

Studying Specific Ion Effects on the Micellization of 1, 2-Hexanediol

by

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Abstract

Specific ion effects on protein interfaces have been observed for many years, but yet comprehensive explanations regarding the mechanism by which ions interact with proteins and more general aqueous interfaces are still under investigation. Realistically, ion specificity on protein stability is due to numerous contributions and interactions between the solution and protein. However, the most important contribution is arguably the hydrophobic effect, specifically the change in free energy when water molecules are liberated from the interfacial region upon protein folding. In the work presented here, the effects of different ions on the critical micelle concentration (CMC) of 1, 2 –Hexanediol were examined to study salt effects on hydrophobicity by the means of fluorescence spectroscopy. Our results show that anions and cations do exhibit the specific effects on hydrophobic interactions. However, the origin of these specific ion effects different for cations and anions. Cation specific effects are caused by their ability to form cavities in solution, while anion specific effects arise from their ability to interact with the interface. These results are of interest to the researchers in the protein folding field, providing significant experimental hydrophobicity data necessary for theoretical biologists that are attempting to predict protein structures.

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Chapter 1

Introduction

1.1 Ion specific effects in biophysics

It has long been known that the addition of salt to aqueous solutions of proteins significantly influences protein stability and dynamics.^{1,2,3,4,5,6} Salts have been shown to not only directly affect the thermodynamic and kinetic properties of the folding process^{7,8,9,10,11}, but to also modulate such diverse properties as protein solubility (salting-out and salting-in)^{12,13,14,15,16,17}, loop and domain motion^{18,19} and enzymatic activity^{20,21,22,23,24, 25}. Besides protein solutions, it has been found that there are consistent salt effects influencing a wide range of physical properties in amino acids^{26,27,28}, surface tension of aqueous interfaces^{29,30,31,32}, micelle formation^{33,34,35,36,37,38,39}, membrane permeability^{40,41}, the phase behavior of monolayers and macromolecules^{42,43}, etc. Studying ion specificity became a longstanding goal of biophysicists because a fundamental comprehensive model which can describe ionic behavior at biologically relevant interfaces has not been developed yet. A wide variety of spectroscopic, thermodynamic, and computational studies have been conducted to study the ion effects at the interfaces.^{44,45,46} Most of those studies involve proteins. The effect of salts on

proteins has been demonstrated using different systems.⁶ For example, salts were shown to affect thermodynamics of association and dissociation of a protein-ligand binding system.⁴⁷ Protein surfaces also were used to model specific salt effects with the aim to elucidate ion absorption at those surfaces.⁴⁸ Unfolded profiles of proteins have been used to show the role of salt concentrations in protein stability⁴⁹. It has been shown that the effect of different salts on the properties of proteins varies based on the nature of ionic species present and its concentration.^{12,50} For example, protein solubility can change by addition of salts to the solution. Ammonium sulfate or sodium sulfate at high concentration can be used to purify or crystallize proteins by “salting out”, which is a very important aspect of protein crystallization.^{51,52,53} One can also “salt in”, or denature a protein by addition of concentrated urea or guanidine hydrochloride.^{54,55,56} Interaction of salts with proteins is highly specific and different salts affect proteins differently. Perhaps the primary and the most systematic example of specific ion effects on the solubility of proteins are the Hofmeister series.

1.1.1 Hofmeister series

The work of the Franz Hofmeister on the specific salt effects dates back to the 19th century, when he shown that various salts affect protein solubility differently.^{2,12} In his experiments Hofmeister observed that proteins would precipitate from a solution at different concentrations of these salts, with some salts being much more effective precipitants than others.¹² Salt effects on the proteins were ranked based on the effectiveness of both anions and cations in precipitating of the proteins. This ranking gave a rise to what are known today as Hofmeister series or specific salt effects. But an

interesting fact arises regarding all the published data on the Hofmeister series, it turns out that these effects are highly system specific. Some ions can change position in the series or even be completely absent or you can also find an inverse series depending on the system being examined.^{57,58,59,60} Cremer's work provide a good example on the inverse and direct Hofmeister series for lysozyme. He showed that protein aggregation in solution followed 2 distinct Hofmeister series depending on salt concentration. At low salt concentration lysozyme displayed an inverse Hofmeister series. As the concentration of salt was raised, the order reverted back to a direct Hofmeister series.¹³² Kunz in his recent book¹³¹ attempted to summarize the more current knowledge regarding to Hofmeister series ordering.³

