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Assemblies of amphiphilic compounds over rigid polymers: 2. Interaction of sodium dodecylbenzenesulfonate with chitosan–scleraldehyde gels

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Gels based on chitosan cross-linked with scleraldehyde were prepared. The swelling behavior due to pH variation and to equilibration with solutions of sodium dodecylbenzene–sulfonate (SDBS) or sodium *p*-toluenesulfonate (*p*-STS) was investigated. Binding isotherms and the solubilization of Sudan III by gels equilibrated with SDBS were also determined. Gel collapse was at a SDBS concentration one order of magnitude smaller than the critical micelle concentration and was accompanied by moderately cooperative binding with K equal to $4120\,M^{-1}$. Binding of *p*-STS, which is characterized by a similar value of K but much smaller cooperativity parameter, was not accompanied by gel collapse. The formation of micelle-like aggregates of SDBS within the gel is discussed in terms of recent theoretical and experimental results.

(Keywords: polysaccharides; gels; assemblies; collapse)

INTRODUCTION

We have recently reported the formation of spherical assemblies characterized by a core composed of a rigid polyelectrolyte, or a charged gel, and a skin membrane formed by complexation of two oppositely charged polyelectrolytes[1]. These assemblies were obtained by letting drops of one polyelectrolyte solution (e.g. chitosan) to free-fall into a solution of an oppositely charged polyion (e.g. sclerox, the acid form of oxidized scleroglucan). Encapsulated gels were based on chitosan cross-linked with the polyaldehyde obtained from controlled periodate oxidation of scleroglucan[1]. Chitosan is the rigid component of the network since the triple helical structure of scleroglucan is lost during the oxidation reaction.

A schematic representation of these assemblies (diameters up to $500\,\mu m$) is given in *Figure 1a*. The possible structure of cross-linked gels is given in figure 2 of reference 1.

The skin of the assemblies was shown to act as a semipermeable membrane allowing the entry of dye molecules such as fluoresceine, or the release of drugs such as disodium cromoglycate. This suggested the possibility of transferring amphiphilic molecules within the interior of the assembly, eventually forming a doubly-assembled supramolecular structure composed of a micelle encapsulated within the primary spherical skin. The resulting assembly (schematized in *Figure 1b*) could serve as a model for the organization of cells containing a nucleus, and might be used to perform a variety of reactions. For instance, by encapsulating enzymes such as lipase, the self-reproduction of the micellar nucleus could be achieved extending the scheme of the autopoietic system endowed of 'minimal life' described by Luisi[2]. Slow release of drugs, produced *within* the assembly also appears conceivable.

As a first step towards these goals, we present here a study of the fundamental core interaction occurring between the chitosan–scleraldehyde gel and an amphiphile such as sodium dodecylbenzene–sulfonate (SDBS), avoiding at this time the use of the external skin. We attempt to describe the results in terms of recent theoretical and experimental investigations concerning the formation of micellar structures within polyelectrolyte networks. In order to obtain a clearer identification of the contributions of electrostatic and hydrophobic interaction we compare the behavior of SDBS with that of sodium *p*-toluenesulfonate (*p*-STS) which has the same charged group but lacks the long apolar tail of the surfactant and, therefore, has no strong tendency to self-aggregation.

