Abstract

The interaction of cetyltrimethylammonium bromide (CTAB) and its gemini homologue (butanediyl-1, 4-bis (dimethylcetylammonium bromide), 16-4-16 with biocompatible polymer sodium alginate (SA) has been investigated in aqueous medium. Addition of K$_2$CO$_3$ influences viscoelastic properties of surfactant impregnated SA via competition between electrostatic and hydrophobic interactions. Viscosity of these polymer-surfactant systems increases with increase in concentration of K$_2$CO$_3$, and a cryogel is formed at about 0.5M K$_2$CO$_3$ concentration. The thermal stability of gel (5% SA + 0.5M K$_2$CO$_3$) decreases with increase in surfactant concentration, a minimum is observed with increase in 16-4-16 concentration. The impact of surfactant addition on the alginate structure vis-à-vis its drug loading capability and release thereof was studied using Ibuprofen (IBU) as the model drug. The hydrogel with 16-4-16 exhibits higher IBU encapsulation and faster release in comparison to the one containing CTAB. This higher encapsulation cum-faster release capability has been related to micelle mediated solubilization and greater porosity of the hydrogel with gemini surfactant.

**Key words:** K$_2$CO$_3$, sodium alginate, Ibuprofen, 16-4-16, Hydrogel.
3.1 Introduction

The interaction of polyelectrolytes with oppositely charged surfactants in aqueous media has received considerable attention owing to their wide range of applications in many industrially important processes and products, such as water treatment, detergency, and oil recovery. The irreversible macroscopic phase separation occurs in many cases by electrostatic and hydrophobic interactions between polyelectrolytes and oppositely charged surfactants. In a surfactant/polyelectrolyte solution, the addition of salt is therefore expected to have a significant effect on these interactions.

Sodium alginate (SA), a polyelectrolyte biopolymer, extracted from brown seaweeds is considered a biodegradable block copolymer. It can be characterized as an anionic copolymer consisting of 1-4 linked α-L-guluronic (G) and β-D-mannuronic (M) acid residues and is widely used as a gelling agent in food and pharmaceutical applications. Alginate is known to form gel in the presence of divalent cations, such as Ca$^{2+}$ which act as cross linkers between the functional groups of alginate chains, nicely described by the egg box model. This ability of alginate to form gels in presence of the multivalent cations in combination with their specific biological properties like biocompatibility and low toxicity have led to important developments in food, medicine, and pharmacy. These materials have been used for many years to prepare wound dressings, dental impression material and to improve and modify the texture of foods because of their stabilizing, viscosifying and gelling properties. Besides gelation induced by divalent cations, acid gels of alginate are formed at low pH. The aggregation between alginate and sodium dodecylsulphate at low pH has been reported in dilute aqueous solution owing to hydrophobic interactions. Yang et al. have reported electrostatic attractions at higher pH and electrostatic/hydrophobic interactions at lower pH between anionic sodium alginate and cetyltrimethylammoniumbromide (CTAB). However, interactions between polyelectrolytes and the gemini surfactants having lower cmc and better wetting/solubilizing properties compared to corresponding single-tailed surfactants have been scarcely investigated. Moreover, a comparative study on the
interaction of polyelectrolytes with the single and double-tailed surfactants with similar hydrophobic chain length has not been attempted so far.

The properties of polyelectrolyte–surfactant solutions are known to be affected by electrolytes. The addition of NaBr is known to decrease critical aggregation concentration (cac) in sodium carboxymethylcellulose dodecytrimethylammonium bromide system. A larger cac has also been reported by the reduction of electrostatic interaction between surfactants and polyelectrolytes. Addition of large amount of electrolyte can completely screen the electrostatic interactions and prevent the formation of polyelectrolyte/surfactant complexes. Although many studies have been devoted to understand electrolyte effect on surfactant (single chain)–polyelectrolyte systems, a few of such reports are known for gemini–polyelectrolyte systems. Specific binding of multivalent cations has a prominent effect on the gelation behavior of alginates. However, the literature regarding the gelation of alginate in the presence of monovalent cation is limited, although it is well known that K$_2$CO$_3$ is able to drive some specific interactions between the alginates with a prominent effect on its behavior, chain conformation, solubility and the solution properties. Seale et al. reported that monovalent cation can interact with alginate according to three different modes: ion pair formation, specific site binding and cooperative “egg box” binding. Karakasyan et al. using calorimetric and viscoelastic experiments reported the thermoreversible gelation of the alginates in aqueous solution of K$_2$CO$_3$.

In view of the above, it would be highly interesting to explore the effects of monovalent cation on the interaction of sodium alginate with cationic single chain and gemini surfactants, which to the best of our knowledge has not been reported in the literature. The present investigation, therefore, deals with comparative study of interaction of sodium alginate with cationic single tailed surfactant CTAB and its gemini homologue 16-4-16. The main focus was to evaluate the effect of K$_2$CO$_3$ on the rheological behavior of sodium alginate–surfactant (CTAB or 16-4-16) complexes. More specifically, the influence of varying surfactant/K$_2$CO$_3$ concentrations at a fixed K$_2$CO$_3$/surfactant concentration on the sol/gel transitions and
viscoelastic properties of alginate gels was studied. It was observed that at a given 
$K_2CO_3$ concentration the addition of surfactants significantly modifies the properties 
of alginate gels and hence were explored for drug (Ibuprofen) encapsulation and 
release behavior.

