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A non-covalently cross-linked chitosan based hydrogel

Lorna Noble, Alexander I. Gray, Lubna Sadiq, Ijeoma F. Uchegbu *

Department of Pharmaceutical Sciences, Strathclyde Institute for Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK

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Abstract

Hydrogels are normally formed by the covalent cross-linking of linear polymers. In the case of chitosan based hydrogels this cross-linking is often achieved with glutaraldehyde, glyoxal or other reactive cross-linking agents. Such hydrogel materials have limited biocompatibility and biodegradability. However by the attachment of hydrophobic palmitoyl groups to glycol chitosan, a water soluble chitosan derivative, we have produced a version of the amphiphilic vesicle forming polymer-palmitoyl glycol chitosan (Uchegbu et al., 1998, J Pharm Pharmacol 58, 453–458). The level of palmitoylation in this variant of the polymer (GCP11), as determined by proton neutron magnetic resonance spectroscopy, is $19.62 \pm 2.42\%$ ($n = 4$). GCP11 has been used to prepare soft, slowly eroding hydrogels suitable for drug delivery by simply freeze-drying an aqueous dispersion of the polymer. Non-covalent cross-linking to form the gel matrix is achieved by the hydrophobic interactions of the palmitoyl groups. The resulting material, as examined by scanning electron microscopy, is porous and may be hydrated to up to $20\times$ its weight in aqueous media without any appreciable change in volume—transforming from an opaque to a translucent solid. The slow erosion of this material in aqueous environments gives a biodegradable and ultimately more biocompatible material than covalently cross-linked hydrogels. Unlike most chitosan-based gels, the gel is hydrated to $20\times$ its weight at alkaline pH but only $10\times$ its weight at neutral and acid pH. This is as a result of the gradual erosion of the gel at lower pH values. Hydration is also reduced from $20\times$ the dry gel weight in water to $10\times$ the dry gel weight in the presence of dissolved salts such as sodium chloride. GCP11 hydrogels have been loaded to 0.1% w/w with a model fluorophore, rhodamine B, by simply freeze-drying an aqueous dispersion of GCP11 in the presence of a solution of rhodamine B dissolved in either water or phosphate buffered saline (PBS, pH = 7.4). The release of this model fluorophore was retarded by between 8 and 12% when PBS was contained in the gel in accordance with the hydration profiles. Rhodamine B release was also reduced by between 13 and 25% in the presence of acid as a result of the reduced solubility of rhodamine B at acid pH.

Keywords: Chitosan; Glycol chitosan; Hydrogels; Rhodamine B

1. Introduction

Hydrogels are usually formed by the covalent cross-linking of linear hydrophilic polymers to form a network of material capable of absorbing

* Corresponding author. Fax: +44-141-552-6443.
E-mail address: i.f.uchegbu@strath.ac.uk (I.F. Uchegbu)

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water, yet still remaining insoluble (Graham and McNeil, 1984). Heterogeneous polymer mixtures may also be used to form hydrogels without the need for covalent cross-linking (Bae and Kim, 1993). Chitosan is the deacetylated derivative of the natural polymer chitin (derived from crab and prawn shells) and hydrogels formed from this material are usually covalently cross-linked. Cross-linking agents such as glutaraldehyde (Nakatsuka and Andrady, 1992; Deyao et al., 1994), glyoxal (Nakatsuka and Andrady, 1992) and diethyl squarate (DeAngelis et al., 1998) have been employed in the fabrication of chitosan hydrogels. In addition semi-interpenetrating polymer network (semi-IPN) hydrogels have been produced between chitosan/ polyethylene oxide diacrylate—cross linked by U.V. irradiation (Lee et al., 1997), glyoxal cross-linked chitosan/ polyethylene oxide (Patel and Amiji, 1996) and glutaraldehyde cross-linked chitosan combined with silk fibroin (Chen et al., 1997) or polyacrylic acid (Wang et al., 1997). Heterogeneous interpolymer hydrogen bonds are the main source of interpolymer cohesion in these semi-IPN hydrogels. Chitosan has also been covalently cross-linked with the carbohydrate sceleroglucandialdehyde to form what is termed a co-network of two cross-linked polymers (Crescenzi et al., 1995). The obvious drawback with these kinds of systems is their limited biodegradability.

