ELSEVIER

Biophysical Chemistry 117 (2005) 263 – 273

Biophysical
Chemistry

http://www.elsevier.com/locate/biophyschem

# Adsorption of single chain Zwitterionic phosphocholine surfactants: Effects of length of alkyl chain and head group linker

M. Yaseen [a], J.R. Lu [a,*], J.R.P. Webster [b], J. Penfold [b]

[a]*Biological Physics Group, School of Physics and Astronomy, the University of Manchester, Sackville Street Building, Sackville Street,*
*Manchester M60 1QD, UK*
[b]*ISIS, Rutherford Appleton Laboratory, CLRC, Chilton, Didcot, OX11 0QZ, UK*

Received 29 March 2005; received in revised form 3 June 2005; accepted 3 June 2005
Available online 29 June 2005

## Abstract

The adsorption of a range of single chain zwitterionic phosphocholine surfactants ($C_nP_mC$) at the air/liquid interface has been studied by a combination of surface tension and neutron reflectivity. The critical micellar concentration (CMC) for $C_nPC$ (or $C_nP_2C$), where $n$ varied from 12, 14 to 16, was found to be 0.91, 0.14, and $1.2 \times 10^{-2}$ mM respectively, and followed the same trend as observed for other zwitterionic and non-ionic surfactants. The area per molecule at the CMC, $A_{\mathrm{cmc}}$, for $C_nPC$ was found to remain constant between 50 and 53 Å$^2$, indicating that the increase in the alkyl chain length had little effect on $A_{\mathrm{cmc}}$ at the interface. The neutron reflection measurement also showed an almost constant layer thickness ($\tau$) of $20 \pm 2$ Å from all the alkyl chain deuterated PC surfactants ($dC_nhPC$) in null reflecting water (NRW), suggesting that the alkyl chains of the surfactant responded to changes in either chain length or solution concentration by varying their angle of tilt. In contrast, increasing the length of head group linker between P and N atoms in $C_{12}P_mC$, where $m=2$, 4, to 6, resulted in a much slower decrease of CMC from 0.91, 0.7, to 0.5 mM, consistent with a different contribution to the free energy of micellization. $A_{\mathrm{cmc}}$ for $C_{12}P_mC$ did not vary when m was increased from 2 to 4, and this observation together with the thickness of the head group region indicated an almost perpendicular projection of the head group in $C_{12}P_2C$ and $C_{12}P_4C$. A further increase in m to 6 resulted in an $A_{\mathrm{cmc}}$ of 70 Å$^2$. This increase in $A_{\mathrm{cmc}}$ however did not result in any change in either the total layer thickness or the fraction of the head group region submerged in the aqueous subphase, suggesting that the head group in $C_{12}P_6C$ was bent away from the surface normal direction. Both increase in temperature from 25 to 40 °C and the addition of 0.1 M NaCl had little effect on the area per molecule or the thickness of $C_{12}P_mC$ surfactant layer, showing that the $C_{12}P_mC$ series behaved like $C_nP_2C$ series. The main conclusion from this study is that for all the $C_nP_mC$ surfactants studied, change in $m$ or $n$ has little effect on the total thickness, the thickness of the alkyl chain or that of the head group region.

*Keywords:* Zwitterionic surfactants; Surface adsorption; Biointerface; Phospholipid; Surface tension; Neutron reflection

## 1. Introduction

In many commercial formulations mixed surfactants are widely used because they offer favourable interfacial properties and act synergistically [1]. In contrast, zwitterionic surfactants, characterised by both a positive and a negative charge chemically bonded in the same head group, offer a number of interesting properties. They have a critical micellar concentration (CMC) intermediate between ionic and non-ionic surfactants. They display surface and interfacial adsorption that are virtually independent of solution conditions such as temperature, salt and pH [2,3]. Zwitterionic surfactants are electrically neutral but the charges they carry in the zwitterionic head group do influence their hydrophilicity, making them different from non-ionic surfactants.

In a systematic study of surface and interfacial behaviour of surfactants, we have previously used a combination of surface tension and neutron reflectivity to investigate the characteristics of surface monolayers adsorbed from anionic, cationic and non-ionic surfactants [4–6]. More

---

* Corresponding author. Tel.: +44 161 2003926.
*E-mail address:* j.lu@manchester.ac.uk (J.R. Lu).

0301-4622/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2005.06.001

recently we have extended this work to study the main features of single chain phosphocholine surfactants, $C_nPC$, where $n = 12$ and 16 [7]. These PC surfactants show similar adsorption behaviour to zwitterionic sulfobetaine [8] and carboxybetaines [9]. However the structure of the surface layer showed a different dependence to temperature change and salt addition from conventional ionic (for example, $C_nTAB$) surfactants and non-ionics (for example, $C_nEO_m$). In this work, we report a recent study that examines how the increase in alkyl chain length, $n$, and in the number of methylene units in the head group, $m$, of the PC surfactant affects the structural features of the surface monolayers. The general formula of the surfactants is $C_nH_{2n+1}PO_4^-C_mH_{2m}N^+(CH_3)_3$, which will be referred to as $C_nP_mC$, where n will be varied from 10, 12, 14, 16 to 18 and m from 2, 4 to 6. To examine the effect of the alkyl chain length, m has been fixed at 2 and the series is referred to as $C_nPC$ (or $C_nP_2C$). To assess the effect of the length of head group linker, n has been fixed at 12 and the series is referred to as $C_{12}P_mC$.

The effect of the length of straight alkyl chain on the critical micellar concentration (CMC) for both ionics and non-ionic alkyl ethoxylates ($C_nEO_m$) has been extensively studied. A linear relationship between log[CMC] and the number of carbon atoms ($N_c$) in the alkyl chain is empirically applicable in most cases [10]. If the area per molecule at the CMC, $A_{cmc}$, is entirely controlled by the bulky size of the head group associated with charge and hydration, increase in alkyl chain length would not be expected to alter $A_{cmc}$. However, some extent of variation is often observed due to the changing hydrophobic interaction between neighbouring alkyl chains. The effect of alkyl chain length has been extensively studied from the adsorption of cationic n-alkyl-trimethylammonium bromide ($C_nTAB$) at the air/water interface with n from 12 to 18 [6,11–15]. Neutron reflectivity measurements revealed that the thickness of the surfactant layers stayed almost constant at 18–20 Å for all the chain lengths, when modelled as a uniform layer, with $A_{cmc} = 44$ to 48 Å$^2$. The lack of layer thickness variation with alkyl chain length is in part due to the capillary wave contribution to the effective thickness of the adsorbed layer but this observation also suggests an increased tilting of the alkyl chains away from the surface normal direction, resulting in the increase in layer packing density. The increased fluidity was consistent with the slight lowering in $A_{cmc}$, indicating an increased hydrophobic interaction within the surface layer. For $C_{10}TAB$, the thickness at the CMC was still similar, but $A_{cmc}$ increased to some 60 Å$^2$ suggesting that as alkyl chain became shorter, they were stiffer, the hydrophobic attraction became less significant and the chains were closer to the surface normal than for the other longer chain $C_nTAB$'s.

The non-ionic surfactants, $C_{12}H_{25}(OC_2H_4)_mOH$, $C_{12}(EO)_m$, have been used to examine the effect of head group size [4,16–18] on adsorption . It was found that for $m = 2$ and 4 the effective layer thickness became smaller with increasing area per molecule. The extent of the ethoxylated head group layers also became smaller than their fully extended length, suggesting that ethoxylates were bent or coiled with increasing head group size. $A_{cmc}$ increased from 33 Å$^2$ for $m = 2$ to 44 Å$^2$ at $m = 4$, showing the dominant contribution of the ethoxylate head group to $A_{cmc}$ [4]. An interesting observation was the occurrence of a strong intermixing between alkyl chains and ethoxylates within the interfacial layer as the size of the head groups increased. Further increase in m for $C_{12}EO_m$ series resulted in a steady increase in $A_{cmc}$, indicating the different packing adopted by the EO head groups from the alkyl chain and different interactions involved. Chain branching may however result in considerably different surface packing compared to the straight chain [19].

Changes in the molecular architecture of surfactant molecules have a profound implication on surface monolayer structure and related adsorption properties. An example of this is the interesting features revealed for the di-chain Gemini $C_{12}TAB$ surfactants with different length of linking spacer between the two cationic heads [20]. Within the monolayers, the chain–chain hydrophobic interaction tends to lower the polar head surface area on the surface of water, but these interactions cannot be considered on their own. These forces compete with the hydrophilic forces between the head groups and between the head group and water. In addition, as the length of linker increases, structural flexibility may arise from the increased hydrophobic interaction between the head group and the alkyl chain, causing the head groups to adopt a different conformation. Although the $C_nP_mC$ series to be studied in this work has very different molecular configuration, the balance of interactions between the hydrophobic tail and hydrophilic head should be considered to be similar.

