Journal Article

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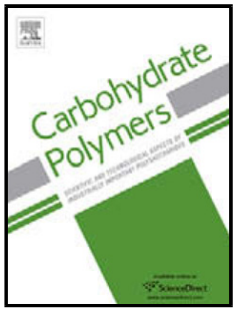

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Synthesis of cationic alkylated chitosans and an investigation of their rheological properties and interaction with anionic surfactant

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Abstract

Two methods were used to alkylate high M_W chitosan with glycidyltrimethylammonium chloride (GTAC) in order to produce chitosan derivatives that are water-soluble throughout the pH range. In addition, a novel chitosan derivative was created by alkylating one of the products with the GTAC analogue Quab 342 containing C12 alkyl chains. The phase behaviour and rheological characteristics of the chitosan derivatives were studied in the presence of anionic surfactant. The derivatives were found to form soluble complexes at low and high SDS concentrations and the Quab 342 derivative was able to form gels.

Keywords: chitosan, quaternisation, rheology, anionic surfactant interactions

1. Introduction

Chitin (poly β-(1→4)-*N*-acetyl-D-glucosamine) is found in arthropod shells (Mao et al., 2017) and the cell walls of yeasts and fungi. It is the second most abundant natural polysaccharide after cellulose (Dutta et al., 2004), and it is in increasing demand as a raw material for many sophisticated applications in medicine, agriculture and other areas (Dutta et al., 2004), (Xia et al., 2011), (Pillai et al., 2009), (Hayes et al., 2008b), (Kumar et al., 2004). Chitin's desirable properties include biocompatibility, biodegradability to normal body constituents, safety, non-toxicity, binding to mammalian and microbial cells, and antimicrobial activity against bacteria and fungi (Bellich et al., 2016), (Sahariah and Másson, 2017). These properties are shared by its acid-soluble derivative chitosan, which is prepared by removing at least 50% of the N-acetyl groups, and also by a wide variety of chemical derivatives.

Chitin is generally extracted from marine sources, such as shrimp shells and other shellfish by-products, although there is also interest in fungal and insect chitin (Sajomsang and Gonil, 2010). The extraction process (reviewed by Hayes et al. (2008a) and Younes and Rinaudo (2015)) consists of demineralisation, deproteination, decolourisation, and in the case of chitosan, deacetylation. It generally involves strong acids and alkali, and may be extended to depolymerise the chitosan if low M_W products are desired (Mohammed et al., 2013). Alternatively a specific depolymerisation step may be added, such as ultrasound or enzyme hydrolysis (Lodhi et al., 2014).

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Chemically, chitosan is a linear polyamine, basic, carrying reactive amino and hydroxyl groups, capable of chelating transition metal ions, and soluble in water below pH 6.5. Its hydroxyl and amino groups can be acylated or alkylated, which is very useful, because its uses under physiological conditions are limited by the fact that it precipitates when the pH is raised above 6.5 (e.g. (Snyman et al., 2002), (Lim and Hudson, 2004) (Tungtong et al., 2012)). This problem can be solved by adding polar or charged groups to the polysaccharide backbone. Hydrophobic groups such as dodecyl moieties are also sometimes added to make chitosan soluble in organic solvents (Mourya and Inamdar, 2008) , or enable it to bind to plastics as a biodegradable component (Kumar et al., 2004). Chemical derivatives of chitosan have been comprehensively reviewed by Mourya and Inamdar (Mourya and Inamdar, 2008) while Sahariah and Másson discuss their antibacterial activity (Sahariah and Másson, 2017).

One potentially extremely useful modification is to convert the 2-amino group into a quaternary amine (Mourya and Inamdar, 2008), (Sahariah and Másson, 2017). The quaternary amine remains charged throughout the pH range and if the degree of substitution (D.S.) is high enough it can render even high M_W chitosans completely water-soluble. The simplest quaternised chitosan is N,N,N-trimethyl chitosan, synthesised by reductive alkylation (Guo et al., 2007), which has very promising antifungal (Snyman et al., 2002) and antibacterial activity (Sahariah and Másson, 2017). However, because the reductive methylation synthesis requires iodomethane and N-methyl pyrrolidine as a solvent, an alternative reaction which can be carried out in aqueous solution is often preferred. Glycidyl trimethylammonium chloride (GTAC) alkylates the amino groups via its epoxide ring and it already carries a quaternary amine group. The GTAC alkylation is well studied, and typically carried out under neutral conditions at temperatures of 70°C – 100°C (Kim et al., 2003), (Lim and Hudson, 2004), (Nam et al., 1999) and (Ruihua et al., 2012), and the resulting quaternised chitosan also has antimicrobial activity (Sahariah and Másson, 2017), (Kim et al., 2003), (Lim and Hudson, 2004) and (Nam et al., 1999).

With its wide solubility range, quaternised chitosan has obvious potential applications in a broad range of commercial products, including pharmaceuticals, neutraceuticals, cosmetics and personal care products. As Dutta *et al* point out, chitosan can form a clear elastic skin on hair (which is negatively charged), and it can form gels in aqueous alcohol solvents (many types of cosmetics, skincare products and pharmeuticals are applied as gels) and furthermore, high M_W chitosans do not pass through the skin barrier (Dutta et al., 2004). If quaternised chitosans share all these chitosan traits, they would be desirable components for these formulations. In the case of shampoos it would also be desirable for the chitosans to have foaming and emulsifying properties, either by themselves or when combined with surfactants in a formulation.

This study was undertaken to synthesise quaternised chitosans with high D.S. using GTAC. Two synthetic methods were attempted. 1) Heterogeneous GTAC alkylation at high pH to alkylate both the amino and hydroxyl groups on the chitosan backbone. Our first hypothesis was that at high pH, the 3– and 6– hydroxyl groups may be alkylated as well, increasing the D.S. and the charge density by up to three times. 2) Homogeneous GTAC alkylation in dilute perchloric acid by Ruihua's method (Ruihua et al., 2012). In addition, a second alkylating agent was tested: Quab 342, a GTAC analogue which carries a dodecyl chain in place of one of the quaternary amine's methyl groups.The second hypothesis was that this quaternised chitosan derivative (with hydrophobic groups in addition to the positively charged substituents) would have enhanced rheological characteristics due to intermolecular hydrophobic interaction and that the interactions could be enhanced by the presence of anionic surfactants. Hydrophobically associating polymers, which are predominately non-ionic or anionic, are finding increasing application in commercial

formulations in many industrial sectors and since the formulations invariably include surfactants a knowledge of the polymer-surfactant interactions is important (Williams, 2003), (Goddard and Ananthapadmanabhan, 1998), (Langevin, 2009).