Non-covalent cross-linking has been used to prepare chitosan-based gels by the *O*- and *N*-acetylation of chitosan (Kristl et al., 1993) and the attachment of C_{10}-alkyl glycosides to chitosan (Holme and Hall, 1991). However the former yield either 'rigidly solidified' or 'fragile' gels with high and low molecular weight chitosan respectively with accompanying gel syneresis (Kristl et al., 1993). While the latter form gels when in acid media at elevated temperature ($>50°C$) and are solutions in acid media at lower temperatures (Holme and Hall, 1991). Both these factors limit the use of these materials as drug delivery gels. Chitosan-ethylene diamine tetraacetic acid (EDTA) conjugate gels have also been reported in which the carboxylic acid groups of EDTA are covalently linked to the amino groups of chitosan (Bernkop-Schnürch et al., 1997; Valenta et al., 1998). Covalent cross-linking cannot be ruled out in these systems. Finally non-covalent cross-linked chitosan gel mixtures can be prepared by the use of polyelectrolyte complexes of chitosan and polymers such as carboxymethylcellulose (Long and VanLuyen, 1996).

In an attempt to improve the pharmaceutical acceptability of chitosan hydrogels we have prepared a non-covalently cross-linked chitosan hydrogel from a chitosan derivative which is a version of the vesicle forming amphiphilic polymer-palmitoyl glycol chitosan (Uchegbu et al., 1998). This hydrogel is designed to slowly erode and hence should have improved biocompatibility. Glycol chitosan, a water soluble derivative of chitosan is the starting material which on attachment of a strategic number of hydrophobic groups yields a soft, slowly eroding gel on freeze drying. This gel is suitable for buccal delivery as a result of its softness and hence good feel in the mouth. Also because of the slow erosion of the gel the dosage form does not have to be retrieved after delivery of the dose. The use of an amphiphilic polymer to prepare hydrogels has the added advantage of potentially improving the permeation of drug substances through biological membranes. This chitosan-based hydrogel has been characterised in terms of hydration capacity and release profile in various media using the fluorophore rhodamine B as a model compound.

2. Materials and methods

2.1. Materials

Glycol chitosan ($M_w = 164\,000$), palmitic acid *N*-hydroxysuccinimide, rhodamine B and deuterated solvents were all supplied by Sigma Chemical Co, UK. All organic solvents and other reagents were supplied by Merck Chemical Co, UK.

2.2. Synthesis of palmitoyl glycol chitosan (GCP11) and preparation of GCP11 gels

Synthesis was carried out as shown in Scheme 1. Glycol chitosan (250 mg) was dissolved in sodium bicarbonate solution (4.75 mg ml^{-1}, 40 ml) to which was added absolute ethanol (25 ml).

To this solution was added drop wise over 1h an ethanolic solution of palmitic acid *N*-hydroxysuccinimide (1.32 mg ml^{-1}, 300 ml) and the mixture left stirring for 72 h protected from light. At the end of this period acetone (50 ml) was added to the reaction mixture and the reaction mixture evaporated under reduced pressure at 45°C to a reduced volume of approximately 50 ml. The resulting aqueous dispersion of the polymer was extracted 3 times with 2 volumes each of diethyl ether and left to stand for 2 h in a fume hood for residual ether to evaporate. Exhaustive dialysis of this dispersion was then carried out by placing the dispersion in Visking tubing (molecular weight cut off = 12–14 kD) and dialysing against 5 l of water with 6 changes over 24 h. The dispersion was then lyophilised in 0.15 ml portions in 96 well plates to give white solid spongy discs (5 mm in diameter, 4 mm thick).

2.3. ^1H NMR

^1H NMR (with integration) and ^1H correlation spectroscopy experiments (Bruker AMX 400 Mhz spectrometer) were performed on solutions of glycol chitosan in D_2O, GCP11 in $(CD_3)_2SO$ to assign non-exchangeable coupled protons.

2.4. Scanning electron microscopy

All specimens were mounted on stubs and gold coated for scanning electron microscopy. Images were recorded digitally at an accelerating voltage of 3 Kv using a Philips SEM 500.

