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European Journal of Pharmaceutics and Biopharmaceutics 55 (2003) 35–45

European Journal of Pharmaceutics and Biopharmaceutics

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Research paper

Sustained buccal delivery of the hydrophobic drug denbufylline using physically cross-linked palmitoyl glycol chitosan hydrogels

Lee Martin[a], Clive G. Wilson[a], Fariba Koosha[b], Ijeoma F. Uchegbu[a,*]

[a]*Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK*
[b]*GlaxoSmithKline, Harlow, Essex, UK*

Received 14 December 2001; accepted in revised form 1 July 2002

Abstract

A physically cross-linked palmitoyl glycol chitosan hydrogel has been evaluated as a controlled release system for the delivery of hydrophobic drugs via the buccal route. Samples of palmitoyl glycol chitosan (GCP) with diminishing hydrophobicity (GCP12 > GCP11 > GCP21) were synthesized, characterized by ^{1}H nuclear magnetic resonance and hydrogels prepared by freeze-drying an aqueous dispersion of the polymer in the presence of a model hydrophobic drug denbufylline and in some cases the soluble detergent sodium glycodeoxycholate (GDC). GDC was employed as a penetration enhancer. Gels were analysed for hydration, erosion, mucoadhesion and imaged by scanning electron microscopy. The buccal absorption of denbufylline from GCP12, denbufylline, GDC (20:12:1.5) formulations was also investigated in the rabbit model with Carbopol 974NF (CP), denbufylline, GDC (60:36:4) tablets used as controls. Denbufylline reduced the porosity, erosion and hydration of the gels while GDC increased the hydration and erosion. All gels were mucoadhesive but less so than the control CP tablets. Denbufylline was detected 0.5 h after dosing with the GCP12 formulation and delivery was sustained for at least 5 h after dosing. In comparison delivery from the CP tablets was not sustained and was first detected 1 h after dosing.

Keywords: Palmitoyl glycol chitosan; Hydrogel; Buccal; Denbufylline; Chitosan; Mucoadhesion

1. Introduction

The buccal route has been advocated as a possible route of administration for drugs which undergo extensive hepatic first pass metabolism or which are susceptible to degradation in the gastrointestinal tract [1–3]. This route is well vascularized with venous blood draining the buccal mucosa reaching the heart directly via the internal jugular vein. Although drug fluxes via this route are inferior to those obtained via the sublingual mucosa due to a permeability barrier [4], the relative immobility, when compared to the sublingual route, of the buccal musculature makes this site ideally suited for mucoadhesive sustained release dosage forms [2,4]. Drug fluxes across the buccal mucosa are poor however and typical peak plasma levels of between 0.0004% [5] and 0.05% [6] of the administered dose have been obtained even in the presence of penetration enhancers [6] in the case of peptides and peak plasma levels of 0.1% of the administered dose have been obtained in the case of morphine [7]. These percentages are arrived at by assuming a total plasma volume of 4% of the animal/human weight. The most studied penetration enhancers are the bile salts [6,8–10] although both the use of unsaturated fatty acids [11] and the conversion of drugs to lipophilic derivatives [12] have been employed.

A number of studies have documented the use of controlled release buccal drug delivery systems for the delivery of various hydrophilic drugs e.g., oxytocin [5], codeine phosphate [13] and hydrophobic drugs such as buprenorphine [14]. The delivery of hydrophobic drugs is usually accomplished using polymer mixtures e.g. polyisobutylene, polyisoprene and Carbopol 934P [14] and not normally hydrogels. There is a paucity of literature evidence documenting the delivery of hydrophobic drugs from hydrogels. This is despite the fact that an increase in hydrophobicity improves drug transport across the buccal mucosa [12] and also that a hydrogel platform may offer a means of sustaining drug delivery. The lack of hydrogel delivery devices for hydrophobic drugs could be due to scant attention paid to the incorporation of relatively hydrophobic drugs into hydrogels and only four such studies could be found in the literature namely the loading of vephylline (a xanthine bronchodilator)

* Corresponding author. Department of Pharmaceutical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK. Tel.: +44-141-548-3895; fax: +44-141-552-6443.
E-mail address: i.f.uchegbu@strath.ac.uk (I.F. Uchegbu).

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doi:10.1016/S0939-6411(02)00118-2

in both poly(malic acid polyethylene glycol) gels and hydrophobized polyethylene glycol based hydrogels [15], the incorporation of ibuprofen into poly(*N*-isopropylacrylamide) based gels [16], the incorporation of progesterone into poly(*N*-isopropylacrylamide) based gels [17] and the incorporation of cyclosporine in polyvinyl pyrrolidone–polyhydroxyethylmethacrylate hydrogels [18]. The current study examines the formulation of the model hydrophobic (aqueous solubility 0.5 mg ml^{-1}) phosphodiesterase-4 inhibitor denbufylline [19], a xanthine derivative (Fig. 1) developed for the treatment of cerebrovascular diseases, as a sustained release chitosan based hydrogel for buccal delivery. The aqueous solubility of denbufylline fits the description of 'very slightly soluble' in water given to drugs by the British Pharmacopoeia, since it has an aqueous solubility of between 0.1 mg ml^{-1} and 1 mg ml^{-1} [20]. For example, prednisolone is characterized by the British Pharmacopoeia 1998 as 'very slightly soluble' [20].

The desirable attributes of a buccal absorption system for prolonged systemic delivery are a high drug loading capacity, good mucoadhesion, non-irritancy, good feel in the mouth, tastelessness and sustained drug delivery. An erodible formulation has the added advantage of not requiring retrieval after delivery of the dose.

Hydrogels are formed from cross-linked polymers and may be hydrated in an aqueous environment without dissolution [21], acting as drug delivery systems by physically entrapping biomolecules, which are then slowly released on gel hydration. Chitosan is a linear polysaccharide made of glucosamine units and chitosan hydrogels have been prepared for drug delivery by chemically cross-linking this polymer using tripolyphosphate pentasodium salt [22] or physical cross-linking via hydrophobic associations of grafted polylactic [23] or palmitic acid [24,25] units. Alternatively chitosan based hydrogels for drug delivery have been prepared where gelation occurs on reacetylation of high molecular weight chitosan [26]. Chitosan is biodegradable [26–28], biocompatible [28] and its use as a pharmaceutical excipient has been investigated and advocated [29]. This polymer is also mucoadhesive [30] and has been reported to enhance drug penetration across the nasal [31] and buccal [32] mucosa. This communication details the use of palmitoyl glycol chitosan (GCP) hydrogels [24,25] for the buccal delivery of the model hydrophobic drug denbufylline, a drug that is extensively metabolized via the oral route [33].

