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A novel in situ gel for sustained drug delivery and targeting

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Abstract

The objective of this study was to develop a novel chitosan-glyceryl monooleate (GMO) in situ gel system for sustained drug delivery and targeting. The delivery system consisted of 3% (w/v) chitosan and 3% (w/v) GMO in 0.33 M citric acid. In situ gel was formed at a biological pH. In vitro release studies were conducted in Sorensen's phosphate buffer (pH 7.4) and drugs were analyzed either by HPLC or spectrophotometry. Characterization of the gel included the effect of cross-linker, determination of diffusion coefficient and water uptake by thermogravimetric analysis (TGA). Mucoadhesive property of the gel was evaluated in vitro using an EZ-Tester. Incorporation of a cross-linker (glutaraldehyde) retarded the rate and extent of drug release. The in vitro release can further be sustained by replacing the free drug with drug-encapsulated microspheres. Drug release from the gel followed a matrix diffusion controlled mechanism. Inclusion of GMO enhanced the mucoadhesive property of chitosan by three- to sevenfold. This novel in situ gel system can be useful in the sustained delivery of drugs via oral as well as parenteral routes.

Keywords: Chitosan; Glyceryl monooleate; Mucoadhesion; Swelling; Cross-linking

1. Introduction

Intimate contact of a delivery system with an absorbing membrane maximizes not only drug absorption, but also influences the rate of drug absorption (Ch'ng et al., 1985). Though several approaches such as particle density (Bechgaard and Ladefoged, 1978), particle size (Sieg and Triplett, 1980) and the use of fibrous materials (Ch'ng et al., 1985) have been reported to prolong GI transit time, the use of bioadhesive polymers have been most investigated (Ch'ng et al., 1985). A recent study by Lagow and Carson has shown that MUC1 gene encodes a transmembrane mucin glycoprotein that is overexpressed in human breast cancer (Lagow and Carson, 2002). This over production of mucin in breast cancer patients can also be utilized as a targeting strategy for the development of a novel drug delivery system for therapy. Therefore, the overall objective of this study was to enhance the mucoadhesive property of an in situ gel delivery system which can be used in sustained delivery and targeting of drugs.

Chitosan (poly(*N*-deacetyl glucosamine)) is a natural, biodegradable and biocompatible polymer with low toxicity (Chandy and Sharma, 1990). It has a low allergenic property with moderate immunostimulating effects and is metabolized by lysosomes (Chobot et al., 1995). It is derived from alkali deacetylation of chitin, which is a principal component of crustascean life forms including crab shells and cell walls of bacteria and mushrooms (Pelletier et al., 1990). Chitosan has been reported to have a very stable crystalline structure and is normally insoluble in neutral

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or alkaline pH. However, in dilute acids (pH $\leq$ 5.0), free amino groups become protonated (RNH_3^+) and its aqueous solubility increases. Solubility of chitosan in acidic medium is also dependent on its molecular weight (Imai et al., 1991). Acidic solutions of chitosan when subjected to alkaline pH lose this charge and form viscous gels (Onsoyen and Skaugurd, 1991). This in situ gel formation of chitosan has been utilized to sustain the release of many drugs (Miyazaki et al., 1988; Sawayanagi et al., 1982). Positive charges on chitosan may give rise to a strong electrostatic interaction with mucus or a negatively charged mucosal surface (He et al., 1998). The mucoadhesive properties of chitosan and its application in drug delivery system design have been reported (Fiebrig et al., 1995; Takeuchi et al., 1996). The mucoadhesive property of chitosan is believed to prolong residence time of drug in the gastrointestinal tract and improve drug absorption and bioavailability (Leußen et al., 1997).