2.5. Hydration of GCP11 gels

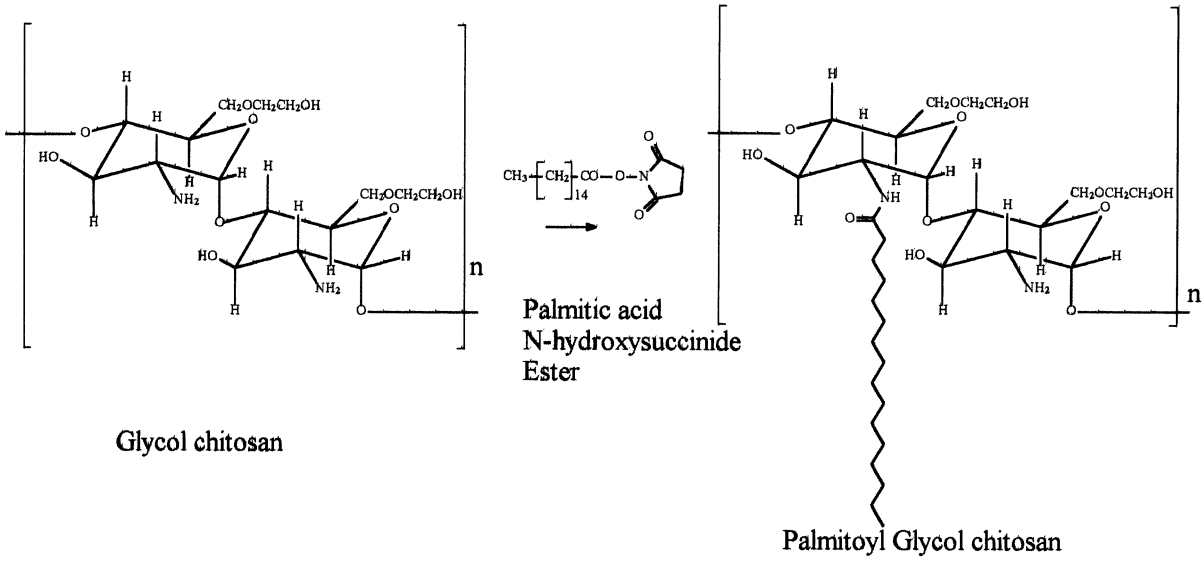
GCP11 gel discs, 5 mm diameter, 4 mm thick and weighing approximately 1.5 mg were placed in hydration media (25 ml), removed and weighed at various time intervals. The weight was recorded and the hydration ratio recorded as follows:

$$\text{Hydration Ratio} = \frac{W_t}{W_0}$$

where W_t = weight of hydrated gel at time t and W_0 = initial weight of dry gel.

2.6. Loading gels with rhodamine B

To 1 ml of the aqueous dispersion of GCP11 obtained from the synthesis step, a solution of rhodamine B (0.05 mM, 0.25 ml) was added. Two types of rhodamine B solution were used rhodamine B dissolved in water and rhodamine B



Scheme 1. The synthesis of GCP11

dissolved in phosphate buffered saline (PBS, pH = 7.4). A total of 0.15 ml portions of these solutions were then lyophilised in 96 well plates to give pink solid discs of rhodamine B loaded gel (5 mm in diameter and 4 mm thick).

2.7. Assay of rhodamine B loaded GCP11 gels

Gels were assayed for rhodamine B content by probe sonicating (Soniprobe Instrument) a weighed piece of gel (~ 1 mg) material in HCL (0.1 M, 2 ml). The dispersion was then centrifuged (150 000 g × 1 h, MSE 75 superspeed) and the supernatant separated and assayed for rhodamine B content fluorimetrically (exc. = 533 nm, em. = 614 nm—Perkin Elmer 650-40 spectrofluorimeter). Standard solutions of rhodamine B were prepared by dissolving rhodamine B in HCL (0.1 M).

2.8. The release of rhodamine B from GCP11 gels

Rhodamine B loaded GCP11 gel discs (~ 1.5 mg in weight) and PBS (pH = 7.4, 1 ml) were placed in 10 cm lengths of Visking tubing (molecular weight cut off = 12 000–14 000 Da) which were sealed at both ends. The gels were then dialysed against PBS (pH = 7.4, 70 ml), the dialysate sampled at various time intervals and assayed for rhodamine B fluorimetrically as described above.

2.9. Statistics

Statistical significance was assessed using one way analysis of variance (ANOVA) via the statistics software package Minitab™ 10.5 for Windows or the student's *t*-test via the graphics/statistics software package Microcal Origin™.

3. Results

3.1. Synthesis of GCP11

By the attachment of a strategic number of palmitoyl groups to glycol chitosan (an aqueous soluble derivative of chitosan) an amphiphilic polymer was produced (GCP11) in a single step procedure, which on freeze-drying yields a soft porous white solid.