2. Materials and Methods

2.1. Materials

High molecular weight chitosans Chitopharm ^{TM} S (S#2265, 17% Degree of Acetylation, DA) and Chitopharm ^{TM} L (L#2272, 16% DA) were supplied by Chitinor AS, Norway. Quab 342 (3-chloro-, 2-hydroxypropyl-*N,N,N*-dimethyllaurylammonium chloride) was a gift from Croda Ltd UK. Glycidyl trimethylammonium chloride (GTAC) was obtained from Sigma Aldrich; sodium dodecyl sulphate and all other chemicals were from Sigma-Aldrich or Fisher.

2.2. Alkylation of chitosan with Quab reagents

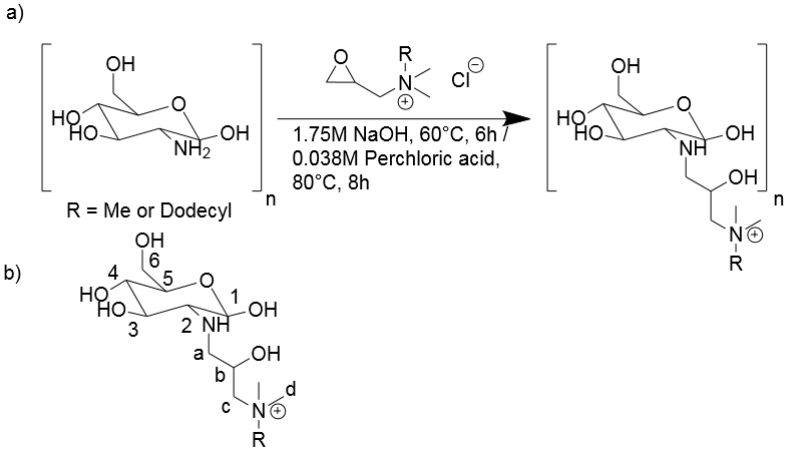


Figure 1: a) Reaction of GTAC or Quab 342 with chitosan monomer either by the high pH method: 1.75M NaOH, 60°C, 6h or Ruihua method: 0.038M perchloric acid, 80°C, 8h.b) Location of protons on the GTAC- or Quab 342- alkylated glucosamine monomer (R = Methyl, R = Dodecyl, respectively).

For the high pH GTAC alkylation reaction, the following method was used: 20g of high molecular weight chitosan S#2265 was suspended in 400g deionised water under mechanical stirring. 35 g sodium hydroxide pellets were dissolved in 100g distilled water, which was then added dropwise to the chitosan slurry. The vessel was then purged with inert nitrogen gas and the temperature raised to 60 °C. 12.08g of GTAC was added via a pressure equalising funnel over 20 minutes at 0, 2 and 4 hours. At 6 hours the sample was allowed to cool, and neutralised with 32% HCl. The sample (G-2265) was subsequently washed with isopropanol.

To produce the G- and GQ-chitosan samples (see Table 2), the method of Ruihua et al (Ruihua et al., 2012) was employed. 5g of chitosan L#2272 was suspended in 750ml ultrapure water, and dissolved by dropwise addition of 4.75ml perchloric acid, with stirring. The sample was then heated to 60°C, with mechanical stirring. 12.5g of GTAC was added at 0, 30 and 60 minutes, then the temperature was raised to 80°C and the reaction continued for 8 hours. For the

G-chitosan, the product was then extracted by precipitation in acetone. For the GQ-chitosan, the pH was raised to 11.2 with 1M NaOH and the alkylation procedure was repeated, using Quab 342 reagent in place of GTAC. The reactions are shown in Figure 1.

2.3. Characterisation of derivatised chitosans

FT-IR spectra of chitosans and chitosan derivatives were measured by the KBr disc method on a Perkin Elmer Spectrum RX1 FT-IR Spectrophotometer. Proton NMR spectra were recorded in D_2O on a Bruker Spectrophotometer at 400MHz, 298.2K, 256 scans and the fid files were analysed in MestReNova 9.0 software for Windows. Noise was removed by apodisation along t1 (Exponential 0.3 and Gaussian 5.0) and background correction by Whitaker Smoother. Phase correction was applied as necessary. Peaks were integrated manually and normalised to the chitosan N-acetyl peak at 1.94 ppm. The degree of acetylation (DA%) was calculated from the areas of the N-acetyl group and the combined areas of H_2 and H_{3-6} in equation 1.

$$\frac{\delta H_{NAc}/3}{\delta H_{2-6}/6} = DA\% \tag{1}$$

The degree of substitution of GTAC (DG%) for the G-chitosan was calculated from the areas of the N-acetyl protons and the single methine proton (b) on the 2-hydroxypropyl moiety in equation 2.

$$\frac{\delta H_b}{\delta H_{NAc}/3} * DA\% = DG\% \tag{2}$$

The degree of substitution of Quab 342 (DQ%) for the GQ-chitosan was calculated from the areas of the N-acetyl protons and the methylene protons of the dodecyl chain in equation 3.

$$\frac{\delta H_{methylene}/18}{\delta H_{NAc}/3} * DA\% = DQ\% \tag{3}$$

The degree of substitution of GTAC (DG%) for the GQ-chitosan calculated from the areas of the N-acetyl protons and the single methine proton (b) on the GTAC 2-hydroxypropyl moiety, with the methine proton of the Quab 342 2-hydroxypropyl group substracted (equation 4). (It was assumed to be the equivalent of $1/18_{th}$ of the $\delta H_{methylene}$ signal.)

$$\frac{\delta H_b - (\delta H_{methylene}/18)}{\delta H_{NAc}/3} * DA\% = DG\% \tag{4}$$

2.4. Molecular mass determination

The molar mass of the chitosan samples was determined using Gel Permeation Chromoatography (GPC). The system consisted of a TSK G5000 PWxL and TSK G6000 PWxL column connected in series, with a TSK G3000 PWxL guard cartridge, equipped with an Optilab DSP interferometric refractometer and a Dawn EOS enhanced Multi Angle Laser Light Scattering detector (Wyatt Technology, Santa Barbara). The elution buffer was 0.1M sodium acetate, adjusted to pH4.8 with 0.2M acetic acid, and the flow rate was 0.5ml/min. Chitosan samples were dissolved in 1% acetic acid (10mg/ml) and diluted in 1:1 0.1M sodium acetate / 0.2M acetic acid. M_W values were calculated in Astra 4.9 software using a Debye model using first order polynomial results fitting (measured dn/dc value of 0.151)(Mohammed et al., 2013).