Glyceryl monooleate (GMO) forms liquid crystals in the presence of water (Engstrom, 1990). In the presence of excess water, the system forms viscous gels known as the cubic phase. When GMO is added to water, it undergoes various phase transformations depending on the water content in the matrix. When the water content is ~0–5% (w/w), it forms a reverse micellar phase, followed by a lamellar phase (~5–20% w/w water), and finally forms the cubic phase (35% w/w water) (Hyde et al., 1984). The cubic phase has a transparent, stiff, gel-like appearance and constitutes a three-dimensional network of curved lipid bilayers separated by a network of congruent water channels (Engstrom, 1990; Hyde et al., 1984). This property of GMO has been used to sustain the delivery of various water-soluble and water-insoluble drugs (Larsson, 1989; Wyatt and Dorschel, 1992). The in situ gel formation by GMO has also been employed as a controlled drug delivery strategy for various compounds (Sadhale and Shah, 1999; Shah et al., 2001). GMO posses bioadhesive properties and thus can be used to enhance the therapeutic efficacy of the dosage form by increasing the contact time at the site of action (Dash et al., 1990; Nielsen et al., 1998). The exact mechanism for this mucoadhesion is still unknown, and possibly involves dehydration of mucosa (Nielsen et al., 1998). The objective of this study was to develop an in situ chitosan-GMO delivery system for sustained drug delivery and targeting. The novelty of this in situ gel system is its versatility in sustaining the release of both hydrophilic and hydrophobic drugs and targeting to cells producing mucin. We hypothesize that the synergistic effect of both mucoadhesives and formation of a liquid crystalline phase by GMO in the presence of water can be utilized for successful targeting and sustaining of drug. The self-emulsifying property of GMO can be an added advantage for enhancing the solubility of a hydrophobic drug in the formulation.

2. Materials and methods

2.1. Materials

Chitosan with a viscosity average molecular weight of 50,000–190,000 and with a viscosity average molecular weight of 190,000–375,000 were obtained from Sigma, MO, USA. Lidocaine, lidocaine hydrochloride, mucin (Type III) (Sigma), ketoprofen and dexamethasone (Professional Compounding Centers of America, TX, USA), GMOrphic-80 (Eastman, TN, USA), glacial acetic acid (Mallinckrodt, KY, USA), citric acid, L(+)-tartaric acid (Acros, NJ, USA), lactic acid, sodium phosphate dibasic, potassium phosphate monobasic, glutaraldehyde and methylene chloride (Fisher Sci., NJ, USA), methylcellulose (Dow Chemical Company, FL, USA), and ethylcellulose (Aqualon, DE, USA) were used as received. The 0.45 μm syringe filters were from Millipore (Millipore Inc., MA, USA).

2.2. Formulation of the delivery system

Solutions (1, 2, 3, 4, and 5% w/v) of chitosan in 1 M acetic acid were prepared. GMO (2 and 3% w/v) was melted at 45 °C and added to the chitosan solution with sonication for 50 min. One milliliter of this solution was added to 40 ml Sorensen's phosphate buffer (pH = 7.4). The in situ gels formed instantaneously were characterized and used for in vitro release studies. Besides acetic acid, other acids investigated were citric, lactic and tartaric. Lidocaine hydrochloride (aqueous solubility 0.68 g/ml at 25 °C), ketoprofen (aqueous solubility 0.3 g/ml at 25 °C; log P = 2.8), dexamethasone (aqueous solubility 100 μg/ml at 25 °C; log P = 2.04) and dexamethasone in ethylcellulose microspheres were used as model compounds.

2.3. Preparation of dexamethasone microspheres

The dexamethasone in ethylcellulose microspheres were prepared by the emulsion-solvent evaporation method (Dash, 1997). Dexamethasone (1.5 g) was dissolved in 1% (w/v) ethylcellulose solution in methylene chloride (10 ml). The solution was added to 25 ml of aqueous methylcellulose solution (0.25% (w/v)) and homogenized using a hand homogenizer (Model M133/1280-0, Biospec Products, OK, USA). The emulsion formed was added to 100 ml of aqueous methylcellulose solution (0.25% (w/v)) and stirred using a magnetic stirrer until all the methylene chloride evaporated (∼3–4 h). The microspheres were centrifuged at 2000 rpm for 5 min. The supernatant was decanted and the sediment was washed with distilled water, filtered and air-dried. The theoretical drug load in these formulations was 60% (w/w).

2.4. Determination of drug load

(a) In the delivery system: the theoretical drug load of lidocaine hydrochloride and ketoprofen in the delivery system was 1% (w/v). However, dexamethasone concentration was 0.03% (w/v). Different drug loads were needed to maintain sink conditions during the in vitro release studies. One milliliter of this solution was added to 50 ml of buffer or appropriate solvent and sonicated for 10–15 min. The solution was filtered through a Nylon syringe filter (0.45 μm) and the concentration of drug in the solution was measured either spectrophotometrically or by HPLC.
(b) In microsphere formulation: a known amount of dexamethasone microspheres (∼10 mg) was weighed in a volumetric flask and 3 ml of methylene chloride was added. After 1 min of sonication, the volume was adjusted to 100 ml with mobile phase. The solution was filtered through a Nylon syringe filter (0.45 μm). Dexamethasone content in the solution was measured by HPLC.