HOFMEISTER SERIES

Cations

$\text{N}(\text{CH}_3)_4^+$ NH_4^+ Cs^+ Rb^+ K^+ Na^+ Li^+ Mg^{2+} Ca^{2+}



SO_4^{2-} HPO_4^{2-} OAc^- cit^- OH^- Cl^- Br^- NO_3^- ClO_3^- BF_4^- I^- ClO_4^- SCN^- PF_6^-

Anions

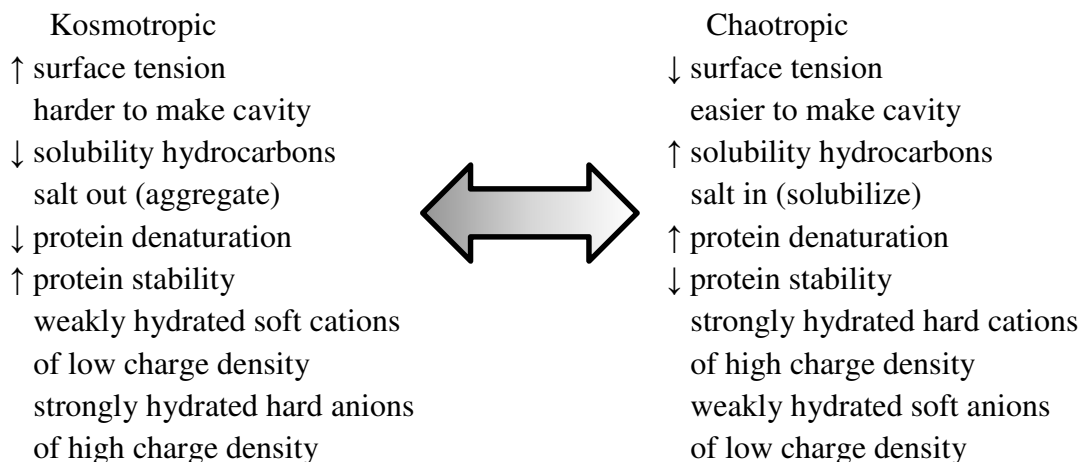


Figure 1-1. Typical ordering of cations and anions in a Hofmeister series.³

As seen in Figure 1-1 both series contains monovalent and divalent ions and monatomic and polyatomic ions. For anions, species to the right of Cl^- are known as chaotropes, and the ions to its left are known as kosmotropes. The chaotropic anions are known to destabilize folded state of the protein, decrease surface tension and result in salting-in behaviour. In contrast, the kosmotropic anions have the opposite effects and salt-out proteins from solution, stabilizing the folded state of the protein. Generally, larger and more polarizable anions tend to be chaotropic, and the smaller anions, which are strongly hydrated, behave as kosmotropes. Cations ranked from weakly hydrated soft cations with least precipitating properties on the left to strongly hydrated hard cations with the most precipitating properties on the right.^{61,12} The Hofmeister effect was originally explained by the ability of various ions to “make” or “break” bulk water structure.⁵⁹ Kosmotropes were considered as water structure makers that strengthen hydrogen bonds in aqueous solution while chaotropes were considered as water structure breakers that break hydrogen bonds in aqueous solution. Therefore, by affecting the water structure around them, ions would influence the chemistry of the solution. Despite the long research history Hofmeister effects at the interfaces are still poorly understood. One of the major reasons for that is the complexity of salt effects on the proteins. It is known that proteins involve lots of specific interactions such as van der Waals interactions, electrostatic interactions, hydrogen-bonding interactions, hydrophobic interactions, etc.^{62,63,64} Our goal is to simplify this complex issue. With this in mind we will consider one of the major stabilizing interactions in proteins, namely hydrophobic effect.

1.2 Salt effects on hydrophobicity

The hydrophobic effect, namely, the observed tendency of nonpolar molecules to aggregate in aqueous solutions and thus exclude water molecules, is one of the most important interactions stabilizing biological molecules.^{65,66,67} For example, there are hydrophobic interactions that stabilize the structure of proteins⁶⁸, form biological membranes⁶⁹ and provide the driving force for the interaction of many ligands with their bio-molecular targets.⁷⁰ However, although biophysicists have known the existence of hydrophobicity for a long time, a complete molecular description of this interaction still eludes characterization. One major challenge in understanding hydrophobicity lies in the fact that most experimental measurements of the process have been performed on simple molecules such as methane dissolved in pure water.^{71,9} As a matter of fact a sizable number of biologically relevant hydrophobic interactions exist between large and complex molecules, and, more importantly, occur in aqueous cellular media that are crowded with co-solutes such as salts, osmolytes, proteins and nucleic acids. In order to gain a better understanding of how salts affect biologically relevant hydrophobic interactions one way to look at it is from the interfacial standpoint. First we will look at the ion-interface interaction from the classical electrostatic theory standpoint. And then consider other theories that attempt to explain the effect of ions on hydrophobic interactions.

