

Biogenic amine – surfactant interactions at the air-water interface

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Abstract

The strong interaction between polyamines and anionic surfactants results in pronounced adsorption at the air-water interface and can lead to the formation of layered surface structures. The transition from monolayer adsorption to more complex surface structures depends upon solution pH, and the structure and molecular weight of the polyamine.

The effects of manipulating the polyamine molecular weight and structure on the adsorption of the anionic surfactant sodium dodecyl sulphate at the air-water interface are investigated using neutron reflectivity and surface tension, for the biogenic amines putrescine, spermidine and spermine.

The results show how changing the number of amine groups and the spacing between the amine groups impacts upon the surface adsorption. At lower pH, 3 to 7, and for the higher molecular weight polyamines, spermidine and spermine, ordered multilayer structures are observed. For putrescine at all pH and for spermidine and spermine at high pH, monolayer adsorption with enhanced surfactant adsorption compared to the pure surfactant is observed. The data for the biogenic amines, when compared with similar data for the polyamines ethylenediamine, diethylenetriamine and triethylenetetramine, indicate that the spacing between amine groups is more optimal for the formation of ordered surface multilayer structures.

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Introduction.

Biogenic amines or polyamines, such as putrescine, spermidine and spermine, important in a range of biochemical functions (1-3). Polyamines are small flexible polycations, and are involved in a variety of physiological roles associated with cell growth and proliferation. Their interaction with different charged species in bio-macromolecules such as DNA and in the cell membrane is of central importance. As such they are associated with DNA compaction and precipitation (4-6), protein folding and unfolding (7) and have been exploited in the formation of polyplexes for potential gene delivery vehicles associated with gene therapy (8-10).

The focus of this paper is on the surface interaction between the biogenic amines and the anionic surfactant sodium dodecyl sulphate, SDS. Although this focus is rather specific, how the surface interaction is affected by the structure and molecular weight, MW, of the polyamine has much broader biological and technological significance and implications. In this broader context the poly(ethyleneimine), PEI, based polymers and the polyamines are important polyelectrolytes because of their widespread applications, and so have been extensively studied (11). Hence the surface adsorption behaviour of polyelectrolyte / surfactant mixtures has also been extensively studied (12, 13). Depending upon the nature of the polyelectrolyte-surfactant interaction enhanced absorption in the form of a monolayer occurs down to relatively low surfactant concentrations due to polyelectrolyte-surfactant surface complexation. In many cases, close to charge neutralisation where the solutions are cloudy and precipitation / coacervation occurs, the surface structure is more complex and ordered layered structures from a trilayer to multiple bilayer structures are formed or adsorb at the interface. Recent studies attributed the surface multilayer formation to a wetting of the surface by a more surface active concentrated precipitated / coacervated phase which is highly surface active and has a lower surface tension than the coexisting dilute phase (14). This surface ordering phenomenon and the systems where only monolayer adsorption accompanied by the partial desorption that occurs in the region of charge neutralisation are now described by a full thermodynamic treatment (15).

PEI is a particularly important polyelectrolyte, in which the nature of its interaction with surfactant varies with pH, MW, and polymer architecture (branched or linear) (16-18). In combination with SDS PEI exhibits the full range of surface properties summarised in the previous paragraph. However, a notable feature is that the PEI-SDS interaction is strong at low pH when the polyelectrolyte is highly charged, and is equally strong at high pH when the polyelectrolyte is essentially neutral (16). From surface adsorption studies on SDS with a range of oligoamines (from ethylenediamine to pentaethylenhexamine) Penfold et al (19) attributed the nature of the interaction at high pH to a combination of an ion-dipole interaction between the sulphate headgroup of the SDS and the amine nitrogen group and the inter-alkyl chain interaction between neighbouring SDS molecules. Similar observations were made by Winnik et al (20, 21), Sherstenin et al (22), who reported evidence for electrostatic and non-electrostatic contributions to the binding of SDS to PEI. This is further supported by Jon and Chang (23) who described an additional interaction between SDS and amine modified polymers in the form of a ion-dipole interaction, and Ogawa et al (24) who discussed the role of cation-dipole interactions in the layer-by-layer assembly of poly(lactic) acid and polylysine films.

Of direct relevance to the study reported here, the recent measurements (25-27) on a range of small linear and branched oligoamines with SDS have illustrated the importance of the oligoamine architecture, MW and charge density on the oligoamine / SDS adsorption. In particular the results demonstrate the extent to which these parameters affect the occurrence of monolayer and multilayer adsorption at the interface. The lower charge density and greater rigidity of the branched oligoamines results in multilayer formation at the interface with SDS at low pH; whereas for the linear oligoamines multilayer formation occurs at higher pH (25). For the linear oligoamines with SDS multilayer formation is strongly dependent upon the oligoamine MW. If the MW is too low multilayer formation is not optimal due to a lack of cooperativity, and does not occur at much higher MW's because of the greater entropic penalty arising from the flexibility of the polymer chain (26). At the lower MW's, ethylenediamine to pentaethylenetetramine, there is a distinct switch in the pH dependence of the surface multilayer formation with MW (27). For ethylenediamine, diethylenetriamine, and triethylenetetramine, multilayer formation occurs at pH 3, and not

at pH 7 and 10. For the slightly higher MW oligoamines, tetraethylenepentamine and penatethylenhexamine, multilayer formation is predominantly observed at pH 7 and 10, and at pH 3 only monolayer adsorption occurs. This illustrates the importance of the balance between the electrostatic attraction between the SDS headgroup and the amine nitrogen groups, the repulsion between neighbouring SDS headgroups and the cooperative interaction between the neighbouring alkyl chains of the bound SDS molecules. It is this aspect which is explored in more detail here in this paper, by varying the distance between the amine groups in equivalent oligoamines and biogenic amines. By comparing previous results for ethylenediamine, diethylenetriamine, and triethylenetetramine (25-27) with those for putrescine, spermidine and spermine reported here, the importance of the competition between the major factors controlling the structure of the adsorbed layer is probed in more detail.