2.5. In vitro release studies

The delivery system consisting of 3% (w/v) chitosan, 3% (w/v) GMO, in 0.33 M citric acid, and the appropriate amount of drug (as stated earlier) was prepared in a glass beaker. One milliliter of this delivery system was added to 40 ml of Sorensen's phosphate buffer (pH = 7.4) in an Erlenmeyer flask. The gels formed were shaken in a bath incubator at 80 rpm and 37 °C. One milliliter of sample was collected at predetermined time intervals and replaced with fresh buffer. Drug concentration was analyzed spectrophotometrically or by HPLC.

2.6. Effect of cross-linker concentration on in vitro release

Glutaraldehyde (50% v/v) was used as a cross-linker at concentrations of 0.1, 0.2, 0.5 and 2.0% (v/v). The in vitro release of ketoprofen and dexamethasone from the cross-linked matrix was monitored over a period of 6 h and the drug concentration was determined by HPLC.

2.7. Effect of reaction time on in vitro release

Cross-linker (glutaraldehyde 0.2% (v/v)) was added to the formulation and stored at room temperature for 0.5, 1.0, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 h. Release of ketoprofen from these formulations over 6 h was determined. All studies were performed in triplicate.

2.8. Analysis of drugs

Lidocaine hydrochloride was analyzed using a spectrophotometer at $\lambda_{max} = 263$ nm. Ketoprofen and dexamethasone were analyzed using HPLC. The chromatographic separation was achieved on a Beta-Basic C-8, 150 mm × 4.6 mm column (Keystone, PA, USA) with UV detection at 260 nm for ketoprofen and 240 nm for dexamethasone. The mobile phase consisted of methanol and pH 7.4 Sorensen's phosphate buffer (50:50) at a flow rate of 1.0 ml/min. Lidocaine was used as the internal standard. Ketoprofen eluted at 2.0 min, dexamethasone at 3.8 min and lidocaine at 7.3 min.

2.9. Measurement of diffusion coefficient

Chitosan solution (3%) in 0.33 M citric acid was stored overnight for complete hydration. Medium molecular weight (190–375 K) chitosan was used in this study. Air bubbles, if any, were removed by vacuum (160 mmHg for 2 h). Membranes of different

thickness were prepared by casting the solution on glass plates using appropriate spacers (0.5–1 mm) and heating at 60 °C for 2 h in an oven. A caliper was used to measure the final thickness of the membrane. The membranes were used in side-by-side diffusion cells with a 3 cm^3 volume and 1.13 cm^2 surface area for diffusion studies. The donor side contained a saturated solution of lidocaine hydrochloride in methanol at 37 °C. Ten microliters of sample was collected from the receiver side and the drug concentration was measured using a spectrophotometer. The diffusion of lidocaine hydrochloride in membranes containing GMO and cross-linker was also evaluated. All studies were performed in triplicate.

2.10. Measurement of mucoadhesive properties

The mucoadhesive property of the gel delivery system was evaluated using 10% (w/v) of mucin Type III partially purified from porcine stomach containing 1% bound sialic acid in deionized water at room temperature. Mucoadhesion was determined in triplicate in pairs using yield stress and peel strength measurements (EZ-Tester, Shimadzu, Kyoto, Japan).

2.10.1. Measurement of yield stress

An appropriate solution pair was placed between plates (8.5 cm × 3 cm) with surface area of 25.5 cm^2. A 50 g weight was placed on the plates for 20 min, 1 h or 2 h. The plates were then loaded on to the EZ-Tester and the yield stress (the force at which the plates just start to slide over one another) was determined.

2.10.2. Measurement of peel strength

Test solution was placed on each of two circular plates (2.54 cm diameter). The plates were kept in contact for 20 min, 1 h, or 2 h and mounted on to the