Other workers have used the two-chained phospholipid monolayers formed from 1,2 - dihexadecyl-sn-glycero-3-phoshocholine (DHPC) to investigate the head and tail interactions by Langmuir troughs and X-ray reflection [21]. Ethylene oxide (EO) groups, of 1, 2, and 3 units were incorporated between the chain and the phosphate group. This type of structural manipulation did not change the phosphocholine zwitterionic character but effectively increased the hydrophilic character of the head. The results have showed that as the head group length increases the influence of the head group on the chain lattice is reduced but its hydration is promoted. Others [22] have compared $C_{10}PC$ with a $C_{10}$ analogue in which the choline head was replaced at the P-O position by two fixed $N(CH_2CH_2)_2O$ groups. This approach increased the number of carbons in the head, and hence increased the hydrophobicity. Such structural changes resulted in the removal of the important zwitterionic character of the head group, but had little influence on the hydrophobicity, the CMC, or $A_{cmc}$. Holmberg et al. have replaced a methyl group on the *N*-choline of $C_nP_mC$ by straight chain hydrocarbon. The substitution showed a drop in CMC by orders of magnitude depending on the length of the alkyl chain [23]. Their results also revealed a substantially reduced area per molecule for *N*-alkyl substituted surfactants, indicating the strong interactions within the surfactant layer.

It is useful to note that our $C_nP_mC$ only has a single straight alkyl chain and is structurally different from the di-chain Gemini cationic or PC zwitterionic surfactants as described above. In addition, all our single chain $C_nP_mC$ surfactants are water-soluble. The lack of a hydrophobic anchoring at the other end of the head group may result in structural reorientation of $P_mC$ group, which is to a large extent dependent on surface layer packing density. The aim of this work is to reveal the main adsorption features from these single chain zwitterionic phosphocholine surfactants using a combination of surface tension and neutron reflectivity and to compare their structural characteristics with the non-ionic and cationic surfactants.

## 2. Experimental

### 2.1. Physical measurements

Neutron reflection was performed on the SURF reflectometer at Rutherford Appleton Laboratory near Oxford, UK [24] using wavelength range of neutron beam between 0.5 and 6.5 Å. The beam incidence angle was inclined at 1.5° to the horizontal liquid surface. Each sample solution was poured into a Teflon trough with a positive meniscus so that the incoming and exiting beam would not hit the trough edges. The beam illuminated area was *ca* 10cm × 3cm, adjusted by sets of vertical and horizontal slits before the liquid trough. The absolute beam intensity was calibrated with respect to clean $D_2O$ and a flat background determined by an extrapolation to the high momentum transfer, $\kappa$. The average reflectivity above 0.3 $Å^{-1}$ was taken as the flat background.

Neutron reflectivity measurements were made from alkyl chain deuterated surfactants $dC_nhP_mC$ adsorbed at the air/water interface to determine the surface excess. To optimise the signal from the adsorbed surfactant layer, the scattering length density of the bulk water was made zero, achieved by mixing $D_2O$ into $H_2O$ in a molar ratio of 1 to 11, equivalent to a volume fraction of 8.1%. This mixture of $D_2O$ and $H_2O$ does not contribute to specular reflection at the air/water interface and was termed null reflecting water (NRW). Under this condition the specular signal arises only from the adsorbed surfactant layer only. The reflectivity (R), defined as the ratio of the reflected to incident beam intensities, is plotted as function of wave vector, $\kappa$, perpendicular to the reflecting interface where

$$\kappa = \frac{4\pi\sin\theta}{\lambda} \tag{1}$$

where $\theta$ is the incidence angle and $\lambda$ the wavelength of the incidence neutron beam [24]. The measured reflectivity was analysed quantitatively using a programme based on the optical matrix method [25,26]. A physical model was first assumed for the adsorbed surfactant layer and the reflectivity was calculated accordingly. This was followed by comparing the measured and calculated profiles and the process was repeated until a physically meaningful best fit was obtained. The structural parameters used in the fitting were the number of layers, thickness ($\tau$), and the corresponding scattering length density ($\rho$) for each layer. For a uniform layer adsorbed at the air/NRW interface, the area per molecule ($A$) for the surfactant was calculated from

$$A = \frac{\sum mb}{\rho\tau} \tag{2}$$

where $\sum mb$ is the total scattering length for $dC_nhP_mC$ and the values for each surfactant is as shown in Table 1 together with other physical constants. The scattering length (b) was from Ref. [27]. The scattering length density ($\rho$) was calculated by using the volume and fully extended length for the chain and PC head group estimated from Ref. [28].

Surface tension measurements were performed on a Kruss K10 tensiometer using the platinum–iridium du Nouy ring and the surface tension readings were calibrated using the formula from Harkins and Jordon [29] to take into account the density and measurement geometry. The temperature of the sample was controlled thermostatically by circulating water from a Haake bath. The surface tension of pure water was initially obtained for each experiment to check the purity of the water and for instrument calibration. Between the measurement runs, the ring was initially cleaned with ultrapure water (UHQ) and flamed. The surface tension ($\gamma$, in mN/m) was plotted against the natural logarithm of concentration (lnC, in molar), a plot conforming to the Gibbs equation. The tension profile measured below the critical micellar concentration (CMC) fitted well

Table 1
Physical constants for $dC_nhP_mC$

| Unit | Scattering length $b/10^{-5}/Å$ | Total extended length $l_e/Å$ | Volume $V/Å^3$ | Scattering length density $\rho/10^{-6}\ Å^{-2}$ |
|---|---|---|---|---|
| ***$C_{12}PC$*** | | | | |
| hPC head | 22.3 | 11 | 297 | 0.75 |
| $dC_{12}$ chain (98% D) | 241.4 | 16.7 | 350 | 6.90 |
| $dC_{12}hPC$ | 263.7 | 27.7 | 647 | 4.08 |
| ***$C_{14}PC$*** | | | | |
| $dC_{14}$ chain (95%) | 271.4 | | 404 | 6.72 |
| $dC_{14}hPC$ | 293.8 | 30 | 701 | 4.2 |
| ***$C_{16}PC$*** | | | | |
| $dC_{16}$ chain (97%) | 316.2 | | 458 | 6.90 |
| $dC_{16}hPC$ | 338.5 | 33 | 755 | 4.48 |
| ***$C_{12}P_6C$*** | | | | |
| $hP_6C$ head | 19 | 17 | 404 | 0.47 |
| $dC_{12}$ chain (96%) | 235.4 | | 350 | 6.72 |
| $dC_{12}hP_6C$ | 254.4 | 33.7 | 754 | 3.37 |

to a second order polynomial where $\gamma = a(\ln C)^2 + b(\ln C) + c$. Input of its differential into the Gibbs equation gives

$$\Gamma = \frac{2a(\ln C) + b}{RT} \tag{3}$$

where $\Gamma$ is the surface excess (in mol m$^{-2}$) and a and b are the constants obtained from the curve fitting to measured surface tension below the CMC. For these zwitterionic surfactants the prefactor was taken to be 1. The limiting area per molecule ($A$, in Å$^2$) could be calculated from $\Gamma$ from

$$A = \frac{10^{20}}{N_a \Gamma} \tag{4}$$

where $N_a$ is Avogadro's number. As already indicated previously [30,31], the resultant $\Gamma$ or $A$ as a function of $C_{12}$PC concentration was compared directly with the neutron results.

### 2.2. Synthetic procedure

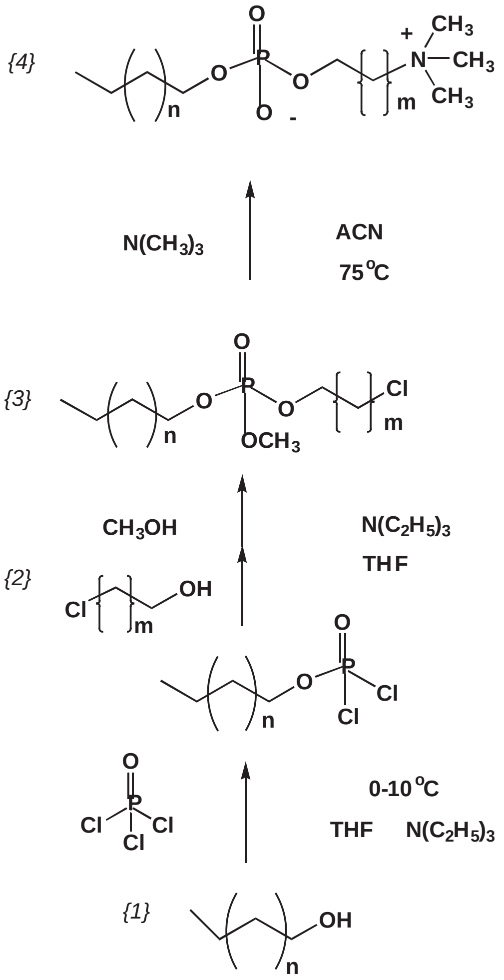
The synthesis for producing phosphocholine surfactants of variable alkyl chain length has already been described previously [7]. The route to obtaining different spacer isomers is shown in Scheme 1.