3.2. ^1H NMR

Proton assignments for glycol chitosan in D_2O (relative to HDO = δ 4.70): δ 2.01 ppm = CH_3 (residual acetyl), δ 2.66 = CH (C2 sugar proton), δ 3.20–4.20 = non-exchangeable sugar protons, δ 4.40 = CH (C1 sugar proton). Proton assignments for GCP11 in $(CD_3)_2SO$ (relative to dimethylsulphoxide δ 2.50): δ 0.85 = CH_3 (Palmitoyl), δ 1.23 = CH_2 (palmitoyl), δ 1.45 = CH2 (palmitoyl deshielded by and β- to carbonyl), δ 1.83 = CH3 (residual acetyl), δ 2.20 = CH2 (α- to carbonyl), δ 2.90–4.00 = non-exchangeable sugar protons, δ 4.4 = CH (C1 sugar proton). The level of palmitoylation was estimated by determining the ratio of acetyl protons (δ 2.01 ppm) to C2 sugar protons (δ 2.66 ppm) in the glycol chitosan spectra followed by an estimation of the ratio of acetyl protons (δ 1.83 ppm) to palmitoyl protons (δ 0.85 ppm) in the GCP11 spectra. The level of palmitoylation was thus found to be 19.6 ± 2.4 mole% ($n = 4$) and ranged from 17.0 to 22.1 mole%. The batch of glycol chitosan used was also found to be 16 mole% acetylated.

3.3. Scanning electron microscopy

Scanning electron micrographs (Fig. 1) reveal the gel to have an extremely porous structure as a result of formation of ice crystals during the freeze drying process. The pore size is approximately 80 μm for plain GCP11 gels and approximately 180 μm for rhodamine B loaded GCP11 gels.

3.4. Hydration of GCP11 gels

On placing these GCP11 gels in aqueous media, they are transformed from an opaque white solid

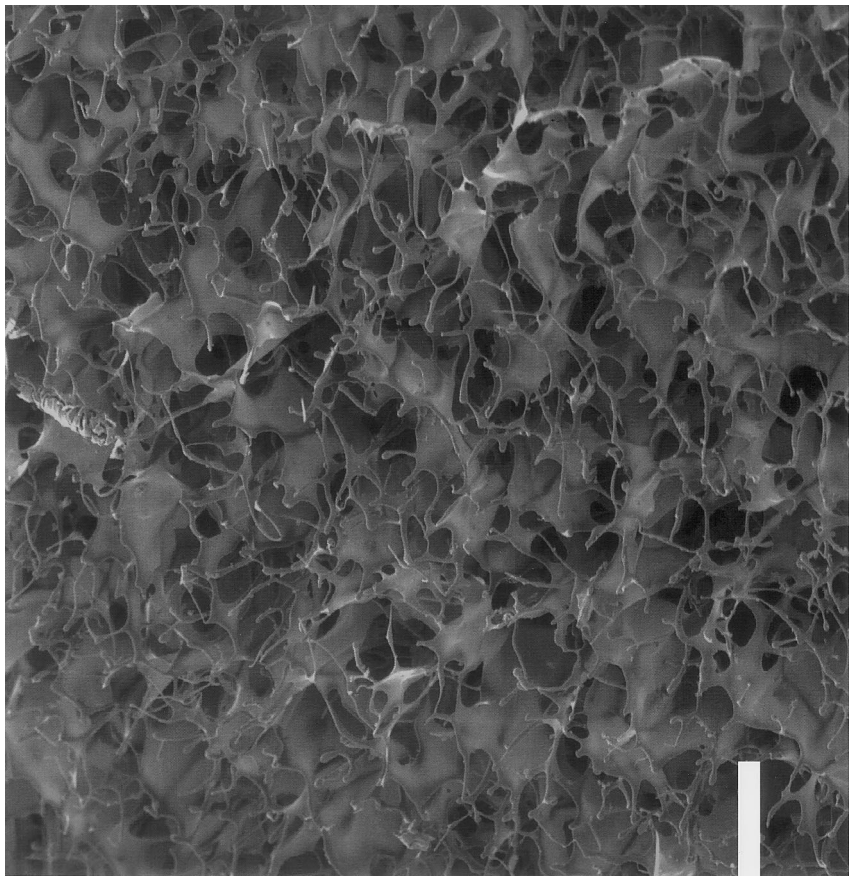


Fig. 1. X240mag scanning electron micrographs of (a) plain GCP11 gels; and (b) GCP11 gels loaded with rhodamine B in PBS (pH = 7.4), bar = 100 μm.

to a translucent gel. GCP11 gels are well hydrated in aqueous media over a 20–40 min time period (Fig. 2) without an appreciable gain in volume. Hydration is significantly retarded in the presence of salts with the gel hydration falling from 20 × the dry gel weight in water to about 10 × the dry gel weight in the presence of sodium chloride. This is as a result of the preferred hydration of soluble salts—a salting out of the gel (Fig. 2). While the presence of salt within the medium dramatically alters the hydration profile, higher levels of salt appear to give slightly higher levels of hydration than lower concentrations although this is not statistically significant (Fig. 2).

Hydration is significantly diminished in acid and neutral media but increased in alkaline media such as PBS (pH = 9.0) and sodium tetraborate solution (pH ~ 9.0) (Fig. 3). The gel is only hydrated to 10 × the dry gel weight in neutral or acid media and 20 × the dry gel weight in alkaline media.