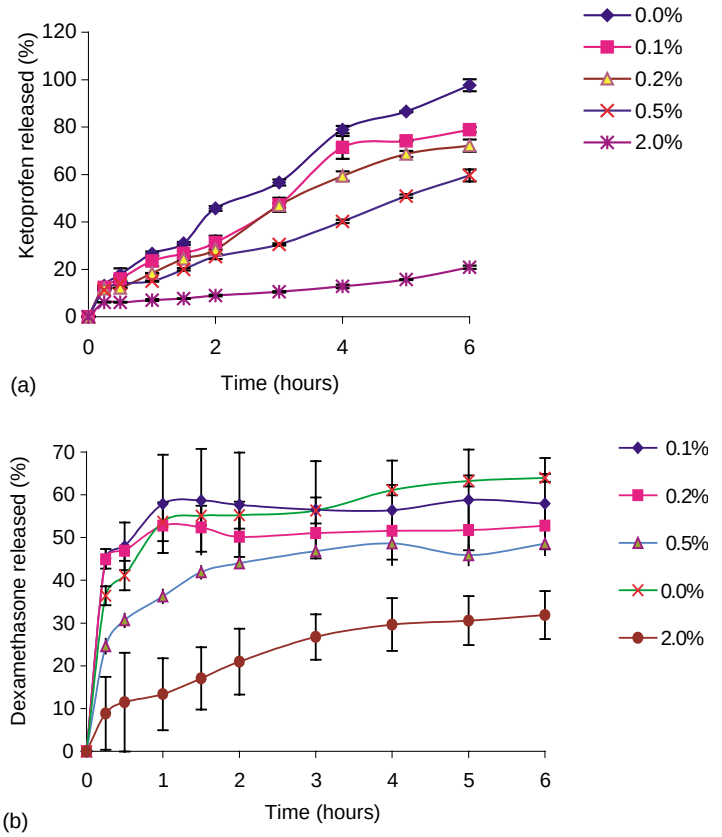

Fig. 1. (a) Effect of cross-linker (glutaraldehyde) on the release of a hydrophilic drug (ketoprofen). The data represent mean ± S.D.; $n = 3$. (b) Effect of cross-linker (glutaraldehyde) on the release of a hydrophobic drug (dexamethasone). The data represent mean ± S.D.; $n = 3$.

EZ-Tester. The yield point (the force at which contact between the plates was lost) was determined.

2.11. Measurement of water uptake by the gel

The water uptake by the gel was determined using a Thermogravimetric Analyzer (TGA-50, Shimadzu, Kyoto, Japan). The in situ gels formed in 40 ml of Sorensen's phosphate buffer were used for this study. At periodic time intervals a portion of the gel was carefully removed. The sample was immediately loaded onto a TGA pan after removal of surface water by an absorbing tissue. The sample was subjected to a controlled temperature program (10 °C/min). The weight loss (% (w/w)) on heating was measured over 30–200 °C. Water uptake of in situ gels containing various cross-linker concentrations and different reaction times was examined over 6 h. All studies were carried out in triplicate.

3. Results and discussion

3.1. Formulation of delivery system

Preliminary studies were carried out to determine the chitosan concentration in acidic solution necessary for in situ gel formation with acceptable consistency for drug delivery. At a chitosan concentration of ≥4% (w/v) the viscosity of the solution was inappropriate for oral or parenteral delivery. At lower concentrations (<2% (w/v)), there was improper gelation and time required for gelation. The optimum chitosan concentration was 3% (w/v). Preliminary studies investigated the effect of four organic acids on the formation of an in situ gel in alkaline pH. Lactic and tartaric acids exhibited slower gel formation than citric and acetic acids. Interestingly, the in situ gel formed using citric acid had better adhesive properties for both glass and a cellulose membrane than acetic acid. This effect may possibly be explained by the greater hydrogen bond capability of citric acid (Cho et al., 2000). Therefore, 3% (w/v) chitosan solution in 0.33 M citric acid was used for the study. The pH of this solution was 3.2. The viscosity of the delivery system was determined using a Brookfield viscometer (Model-DV-I, Brookfield Eng. Lab., MA, USA). The system was pseudoplastic which is advantageous for passage through a hypodermic needle and for oral delivery.

3.2. In vitro release studies with lidocaine hydrochloride

In vitro release of lidocaine hydrochloride was rapid from chitosan gels with >80% of the drug released within 30 min. Incorporation of GMO retarded this release (84.5–66.8%). This reduction in the burst effect may be due to formation of cubic phases with higher viscosity. The TGA results indicated that more than 35% (w/w) water penetrates into the matrix in less than 30 min. GMO forms viscous cubic phases in the presence of ≥35% (w/w) water (Hyde et al., 1984). For GMO at >3% (w/v) a semi-solid mass formed immediately. GMO concentrations lower than 2% (w/v) did not retard drug release (data not shown). Thus, 3% (w/v) chitosan and 3% (w/v) GMO in 0.33 M citric acid were used in the drug delivery system.