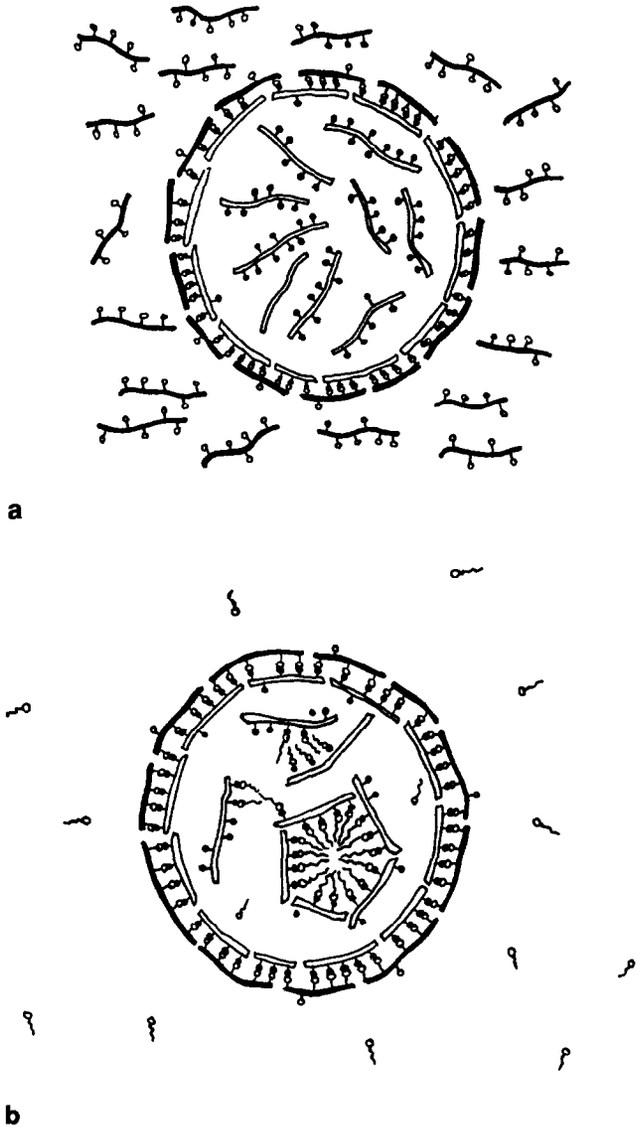

Figure 1 Schematic representation of a spherical assembly formed by oppositely charged polyelectrolytes (a), and of the same including a micelle (b). The charge on the micelle has opposite sign to that of polyelectrolyte enclosed in the spherical assembly

EXPERIMENTAL

Materials

Scleroglucan (Actigum CS-11, obtained from SANOFI Bio-Industries) was sonicated for 60 min at ~100 W to reduce its molecular weight to 5.3×10^5, as estimated from the intrinsic viscosity–molecular weight relation reported by Yanaki *et al.*[3]. Its complete oxidation with $NaIO_4$ under strictly controlled conditions[4] resulted in scleraldehyde (CS-CHO-100). The extent of oxidation was confirmed by titration of the aldehyde[5] and of the carboxylic derivative[6]. Two chitosan samples obtained from Fluka (Chito-FH, $M_w \sim 2 \times 10^6$ and Chito-FL, $M_w \sim 7 \times 10^4$; values given by Fluka) had degrees of acetylation (*d.a.*) 0.14 and 0.20, respectively, as obtained from conductimetric titration. Sodium *p*-toluenesulfonate (*p*-STS) was obtained by treating with NaOH the corresponding acid, *p*-toluenesulfonic monohydrate, obtained from Fluka (purity > 98%). The salt was isolated, recrystallized from a water/ethanol mixture, and dried at 60°C. Sodium dodecylbenzene–sulfonate (SDBS) obtained from Fluka (purity 85%) was further purified by repeated washing with acetone until its yellowish color disappeared and finally dried at 60°C. Sudan III obtained from Fluka was used as received.

Sample preparation

Gels composed of CS-CHO-100 and Chito-FH were prepared starting from a solution of 50 mg (0.162 meq. of CHO groups) of the polyaldehyde, 1.60 ml NaOH 0.1 M and 0.85 ml distilled water (the polymer dissolved under stirring in 3–5 min). To this solution was added 5.88 g of 2% (w/v) chitosan solution in 1.25% HCl (corresponding to 0.597 meq. of NH_2 groups and giving a molar ratio $R = NH_2/CHO = 3.7$). The mixture was homogenized under vigorous agitation until it gellified. Following elimination of air bubbles under vacuum, portions of the gel were cut and immersed for 24 h in 150 ml of 0.02 M NaOH (where deswelling occurred). Samples were then dialyzed and equilibrated in an excess of distilled water first and bidistilled water later, where they were stored. Water in swollen gels was 97%, as determined by drying at 60°C.