### 3.2. Experimental

#### 3.2.1 Materials

Sodium alginate (SA) from brown algae was purchased from Sigma Aldrich (Fluka, 
biochemika, 71238-Norway). The intrinsic viscosity ($[\eta] = 472.02$ mL/g) was 
determined by ubbelohde viscometer.\textsuperscript{39} Using Mark–Houwink equation, $[\eta] = 4.85 \times 10^{-3}M_w^{0.97}$, molecular weight (Mw) was determined to be 138,836 g/mol, fairly in 
conformity with reports in the literature.\textsuperscript{40} The mole fraction of mannuronic 
acid (FM), guluronic acid (FG), and their ratio (M/G) was found to be 0.46, 0.54 and 0.85, 
respectively. The diad sequences FGG, FMM and FMG were found to be 0.48, 0.40, 
and 0.06, respectively. The composition of alginate has been determined from its 1H-
NMR (Fig.2.1.) using standard procedures.\textsuperscript{41}Cetyltrimethylammonium bromide 
(CTAB) was a Aldrich product. Ibuprofen (IBU, >98%) and potassium 
carbonate ($K_2CO_3$) were obtained from Himedia laboratories (India), calcium chloride ($CaCl_2 > 
98\%$) was of analytical grade. The Gemini surfactant butanediyl-1, 4-bis 
(dimethylcetylammonium bromide) (16-4-16) was synthesized and characterized as 
described elsewhere.\textsuperscript{42} All the chemicals were used as received and the solutions 
were prepared in triple distilled water. The structures of the surfactants and the 
polymer used are shown in Scheme.3.1.
Sodium alginate

Scheme 3.1: Structure of surfactants and polymer

3.2.2 Methods

3.2.2.1 Preparation of solutions

Aqueous solutions of SA (5wt%) with various concentrations of the surfactants (CTAB and 16-4-16) and salt (K$_2$CO$_3$) were prepared by dispersing the requisite quantities of SA powder, surfactant, and the salt in water. The samples were stirred for at least 6 h at room temperature to ensure homogenization. The SA concentration was maintained constant at 5% in all cases. In absence of salt, the surfactant concentrations added to SA solution were varied from 0 to 4mM in case of CTAB and from 0 to 0.1mM for 16-4-16. The pH value of surfactant polymer aggregates was determined by pH meter and was found to be 6.94 differing slightly from the value of 6.48 of pure SA solution. Initially the concentration of surfactants was fixed (0.1mM) and K$_2$CO$_3$ concentration varied from 0 to 0.5M and then salt concentration was fixed at 0.5M and concentrations of CTAB and 16-4-16 varied from 0 to 4mM and 0–0.1mM, respectively. When necessary, solutions were kept in a water bath maintained at 50±0.1 °C for 10 min. to complete the dissolution process.
3.2.2.2 Rheology

Steady and dynamic rheological experiments were performed on Anton Paar MCR-102 Rheometer equipped with a peltier temperature control system with an accuracy of ±0.01 °C. Cone-plate geometry (diameter of 25cm with cone angle of 1.998°) was used for the measurements. Experiments were carried out in duplicate with good reproducibility. To prevent dehydration during rheological measurements, a thin layer of silicone oil was placed on the periphery surface of the sample held between the plates. The test methods employed were steady flow, oscillatory temperature, frequency and time sweeps at a range of surfactant and salt concentrations as mentioned above. The experiments were carried out in the linear viscoelastic regime determined for each sample by a preliminary stress sweep at 1 Hz. The flow properties were determined at 25 °C by steady shear tests using shear rates in the range of 0.01 to 1000 s⁻¹. Thermal behavior of samples was studied by temperature sweep; the samples were subjected to a temperature ramp in the range of 0–60 °C with a heating rate of 2 °C min⁻¹ and, thermo-reversibility was checked by cooling the samples in reverse cycle at the same rate. To ensure that the experimental conditions did not interfere with the gelation process, temperature ramp was performed at a low oscillation frequency (1 Hz) and a shear stress of 2 Pa applied in the linear viscoelastic plateau. The samples were also subjected to a frequency sweep in the linear viscoelastic region (stress of 2 Pa) to study the viscoelastic performance over a wide range of frequencies (0.01 to 100 Hz). In linear viscoelastic region (LVR) the evolution and dynamics of elastic storage modulus (G') and the viscous loss modulus (G'″) with time was monitored at a constant shear frequency of 1 Hz.

3.2.2.3 FT-IR spectroscopy

The structural properties of sodium alginate gels at high concentration of K₂CO₃ (0.5M) in absence and presence of CTAB and 16-4-16 were examined using a Perkin-Elmer FT-IR spectrophotometer fitted with a Universal ATR sampling accessory. Spectra were recorded in the 4000–400cm⁻¹ range using KBr pellets, acquiring 64 scans with a resolution of 1 cm⁻¹.
3.2.2.4 Scanning electron microscopy (SEM)

The morphology of alginate gels was characterized using Hitachi S-3000H Scanning Electron Microscope equipped with a digital camera. Samples of the xerogels were prepared by placing a small aliquot of hydrogel on carbon tape placed over sample stub, coated with gold in a Hitachi Fine Coat Ion Sputter.