3.5. Rhodamine B release form GCP11 gels

Gels were loaded to the level of 0.1% w/w rhodamine B by using rhodamine B dissolved in water or PBS (pH = 7.4). The release of the model fluorophore rhodamine B was virtually complete in 5 h (Figs. 5 and 6). Rhodamine B release was reduced at acid pH by approximately 23% in gels prepared from rhodamine B dissolved in PBS and by approximately 14% in gels prepared from rhodamine B dissolved in water (Fig. 4). This was as a result of the reduced solubility of rhodamine B

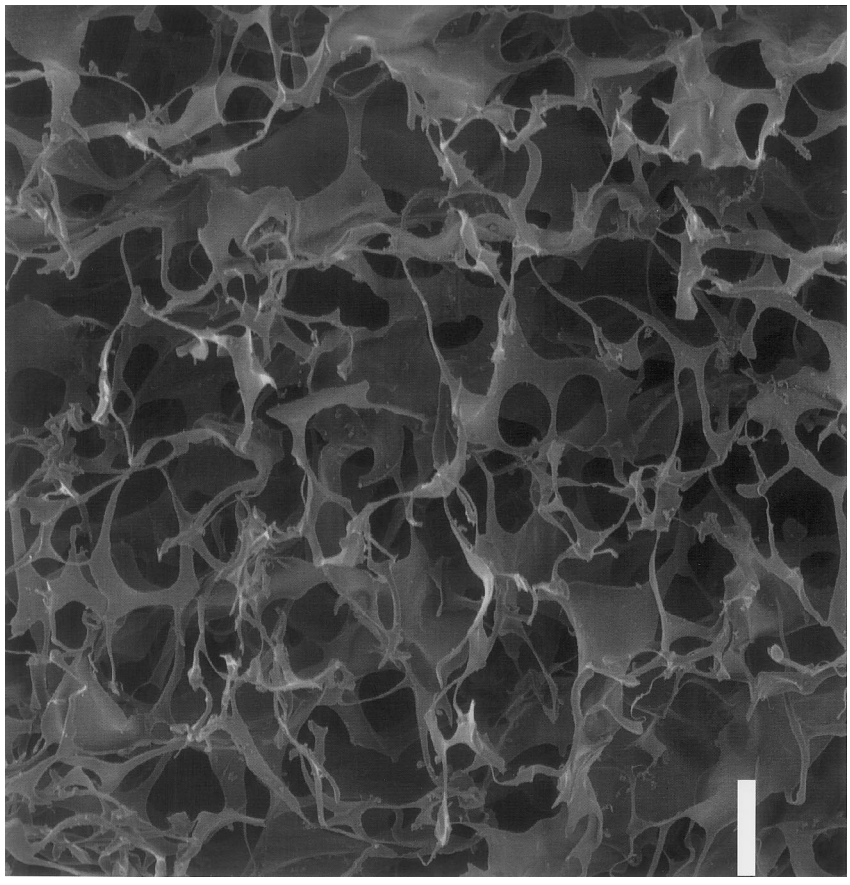


Fig. 1. (*Continued*)

in acid media which overrides the increased erosion of GCP11 gels at this pH. However more importantly was the fact that if rhodamine B was loaded onto the gel as a solution in PBS (pH = 7.4), release from the gel was retarded by about 9% (Fig. 5).

4. Discussion

Gelation in GCP11 gels is achieved by the hydrophobic interaction of the palmitoyl groups (Fig. 6) in a similar manner to that reported for the thermal gelation (> 50°C) of chitosan C10-alkyl glycoside derivatives (Holme and Hall, 1991). The initial glycol glucosamine, palmitoyl ratio was 1:1, however a palmitoylation level of

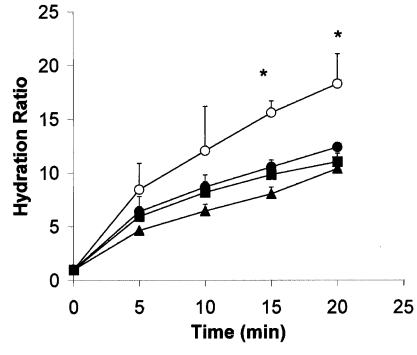


Fig. 2. The effect of the ionic strength of the hydration media on the hydration of GCP11 gels; ○ = water, ▲ = 0.15 M NaCl, ■ = 1.0 M NaCl, ● = 2.0 M NaCl, data points = mean ± S.D., $n = 2$ for NaCl solutions, $n = 3$ for water, * = statistical significance ($P < 0.05$).

