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Quaternary ammonium palmitoyl glycol chitosan—a new polysoap for drug delivery

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Abstract

A new polysoap, quaternary ammonium palmitoyl glycol chitosan (GCPQ, $M_w = 178{,}000$ g mole^{-1}) with drug solubilising potential has been synthesised and characterised. In solution hydrophobic domains of GCPQ polymeric micelles were identified by the hypsochromic shift in the λ_{max} of methyl orange and by the increase in the ratio of the fluorescence emission intensity of the third and first pyrene vibronic peaks (I_3/I_1). At high aqueous concentrations (> 10 mg ml^{-1}) GCPQ presents as a gel which solubilises pyrene (2.5 mM, normal solubility in water ~ 2 μM) on probe sonication. Dilution of the gel to a liquid solution of polymeric micelles (≤ 3.75 mg ml^{-1} of GCPQ), results in the observation of fluorescent pyrene excimers (excited dimers) and a high excimer to monomer fluorescence ratio (I_E/I_M). However, attempts to solubilise pyrene at a concentration of 2.5 mM in a liquid solution of GCPQ (3.75 mg ml^{-1}) results in a reduced I_E/I_M value and pyrene precipitation. Viscometry measurements show a more compact conformation for the polymer solubilising pyrene than the polymer alone. The polymer is non-haemolytic when present as the liquid solution and relatively non cytotoxic. In conclusion, a new biocompatible polysoap (potential drug solubiliser) is described which forms hydrophobic domains in solution and shows hysteresis in its solubilisation of pyrene.

Keywords: Chitosan; Polysoap; Pyrene; Quaternary ammonium palmitoyl glycol chitosan; Solubilisation; Viscometry

1. Introduction

Soluble polymers bearing pendant amphiphilic or hydrophobic groups, commonly known as polysoaps have been studied for a number of years and numerous applications proposed (Laschewsky, 1995) based on exploiting their solubilisation capacity for hydrophobic molecules (Yang and Engberts, 1992; Anton and Laschewsky, 1994). These compounds form intramolecular micelles (Yang and Engberts, 1991; Cochin et al., 1992) usually several per molecule

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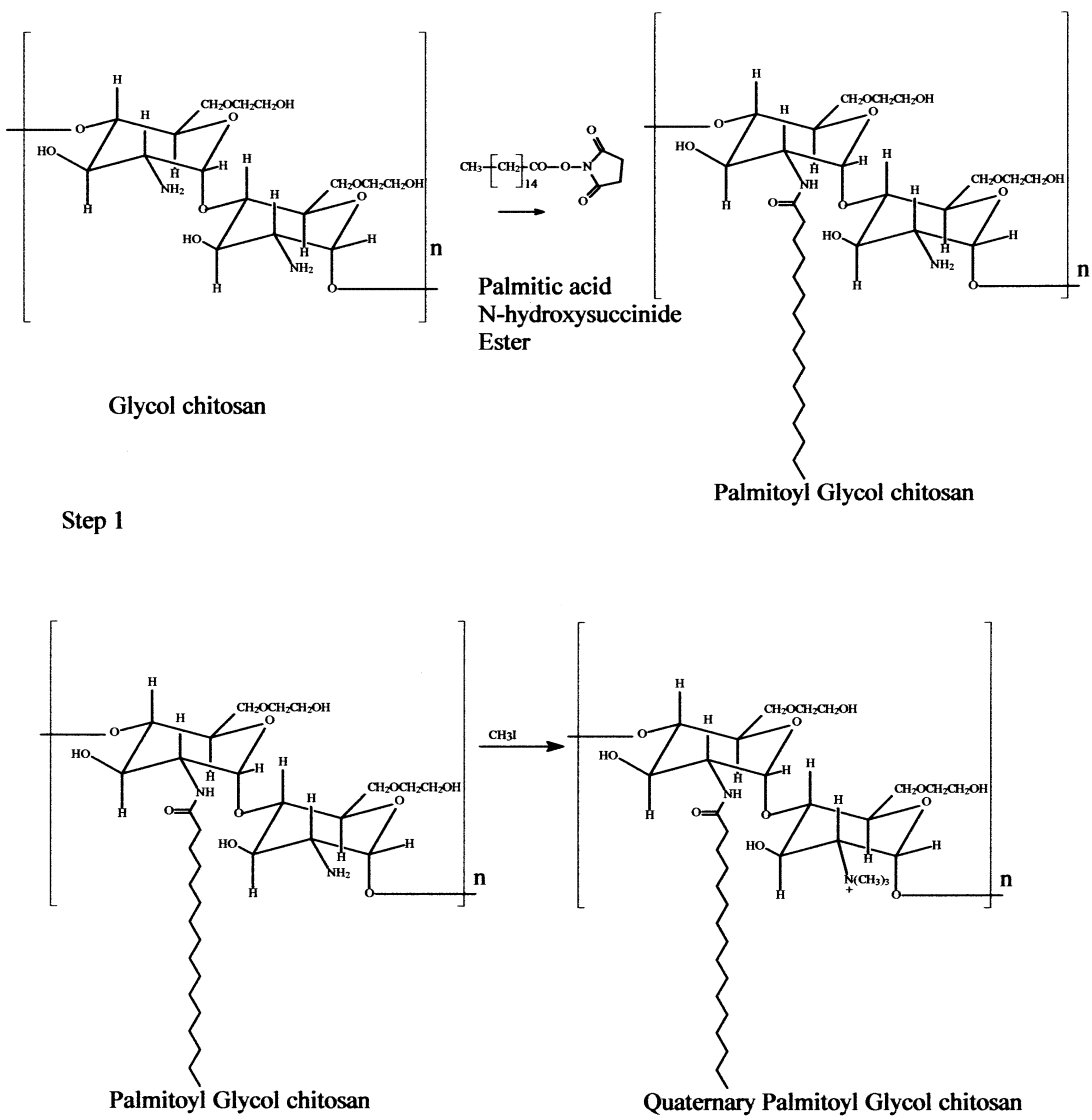
(Binanalimbele and Zana, 1990) and their solubilisation capacity is not lost on dilution (Laschewsky, 1995) unlike small molecular weight micelles (Florence and Attwood, 1998) making them especially useful as solubilisers. Polymeric micelles, prepared from block co-polymers have been used to improve the efficacy of drugs (Kataoka et al., 1993; Batrakova et al., 1996; Inoue et al., 1998) and although solubilising polysoaps have been prepared from *N*-lauryl 6-carboxymethyl chitosan (Miwa et al., 1998), *N*-acyl 6 sulphated chitosans (Yoshioka et al., 1995) and alkylated poly(L-lysine citramide) (Gautier et al., 1999), very few biomedical applications for polysoaps appear in the literature. The present study reports the unusual solubilisation behaviour with a new type of chitosan-based polysoap—quaternary ammonium palmitoyl glycol chitosan (GCPQ) (Scheme 1). This compound, which may be used as a drug solubiliser, is found to form



Scheme 1. The synthesis of quaternary ammonium palmitoyl glycol chitosan.

hydrophobic domains in solution and show hysteresis in its solubilisation behaviour.