3.2.2.5 Swelling studies

Alginate gels with composition 5% SA + 0.5M K₂CO₃ and varying CTAB (0–4mM) and 16-4-16 (0–0.1mM) concentrations were accurately weighed ranging from 100 to 200mg and immersed in 10 ml of 10mM CaCl₂ solution. After a fixed time interval of 30 min the gels were separated from the medium by filtration and were wiped gently and weighed. The dynamic weight change of the gel at time t = 30min was calculated according to the formula:

\[
\text{% weight change} = \left[ \frac{W_t - W_i}{W_i} \right] \times 100 \%
\]  

(1)

Where, \( W_t \) is the weight of the gel at time \( t = 30 \) min and \( W_i \) is the initial weight of the gel.⁴³

3.2.2.6 Estimation of drug loading and encapsulation efficiency

Drug loading (LC) and encapsulation efficiency (EC) were determined as per the literature.⁴⁴ Briefly, 45mg of gel (L) (5% SA + 0.5M K₂CO₃ or 5% SA + 0.5M K₂CO₃ + 0.1mM surfactant) was vigorously stirred for a period of 3 h on a magnetic stirrer at a temperature of 25±0.5 °C in 10 ml vials containing 78mg (L₁) of IBU in 2ml of methanol and sealed with screw caps. The solution was filtered and the concentration of IBU in the methanolic filtrate (L₂) was determined using a Schimadzu spectrophotometer (model UV-1650PC) by comparing absorbance at 220nm with a predetermined calibration curve. Drug loading (LC) and encapsulation (EC) capacities were calculated as

\[
LC = \frac{(L_1 - L_2)}{L} 
\]  

(2)

\[
EC = \frac{(L_1 - L_2)}{L_1} \times 100 \%
\]  

(3)
3.2.2.7 In vitro release study

The release of IBU from the gel was studied by adding the drug loaded gel into 50mL of 10mM CaCl$_2$ as dissolution media at pH 7.4. The solution was continuously stirred at 25±0.5 °C. Aliquots of the release medium were withdrawn at intervals of 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 min and replaced simultaneously with fresh salt solution to maintain a constant volume. The collected samples were assayed with spectrophotometry at a wavelength of 220nm and the drug release profile obtained by plotting a curve of Mt/M$_{eq}$ against t, Mt being the amount of drug released at time t and Meq the amount released once the equilibrium state is reached. All measurements were carried out in triplicate and the results are presented as average.

3.2.2.8 Fluorescence measurements

The fluorescence spectra of IBU in different solvents and surfactant impregnated gels were obtained on Schimazdu spectrofluorimeter (Model RF-5301) operating in the steady-state mode at 25±0.1 °C. Samples loaded in a non-fluorescent cell were excited at 228nm with excitation and emission slit of 3 nm. The fluorescence emission spectra were recorded in the range 250–400 nm.

3.3 Results and discussions

3.3.1 Rheological properties of sodium alginate in presence of surfactants

Fig.3.1. shows the viscosity as a function of shear rate for the pure aqueous 5% SA in absence and in presence of various surfactant concentrations (CTAB: 0–4mM and 16-4-16: 0–0.1mM) at 25 °C. The viscosity profiles of alginate are similar in presence of both the surfactant systems, exhibiting Newtonian behavior at low to intermediate shear rates followed by non-Newtonian behavior at very high shear rates. Zero shear viscosity decreases initially followed by an increase upon surfactant addition (insets
of Fig.3.1). The non-Newtonian shear thinning may be due to disruption of intermolecular junctions at a faster rate than the rate of their reformation leading to

Figure 3.1. Variation of viscosity of 5% sodium alginate with shear rate in presence of varying concentrations of surfactants: (a) CTAB (0-4 mM), (b) 16-4-16 (0-0.1 mM) (c) Variation of viscosity with shear rate for alginate-CTAB complex at varying K₂CO₃ concentration. Insets show the zero-shear viscosity as a function of surfactant (a,b) and K₂CO₃ (c) concentrations for 5% sodium alginate in presence of CTAB and 16-4-16.
decrease in junction density and hence drop in the viscosity at the high shear rates. At very low concentrations, ionic surfactants behave as salts and can reduce the electrostatic repulsions within alginate polymer chains thereby reducing their dimensions and hence viscosity. Such a behavior is reported in literature for polyacrylic acid\textsuperscript{46} and acrylamide/sodium 2-acryl-\textsuperscript{2} methyl propane sulfonate\textsuperscript{47} systems upon addition of DTAB. The decrease in viscosity with increase in surfactant concentration is more pronounced for the system containing gemini 16-4-16 surfactant. Perhaps the higher charge density due to two close head groups leads to enhanced screening of electrostatic repulsion resulting in significant viscosity drop. The viscosity increases with further increase in surfactant concentration owing to the conformational expansion of polymer chains as a result of enhanced electrostatic repulsion among the micelles bound to the polymer backbone. Similar results were obtained in CTAB-MC system\textsuperscript{48} at higher surfactant concentration and explained in terms of enhanced electrostatic repulsions.

Alginate solutions at 25 °C without or with surfactants (CTAB or 16-4-16) show crossover of elastic (G') and loss (G'\textsuperscript{''}) moduli curves as a function of frequency at oscillatory stress amplitude of 2 Pa (\textbf{Fig. 3.2}). The crossover frequency, \(\omega_c\), indicates the frequency at which sol–gel transition occurs and is related to the effective structural relaxation time as:

\[
\tau_R = \frac{1}{\omega_c} \quad (4)
\]

For pure 5wt% SA, \(\omega_c\) is 8.5 Hz with corresponding relaxation time 11.7×10\textsuperscript{-2} s, which gets shifted toward lower relaxation time upon addition of CTAB and 16-4-16 surfactants as shown in \textbf{Table. 3.1}, indicating weakening of the interpolymer network structure of SA. This could possibly be due to screening of interpolymer association upon addition of these positively charged surfactants. With further increase in surfactant concentration crossover frequency remains almost constant. Comparison of \(\omega_c\) and \(\tau_R\) of the two surfactants (\textbf{Table.3.1}) indicates that higher relaxation time is required for reorganization of SA + 16-4-16 systems compared to SA + CTAB systems at all concentrations. This can be explained on the basis of strong co-
operative aggregation of the gemini surfactants compared to its monomeric counterpart owing to higher hydrophobicity of the former surfactant compared to the latter. It is reported that the co-operative aggregation enhances the viscoelasticity of the system, thereby leading to increase in the relaxation time.