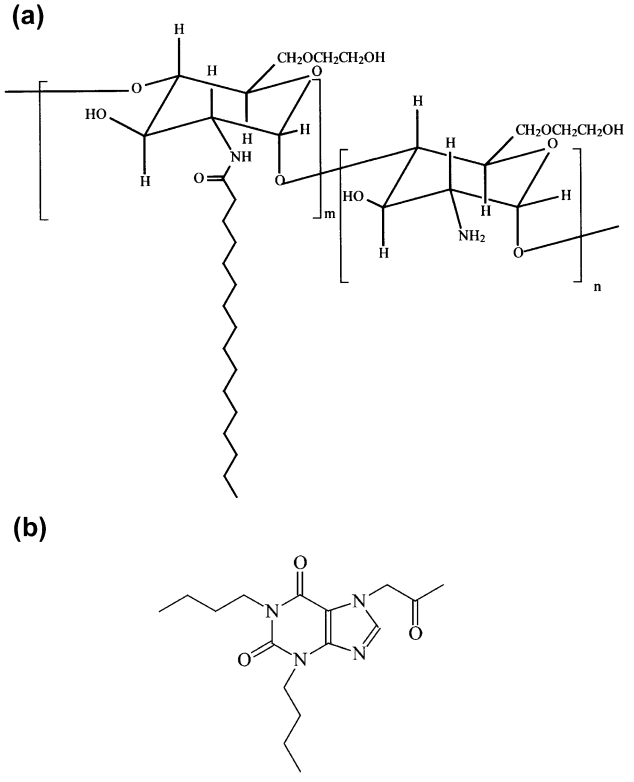

Fig. 1. (a) Palmitoyl glycol chitosan. (b) Denbufylline.

2. Materials and methods

2.1. Materials

All materials were used as received. Glycol chitosan (GC), palmitic acid *N*-hydroxysuccinimide (PNS), sodium glycodeoxycholate (GDC) and phosphate-buffered saline (PBS) tablets were all purchased from Sigma Aldrich Co. Ltd., UK. Sodium bicarbonate was obtained from Fluka, UK. Denbufylline (M_r 320.4) was donated by GlaxoSmithKline, UK. Fresh excised porcine buccal mucosa was obtained from the local abattoir. Female New Zealand white rabbits were purchased from Harlan UK Ltd., UK. Absolute ethanol, acetone and diethyl ether were all purchased from the Department of Pure and Applied Chemistry, University of Strathclyde. Carbopol 974NF was obtained from B.F. Goodrich, USA. Dialysis tubing was obtained from Medicell International, UK. Hypnorm was sourced from Janssen Pharmaceutica, Belgium and Halothane from Roche Ltd., UK. Heparin was obtained from Leo Laboratories, UK and normal saline solution from Animal Care, UK. All gases were from BOC, UK.

2.2. Synthesis of GCP

Synthesis of the polymers (Fig. 1a) was carried out as previously described [24,25]. The ratios of reactants varied as shown in Table 1 to give GCP21, GCP11 and GCP12 of increasing hydrophobicity. Briefly, for the preparation of GCP12, GC (250 mg) was dissolved in water (40 ml) to which was added sodium bicarbonate (190 mg) and absolute ethanol (25 ml). A solution of PNS (792 mg) in absolute ethanol (300 ml) was added drop-wise to the alkaline solution of GC over a 1-h period. After 72 h stirring, protected from light, acetone (50 ml) was added to the reaction mixture and the resulting solution evaporated under reduced pressure at 45 °C to remove organic solvents. The residual mixture was extracted with 3× diethyl ether (100 ml) and

Table 1
Synthesis of various GCP samples

GCP gel formulation	Glycol chitosan (g)	Palmitic acid *N*-hydroxysuccinimide (g)	Mol% palmitoylation (mean ± SD, $n = 3$)
GCP21	0.500	0.396	4.2 ± 2.62
GCP11	0.250	0.396	10.0 ± 7.32
GCP12	0.250	0.792	20.31 ± 2.22

subjected to exhaustive dialysis within dialysis tubing (molecular weight cut-off 12–14 kDa) against water (5 litres) for 24 h with six changes. The polymer dispersion was freeze-dried (see below).

Analysis of the polymer samples was carried out by ^{1}H nuclear magnetic resonance as detailed previously [24].

2.3. Preparation of GCP gels/CP tablets

2.3.1. Plain GCP12 gels

The purified polymer dispersion obtained from above was frozen at −20 °C and freeze-dried in plastic sample bottles of 15 mm in diameter to yield manually compressed discs of 15 mm in diameter and 2 mm in height.

2.3.2. Denbufylline loaded GCP gels

Dried GCP gels (20 mg) obtained from above were dispersed in an ethanol, water (1:9) solution (10 ml) of denbufylline (1.2 mg ml^{-1}) by probe sonication (Lucas Davis Ultrasonics, UK). The resulting dispersion was frozen at −20°C and freeze-dried in plastic sample bottles of 15 mm in diameter (10 ml) or 96-well plates (0.25 ml). The freeze-dried cake was subsequently manually compressed to yield discs of 15 mm diameter and 2 mm thick or uncompressed cylinders of 6 mm in diameter and 9 mm in length. Discs were used for mucoadhesion and scanning electron microscopy studies and cylinders were used for the hydration experiments.

2.3.3. Denbufylline, GDC, GCP12 gels

The dried plain GCP12 gel formulation (20 mg) obtained from above and GDC (1.5 mg) were dispersed in ethanol, water (1:9) denbufylline solutions (1.2 mg ml^{-1}, 10 ml) by probe sonication. After probe sonication, the resulting solutions were freeze-dried (10 ml) in plastic sample bottles of 15 mm in diameter as described above to give GCP12 disc-shaped gels loaded with 36%w/w denbufylline and 4%w/w GDC, which were 15 mm in diameter and 2 mm thick on manual compression. Alternatively the dispersion was freeze-dried within 96-well plates (0.25 ml) to yield cylindrical gels of 9 mm in length and 6 mm in diameter. Discs were used for the in vivo and mucoadhesion studies and cylinders were used for the hydration experiments.