The formation of surface multilayer structures in surfactant, polymer and polymer-surfactant systems is increasingly encountered in a range of biological and technological circumstances. They are implicated in a range of diverse applications and potential applications, in lung surfactants (28, 29), tissue lubrication (30, 31), in fabric and hair conditioning (32), and in the potential for increasingly efficient detergency, soft lubrication, efficient delivery of active agents such as perfumes and anti-microbial/anti-bacterial agents and in encapsulation. Recent studies on the formation of surface multilayer structures in ionic surfactants induced by multivalent counterions (33-36) closely relate to the studies on the effects on surfactant adsorption of the polyamines and the biogenic amines which can be considered as flexible multivalent cations. Hence the results reported here are aimed at extending our understanding of the polyamine-surfactant interaction and the impact upon surface adsorption, and relate to both the biological function of biogenic amines and the diverse potential technological applications.

In this paper the determination of the surface adsorption behaviour of biogenic amine (putrescine, spermidine, and spermine) / SDS mixtures at the air-water interface, measured over a wide range of surfactant and biogenic amine concentrations and pH by NR and ST, are reported.

Materials and Methods.

(i) Materials and measurements made

The biogenic amines putrescine, spermidine and spermine (1,4-diaminobutane, 1,5,10-triazadecane, and 1,12-dodecanedamine) were obtained from Sigma-Aldrich (>97% purity) and used as received. The structure of the biogenic amines is shown in figure 1a, and the corresponding equivalent polyamines ethylenediamine (diamine), diethylenetriamine (triamine) and triethylenetetramine (tetramine) are shown in figure 1b.

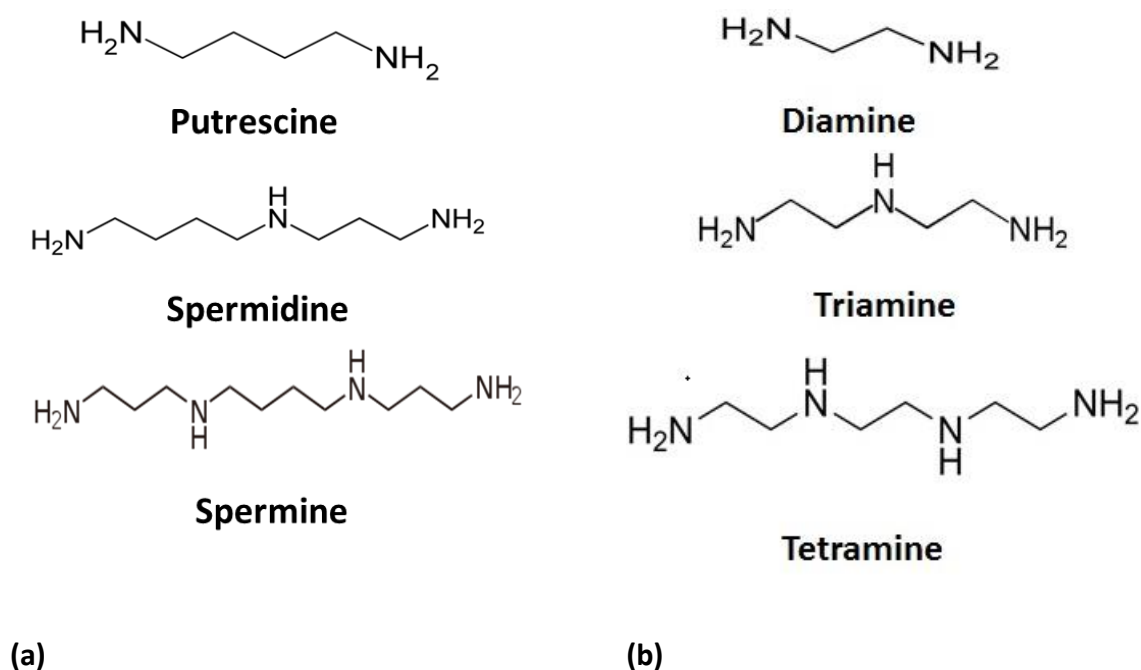


Figure 1. Structure of (a) biogenic amines putrescine, spermidine and spermine, (b) diamine, triamine and tetramine.

The alkyl chain deuterium labelled SDS (d-SDS) was obtained from the Oxford Isotope Facility (37) and purified before use by repeated recrystallization from ethanol (38). The chemical purity of the surfactant was verified by surface tension, ST, where no minimum was observed about the critical micellar concentration, cmc, and from the adsorbed amount

above the cmc measured by neutron reflectivity, NR. Deuterium oxide, D₂O, was obtained from Sigma-Aldrich and high purity water (Elga Ultrapure) was used throughout. The solution pH was adjusted by the addition of aqueous hydrochloric acid (5.0 M) or aqueous sodium hydroxide solution (5.0 M). The glassware and Teflon troughs used in the NR and ST measurements and sample preparation were cleaned in alkali detergent (Decon 90) and rinsed thoroughly in high purity water before use. Stock solutions of the biogenic amines and the d-SDS were prepared and the biogenic amine / SDS solutions mixed from the stock solutions. All solutions were left for at least 12 hours to equilibrate before any measurements were made. The measurements were all made at 25 °C.

The neutron reflection, NR, measurements were all made for the polyamine / d-SDS solutions in null reflecting water, nrw (92 mole% H₂O / D₂O mixture) at pH 3, 7, and 10 for a biogenic amine concentration of 5 mM and SDS concentrations from 10⁻⁴ to 10⁻² M for the biogenic amines putrescine, spermidine, and spermine. For spermine at pH 7 and spermidine at pH 3 and 7 measurements were also made at a fixed SDS concentration of 5 mM and biogenic amine concentrations from 0.5 to 10 mM. The ST measurements were made for 5 mM spermidine / SDS mixtures at pH 7 and 10, and for 5 mM putrescine / SDS mixtures at pH 7 and 10, and over a wide range of SDS concentrations.