Swelling and binding

Weighted amounts of the gel equilibrated in water (~50 mg, surface dried) were put into solutions (~50 ml) of NaOH or HCl at controlled pH, measuring the time variation of the weight of the gel until constant values were obtained. These data were used to construct swelling curves *versus* pH. Swelling and binding with SDBS or *p*-STS were determined starting with gel samples (0.3 g) equilibrated for a few days at pH = 2.80 (where maximum swelling was evident with water uptake, ~99.5%) and then transferred to 5 ml solutions with known initial concentrations (C_i) of surfactant or *p*-STS. After about one week equilibration, the weight of gel (P) and the final concentration of the bathing solution (C_{eq}) were determined. Measurements were performed using SDBS and *p*-STS solutions with controlled pH (pH = 2.80, adjusted with HCl), or at uncontrolled pH resulting from mixing the pH = 2.8 gel with SDBS or *p*-STS solutions. The amount of SDBS or *p*-STS firmly bound to unit weight of the gel was deduced from the difference $(C_i V_i - C_{eq} V_{eq})$ measured for the external solution assuming that C_{eq} represents the concentration of the free solute in the solution both *inside* and *outside* the gel. The release of bound SDBS or *p*-STS as a function of time was determined by placing a known amount of gel (which had been equilibrated for one week with SDBS or *p*-STS) in a 0.04 M NaCl solution and following the corresponding increase of the C_{eq} concentration. A complex of Chito-FL and SDBS was prepared by adding 80 ml of 0.05 M SDBS to 20 g of 2% chitosan solution in 1.25% HCl and stirring over 48 h.

The white solid obtained was washed with water and dried at 50°C. Samples of gels swollen in water (at neutral and acid pH), of gels swollen in acid solution (pH = 2.8) and equilibrated with SDBS solutions having $C_i = 0.0017$ M and of the chitosan–SDBS complex were exposed to fine suspensions of Sudan III prepared by mixing 5.96 mg of the dye with 25 ml H_2O. An analytical balance (±0.00001 g) and a Hewlett-Packard u.v. spectrophotometer (mod. 8452) were used for the determination of the weight of the gels and of the concentration of SDBS or *p*-STS (through calibration curves), respectively. Swelling is characterized by the ratio P/P_0 of the weight of the swollen gel (P) to the corresponding dry weight of the polymeric network (P_0). The polymer weight fraction $w_p = P_0/(P_0 + P_s) = P_0/P$, where P_s is the weight of adsorbed solvent and is alternatively given. All measurements were performed at room temperature (~25°C).

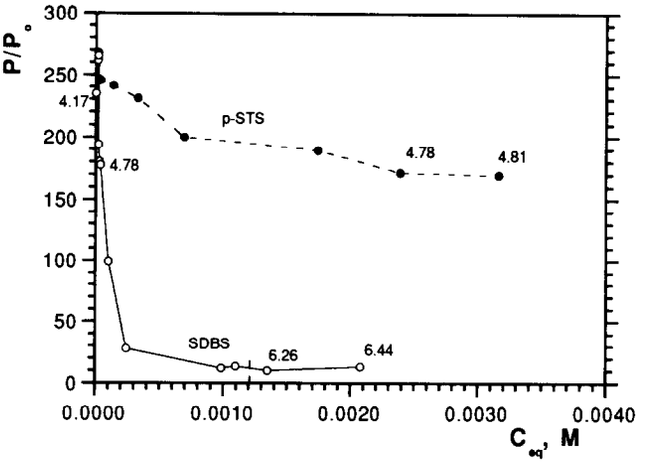

Figure 3 Variation of the swelling of chitosan–scleraldehyde gels with the equilibrium concentration of the SDBS/water and *p*-STS/water excess bathing solution; pH values are indicated. The bar indicates the c.m.c. for SDBS in water