**Figure 3.2**: Elastic ($G'$) and loss ($G''$) moduli as a function of frequency: (a) 5% SA+CTAB (0-4mM) and (b) 5% SA +16-4-16 (0-0.1mM).

Temperature sweeps of 5% SA with varying surfactant concentrations (CTAB, 0–4mM and 16-4-16, 0–0.1mM) at fixed shear stress and frequency of 2 Pa and 1 Hz, respectively (Fig. 3.3) reveal that there is no sol–gel transition either in the pure alginate solution or at different concentrations of the two surfactants during the course of heating (0–60 °C). Since $G''$ is above $G'$ over the entire range of temperature, the surfactant-polymer systems therefore remain in sol form throughout the temperature range. Again the variation of $G'$ and $G''$ with time (Fig. 3.4) for the above mentioned systems at a temperature of 25 °C and a frequency of 1Hz exhibits dominance of loss.
modulus $G''$ over the elastic modulus $G'$, indicating sol form of solution which does not undergo any phase transition with time.

Table. 3.1: Crossover frequency and relaxation time for (a) 5% SA without $K_2CO_3$ at varying concentration of CTAB and 16-4-16, and (b) 5% SA +0.1 mM CTAB and 5% SA+0.1 mM 16-4-16 in presence of varying $K_2CO_3$ concentration.

(a) 5% SA + 0M $K_2CO_3$

<table>
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<th>CTAB(mM)</th>
<th>$\omega_c$(Hz)</th>
<th>$\tau_R$(s$^{-1}$)</th>
<th>16-4-16(mM)</th>
<th>$\omega_c$(Hz)</th>
<th>$\tau_R$(s$^{-1}$)</th>
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<td>0.117</td>
<td>0</td>
<td>8.50</td>
<td>0.117</td>
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<td>0.005</td>
<td>10.78</td>
<td>0.093</td>
</tr>
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<td>0.010</td>
<td>10.63</td>
<td>0.094</td>
</tr>
<tr>
<td>2.0</td>
<td>13.21</td>
<td>0.075</td>
<td>0.015</td>
<td>10.08</td>
<td>0.099</td>
</tr>
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<td>4.0</td>
<td>13.39</td>
<td>0.075</td>
<td>0.100</td>
<td>10.35</td>
<td>0.096</td>
</tr>
</tbody>
</table>

(b) 5% SA + 0.1mM CTAB/0.1mM 16-4-16

<table>
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<tr>
<th>$K_2CO_3$(M)</th>
<th>$\omega_c$(Hz)</th>
<th>$\tau_R$(s$^{-1}$)</th>
<th>$K_2CO_3$(M)</th>
<th>$\omega_c$(Hz)</th>
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<td>0.14</td>
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Figure 3.2: Viscoelastic properties of: (a) 5% SA+CTAB (0-4mM) and (b) 5% SA+16-4-16 (0-0.1mM) respectively in the temperature range of 0-60°C.

Figure 3.4: $G'$ and $G''$ as a function of time for (a) 5% SA+CTAB (0-4mM) and (b) 5% SA+16-4-16 (0-0.1mM).
3.3.2 Effect of K$_2$CO$_3$ on rheological properties of sodium alginate in presence of 0.1M CTAB/gemini 16-4-16

In order to study the influence of K$_2$CO$_3$ on rheological behavior of alginate–surfactant complexes in solution, we investigated the dependence of viscosity on shear rate for 5% SA + 0.1mM CTAB and 5% SA + 0.1Mm 16-4-16 systems in presence of various concentrations of K$_2$CO$_3$ (Fig. 3.1c). At 0.1M K$_2$CO$_3$ viscosity was lowered in both the systems exhibiting a Newtonian behavior at low shear rate followed by shear thinning as observed for the system without salt. At very low shear rate the alginate + CTAB system shows significant increase in viscosity with K$_2$CO$_3$ concentration. However, shear thinning with shear rate is observed from the very beginning without any Newtonian plateau. In alginate + 16-4-16 system similar behavior is observed except that viscosity at 0.3M K$_2$CO$_3$ is lower than in the system without salt and is further lowered at high shear rates. The viscosity decrease at high shear rate is due to breakdown of intermolecular junctions. The zero shear viscosity for 5% SA in presence of similar concentration (0.1mM) of CTAB and 16-4-16 at three K$_2$CO$_3$ concentrations are shown as inset in Fig. 3.1c. At 0.1M K$_2$CO$_3$ both systems show small viscosity decrease followed by a marked increase with increase in K$_2$CO$_3$ concentration at and above 0.3M in the alginate + CTAB system. The gemini containing system, however, exhibits the same viscosity at 0.3M K$_2$CO$_3$ before undergoing an increase at 0.5M K$_2$CO$_3$. The results may be a consequence of competition between electrostatic forces and hydrophobic associations upon addition of K$_2$CO$_3$. Screening of repulsive forces between polymer chains may reduce viscosity of the system with CTAB at low salt concentration. At 0.5M K$_2$CO$_3$ the electrostatic repulsion between surfactant aggregates on the polymer chains are reduced thereby enhancing further the binding of surfactant on the polymer chain with consequent viscosity increase. Both at 0.3M and 0.5M K$_2$CO$_3$ the viscosity of 5% alginate impregnated with 0.1mM CTAB is higher than that of 0.1mM 16-4-16 imbued one. A possible reason may be enhanced association between polymer chains in the former system than in the latter system.
Frequency sweep experiments under LVR conditions were carried on both the alginate–surfactant complexes, the crossover frequencies (ωc) between G’ and G” at different concentrations of K2CO3 are presented in Table.3.1 and Fig 3.5. The values show a small increase with the addition of 0.1M K2CO3 but a significant decrease from 12.86 Hz to 8.11 Hz and from 10.35 Hz to 6.73 Hz for the 5%SA + CTAB and 5%SA + 0.1mM 16-4-16 systems respectively. The corresponding relaxation times increased from 0.077 s⁻¹ to 0.12 s⁻¹ and from 0.096 s⁻¹ to 0.14 s⁻¹ for SA + CTAB and SA + 16-4-16 systems respectively, indicating transition from liquid like polymeric solution to an association structure with more desirable viscoelasticity.51-53