Alcohol *{1}* was reacted with phosphoryl trichloride, $POCl_3$, and stirred for 2.5 h. To this mixture the bromo or chloro *n*-alkyl alcohol *{2}*, the spacer, was added with formation of the phosphonate ester *{3}*. To the purified and dried intermediate {3} trimethylamine was added in a sealed tube and left to react for 24 h at 75 °C to afford the main product *{4}*. The phosphoryl trichloride was used in excess of ca 1.25–1.5 molar ratio to 1 mol of the alcohol, to ensure the complete reaction of the alcohol. A molar ratio of 1.5 to 1 of the bromo or chloro *n*-alkyl alcohol to the alcohol *{1}* was used to improve the reaction yield. The trimethylamine was also used in large excess, ca 3–5 times the molar ratio of the intermediate phosphonate tri-ester, again to improve the yield of the product. Deuterated dodecanol was used for the synthesis of the chain deuterated isomer.

#### 2.2.1. Characterisation

*2.2.1.1. The assignment of NMR for $hC_{12}hP_6C$.* **$^{31}$P NMR** ($D_2O$): 1.08 ppm. **$^1$H NMR** ($D_2O$): (3H, tail end–C**H**$_3$), 0.87–1.0; (18H, tail, C**H**$_2$ x 9), 1.2– 1.35; (4H,–C**H**$_2$C**H**$_2$–spacer), 1.35–1.5; (4H,–C**H**$_2$CH$_2$OPOCH$_2$C**H**$_2$–), 1.5 – 1.7; (2H, NCH$_2$C**H**$_2$–), 1.75–1.85; (9H,–N(C**H**$_3$)$_3$), 3.1–3.25; (2H,–NC**H**$_2$–), 3.3– 3.4; (4H,–C**H**$_2$OPOC**H**$_2$–), 3.75– 3.8. **$^{13}$C NMR** ($D_2O$): (end–**C**H$_3$) 14.7; (tail CH$_2$), 23.47, 26.67, 28.7, 30.65– 30.75, 31.45–31.55, 32.8; (spacer **C**H$_2$), 23.1, 25.5, 26. 10, 30.3– 30.4; (–N(**C**H$_3$)$_3$) 53.67; (–N**C**H$_2$CH$_2$OP) 66.0– 66.25; (–NCH$_2$**C**H$_2$OP) 67.25.

*2.2.1.2. The assignment of $^1$H NMR for $dC_{12}hP_6C$.* **$^{31}$P NMR** ($D_2O$): 1.41 ppm. **$^1$H NMR** ($D_2O$): (4H,–C**H**$_2$C**H**$_2$–spacer), 1.35–1.5; (2H,–OPOCH$_2$C**H**$_2$–), 1.5– 1.7; (2H,–NCH$_2$C**H**$_2$–), 1.75–1.85; (9H,–N(C**H**$_3$)$_3$), 3.1–3.2; (2H,–NC**H**$_2$–), 3.3– 3.4; (2H,–OPOC**H**$_2$–), 3.75– 3.85. **$^{13}$C NMR** ($D_2O$): (**C**H$_2$, spacer), 23.0, 25.35, 25.90, 30.43–30.53; (–N(**C**H$_3$)$_3$), 53.475; (–N**C**H$_2$CH$_2$OP), 65.80 – 65.85; (–NCH$_2$**C**H$_2$OP), 67.05.



Scheme 1. The procedure for the synthesis of phosphocholine surfactants with different spacer lengths within the head group. (THF, Tetra Hydro Furan; ACN, Acetonitrile).

### 2.3. Chemicals and other information

The solvent for all NMR measurements was $D_2O$ except $C_{18}$PC for which deuterated methanol was used. $^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded on a Bruker Advance DPX spectrometer operating at 300, 75.5 and 121.5 MHz, respectively, with $^{13}$C and $^{31}$P spectra being recorded proton-decoupled. Chemical shifts were quoted in p.p.m. downfield from tetramethylsilane ($^1$H and $^{13}$C) or $H_3PO_4$ ($^{31}$P). All $^{13}$C NMR were also obtained in DEPT form. Column chromatography was performed on Merck silica gel 60 (40–60 μm). Thin Layer Chromatography, TLC, developed in a mixture of absolute ethanol (90%), concentrated sulphuric acid (5%) and *p*-anisaldehyde (5%) was run in the same solvent as for column chromatography.

All solvents used for the organic synthesis were dried and distilled before use. $D_2O$ for neutron experiments was purchased from Aldrich, containing 99.9% D and was used as supplied. High quality water for all measurements was purified using Elga Ultrapure (UHQ) PS system. NaCl was bought from Aldrich over 99.9% pure. All glassware were always cleaned by placing them in Piranha solution, composed of 95 parts of concentrated sulphuric acid (98%) and 5 parts of hydrogen peroxide (30%) for at least 1 h. They were then washed in water, dilute 1% neutral Decon, rinsed with water again, then with more distilled water and finally with UHQ water. Non-glassware apparatus was soaked in 5–10% Decon for about 1 h to remove any traces of impurity, followed by washing with water, distilled water and then UHQ water. This last method of washing was also used for the cleaning of Teflon trough in neutron experiments.

## 3. Results and discussion

### 3.1. The effect of alkyl chain length in $C_nPC$

The surface tension measured for fully hydrogenated zwitterionic phosphocholine surfactants $hC_nhPC$ with $n=12$, 14 and 16 is shown in Fig. 1. The absence of a surface tension minimum around the CMC indicates the high purity of the samples. Surface tension measured from alkyl chain deuterated $dC_{14}hPC$ and $dC_{16}hPC$ is also shown in Fig. 1. The consistency shows that, as far as surface activity is concerned, no isotopic effects are present. Fitting to the surface tension measured for each surfactant below the CMC to a second order polynomial and the subsequent use of the Gibbs equation led to the calculation of surface excess ($\Gamma$) below the CMC, and area per molecule at the CMC ($A_{\mathrm{cmc}}$). This together with the CMC and the surface tension at CMC ($\gamma_{\mathrm{cmc}}$) obtained from surface tension measurements are shown in Table 2.

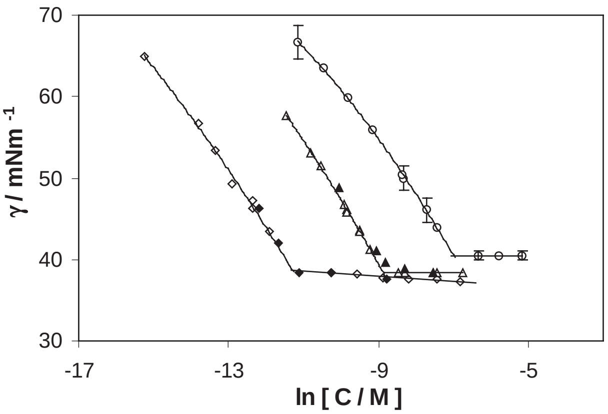


Fig. 1. Surface tension plots shown for $hC_{12}hPC$ (○), $hC_{14}hPC$ (Δ) and $hC_{16}hPC$ (◇) all fitted to second order polynomial fits below the CMC. The chain deuterated $dC_{14}hPC$ (▲) and $dC_{16}hPC$ (◆) are also shown for comparison.

Table 2
Key physical parameters obtained from surface tension data for $C_nPC$ where $n=8$, 10, 12, 14, 16 and 18

| Surfactant | $A_{\mathrm{cmc}}\pm3$Å$^2$ | CMC/mM | $\gamma_{\mathrm{cmc}}\pm0.5$ mN m$^{-1}$ |
|---|---|---|---|
| $hC_8hPC$ |  | 75 (114)±20 |  |
| $hC_{10}hPC$ | 40±10 | 8.5 (11)±2 | 41.5±2 |
| $hC_{12}hPC$ | 53 | 0.91±0.05 | 40.5 |
| $hC_{14}hPC$ | 50 | 0.14±0.02 | 38.5 |
| $hC_{16}hPC$ | 54 | $(1.2\pm0.1)\times10^{-2}$ | 38.1 |
| $hC_{18}hPC$ | 51±5 | $(3.5\pm2)\times10^{-4}$ | 38.1 |

The CMC of 114 and 11 mM for $C_8PC$ and $C_{10}PC$ are from Ref [32].