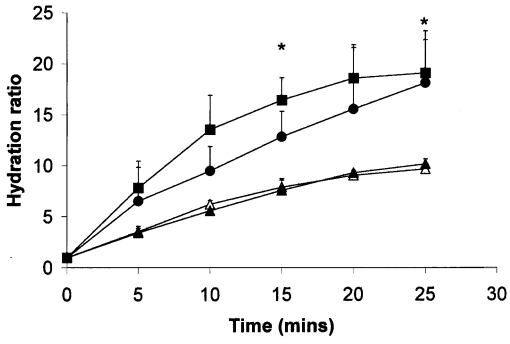


Fig. 3. The effect of the pH of the hydration media on the hydration of GCP11 gels; ● = PBS (pH = 9.0), ■ = sodium tetraborate solution (pH ~ 9.0), ▲ = PBS (pH = 7.4), △ = PBS (pH = 4.0), data points = mean ± S.D., $n = 2$ for PBS solutions, $n = 4$ for sodium tetraborate, * = statistical significance ($P < 0.05$).

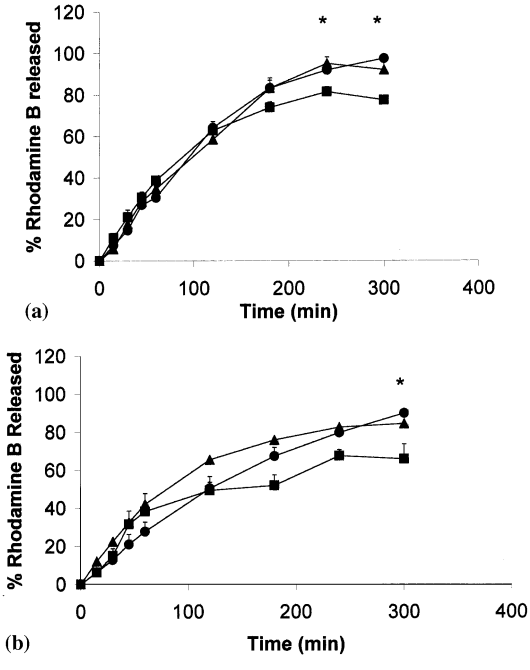


Fig. 4. The influence of the pH of the hydration medium on the release of rhodamine B from GCP11 gels; ● = PBS (pH = 7.4), ■ = PBS (pH = 4.0), ▲ = PBS (pH = 9.0): (a) = rhodamine B loaded as a solution in water; and (b) = rhodamine B loaded as a solution in PBS (pH = 7.4), $n = 3$ except for rhodamine B loaded as a solution in PBS and release monitored at pH = 7.4 where $n = 2$, * = statistical significance ($P < 0.05$).

only 1 in 5 glycol glucosamine units (~20 mole%) was achieved. Previously 1 in 6 glycol glucosamine units were palmitoylated (~15 mole%) (Uchegbu et al., 1998) in the synthesis of GCP41, a version of this amphiphilic polymer in which the initial glycol glucosamine, palmitoyl ratio was 4:1. GCP41 has been used to make unilamellar drug delivery vesicles in the presence of cholesterol (Uchegbu et al., 1998) The level of palmitoylation achieved on increasing the initial amount of palmitoyl groups four fold indicates

