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Amperometric method for determining the degree of complexation of polyelectrolytes with cationic surfactants

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Abstract

The complexation of sodium polystyrene sulfonate with monovalent cationic surfactants at a microsized liquid/liquid interface has been studied using electrochemistry. The method is based on measurement of surfactant ion transfer across the interface between two immiscible electrolyte solutions (ITIES). The complexation of various cationic surfactants (alkylpyridinium- and trimethylammonium-) with oligosized polystyrene sulfonate was measured. Binding isotherms were used to determine the degree of binding as a function of the surfactant chain length and type of head group. It was found that the hydrophobicity of the surfactant was the predominant factor. The effect of the polyelectrolyte chain length on the binding mechanism was studied using cetylpyridinium chloride as a complexing agent. It was found that binding affinity, as well as cooperativity of the binding process, decreases with decreasing polyelectrolyte chain length. Thermodynamics of surfactant binding was measured using titration microcalorimetry. The thermodynamic data obtained show that the enthalpy of surfactant binding is not dependent on polymer chain length, but an increase in chain length makes the binding process entropically more favorable.

Keywords: Polyelectrolyte complexation; Cationic surfactants; Ion transfer voltammetry; Isothermal titration calorimetry

1. Introduction

The interaction between polyelectrolytes and surfactants of opposite charge has received much attention both experimentally and theoretically [1,2]. In most cases, the polyelectrolytes studied have been high-molecular-weight linear macromolecules. However, behavior of oligosized polyelectrolytes is becoming increasingly important because of their potential use in novel pharmaceutical applications. For instance, oligonucleotides (anionic oligoelectrolytes) have been investigated as potential new drugs [3].

Electroanalytical techniques have been used successfully to determine the binding isotherms for surfactant–polymer complexation. A surfactant-selective membrane electrode has been used to potentiometrically determine the concentration of free surfactant throughout the binding process to obtain the degree of binding [4,5]. In this study, we present a method for monitoring the complexation reaction where the free surfactant concentration is measured amperometrically using ion transfer voltammetry at a microsized interface between two immiscible liquids. Ion transfer voltammetry at microsized interfaces has earlier been used for monitoring facilitated cation binding to DNA [6]. The microinterface was realized using a micropipette filled with the organic phase immersed in the aqueous phase or by placing a polymer film with a laser-drilled microhole between the organic and aqueous phases. All of the measurements were carried out at low surfactant concentrations, below the critical micelle concentration (cmc).

Initially, experimental procedures were optimized by evaluating the binding efficiency of a series of monovalent cationic surfactants. The degree of binding between a polyanion and cationic surfactant is governed by both electrostatic and hydrophobic interactions; therefore both the surfactant head group and the alkyl chain length were varied. From the surfactants studied, cetylpyridinium chloride showed the strongest binding affinity to sodium polystyrene sulfonate (molar mass 5400 g mol$^{-1}$). This was used subsequently in studies concerning the complexation of sodium polystyrene sulfonate samples of differing chain lengths. Binding isotherms, where the degree of binding is given as a function of the concentration of free surfactant, were used to determine the binding parameters for the cooperative binding isotherm.
binding process. The results from supplementary titration calorimetry experiments have been used to establish the thermodynamics of polyelectrolyte surfactant binding.

2. Method: ion transfer voltammetry

The transfer of surfactant ions across the liquid/liquid interface was induced electrochemically. The electrochemical cell used is shown in Scheme 1, where || refers to the polarizable interface under study.

Sodium chloride and tetraphenyl arsonium tetrakis-4-(chloro)phenyl borate (TPA$_2$TPBCl$_4$) were used as aqueous and organic phase supporting electrolytes, respectively. The organic solvent used was 1,2-dichloroethane in the case where the microinterface was supported at the tip of a micropipette and o-nitrophenyl octyl ether immobilized with 5% polyvinyl chloride (PVC) in the case where a microhole was used. Two electrodes located in opposing phases controlled the applied potential across the liquid–liquid interface. As the current flow across the interface is in the nA range, this two-electrode arrangement is sufficient, with each electrode acting as both counter and reference electrode for the respective phase. An ion-selective organic reference electrode (Ag/AgCl/5 mM TPA$_2$Cl/) was used.