3.3. Effect of cross-linker

The effect of glutaraldehyde (50% (v/v)) as a cross-linker on the in vitro release of ketoprofen and dexamethasone are shown in Fig. 1a and b, respectively. For both drugs, an increase in the concentration of cross-linker decreased both the rate and extent of release.

Glutaraldehyde undergoes a Schiff's reaction to cross-link with chitosan (Monteiro and Airoldi, 1999). Cross-linking of the gel lowers the diffusivity of the drug in the matrix. At >2% (v/v) glutaraldehyde the

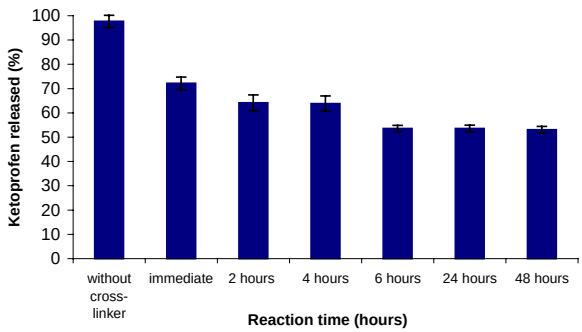

Fig. 2. Effect of cross-linking reaction time on the in vitro release of ketoprofen over 6 h. The vertical bars represent mean ± S.D.; $n = 3$.

gel became a rigid mass that was unacceptable for the proposed application. Concentrations below 0.2% (v/v) did not significantly retard drug release. Hence, the lowest concentration that significantly retarded release (0.2% (v/v)) was considered optimal.

The effect of time of cross-linking on the in vitro release of ketoprofen was then investigated. After addition of the cross-linker (0.2% (w/v)), the solutions were allowed to stand for 2, 4, 6, 24 and 48 h. The amount of ketoprofen released after 6 h was determined (Fig. 2). No further decrease in ketoprofen release was found after 6 h of cross-linking ($p > 0.05$). However, there was a significant difference in ketoprofen release within 4 h ($p < 0.05$). An acidic pH can adversely affect the cross-linking reaction (Monsan et al., 1975). However, an acidic pH does not prevent glutaraldehyde from interacting with the free amino groups of chitosan (Monteiro and Airoldi, 1999). Our results also indicate that this cross-linking reaction can proceed in acidic conditions and the reaction time can affect in vitro drug release.

The amount of ketoprofen released was plotted against the square root of time (not shown). A linear relationship ($r^2 > 0.96$) indicated that drug release