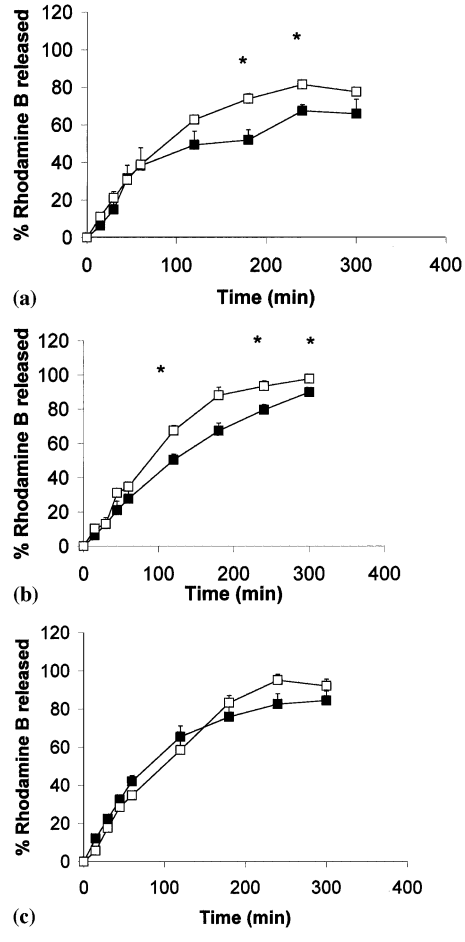


Fig. 5. The influence of PBS on rhodamine B release from GCP11 gels; □ = rhodamine B loaded as a solution dissolved in water, ■ = rhodamine B loaded as a solution dissolved in PBS (pH = 7.4): (a) hydration media = PBS (pH = 4.0); (b) hydration media = PBS (pH = 7.4); and (c) hydration media = PBS (pH = 9.0), $n = 3$ except for samples where rhodamine B loaded as a solution in PBS and release monitored at pH = 7.4 where $n = 2$, * = statistical significance ($P < 0.05$).

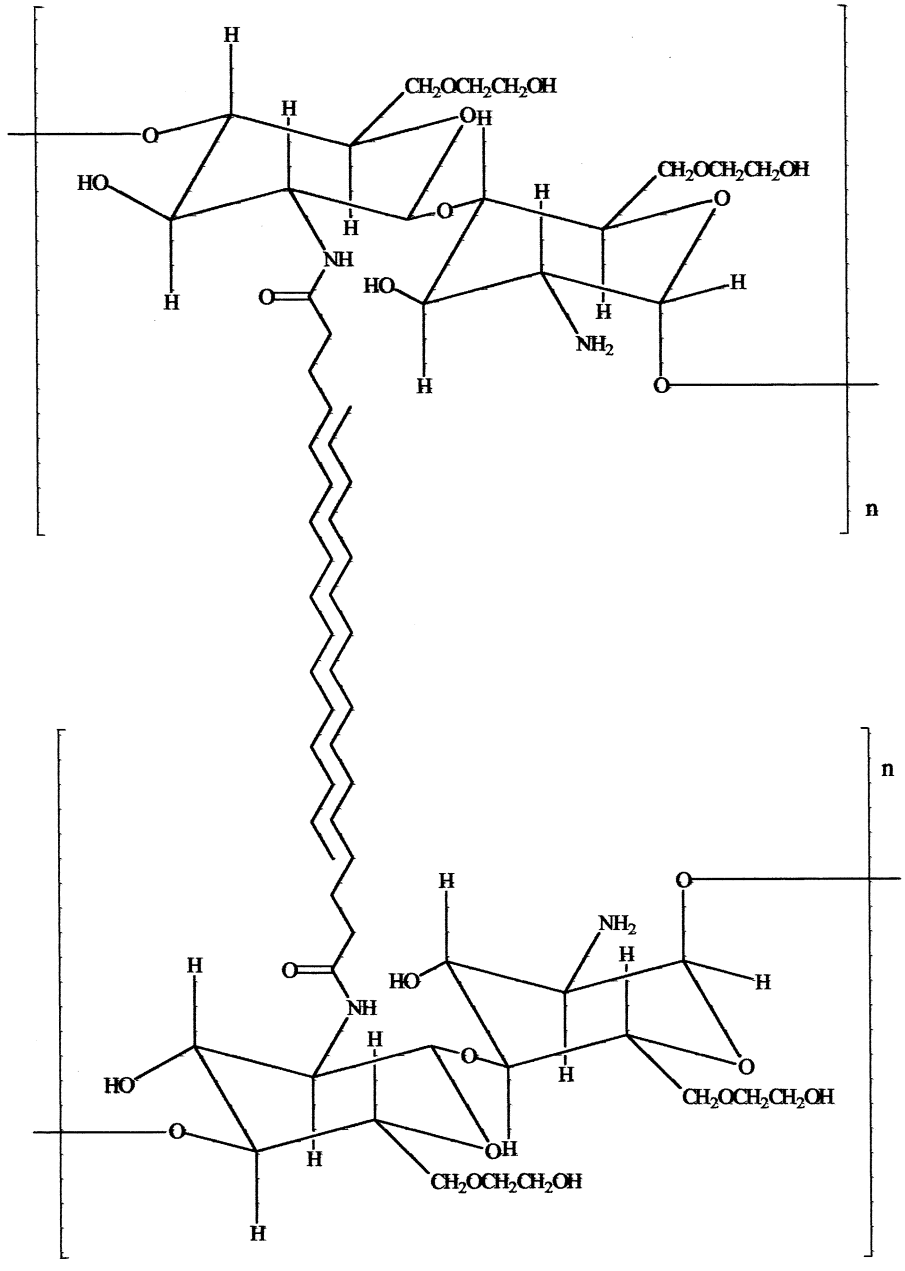


Fig. 6. Proposed mechanism of gel formation in GCP11 gels involving hydrophobic interactions between neighbouring palmitoyl groups.

that high levels of palmitoylation may be sterically hindered. GCP41 is less hydrophobic than GCP11 and although gels may be formed from this material, erosion in aqueous media is too rapid for it to be useful for controlled drug release.