1.2.1 Salt effects on hydrophobicity: interfacial theories

a) Classical electrostatic description of ion-interface interactions

The simplest aqueous interface is that of air and water. Classical thermodynamics shows that if salt added to an aqueous solution – the surface tension of that solution is going to change, as well as the concentration at the interface.⁷² The relationship between surface

tension increment $\left(\frac{d\gamma}{da_i}\right)_{T,P}$ and excess surface concentration can be described by the

Gibbs absorption law⁷³:

$$\Gamma_i = -\left(a_i/RT\right)\left(\frac{d\gamma}{da_i}\right)_{T,P} \quad (1-1)$$

In which Γ_i is the surface excess concentration, a_i is the activity of a particular ion; while R , T and P are the gas constant, temperature and pressure.

The surface tension increment is a key quantity in dealing with the role of ions at the interface. Applying this equation to the experimentally observed data it can be concluded that different salts will have different effects on surface tension, so there is ion specificity observed on the air/water interface.^{74,75,76} How does theory explain this observation? The simplest description of an ion interaction with an interface can be given by a classical electrostatic theory. In this description ions considered as a simple hard spheres with a point charges located at the center. This is the basis of Debye-Hückel (DH) theory⁷⁷. Based on this theory, all monovalent cations are considered to be spheres with same positive charge in the center and all monovalent anions are considered to be spheres with same negative charge in the center. Air and water are considered to be continuum media

with dielectric constants $\epsilon=1$ and $\epsilon=80$ respectively and separated from each other by a perfectly flat interface. Figure 1-2 represents the behaviour of the ion at the interface of two dielectrics. When the ion approaches the interface it is moving from the bulk solution with high dielectric constant to the interface with low dielectric constant. The ion would prefer to be in the high dielectric media rather than in the low. So the ion is being repelled by the interface. It can be modeled as an existence of a fictitious ion referred to as an image charge with the same charge present in the air repelling it.

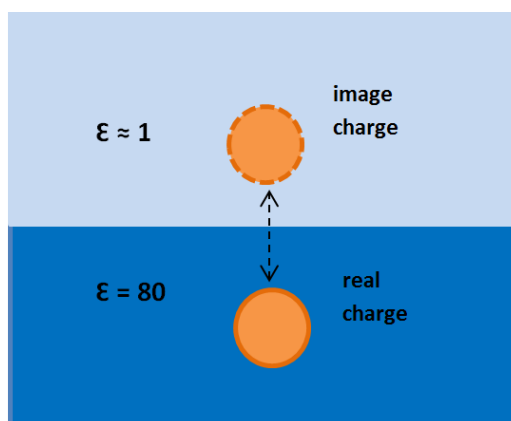


Figure 1-2. Continuum dielectric model of an ion at an aqueous interface.

Classical electrostatic theory predicts that ions of the same charge - fluorine and chlorine for example - will be distributed in the same way between the bulk solution and the solute interface, in other words they both will be repelled from the interface. In the real world, monovalent anions vary from the small, strongly hydrated and usually protein stabilizing (F^-) to the large, very weakly hydrated and usually protein denaturing (SCN^-). Debye-Hückel (DH) theory became a basis for further developing models and was advanced to calculate surface tension of electrolytes. The first person who studied the effects of salts on surface tension of the aqueous solution was Wagner, who applied Gibbs' absorption isotherm to calculate surface tension of electrolyte solutions.¹²⁹ Later