\[
\begin{array}{c}
\text{Frequency (Hz)} \\
1 & 10 & 100 \\
G' & G'' \\
0M K_2CO_3 & 8.11Hz \\
0.1M K_2CO_3 & 12.95Hz \\
0.3M K_2CO_3 & 6.73 Hz \\
0.5M K_2CO_3 & 11.22 Hz \\
1M K_2CO_3 & 10.35Hz \\
\end{array}
\]

\[
\begin{array}{c}
\text{Frequency (Hz)} \\
1 & 10 & 100 \\
G' & G'' \\
0M K_2CO_3 & 12.86Hz \\
0.1M K_2CO_3 & 10.35Hz \\
0.3M K_2CO_3 & 8.11Hz \\
0.5M K_2CO_3 & 6.73 Hz \\
1M K_2CO_3 & 11.22 Hz \\
\end{array}
\]

**Figure 3.5**: Elastic (G') and loss (G") moduli as a function of frequency: (a) SA + CTAB (0.1mM) and (b) SA + 16-4-16 (0.1mM) with varying K2CO3 concentration.

The influence of temperature on structural aspects of the two alginate–surfactant complexes (5% SA + 0.1mM CTAB/16-4-16) were investigated using temperature sweep measurements (Fig. 3.6), revealing the variation of dynamic moduli in the course of heating and cooling at frequency of 1Hz and heating/cooling rate of 2 °Cmin⁻¹. It is observed that G’ as well as G” of the two alginate complexes with 0.1M...
and 0.3 M K$_2$CO$_3$ gradually decreased with increasing temperature, $G'$ being lower than $G''$ over the entire range of temperature. This demonstrates that such complexes are in solution state in this temperature range without undergoing any phase transition on heating/cooling. At 0.5 M K$_2$CO$_3$, $G'$ overtakes $G''$ and the systems display a gel like viscoelastic behavior at low temperature while exhibiting a solution behavior ($G'< G''$) at higher temperatures. Thus the gel can be categorized as cryogel that transforms from sol to gel upon cooling. During the heating process, the crossover temperatures, Tgs, denoting gel to sol transition were observed to be 48 °C and 43 °C for the alginate complex with CTAB and 16-4-16, respectively. In the cooling process, both moduli increase, crossing over at about 2.85 °C and 1.95 °C, respectively, in the two systems indicating sol to gel transition, Tsg. The higher Tgs and hence higher thermal stability of 5% SA + 0.1 mM CTAB over 5% SA + 0.1 mM 16-4-16 could be a result of stronger interpolymer associations in the former system than in the latter. The possible reason might be the higher charge density of 16-4-16 which effectively screens the interpolymer association, resulting in lower cross over temperature and lower thermal stability of the alginate–gemini gel.

The time sweep profiles of $G'$ and $G''$ for 5% alginate complexed separately with same concentration (0.1 mM) of CTAB or 16-4-16 (Fig. 3.7) at 25 °C and frequency of 1 Hz over a range of K$_2$CO$_3$ concentrations reveal that the viscous properties dominate over the elastic properties, $G''$ being larger than $G'$ throughout the time sweep in both the systems when the concentration of K$_2$CO$_3$ is within 0 - 0.1 M. As the concentration of K$_2$CO$_3$ is increased to 0.3 M, $G'$ overtakes $G''$ resulting in crossover at about 2014 s and 1777 s, respectively for the system containing CTAB and 16-4-16 referred to as gelation time. The lower gelation time of gemini containing system may be traced to its higher charge density than that of the CTAB system. At 0.5 M K$_2$CO$_3$, $G'$ maintains its dominance over $G''$ indicating gel behavior throughout the time sweep without any sol–gel transition. Thus, it is clear that alginate gelation is highly sensitive to K$_2$CO$_3$ concentration forming a three dimensional gel like structure at 0.5 M K$_2$CO$_3$ in presence of 0.1 mM concentration of either of the two surfactant in whole frequency range. Therefore, the concentration of K$_2$CO$_3$ was fixed.
Figure 3.6: Viscoelastic properties of (a) 5% SA+CTAB (0.1mM) + K$_2$CO$_3$ (0-0.5M) (b) 5% SA +16-4-16(0.1mM) + K$_2$CO$_3$ (0-0.5M) as a function of temperature

Figure 3.7: G' and G" as a function of time for (a) 5% SA+CTAB (0.1mM) and (b) 5% SA +16-4-16(0.1mM) with a varying K$_2$CO$_3$ concentration
at 0.5M to study the effect of varying concentrations of CTAB/16-4-16 on rheological behavior of the alginate gel.