When surface tension was measured for $hC_8hPC$, $hC_{10}hPC$, it was found that these two short chain surfactants were impure, as indicated by the appearance of tension minimum around the CMC. In the case of $C_{18}PC$, however, the CMC also suffered from a large error due to the handling of very low concentration. However, estimated CMC values for these PC surfactants are also listed in Table 2 for comparison.

It can be seen from Table 2 that apart from the two short chain PC surfactants which were contaminated with surface active impurities, the area per molecule at CMC for all the long chain PC surfactants was constant at 51±3 Å$^2$. The value of $\gamma_{\mathrm{cmc}}$ shows a small decrease from $C_{12}PC$ to $C_{14}PC$, but remains constant for any subsequent chain increase. For straight chain ionic surfactants, the CMC is usually lowered by 50% upon increase of the alkyl chain by a single methylene. In contrast, the CMC of non-ionic and zwitterionic surfactants is reduced to a much greater extent, usually by a factor of 10 for each methylene increase in chain length. The lowering of the CMC with the number of carbon atoms, $N_c$, on a surfactant chain can be expressed by the following empirical equation [33]

$$\log CMC = -BN_c + A \qquad (5)$$

where $A$ is a constant for a particular hydrophilic head group at a given temperature and $B$ is a constant ≈0.3 for ionic surfactants and ≈0.5 for non-ionics and zwitterionics.

In Fig. 2 the plot of $\log_{10}$CMC against $N_c$ has been plotted to show if the constant $A$ and $B$ obtained adhere to the prediction of Eq. (5). The value for constant $B$, the gradient, was found to be 0.47 and this compares well with the value of 0.5 as cited for other non-ionic and zwitterionic surfactants [33,34]. The intercept, $A$, for the PC surfactants was found to be 2.6. It has been reported that for non-ionic surfactants $A$ is about 1.8 and for zwitterions such as betaines, $R{-}N^+(CH_3)_2\,CH_2COO^-$, it is about 3.1 [35]. This demonstrates the analogous behaviour of PC surfactants to non-ionic and zwitterionic surfactants rather than the ionic surfactants. An analogous CMC plot obtained by florescence technique [36] resulted in similar values for $A$ of 2.8, and $B$ of 0.52, in good agreement with our data.

Short chain surfactants $C_8PC$ and $C_{10}PC$ were found to be difficult to purify using the same purification procedures as for the long chain ones. This suggests that either surface

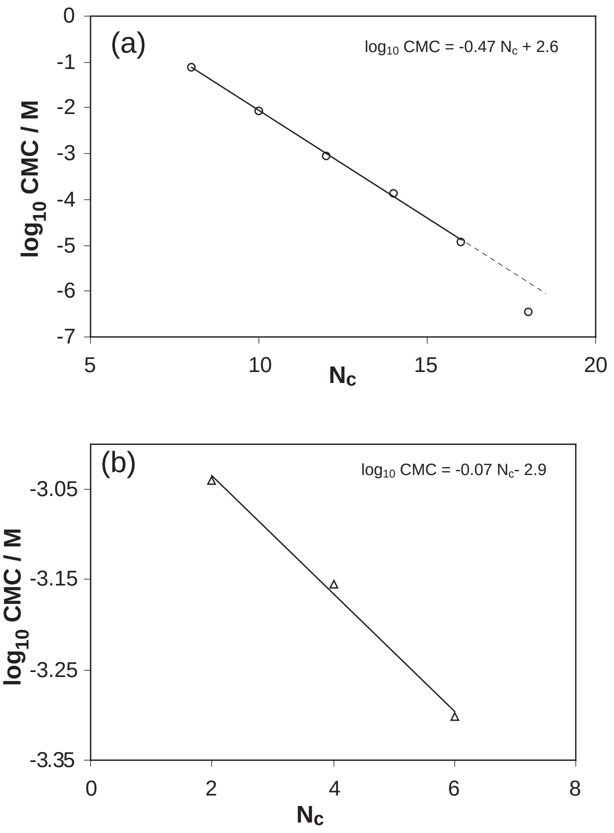


Fig. 2. (a) Plot of $\log_{10}$ CMC against the number of carbon, $N_c$ , increase in the alkyl chain of $C_n$PC (○) with a gradient of − 0.47. In (b) plot for the carbon increase in the head group linker of $C_{12}P_mC$ (Δ) is shown with a gradient of − 0.07.

active impurities remain in the samples or they are regenerated through sample deterioration. As in the case of sodium dodecyl sulphate [37], the alcohol chains were coupled to phosphorus through ester bonding for PC surfactants. A slight amount of octanol or decanol produced through sample destabilisation could explain the minimum around the CMC for $C_8$PC and $C_{10}$PC from surface tension measurements. The samples were purified by silica columning, but repeated column purification was found not to improve the purity. This was in contrast to the easy removal of surface active impurities for $C_{12}$PC and the longer chain PC surfactants. This observation may suggest a relationship between the alkyl chain length and the stability of ester bonding or the dependence of this stability on the hydrophobic and hydrophilic balance within the molecule. Although it was difficult to determine the CMC from $C_8$PC due to the large surface tension minimum, it was estimated to be about 8.5 mM for $C_{10}$PC. Furthermore, the limiting area per molecule at the CMC was about 40 ± 10 Å$^2$. The surface tension at the minimum was 30.5 mN/m at 7 mM but beyond it the tension rose to a constant value of 41.5mN/m, well in line with $\gamma_{\text{cmc}}$ of 40.5 mN/m for $C_{12}$PC. It is interesting to mention that the surface tension for $C_{10}$PC has been reported [22], with $A_{\text{cmc}}$ estimated to be 40 Å$^2$ and the CMC of 20mM. However, the corresponding $\gamma_{\text{cmc}}$ is 30 mN/m only and is the same as our tension value at its minimum, thus suggesting the same issue of contamination. A CMC of 11 mM for $C_{10}$PC has also been quoted [32], close to our estimated value from the tension data. In summary, it is our belief that none of the existing studies obtained sufficiently pure $C_8$PC and $C_{10}$PC. This may be partly due to the inappropriate methods of sample synthesis, partly due to the intrinsic nature of unstable ester bonding related to short alkyl chains.

Neutron reflectivity was measured using alkyl chain deuterated surfactants adsorbed from NRW. The neutron reflectivity measurements provide an estimate of how the thickness of the adsorbed layer changes with alkyl chain length. In addition, the neutron data provides an independent verification of the area per molecule with that determined from surface tension. When measured from $dC_nhPC$ in NRW, the neutron signal mainly arises from the deuterated chain but contains a small contribution from the hydrogenated head. The measurements thus provide a good estimate of the thickness of the alkyl chain layer. Fig. 3 shows a set of reflectivity profiles measured from $dC_{14}hPC$ in NRW over a range of surfactant concentrations. The main feature of Fig. 3 is that reflectivity profiles shift in level but not in shape in response to concentration variation. This means that lowering in bulk solution reduces surface excess but not the thickness of the adsorbed layer.

Quantitative analysis of the measured reflectivity profiles was performed using the optical matrix model as previously described [25,38–40]. A single uniform layer model was found to fit the data well, resulting in layer thickness ($\tau$) and scattering length density ($\rho$). The area per molecule ($A$) is related to these two parameters through Eq. (2). $A_{\text{cmc}}$ was calculated to be 50 ± 3 Å$^2$ for $dC_{12}hPC$, 51 ± 3 Å$^2$ for

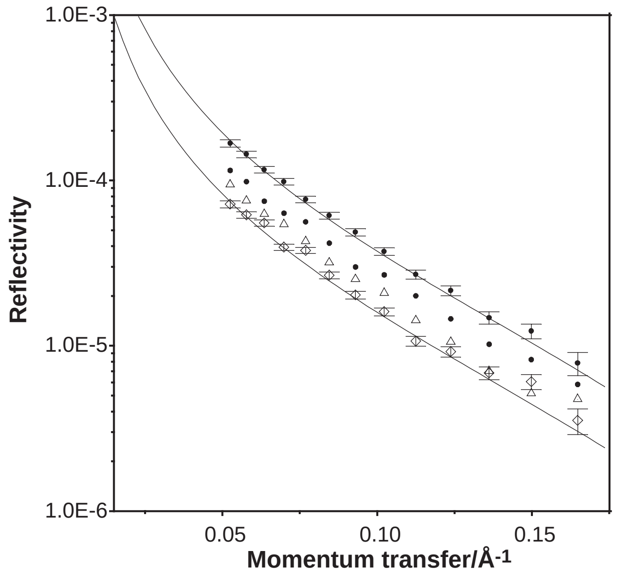


Fig. 3. Neutron reflectivity is plotted against $\kappa$ for $dC_{14}hPC$ adsorbed from NRW at the concentration of 0.135 (●) 0.02 (○) 0.01 (Δ) and 0.005 (◇) mM. The two continuous lines represent the best uniform layer fits to the reflectivity profiles measured at the lowest and highest concentrations.