2. Materials and methods

2.1. Materials

Glycol chitosan, palmitic acid *N*-hydroxysuccinimide, sodium iodide, sodium bicarbonate, methyl iodide, *N*-methyl-2-pyrrolidone, PBS tablets, cholesterol, pyrene and deuterated solvents were all supplied by Sigma-Aldrich Company, UK. Amberlite IRA-93 was obtained from Merck Co, UK.

2.2. Synthesis of GCPQ

The synthesis of palmitoyl glycol chitosan was carried out as described earlier (Noble et al., 1999). Briefly glycol chitosan (500 mg) and sodium bicarbonate (376 mg) were dissolved in a mixture of absolute ethanol (24 ml) and water (76 ml). To this glycol chitosan solution was added drop wise a solution of palmitic acid *N*-hydroxysuccinimide (792 mg) dissolved in absolute ethanol (150 ml), with continuous stirring over a period of 1 h. The mixture was then stirred for 72 h and the product isolated by evaporating off most of the ethanol and extracting the remaining aqueous phase with diethyl ether (3×100 ml). The aqueous mixture of the polymer was exhaustively dialysed against water (5 l) with six changes over a 24 h period and the resultant product freeze-dried to give a white cotton-like solid.

Quaternisation was carried out using essentially the same method as reported by Domard and others (Domard et al., 1986). Briefly palmitoyl glycol chitosan (300 mg) was dispersed in *N*-methyl-2-pyrrolidone (25 ml) overnight for 12 h at room temperature. Sodium hydroxide (40 mg), methyl iodide (1.0 g) and sodium iodide (45 mg) were added and the reaction stirred under a stream of nitrogen at 36 °C for 3 h.

The quaternary ammonium product was recovered by precipitation with diethyl ether, filtered and washed with copious amounts of absolute ethanol followed by copious amounts of diethyl ether to give a brown hygroscopic solid. The solid was dissolved in water (100 ml) to give a yellow viscous solution. The resultant aqueous solution was exhaustively dialysed against water (5 l) with six changes over a 24 h period and the product freeze-dried to give a white cotton-like solid which was present as the iodide salt The quaternary ammonium iodide was then dissolved in water (150 ml) to give a clear solution and the solution passed through a column (1×6 cm) packed with Amberlite IRA-93 Cl^{-1}. The column was packed with one volume of the resin (30 ml) and subsequently washed with hydrochloric acid solution (90 ml, 1 M) followed by distilled water (500 ml) to give a neutral pH. The clear eluate from the column was freeze-dried to give GCPQ as a transparent fibrous solid.

2.3. ^1H NMR Studies

^1H NMR and ^1H correlation spectroscopy experiments (Bruker AMX 400 MHz spectrometer, Bruker Instruments, UK) were performed on GCPQ solutions in CD_3OD and on pyrene-polymer solutions in either D_2O or CD_3OD prepared as described above. Solutions were clarified by centrifugation ($1000 \times g$) if necessary. Pyrene and polymer spectra were recorded under the same conditions. ^{13}C NMR experiments were also performed on GCPQ solutions in CD_3OD on the same instrument. $^1H-^{13}C$ heteronuclear multiple bond coherence experiments were also carried out on solutions of GCPQ in CD_3OD in order to assign peaks. The level of palmitoylation was calculated by comparing the ratio of palmitoyl methyl protons ($\delta = 0.89$ ppm) to sugar protons ($\delta = 3.5$–4.5 ppm) and the level of quaternisation calculated by comparing the ratio of quaternary ammonium ($\delta = 3.45$ ppm) to sugar protons.

2.4. Light scattering

The molecular weight of GCPQ was determined by multi-angle laser light scattering and gel permeation chromatography (GPC/MALLS, MiniDawn, Wyatt, USA, equipped with a 20 mV semiconductor diode laser, vertically polarised, $\lambda = 690$ nm). Fractionation was performed over

one Styragel HR 4E (Waters, exclusion limit for polystyrene = 50–1,000,000) and one Styragel HR SE (Waters, exclusion limit for polystyrene = 2000–40,000,000) column. The mobile phase was dimethylsulphoxide. About 200 μl samples were injected at a loading concentration of 1 mg ml^{-1} using a Waters 717 plus autosampler and all determinations were carried out at room temperature.

Refractive index increments (dn/dc) of GCPQ solutions in dimethylsulphoxide were measured with a Waters 2410 refractive index detector (λ = 850 nm) and data was processed using DNDC for Windows 5.10 software.

2.5. Fluorescence spectroscopy

A solution containing GCPQ (15 mg ml^{-1}) and pyrene (2.5 mM) was prepared in water by probe sonication (2×2 min, Soniprep Instruments, UK). The resulting viscous solution (resembling a gel) was diluted (3.75 mg ml^{-1}), filtered (0.45 μm), the filtrates scanned (350–600 nm) on excitation at 340 nm and the fluorescence emission recorded (Perkin Elmer LS50-B). A dilute solution of GCPQ (3.75 mg ml^{-1}) and pyrene (2.5 mM) was also prepared, undissolved pyrene was filtered out (0.45 μm) and the filtrate scanned fluorometrically as described above. Various dilutions from these mother solutions were prepared and also scanned as described above. The fluorescence intensity of the excimer (474 nm) and monomer (394 nm) peaks were recorded and the excimer to monomer ratio (I_E/I_M) computed.

A dilute aqueous solution of pyrene (2 μM) was prepared by initially dissolving pyrene in ethanol (0.4 mg ml^{-1}). About 100 μl of this solution was pipetted into a volumetric flask (l00 ml) and the ethanol dried under a stream of nitrogen gas. The solution was then made up in distilled water. Using the aqueous pyrene solution as the solvent, polymer solutions were made at the high level (15 mg ml^{-1}) by probe sonication and diluted to 3.75 mg ml^{-1}. Again using the aqueous pyrene solution as the diluent, polymer solutions were also made at the level of (3.75 mg ml^{-1}) by probe sonication without dilution. The fluorescence emission spectra were recorded (340–600 nm) at an excitation wavelength of 335 nm for the pyrene polymer samples from above as well as dilutions there from. All dilutions were performed using the aqueous pyrene solution as the diluent and the aqueous pyrene solution also served as a control. The I_3/I_1 ratio was calculated from the intensity of the third (383 nm) and first (375 nm) vibronic peaks in the pyrene emission spectra (Kalyanasundaram and Thomas, 1977). In this second set of fluorescence experiments, the pyrene concentration was kept low in order to exclude complications due to the formation of excimers.

2.6. UV-visible spectrophotometry

A solution of methyl orange (25 μM) was prepared in borate buffer (0.02 M, pH 9.4) and used as a diluent in preparing a high concentration of GCPQ (15 mg ml^{-1}) by probe sonication. This solution was subsequently diluted with the aqueous methyl orange solution to 3.75 mg ml^{-1}. The aqueous methyl orange solution was also used to prepare a low concentration of the polymer (3.75 mg ml^{-1}) by probe sonication. This solution was undiluted. Polymer solutions (3.75 mg ml^{-1}) were incubated for 1 h at 25 °C before their absorption were recorded (350–600 nm). The aqueous methyl orange solution served as a control.