### 3.3.3 Effect of surfactant (CTAB/16-4-16) concentrations on the rheological properties of sodium alginate gel in presence of 0.5M K₂CO₃

From the representative viscosity vs shear rate profiles of alginate gel with composition 5%SA + 0.5M K₂CO₃ (Fig 3.8), no general trend was found with varying surfactant concentration (CTAB (0-4mM)/16-4-16 (0-0.1mM)), but a non-Newtonian behavior was observed from the low shear rate to the high shear rate regime. The frequency sweep experiments (Fig. 3.9) however, show that it always remain as gel with varying surfactant concentration as \( G' \) is higher than \( G'' \) throughout the frequency sweep. Moreover, the time profiles (Fig. 3.10) reveal that the gel form is stable throughout the time sweep with perceptible increase in gel strength with time. Temperature sweep measurements reflect the temperature dependent structural changes of a thermo-reversible gel. As a prototype temperature dependence of \( G' \) and \( G'' \) of 5%SA + 0.5M K₂CO₃ system in absence of surfactant are depicted in Fig. 3.11. At low temperature \( G' > G'' \) categorizing the system as a cold set gel. Upon heating both \( G' \) and \( G'' \) decrease gradually indicating the weakening of the gel network, as has been reported for Gallic acid-Xyloglucan (GA-TSX) system.\(^{54}\) However, the decrease of \( G' \) takes over that of \( G'' \) and at \( \approx 55 \, ^\circ \text{C} \) denoted by Tgs, \( G'=G'' \) beyond which gel to sol transition takes place. At high temperature the system acts as a sol that sets into gel upon cooling in the reverse cycle similar to the polysaccharide based systems.\(^{55}\) The sol–gel transformation occurs at Tsg which is much lower compared to Tgs. During the cooling process, increase in \( G' \) and \( G'' \) with decreasing temperature occurs because of thermal contraction rather than new joint formation as reported in GA-TSX system.
Figure 3.8: Viscosity as a function of shear rate: (a) 5% SA+K₂CO₃ (0.5M) with varying concentration of CTAB; (b) 5% SA+K₂CO₃ (0.5M) with varying concentration of 16-4-16.

Figure 3.9: Prototype plot of elastic moduli (G') and loss (G'') moduli as a function of frequency for 5% SA+K₂CO₃+CTAB or 16-4-16.
Figure 3.10: $G'$ and $G''$ as a function of time for (a) 5% SA+0.5MK$_2$CO$_3$/CTAB (0-4 mM) and (b) 5% SA+0.5MK$_2$CO$_3$+16-4-16 (0-0.1mM).

Figure 3.11. Viscoelastic properties of alginate gel (5% sodium alginate+0.5M K$_2$CO$_3$) as a function of temperature
Rheological measurements as depicted in Table 3.2 show that both the crossover temperatures, Tgs and Tsg, are affected by the addition of surfactant. Tgs was found to decrease with increase in CTAB concentration, but with increasing 16-4-16 concentration it first decreases, passes through a minimum and then increases reaching finally to a constant value. The breaking and making of gels upon heating and cooling are related to the network structures as previously studied by^56^, where it was found that the hydrogen bond formation and dissociation cause the gel to form and to break upon cooling and heating respectively. In the present study, decrease in Tgs on small surfactant addition might be due to screening of interpolymer association of alginate chains at high K$_2$CO$_3$ concentration. With increasing CTAB concentration the degelation temperature (Tgs) decreases further till the system becomes a permanent sol due to increased electrostatic repulsions between the surfactant bound polymer aggregates causing disruption of connectivity between polymer chains with rise in temperature. The results obtained are different for the gemini containing system with significant decrease in crossover temperature even at low 16-4-16 concentration. 16-4-16 with higher charge density might lead to enhanced screening of interactions compared to CTAB. With further increase in surfactant concentration the thermal stability increases probably because of more cooperative binding of gemini than CTAB. Thus, thermal stability of 5% SA + 0.5mM K$_2$CO$_3$ gel can be tuned by varying surfactant concentration. This surfactant induced tunability of biocompatible alginate gels makes them suitable materials for numerous biomedical applications like bioavailability enhancement and controlled release of hydrophobic drugs.

### 3.3.4 Structural characterization

The IR spectra of 5% SA, 5% SA + 0.5M K$_2$CO$_3$, 5% SA + 0.5M K$_2$CO$_3$ + 0.1mM CTAB and 5% SA + 0.5M K$_2$CO$_3$ + 0.1mM 16-4-16 surfactant are given in Fig.3.12. SA shows bands at 3438.5 cm$^{-1}$ and 2065 cm$^{-1}$ due to O-H and C-H stretching vibrations respectively. The peak at 1633 cm$^{-1}$ due to asymmetric stretch while that at 1410 cm$^{-1}$ due to symmetric stretching vibration of carboxylate group. The band at
Table 3.2: Characteristic degelation, $T_{gs}$ and gelation, $T_{sg}$ temperature of 5% SA + 0.5M $K_2CO_3$ at different surfactant concentrations.