$dC_{14}hPC$ and 53 ± 3 Å$^2$ for $C_{16}hPC$, in excellent agreement with those obtained from surface tension measurements. As the surface tension of $dC_{10}hPC$ showed a tension minimum around the CMC, $A_{cmc}$ was measured well above the CMC, at 40 mM, where the surface tension was constant. $A_{cmc}$ for $dC_{10}hPC$ was found to be 50 ± 3 Å$^2$, suggesting that at the surfactant concentration well above the surface tension minimum the surface layer is free from any surface active contaminants. This result is in general consistent with the previous observation reported from SDS/dodecanol mixture [37] where increase in SDS concentration well above its CMC reduced dodecanol surface excess to a negligible level. This effect was attributed to the formation of SDS micelles that helped to solubilize dodecanol. The data altogether shows that the limiting area per molecule at the CMC does not vary with the alkyl chain length in $C_nPC$ series.

The thickness of the layers for all the $dC_nhPC$ samples ($n$ = 10, 12, 14, 16 and 18) in NRW at the CMC was found to be about 20 ± 2 Å$^2$, showing that the thickness of the alkyl chain region does not increase with alkyl chain length. Further measurements were made at surfactant concentrations below the CMC to examine the possible variation of layer thickness with surface coverage. For $dC_{14}hPC$ at $5x10^{-6}$ M, $\tau$ was found to be 21 ± 3 Å at $A$ = 78 ± 3 Å$^2$. For $dC_{16}hPC$ at $2x10^{-6}$ M, $\tau$ was found to be 20 ± 3 Å at $A$ = 79 ± 3 Å$^2$. Over all the lower concentrations studied for different $dC_nhPC$ samples, a similar layer thickness of 20 ± 2 Å$^2$ was obtained, showing that the layer thickness for the alkyl chain region does not vary much with surface packing density, as shown in Table 3.

Table 3
Physical parameters for $dC_{12}hPC$, $dC_{14}hPC$ and $dC_{16}hPC$ layers adsorbed in NRW obtained from uniform layer model fitting

| $C/10^{-3}$ M | $\rho/10^{-6}$ Å$^{-2}$ | $\tau \pm 2$ Å | $A \pm 3$ Å$^2$ | $\Gamma \pm 0.25/10^{-6}$ mol m$^{-2}$ |
|---|---|---|---|---|
| ***$C_{12}PC$*** | | | | |
| 1.46 | 2.71 | 20 | 50 | 3.35 |
| 0.73 | 2.22 | 20 | 59 | 2.79 |
| 0.292 | 1.81 | 21 | 69 | 2.39 |
| 0.073 | 1.95 | 18 | 75 | 2.21 |
| 0.0365 | 1.51 | 20 | 87 | 1.91 |
| ***$C_{14}PC$*** | | | | |
| 0.25 | 2.87 | 20 | 51 | 3.29 |
| 0.135 | 2.87 | 20 | 51 | 3.26 |
| 0.05 | 2.65 | 20 | 55 | 2.99 |
| 0.02 | 2.35 | 20 | 62 | 2.66 |
| 0.01 | 2.1 | 20 | 70 | 2.37 |
| 0.005 | 1.87 | 20 | 78 | 2.11 |
| ***$C_{16}PC$*** | | | | |
| 0.024 | 2.87 | 22.5 | 53 | 3.17 |
| 0.012 | 2.81 | 22.5 | 53 | 3.11 |
| 0.0084 | 2.83 | 20.5 | 58 | 2.85 |
| 0.0024 | 2.35 | 21 | 68 | 2.42 |
| 0.002 | 2.15 | 20 | 78 | 2.11 |
| 0.0012 | 0.83 | 20.5 | 197 | 0.84 |

It is worth mentioning here that the thickness is affected by the mean angle of tilt of the chain with respect to the surface normal and the roughness of the surface [16]. The contribution from roughness represents a combination of both static disorder and thermal capillary wave fluctuations. Since the latter is related to surface tension [1], the retaining of an almost constant total layer thickness must imply a possible adjustment in the tilting of the alkyl chain when change in surface tension is substantial.

The variation of surface coverage with bulk concentration obtained from neutron reflectivity below the CMC is shown in Fig. 4 for $dC_{14}hPC$. The same data from surface tension measurements are also shown for comparison. The continuous line was calculated from Eq. (3) with constants a and b obtained from the best second order polynomial fit to the surface tension measured below the CMC (Fig. 1). The same comparison is given in Fig. 5 for $dC_{16}hPC$ from neutron reflection and $hC_{16}hPC$ from surface tension. The overall agreement is good, but at lower concentration some deviations are evident as a result of the very low concentrations that were measured.

### 3.2. The effect of head group spacer in $C_{12}P_mC$

Pure surfactants $C_{12}P_mC$, with $m$ = 2, 4 and 6, were prepared following the synthetic and purification procedures as outlined in Scheme 1, the Experimental Section. Their surface tension data are shown in Fig. 6. The absence of surface tension minimum from each of these curves also indicates very high purity of the samples. The area per molecule, CMC and $\gamma_{cmc}$ obtained are shown in Table 4. We discuss how these parameters change with m in the following paragraph.

An increase in m from 2 to 4 reduces the CMC from 0.9 mM to 0.7 mM. At the same time, $\gamma_{cmc}$ decreases from 40.5 mN/m to 38.8 mN/m, showing a typical trend of effect of increasing hydrophobicity as expected. It is useful to compare the changes in these parameters for variation in m and n. The extent of reduction in CMC is much smaller than observed from increase in the length of alkyl chain from $hC_{12}hPC$ to $hC_{14}hPC$ (see Figs. 1 and 2). However, the range of variation in $\gamma_{cmc}$ is almost identical and the area per molecule for $C_{12}P_4C$ is remarkably at 50 ± 4 Å$^2$ and is close to 53 ± 4 Å$^2$ for $hC_{12}hPC$. The almost constant $A_{cmc}$ suggests that the head group of $hC_{12}hP_4C$ is likely to project normal to the surface as in the case of the PC ($P_2C$) group and the increase in the head group size by two extra methylene units has not resulted in any measurable structural bending or curling. It is relevant to comment here that in the case of cationic Gemini $C_{12}TAB$ surfactant with two dodecyl chains [20], increase in the linking spacer from $C_3$ to $C_4$ results in the increase in $A_{cmc}$ from *ca* 65 Å$^2$ to 82 Å$^2$. This difference indicates that the $P_2C$ and $P_4C$ head groups adopt different orientational conformations.

A further increase in m to 6 resulted in further reduction in CMC, and interesting variations in $A_{cmc}$ and $\gamma_{cmc}$ for

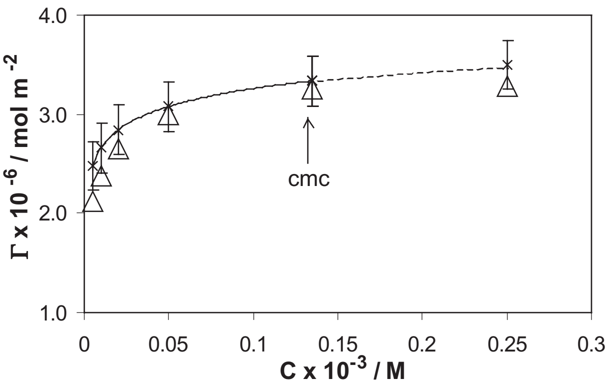


Fig. 4. Comparison of surface excess obtained from surface tension (×) and neutron reflectivity (Δ) measurements for $C_{14}PC$.

$hC_{12}hP_6C$ with $A_{cmc}=70 \pm 4$ Å$^2$ and $\gamma_{cmc}=41$ mN/m. An increase in $A_{cmc}$ can only occur if the head group is now bent away from the surface normal direction. An increase in m to 6 provides more flexibility in the linking spacer accompanied by an increased hydrophobicity. A further factor that could contribute to the bending is the attraction between opposite charges between P and N atom centres between the neighbouring head groups in the layer. The interplay between these interactions may mean that with increasing spacer length the N atom charge centres are brought back to the outer surface. The structural reorientation is accompanied by the shift of $\gamma_{cmc}$ from 38.8 mN/m for $C_{12}P_4C$ to 41 mN/m for $C_{12}P_6C$, which is even closer to 40.5mN/m for $C_{12}P_2C$. When compared with $C_{16}PC$ surfactant it indicates that the increase in the linking spacer chain in the head group has a very different implication to the structural alignment in the surface layer and the surface activity of the molecules.