his idea was extended by Onsager and Samaras when they suggested a universal limiting law for the dependence of surface tension on the salt concentration.¹³⁰ In their theory Onsager and Samaras provided an explanation for why salts increase surface tension, and argued that the increase in surface tension they observed was independent of the ion type. Contrary to this explanation, further investigations on the ion-specific interactions between ions and interfaces, and their role in changing surface tension of electrolyte solution have shown that there are ions present at the air-water interface.^{78,79,80,81} Later several improvements have been made to the electrostatic theories, taking into account finite ion sizes, ion hydration, polarizability, dispersion forces and variation of dielectric constant.^{82,83,84,85} However, these continuum approaches are still oversimplified, as they neglect discrete nature of water, especially its geometry around ions and at surfaces.⁸⁶ Nowadays, computer simulations such as molecular dynamics (MD) and Monte-Carlo simulations have been shown to give promising results to describe ion specific effects of relatively simple system such as air/water interface, which include properties of water explicitly.^{87,44,79} However, they have their own limitations due to their complexity and a strong dependence on the fitting parameters, which often have to be adjusted for each salt.⁸⁸ With all the progress made in the field, the solution to the problem of the ion behavior at the interfaces still seems not to come into reach. Classical electrostatic models require improvements because they provide an incomplete picture on what is happening at the interfaces and cannot be used to study ions at the air-water interface let alone at any other hydrophobic interface.

b) Solute partitioning model

Given that it is hard to theoretically understand the mechanism by which ions interact with air/water interface on the microscopic level as seen in the previous section, perhaps we might consider to use a macroscopic model to explain ions behaviour at the interfaces. Pegram and Record hypothesize that ion-specific effects seen at the air/water interface might be similar to those seen at hydrocarbon/water interfaces and the air at the air/water interface can be considered to be an ultimate hydrophobic surface. Based on this hypothesis they suggested a macroscopic model called Solute Partitioning Model (SPM).⁸⁹ The main premise of this model is that if you have an interface and water, the water molecules can be considered to be divided into two populations – the bulk phase and the interfacial phase (Figure 1-3).

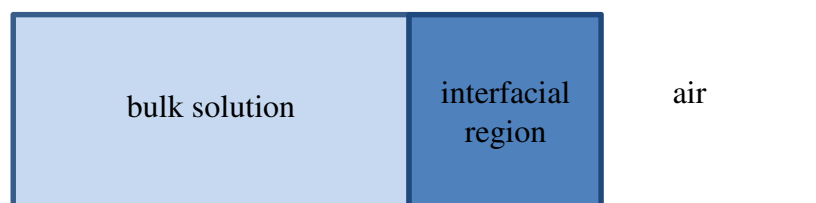


Figure 1-3. Distribution of the water molecules at the hydrophobic interface.

Pegram and Record proposed that ions will partition differentially between those two phases. Some of them will be preferably partitioned into the interfacial phase while other ions will be repelled from the air-water interface accumulating in the bulk solution.⁹⁰ Partitioning of ions between the bulk water and the air-water interface is quantified by partition coefficient K_p . The partitioning coefficient is the ratio of local to bulk solute concentration which shows the extent to which ion accumulates at the interface, replacing local water or the extent to which ion is excluded from the interface, leaving it hydrated. Based on the literature surface tension data available, Pegram and Record obtained

partitioning coefficients K_p for both anions and cations, according to the following equation⁹¹:

$$\frac{d\gamma}{dm_2} = -\frac{RTb_1^\sigma v}{m_1^\square} (1 + \varepsilon_\pm^b) \left[\left(\frac{v_+K_{p,+} + v_-K_{p,-}}{v} \right) - 1 \right] \quad (1-2)$$

Where $\frac{d\gamma}{dm_2}$ is the surface tension increment, m_2 is the molal salt concentration, $m_1^\square$ is the solvent molality (55.5 mol/kg H₂O), b_1^σ is the number of water molecules per unit surface area in the surface phase, $\varepsilon_\pm^b$ is the nonideality correction term, v is the number of ions per formula unit of the salt, $K_{p,i}$ are apparent equilibrium constants for partitioning of each ion between surface and bulk. For the low molal salt concentrations (below 3M) the above derivative $\left(\frac{v_+K_{p,+} + v_-K_{p,-}}{v} \right)$ can be rewritten in terms of concentration using simple conversion factor. The ion partition coefficients ($K_{p,+}K_{p,-}$) are related to the partition coefficient obtained by treating the salts as an electroneutral component ($vK_{p,2}$):

$$vK_{p,2} \equiv v_+K_{p,+} + v_-K_{p,-} \quad (1-3)$$

For most Hofmeister salt ions, K_p values quantifying accumulation ($K_p > 1$) or exclusion ($K_p < 1$) from the air–water interface and molecular hydrocarbon surface were found to be similar⁹². It has been shown that ions that partition favourably towards the interface will lower the surface tension, while those that partition into the bulk solution at a higher rate will increase the surface tension⁹⁰. Pegram and Record were theoreticians and results they obtained were based on the literature data available at that moment. So based on their results some correlation between obtained partitioning coefficients and Hofmeister series have been showed, but Pegram and Record did not look at all at the divalent cations. And still there is a lack of a complete picture of how ions interact with interfaces.