(ii) Surface tension

The surface tension, ST, measurements were made on a Kruss K11 maximum pull digital tensiometer, using a platinum-iridium ring and the deNouy method. Before each measurement the ring was rinsed in high purity water and dried in a Bunsen flame. The tensiometer was calibrated to a value of 72 mN/m in high purity water and the temperature controlled to 25 ± 0.2 °C. The solutions were partially covered to reduce evaporation. Repeated measurements were made, for a lapse time of up to ~ 2 hours depending upon the solution, until the variation in the ST was < 0.2 mN/m.

(iii) Neutron reflectivity

The neutron reflectivity measurements were made on the SURF (39) and INTER (40) reflectometers at the ISIS neutron source. The reflectivity was measured over a wide wave vector transfer, Q , range (where $Q=(4\pi\sin\theta)/\lambda$, θ is the grazing angle of incidence and λ the

neutron wavelength). On INTER the usable Q range was ~ 0.03 to 0.3 \AA^{-1} , with a θ of 2.3° and a λ range of ~ 1 to 15 \AA . On SURF a slightly different Q range ~ 0.045 to 0.5 \AA^{-1} was used, for a θ of 1.5° and a λ range of ~ 0.5 to 7 \AA . The reflectivity was calibrated with respect to the direct beam intensity and from the reflectivity from a D_2O surface. The measurements were all made in nrw in sealed Teflon troughs at 25°C and with sample volumes $\sim 25 \text{ mL}$. Each NR measurement took ~ 15 to 30 minutes, and the measurements were made sequentially in a 5 or 7 position sample changer. The measurements were repeated ~ 3 to 4 times, which represented a total lapse time of 4 - 6 hours, by which time the reflectivity profiles had reached a steady-state.

In the kinematic approximation (41) the reflectivity is related to the square of the Fourier transform of the scattering length density profile $\rho(z)$ normal to the surface ($\rho(z) = \sum_i n_i(z) b_i$, where n_i and b_i are the number density and scattering length of the i^{th} component, and $\rho(z)$ is related to the neutron reflective index by $n(z) = 1 - \frac{\lambda^2 \rho(z)}{2\pi}$). Hence, the reflectivity can be written as,

$$R(Q) = \frac{16\pi^2}{Q^2} \left| \int \rho(z) e^{-iQz} dz \right|^2 \quad (1)$$

$\rho(z)$ can be manipulated by deuterium labelling (H and D have different scattering lengths, $-3.7 \times 10^{-6} \text{ \AA}$ for H and $6.67 \times 10^{-5} \text{ \AA}$ for D) such that the reflectivity can provide directly information about adsorption at the interface and the structure of the adsorbed layer. This approach has been extensively exploited for a wide range of surfactants (41) and polymer-surfactant (12) systems. The scattering lengths of the different components used in this study are listed in table 1.

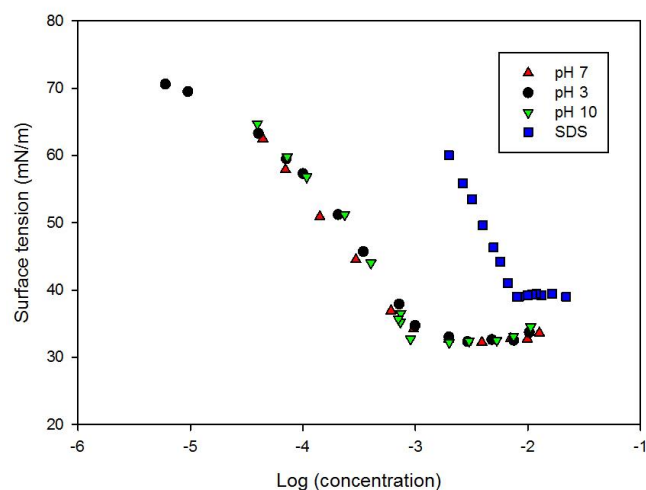
Table 1. Scattering lengths for the different components in this study

Component	Scattering length, $\sum b \text{ (\AA)}$
nrw	0.0
D_2O	1.92×10^{-4}
d-SDS	2.76×10^{-3}
putrescine	0.03×10^{-4}
spermidine	0.33×10^{-4}
spermine	0.67×10^{-4}

Results and Discussion

(i) Surface tension

Surface tension measurements were made for SDS mixed with 5 mM putrescine and 5 mM spermidine at pH 3, 7 and 10, and are shown in figure 2.



(a)

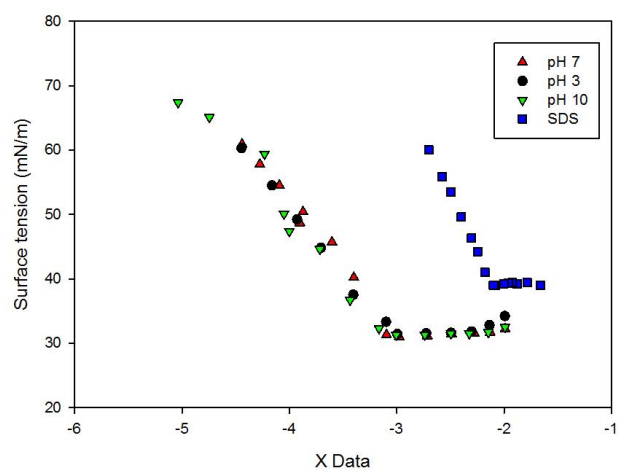


Figure 2. Surface tension for (a) SDS / 5 mM putrescine, (b) SDS / 5 mM spermidine, at (●) pH 3, (▲) pH 7, (▼) pH 10, and (■) for SDS in the absence of biogenic amines. The errors in the ST values (not shown) are <0.2 mN/m.