The Degree of Polymerisation (DP) was calculated for the S#2265, L#2272, G_2265 and G-chitosans from the chitosan M_W divided by the monomer masses of N-acetylglucosamine (203

Da), glucosamine (161 Da), and GTAC-labelled glucosamine (277 Da), multiplied by their respective monomer percentages DA%, DD% and DG%.

$$DA\% * 203 + DD\% * 161 + DG\% * 277 = M_W(Monomer) \quad (5)$$

$$\frac{M_W}{M_W(Monomer)} = DP \quad (6)$$

2.5. Rheology

The steady shear viscosities and storage and loss moduli (G' and G") were measured on a TA Advanced Rheometer AR2000 (TA Instruments, New Castle, DE), using standard sized recessed end concentric cylinders (for dilute solutions) and 6 cm diameter 2° stainless steel cone and plate geometry (for gels). Viscosity measurements included a 3 minute preconditioning step at 25°C, followed by a single measurement at 1 s^{-1} or a stepped flow measurement from 0.01 - 1000 s^{-1}. Mechanical spectra were recorded from 0.1 - 100 Hz at 25°C, 10% strain, which was determined to be in the linear viscoelastic region by performing a strain sweep. Solution pH was adjusted to 3 using 1M HCl, or to 10 using 1M NaOH.

2.6. Interaction with anionic surfactant

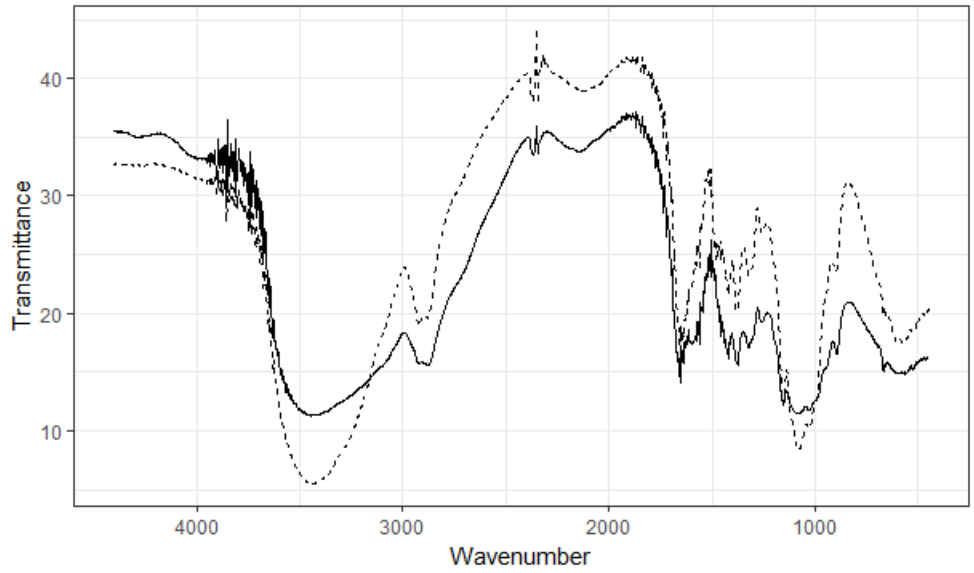
Aqueous solutions of G-Chitosan and GQ-Chitosan at pH∼6.5 were added to aqueous SDS solutions to obtain a range of concentrations from 0.02% to 1% chitosan and 0.1mM to 350mM SDS. The phase behaviour of the mixtures were observed visually (solution, gel or precipitate), and viscosities, G' and G" were subsequently measured at 1 s^{-1} and 1 Hz, 10% strain if the mixture formed a solution or a gel.

3. Results

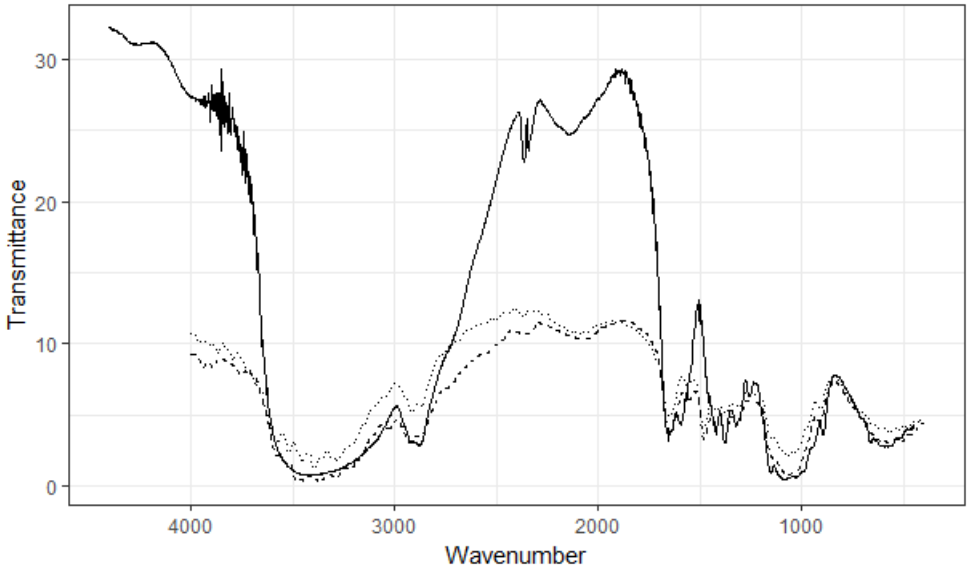
3.1. Synthesis of G-2265, G- and GQ-chitosan