2.3.4. Denbufylline, GDC, CP tablets

Denbufylline (36 mg), CP (60 mg) and GDC (4 mg) were blended together for 30 min in a Turbula Mixer (Glen Creston, UK) prior to compression. The resulting powder was directly compressed at a weight of 100 mg, diameter 10 mm, using a single-punch tablet press (Model E2, Manesty, Liverpool, UK). Compressed tablets were fully characterized for weight, diameter, thickness and hardness (Erweka TGB30 tablet tester, Copley Instruments, Nottingham, UK). Tablets ($n = 10$) were an average weight of 100 mg, 10 mm in diameter, 2 mm in thickness and had a mean hardness of 6 kp. The CP tablets, loaded with 36%w/w (36 mg) denbufylline and 4% w/w (4 mg) GDC, were used as control formulations during in vivo testing.

2.4. Characterization of the GCP gels/CP tablets

2.4.1. Hydration and erosion studies

Dried cylindrical gels (9 mm in length × 6 mm in diameter) were weighed and immersed in water (25 ml) contained within stoppered bottles that were in turn placed in a shaking water bath set at 35 °C. At various time points gels were removed drained and their weights recorded. Swelling (weight gain) ratio was recorded as

$$\frac{W_t}{W_0}$$

where W_0 is the weight of the dried hydrogel and W_t the weight of the hydrated hydrogel at time t. Experiments were performed in triplicate.

The gradual erosion of GCP gels was also studied by observing the time at which W_t began to decrease. Experiments were performed in triplicate. To minimize erosion due to gel handling, a separate pre-weighed gel was used to generate each data point.

2.4.2. The in vitro release of denbufylline from denbufylline loaded GCP gels

The in vitro release of denbufylline from GCP gels was studied by placing individual weighed pieces of denbufylline GCP gel within sealed Visking dialysis tubing (M_r cut-off 12–14 kDa) containing phosphate-buffered saline (PBS, pH 7.4, 5 ml). This was then submerged into a universal bottle containing PBS (pH 7.4, 15 ml), which was placed in a shaking water bath at 35 °C. At pre-determined time points (0, 5, 15, 30, 60, 120, 180, 240, 300 and 1440 min), aliquots of the PBS surrounding the dialysis tubing (1 ml) were removed, reserved for analysis and replaced with the same volume of fresh PBS. The 1-ml sample collected above was placed in a cuvette to obtain a reading using a Unicam U.V.1

UV-visible spectrometer, set at a wavelength of 296 nm. Quantification was carried out with reference to a standard curve for denbufylline (0.006–60 μg ml^{-1}, $r = 0.998$) prepared by diluting a stock solution of denbufylline in water and measuring the absorbance at a wavelength of 296 nm.

2.4.3. Scanning electron microscopy

Compressed disk shaped gel formulations were fast frozen in liquid propane and freeze-dried on coverslips at −80 °C overnight under a vacuum of 10^{-6} Torr. Dried specimens were gold-coated on a Peltier-cold stage sputter-coater and examined using a Phillips 500 scanning electron microscope at 3 or 6 kV accelerating voltage. 1 K × 1 K images of the cross-sectional morphology of the various freeze-dried GCP gel formulations were captured using a digital interface, and recorded onto CD.

2.4.4. Mucoadhesion studies

Control tablets consisting of HPMC, CP (7:3) were prepared. Excipients were weighed to give the desired composition and the powders blended for 30 min in a Turbula Mixer prior to compression. After blending, the resulting powder was directly compressed at a weight of 100 mg and at a diameter of 7 mm, using a single-punch tablet press (Model E2, Manesty). Control tablets were fully characterized for weight, diameter, thickness and hardness (Erweka TGB30 tablet tester). Control tablets ($n = 5$) had an average weight of 95 mg and were 7 mm in diameter, 3 mm thick and with a mean hardness of 6 kp.

Freshly excised porcine buccal mucosa was obtained from male/female pigs with an average weight of 65 ± 6 kg, from the local slaughterhouse. Pigs were 6.5 ± 0.5 months of age at the time of death. The animals were killed by electroshock followed by exsanguination. The tissue was then removed, placed in PBS solution (pH 7.4, 300 ml) and used within 2 h of the animal's death.

Disc-shaped gels, 15 mm in diameter and 2 mm in height, were manually attached to the texture analyser probe (Stable Micro Systems TA–XT2 Texture Analyser). The force of detachment of the gels and tablets from a piece of porcine mucosa was recorded by lowering tablets or gels (affixed to the instrument's perspex support) at a speed of 2 mm s^{-1} until contact with the tissue was achieved. A predetermined contact force of 0.02 N and contact time of 60 s was used. The probe was then raised (in an attempt to detach the gel/tablet from the mucosa) to a distance of 15 mm and the force of detachment recorded as a measure of the mucoadhesion of the formulation.

2.5. In vivo absorption experiments

2.5.1. Animal studies

Animal studies were conducted under United Kingdom Home Office Personal and Project licences. Six-month-old male New Zealand White rabbits (mean weight ± SD 2.59 ± 0.37 kg) were anaesthetized by an intramuscular injection of Hypnorm followed with an inhalation anaesthetic dose of halothane and medical oxygen gas for the duration of the experiment. An intravenous cannula (Braun Melsungen AG, Melsungen, Germany) with a three-way stopcock (Bentley Laboratories, Europe BV, Netherlands) was placed into the marginal ear vein for blood sample collection. A solution of heparin (5000 U ml^{-1}) in normal saline solution was used to flush out the three-way stopcock to keep the cannula patent throughout the experiment. A blank blood sample (1 ml) was obtained prior to the application of the drug ($t = 0$). Two groups of rabbits were tested. The first group of three rabbits each received two 12-mg doses of denbufylline as GCP12, denbufylline, GDC (20:12:1.5), with one applied to each side of the buccal mucosa. The second group of three rabbits each received two 36-mg doses of denbufylline as CP, denbufylline, GDC (60:36:4), with one applied to each side of the buccal mucosa. Blood samples (1 ml) were collected 30, 60, 120, 180, 240 and 300 min after application. Animals received a higher dose of denbufylline from the CP formulation because preliminary investigations had revealed a difficulty in detecting denbufylline from CP formulations at certain time points. This is despite the fact that denbufylline is an extremely weak base with a pK_a estimated at 1.4 and hence would be unlikely to interact electrostatically with CP.