The ST data for SDS / putrescine and SDS / spermdine are broadly similar, and independent of pH. Compared to the data for SDS in the absence of biogenic amine the initial decrease in the ST in the prescence of putrescine or spermidine is shifted to lower SDS concentrations and the plateau in the ST is at a lower value, ~ 30 mN/m, compared to ~ 40 mN/m for SDS. This implies a strong interaction between the SDS and biogenic amine which is largely independent of pH and MW. The initial slope of the ST curve is also more gradual in the presence of the biogenic amine than is observed for pure SDS. At higher SDS concentrations, towards ~ 10 mM, the ST shows a gradual increase towards the value for SDS in the absence of biogenic amine. The ST data presented here are different to that previously observed for PEI/SDS (16-18) and oligoamine / SDS mixtures (19, 26, 27). The ST data has broad similarities to that reported by Xu et al (35, 36) for the impact of the trivalent counterion Al^{3+} on the surface tension of the anionic surfactant SLES. The upturn in the ST in the presence of biogenic amines at higher SDS concentrations is not usually observed in measurements in the presence of electrolytes such as NaCl, which are usually made at much higher electrolyte concentrations (~ 100 mM). The upturn is a direct consequence of the measurements being made at a low fixed biogenic amine concentration, 5 mM; and at the higher SDS concentrations the SDS is in significant excess compared to the biogenic amines, as discussed by Xu et al (35, 36).

In addition to the ST measurements the physical state of the solutions was observed and recorded. For the SDS / spermidine mixtures the solutions were clear at pH 3 and 7 over the SDS concentration range measured, and at pH 10 cloudy over a narrow concentration range, from 1.83 to 3.2 mM. For the SDS / putrescine mixtures the solutions were cloudy at all 3 pH values and over SDS concentration ranges of 2.91 to 7.22 mM at pH 3, 1.98 to 9.9 mM at pH 7, and at 2.0 to 5.29 mM at pH 10.

(ii) Neutron reflectivity.

Neutron reflectivity measurements were made for d-SDS / biogenic amine mixtures at the air-nrw interface. The measurements were made for 5 mM putrescine, spermidine, and spermine at pH 3, 7 and 10, over a range of SDS concentrations from 10^{-4} to 10^{-2} M. Further measurements were made at a fixed SDS concentration of 5 mM and a range of spermidine and spermine concentrations, from 0.5 to 10 mM, at pH 3 and 7 for spermidine and at pH 7

for spermine. The data are characterised by two distinctly different forms of reflectivity which are observed, as represented in figure 3 for 5 mM SDS / 10mM spermidine at pH 7 and for 5mM SDS / 2 mM spermidine at pH 7.

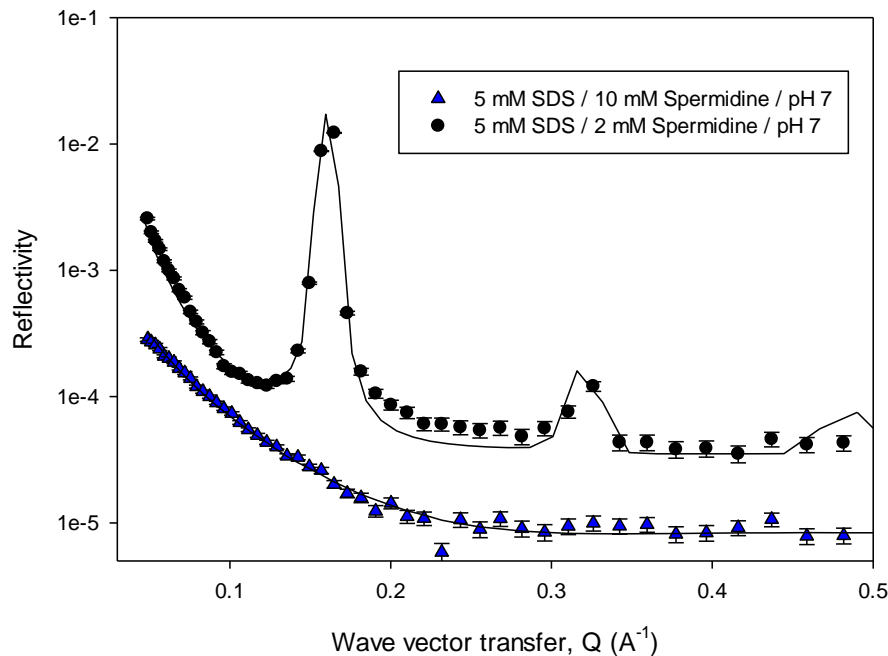
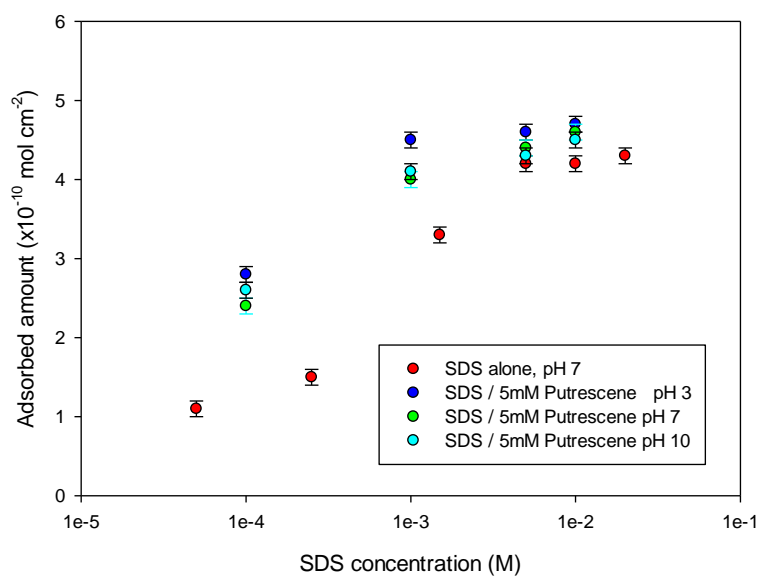