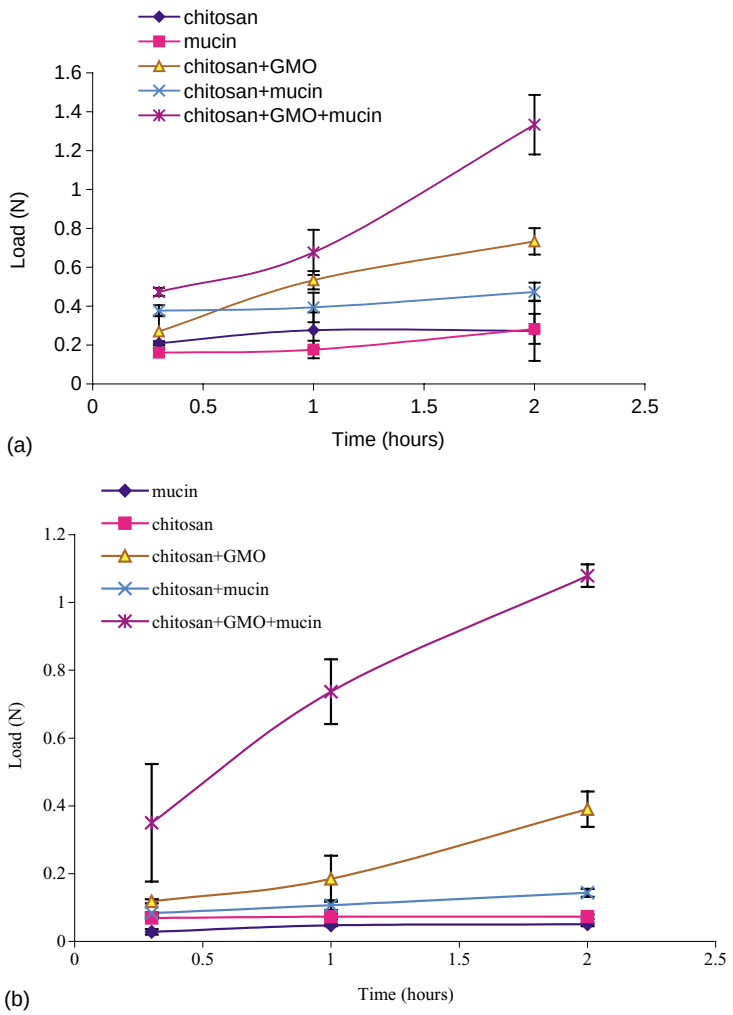

Fig. 3. (a) Determination of yield stress. The *x*-axis represents contact times for both test solutions before pulling. The data represent mean ± S.D.; $n = 3$. (b) Determination of peel strength. The data represent mean ± S.D.; $n = 3$.

Table 1
Diffusion coefficient of lidocaine hydrochloride in chitosan membranes

Type of membrane	Thickness of membrane (cm)	Lag time* (min)	Diffusion coefficient* (cm^2/s) ($\times 10^{-8}$)
Chitosan	0.07	87.7 ± 2.52	15.5 ± 0.45
Chitosan-GMO	0.04	89.3 ± 1.16	4.97 ± 0.06
Chitosan-GMO with 0.2% cross-linker	0.04	131 ± 9.02	3.39 ± 0.29

* mean ± S.D.; $n = 3$.

from the in situ gel follows a diffusion-controlled mechanism (Higuchi, 1961).

3.4. Determination of diffusion coefficient of lidocaine hydrochloride from chitosan membranes

The diffusion coefficient of lidocaine hydrochloride from chitosan membranes with and without cross-linker was determined (Table 1) from a lag-time equation Eq. (1) (Cerchiara et al., 2002). The diffusivity was different in chitosan and chitosan-GMO membranes even though lag-times were similar because of different thicknesses.

$$D = \frac{h^2}{6t_{\text{Lag}}} \quad (1)$$

where D is the diffusion coefficient of the drug (cm^2/s), h is the thickness of the membrane (cm), and t_{Lag} is the lag time (s).

The diffusivity of lidocaine hydrochloride in the cross-linked membrane was 1.5 times lower than in the non-cross-linked membrane containing GMO. Incorporation of GMO into a chitosan membrane without a cross-linker also lowers the diffusivity of lidocaine hydrochloride by >1/3. Thus, incorporation of glutaraldehyde as well as GMO decreases the diffusivity of the drug in the matrix.

3.5. Mucoadhesive studies

The mucoadhesive properties of chitosan have been attributed to its polycationic nature (He et al., 1998). However, the bioadhesive property of GMO remains unclear, but seems to involve dehydration of the mucosa (Nielsen et al., 1998). We hypothesized that a combination of these bioadhesives will increase mucoadhesion and GI residence time or be a better adjuvant for drug targeting for mucin. The rheograms for yield stress and peel strength are shown in Fig. 3a and b, respectively. Yield stress studies show that there is an increased adhesion of mucin to chitosan. However, when 3% (w/v) GMO was incorporated into chitosan, there was a threefold further enhancement of mucoadhesive property. Peel strength data resulted in a similar conclusion. GMO increased the peel load six- to eightfold. These studies suggested a synergistic increase in mucoadhesion of chitosan by GMO. Hence, this increase can be used as an index of bioadhesion bond strength (Hassan and Gallo, 1990).

3.6. Water uptake studies

Release of a drug from a polymeric matrix is dependent on the amount of water associated with the system and hence its swelling properties (Khalid et al., 2002). The release of drugs from a polymeric matrix may involve the penetration of water into the matrix and simultaneous release of drugs via diffusion, as governed by Fick's Law (Kim et al., 1992). The water associated with the formulation at any point in time in the release medium was studied by TGA. The percentage of weight loss was thought to be due to water loss during heating. TGA was also used to study the effect of cross-linking on water uptake by the gels. The results are shown in Fig. 4. There was a sudden increase in water uptake followed by a decrease. This decrease is particularly prominent for gels without cross-linker and has been observed in lower concentrations of cross-linker (0.1% v/v) (data not shown). This decrease in water uptake can be explained by the collapsing of gels with time. There was also a decrease in water uptake by the gels with cross-linker. The formation of cross-linked networks provided an additional barrier to water penetration. As the concentration of the cross-linker in the delivery system increased, the time taken to reach maximum water uptake increased.