When the electrochemical potential of the aqueous phase was positive relative to the organic phase, the cationic surfactant molecules transferred from the aqueous to the organic solution. The potential at the interface was controlled via two Ag/AgCl electrodes, one placed in each phase, such that the rate at which the cations transferred, measured as the current flow, was controlled by the rate of surfactant diffusion to the interface. For a microsized interface, where diffusion is hemispherical, the relationship between the steady state current $i$ and the concentration of free surfactant is given by the equation [7]

$$i = 4nFDrc_f,$$

where $D$ is the diffusion coefficient and $c_f$ is the concentration of free surfactant ions, $F$ is the Faraday constant, $n$ is the ionic charge, and $r$ is the radius of the microhole or micropipette tip. Within the available potential range (determined by the supporting electrolytes used), the surfactant molecules were the only species transferring across the interface. Therefore, the measured steady state current was directly proportional to the concentration of free surfactant and thus the aqueous phase concentration could be assumed to be unchanged. A schematic drawing of the experimental setup is shown in Fig. 1.

3. Experimental

3.1. Chemicals

All organic solvents, surfactants, and polymers were used as purchased without further purification. All aqueous solutions were prepared in distilled Milli-Q water.

3.1.1. Studied surfactants

Cetylpyridinium chloride (CPC) (p.a. 99%) and dodecylpyridinium chloride (DPC) (p.a. 94%) were purchased from Merck. Cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DoTAB), and tetra decyltrimethylammonium bromide (TTAB) (all 99% assay) were purchased from Sigma/Aldrich.

3.1.2. Studied polyelectrolytes

Sodium polystyrene sulfonate (NaPSS) was purchased from Polysciences Inc. Three different molecular weights were used, 1800, 5400, and 18,000, corresponding to polymer chain lengths of 9, 26, and 87 monomer units, respectively. Polydispersity ($M_w/M_n$) of the longer chain length polymers (5400, 18,000) was 1.1 and polydispersity was 1.25 for the short polymer (1800).

3.1.3. Electrolytes for the electrochemical cell

The organic solvents used were 1,2-dichloroethane (DCE, HPLC-grade) from Rathburn and o-nitrophenyl octyl ether (NPOE, Selectophore) from Fluka. Poly(vinyl chloride)
(PVC) (Sigma, very high molecular weight) was used as a gelling agent. The organic supporting electrolyte was tetraphenyl arsonium tetrakis-4-(chloro)phenyl borate (TPAsTPBCl4), which was synthesized from tetraphenylarsionium chloride (TPAsCl, 97%) and potassium tetrakis-4-(chloro)phenyl borate (KTPBCl4) as described earlier [8]. Both of them were purchased from Aldrich. The aqueous supporting electrolyte was sodium chloride (NaCl, p.a.) purchased from Merck.

3.2. Microinterface preparation

Micropipettes for the electrochemical measurement were prepared as described previously [9]. The pipette was filled by drawing solution through the tip using a syringe attached at the other end. Initially, an aqueous reference phase was drawn inside the capillary, followed by an organic phase, so that the aqueous reference phase was situated on top of the organic phase. The Ag/AgCl electrode was placed in the upper aqueous phase. Placing the filled pipette in an aqueous solution resulted in a well-defined 25-µm-diameter liquid/liquid interface being established at the tip of the capillary between the organic and aqueous phases. The second Ag/AgCl electrode was placed in the aqueous phase.

The microholes used in this study were a kind gift from Professor H.H. Girault, EPL, Switzerland. The preparation and assembly of the microelectrodes was as described previously [10]. A 5% PVC/NPOE gel was used as the organic phase. The gel was prepared as described previously [11]. As for the micropipette case, the aqueous reference phase was situated on top of the NPOE gel, an Ag/AgCl electrode was placed in it, and the second Ag/AgCl electrode was placed in the aqueous phase (for details, see Fig. 1).

3.3. Titration procedure

The complexation of NaPSS was realized either by stepwise addition of the polyanion to a solution of surfactant or by stepwise addition of surfactant to a solution of the polyanion. In the former case, a microhole was used to support the microinterface, while a micropipette was used in the latter case. A high concentration of the surfactant decreased the interfacial tension, making it difficult to maintain an interface of constant size at the tip of the micropipette. This difficulty was avoided by using the microhole-supported interface when the polyanion was added to a solution of surfactant.