Blood samples were centrifuged at 3000 × *g* for 20 min (BTC bench centrifuge), the supernatant plasma drawn off, placed into an Eppendorf tube and frozen immediately in liquid nitrogen. Frozen plasma samples were then stored (−20 °C) until analysis could be performed.

2.5.2. Determination of drug content in plasma samples

Denbufylline content of the plasma samples was determined by high-performance liquid chromatography (HPLC) using a Spectra Physics P100 pump, Waters 717 autosampler, Waters μBondapak C-18 reverse phase column (3.9 × 150 mm), Waters 486 variable wavelength absorbance detector ($\lambda = 274$ nm) and a Waters 746 data module. The mobile phase consisted of potassium dihydrogen orthophosphate buffer (0.05 M, pH 2.5, 450 ml), acetonitrile (400 ml) and ethanol (50 ml). The flow rate was 2 ml min^{-1} and the injection volume 20 μl.

To thawed plasma samples (0.5 ml) was added distilled water (0.4 ml) and carbonate–hydrogen carbonate buffer (1 M, pH 10.1, 50 μl). TC2 cartridges (Waters, UK) were primed with methanol (1 ml) and water (1 ml) before the plasma solution was added. The cartridge was washed with water (2 ml) followed by sample elution with 0.5 ml chloroform, propan-2-ol (95:5) solution. The collected samples were evaporated to dryness at room temperature, under a stream of nitrogen. To the dried residue was added 0.1 ml of a solution of water, acetate buffer (1 M, pH 5.6), acetonitrile (89.75:0.25:10). Twenty microlitres of this solution were injected onto the HPLC column. Quantification was carried

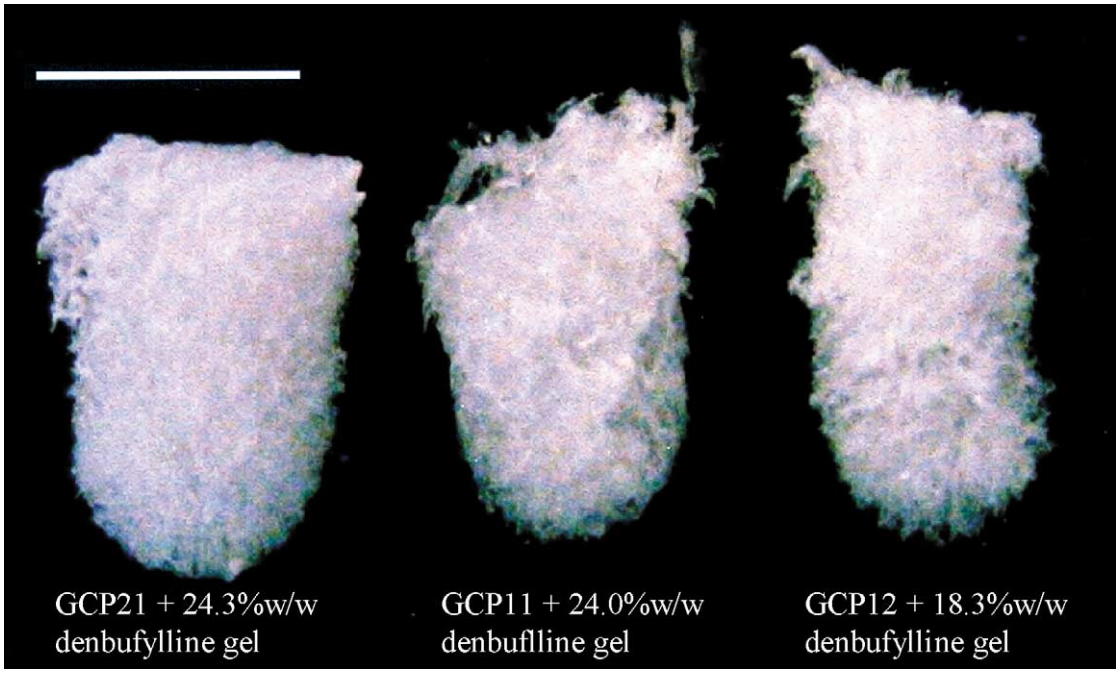

Fig. 2. GCP gel formulations loaded with denbufylline. From left to right: GCP21 containing 24.3%w/w denbufylline, GCP11 containing 24.0% denbufylline, GCP21 containing 18.3%w/w denbufylline. Bar: 6 mm.

out with reference to a denbufylline standard curve derived from spiked plasma samples ($r = 0.97$). The limit of detection was 6.4 ng.

2.6. Statistics

Analysis of variance and Student's *t*-tests were performed on data sets using the Mintab 7.1 software package. Statistical significance was set at $P < 0.05$.

3. Results

3.1. GCP gel preparation

Palmitoyl glycol chitosan dispersions on freeze-drying produce a spongy material, which on hydration forms a hydrogel due to the physical cross-linking of palmitoyl pendant groups [24]. GCP12, GCP11 and GCP21 gels have decreasing levels of hydrophobicity (Table 1) and the incorporation of denbufylline into each of these gels gives rise to cotton wool like material (Fig. 2), which still hydrates in aqueous media (Fig. 3).