2.7. Viscometric measurements

A solution containing GCPQ (15 mg ml^{-1}) and pyrene (2.5 mM) was prepared in water by probe sonication. The resulting gel was diluted to a polymer concentration of 3.75 mg ml^{-1} and filtered (0.45 μm). A solution of the polymer was also prepared containing GCPQ (3.75 mg ml^{-1}) and pyrene (2.5 mM). The efflux times of these solutions (and dilutions there from) through a capillary viscometer were measured in a thermostatted (24.8 ± 0.05 °C) water bath and the efflux time of pure filtered water measured as a control. Reduced viscosity was calculated using the equation

$$\frac{\eta_{rel} - 1}{c} = \eta_{sp}$$

where $\eta_{rel} = t_1/t_0$ and t_1 = the efflux time of the polymer solution and t_0 = the efflux time of water at the same temperature. The mean of five determinations was used to compute the relative viscosity value and the experiment was performed thrice. To ensure that the filtration of the polymer caused no actual loss of material prior to viscometric measurements, a solution of the GCPQ (2 ml, 3.75 mg ml^{-1}) was filtered (0.45 μm), freeze dried and the amount of GCPQ solid weighed. An unfiltered sample was also freeze dried and the remaining solid weighed as a control. The loss of material to the filtration step was thus calculated.

2.8. Haemocompatibility

Fresh human blood (~ 5 ml) was centrifuged ($1000 \times g$ for 10 min). The erythrocyte pellet was isolated, washed twice with phosphate buffered saline (PBS, pH 7.4) at 4 °C by resuspending the pellet in PBS (pH 7.4) followed by centrifugation ($1000 \times g$ for 10 min). The pellet was weighed and a 3% w/w dispersion of the erythrocytes prepared in PBS (pH 7.4). Various concentrations of the polymer solution (100 μl) were added to the erythrocyte suspension (100 μl) in 96 well microtitre plates. PBS and Triton X-100 (1% v/v) served as negative and positive controls, respectively. After incubation for 4 h, the microtitre plate was centrifuged ($1000 \times g$ for 10 min), the supernatant (100 μl) transferred to a new microtitre plate and the absorbance measured at 570 nm. The haemolysis given by the triton-X solution was considered to be 100% while the haemolysis given for the PBS solution was taken as 0%. The results were thus expressed as percentage haemolysis.

2.9. Cytotoxcicity

A human epidermoid carcinoma cell line (A431, ATCC CR L-1555) was maintained in Dulbecco's minimum essential medium (DMEM) supplemented with 10% foetal calf serum (FCS) and 2 mM glutamine (GibcoBRL, UK) at 10% CO_2 and 37 °C.

The cytotoxicity was assessed by the measurement of the IC50 value in a standard MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide thiazolyl blue-indicator dye) assay (Freshney, 1994). Briefly, 96-well microtitre plates were seeded with 700 cells per well and incubated for 72 h. A solution of the polymer (10 mg ml^{-1}) was prepared in water as described above and dilutions of the polymer (100 μl) in tissue culture medium (Opti-Mem) were incubated with the cells for 4 h.

The samples were then replaced with fresh DMEM daily and incubated for 72 h. After this period the indicator dye (50 μl, 50 mg ml^{-1}) was added to each well and incubated with the cells for 4 h in the dark. The medium and indicator dye were then removed and the cells lysed with dimethylsulphoxide (200 μl). After addition of Sorensen's glycine buffer (25 μl), the absorption was measured at 570 nm. Values were expressed as a percentage of the control to which no polymer was added.

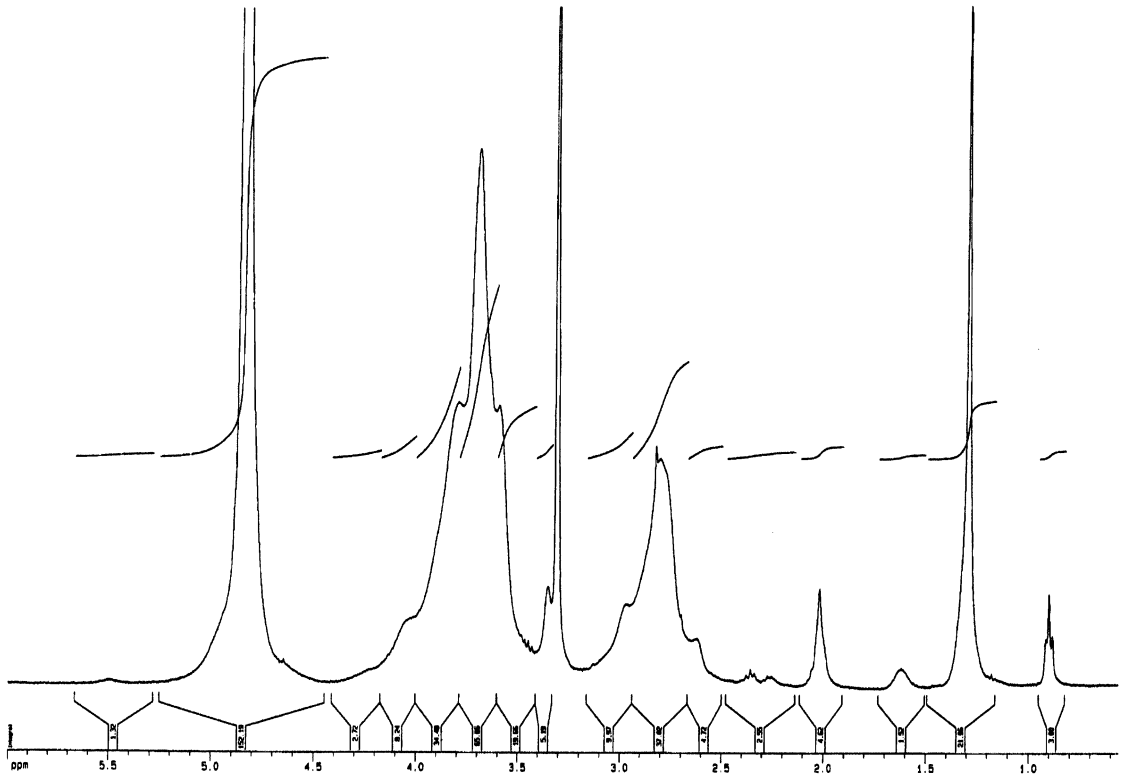
3. Results

3.1. Synthesis and characterisation of GCPQ

The yield of palmitoyl glycol chitosan was 332 mg (41%) while the yield of GCPQ was 250 mg (74%). The synthesis of quaternary ammonium palmitoyl glycol chitosan was confirmed by NMR (Fig. 1). Proton assignments, $\delta_{0.89} = CH_3$ (palmitoyl), $\delta_{1.29} = CH_2$ (palmitoyl), $\delta_{1.52} = CH_2$ (palmitoyl deshielded by carbonyl), $\delta_{2.02} = CH_3$ (acetyl-glycol chitosan), $\delta_{2.25-2.40} = CH_2$ (adjacent to carbonyl protons), $\delta_{2.50-3.10} = CH_3$ (dimethylamino-glycol chitosan), $\delta_{3.45} = CH_3$ (trimethylamino-glycol chitosan), $\delta_{3.60-4.3}$ (sugar protons), $\delta_{3.31}$ = methanol protons, $\delta_{4.4}$ = water protons.