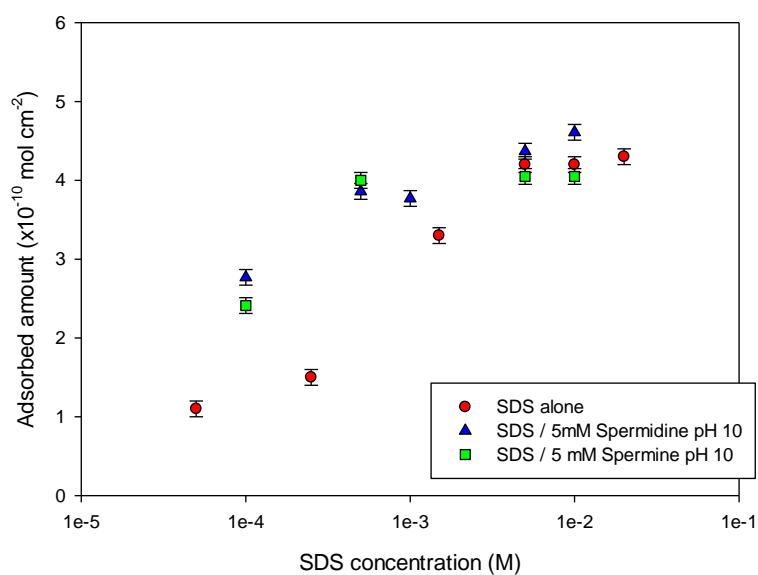
Figure 3. Neutron reflectivity data for (▲) 5 mM d-SDS / 10 mM spermidine at pH 7 and (●) 5 mM d-SDS / 2 mM spermidine at pH 7. The solid lines are model calculations, as described in the main text.

The NR data for 5 mM SDS / 2 mM spermidine at pH 7 are consistent with a thin uniform layer of predominantly SDS adsorbed at the interface. The data in this form are analysed using the simplest model consistent with the data, and fitted by a least squares algorithm using calculated reflectivities based on the exact optical matrix method (41, 42). In this case a single layer of uniform composition is sufficient to describe the data, and this provides a thickness, d , and a scattering length density, ρ . On the assumption that the reflectivity is dominated by the d-SDS adsorbed at the interface, and that the contribution from the polyamine is small (18, 25-27), the area/molecule, A , of the SDS in the surface layer is given by $A = \sum b / d\rho$, where $\sum b$ is the scattering length for d-SDS as listed in table 1, and the adsorbed amount is $\Gamma = 1/NaA$ (Na is Avogadro's number). At an area/molecule $\sim 50 \text{ \AA}^2$ the typical statistical / systematic error is $\sim \pm 2 \text{ \AA}^2$ (41), and the errors quoted here are on that

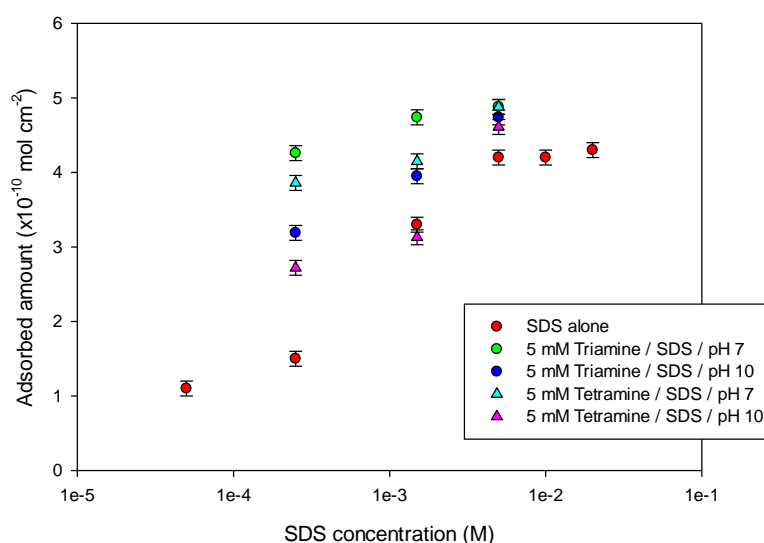
basis. There will be a finite but small contribution from the polyamine component at the interface, which can be largely neglected. For example, a stoichiometry of one SDS molecule per polyamine results in an ~2% contribution from spermine (which has the largest Σb values, see table 1, of the biogenic amines studied) when compared to the Σb for d-SDS. Hence in general the contribution will be $\ll 2\%$.



(a)



(b)



(c)

Figure 4. Variation in SDS adsorption for (a) SDS / 5 mM putrescine, at pH 3, 7 and 10, (b) SDS / 5 mM spermidine (spermine) at pH 10, (c) SDS / 5mM triamine (tetramine) at pH 7 and 10; see legend for details

The NR data in figure 3 for 5 mM SDS / 2mM spermidine at pH 7 gave rise to d and p of 18 ± 1 Å and $4.2 \pm 0.2 \times 10^{-6}$ Å² respectively; resulting in an area/molecule and adsorbed amount of 38 ± 2 Å² and $4.4 \pm 0.1 \times 10^{-10}$ mol cm⁻² respectively. The data for putrescine / SDS resulted in monolayer formation at all the SDS concentrations and pH values measured, and the variation in the adsorption with SDS concentration and pH are illustrated in figure 4a. In figure 4b the variation in the adsorption for SDS / 5mM spermidine and spermine with SDS concentration at pH 10 are plotted, for the data where only monolayer adsorption is observed.