<table>
<thead>
<tr>
<th>CTAB (mM)</th>
<th>$T_{gs}$(°C)</th>
<th>$T_{sg}$(°C)</th>
<th>16-4-16 (mM)</th>
<th>$T_{gs}$(°C)</th>
<th>$T_{sg}$(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
<td>5.28</td>
<td>0</td>
<td>55</td>
<td>5.28</td>
</tr>
<tr>
<td>0.1</td>
<td>48</td>
<td>2.86</td>
<td>0.005</td>
<td>38</td>
<td>1.98</td>
</tr>
<tr>
<td>0.5</td>
<td>34</td>
<td>1.9</td>
<td>0.010</td>
<td>40</td>
<td>2.01</td>
</tr>
<tr>
<td>2.0</td>
<td>27</td>
<td>0.9</td>
<td>0.015</td>
<td>43</td>
<td>2.38</td>
</tr>
<tr>
<td>4.0</td>
<td></td>
<td></td>
<td>0.100</td>
<td>43</td>
<td>2.32</td>
</tr>
</tbody>
</table>

1025 cm$^{-1}$ is a result of C-O-C anti symmetric stretch. These prime bands exhibit shifts in presence of salt as well as surfactants and in general the bonds existing in SA are weakened by both salt and surfactant. The O-H stretching band is shifted from 3438.5 cm$^{-1}$ to 3443 cm$^{-1}$, that of C-H stretch from 2065 cm$^{-1}$ to 2093 cm$^{-1}$, the peak at 1633 cm$^{-1}$ is shifted to 1638 cm$^{-1}$ while the one at 1410 cm$^{-1}$ to 1392 cm$^{-1}$ in presence of the salt. K$^+$ ions which bring about the gelation of SA by chain–chain association decrease the strength of the bonds within the individual polymeric chain in general. The surfactants used being positively charged destabilize the gellified network of Sodium alginate and the salt, so the bands obtained also show some irregular trends where in the band due to O-H stretch occurs at lower wave number and the rest of the characteristic bands occur at higher wave number in presence of CTAB. In presence of the gemini surfactant system also irregular band shifts were observed indicating that in general bond strength of polymeric chain decreases by its interaction with surfactants. The latter adsorb on the polymeric chain of the gel and also repel K$^+$ ions embedded within the network thereby decreasing the elasticity and strength of the gel.
3.3.5 Morphology of the alginate aggregates in presence of K$_2$CO$_3$ and surfactants (CTAB/16-4-16)

SEM images can be used to study the surface morphology and describe the pattern formation of materials. To have a direct observation of the morphology of the polymer-surfactant aggregates in presence of salt and surfactant, the SEM images of 5% SA, 5% SA + 0.5M K$_2$CO$_3$, 5% SA + 0.5M K$_2$CO$_3$ + 0.1mM CTAB, 5% SA + 0.5M K$_2$CO$_3$ + 0.1mM 16-4-16 were taken and are shown in Fig.3.13. Pure sodium alginate has porous structure with rougher morphology as reported in other studies $^{57-58}$ but in presence of high concentration of salt the microstructures appear like globular bushes with loose connectivity between the individual clustered spheres. Such
assemblies frequently occur in highly cross-linked gels and the morphology appears rougher, denser and less porous because of increase in cross linking between the polymer chains. Upon interaction with either CTAB or 16-4-16, the morphology changed to a smoother sheet like structure with lower porosity. The porosity of gel with 16-4-16 appears more than that of the gel with CTAB which might be because of the loose polymer network in presence of the former.

**Figure. 3.13.** SEM images of: (a) 5% SA; (b) 5% SA+0.5M K$_2$CO$_3$; (c) 5% SA+0.5M K$_2$CO$_3$+0.1 mM CTAB (d) 5% SA +0.5M K$_2$CO$_3$+0.1mM 16-4-16.

### 3.3.6 Swelling measurements

The change in the swelling percentage of the alginate (5% SA + 0.5M K$_2$CO$_3$) gel when immersed in the divalent metallic salt solution (10mM CaCl$_2$) with varying surfactant concentrations is shown in **Fig. 3.14.** The results indicate that the degree of swelling decreases initially with addition of surfactant followed by an increase. The swelling behavior of the ionically cross linked gels is affected by: (i) the osmotic pressure difference between the gel and the outer solution and, (ii) the interaction of
carboxyl and hydroxyl groups with water molecules which leads to opening of alginate structure, making the gel to swell.\textsuperscript{60,61} As already stated, at low concentration the surfactant behaves as a salt and hence screen the electrostatic repulsion between the polymer chains of the gel resulting in reduced dimensions of the gel. Further addition of surfactant leads to its aggregation on the polymer chains, stretching them out due to repulsions between similarly charged aggregates. As a consequence, the polymer networks swell and imbibe more solvent. It could be inferred from Fig. 3.14, that micelles begin to form on the polymer chains above 0.5mM and 0.025mM concentration of CTAB and 16-4-16 surfactants, respectively. Gemini micelles adsorbed on SA chains possess stronger electrostatic repulsion because of higher charge density thereby producing more swelling compared to the single headed CTAB micelles. The morphological results also support the enhanced swelling of the gel with high porosity (SA + 16-4-16) than the gel with low porosity (SA + CTAB).

\textbf{Figure 3. 14.} Swelling percentage of the alginate gels (5\% sodium alginate + 0.5M $K_2CO_3$) with varying surfactant concentrations (a) CTAB (0-4mM) (b) 16-4-16 (0-0.1mM)) in the divalent metallic salt solution (10mM $CaCl_2$) at a pH of 7.4.
3.3.7 Encapsulation and partitioning of IBU in alginate gels

The drug (IBU) encapsulation (6.05%) and loading capacity (0.012) (calculated using Eqs. (2) and (3)) of the alginate gel (SA + 0.5M K₂CO₃) in absence of surfactant decreased to 3.2% and 0.01, respectively, upon addition of 0.1mM CTAB. However, addition of 0.1mM 16-4-16 enhanced the encapsulation and loading capacity of the alginate gel to 22% and 0.06, respectively. Reduction of encapsulation/loading of the 5%SA + 0.5M K₂CO₃ system in presence of 0.1mM CTAB can be explained on the basis of its reduced dimensions as supported by the decrease in viscosity (Fig. 3.1.) and its lower swelling ability (Fig.3.14) compared to that of the systems in absence and presence of 0.1mM 16-4-16. Enhancement of IBU encapsulation/loading of the alginate gel in presence of 0.1mM 16-4-16 (cmc = 0.028mM) can be ascribed to presence of the micellar aggregates on alginate chains which not only leads to swelling of the gel creating more space for the drug but also aid the solubilization of more IBU molecules. The micelle enhanced solubilization is ruled out in alginate gel with 0.1mM CTAB as its viscosity and swelling profiles at this concentration of surfactant points to the absence of micelles on the polymer network.