RESULTS

Figure 2 illustrates the pH variation of gel swelling in the absence of SDBS or *p*-STS. The amount of swelling characterized by a value of $w_p \sim 0.03$ at neutral pH increases to a maximum of $w_p \sim 0.006$ at pH = 2.80. It takes more than 72 h equilibration to attain the latter value upon changing the pH of the external solution from 6.5 to 2.80. However, if the gel at pH = 2.80 with $w_p \sim 0.006$ is transferred to NaOH solutions at pH = 10.7, deswelling to a value of $w_p \sim 0.03$ occurs in only 30 min. Moreover, if the gel at pH = 2.80 with $w_p \sim 0.006$ is transferred to pure water under conditions in which a pH = 4.2 is attained, swelling increases to a value of $w_p \sim 0.0043$ (compare the maximum swellings in *Figures 2* and *3*). These peculiar variations are not simply explained, but we feel that the rate at which the gel responds to pH variation is controlled not only by the ionization equilibrium of NH_2 groups, but also by the slow melting of residual crystalline regions within uncharged chitosan. The maximum degree of charged NH_3^+ groups may in fact

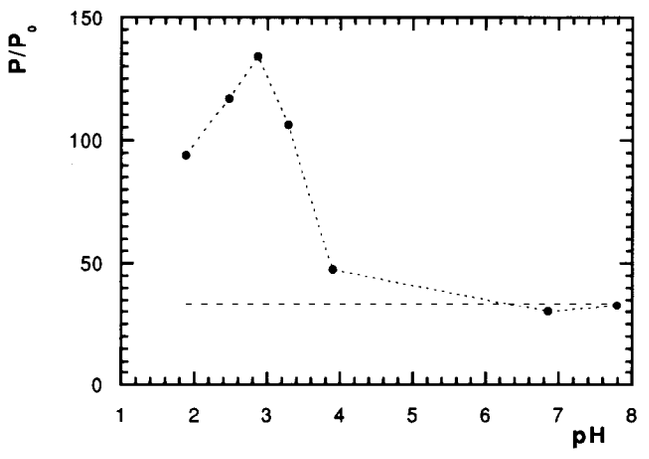

Figure 2 Variation of the swelling of chitosan–scleraldehyde gels with pH

be reached at a pH somewhat larger than 2.80 for the completely amorphous gel. It is worth remembering that the *d.a.* of the chitosan sample used for the gels is 0.14 and from the theoretically possible 86% NH_3^+ groups, 23% are excluded due to the cross-linking reaction with scleraldehyde (the ratio R of NH_2/CHO groups is 3.7).

Figure 3 illustrates the variation of swelling with the concentration of SDBS or *p*-STS. Before transfer to each surfactant or *p*-STS solution, gels were conditioned at pH = 2.80 in order to attain complete charging, melting and swelling. The deswelling caused by *p*-STS is much less pronounced than that caused by SDBS and the comparison of the two curves affords evidence for the reinforcement of the electrostatic interaction between NH_3^+ and SO_3^- groups, present in both compounds, by the aliphatic tail of SDBS. The deswelling due to SDBS is catastrophic (gel collapse) with w_p increasing from ~0.005 to ~0.05 already at $C_{eq} < 0.0002$ M, which is smaller than the c.m.c. of the surfactant in pure water. With SDBS, values of w_p even smaller than those observed in pure water at neutral or alkaline pH are obtained. Note, however, that even at the largest SDBS concentration investigated the gel retains large amounts of diluent ($w_p < 0.1$).

Not much difference is seen between the swelling behavior corresponding to situations under which the pH is kept at the 2.80 value or when the gel is transferred to SDBS or *p*-STS solutions at their natural pH values (~6.6 and 4.8, respectively). At very low SDBS or *p*-STS concentrations a small increase of swelling (preceding the collapse) is seen. A similar effect, which is poorly understood, was noticed with collagen/salt[7] and collagen/SDS[8] systems under non-isoelectrical conditions.