c) Sticky ions in biological systems

A useful and very simple concept on the interaction of ions with hydrophobic surfaces was introduced by Kim D. Collins in 1995. He performed aqueous gel sieving chromatography on Sephadex G-10 of various solutions of salts.⁹³ Sephadex G-10 column separates solutes based on their molecular weight by gel sieving: large molecules are excluded by the small pore size of the beads, taking a short path and emerging early, while small molecules enter the beads, taking a long path through the column and emerging late. The results showed that small monovalent ions, such as Na^+ and F^- passed through the column quickly – they were effectively repelled from the hydrophobic column. On the other hand, large monovalent ions such as Rb^+ and I^- were actually adsorbed to the hydrophobic column. This can be observed in Figure 1-4. To explain the different types of interaction, Collins used the term of hydration. It has been pointed out that the transition from strong to weak hydration occurs at a radius of about 1.78 Å for monovalent anions, compared with a radius of about 1.06 Å for the monovalent cations, suggesting that the anions are more strongly hydrated than cations for a given charge density. In terms of hydrophobicity this means that ions which have small ionic radii will be enhancing hydrophobicity. On the other hand, ions that have large ionic radii will be weakening hydrophobicity.⁹³

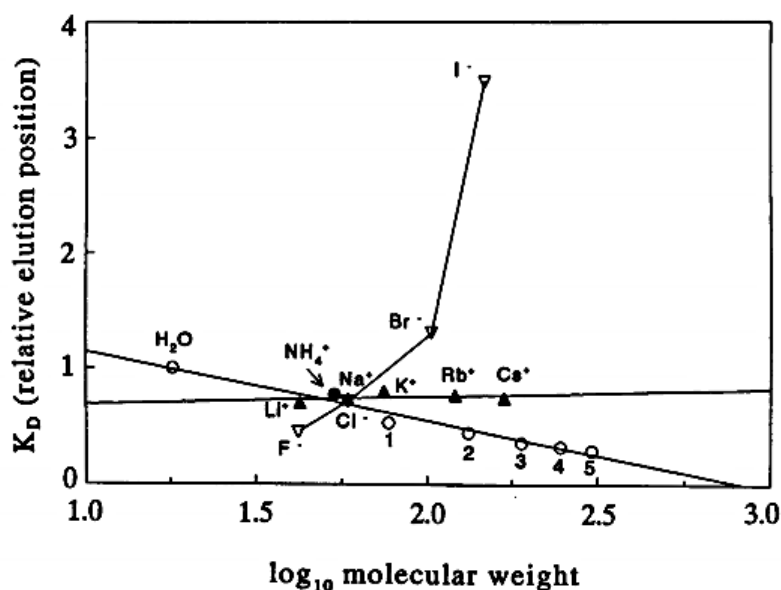


Figure 1-4. Aqueous gel sieving chromatography on Sephadex G-10 of the Group IA cations (Li^+ , Na^+ , K^+ , Rb^+ , Cs^+) plus NH_4^+ and the halide anions (F^- , Cl^- , Br^- , I^-). Reprinted with permission from ref 93. Copyright 1995 National Academy of Sciences, U.S.A.

d) Ion behaviour near air/macromolecule/water interface

Solute partitioning at the air/water interfaces was experimentally studied by Cremer. In his work he investigated specific ion effects on interfacial water structure near macromolecules by the use of vibrational sum frequency spectroscopy (VSFS).⁹⁴ In a typical VSFS setup, two beams of laser light, one having a fixed frequency and the other tunable in an infrared region, must access the surface at the same location at the same time so that they combine and generate an output frequency, which will be equal to the frequency sum of those two input beams. This sum frequency will leave the surface and will be picked up by detector, which will measure its intensity. VSFS is extremely surface/interface specific, allowing probing and analyzing surface/interface regions at a specific location, which makes it unique and a very powerful technique.⁹⁵ (Figure 1-5)