The values of $\gamma_{cmc}$ for the PC surfactants are close to that for SDS and single chain $C_{12}TAB$, at about 40 mN/m. Given that the molecular structure and charge interaction are different between $C_{12}P_mC$ and the di-chain Gemini $C_{12}TAB$, $\gamma_{cmc}$ is also around 40 mN/m. For the Gemini $C_{12}TAB$ surfactants, $\gamma_{cmc}$ is 37 mNm$^{-1}$ when the linking spacer has 3 methylene units but then increase to 41 mN/m for $m=4$ and 42 mN/m for $m=6$ [20]. This steady increase in $\gamma_{cmc}$ is contrasted to the initial decrease in $\gamma_{cmc}$ which then rises again in the case of $C_{12}P_mC$ surfactants. This illustrates the different interfacial interactions involved in bending the head groups toward the hydrophobic tail region and minimising the effective hydrophobic area in contact with water. The difference in $\gamma_{cmc}$ is broadly consistent with the observed variation in $A_{cmc}$.

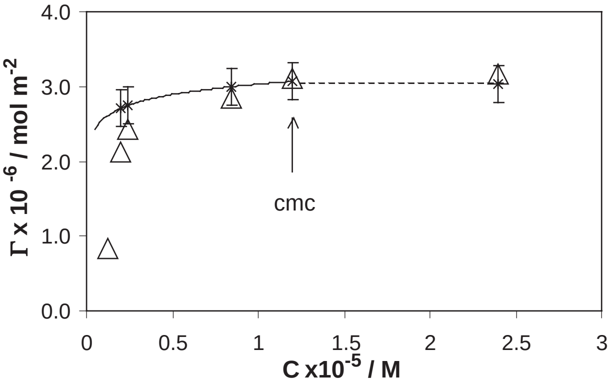


Fig. 5. Comparison of surface excess obtained from surface tension (×) and neutron reflectivity (Δ) measurements for $C_{16}PC$.

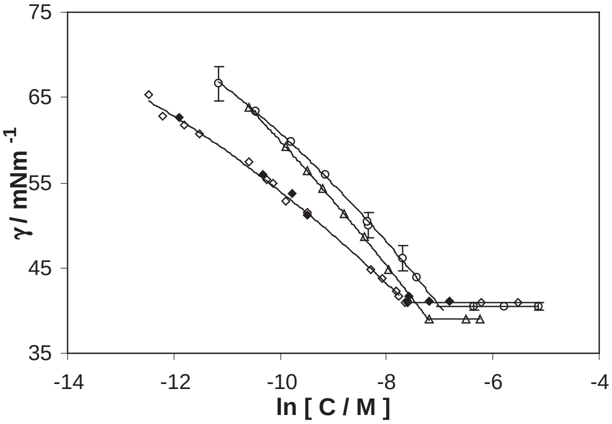


Fig. 6. The surface tension plots shown for $C_{12}P_mC$, with $m=2$ (○), 4 (Δ) and 6 (◇), with continuous lines below the CMC representing the best second order polynomial fits. Surface tension measured from $dC_{12}hP_6C$ (◆) is compared with that from $hC_{12}hP_6C$ and the good agreement suggests no isotopic effect.

The CMC for $C_{12}P_mC$ shows a steady decrease, indicating that a slightly different packing and energetic minimization in solution micellization as compared with surface adsorption. This trend is broadly similar to the di-chain Gemini $C_{12}TAB$'s [20] but is opposite to that observed for the non-ionic surfactants [4].

Fig. 2 (b) also shows that the gradient of the dependence of CMC on the increase of methylene groups in the head group linker. Interestingly, the CMC gradient for the non-ionic $C_{12}EO_m$ surfactants when increased from $m=1$ to 4 is about the same as that for $C_{12}P_mC$, but with opposite sign. For the $C_{12}EO_m$ surfactants, the hydrophilicity shows a slight increase with m whilst the opposite is true for the $C_{12}P_mC$ series. In all cases, the gradients related to CMC changes caused by methylene increase in the head group linker are significantly lower than that observed from increase in n, in the alkyl chain.

Table 4
Key physical parameters obtained from surface tension for $C_{12}P_mC$ at 25 °C and 40 °C

| Surfactant | $A_{cmc}$ ±4 Å$^2$ | CMC/10$^{-3}$ M±0.1 mM | $\gamma_{cmc}$±0.5 mN m$^{-1}$ |
|---|---|---|---|
| ***25 °C*** | | | |
| $hC_{12}hP_2C$ | 53 | 0.91 | 40.5 |
| $hC_{12}hP_4C$ | 51 | 0.7 | 38.8 |
| $hC_{12}hP_6C$ | 70 | 0.5 | 41 |
| ***40 °C*** | | | |
| $hC_{12}hP_6C$ | 71 | 0.5 | 41 |

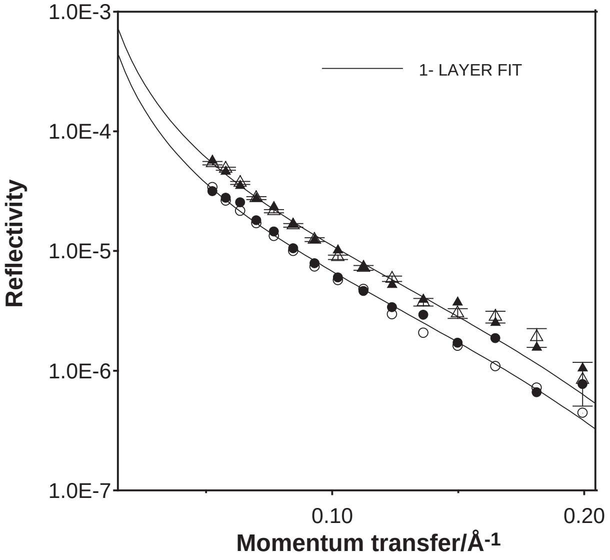


Fig. 7. Neutron reflectivity profiles for $dC_{12}hP_6C$ at a concentration of 0.5 mM and at temperature of 25 °C (Δ) and (▲) 40 °C in NRW. Also at the lower concentration of 0.0025mM at 25 °C (○) and (●) at 40 °C, both in NRW. Continuous lines represent the best uniform model fits with structural parameters listed in Table 5.

Neutron reflectivity measurements were also made from $dC_{12}hP_6C$ in NRW to determine layer thickness and area per molecule (Fig. 7). $A_{\text{cmc}}$ was found to be 78 ± 3 $Å^2$ and compares well with surface tension. The total thickness of the layer ($\tau$ ) at the CMC was again 21.5 ± 2 Å and showed little measurable thickness variation in the alkyl chain region with the linker length. This further supports the view of a bent configuration for the head group. At the lower concentration of 0.025mM, $A$ increased to 100 ± 4 $Å^2$. However, similar to $C_nPCs$, the total thickness was constant at 21.5 ± 2 Å. The fitted curves in Fig. 7 show how change in concentration or temperature has little effect on thickness of $dC_{12}hP_6C$.

Fig. 7 also shows reflectivity profiles measured at 40 °C, which are within experimental error identical to those measured at 25 °C. Because the area per molecule is dominated by the conformational structure of the $P_6C$ head group, the insensitivity of the layer structure to temperature variation is likely to indicate little dehydration or any other structural changes within the head group region.

Analysis of this surfactant adsorption by neutron reflectivity was further enhanced by the use of isotopic substitution and contrast manipulation of the surfactant and the sub-phase respectively. The chain deuterated surfactant, $dC_{12}hP_6C$, in $D_2O$ (dh / $D_2O$) at CMC confirmed a similar total thickness of 21 ± 2 Å and area per molecule of 78 $Å^2$ compared to $dC_{12}hP_6C$ in NRW (dh / NRW) as shown in Table 5. Fig. 8a shows a direct comparison of neutron data for $dC_{12}hP_6C$ in

Table 5
Physical parameters for $C_{12}P_6C$ layers adsorbed in NRW and $D_2O$ at 25 °C obtained from uniform layer model fitting

| Contrast | $C/10^{-3}$ M | $\rho/10^{-6}$ $Å^{-2}$ | $\tau$ ± 2 Å | A ± 3 $Å^2$ | $\Gamma$ ± 0.25/$10^{-6}$ mol $m^{-2}$ |
|---|---|---|---|---|---|
| dh/NRW | 0.75 | 1.58 | 21.5 | 76 | 2.20 |
| dh/NRW | 0.5 | 1.51 | 21.5 | 78 | 2.14 |
| hh/$D_2O$ | 0.5 | 4.51 | 14 | 77 | 2.16 |
| dh/$D_2O$ | 0.5 | 4.32 | 21.1 | 78 | 2.14 |
| dh/NRW | 0.15 | 1.35 | 21.5 | 87 | 1.90 |
| dh/NRW | 0.025 | 1.18 | 21.5 | 100 | 1.65 |
| hh/$D_2O$ | 0.025 | 4.82 | 14 | 100 | 1.65 |
| dh/$D_2O$ | 0.025 | 4.58 | 20 | 101 | 1.64 |
| dh/NRW | 0.0075 | 1.01 | 21.5 | 117 | 1.41 |