Figure 4 illustrates the amount of SDBS or *p*-STS bound per gram of gel plotted as a function of the supernatant equilibrium solution C_{eq}. The amount of SDBS adsorbed is much larger than that of *p*-STS and

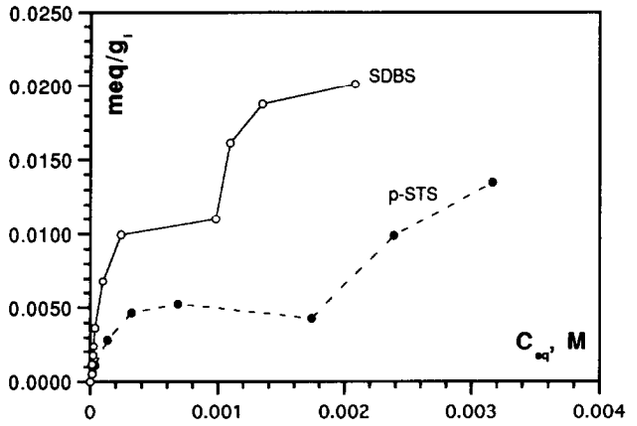

Figure 4 Amount of SDBS and *p*-STS bound to chitosan–scleraldehyde gels equilibrated with corresponding SDBS and *p*-STS aqueous solutions

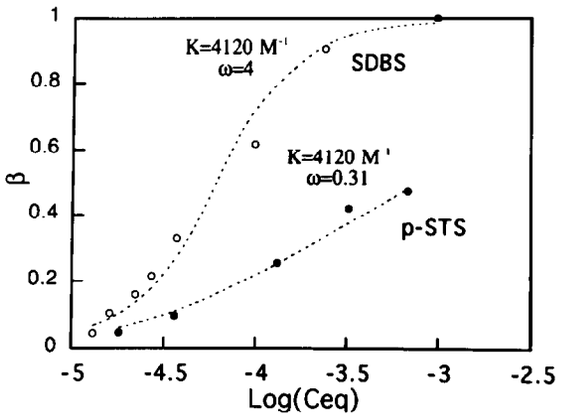

Figure 5 Binding isotherms calculated from equation (2) using the data in *Figure 4*

shows two consecutive binding steps characterized by a steep cooperative-type rise and a saturation plateau.

To evaluate equilibrium constants and cooperativity parameters, data were analyzed using the relationships:

$$K = \frac{v/n}{(1 - v/n)C_{eq}} \quad (1)$$

$$\beta = \frac{1 + (K\omega C_{eq} - 1)/[(1 - K\omega C_{eq})^2 + 4KC_{eq}]^{1/2}}{2} \quad (2)$$

In equation (1)[9,10], v is the average number of SDBS bound per mole of chitosan having a total number (n) of binding sites. K and n can be deduced from plots of v/C_{eq} *versus* v and a linear relationship is expected only if there is one type of sites and no cooperativity. In fact these plots did not allow a satisfactory determination of K.

In equation (2)[11], β is the fractional binding saturation and ω is the cooperativity parameter, so that $K\omega$ represents the equilibrium constant for binding to a site adjacent to an occupied one. The concentration at the midpoint saturation ($\beta = 0.5$) obeys the equalities $C'_{eq} = (K\omega)^{-1}$ and $d\beta/d\ln(C'_{eq}) = (\omega^{1/2})/4$. Plots of β *versus* the log of the free SDBS concentration, given in *Figure 5*, reveal the good fitting between theory and experiment. For these calculations, only data corresponding to the first binding step were considered and the values of β are not affected by normalization using the plateau value or the known number of NH_3^+ binding sites with a 1:1 stoichiometry. The best values of K and ω (respectively, 4120 M^{-1} and 4 for SDBS, and 4120 and 0.31 for *p*-STS) were obtained by iteration using the product $K\omega$ at midpoint saturation and the initial slopes yielding ω. The product $K\omega$ for SDBS is comparable to that reported by Wei and Hudson[12] (~22 000 M^{-1}) for soluble chitosan and SDS. It appears that the data for SDBS and *p*-STS can be fitted with the same value of K, the difference residing only in the larger cooperativity shown by SDBS with respect to *p*-STS.