Carbon assignments, $\delta_{14.59} = CH_3$ (palmitoyl), $\delta_{23.38} = CH_3$ (acetyl-glycol chitosan), $\delta_{23.89}$, $\delta_{30.95}$ and $\delta_{33.50} = CH_2$ (palmitoyl), $\delta_{42.87} = CH_3$ (dimethylamino-glycol chitosan), $\delta_{54.5}$ CH_3 (trimethylamino-glycol chitosan), $\delta_{58.5}$ = C2 (glycol chitosan), $\delta_{62.37}$, $\delta_{71.53}$, $\delta_{73.83}$, $\delta_{74.25} = CH_2$ (C6 on glycol chitosan), $\delta_{70.07}$, $\delta_{76.25}$, $\delta_{80.05}$ = C3, C4 and C5 (glycol chitosan), $\delta_{98.50}$ = Cl (glycol chitosan).

The level of palmitoylation, calculated from the ^1H NMR spectrum was determined as 5.9 mole%



(a)

Fig. 1. (a) ^{1}H NMR of GCPQ in CD_3OD. (b) ^{13}C NMR of GCPQ in CD_3OD.

and the level of quaternisation was calculated as 4.0 mole%. The dn/dc of GCPQ was 0.12 ml g^{-1}, the weight average molecular weight (M_w) of GCPQ was 176 800 g $mole^{-1}$ and the polydispersity (M_w/M_n) was 3.702. A high polydispersity is recorded due to the randomness of acylation by the palmitic acid ester and alkylation by methyl iodide. Additionally the possibility of chain cleavage during the alkaline conditions of the alkylation reaction can not be ruled out.

3.2. UV-visible spectroscopy

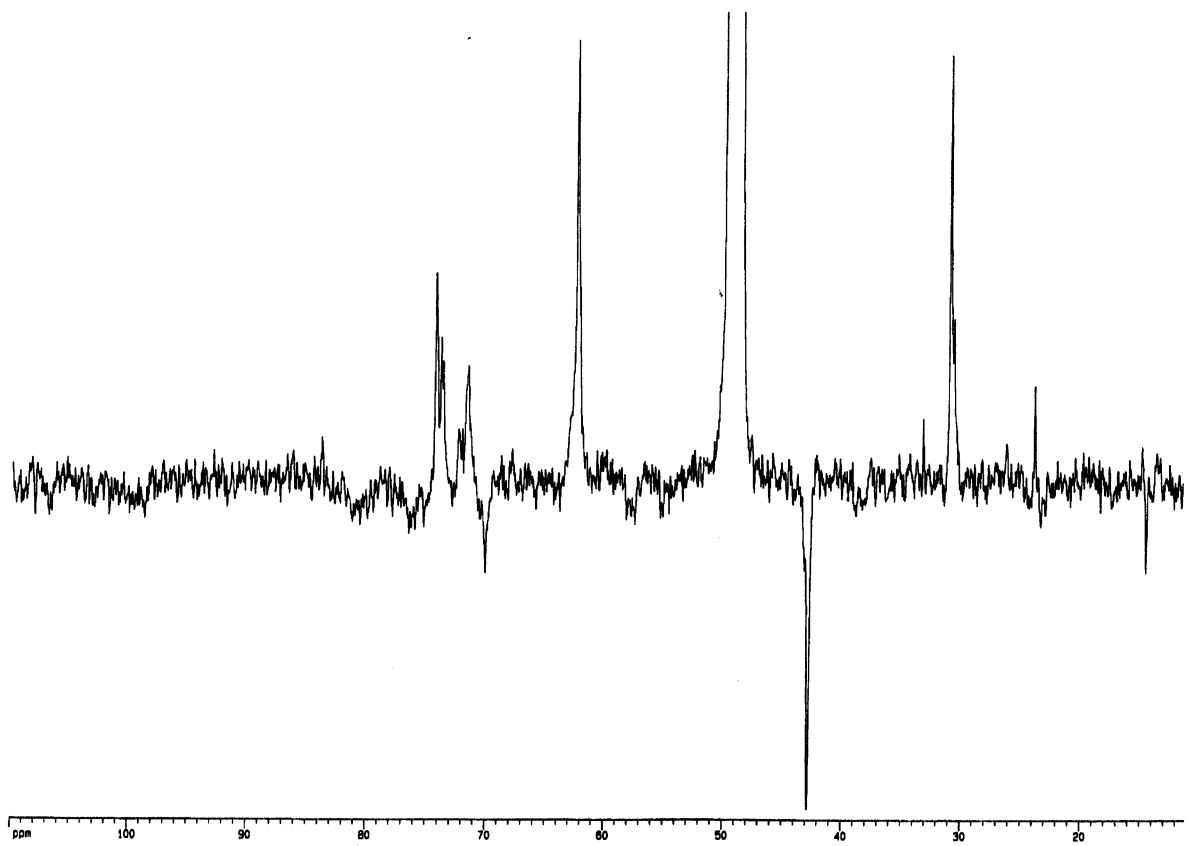
The λ_{max} (wavelength of maximum absorbance) of methyl orange is known to undergo a hypsochromic shift on being solubilised within the hydrophobic core of micelles (Lieske and Jaeger, 1999). The shift from 465 to 427 nm in the λ_{max} of methyl orange is observed in Fig. 2 when a methyl orange solution (25 μM, pH 9.5) is used to solubilise the polymer at a concentration of 3.75 mg ml^{-1} thus confirming the presence of hydrophobic domains within the solution state of the polymer. The methyl orange probe is unable to detect any differences in the λ_{max} when GCPQ was diluted from the gel (15 mg ml^{-1}) or prepared as a liquid solution (3.75 mg ml^{-1}) although the absorbance intensity was higher in the former case, indicating a higher degree of solubilisation of methyl orange within GCPQ micelles diluted from the higher concentration of 15 mg ml^{-1}.

3.3. Fluorescence spectroscopy

GCPQ forms a gel at high aqueous concentration (15 mg ml^{-1}, Fig. 3). The dissolution of pyrene within this gel at the level of 2.5 mM results in the observation of excimers on a 1:4

dilution of the gel to a liquid solution (Figs. 3 and 4). Attempts to dissolve the same level of pyrene in a liquid solution of GCPQ (3.75 mg ml^{-1}) results in the observation of pyrene crystals and a reduced excimer emission (Fig. 4). GCPQ dilutions arising from the gel show a fall in the I_E/I_M value, whereas dilutions arising from the GCPQ liquid solution formulation show a low and unchanged I_E/I_M ratio (Fig. 4). The I_E/I_M values for the dilutions arising from the initial gel do not attain the low levels shown by the dilutions arising from the initial solution. This demonstrates a differential in the solubilising capacity of the gel and liquid solution formulations of GCPQ and a persistence in the higher solubilising capacity of the gel formulation even on dilution to a liquid solution.