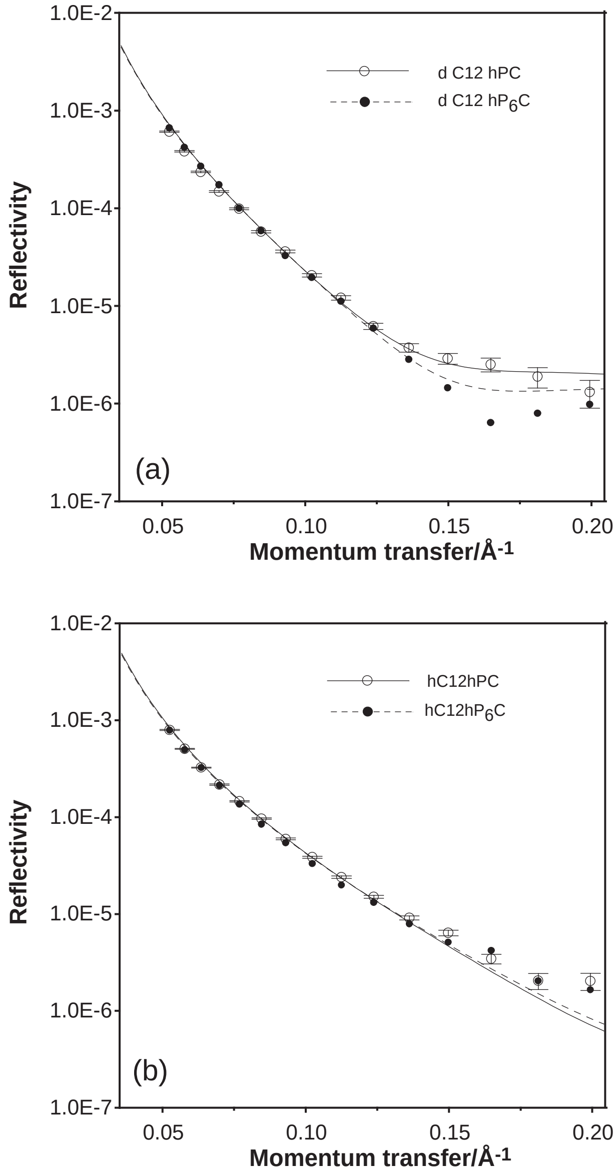


Fig. 8. a. A comparison of neutron reflectivity profiles for $dC_{12}hP_6C$ (●) and $dC_{12}hPC$ (○) both in $D_2O$ at their CMC's and at a temperature of 25 °C. The continuous and dash lines represent the best uniform model fits with structural parameters listed in Table 5. b. A comparison of neutron reflectivity profiles for $hC_{12}hP_6C$ (●) and $hC_{12}hPC$ (○) both in $D_2O$ at their CMC's and at a temperature of 25 °C. The continuous and dash lines represent the best uniform model fits with the structural parameters listed in Table 5.

$D_2O$ with $dC_{12}hPC$ in $D_2O$ at CMC. Both sets of the measured data are close except over the high wave vector region where the data has a significant contribution from the background scattering. It should be noted that the $dC_{12}hPC$ and $dC_{12}hP_6C$ have different scattering length densities because the areas per molecule are not the same. A further contrast pair, $hC_{12}hPC$ in $D_2O$ and $hC_{12}hP_6C$ in $D_2O$ (hh / $D_2O$) at CMC, as shown in Fig. 8b, provides conclusive evidence that the hydrophilic part of the surfactant immersed in water has the same thickness, thereby indicating the change of the head group conformation. The thickness for this submerged region was found to be 14 Å, close to 15 Å obtained from $hC_{12}hPC$. Note that the fully extended length for the hydrophilic part, $hP_6C$, is 17Å, as shown in Table 1. In Fig. 8b the contrast of $hC_{12}hP_6C$ in $D_2O$ is again directly compared with $hC_{12}hPC$ in $D_2O$ and shows a similarity in the reflectivity shown by the parallel uniform layer fits, thus concluding a similar thickness. For both $dC_{12}hP_6C$ and $hC_{12}hP_6C$ isotopic contrasts the thickness is unaltered with change in the concentration.

The addition of salt or a change in temperature or a combination of these had little measurable effect on layer thickness or area per molecule. This observation is the same as found for $C_{12}PC$, showing that increasing head group linker length does not alter the general zwitterionic nature of these surfactants.

## 4. Conclusions

One of the most interesting results obtained from this work is the thickness of surfactant layers constant at 21 ± 2Å, for both $C_nPC$ and $C_{12}P_mC$. The thickness for the chain deuterated surfactants is some 20 Å and is close to the whole layer thickness. The similarity between the two values arises partly from the contribution of the hydrogenated PC head to the thickness obtained from the alkyl chain deuterated layer and partly reflects the extent of mixing within the layer. In addition, the thickness of the head group region immersed in water is 14 Å. A reasonable corroboration of these data is to assume the high extent of intermixing between the alkyl chain and the head group and the submersion of some fraction of the alkyl chain into water. Mixing of alkyl chain with water is well illustrated from more conventional ionic and non-ionic surfactants in our previous neutron work [40].

In the case of $C_nPC$ where n was increased from 10 to 18, the constant layer thickness indicates a progressive tilting of alkyl chains away from the surface normal direction, thereby increasing the packing density of the layer. For a particular $C_nPC$, constant thickness was also observed as the concentration of surfactant was lowered, suggesting that as the layer coverage is reduced, the average tilt angle of the alkyl chains varies little. This may have direct implication to the packing of the surfactant molecules within the surface layer.

In the case of $C_{12}P_mC$ where m varies from 2 to 6, the constant layer thickness means that the angle of tilting of the dodecyl chains varies little whist the orientational configuration of the head groups is altered. This reconfiguration results in a change of $A_{cmc}$ for the surfactant.

$A_{cmc}$ was found to be ca 50 ± 3 Å$^2$ for all the chain isomers from $C_{10}PC$ to $C_{18}PC$. Very good agreement was obtained in area per molecule between surface tension and neutron reflectivity. The constant $A_{cmc}$ suggests that the limiting area per molecule is occupied by the bulky PC head group. In the case of $C_{12}P_mC$, $A_{cmc}$ was also constant around 50 Å$^2$ when m increased from 2 to 4. However, as m was increased to 6, $A_{cmc}$ became some 70 Å$^2$, suggesting that further increase in the size of the head group caused a major change in the orientational configuration.

Increase in alkyl chain length reduced the CMC for the $C_nPC$ series and the gradient of the plot of logCMC versus the number of increase of methylenes was found to be close to those obtained from non-ionic and zwitteroinic betaine surfactants. But increase in the number of carbons in the hydrophilic part of the surfactant reduced the rate of CMC lowering by a factor of 7–10, showing different roles played by the alkyl chain and head group in the micellization. The gradient of CMC lowering for $C_{12}P_mC$ was similar to that observed from non-ionic $C_{12}EO_m$ surfactants but with opposite sign, suggesting different causative effects on amphiphilicity.

Increasing the temperature to 40 °C or the addition of salt showed no effect on the area per molecule or thickness of the surfactant. Also the separation of the zwitterionic charges by the introduction of methylene units has not altered the capability of the surfactant to resist environmental changes. A possible explanation for this stability is that as the linker length increases the structural flexibility between P and N charge centres increases. The zwitterionic nature is sustained through improved interaction between neighbouring head groups. These specific structural features may provide PC surfactants unique properties in the solubilization of glycoproteins and drug molecules that are difficult to dissolve in most conventional surfactants.

## Acknowledgements

We thank EPSRC for supporting the access to ISIS Neutron Facility. Thanks to Haji M. Sharif and Javid Ali for their financial assistance and helpful discussions. Thanks also to T J Su for her helpful discussions.

## References

[1] R.K. Thomas, Neutron reflection from liquid interfaces, Annu. Rev. Phys. Chem. 55 (2004) 391.

[2] R.J.M. Tausk, J. Karmiggelt, C. Oudshoorn, J.Th.G. Overbeek, Physical chemical studies of short-chain lecithin homologues: I. Influence of the chain length of the fatty acid ester and of electrolytes on the critical micelle concentration, Biophys. Chem. 1 (1974) 175.

[3] A. Pinazo, C.H. Chang, E.I. Franses, Dynamic surface-tension behavior of aqueous-solutions of *N*-Dodecyl-*N*,*N* Dimethyl Aminobetaine Chlorhydrate, Colloid Polym. Sci. 272 (1994) 447.