3.2. Hydration and erosion

All GCP, denbufylline gels hydrated without an appreciable change in volume or shape in the early hydration phase and were able to hydrate to up to 26 times their own weight. There was no significant difference in the hydration rate for all the gels although the more hydrophobic GCP12 and GCP11 gels all hydrated to a lesser extent than the plain

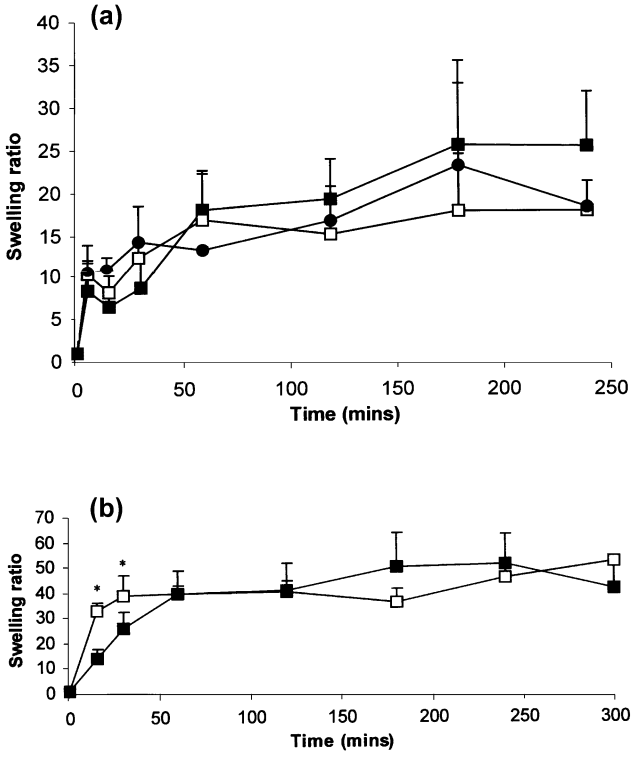

Fig. 3. (a) The swelling ratio of GCP gels. □, GCP21 + 22.3%w/w denbufylline; ●, GCP11 + 24.1%w/w denbufylline; ■, GCP12 + 18.4%w/w denbufylline. Data points represent the mean ± SD of three experiments. (b) The swelling ratio of GCP12 gel formulations, ■, 100% GCP12; □, GCP12, GDC, denbufylline (20:1.5:12). *Significant difference between formulations ($P < 0.05$). Data points represent the mean ± SD of three experiments.

gels prepared without the inclusion of denbufylline. Plain GCP12, GCP11 and GCP21 gels could be hydrated to 50, 95 and 15 times their original weight, respectively, over a 4-h period [25]. While denbufylline loaded GCP12, GCP11 and GCP21 gels hydrated to up to 26, 23 and 18 times their original weight, respectively, over the same time period (Fig. 3a). The incorporation of the soluble surfactant GDC in the gels (Fig. 3b) increased the rate of hydration when compared to plain GCP12 gels and GCP12, GDC, denbufylline gels (20:1.5:12) were hydrated faster than the plain gels. The presence of denbufylline in the former gels is unlikely to be responsible for this increased hydration.

On hydration all GCP, denbufylline gels gradually erode with time and the onset of discernable hydration is given in Table 2. The hydrophobicity of the gel material governed the speed of erosion for the GCP, denbufylline gels and the incorporation of denbufylline decreased the speed of erosion as discernable erosion onset values for the plain gels were 4, 6 and 48 h for GCP21, GCP11 and GCP12, respectively [25]. While similar values for the denbufylline-loaded gels were 18, 22 and above 76 h, respectively (Table 2). The incorporation of the soluble surfactant GDC into GPC12, denbufylline gels increased the rate of erosion (Table 2).

3.3. In vitro release of denbufylline

The release of denbufylline was essentially controlled by the poor solubility of the drug with high loading levels giving rise to slower percentage release levels (Fig. 4 and Table 3). This is despite the fact that all experiments were conducted under conditions where 100% release of denbufylline would have yielded drug levels at least one order of magnitude below the saturation solubility of the drug (saturation solubility of denbufylline = 0.5 mg ml^{-1}). There were some exceptions to the trend for gels containing less denbufylline to release a higher percentage of the load, namely the lightly loaded GCP21 sample (loaded with 7.3% w/w of denbufylline) and two of the lightly loaded GCP12 samples (loaded with 5.3% w/w and 6.3% w/w of denbufylline, respectively). In these gels we speculate that the hydrophobicity of the drug may have made a contribution to the gel structure such that gel hydration, drug release and gel erosion were comparatively retarded

Table 2
Weight and time of erosion onset for GCP gels ($n = 3$)

GCP gel formulation	Denbufylline content (%w/w)	Time of erosion onset (h)
GCP21, denbufylline	22.3	18
GCP11, denbufylline	24.1	22
GCP12, denbufylline	18.4	> 76
GCP12, denbufylline, GDC (20:12:1.5)	36.0	16

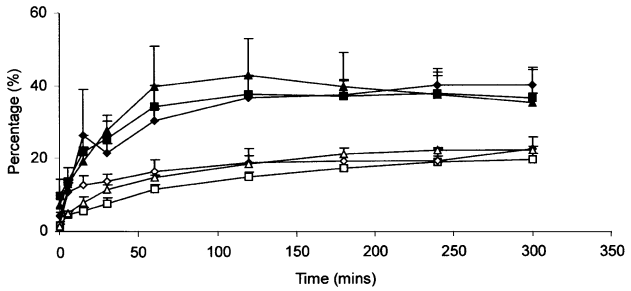

Fig. 4. The effect of denbufylline load on the release of denbufylline from GCP gels. Filled symbols, low levels of denbufylline; open symbols, high levels of denbufylline. ■, GCP21 + 10%w/w denbufylline; ▲, GCP11 + 8.6%w/w denbufylline; ◆, GCP12 + 6.3%w/w denbufylline; □, GCP21 + 22.3%w/w denbufylline; △, GCP11 + 24.1% denbufylline; ◇, GCP12 + 18.4%w/w denbufylline. Data points represent the mean ± SD of three experiments.

3.4. Scanning electron microscopy

GCP gels are porous due to the formation of ice crystals when freezing [24,25]. The incorporation of the hydrophobic drug denbufylline into GCP12 gels reduces gel porosity (Fig. 5a,b) and increasing the level of denbufylline abolishes the pores altogether (Fig. 5c,d).

3.5. Gel mucoadhesion

While the GPC gels were mucoadhesive, the CP formulations were significantly more mucoadhesive than all GPC formulations (Tables 4 and 5). The incorporation of denbufylline (18.4% w/w) into GCP12 gels did not alter the mucoadhesion whereas formulations containing GDC (4.5% w/w) and a higher level of denbufylline (36% w/w) showed increased mucoadhesion.