The initial GTAC alkylations tested a method designed for the synthesis of N,N,N-trimethyl-3-amino-2-hydroxypropyl glucosamine, i.e. for the derivatisation of the monosaccharide on the high M_W polysaccharide. The protocol is similar to established methods applied to chitosan oligosaccharides (Kim et al., 2003), and polysaccharides (Nam et al., 1999) and (Lim and Hudson, 2004)), except that sodium hydroxide was used to deprotonate the hydroxyl groups and a nitrogen atmosphere was used to prevent oxidation. This method produced products (e.g. G-2265) which were soluble in acid solution but not in water, in contrast to the neutral GTAC alkylations in the reports listed above. They were observed to precipitate when the pH was raised above 6.5, as was the case of the unmodified chitosans. By contrast, the method of Ruihua *et al* (Ruihua et al., 2012) yielded derivatised chitosan samples (G- and GQ-chitosan) which were soluble at all pH values tested, from pH3 - 11.This suggested that the Ruihua method had produced chitosans with a degree of substitution (DG%) higher than the solubility threshold, but that the alkaline method failed in this regard. This is probably due to the fact that the chitosan substrates were soluble in the reaction medium, as opposed to the high pH alkylation method, where they were merely dispersed.

Figures 2(a) and 2(b) show the FT-IR spectra of the unmodified chitosans S#2265 and L#2272 and their GTAC derivatives. The most important peaks in the chitosan IR spectrum are listed by Kasaai (Kim, 2010). The -NH_2 peak at 1590 cm^{-1} is clearly visible in the S#2265 spectrum of Figure 2(a) (black line) but in the spectrum of GTAC-derivatised S#2265 it has

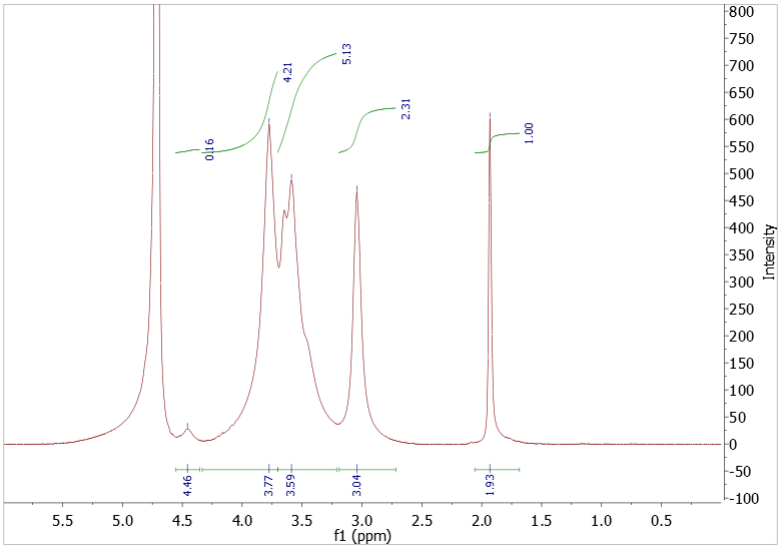


(a)

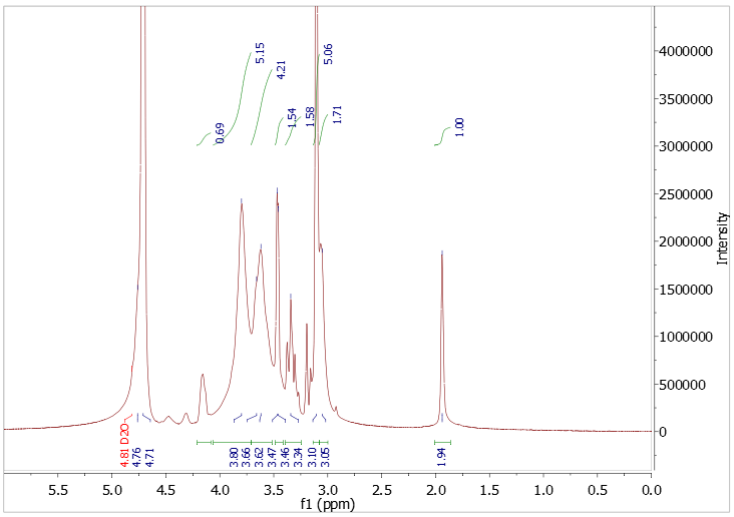


(b)

Figure 2: a) FT-IR spectra of unmodified chitosan S#2265 (solid), and GTAC-modified chitosan S#2265 (G-2265) (dashed). b) FT-IR spectra of unmodified chitosan L#2272 (solid), G-chitosan (dashed) and GQ-chitosan (dotted). The C-H stretch is at 2930 cm^{-1} and the amide I and chitosan NH_2 bands at 1650 and 1590 cm^{-1}, respectively.