[4] J.R. Lu, Z.X. Li, T.J. Su, R.K. Thomas, J. Penfold, Structure of adsorbed layers of ethylene–glycol monododecyl ether surfactants with one, two, and four ethylene-oxide groups, as determined by neutron reflection, Langmuir 9 (1993) 2408.

[5] J.R. Lu, A. Marrocco, T.J. Su, R.K. Thomas, J. Penfold, Adsorption of dodecyl sulfate surfactants with monovalent metal counterions at the air–water interface studied by neutron reflection and surface tension, J. Colloid Interface Sci. 158 (1993) 303.

[6] D.J. Lyttle, J.R. Lu, T.J. Su, R.K. Thomas, J. Penfold, Structure of a dodecyltrimethylammonium bromide layer at the air–water interface determined by neutron reflection— comparison of the monolayer structure of cationic surfactants with different chain lengths, Langmuir 11 (1995) 1001.

[7] M. Yaseen, Y. Wang, T.J. Su, J.R. Lu, Surface adsorption of zwitterionic surfactants: *n*-alkyl phosphocholines characterised by surface tensiometry and neutron reflection, J. Colloid Interface Sci. 288 (2005) 361.

[8] F.H. Florenzano, L.G. Dias, Critical micelle concentration and average aggregation number estimate of zwitterionic amphiphiles: salt effect, Langmuir 13 (1997) 5756.

[9] J.D. Hines, P.R. Garrett, G.K. Rennie, R.K. Thomas, J. Penfold, Structure of an adsorbed layer of *n*-Dodecyl-*N*,*N*-dimethylamino acetate at the air/solution interface as determined by neutron reflection, J. Phys. Chem., B 101 (1997) 7121.

[10] R. Zana, Ionization of cationic micelles— effect of the detergent structure, J. Colloid Interface Sci. 78 (1980) 330.

[11] E.M. Lee, R.K. Thomas, J. Penfold, R.C. Ward, Structure of aqueous decyltrimethylammonium bromide solutions at the air water interface studied by the Specular Reflection of Neutrons, J. Phys. Chem. 93 (1989) 381.

[12] J.R. Lu, M. Hromadova, E.A. Simister, R.K. Thomas, J. Penfold, Neutron reflection from hexadecyltrimethylammonium bromide adsorbed at the air/liquid interface: the variation of the hydrocarbon chain distribution with surface concentration, J. Phys. Chem. 98 (1994) 11519.

[13] J.R. Lu, E.A. Simister, R.K. Thomas, J. Penfold, Some aspects of the structure of amphiphilic monolayers at the air–liquid interface determined by neutron reflection, J. Phys.: Condens. Matter 6 (1994) A403.

[14] J.R. Lu, Z.X. Li, J. Smallwood, R.K. Thomas, J. Penfold, Detailed structure of the hydrocarbon chain in a surfactant monolayer at the air/water interface: neutron reflection from hexadecyltrimethylammonium bromide, J. Phys. Chem. 99 (1995) 8233.

[15] J.R. Lu, E.A. Simister, R.K. Thomas, J. Penfold, Structure of an octadecyltrimethylammonium bromide layer at the air/water interface determined by neutron reflection: systematic errors in reflectivity measurements, J. Phys. Chem. 97 (1993) 6024.

[16] J.R. Lu, M. Hromadova, R.K. Thomas, Neutron reflection from triethylene glycol monododecyl ether adsorbed at the air–liquid interface— the variation of the hydrocarbon chain distribution with surface concentration, Langmuir 9 (1993) 2417.

[17] J.R. Lu, Z.X. Li, R.K. Thomas, E.J. Staples, L. Thompson, I. Tucker, J. Penfold, Neutron reflection from a layer of monododecyl octaethylene glycol adsorbed at the air–liquid interface: the structure of the layer and the effects of temperature, J. Phys. Chem. 98 (1994) 6559.

[18] J.R. Lu, R.K. Thomas, E.M. Lee, J. Penfold, S.L. Flitsch, Direct determination by neutron reflection of the structure of triethylene glycol monododecyl ether layers at the air–water interface, Langmuir 9 (1993) 1352.

[19] S.R. Green, T.J. Su, J.R. Lu, J. Penfold, The monolayer structure of the branched nonyl phenol oxyethylene glycols at the air–water interface, J. Phys. Chem., B. 104 (2000) 1507.

[20] Z.X. Li, C.C. Dong, J.B. Wang, R.K. Thomas, J. Penfold, Unusual surface structure in layers of cationic gemini surfactants adsorbed at the air/water interface: a neutron reflection study, Langmuir 18 (2002) 6614.

[21] H. Baltes, M. Schwendler, C.A. Helm, H. Möhwald, Tail and Head Group Interactions in phospholipid monolayers, J. Colloid Interface Sci. 178 (1995) 135.

[22] F. Giulieri, M.P. Krafft, Can the formation of vesicles from single-chain perfluoroalkylated amphiphiles be predicted, Colloids Surf., A Physicochem. Eng. Asp. 84 (1994) 121.

[23] A. Kumar, E. Alami, K. Holmberg, V. Seredyuk, F.M. Menger, Branched zwitterionic gemini surfactants micellization and interaction with ionic surfactants, J. Colloid Surf. A, Physicochem. Eng. Asp. 228 (2003) 197.

[24] J. Penfold, Neutron, X-ray and Light Scattering, in: P. Lindner, Th. ZembElsevier, 1991, p. 223;
J. Penfold, Rutherford Appleton laboratory internal report, RAL-88-088, 1988.

[25] J.B. Hayter, R.R. Highfield, B.J. Pullman, R.K. Thomas, A.I. McMullen, J. Penfold, Critical reflection of neutrons— a new technique for investigating interfacial phenomena, J. Chem. Soc., Faraday Trans. I 77 (1981) 1473.

[26] J.R. Lu, R.K. Thomas, Neutron reflection from wet interfaces, J. Chem. Soc., Faraday Trans. 94 (1998) 995.

[27] V.F. Sears, Neutron News 3 (1992) 26.

[28] C. Tanford, Micelle shape and size, J. Phys. Chem. 76 (1972) 3020.

[29] W.D. Harkins, H.F. Jordan, A method for the determination of surface and interfacial tension from the maximum pull on a ring, J. Am. Chem. Soc. 52 (1930) 1751.

[30] J.R. Lu, A. Simister, E.M. Lee, R.K. Thomas, A.R. Rennie, J. Penfold, Direct determination by neutron reflection of the penetration of water into surfactant layers at the air/water interface, Langmuir 8 (1992) 1837.

[31] J.R. Lu, T.J. Su, Z.X. Li, R.K. Thomas, E.J. Staples, I. Tucker, J. Penfold, Structure of monolayers of monododecyl dodecaethylene glycol at the air–water interface studied by neutron reflection, J. Phys. Chem., B. 101 (1997) 10332.

[32] Anatrace Inc. Catalog, Maumee, OH 43537. 1999–2000.

[33] M.J. Rosen, Surfactants and Interfacial Phenomena, II ed., Wiley, New York, 1988.

[34] K. Meguro, Y. Takasawa, N. Kawahashi, Y. Tabata, M. Ueno, Micellar properties of a series of octaethyleneglycol-*n*-alkyl ethers with homogeneous ethylene oxide chain and their temperature dependence, J. Colloid Interface Sci. 83 (1981) 50.

[35] A. Cipiciani, S. Primieri, The effects of zwitterionic surfactant systems upon aromatic nucleophilic-substitution, J. Chem. Soc., Perkin Trans. 2 (1990) 1365.

[36] P.M. Macdonald, J.R. Rydall, S.C. Kuebler, F.M. Winnik, Synthesis and characterization of a homologous series of zwitterionic surfactants based on phosphocholine, Langmuir 7 (1991) 2602.

[37] A.M. Poskanzer, F.C. Goodrich, Surface viscosity of sodium dodecyl sulfate solutions with and without added dodecanol, J. Phy. Chem. 79 (1975) 2122;
J.R. Lu, I.P. Purcell, E.M. Lee, E.A. Simister, R.K. Thomas, A.R. Rennie, J. Penfold, The composition and structure of sodium dodecyl–sulfate dodecanol mixtures adsorbed at the air–water interface— a neutron reflection study, J. Colloid Interface Sci. 174 (1995) 441.

[38] M. Born, E. Wolf, Principles of Optics, University Press, Cambridge, 1997.

[39] T.L. Crowley, A uniform kinematic approximation for specular reflectivity, Physica A 195 (1993) 354.

[40] J.R. Lu, R.K. Thomas, J. Penfold, Surfactant layers at the air/water interface: structure and composition, Adv. Colloid Interface Sci. 84 (2000) 143.