The NR data for 5 mM SDS / 2 mM spermidine at pH 7 in figure 3 have a completely different form, and are not consistent with monolayer adsorption at the interface. The data are dominated by two ‘Bragg’ peaks at Q values ~ 0.16 and 0.32 Å⁻¹; and are consistent with the formation of a multilayer structure at the interface. This is broadly similar to the multilayer structures observed in some polyelectrolyte / surfactant mixtures (12), polyamine / surfactant mixtures (25-27) and surfactant / electrolyte mixtures (33-36). In this case the data are analysed using a surface multilayer model based on the kinematic approximation

(43, 44), and the use of this approach for these types of systems is described in detail elsewhere (17-19, 25-27, 33-36). The key model parameters are the bilayer thickness, d_t , where the thicknesses of the alkyl chain and headgroup regions of the bilayer structure are d_1 and d_2 such that $d_t = d_1 + d_2$, the scattering length densities of the two regions ρ_1 and ρ_2 , and N the number of bilayers at the interface. There is a limited number of Bragg peaks visible in the data (two in the data in figure 3, but mostly only one over much of the data range where multilayers occur) the modelling is most sensitive to N , d_t , and $\Delta\rho$ ($\rho_1 - \rho_2$). The number of bilayers, N , is relatively large and is related to the width of the Bragg peak (width of Bragg peak is $\sim 1/N$) convolved with the instrumental resolution, $\Delta Q/Q$. The absence of subsidiary interference fringes between the Bragg peaks also means that the total thickness, and hence N , is sufficiently large that their visibility is reduced by the finite instrumental resolution ($\sim 5\%$ in $\Delta Q/Q$). For the data in figure 3 the values of d_t , d_1 , d_2 , ρ_1 , ρ_2 , $\Delta\rho$ and N are 38, 22, 16 Å, 4.5×10^{-6} , 1.5×10^{-6} , 3×10^{-6} Å², and 50 respectively; and are typical of the parameters for all the multilayer structures observed in this study. The visibility of the Bragg peaks in figure 3, as determined predominantly by the combination of N and $\Delta\rho$, indicates a high lateral coverage, and a relatively low uncertainty in the N and $\Delta\rho$ values. The width of the first order Bragg peak in figure 3 is determined entirely by the number of bilayers and the instrumental resolution, and this is a further indication of the high degree of surface coverage and ordering. In general the width and visibility of the Bragg peaks will be affected by lateral disorder and disorder in the z direction, perpendicular to the interface. As the widths of the Bragg peaks observed here are determined predominantly by $1/N$ and $\Delta Q/Q$ any lateral disorder is relatively low. In figure 3 the model predicts that a 3rd order Bragg peak should be just visible. Vertical disorder, in the z direction, will progressively dampen out the higher order Bragg peaks. This has not been included here as the 3rd order Bragg peak is on the extreme edge of the data range. Surface multilayer formation occurs here for spermidine and spermine predominantly at pH 3 and 7 and in the SDS concentration range of 10^{-3} to 10^{-2} M. Outside that range of pH and at the lower SDS concentrations investigated monolayer adsorption occurs.

(iii) Discussion

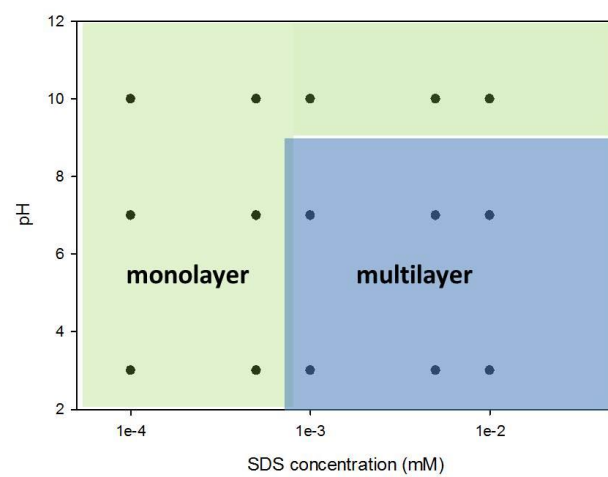
The ST data for SDS / putrescine and SDS / spermidine are largely independent of pH and the increase in the biogenic amine MW, from putrescine to spermidine, does not significantly impact upon the ST. The form of the ST data is more closely aligned to that observed for surfactants in the presence of multivalent counterions (35, 36), than previously observed for PEI / SDS (16-18) and oligoamine / SDS mixtures (19, 26, 27). However and importantly the ST data provides no real indication of the transition in the adsorption behaviour with pH and biogenic amine MW, from monolayer to multilayer adsorption, That is critically observed in the NR data. Furthermore the physical appearance of the solutions does not necessarily correlate with the changes in the surface behaviour.

The results in figure 4 a and b show the comparison of the adsorption of SDS in the presence of putrescine, spermidine and spermine in the regions of pH where only monolayer adsorption occurs. For putrescine, spermidine and spermine the SDS adsorption is enhanced at surfactant concentrations $<10^{-2}$ M compared to the adsorption of pure SDS. This arises from the strong surface interaction between the polyamine and SDS, as was originally observed for PEI-SDS mixtures (16). For SDS in the presence of putrescine monolayer adsorption occurs over the entire pH range measured, pH 3 to 10, and the adsorption is largely independent of pH. For SDS in the presence of spermidine and spermine monolayer adsorption occurs only at pH 10, and the adsorption is similar for both polyamines. Comparison with the adsorption data in figure 4c for SDS with triamine and tetramine at pH 7 and 10 (27) shows that the enhancement in the adsorption is greater for tetramine and triamine at pH 7 and comparable at pH 10. It has been postulated previously (19) that the nature of the polyamine-surfactant interaction which is responsible for the enhanced adsorption arises from two different mechanisms. At low pH, where the polyamine is highly charged, there is a strong electrostatic attraction between the amine group and the surfactant headgroup. At high pH the polyamine is essentially neutral and yet the surface interaction is still significant. This was attributed to a combination of an ion-dipole interaction between the surfactant headgroup and the nitrogen group on the amine and an attraction between neighbouring attached surfactant alkyl chains. The results presented here further support the hypothesis. Penfold et al (19, 27) studied the adsorption of SDS in the presence of polyamines, from diamine to hexamine, and demonstrated that