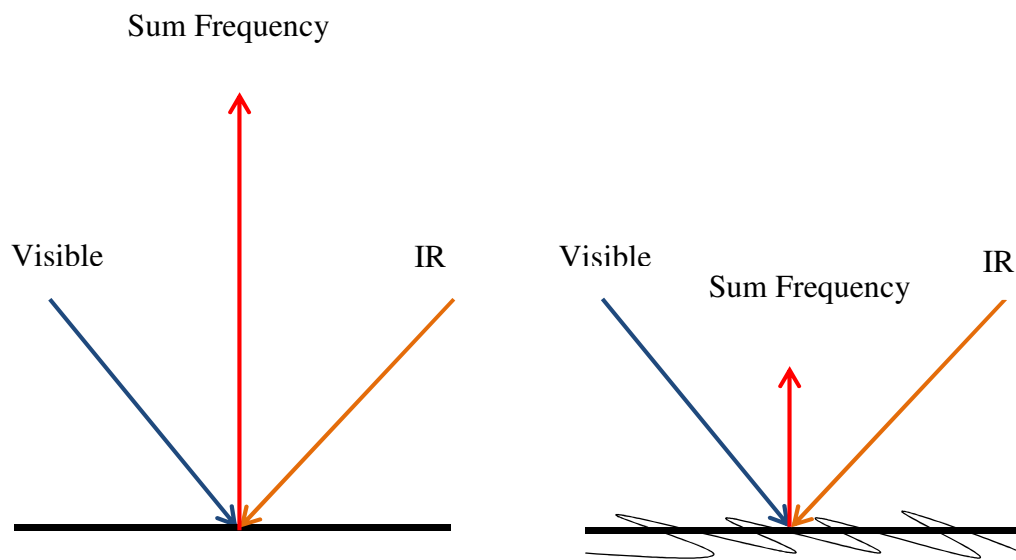


Figure 1- 5. Schematic diagram of the VSFS probing an air/water interface, on the left – no ionic species present at the interface, on the right – ionic species are present at the interface, absorbing light and resulting in the change of the measured SF intensity.

Cremer used a polymer poly-(N-isopropylacrylamide) (PNIPAM) to form a Gibbs monolayer at the air/water interface and then introduced various salts into aqueous subphase to probe the interface by VSFS. Measured VSFS spectrums showed that the water IR absorbance peaks at 3200 cm^{-1} , which are associated with the interfacial water molecules were significantly influenced by the presence of anions (Figure 1-6). As it can be observed, addition of 1M NaSCN resulted in the induction of the 3200 cm^{-1} water peak by approximately 2 orders of magnitude compare to a pure water subphase. In the case of cations, measured spectra were essentially identical suggesting that cations did not associate with the PIPAM interface.

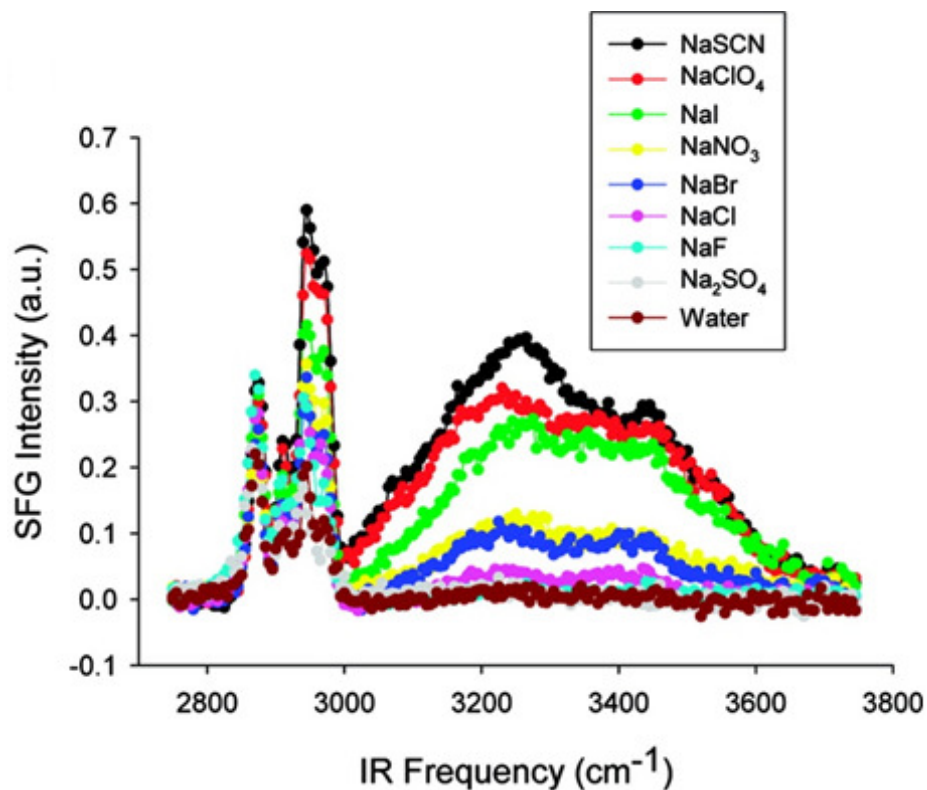


Figure 1-6. VSFS spectra show specific anion effects on PNIPAM absorbed at the air/water interface. Reprinted with permission from ref 94. Copyright 2007 American Chemical Society.