3.6. Drug content in plasma samples

Samples taken prior to dosing contained no denbufylline. These samples served as negative controls for the experiment. Denbufylline was detectable in the plasma within 0.5 h of application of the GCP12 formulations but not until 1 h after application of the CP tablet formulations, and while denbufylline was detected at all time points from the GCP12-dosed animals it was not detectable at 0.5, 3 and 5 h from the CP-dosed animals (Fig. 6). Delivery from both formulations peaked at 4 h with 2.4% of the dose delivered from the GCP12 formulation and 1.2% of the dose delivered from the CP formulation. GCP12 gel formulations were easy to apply and remove from the buccal mucosa and remained intact and attached to the buccal mucosa for the duration of the experiment. The CP tablets also remained intact for the duration of the experiment, but more force was required to remove them from the buccal mucosa and there was evidence of epithelial damage on removal of the CP tablets.

Table 3
The release of denbufylline from GCP gels after 24 h

GCP gel formulation/% denbufylline in gel (w/w)	Average weight of denbufylline gel (mg)	Original amount of denbufylline loaded (mg)	Mean% of denbufylline released (±SD)
(a) GCP21 gels			
22.3	1.7	0.38	19.12 ± 1.47
13.6	1.4	0.19	23.71 ± 2.25
10.0	1.7	0.17	37.34 ± 4.99
9.0	2.0	0.18	39.41 ± 11.90
7.3	1.9	0.14	15.39 ± 3.71
(b) GCP11 gels			
24.1	1.7	0.41	21.72 ± 0.94
14.4	1.8	0.26	23.39 ± 1.68
12.3	1.7	0.21	49.63 ± 9.86
8.6	2.2	0.19	42.37 ± 10.16
2.3	2.2	0.05	67.57 ± 10.33
(c) GCP12 gels			
25.7	0.7	0.18	10.57 ± 1.78
18.4	2.5	0.46	21.93 ± 3.25
16.0	1.0	0.16	79.76 ± 13.97
6.3	2.4	0.15	39.67 ± 4.75
5.3	1.7	0.09	45.08 ± 6.39

4. Discussion

Hydrogels for drug delivery have been prepared by the physical cross-linking of the amphiphilic polymer GCP [24,25] and have been tested as vehicles for the controlled delivery of hydrophobic drugs using the model phosphodiesterase 4 inhibitor denbufylline. Hydrophobic cross-linking is facilitated by dehydrating (freeze-drying) a dispersion of the polymer and this facile mode of preparation allows for high drug loads as the drug may be incorporated into the polymer dispersion prior to freeze-drying [24,25]. GCP12 gel formulations were easily loaded with 36% w/w denbufylline (M_r 320.4) and 4% w/w GDC (M_r 471.6) by simply freeze-drying a dispersion of the GCP12 polymer in the presence of denbufylline and GDC.

The hydrophilic parts of the gel (glycol chitosan) and in some cases GDC enable gel hydration which is important for gel mucoadhesion [34] as discussed below, while the hydrophobic parts of the gel–palmitoyl units and denbufylline prevent solubilisation of the gel but allow the gel to erode as the cross-linking is by physical and not covalent means. The gel erodes as the hydrophilic parts of the gel are hydrated and the hydrostatic pressure diminishes the strength of hydrophobic cross-links.

The porosity of the gel structure was diminished by an increase in the level of the hydrophobic drug due to a diminished ability to form ice crystals from less polymer bound water during the freezing step, prior to freeze-drying (Fig. 5). This diminished porosity led to gels, which became visibly denser in appearance as the level of denbufylline increased.

With the plain gels it was found that gel hydrophobicity (GCP12 > GCP11 > GCP21) governed both hydration and erosion [25], with the more hydrophobic gel undergoing less hydration and erosion. In the current study the presence of the hydrophobic drug reduced hydration/erosion overall such that hydration differences due to gel polymer hydrophobicity were not observed (Fig. 3a). However, compared to the plain gels hydration of the more hydrophobic gels (GCP12 and GCP11) was generally diminished by the incorporation of denbufylline (Fig. 3a and Ref. [25]). Furthermore, it can be concluded that the incorporation of the soluble detergent GDC into the more hydrophobic gel GCP12 increased both the rate of hydration (Fig. 3b) and speed of erosion (Table 2). The more hydrophilic gel GCP21 actually showed higher hydration levels with the incorporation of denbufylline due to a decrease in the rate of erosion. The erosion of this gel usually leads to an underestimation of gel hydration [25].

Drug release from hydrogels usually follows gel hydration. However, the relative influences of gel hydration, polymer chain relaxation and drug diffusion on hydrophobic drug release are not clear-cut. Hydrophobic drugs remain bound to hydrophobic gels by non-polar interactions [16] and this binding may be increased by an increase in the level of hydrophobic modification of the gel network [35]. Manipulations in the level of gel hydrophobicity have been exploited to control the release of hydrophobic drugs such as cyclosporine [18] and hydrophilic molecules such as FITC-dextran [25]. It is thus envisaged that a reduced level of hydration would give rise to a slow release of the drug even simply due to polymer chain relaxation effects alone.

To evaluate drug delivery from the current gels, in vivo tests were thus selected since hydrophobic drug release from