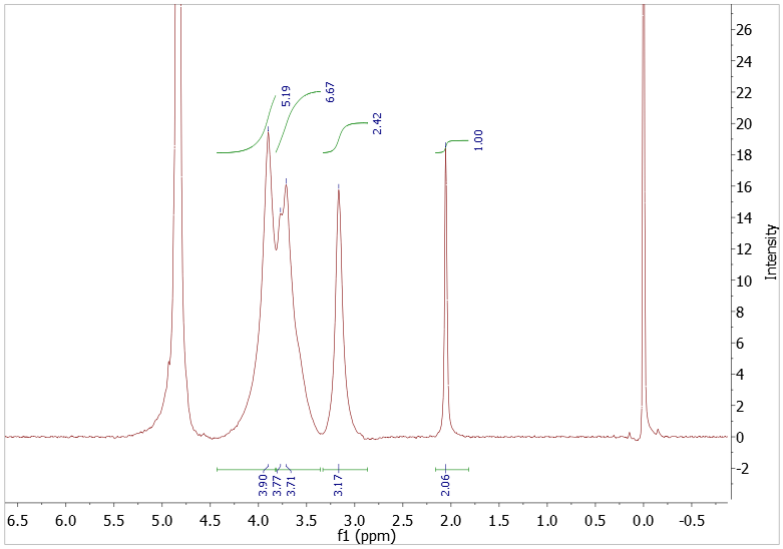


(a) Chitosan S#2265

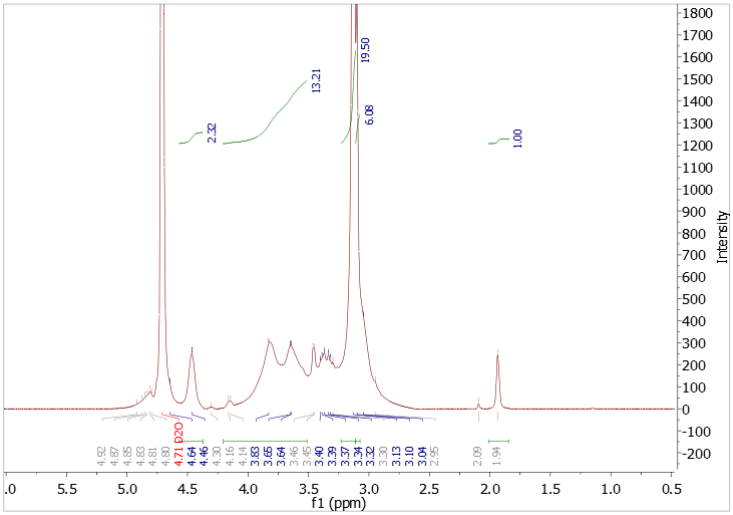


(b) G-2265

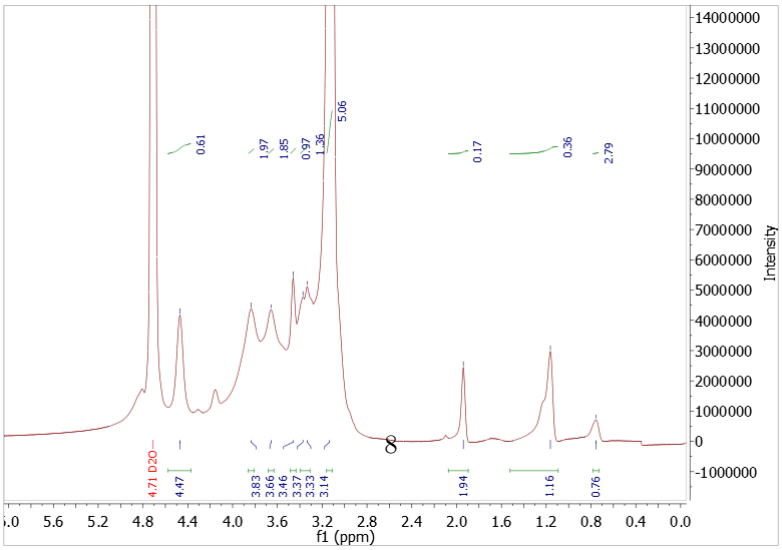
Figure 3: ^{1}H-NMR spectra for (a) unmodified S#2265 and (b) G-2265.



(a) Chitosan L#2272



(b) G-chitosan



(c) GQ-chitosan

Figure 4: ^{1}H-NMR spectra for (a) unmodified L#2272, (b) G-chitosan, and (c) GQ-chitosan

diminished to a shoulder (dashed line). The initial GTAC alkylation method has reduced the amount of -NH_2 detectable, probably by alkylating the 2-amino group. When Ruihua's method was used, the 2-amino peak vanished entirely (Figure 2(b), dashed and dotted lines). Also of interest is the 2930 cm^{-1} C–H stretch, which shows a noticeable increase in the GTAC-modified chitosan in Figure 2(a) (dashed) and the G-chitosan in Figure 2(b) (dashed) due to the presence of the trimethyl-ammonium groups. The GQ-chitosan in Figure 2(b) (dotted line) has a greater peak at 2930 cm^{-1} than the G-chitosan, due to the presence of the dodecyl group. This data suggests that the reason the G- and GQ-chitosans had become soluble at neutral and alkaline pH was that they were more highly substituted with quaternary amine than the G-chitosan S#2265, because the second derivatisation method was more efficient than the first.

NMR spectra are shown in Figures 3 and 4, NMR peaks and peak areas in Table 1 and the structure of the G-chitosan monomer in Figure 1. GTAC δH_b was chosen for the DG% calculations because it was relatively isolated from the chitosan peaks, unlike GTAC δH_d, which occurs between the H_{3-6} complex and H_2 of the glucosamine monomer.

Peaks	ppm	protons	I(G-chitosan)	I(GQ-chitosan)	I(G-2265)
Chitosan δH_{NAc}	1.94	3	1.00	1.00	1.00
Chitosan $\delta H_{2(GluNH)}$	3.1	1	6.08	5.88	1.71
Chitosan $\delta H_{2(GluNAc)}, \delta H_{3-6}$	3.83, 3.65	5	13.21	16.42	9.36
GTAC δH_b	4.46	1	2.32	3.28	0.69
GTAC δH_d	3.13	9	19.5	21.21	5.06
Quab 342 $\delta H_{methylene}$	1.17	18		2.34	

Table 1: ^{1}H-NMR peak intensity (I) used to calculate degrees of substitution for G- and GQ-chitosans.

Table 2 shows the degrees of substitution for the derivatised chitosans, their molecular weights (as calculated from GPC results using the Debye method), and DP values.

Chitosan	DA%	DG%	DQ%	M_W (Da)	Monomer average (Da)	DP
L#2272	16%	N.A.	N.A.	1.70 x10^5	167.7	1013.7
S#2265	17%	N.A.	N.A.	1.68 x10^5	168.1	999.4
G-chitosan	10.4%	72.4%	N.A.	1.69 x10^5	250.1	676
GQ-chitosan	9.0%	85.1%	3.5%		273.8	
G-2265	18.1%	23.0%	N.A.	5.86 x10^4	195.3	300

Table 2: DA%, DG%, DQ% and M_W for chitosans alkylated by the perchloric acid method (G- and GQ-chitosan) and the alkaline method (G-2265). Equations 5 and 6 give the average monomer size and DP.

The NMR data corroborates the solubility data and the FT-IR data. The perchloric acid method has produced higher yields in terms of substituted chitosan monomer : >70% compared to 23%, and the result is a derivatised chitosan polysaccharide soluble throughout the aqueous pH range. The DA% appears to have declined for the G- and GQ-chitosans. It was difficult to determine the relative abundances of H3 and H6 from the NMR spectra, but the fact that DQ% remained low and DG% did not increase above 100% in the GQ-chitosan suggests that few hydroxyl groups were alkylated at high pH. The intensity of the alkylated chitosans increased dramatically, presumably due to their increased solubility.