The fluorescence spectra of IBU in some model solvents H₂O, CH₃OH, (C₂H₅)₂O and CCl₄ differing in their polarities are given in Fig.3.15 (a). The results reveal that the maximum emission wavelength increases with decrease in solvent polarity. The ground state of IBU is stabilized by polar solvents as it involves hydrogen bonding and hence exhibits a negative solvatochromism. Fig.3.15. (b) and (c) depict the changes in emission spectrum of the drug when the gel (5% SA + 0.5M K₂CO₃ + 0.1mM CTAB) or (5% SA + 0.5M K₂CO₃ + 0.1mM 16-4-16) is added to the solution of the drug in methanol. A small addition of the gel, causes increase in IBU emission intensity corresponding to the wavelength of 298nm and at the same time a new band appears at around 370 nm. The results show that the gel brings about solvation of the drug resulting in increase in emission intensity. Moreover, simultaneous appearance of new peak at 370nmis believed to be due to the solubilization of some fraction of IBU molecules within the non-polar regions of the alginate hydrogel. Addition of increasing amounts of the hydrogel increases the intensity of the peak at 370nm with a
corresponding decrease in the peak at 289nm (Fig.3.15). This could be explained on the basis of partitioning of higher fractions of IBU into the non-polar regions of the hydrogel. The increase in peak intensity at 370nm is more in the gemini impregnated gel than the one containing CTAB again indicating more encapsulation of drug in the former. The results highlight the fact that the gel is a better solubilization medium for IBU than methanol and inside the gel IBU has more propensity toward the hydrophobic sites.

3.3.8 Drug release kinetics

The release kinetics of IBU from the alginate gels (5%SA + 0.5M K₂CO₃) in absence and presence of 0.1mM CTAB/16-4-16 was analyzed by plotting cumulative release data versus time as shown in Fig.3.15 (d), fitting the data to Higuchi model as per the equation:

\[ F_t = K_H t^{1/2} \]

where \( K_H \) is the Higuchi dissolution constant and \( F_t = M_t / M_{eq} \) is the amount of drug released in time \( t \). The values of dissolution constant (\( K_H \)) for the alginate gels containing 0mM surfactant, 0.1mM CTAB and 0.1mM 16-4-16 are 0.108, 0.079 and 0.105 min\(^{-1/2}\), respectively, with a regression coefficient >0.97. These results might be due to more crosslinking density and hence low loading capacity of the gel having CTAB than the gel with 16-4-16. It has been found that more crosslinking causes contraction of the polymer chains leading to reduced free void spaces for transport of drug molecules and hence less loading capacity as well as slow release of the drug. Besides this, as the model is based on diffusion, the drug diffuses faster when the gel is in swollen (porous) form than when in shrunken form. The same inference is borne out by the present study as the gel without any surfactant and the one containing gemini with higher degree of swelling allows more diffusion of IBU and a consequent larger dissolution constant than the gel with CTAB.
Figure 3.15. Fluorescence emission spectra of IBU in (a) model solvents (b) in methanol solution with varying amount of the alginate gel having composition 5% SA + 0.5M K$_2$CO$_3$ + 0.1mM CTAB (c) 5% SA + 0.5M K$_2$CO$_3$ + 0.1mM 16-4-16. and (d) Release behavior of IBU from alginate gels with composition 5% sodium alginate + 0.5M K$_2$CO$_3$: without surfactant, with 0.1mM CTAB and 0.1mM 16-4-16. Inset shows fit to release data as per Higuchi model for 0.1mM CTAB.

3.4 Conclusions

The previous reports on biocompatible alginate hydrogels in conjunction with present prototype comparative report on influence of cationic single head-single tailed (CTAB) and double head-double tailed (16-4-16) surfactants have significance to make such materials task specific by tailoring their physicochemical behavior. Rheological investigations revealed that the viscosity of sodium alginate first
Chapter-3  Hydrogels of sodium alginate in cationic..............

decreases and then increases with increase in CTAB/16-4-16 concentration. The viscosity drop upon addition of the surfactants is more in gemini containing alginate system than that of CTAB one, attributed to more charge density of former which lead to more screening of interpolymer association. Moreover, the viscosity of the SA + CTAB as well as SA + 16-4-16 is greatly affected by the addition of salt to the extent that the relative magnitude gets reversed due to more interpolymer association in former system than latter. The increase in salt concentration also results in cryogel formation, which has high thermal stability. The addition of the two surfactants affects the alginate gel differently, gemini improves drug encapsulation and loading but doesn’t improve release behavior while as addition of CTAB reduces the encapsulation and loading capacity but improves the release behavior when compared to alginate gel without surfactant. Therefore, this study highlights the importance of amphiphile chemical structure on gelation characteristics of sodium alginate and encourages the use of wide range of amphiphiles to optimize the alginate gels for desired applications like high encapsulation and delayed release of feeably soluble hydrophobic drugs.
References