The I_3/I_1 value for pyrene (2 μm) solubilised in water is 0.71, a value close to that of 0.64 reported earlier (Kalyanasundaram and Thomas, 1977). The increasing intensity of the third vibronic peak (I_3) in relationship to the first vibronic peak (I_1) is experienced by pyrene on encountering a more hydrophobic local environment leading to an increase in the I_3/I_1 value (Kalyanasundaram and Thomas, 1977). The I_3/I_1 ratio for pyrene when solubilised by the polymer is shown to increase relative to that shown by the fluorophore in water (Fig. 5, Table 1), evidence of pyrene being solubilised within the hydrophobic domains of the polymeric micelle system. This high hydrophobicity persists over a concentration difference of two orders of magnitude showing that these solubilising hydrophobic domains persist on dilution of GCPQ.



(b)

Fig. 1.

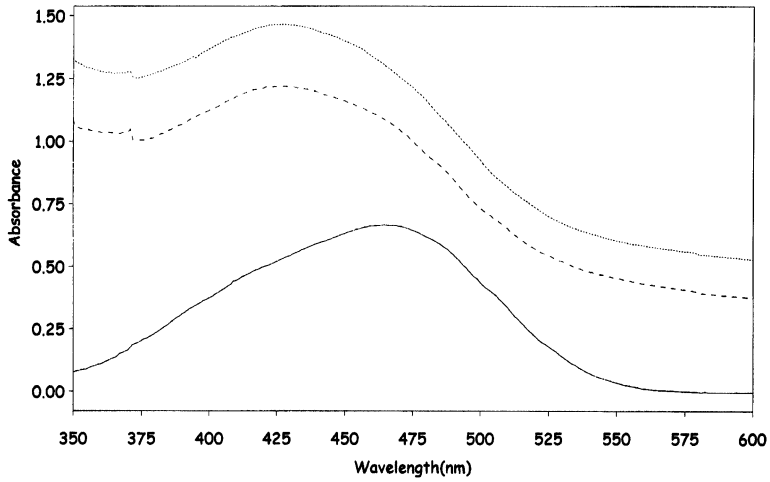


Fig. 2. The absorption of Methyl orange (25 μM) in the presence of GCPQ: (—) = methyl orange in water, (----) = methyl orange + GCPQ (3.75 mg ml^{-1}) undiluted, (· · · ·) = methyl orange + GCPQ (3.75 mg ml^{-1}) diluted from 15 mg ml^{-1}.

3.4. ^1H NMR

^1H NMR data reveal an absence of the pyrene aromatic peaks at $\delta_{8.00-8.22}$ when GCPQ solubilises pyrene in D_2O (Fig. 6a), despite the detection of fluorescent excimer emissions for these samples. A ^1H NMR scan of the same mixture dissolved in CD_3OD is shown as a control (Fig. 6b). The absence of pyrene peaks in the aqueous solution is indicative of the fact that pyrene is solubilised within GCPQ apolar domains and hence inaccessible to the aqueous solvent.

3.5. Viscometric measurements

The loss of GCPQ on filtration of GCPQ solution was determined as 1.38% w/w, a negligible loss. Viscometry data points to GCPQ adopting a more compact conformation in solution once it solubilises pyrene compared with the polymer alone (Fig. 7), evidence that the pyrene molecules promote association of the hydrophobic chains and thus cause a relative contraction of the GCPQ polymer coil.

3.6. Haemacompatibility

The liquid solution formulation of the polymer is non-haemolytic (Fig. 8) and we hypothesise that although the polymer is a soluble amphiphile, the high molecular weight of the polymer prevents the effective partitioning into and hence solubilisation of the erythrocyte membranes. Haemolysis is seen when concentrated solutions (gel-like) are applied to the erythrocytes, however, presumably due to the high osmotic stress caused by the gel. Application of the gel formulation to the erythrocytes is a situation unlikely to be encountered in vivo.

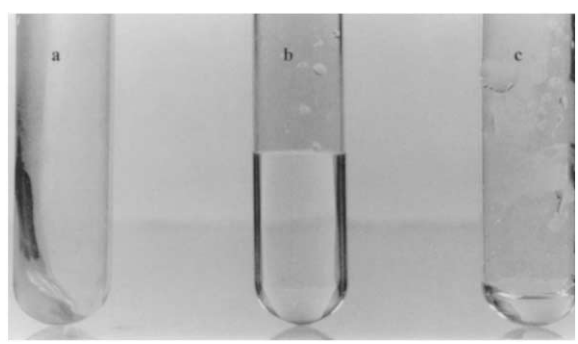


Fig. 3. GCPQ gel (15 mg ml^{-1}, sample a), solution diluted from gel (3.75 mg ml^{-1}, sample b), undiluted solution (3.75 mg ml^{-1}, sample c).

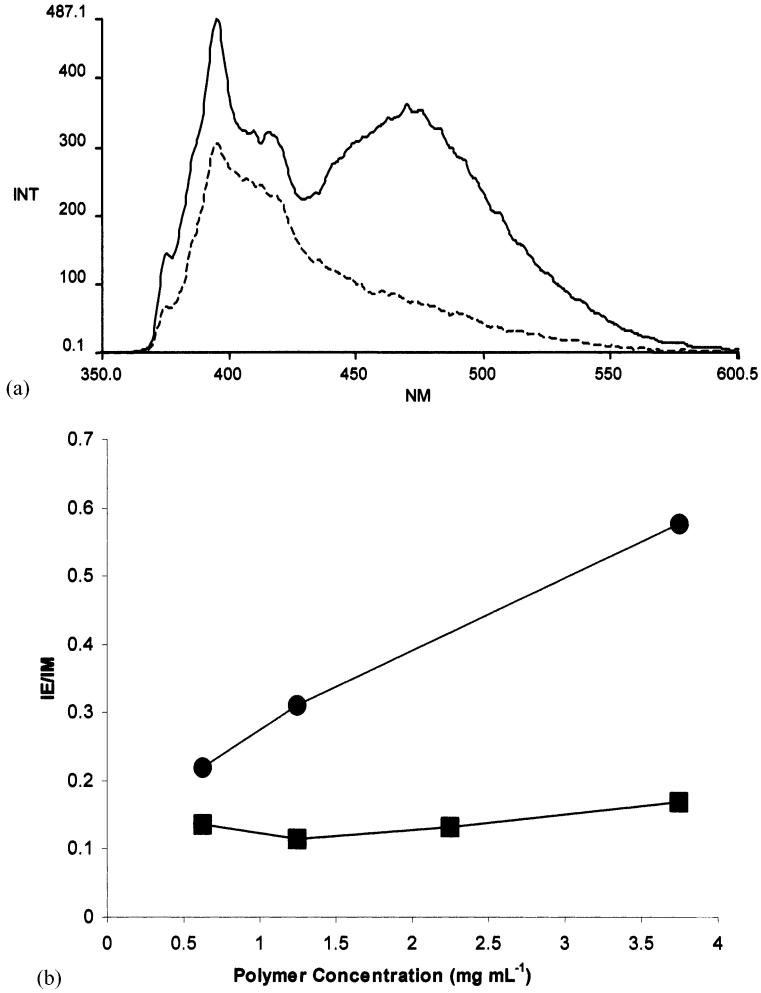


Fig. 4. (a) Fluorescence spectroscopy emission scan on pyrene (Excitation 340 nm, initial concentration 2.5 mM): (—) = pyrene solubilised in 15 mg ml^{-1} GCPQ which was diluted to 3.75 mg ml^{-1}, and (----) = pyrene solubilised in 3.75 mg ml^{-1} GCPQ which was scanned undiluted. (b) The ratio of pyrene (initial concentration 2.5 mM, excitation = 340 nm) excimer emission ($\lambda = 474$ nm) to monomer emission ($\lambda = 375$ nm) when solubilised in GCPQ: ● = GCPQ diluted from an initial polymer concentration of 15 mg ml^{-1}, $n = 3$, ■ = GCPQ diluted from an initial polymer concentration of 3.75 mg ml^{-1}, $n = 1$. All solutions were filtered (0.45 μm) before analysis.