Figure 6 shows how strongly bound to the gel

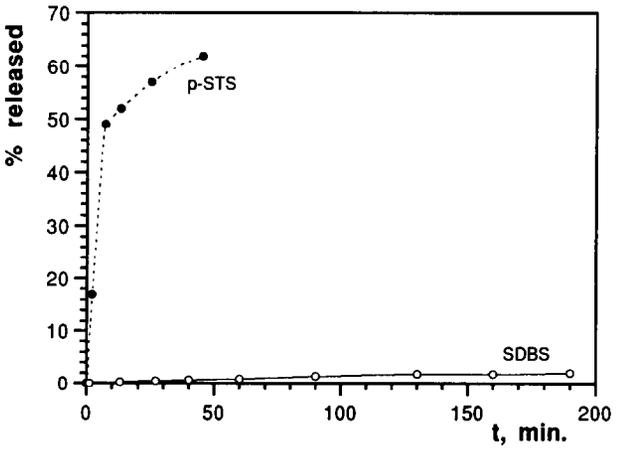

Figure 6 Time course of the release of *p*-STS and SDBS in excess 0.04 M NaCl aqueous solution following the equilibration indicated in *Figure 4*

structure SDBS has become with respect to *p*-STS. Less than 2% SDBS is released after 3 h exposure to a surfactant-free dilute NaCl solution. Over 50% of *p*-STS adsorbed is instead released during the first 10 min exposure to the *p*-STS-free solution.

The result of the exposure of the gels to a suspension of Sudan III revealed an intense orange coloration for gels containing SDBS (these gels were extensively washed with water before and after exposure to the dye and then surface dried to prevent any spurious effect due to deposition of free micelles over the gel surface). Similar effects were shown by a chitosan–SDBS complex. A similar but less intense coloration was shown by gels swollen in water (without SDBS) at neutral pH. No coloration was instead evident for gels at pH = 2.8 without SDBS.

DISCUSSION

It is appropriate to analyze the present data in the light of results pertaining to the binding and organization of surfactants over polyelectrolytes. Micellization of amphiphilic compounds over a charged polymer substrate has been described by several authors both in