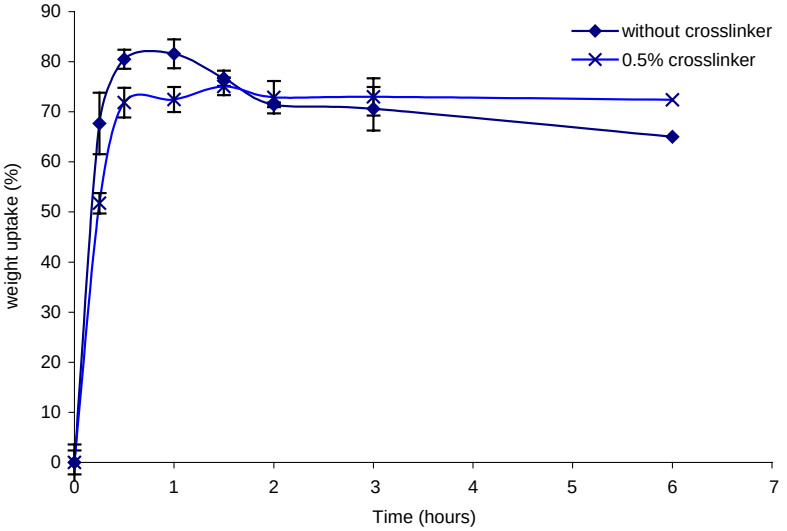

Fig. 4. Effect of cross-linker (glutaraldehyde) on water uptake by the in situ gels. The data represent mean ± S.D.; $n = 3$.

At a higher cross-linker concentration the collapsing of the gel was negligible compared to gels without a cross-linker.

3.7. Drug release from dexamethasone loaded microspheres

To further sustain the release of the drug from these gels, some of the free drug was replaced with drug-loaded microspheres. Dexamethasone loaded ethylcellulose microspheres with a theoretical drug load of 62 ± 1.6% (w/w) were used. A physical mixture of free dexamethasone and dexamethasone microspheres (50:50 (w/w)) was incorporated into the delivery system. The in vitro release profiles of dexamethasone from these delivery systems are depicted in Fig. 5. The use of drug loaded microspheres can further sustain the rate and extent of drug release. This may be explained by the additional barrier to drug diffusion in the microspheres.

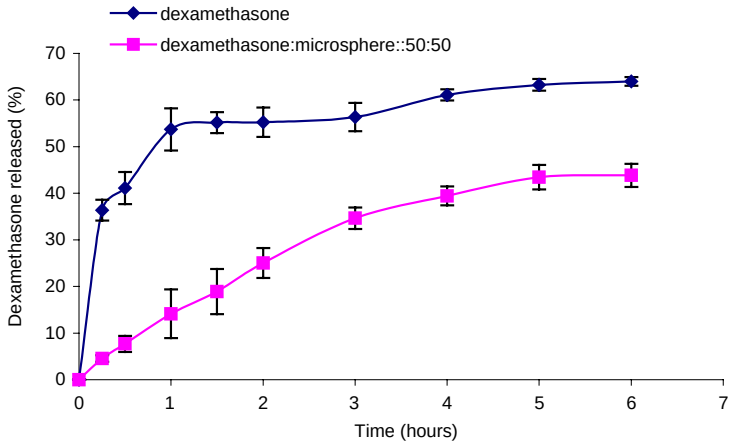

Fig. 5. In vitro release of dexamethasone from in situ gels containing free drug and drug-loaded microspheres. The data represent mean ± S.D.; $n = 3$.

4. Conclusions

This study reports the development and characterization of a novel in situ delivery system. For the various drugs tested, the optimal formulation consisted of 3% (w/v) chitosan with 3% (w/v) GMO in 0.33 M citric acid. In vitro release of both hydrophilic and hydrophobic drugs from this gel was very quick. Incorporation of 0.2% (v/v) glutaraldehyde (50% (v/v)) as a cross-linker can retard drug release. The drug release can be further sustained by incorporation of drug-loaded microspheres into the delivery system which followed a diffusion-controlled mechanism. Addition of GMO to chitosan enhances its mucoadhesive property by more than threefold. Interestingly, enhancement of mucoadhesion can be utilized as a drug targeting strategy for oral or parenteral delivery.

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