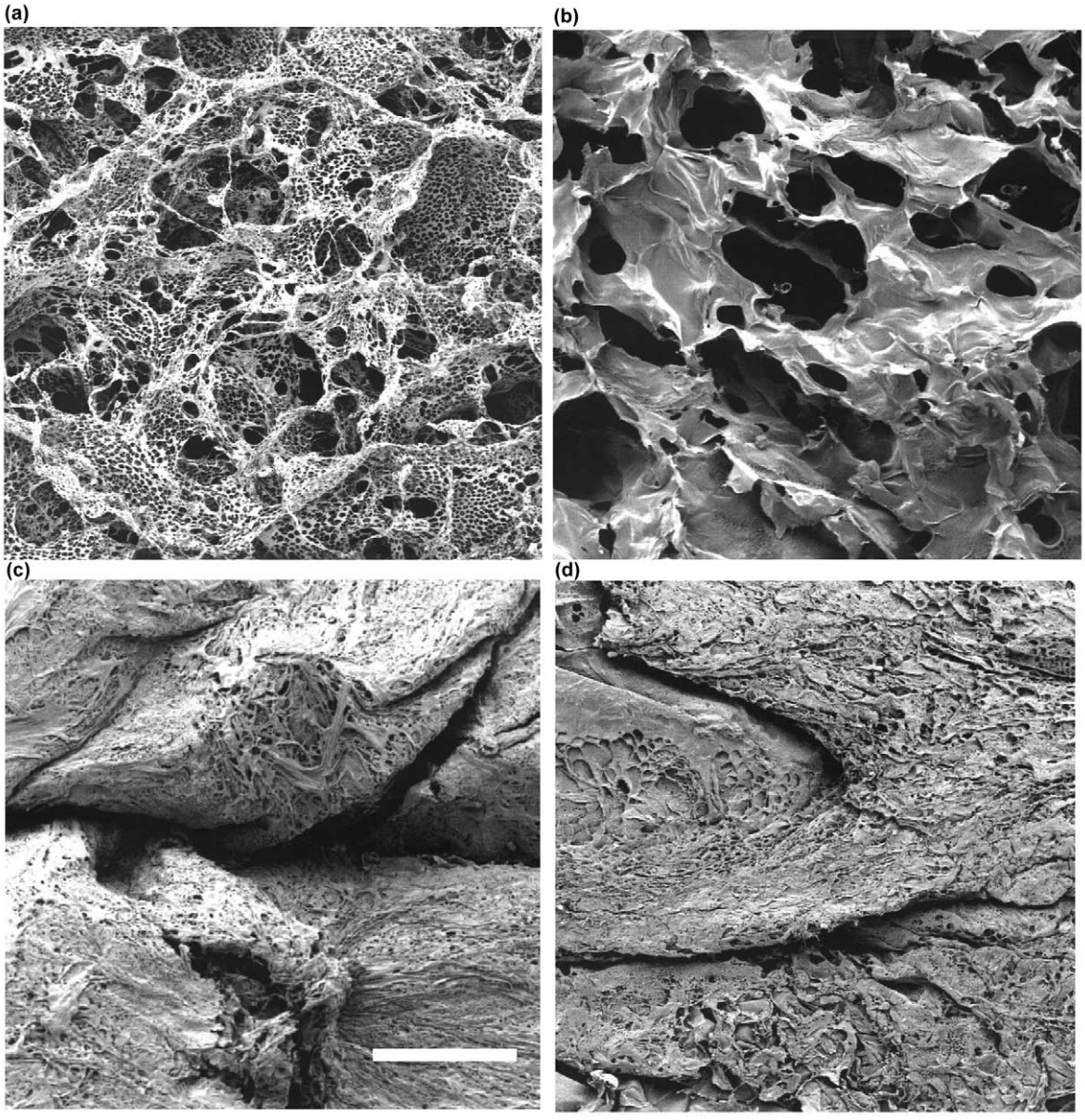

Fig. 5. Scanning electron micrographs (SEMs) of GCP gels. (a) 100% GCP12; (b) GCP12, denbufylline (82:18); (c) GCP12, denbufylline, GDC (60:36:4); (d) GCP12, denbufylline (54:46). Bar: 200 μm.

hydrogels is not easy to measure in aqueous media as low drug solubility exerts an overriding influence on the amount of drug detected in the release medium (Fig. 4 and Table 3).

Mucoadhesion is desirable for a buccal drug delivery system designed for sustained delivery. Porcine buccal mucosa was used as the model mucosal layer, and this tissue

Table 4
Mucoadhesion of GCP–denbufylline gels

Formulation	Detachment force (N cm^{-2}) (mean ± SD, $n = 3$)[a]
GCP21, denbufylline (77.7:22.3)	0.074 ± 0.0192
GCP11, denbufylline (75.9:24.1)	0.105 ± 0.037
GCP12, denbufylline (81.6:18.4)	0.029 ± 0.0017*
HPMC, CP (70:30)	0.416 ± 0.023*

[a] *Significant difference between gel and all other formulations shown in Table 3a ($P < 0.05$).

Table 5
Mucoadhesion of GCP–denbufylline, GDC gels

Formulation	Mean detachment force (N cm^{-2}) (mean ± SD, $n = 3$)[a]
GCP12	0.028 ± 0.014*
GCP12, denbufylline, GDC (20:12:1.5)	0.077 ± 0.039*
CP, denbufylline, GDC (60:36:4)	0.932 ± 0.387*

[a] Significant difference between gel and all other formulations shown in Table 3b ($P < 0.05$).

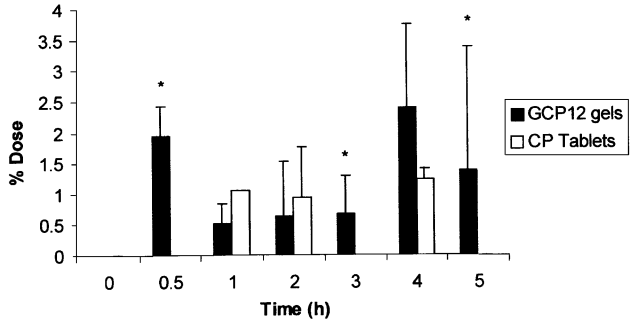

Fig. 6. Plasma levels of denbufylline after buccal administration to the rabbit model ($n = 3$). Black bar, GCP12 gels; white bar, CP tablets. *Significant difference between GCP12 gel and CP tablet ($P < 0.05$). Bars represent the mean ± SD of three animals.

has been evaluated as an in vitro tool and found to provide a 2.5-h window post mortem (on storage at room temperature in PBS) before tissue enzyme activity falls below 80% of the initial value [36]. All experiments were conducted within 2 h of the animals' death. GCP12 gels were not as mucoadhesive as the CP (polyacrylic acid) formulations which have been acknowledged as being extremely mucoadhesive [30,37]. CP polymers are considered so mucoadhesive that they are often incorporated into formulations simply to impart this property on to these formulations [38].