In addition to VSFS experiments, Creamer then measured surface potential differences (SPD) at the air/PNIPAM/aqueous interface with the same set of anions and cations. Surface potential measurement is an extremely precise measurement which assesses the changes in electrical potential at the interface. When salt is added to an aqueous solution and measured SPD is positive, this indicates that ion is excluded from the air/water interface, if SPD is negative – this indicated that ions migrated to the surface and accumulated there. Creamer's results showed that upon addition of anionic salts, the potential became negative (Figure 1-7), while for cationic salts no significant changes in surface potential have been observed.

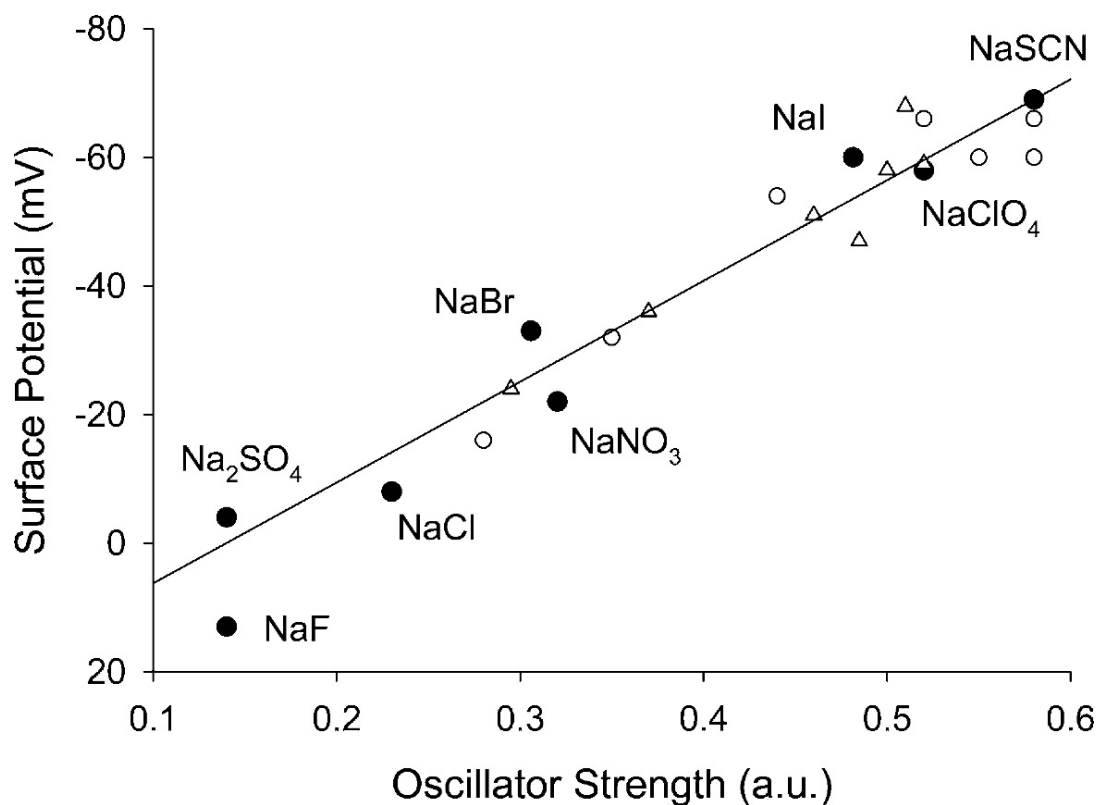


Figure 1-7. Correlation between the surface potential and the oscillator strength for the 3200 cm^{-1} peak. The solid circles are 1 M salt concentrations. The open circles and open triangles represent various concentrations of NaSCN and NaClO₄, respectively. Reprinted with permission from ref 94. Copyright 2007 American Chemical Society.