3.2. Molar Mass

The weight-average molecular weights of the S#2265 and L#2272 chitosans were determined by GPC to be 1.68 x 10^5 Da and 1.70 x 10^5 Da, respectively. The G-chitosan's M_W was found to be 1.69 x 10^5 Da. The average monomer size equation (5) has to take into account the presence of the substituent DG% (72.4% for G-chitosan as shown in Table 2) as well as the DA%. The DP equation (6) gives a figure of 1013.7 monomer units per chain for the unmodified chitosan L#2272, 999.4 for unmodified S#2265, and 676 for the G-chitosan: a significant decline in degree of polymerisation due to GTAC alkylation. The G_2265 chitosan had declined to 5.86 x 10^4 Da. Given an average monomer size of 174.2 $gmol^{-1}$ the DP had declined to 336.4 monomer units per chain. It was not possible to determine the molar mass of GQ-chitosan since it was found to interact with the GPC column substrate.

3.3. Rheology

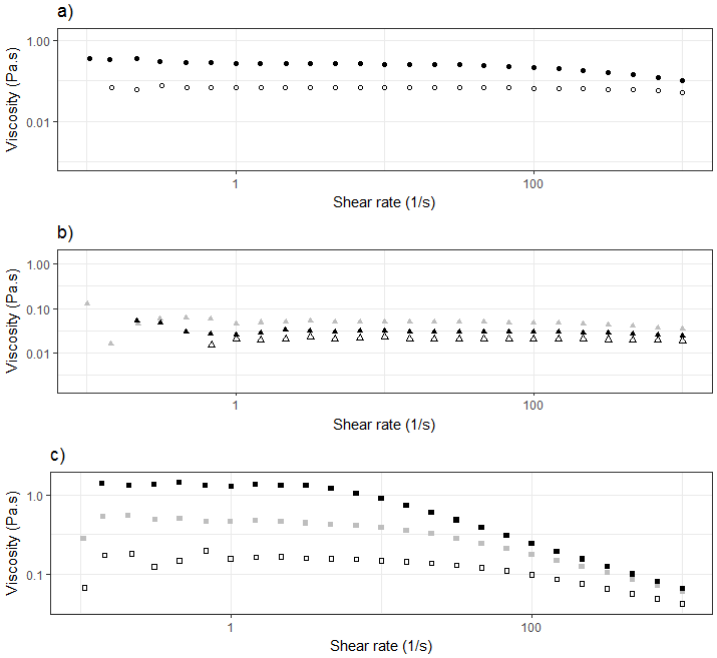


Figure 5: Viscosity v. shear rate for a) L#2272 (closed circles) and S#2265 (open circles); b) G-chitosan (triangles) at pH 3 (white), pH 6 (grey) and pH 10 (black); c) GQ-chitosan (squares) at pH 3 (white), pH 6 (grey) and pH 10 (black).

The steady shear viscosities for 1% solutions of chitosans L#2272 and S#2265 at pH3 (the samples are insoluble at neutral and alkaline pHs) are plotted as a function of shear rate in Figure 5 a) Similar plots for the G-chitosan and the GQ-chitosan at pH 3, 6 and 10 are shown in Figure 5 b) and c). The unmodified chitosans and G-chitosan have very low viscosities and are essentially Newtonian in behaviour.The fact that the viscosity of the G-chitosan is lower than the parent chitosan is due to the fact that it has a lower DP as shown in Table 2. In the case of the GQ-chitosan, the solutions have higher viscosities than the parent chitosans despite that fact that the DP is reduced slightly and exhibit shear thinning as the shear rate

is increased. This is evidence of intermolecular hydrophobic association which gives rise to a weak three-dimensional intermolecular network. Interestingly the viscosity of the GQ-chitosan increases with increasing pH. This may be due to a slight increase in the ionic strength caused through pH adjustment which would inhibit intermolecular electrostatic repulsions and promote intermolecular hydrophobic association.

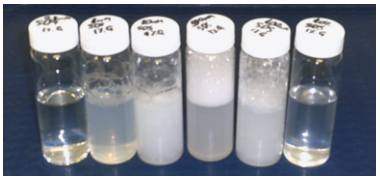
3.4. Interaction with anionic surfactant

The phase behaviour of the highly substituted G- and GQ-chitosans is summarised in Figure 6. For the G-chitosan (Figure 6 a) and c)) solutions containing up to 1% (w/v) G-chitosan and 0.5mM SDS remained as clear solutions. At higher SDS concentrations precipitation occurred and then as the concentration of SDS increased even further (to 2mM to $>$ 100mM depending on the G-chitosan concentration) a clear solution was observed. The GQ-chitosan shows a similar behaviour (Figure 6 b) and d)), but with the presence of a gel phase rather than a precipitate when the GQ-chitosan concentration was above 0.2%.

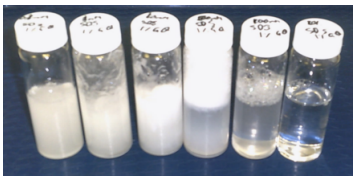
The mechanical spectra for selected samples are presented in Figure 7. Figure 7 a) shows G" values only as a function of frequency for 1% G-chitosan systems in the presence of 0.1 mM and 350mM SDS which were seen to be clear solutions. The values are very low and typical of a low viscosity solution. The G' values were close to zero and are not included in the plot. Figure 7 b) shows G'(filled squares) and G" (open squares) as a function of frequency for 1% GQ-chitosan systems in the absence of SDS, which was a clear solution, and in the presence of 0.1mM, 1mM, 10mM and 350mM SDS which were seen to be a clear solution, gel, gel and clear solution respectively. At 0.1mM SDS (black tiny squares), the system showed weak gel characteristics in that G' was slightly higher than G" but both varied with frequency. At 1mM SDS (blue small squares), the value of G' was two orders of magnitude higher than G" and was independent of frequency thus indicating that the system exhibited the properties of a gel. At 10mM SDS (green medium squares), G' had similar values to the system in the presence of 1mM SDS but G" was significantly higher, about one order of magnitude lower than G'. At very much higher SDS concentration (350mM, red large squares) G" is very low and strongly dependent on frequency and G' is close to zero (not included in the plot) and displays typical behaviour for a dilute polymer solution.

