at the end of 192 h when the swelling profile has reached a plateau, Gelx gel has released 82% of the total BTB loaded where as [Gelx-NaCMC] gels released 98%.

Dye Release from Eroding Discs

The rate of drug release from the matrix depends on the swelling characteristics of the matrix. The release of drug into the external medium is concomitant with the penetration of the water molecules into the glassy matrix. The relative contributions of the polymer relaxation and dye diffusion through the swollen gel network determine the exact nature of the release kinetics. However the release rate decreases with the time, as the surface

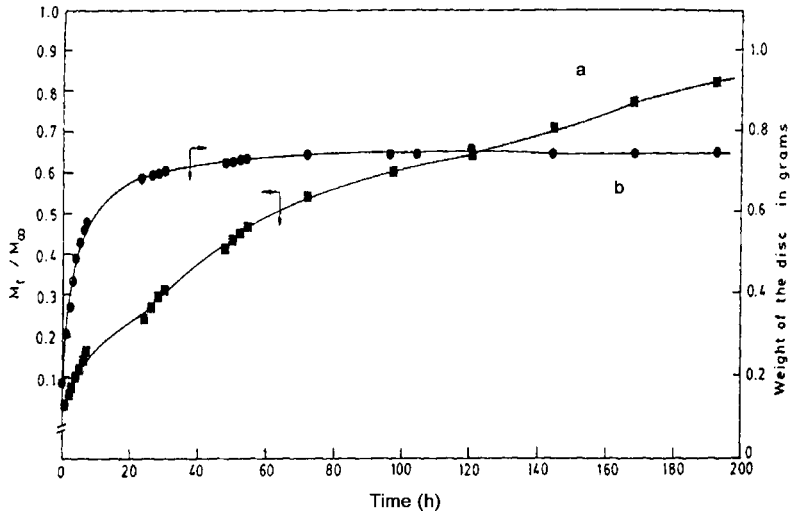

Figure 3. a) Release of BTB from Gelx gels in buffer of pH. 7.4, b) Change in swollen weight of Gelx discs.

concentration of the drug decreases because the drug has to diffuse from the core to surface, prior to release into the medium. This flaw could be avoided if the surface itself can be made to erode at a rate comparable to the substrate diffusion. Hence, we made an attempt to monitor trypsin mediated BTB release from [Gelx-NaCMC].[15]

The release studies for [Gelx-NaCMC] gels were carried out in tris buffer at 44°C, because trypsin is active at that temperature. The results confirm that 20 mg of

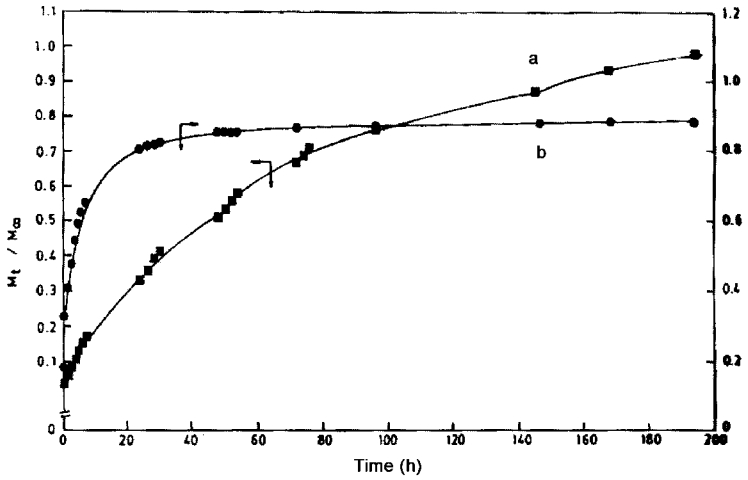

Figure 4. a) Release of BTB from [Gelx-NaCMC] of set B in buffer of pH. 7.4, b) Change in swollen weight of [Gelx-NaCMC] discs.

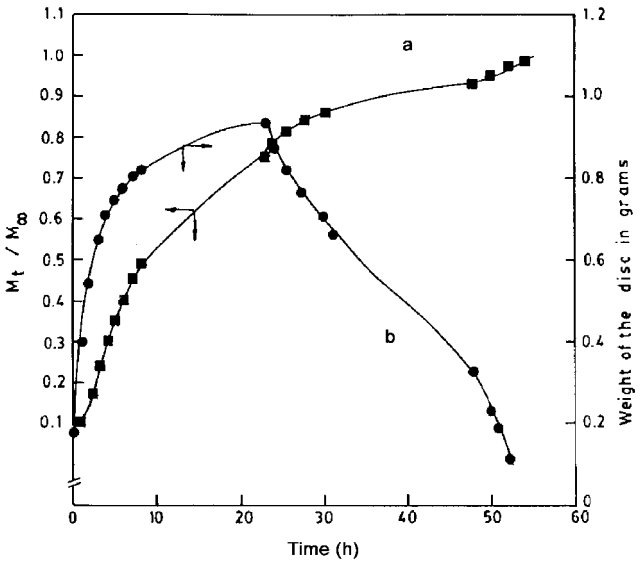

Figure 5. a) Release of BTB from [Gelx-NaCMC] of set B in presence of an enzyme (trypsin) 25 mg/100 g of disc, b) Change in swollen weight of [Gelx-NaCMC] discs.