3.7. Cell cytotoxicity

The IC50 for the polymer against the A431 cell line was found to be 1.28 mg ml^{-1} (Fig. 9), which compares favourably with that reported by the cationic polymers poly-L-lysine (0.007 mg ml^{-1}) and poly-L-ornithine (0.004 mg ml^{-1}) in the same cell line (Brown et al., 2000).

4. Discussion

Polysoaps are water-soluble amphiphilic polymers with hydrophobic pendant side chains, which aggregate in solution (Wang and Engberts, 1994; Damas et al., 1995; Kramer et al., 1996) forming either a necklace of intrachain spherical micelles (Binanalimbele and Zana, 1990; Borisov

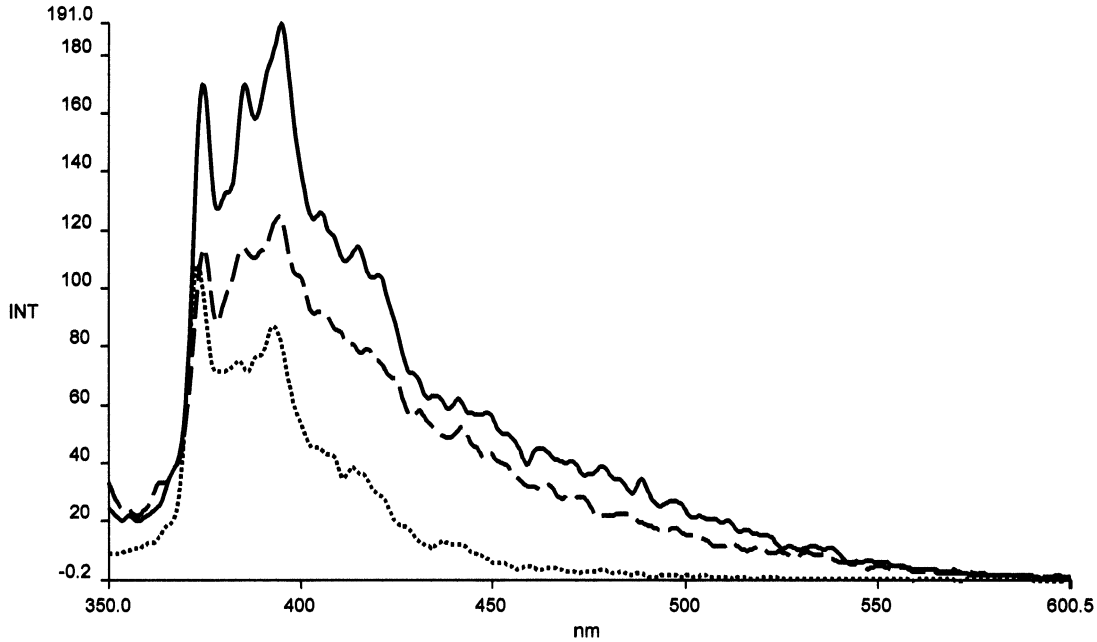


Fig. 5. Fluorescence spectroscopy emission scan on pyrene (Excitation 340 nm, aqueous concentration 2 μM): (·····) = pyrene solubilised in water, (— —) = pyrene solubilised in 3.75 mg ml^{-1} GCPQ which was scanned undiluted, (—) = pyrene solubilised in 15 mg ml^{-1} GCPQ which was diluted to 3.75 mg ml^{-1}.

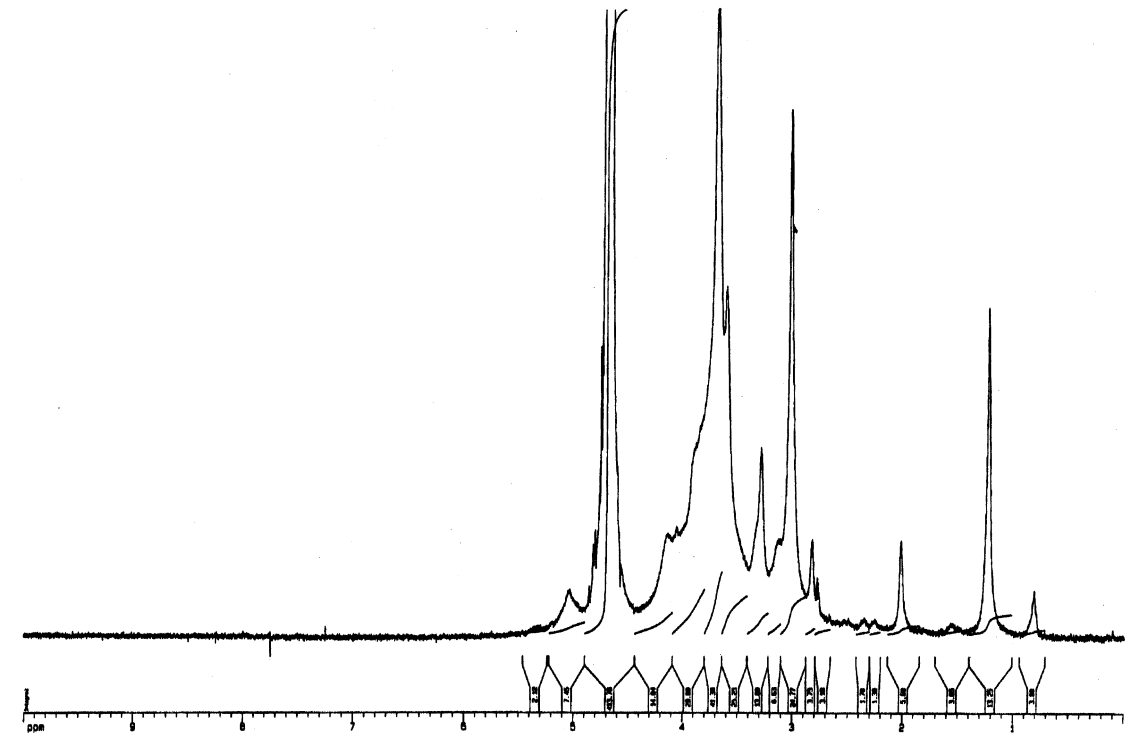
and Halperin, 1996, 1997) or larger cylindrical micelles Turner and Joanny, 1993). Aggregation behaviour is promoted by an increased level of hydrophobic modification (Yang and Engberts, 1991; Mccormick et al., 1992; Wang and Engberts, 1994, 1995a,b; Damas et al., 1995; Kramer et al., 1996; Wang and Engberts, 1996) and an increase in hydrophobic group chain length (Mccormick and Chang, 1994; Benjelloun et al., 1996; Wang and Engberts, 1996) with a minimum length of eight carbons being necessary for an aggregating polymer and a chain length in excess of 18 carbons decreasing aggregation due to a decreased flexibility of the molecule (Laschewsky, 1995). GCPQ with a hydrophobic group chain length of 16 carbons behaves like a polysoap in solution when solubilising pyrene (Figs. 4 and 5) and methyl orange (Fig. 2) with a consequent contraction of the polymer coil in solution (Fig. 7). The contraction of the polymer is evidence of its flexibility. It is believed that GCPQ attains the gel state at high concentration due to the interchain hydrophobic associations as shown schematically in Fig. 10a.