Mucoadhesion is thought to be promoted by high polymer molecular weight [39], polymer flexibility [2], hydrophilicity [2] and any monomer chemistry which promotes hydrogen bonding [2,34]. The interaction of mucin with chitosan increased as the molecular weight of chitosan increased due to an increase in multivalent attachments [36]. The physical state of the mucoadhesive also plays a major role in the strength of mucoadhesion with the ability of the gel to absorb moisture from the mucosal surface contributing to good mucoadhesion [34]. Contact of the gel with the mucosal layer would be followed by dehydration of the mucosal layer, interpenetration of the polymer into the mucosal layer and subsequent hydrogen bonding between the mucosal layer and the polymer chains. Presumably the GCP12, denbufylline GDC formulations used in the current study would immediately hydrate on contact with the mucosal surface and this would promote mucoadhesion. Previous studies have shown that the inclusion of GDC (a penetration enhancer) [9] in CP containing formulations does not increase mucoadhesion [40] although GDC may be implicated in the enhancement of mucoadhesion in GCP12–denbufylline formulations (Tables 4 and 5). The influence on mucoadhesion of the higher concentration of denbufylline, in this latter GCP12–GDC formulation, was not tested although it is clear that the inclusion of GDC within denbufylline gels also increases hydration (Fig. 3b) and this parameter would contribute to mucoadhesion. Hence from our studies it appears possible that the incorporation of soluble surfactants within buccal drug delivery gels may be used to enhance mucosal adherence. The same argument could be extended to explain the superior mucoadhesion of the less hydrophobic GCP11 gel when compared to the more hydrophobic GCP12 gel (Table 3a). Conceivably, hydration of the least hydrophobic and most easily erodible [25] GCP21 gel would yield too mobile a material to establish good mucoadhesion and this would explain the low mucoadhesion value obtained with GCP21. A similar trend in mucoadhesion values was reported previously with plain GCP gels and GCP11 showed the highest hydration levels in these earlier studies [25]. In vivo the CP formulations were found to be too mucoadhesive and removal from the mucosa at the end of the experiment was difficult, with there being macroscopic evidence of continued adherence of the mucosal epithelia to the tablets after removal. This has been observed microscopically with CP containing systems previously [40] but was not observed macroscopically with the GCP12 gel formulations. For practical purposes high levels of CP may not be useful for buccal delivery systems. However, this CP gel was chosen in order to use a formulation with a high degree of mucoadhesion, there being no standard mucoadhesive buccal drug delivery formulation available.

The rabbit is a good animal model for the testing of buccal drug delivery, as this is the only rodent model with a non-keratinized buccal mucosa similar to humans [2]. Both formulations delivered the same peak plasma levels of the dose to the blood (Fig. 6), although the level of denbufylline contained in the CP formulation was three times that contained in the GCP12 formulation. The GCP12 formulation also offered a more sustained delivery profile which was statistically significantly different from the CP delivery profile (Fig. 6). As denbufylline is heavily metabolized it is possible that on blending the solid drug with the CP tablets, a heterogeneous formulation would result which would deliver drug across the mucosa in bursts unlike the more homogeneous formulation formed from freeze-drying a solution of denbufylline with the surfactants GDC (in solution) and GCP12 (as a dispersion). This will explain the erratic delivery observed with the tablet and not with the gel formulation. It is unlikely that the acidic CP interacted with denbufylline due to the fact that denbufylline is a very weak base (pK_a estimated at 1.4). Another important factor in these experiments is that the rabbits were anaesthetized for the duration of the experiment. Anaesthesia will reduce salivary flow as well as the constancy of local blood perfusion.

Two events must occur for drug absorption to take place. These are (a) the drug must partition from the drug delivery device to the epithelium, and (b) the drug must diffuse through the epithelial barrier. The second event is generally the rate-limiting one. Theoretically, on hydration of the gel there will be relaxation of the polymer chains and drug diffusion out of the polymer matrix and into the buccal mucosa, with the hydrophobicity of the buccal mucosa maintaining sink conditions and permitting the egress of drug in the direction of the buccal mucosa. The amount of water available on the buccal mucosa is quite small and both

formulations, on removal, were not seen to be excessively hydrated and showed virtually no change in weight (data not shown).

The buccal permeability barrier consists of the mucus layer on the surface of the buccal epithelium and the upper layers of the buccal epithelium [41]. The intercellular lipids arising from the membrane coating granules have been implicated as being responsible for this epithelial intercellular permeability barrier [41]. However, while the transport of high molecular weight molecules across this permeability barrier is severely restricted [41], the buccal route does allow delivery of low molecular weight drugs such as glyceryl trinitrate and isosorbide dinitrate [42]. The use of amphiphilic [6,8,10] and fatty acid [11] permeation enhancers improves delivery across the buccal mucosa. Despite this, drug fluxes across the buccal mucosa are typically poor, giving peak plasma levels of between 0.0004 and 0.1% of the administered dose [5–7] even in the presence of penetration enhancers [6]. We obtained peak plasma levels of 2.5% of the administered dose (Fig. 6), probably due to the hydrophobicity of denbufylline. Bile salt permeation enhancers such as GDC, used here, appear to act by solubilizing the intercellular lipids or permeating the epithelial membrane [9]. However, GDC must be used with caution as a penetration enhancer since it has been found to cause the formation of vacuoles and the swelling of cells after 4 h of contact with tablets containing this material at a level of 5% w/w [40]. The presence of this surfactant, apart from aiding the penetration of the buccal permeation barrier, would also improve the transport of denbufylline out of the gels/tablets by solubilizing this drug within GDC micelles. Hence, gel hydration would be followed by diffusion of the drug out of the gel and in to the buccal tissue.

Denbufylline was detected in the blood and presumed to originate via the buccal cavity but a note of caution must be sounded, as gastrointestinal delivery cannot be ruled out although the reduced saliva/blood flow would make this less likely. However, what can be concluded from the present studies is that sustained delivery of denbufylline can be achieved with these new GCP gels but not with the CP formulations. Optimization of GCP gels may lead to the development of a new transmucosal delivery system.

Acknowledgements

L.M.M. is in receipt of a BBSRC-GSK CASE Award. The authors acknowledge the expertise of Dr Laurence Tetley, Electron Microscopy Unit, University of Glasgow in the preparation of the images shown in Fig. 5.

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