In the microhole experiments, the concentration of the supporting electrolyte (TPAsTPBCl4) in the organic phase (NPOE) was 2 mM and the concentration of the aqueous supporting electrolyte (NaCl) was 10 mM. A 50 µM surfactant solution was titrated with injections of 25–50 µl of 20 µM NaPSS solution. In the micropipette experiments, the concentration of the supporting electrolyte (TPAsTPBCl4) in the organic phase (1,2-DCE) was 10 mM and the concentrations used for the aqueous supporting electrolyte (NaCl) were 10 and 100 mM. A 5–40 µM NaPSS solution was titrated with injections of 10–20 µl of 3 mM surfactant solution. The volume of the aqueous phase was 2 ml in both cases. Steady state currents were then recorded at a fixed potential after each addition.

3.4. Isothermal titration calorimetry

All titration calorimetric measurements were made using an isothermal titration calorimeter (Microcal VP-ITC, USA) at 25 °C and analyzed with Origin 5.0 software (Microcal, USA). The injections were typically 9 µl and the equilibrium time before each injection was 5 min. The concentration of the CPC was 140 µM and it was chosen to be under critical micelle concentration. The concentrations of the polyanions were c(NaPSS_9) = 2.5 µM, c(NaPSS_26) = 1.0 µM, and c(NaPSS_87) = 0.20 µM. The volume of the cell was 1.4413 ml and the stirring rate was 300 rpm. The heat caused by the dilution of the CPC solution was small and was subtracted from the results.

4. Results and discussion

4.1. Calibration

Cyclic voltammograms of surfactant ion transfer across the liquid–liquid interface are shown in Fig. 2. The surfactant is transferring within the entire potential window, the size of which is determined by the supporting electrolytes used. Fig. 3 shows the steady state current plotted against the total concentration of surfactant c1 in the absence of NaPSS. It can be seen that there is a linear relationship between current and surfactant concentration, the slope of which can be used to determine the concentration of free surfactant in solution.

4.2. Surfactant binding efficiency

The stepwise addition of the polyanion to a solution of surfactant was used to study the binding efficiency of surfac-
Fig. 3. Steady state current as a function of the concentration of CPC at $25^\circ$C in the absence of polyelectrolyte.

Fig. 4. Steady state current as a function of the concentration of NaPSS$_{26}$ for stepwise addition of 20 µM NaPSS$_{26}$ in 10 mM NaCl solution to 2 ml of 100 µM CPC at $25^\circ$C.

This method ensured that the surfactant was initially present in excess, meaning that all of the available binding sites were occupied. Since the surfactant was in excess in the beginning, phase separation did not occur immediately, although all the binding sites were occupied. Some cloudiness was observed during the measurement, which disappeared when polymer addition was continued and surfactant started to dissociate from the complex. The surfactants differed in both the type of head group, either a trimethylammonium ion or a pyridinium ion, and the hydrocarbon chain length, C$_{12}$ to C$_{16}$. Typical voltammetric data are shown in Fig. 4, where the steady state current is plotted against the total concentration of added NaPSS$_{26}$.

Since the relationship between current and the concentration of free surfactant is known, the degree of binding can be calculated using the equation

$$\beta = \frac{c_b}{zc_p} = \frac{(c_t - c_f)}{zc_p},$$

where $c_b$ is the concentration of bound surfactant, $c_p$ is the concentration of NaPSS, and $z$ is the number of charged binding sites of the polyelectrolyte, which in the case of NaPSS is also the number of monomer units. $c_b$ is calculated from the difference between the concentration of total added surfactant, $c_t$, and the concentration of free surfactant, $c_f$.

Fig. 5. Binding constant $\beta$ as a function of the concentration of NaPSS$_{26}$ for different surfactants in 10 mM NaCl solution at $25^\circ$C.