trypsin [10 mg/100 mg of disc (w/w)] failed to bring about protein degradation even after 50 h. On analysis, it was found that the enzyme preparation is impure with very low activity. Hence, we had to administer large quantities of the enzyme to get the desired result. Therefore trypsin was increased [25 mg/100 mg of disc (w/w)] under

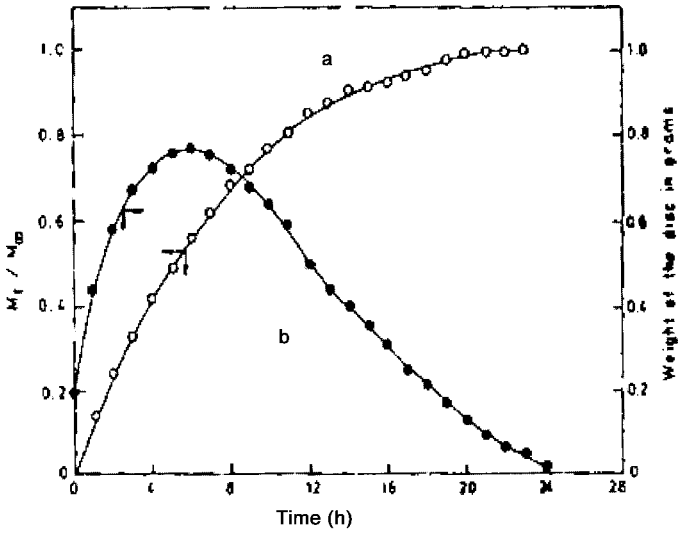

Figure 6. a) Release of BTB from [Gelx-NaCMC] of set B in presence of an enzyme (trypsin) 50 mg/100 mg of disc, b) Change in swollen weight of [Gelx-NaCMC] discs.

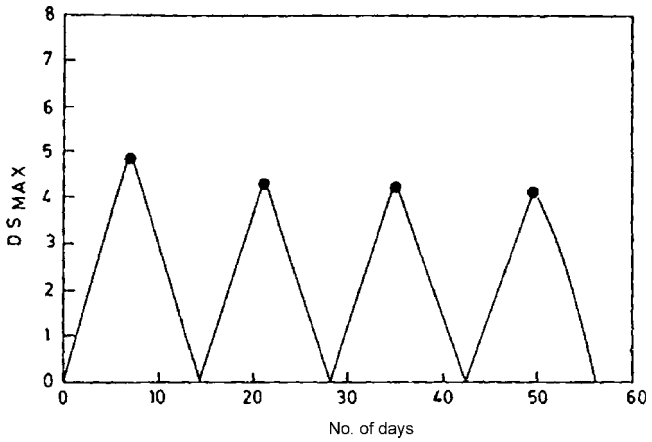

Figure 7. Swelling and drying cycles for [Gelx-NaCMC] gels of set B.

similar conditions. We observed in Fig. 5, after 25 h the weight of the disc has begun to decrease and simultaneously the rate of BTB release has increased. Diffusion of BTB was completed within 55 h, but failed to achieve constant release of BTB. For the data plotted in Fig. 6, 50 mg of the enzyme was used for 100 mg of the disc (w/w). In the initial six hours the disc registered 350% of swelling. Thereafter, the weight of the matrix registered a steady decrease. With an increase in degradation of the matrix the release rate of the BTB has increased and within 24 h the degradation of matrix and release of BTB were completed.

Reproducibility of Swelling Behavior for [Gelx-NaCMC] Gels

Figure 7, represents the repeated swelling and drying cycles for [Gelx-NaCMC] of set B as in Table 1. The data confirms that these gels have good resilience; they could withstand four complete cycles of swelling and drying. Except for the first cycle all other cycles reached the same maximum degree of swelling (DS_{max}). However, [Gelx-NaCMC] gels of higher NaCMC content did not show reproducibility.

CONCLUSION

Our results suggest that it is possible to accelerate substrate release from bioerodible hydrogels. To achieve zero order release from an erodible matrix there has to be a fine tuning among a number of factors such as the initial drug loading, the size and shape of the matrix, swelling kinetics and degradation kinetics of the matrix.

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