Polysoaps exhibit a comparatively low viscosity in solution due to intramicellar aggregation (Cochin et al., 1995; Laschewsky, 1995; Wang and Engberts, 1995a,b). However, at high concentration the presence of hydrophobic units on soluble acrylamide (Biggs et al., 1992), poly(alkylmethyldiallylammonium chlorides) (Wang and Engberts, 1995a,b) and polyethylene glycol (Preuschen et al., 1999) polymers increases the viscosity due to

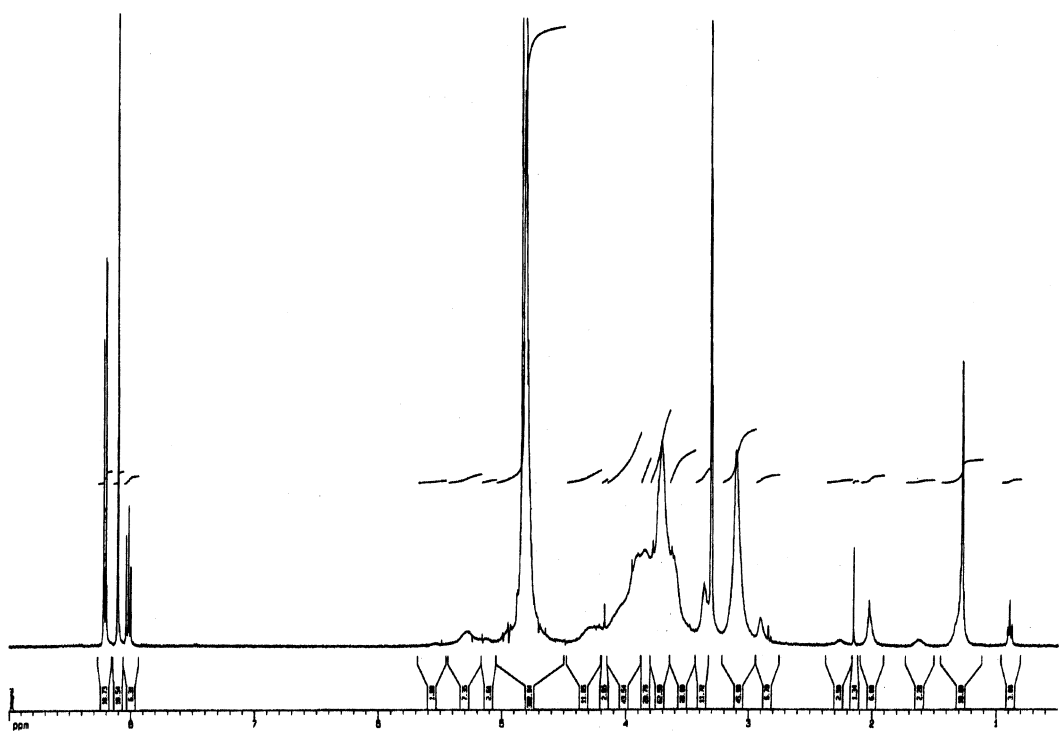
Table 1
I_3/I_1 values obtained from the emission spectra of pyrene solutions (2 μM) in the presence of GCPQ

Polymer concentration	GCPQ (initial concentration = 15 mg ml^{-1})	GCPQ (initial concentration = 3.75 mg ml^{-1})
0	0.71	0.71
3.75	0.97	0.99
2.75	0.96	1.00
1.75	0.91	0.95
0.0875	0.92	0.88

Mean of two values.



(a)



(b)

Fig. 6. (a) ^{1}H NMR of GCPQ (8 mg ml^{-1}) and pyrene (5 mM) in D20. (b) ^{1}H NMR of GCPQ (8 mg ml^{-1}) and pyrene (5 mM) in CD3OD.

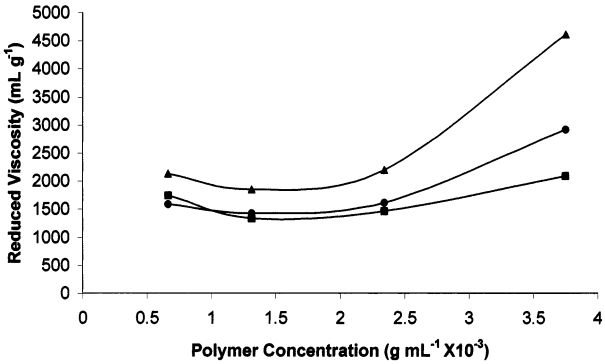


Fig. 7. Reduced viscosity of polymer-pyrene and polymer solutions, ▲ = GCPQ alone diluted from an initial concentration of 3.75 mg ml^{-1}, ● = GCPQ-pyrene diluted from an original polymer concentration of 15 mg ml^{-1} and a pyrene concentration of 2.5 mM, ■ = GCPQ-pyrene diluted from a polymer concentration of 3.75 mg ml^{-1} and a pyrene concentration of 2.5 mM. All samples were filtered (0.45 μm) before analysis.

inter chain interactions and the gel state is sometimes attained (Preuschen et al., 1999). A similar gelation is demonstrated by GCPQ (Fig. 3).

On incorporation of pyrene into GCPQ gels, pyrene is solubilised in hydrophobic domains of the intrachain micelles. On dilution, this micellar conformation is retained and the hydrophobic fluorophore remains solubilised. In effect a stable polymeric micellar aggregate is formed when the hydrophobic fluorophore is introduced into the gel formulation of GCPQ. In the case of methyl orange a higher amount of methyl orange is also solubilised when the polymer is diluted from the gel state. The dilution of gels formed from fluorocarbon amphiphilic polymers also results in the retention of the micellar aggregates (Preuschen et al., 1999). Attempts to solubilise pyrene in the liquid solution formulation of GCPQ result in a decreased solubilisation of pyrene and hence a decreased I_E/I_M, although polymeric micelles are still present when the polymer solution is prepared from the liquid solution state since hydrophobic domains are seen whether the polymer solution is prepared from the gel or the liquid solution state.

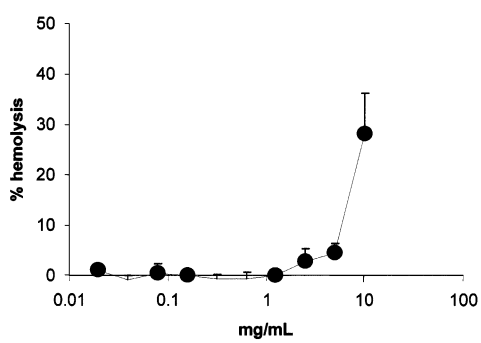


Fig. 8. Haemocompatibility of GCPQ relative to triton-X 100.