Binding data for the complexation of a series of surfactants and NaPSS$_{26}$ are shown in Figs. 5a and 5b. The binding efficiency can be determined from the shape of the isotherms. At the beginning of the experiment the ratio of $c_t$ and $c_p$ is so high that a substantial amount of surfactant is bound to a polymer. This is seen in high $\beta$ at low polymer concentrations in Figs. 5a and 5b. The plateau in the isotherm corresponds to a region where the concentration of free surfactant is decreasing to maintain the same degree of binding. On the other hand, the drop in $\beta$, observed as the concentration of the polyanion $c_p$ increases, corresponds to a region where the bound surfactant is dissociating from the polymer to maintain equilibrium between the bound and unbound states of the surfactant. The extension of the plateau into a region where the ratio of surfactant to NaPSS is small is indicative of the strong binding affinity of the surfactant to the polymer. It can be seen in Fig. 5 that the binding of the longer chain surfactants is more effective as the value of $\beta$ increases with increasing chain length for all concentrations of NaPSS. Also, the binding affinity is seen to increase with increasing chain length, shown by the extended horizontal region in the $\beta$-curve for CTAB, TTAB, CPC, and DPC. The trend is similar to that reported by others [12], i.e., the binding of the pyridinium ions is stronger and the degree of binding decreases with decreasing chain length. Therefore, on comparison of the binding between these surfactants with NaPSS, cetylpyridinium chloride was seen to be the most effective and it was thus chosen for the measurements to determine the binding mechanism.
4.3. Binding mechanism

The stepwise addition of surfactant to a polyelectrolyte solution was used to determine the binding mechanism of CPC to NaPSS. The CPC concentration range chosen (0.01–0.15 mM) is below the critical micelle concentration, which was measured to be 0.18 mM by isothermal titration calorimetry, ensuring that all the added surfactant was present in monomeric form and was available to be transferred across the liquid/liquid interface.

Fig. 6a shows an example of the relationship between current and surfactant concentration in the presence of NaPSS and the corresponding binding isotherm is presented in Fig. 6b. Initially, virtually all of the surfactant is complex-bound and therefore unavailable to transfer and hence the measured current is very small and almost constant. However, once the polymer is saturated with surfactant, further addition gives rise to free surfactant ions and the current starts to increase. At this point, the polyelectrolyte is saturated with surfactant. As previous studies have also shown [13], phase separation occurred before saturation of the polyelectrolyte, resulting in a decrease of the current near the end of the titration. Correspondingly, the decrease in current has an influence on the shape of the isotherm, which bends backward near the saturation point.

Binding occurs due to electrostatic interaction between the positively charged surfactant head group and the negatively charged groups on the polyanion and hydrophobic interactions between the hydrocarbon chains of the surfactant. Hydrophobic interactions are indicative of a cooperative binding mechanism, which is characterized in the binding isotherm by a sharp increase in $\beta$ at a given value of $c_f$. It is common to describe this kind of cooperative binding by a Zimm–Bragg model, where the polyelectrolyte is represented by a linear array of binding sites and where the effect of the cooperative interactions between adjacent bound ligands is taken into account [14]. Satake and Yang developed the model further and showed the binding degree and the free surfactant concentration to have the relationship [15]

$$\beta = 0.5 \left(1 + \frac{K w f - 1}{\sqrt{1 - K w f}^2 + 4 K f}\right),$$  \hspace{1cm} (3)

where $K$ is the intrinsic binding constant and is a function of the electrostatic interaction between the surfactant and the polymer. The position of the isotherm along the $x$-axis gives the binding affinity $K w$. A shift to the right indicates a lower binding affinity, essentially increasing the critical aggregation concentration (cac), the free surfactant concentration at which the onset of binding occurs [16]. For cooperative binding, $K w$ can be calculated using the equation

$$c_f|_{\beta=0.5} = (K w)^{-1},$$  \hspace{1cm} (4)

where $c_f|_{\beta=0.5}$ is the free surfactant concentration at the half-bound point on the isotherm. The slope of the binding isotherm determines a cooperativity parameter, $w$, which is a function of the hydrophobic interaction between adjacent bound surfactant molecules. The experimental binding isotherms with the corresponding fitted Satake–Yang curves are shown in Fig. 7 for three NaPSS samples of different chain lengths and for two different salt concentrations. Only the lower parts of the binding isotherms, typically $\beta < 0.5$,
were used to fit the theoretical curves to the experimental results. As it is difficult to obtain a value for \( w \) directly from the slope of the isotherm, a value for \( K \) is calculated by a least-squares fit of the binding isotherm to Eq. (3). The parameters obtained from the curve fitting are given in Table 1.