4. Discussion

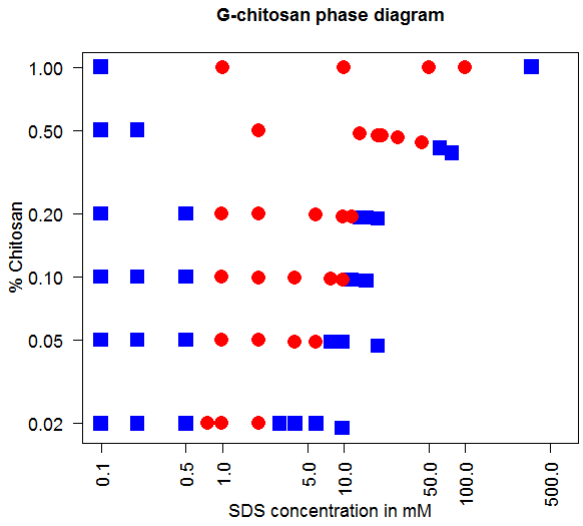
In terms of producing a water-soluble high-molecular weight chitosan, the adaptation of Ruihua's perchloric acid method has been successful. The GTAC alkylation reaction yields are nearly 16 times higher in terms of DS% for the perchloric acid reaction compared to the reaction in alkaline medium. The alkaline method was initially used because of the tendency of GTAC to convert from the epoxide to a relatively inactive chlorhydrin form (3-chloro, 2-hydroxypropyltrimethylammonium chloride) under acid conditions (Goclik et al., 2004). However, the final yield was low (5.4% D.S.). There are several possible reasons. The GTAC epoxide can also react with hydroxyl ions to form the inactive 2,3-dihydroxy product in a side reaction, resulting in potential loss of yield. Also, the chitosan was not dissolved in the reaction medium, but remained in a semi-crystalline form, so that many of the reactive amino groups must have been inaccessible to the GTAC reagent. In the neutral GTAC alkylations reported in the literature (Kim et al., 2003), (Nam et al., 1999), (Lim and Hudson, 2004), the solid chitosan was suspended in a medium less than 2 pH points from the amino pKa (c. 5.6). A sufficient minority



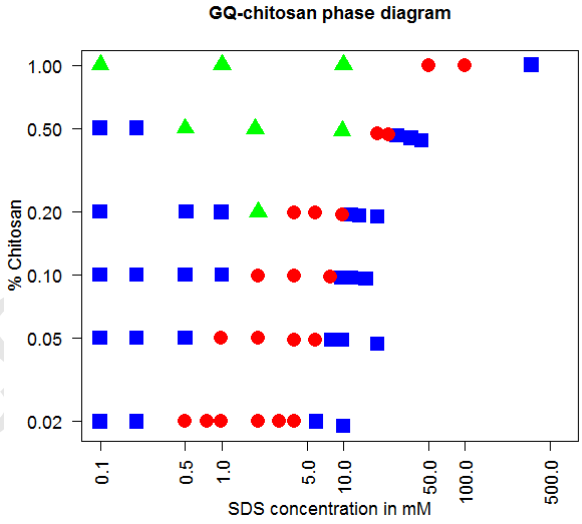
(a) 1% G-Chitosan with increasing SDS



(b) 1% GQ-Chitosan with increasing SDS



(c) G-chitosan plotted against SDS



(d) GQ-chitosan plotted against SDS

Figure 6: a) - b) Effect of SDS concentration on solubility of G- and GQ-chitosans (1% in water). From left to right: 0.1, 1, 10, 50, 100 and 350mM SDS. c) - d) Plot of phase behaviour of G- and GQ-chitosans in the presence of SDS surfactant. Blue squares = solution, red circles = precipitate, green triangles = gel.

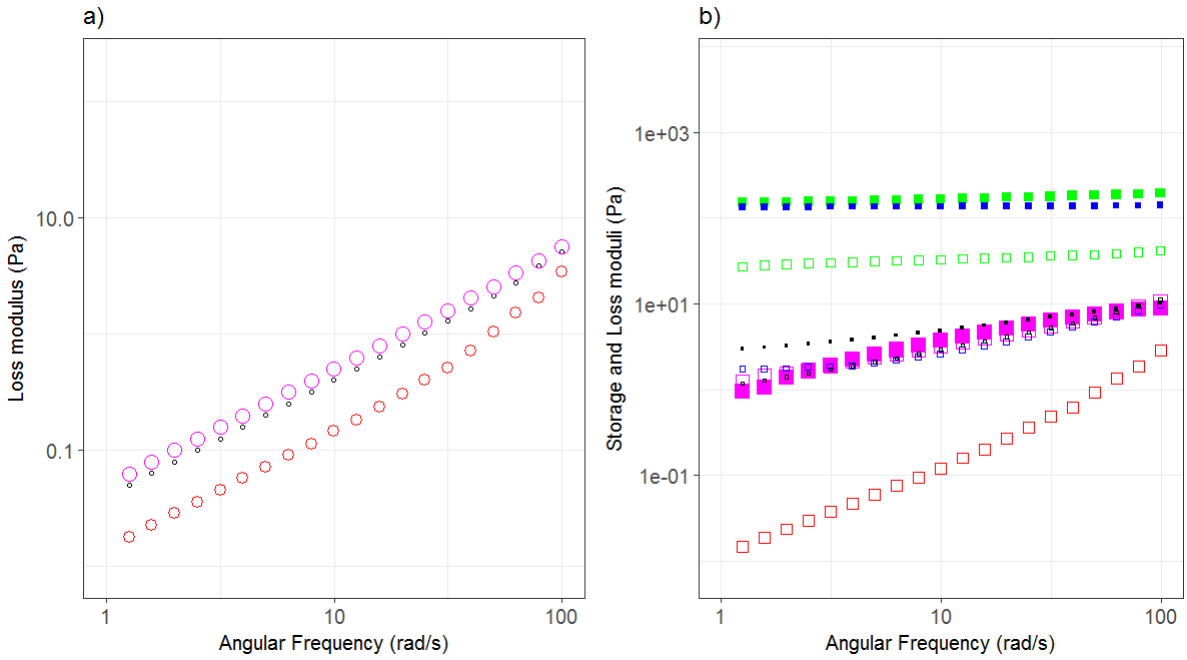


Figure 7: Frequency spectra with G' (filled) and G" (open) for G-chitosan (circles) and GQ-chitosan (squares) in the presence of 0mM (magenta, largest), 0.1mM (black, smallest), 1mM (blue, small), 10mM (green, large) and 350mM SDS (red, larger). a) 1% G-chitosan, SDS 0mM, 0.1mM and 350mM. b) 1% GQ-chitosan, SDS 0mM, 0.1mM, 1mM, 10mM and 350mM.

of glucosamine residues must have been protonated, enough to solvate the surrounding chains and open them up to the GTAC alkylating agent. As the reaction proceeded, the chitosan would become gradually completely dissolved. At pH values of 11 and above, there would be no protonation and therefore little solvation.