Freeze-drying the gel dispersion, providing freezing is slow, gives a porous material as a result

of the presence of ice crystals within the matrix (Fig. 1). The difference in pore size between the loaded and plain gels is not entirely clear although it could reveal a greater level of ice crystal formation during freezing in the presence of the solute rhodamine B. Freeze drying as opposed to the air drying of covalently cross-linked chitosan/polyethylene oxide hydrogels gave these hydrogels a greater porosity and hydration capacity (Patel and Amiji, 1996). In addition covalent cross-linking tends to reduce the hydration capacity of hydrogels (Guo and Cooklock, 1996). Hence a combination of the non-covalent cross-linking and the freeze drying procedure used to prepare these GCP11 hydrogels yields a material of great porosity (Fig. 1) and good hydration capacity (Fig. 2). There is also not a marked increase in gel volume on hydration, making it especially suitable for use by the buccal route.

Drug loading of these non-covalently cross-linked hydrogels simply involves freeze-drying a solution of the drug in the presence of an aqueous dispersion of the gel. This is less deleterious to drug substances than processes in which covalent cross-linking takes place in the presence of the drug (Patel and Amiji, 1996). Covalent cross-linking not only reduces the hydration capacity of hydrogels (Guo and Cooklock, 1996), but also reduces their permeability to drug molecules (Nakatsuka and Andrady, 1992) a fact which ultimately leads to a slower drug release rate. Freeze dried covalently cross-linked chitosan/poly(ethylene) oxide hydrogels by virtue of their porous nature show a faster release rate of entrapped drug than air dried varieties of the gel (Patel and Amiji, 1996). The fast release obtained with the present gels (Figs. 4 and 5) is a result of the non-covalent cross-linking and the freeze-drying procedures used to produce the gels. This is advantageous for delivery to the buccal route where release rates of less than 12 h are desirable in order to ensure patient acceptability.

The reduced hydration of GCP11 in acid and neutral media (Fig. 3) is in contrast to most chitosan based materials which show dramatically increased hydration in acid media (Deyao et al., 1993, 1994; Guan et al., 1996; Patel and Amiji, 1996; Chen et al., 1997) as a result of the protonation of glucosamine amino groups (Deyao et al., 1994) which are subsequently hydrated (Guan et al., 1996). The apparent contradiction found with GCP11 gels is as a result of two factors. Firstly the acylation of glycol chitosan, a material previously found to be 16 mole% acetylated, reduces the number of available amino groups and hence the pH sensitivity of the gel (Fig. 3). Secondly the apparent slight reduction in hydration at acid pH as computed from the W_0 and W_t values is explained by an increase in the erosion of the material at acid pH (which was less so at alkaline and neutral pH). This increased erosion decreased the W_t value and hence the computed hydration ratio. The increased erosion is visible if gels are placed in acid as opposed to alkaline medium. The swelling ratio (computed in a similar manner to the hydration ratio) of a cross-linked chitosan/polyether semi-IPN was found to increase initially and then decline thereafter as a result of the solubilisation/erosion of the gel material with time at acid pH (Peng et al., 1994). There was no such decline in the swelling ratio observed within the same time frame in alkaline medium although it must be stated that the overall swelling ratio of this chitosan based semi-IPN was markedly enhanced at acid pH (Peng et al., 1994). Despite the decreased erosion of GCP11 at acid pH, rhodamine B release is reduced at acid pH (Fig. 4), as a result of the reduced solubility of rhodamine B in acid media.

Rhodamine B release may also be controlled not only by manipulations in environmental pH but also by the addition of dissolved salts to the gel (Fig. 5). The slower release of rhodamine in the presence of dissolved salts is as a result of the diminished hydration of both the gel material and the model compound rhodamine B and the preferred hydration of the salt within the gel.

5. Conclusions

Palmitoyl glycol chitosan (GCP11) prepared by the single step attachment of palmitoyl groups to the water-soluble derivative of chitosan—glycol chitosan may be used to prepare a hydrogel by simply freeze-drying an aqueous dispersion of this

amphiphilic polymer. This hydrogel, cross-linked by the hydrophobic interaction of the palmitoyl groups, is highly porous and well hydrated in aqueous media. The release of a model compound—rhodamine B may be controlled by loading the compound in the presence of dissolved salts or manipulating the environmental pH. The present gel is designed for controlled drug delivery to the buccal cavity and across the buccal mucosa.

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