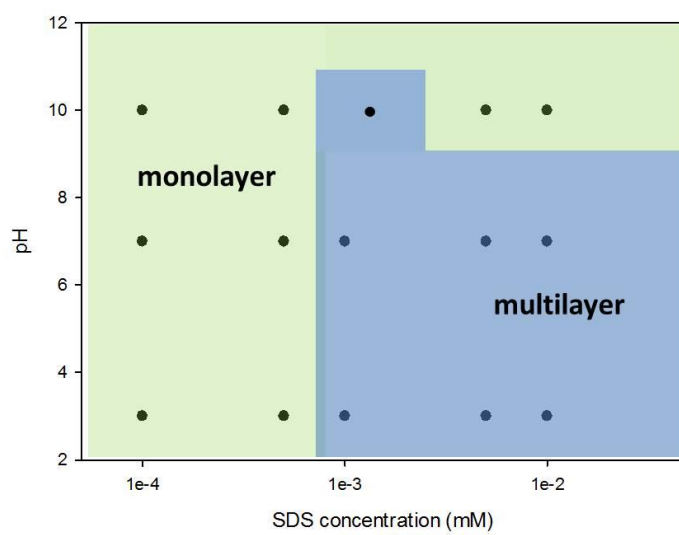
the MW (number of amine groups) affected the cooperativity of the interactions especially at high pH. That is, at a fixed SDS and polyamine concentration and at constant pH the adsorption increases as the number of amine groups increases. The results presented here in general indicate that the enhancement in the SDS adsorption is similar for putrescine, spermidine and spermine, but reduced compared to the polyamines, triamine and tetramine. This implies that the increased spacing between the amine groups of the biogenic amines (see figure 1) results in a reduction in the cooperative attraction between the attached surfactant alkyl chains. However the data in figure 4c for SDS with triamine and tetramine indicate that in the range of parameters measured the effect of partial charge at pH 7 and hence the increased charge interaction can be significant and results in a greater adsorption compared to the data at pH 10. This further illustrates the importance of the relative role of the two interactions in determining the pattern of adsorption, and this will be seen to an important factor in the criteria for surface multilayer formation.

For linear PEI (25k MW) / SDS mixtures at 10^{-4} M SDS (16) the adsorption was largely independent of pH, as observed for putrescine, but the adsorbed amount was much higher. For PEI / SDS mixture it was $\sim 4 \times 10^{-10}$ mol cm $^{-2}$ compared to $\sim 2.5 \times 10^{-10}$ mol cm $^{-2}$ for putrescine at pH 3 to 7 and spermidine at pH 10. This is consistent with the results for SDS / polyamine adsorption at different MW's, where Penfold et al (19) illustrated that adsorption equal to that observed for PEI / SDS was not attained until the MW of hexamine

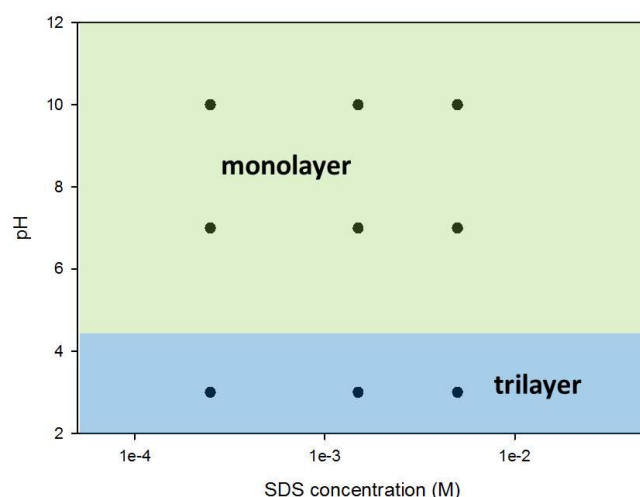
As presented earlier (see figure 3), the adsorption of SDS in the concentration range of 10^{-3} to 10^{-2} M with the addition of spermidine and spermine and at pH 3 and 7 results in multilayer formation. The extent of surface multilayer formation for SDS with spermidine and spermine with pH and concentration is illustrated in figure 5.



(a)



(b)



(c)

Figure 5. Approximate surface phase diagrams for SDS with (a) 5 mM spermidine, (b) 5 mM spermine, and (c) 5 mM triamine (reproduced from reference 27). The points indicate where NR measurements were made.

At pH 3 and 7 SDS in combination with spermidine or spermine forms multilayer structures over the SDS concentration range from 10^{-3} to 10^{-2} M, as shown in figures 5 a and b. Typically the bilayer thickness, d_t , is ~ 38 to 40 Å and the number of bilayers is ~ 50 . In addition for spermine there is a narrow region of SDS concentrations where multilayer formation occurs at pH 10 (see figure 5b). The bilayer thickness for spermidine and spermine is independent of pH, but dependent upon the biogenic amine. It is 38 ± 0.5 Å for spermine and 40 ± 0.5 Å for spermidine. Similar bilayer thicknesses were reported for the polyamines with SDS (19, 25-27). Figure 5 c shows the equivalent surface behaviour for SDS / 5 mM triamine (27), and the direct comparison with spermidine shows that multilayer formation is more extensive for spermidine than for triamine. The surface ordering with triamine occurs only at the lowest pH, pH 3, and the ordering is much less pronounced (27). That is, the reflectivity is described by a single broad interference fringe which corresponds to a trilayer structure at the interface, as described in more detail in earlier studies (19, 27). The results for tetramine / SDS (27), which can be compared directly with the SDS / spermine results presented here, are similar to the SDS / triamine results shown in figure 5c. It was previously shown that surface ordering occurred in polyamine / SDS mixtures at

low pH, pH 3, for polyamine MW's of tetramine and smaller, and for MW's greater than tetramine at high pH, pH 7 to 10. The results presented here for the biogenic amines spermidine and spermine are in general consistent with those observations, except that for the biogenic amines the surface ordering extends up to pH 7.