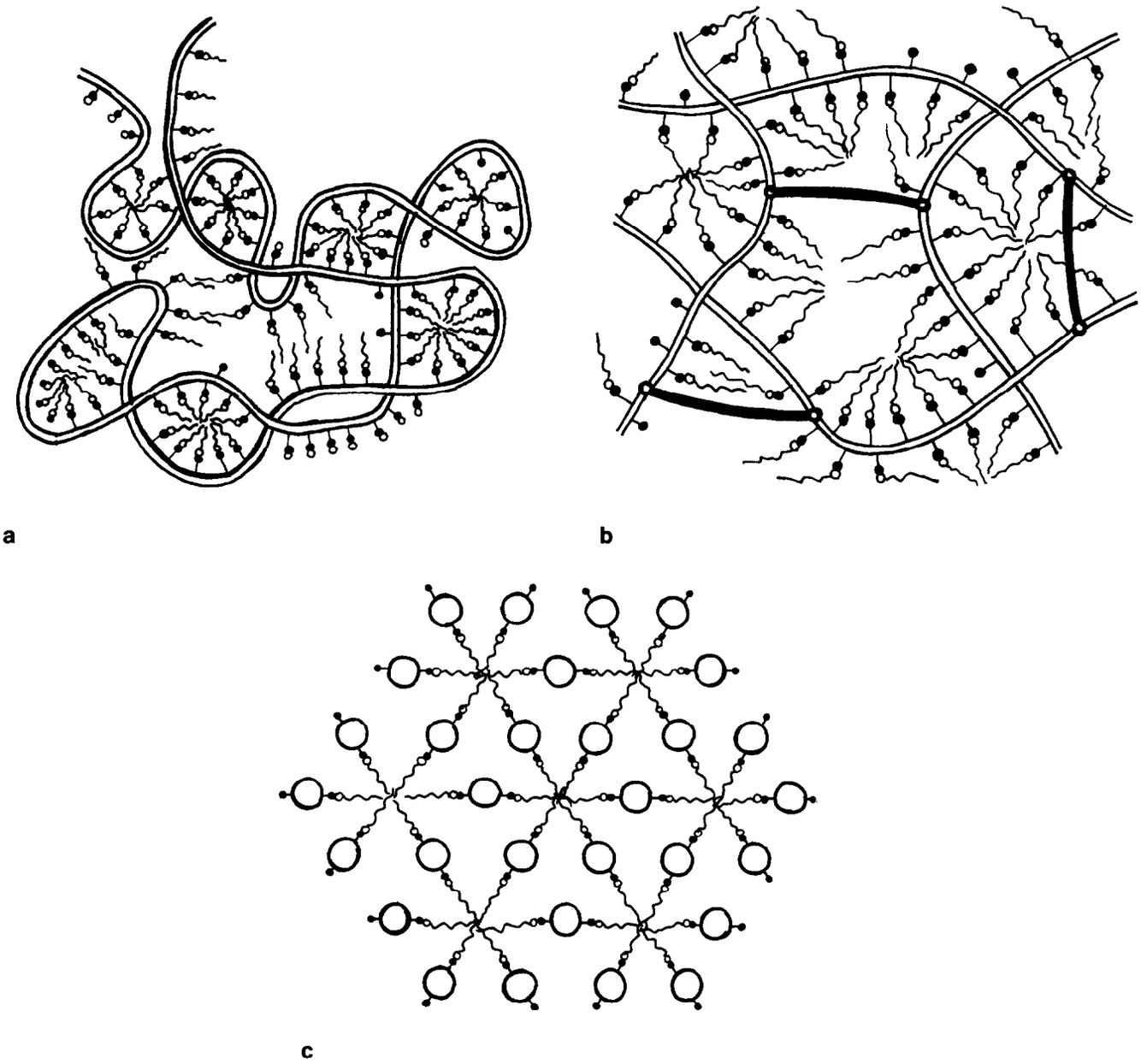

Figure 7 Schematic representation of the assembly of surfactants over charged flexible chains (a), flexible networks (b) and rigid helices (c)

the case of flexible[13–19] and rigid chains[8, 12, 20–23]. In the case of alkyltrimethyl–ammonium bromide and anionic poly(methacrilyc acid) (PMMA), micelle-like aggregates composed of about 100 surfactant molecules per molecule of PMMA were observed at surfactant concentrations 1 to 2 orders of magnitude smaller than its c.m.c. in water[14]. A possible schematization of the clustering of surfactant over a coiling polymer is given in *Figure 7a*. Okuzaki and Osada[17] have compared the SAXD behavior of linear and cross-linked poly[2-(acrylamide)-2-methylpropanesulfonic acid] and N-dodecylpyridinium chloride, and concluded that large clusters having almost twice the usual micellar diameter occur, provided the degree of binding is larger than 67% for the linear polymer and 79% for the gel. Cross-linking appeared to enhance the value of K and reduce the cooperativity parameter. This effect is confirmed by the comparison of the present data with those for soluble chitosan and SDS[12], for which values of K ca. $1000\,M^{-1}$ and $\omega = 23$ were reported.

The charged polymer–surfactant interaction is usually accompanied by a large deswelling or collapse of the gels. Khoklov *et al.*[18] have offered a theoretical description of this collapse in terms of micelle formation within the meshes of polyelectrolyte networks (cf. schematization in *Figure 7b*). When the amphiphilic counterions of the fixed charges become organized in a micellar structure, they no longer contribute to the Donnan effect, and swelling may decrease to the value of the uncharged network. The lack of a transitional entropy loss due to the usual immobilization of counterions around the micelle (when the latter is screened by the fixed charges) also explains a lower value of the c.m.c. within the gels. Micellar formation by cetylpyridinium bromide within gels of copolymers of sodium methacrylate and acrylamide was suggested by Khoklov *et al.*[19].