The interesting thing about this polysoap is the observation that the solubilisation of the apolar fluorophore pyrene in the gel state (Figs. 4 and 8) results in the continued high solubilisation of pyrene on dilution to the liquid solution state. Whereas attempts to solubilise the apolar fluorophore in a dilute solution of GCPQ result not only in pyrene not being solubilised, but also in a comparatively decreased I_E/I_M ratio when compared with the solution diluted from the gel. GCPQ thus appears to remember its solubilisation history. To our knowledge this hysteresis has not been reported earlier. The only report of a hysteresis loop with polysoaps is that shown by hydrophobically modified 6-carboxypullulan which when adsorbed at low pH (pH 2) onto polystyrene films fails to completely desorb when the pH is raised to pH 6, whereas at an initial pH 6, there is virtually no adsorption of these polymers onto polystyrene films (Paris and Stuart, 1999). This hysteresis is more pronounced as the level of hydrophobic modification increases (Paris and Stuart, 1999).

Polysoaps appear to lack a definite CMC (Anton and Laschewsky, 1994) and their solubilisation behaviour (Anton and Laschewsky, 1994) may be exploited for drug delivery. Solubilised material is stable against extreme dilution whereas apolar compounds precipitate out of small molecular weight micelles on dilution (Anton and Laschewsky, 1994; Florence and Attwood, 1998). Generally, the solubilisation capacity of polysoaps may be increased by increasing either hydrophobic load (Anton and Laschewsky, 1994), although above a critical level the reverse is seen as micelles become too compact to be effective solubilisers (Kramer et al., 1996) or alkyl chain length

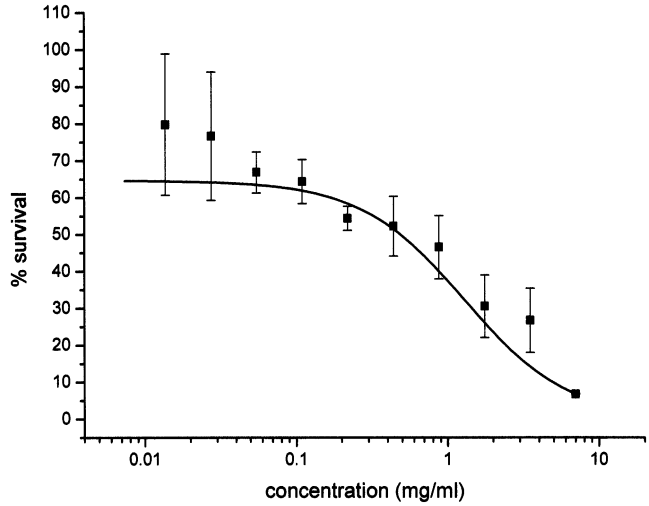


Fig. 9. Cytotoxicity of GCPQ against the A431 cell line.

(Laschewsky, 1995). Solubilisation of a hydrophobic drug within the gel state of GCPQ will result in no loss of hydrophobic load on dilution in vivo.

Polysoap geometry has been classified as either 'head-type' when the hydrophilic head group is located on the main chain or 'tail-type' when the hydrophilic head group is located at the terminal end of the hydrophobic pendant group (Anton et al., 1993; Laschewsky and Zerbe, 1991). The tail end hydrophilic head group geometry in vinylic polymers (Anton et al., 1993; Laschewsky and Zerbe, 1991) is more conducive to the formation of a soluble polymer with polysoap behaviour. However, the incorporation of a main chain spacer in head type polymers improves water solubility (Koberle et al., 1992; Anton and Laschewsky, 1993) and it is thought that for intrachain micellisation to occur in head type polysoaps, in particular, a minimum level of main chain space between amphiphilic groups must exist to prevent crowding of the hydrophobic groups as the aggregates are formed (Borisov and Halperin, 1995). GCPQ according to this classification is a 'head type' polysoap and the low density of hydrophobically modified glycol chitosan units (5.9 mole%) has the effect of a main chain spacer decreasing the crowding of hydrophobic units along the main chain. Chitosan based soluble amphiphiles have been prepared from sulphated *N*-acylated chitosans with C2–C14 pendant groups (Yoshioka et al., 1995) although the gel form reported here was not been reported for these sulphated acyl chitosans. Other chitosan based amphiphiles such as palmitoyl glycol chitosan (Uchegbu et al., 1998) and deoxycholyl chitosan (Lee et al., 1998) have been found to assemble into polymeric vesicles of 500 nm in diameter and aggregate into solid particles of l60 nm in diameter, respectively.

The high molecular weight of the polymer is believed to be responsible for the lack of haemolytic activity and significant cytotoxicity of GCPQ and although polysoaps are known to interact with membranes (Yang et al., 1998), the non-destruction of the erythrocytes is an advantage for biomedical applications.

5. Summary

GCPQ, a new chitosan based polysoap, is able to solubilise pyrene in high local concentrations within apolar domains. Solubilisation is much more efficient in the gel state than in the liquid solution state and the polysoap shows hysteresis in its solubilisation profile, appearing to remember its solubilisation history. The solubilisation of

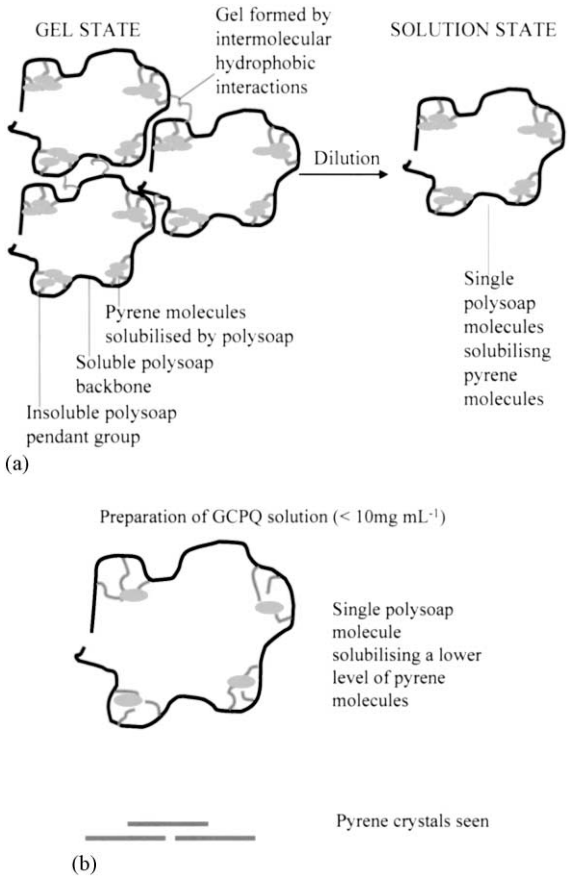


Fig. 10. (a) Schematic representation of the solution properties of the GCPQ polysoap gel formulation. (b) Schematic representation of the solution properties of the GCPQ polysoap liquid solution formulation.

pyrene contracts the polymer coil in solution and on dilution of the gel the hydrophobic compound remains solubilised in the micellar aggregates. Although GCPQ is a soluble amphiphile, it is non-haemolytic when present as a liquid solution and it is believed that this unique physico-chemistry and good haemocompatibility make this polymer suitable for the solubilisation of apolar material for biomedical applications.

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