The comparison of triamine and tetramine with spermidine and spermine also show that only trilayer formation occurs for triamine and tetramine, whereas for spermidine and spermine the surface ordering is much stronger and extensive multilayer formation occurs. The pH dependence of the transition from monolayer to multilayer formation, which changes from low to high pH for polyamine MW's between tetramine and pentamine, is associated with the relative balance between the electrostatic attraction between the SDS and amine nitrogen which decreases as the pH increases, the ion-dipole interaction between the amine nitrogen and the SDS headgroup which is dominant at high pH, and the cooperative van der Waals attraction between the alkyl chains of the attached surfactant molecules. However the results presented here indicate that the increase in the spacing between the amine groups of the biogenic amines compared to the polyamines further favours the multilayer transition at these lower MW's. The interaction energy $U(r)$ for the Coulombic interaction varies as $1/r$, whereas the van der Waals attraction varies as $1/r^6$. The ion-dipole interaction is also more shorter ranged, with a $1/r^4$ dependence when thermal averaging is taken into account. The mean distance between amine groups in the polyamines is ~ 4 Å, and increases to ~ 6 Å for the biogenic amines spermidine and spermine, and ~ 8 Å for putrescine. Hence the increase in the amine group spacing has relatively little impact upon the short ranged interactions, but will reduce any lateral inter-SDS headgroup interactions. The increase in the amine group spacing will also alter the packing conditions associated with monolayer or multilayer formation. In the comparison of the effect of the biogenic amines and polyamines on the SDS adsorption (see figure 4) in the regions of monolayer adsorption, increasing the amine spacing, from the polyamines to the biogenic amines, results in a lower enhancement in the adsorption. In contrast, increasing the amine spacing, from the polyamines to the biogenic amines, results in a greater tendency to promote multilayer formation. For the lowest MW biogenic amine, putrescine the further increase in the spacing and the lower number of amine groups

makes the cooperativity too small to promote multilayer formation at any pH. The balance between the different interactions between the SDS and polyamines and biogenic amines has a different optimisation in multilayer adsorption than in monolayer adsorption. This was also concluded by Halacheva et al (27) from the pH and MW dependence of the monolayer to multilayer transition in oligoamine / SDS mixtures.

The results presented so far are all measured at a fixed polyamine or biogenic amine concentration of 5 mM and variable SDS concentrations. Measurements were also made at a fixed SDS concentration of 5 mM and variable spermidine and spermine concentrations, from 0.5 to 10 mM, at pH 7. For both spermidine and spermine surface multilayer formation at pH 7 and the fixed SDS concentration occurs over a wide biogenic amine concentration range. Monolayer formation occurs only at the extremes of the biogenic amine concentrations measured, ≤ 1.0 mM and ≥ 10 mM. In figure 5 the multilayer formation occurs mostly when the polyamine is in excess, apart from at the highest SDS concentrations. However, the results for a fixed SDS concentration and variable biogenic amine concentrations show that this is not a necessary criterion, and multilayer formation occurs over a wide range of parameters in which the SDS is in excess in the solution.

At low pH the polyamines are often referred to as flexible cations. It is well established that the addition of simple electrolytes, such as NaCl, and multivalent electrolytes, such as CaCl_2 and AlCl_3 , enhance anionic surfactant adsorption and reduce cmc values. Furthermore it has been demonstrated that the multivalent counterions can induce surface multilayer structures, from a trilayer to a multilayer (33-36). Jiang et al (14) showed that the effect of sodium oligoarene sulphonate on the adsorption of dodecyltrimethyl ammonium bromide showed a transition from that of a multivalent ion to that associated with a polyelectrolyte with increasing MW. Jiang et al (45) investigated the effect of amine additives, from diamine to pentamine, on the cmc of ionic surfactants, which included SDS. The addition of the polyamines resulted in a modest reduction in the cmc, which would not account for the variations in the SDS adsorption encountered in this study and related SDS / polyamine studies (19, 25-27). Khan et al (46) investigated the effect of the addition of polyamines on the surface tension of cationic Gemini surfactants, and interpreted the surface tension variations in terms of non-ideal mixing. However, they observed that, from diamine to

pentamine, there was an increasing impact on the surface tension with polyamine MW. The ST data presented here for SDS / putrescine and SDS / spermidine mixtures showed no dependence on solution pH and were broadly similar as the MW and number of amine groups increased from putrescine to spermidine. The general form of the ST data was more closely aligned to that observed for surfactants in the presence of multivalent counterions (35, 36), than for polyelectrolyte / surfactant mixtures (12). However the NR data presented here for the biogenic amines, and in particular the way the surface structure evolves with biogenic amine MW, support the supposition that the polyamines are better described as oligomeric polyelectrolytes rather than as flexible multivalent cations.

Conclusions

The results presented here show that the biogenic amines, putrescine, spermidine, and spermine induce enhanced SDS adsorption over the pH range 3 to 10, broadly similar to that observed in polyamine / SDS (25-27) and PEI / SDS (16) mixtures. The strong interaction between the biogenic amine and SDS arises from electrostatic attraction at low pH and a cooperative ion-dipole interaction at high pH, as discussed by others (19, 22-27). For spermidine and spermine at pH 3 and 7 the strong interaction promotes the formation of surface multilayer structures. The surface ordering is more well developed and over a wider range of parameters (pH and concentration) than was observed for the equivalent polyamines (26, 27). Hence the results show the importance of the spacing between the amine groups and the number of amine groups on the interactions with the SDS and the subsequent adsorption and surface ordering. The results provide a powerful insight into some of the important factors which affect the biological function of biogenic amines (1-3) and the potential applications of polyamines and biogenic amines (5-10).

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