In the case of rigid polyelectrolytes, such as chitosan (used here), surfactant organization may be expected to reflect the difficulty of the fixed charges to loop around spherical micelles. Ciferri[21,22] has discussed the type of supramolecular assembly expected when an amphiphile such as a dodecylpyridinium cation interacts with DNA, based on adsorption isotherms reported by Shirakawa *et al*[20]. These isotherms exhibited two binding steps (analogous to the behavior shown by the present data). The first binding step was attributed to formation of a first layer of hydrophobic tails around each DNA molecule, followed by their interdigitation during the second step, thus producing a cylindrical micelle-like unit able to replicate along the longitudinal and transverse directions (cf. schematization in *Figure 7c*). However, experimental results[8] for the collagen/SDS system revealed strong gel collapse but failed to reveal high cooperativity in the binding process or the occurrence of ordered cylindrical assemblies. This result was attributed to the low charge density of the collagen triple helix, resulting in binding sites too far apart for hydrophobic interaction between the SDS tails to cooperatively develop. Wei and Hudson[12], working with the chitosan/SDS system, had in fact evidenced that the cooperativity parameter of the binding process decreases when the degree of acetylation increased from 0.08 to 0.24, corresponding to an increased separation between fixed charges.

It may be assumed that when the charge density is low the hydrophobic tails may fall back and interact with the less polar section of the polymer chain. This interaction reinforces the binding constant but may prevent a cooperative hydrophobic interaction leading to micellization. Moreover, the reinforced coulombic interaction will reduce Donnan swelling due to an effective reduction of the fixed charges, while additional deswelling is promoted by the increased hydrophobicity of the bound polymer chain. Thus, these interactions provide an explanation of gel collapse induced by surfactants, which is alternative to that proposed by Khoklov *et al.*[18], who based the reduction of Donnan swelling on the micellization of surfactant, rather than on the strong site binding.

The present results reveal that gel collapse due to SDBS is virtually complete at $C_{eq} < 0.0002\,M$, when the first step increase of surfactant adsorption also occurs. From *Figure 5*, the value of β corresponding to $C_{eq} \sim 0.0002\,M$ is $\leqslant 0.9$. Since the value of ω is a relatively modest one, the number of clustered molecules, m, tends to be rather small. In fact, using the equation[11,17,18].

$$m = 2\beta(\omega - 1)/\{[4\beta(1-\beta)(\omega - 1) + 1]^{1/2} - 1\} \qquad (3)$$

a value of $m = 12.6$ can be calculated at $\beta = 0.9$. This is suggestive of small discrete aggregates (m diverges to large values only for $\beta > 0.95$). Therefore, it appears the gel collapse occurs when 90% binding sites are saturated while micelles of the type envisioned in references 20 and 21 may occur past the saturation and during the second step increase of surfactant adsorption.

Other features of the present results are not accounted for by the theoretical description of Khoklov *et al.*[18]. One is the occurrence of strong binding, which amounts to a more effective neutralization of the fixed charges on the polymer than is the case with simple screening. Thus, the micelle is more strongly bound to the polymer than anticipated. Moreover, the extremely slow release of SDBS by the gel reveals a locked-in structure due to an unexpectedly strong binding and the possible formation of crystalline regions involving neutralized chitosan. Another unpredicted feature is the observation that, due to the increased hydrophobicity resulting from SDBS adsorption, swelling attains values *smaller* than those of the uncharged network. Finally, the present network is based on rigid chains and this may affect the shape of the aggregate[21,22]. Verification of the occurrence of micellar aggregation and its morphology should ideally be sought with specific techniques[23], including SAXS[17,24].

In spite of strong binding, gel collapse and the difficulty in releasing the surfactant, the observation that a large amount of water is retained by the gels encourages our project of preparing functional supramolecular assemblies[22] enclosed by a semipermeable skin (cf. Introduction and *Figure 1b*). Work along these lines is in progress and it shall be reported at a later date.

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