The problem was solved by using aqueous perchloric acid as the reaction medium. The chitosan dissolves under conditions of low pH, and the perchloric acid has a low nucleophilicity, so that it does not open the epoxide ring. In the G-chitosan synthesis, both the chitosan and the GTAC were able to react under optimum conditions.

The Quab 342 alkylation was carried out on a chitosan already alkylated with GTAC, under alkaline conditions, because the reagent was supplied in the unreactive chlorhydrin form. The unsatisfactory results with the alkaline heterogeneous GTAC alkylation suggested that the chitosan had to be rendered alkali-soluble first. Accordingly, the DQ% of GQ-chitosan is low, because most of the active sites were already taken by hydroxypropyltrimethylammonium groups. However, the presence of a small 3.5% Quab 342-derived hydrophobic dodecyl chains have made a considerable difference to the physicochemical properties of the GQ-chitosan. Its viscosity is considerably increased compared to the G-chitosan, especially at high pH as the chitosan 2-amino groups are deprotonated. The increase in viscosity is attributed to intermolecular hydrophobic interactions of the C_{12} alkyl chains present along the GQ chitosan backbone as has been reported for other hydrophobically modified polymers (Tanaka et al., 1992). Its phase behaviour with SDS is also altered and it has acquired surfactant properties; it is observed to foam during mixing, unlike its parent compounds, the G-chitosan and the unmodified chitosan L#2272.

There has been considerable interest over many years in the interaction of polymers and surfactants including polyelectrolytes and oppositely charged surfactants (Williams, 2003), (Langevin, 2009). It is generally observed that association occurs at a critical surfactant concentration (critical aggregation concentration) which is much lower than the critical micelle concentration and is due to cooperative binding. A number of studies have been reported on the interaction of unmodified chitosan with SDS under acid conditions (Petrovic et al., 2016), (Onesippe and Lagerge, 2008), (Chiappisi and Gradzielski, 2015), other anionic surfactants (Desbrieres et al., 2010), (Petrovic et al., 2017) (Chiappisi and Gradzielski, 2015), or with polyanions such as carboxymethylcellulose (Rosca et al., 2005). It is expected that binding occurs through electrostatic interaction between the surfactant sulphate groups and the protonated amine group on the chitosan chain.

Chiappisi and Gradzielski (2015) have argued that at very low SDS concentrations some non-cooperative binding occurs but the complexes are soluble and the solution remains clear. At intermediate SDS concentrations, polymer / surfactant aggregation occurs at a critical concentration, the critical aggregation concentration (CAC) as a result of cooperative binding resulting in micellar-like aggregates forming along the polymer chain and that turbidity can be observed. A further increase in SDS concentration results in the saturation of the polymer chain. Onesippe and Lagerge (2008) have reported that, for a 0.05% chitosan solution, the CAC occurred at 1.8mM SDS which is considerably lower than the critical micelle concentration (CMC) which is 8mM SDS. Senra et al. (2018) studied the interaction of cationically modified chitosan with sodium decyl sulphonate and showed through conductiometric measurements that for a 1mM chitosan solution interaction occurred at a concentration of 1mM surfactant which is much lower than its CMC (40mM). For the cationically modified chitosans in our study, at a polymer concentration of 0.02%, turbidity was observed at concentrations of approximately 0.7mM and 0.5mM

SDS for G-chitosan and GQ-chitosans respectively, confirming that significant interaction had occurred at these concentrations. The concentrations correspond to a molar ratio of SDS to G- and GQ-chitosan monomer units of 0.88 and 0.54 at which the electrostatic charge on the complex is expected to be close to zero. Chiappisi and Gradzielski also determined the electrophoretic mobility of chitosan/SDS complexes and reported that for a 0.01% chitosan solution charge neutralisation occurred at an SDS concentration of 0.45mM and at higher SDS concentrations the complexes became negatively charged.

In addition to the interesting phase behaviour, the GQ-chitosan has novel rheological properties. It is noted in Figure 6 that a number of the samples have gel-like characteristics and this is confirmed through the rheological data shown in Figure 7. It is believed that the gels are formed through intermolecular hydrophobic interactions between the C_{12} chains on the GQ-chitosan. This behaviour is typical of 'associative thickeners' and is supported by the fact that G-chitosan, which does not contain C_{12} chains, does not form gels. G' values were found to increase significantly in the presence of SDS up to concentrations close to its CMC (8mM). It is evident that the SDS promotes intermolecular hydrophobic association of the GQ-chitosan polymer chains by increasing the number and/or life-time of the crosslinks as has been reported for other hydrophobically-modified polymers (Tanaka et al., 1992), (Jiménez-Regalado et al., 2000). At higher SDS concentrations, above the SDS CMC each C_{12} chain will be encapsulated within an SDS micelle and hence intermolecular associations will be inhibited and the systems will have the characteristics of a dilute solution.

5. Conclusions

This study has demonstrated that cationic chitosan derivatives with a high DS can be synthesised using GTAC in dilute perchloric acid and that the derivatives are soluble over a broad pH range. Furthermore, it has been shown that the introduction of C_{12} alkyl groups along the chitosan chain leads to the formation of viscoelastic gels in the presence of SDS molecules. These materials have potential application in a range of commercial formulations, including cosmetics, pharmaceuticals and personal care.

6. Acknowledgements

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Highlights

- Chitosan was alkylated with GTAC to form water-soluble G-chitosan.
- The G-chitosan was alkylated with Quab 342 to form the novel GQ-chitosan derivative.
- GQ-chitosan has increased viscosity compared to G-chitosan and unmodified chitosan.
- The derivatives form complexes with the anionic surfactant SDS.
- The GQ-chitosan-SDS complex has a gel phase, due to its long alkyl chains.