Summarizing the binding in terms of the \( Kw \) and \( w \) values, it can be seen that the binding parameters for cooperative binding vary with the degree of polymerization as well as with the salt concentration. At low salt concentration, \( Kw \), which is essentially a measure of the binding affinity, is increasing with increasing polymer length. Also, the slope of the isotherm becomes less steep with decreasing polymer chain length, which is seen as decreasing values of \( w \) in our work. Similar results have previously been obtained for a binding of relatively low-molecular-weight polyphosphate and polyaspartate with alkylpyridinium chlorides [17,18]. However, in these works the effect of the polymer size is not included in the cooperativity parameter, which is assumed to be a function of the hydrophobic interaction only, and \( w \) is assumed to remain the same for all polymer sizes under study. If our results are compared with those obtained by Shirahama et al. [19] for complexing very-high-molecular-weight polystyrene sulfonate with dodecylpyridinium chloride, it can be seen that the longer the chain length the more cooperative is the binding mechanism. In 80 mM NaCl solution, Shirahama et al. have obtained values 23 and 100,000 M\(^{-1}\) for \( w \) and \( Kw \), respectively.

The shortest polyelectrolyte, NaPSS_9, behaves differently from the longer ones. The cooperativity parameter, \( w \), is substantially lower for NaPSS_9, while the intrinsic binding constant, \( K \), is essentially the same for all polymer lengths. This means that low binding affinity in the case of very short polyelectrolytes is mainly a consequence of low cooperativity due to the small number of monomeric groups in a polymer chain. In this case probably more than one polymer chain might take part in micelle formation, which has been proposed previously by Svensson et al. [20].

Increasing the salt concentration has the same effect as decreasing the chain length: both binding affinity and cooperativity decrease. This is due to the ion condensation that occurs at high salt concentration. It has been shown that the effective charge number and thus the charge density of the polyelectrolyte decrease markedly with increasing salt concentration [21,22]. Hence, the bound surfactant molecules are situated rather far from each other. This makes micelle formation more difficult.

### 4.4. Calorimetric results

Microcalorimetric measurements were carried out to study the effect of the chain length on the binding efficiency. An order of magnitude more dilute solutions were used than in amperometric measurements. This was due to surfactant micelle deformation under titration procedure, which causes undesired heat effects. Therefore the concentration of surfactant in a titration unit was kept under the cmc.

The enthalpy data obtained from the calorimetric measurements are given in Fig. 8 and parameters evaluated from the measurements are presented in Tables 2 and 3. Table 2 shows Gibbs energies calculated from the cooperativity equilibrium constants using enthalpy values from the calorimetric measurements. The values in Table 3 were obtained using Microcal’s Origin 5.0 software. The values of Gibbs free energy obtained from amperometric studies are smaller than those obtained from calorimetric studies. That is simply a consequence of the different concentrations used in different methods, since the equilibrium constant is related to the concentration of the free surfactant ions (Eq. (4)) and the critical aggregation concentration of the surfactant ion is strongly related to the concentration of the polymer [23].

The thermodynamic data show that the enthalpy of the surfactant binding to the polyelectrolyte is basically the same for all polymer chain lengths and the values measured were similar to those obtained previously [24]. However, it is evident that an increase in the polymer chain length makes the binding process entropically more favorable. Since micelle formation is known to be driven by entropy [25], this could mean that binding to longer polymer chains is mainly driven by micelle formation, while polymers with shorter chains form looser micelle-like complexes, the formation of which is mostly driven by electrostatic interaction.
Table 2
Amperometric data combined with the enthalpy data

<table>
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<tr>
<th>Polymer</th>
<th>(c) (µM)</th>
<th>(\Delta G_{\text{amp}}) (kJ mol(^{-1}))</th>
<th>(\Delta H_1) (kJ mol(^{-1}))</th>
<th>(-T\Delta S_1) (kJ mol(^{-1}))</th>
<th>(\Delta S_1) (J mol(^{-1}) K(^{-1}))</th>
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<tr>
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<td>5.1</td>
<td>-31.4</td>
<td>-18.4</td>
<td>-13.0</td>
<td>43.4</td>
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Table 3
Calorimetric data

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<tr>
<th>Polymer</th>
<th>(c) (PSS) (µM)</th>
<th>(\Delta G_{\text{cal}}) (kJ mol(^{-1}))</th>
<th>(\Delta H_1) (kJ mol(^{-1}))</th>
<th>(-T\Delta S_1) (kJ mol(^{-1}))</th>
<th>(\Delta S_1) (J mol(^{-1}) K(^{-1}))</th>
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