The absorption behaviour of anions was viewed as a partitioning between the interface and the bulk solution and was shown to follow Hofmeister ranking. On the contrary in the presence of cations interfacial water structure has been shown to remain unchanged suggesting that cations exhibited no effects on the interface.⁹⁴ Cremer's cationic data is in contradiction with Collin's hypothesis on how cations interact with interfaces, introducing a doubt on the validity of both suggested models. The goal of our work will be to investigate both of the suggested hypotheses by applying it on our model system.

1.2.2 Salt effects on hydrophobicity: bulk theories

a) Electrostriction theory

All of the above theories consider salt effects on hydrophobicity from the interfacial standpoint. Alternative theories exist, which take into account bulk properties of the solution when considering specific ion effects. One of the earliest theories which described ion-solvent interactions is the theory of electrostriction.

When an ion is introduced to the aqueous solution, electric field exerted by that ion is going to have an effect on the volume of the solution.⁹⁶ That effect is called electrostriction. Electrostriction in general means the contraction of the material under the influence of the electric field. In terms of solvation electrostriction defined as an apparent decrease in the volume of the ions due to the ion-solvent interactions. Mathematically electrostriction might be expressed as following:⁹⁷

$$\Delta V = \tilde{V} - V_{int} \quad (1-4)$$

Where V_{int} is the intrinsic or actual volume occupied by ion in the solution and $\tilde{V}$ is the partial molar volume of the ion. Partial molar volume is the volume change which occurs when a molecule is added to the solution; in this case it represents both: the intrinsic volume and the interaction between the ion and water.

Electrostriction relation to hydrophobicity can be seen through the specific salt effects. In general, it is known that salts that reduce the hydrophobic effect are the salting-in species (urea, guanidinium chloride), and the ones that increase hydrophobic effect are salting-out species (NaCl, LiCl).⁹⁸ The salting-out effect on hydrophobicity is seen when a salt molecule dissolved in water is going to cause a volume contraction,

electrostriction, due to collapse of the water around the ion in order to solvate it. Thus, there will be less empty space for hydrophobe, and that will cause the increase of the energy required to create a cavity to accommodate that hydrophobe. Therefore, electrostriction increases the energy cost for creating a cavity. Salting-in effect on hydrophobicity has been explained in terms of two contributions: the ability of large ions to disrupt the water structure and being able to improve the solubility of hydrophobe in some way, which makes cavity formation easier.⁹⁹

b) Scaled particle theory

Scaled particle theory (SPT) was originally developed to explain solvation of particles in water. According to this theory any type of solvation has two major components. The first component is formation of the cavity: when a solute introduced to water, a cavity of adequate size has to be created to accommodate that solute; in order to create that cavity work has to be done on the solvent. The second component is the solute/solvent interaction, because once the solute molecule is inside the cavity it will be interacting with the solvent.¹⁰⁰ SPT offers a methodology to calculate a free energy of cavity formation, which will allow us to analyze and quantify salt specific effects on hydrophobicity. Originally, the hydrophobic effect was expressed as a solubility of nonpolar molecules (hydrocarbons) in water and the thermodynamics of the hydrocarbon solvation process were used to predict the origin of the hydrophobicity. Hydrocarbon molecules have very low solubility in water and when they are added to water, they will aggregate together. When salt is added to that solution it will decrease or increase the

solubility of hydrophobes. Salt effects on the solubility of hydrocarbons are empirically represented by the Setschenow equation:¹²⁸

$$\log \frac{S_0}{S} = k_s c \quad (1-5)$$

Where S_0 is the solubility of the non-electrolyte in pure water, S is its solubility in the salt solution of a certain concentration c , and k_s is the salting coefficient.

Various experimental data provide different values of the Setschenow constant, suggesting its ion specificity.^{101,102, 103} Theoretically SPT provides a way to analyze these salt specific effects, which after will be applied to analyze our experimental hydrophobicity data. Masterton and Lee applied SPT to the Setschenow equation and derived the following equation for the salting coefficient¹⁰³:

$$k_s = \left[\frac{d(\bar{g}_1^h)/2.3kT}{d(c)} \right]_{c \rightarrow 0} + \left[\frac{d(\bar{g}_1^s)/2.3kT}{d(c)} \right]_{c \rightarrow 0} + \left[\frac{d \log \sum p_i}{d(c)} \right]_{c \rightarrow 0} \quad (1-6)$$

Where $\bar{g}_1^h$ is the free energy change when a cavity is formed in the solution, $\bar{g}_1^s$ is the free energy change when the nonelectrolyte is introduced to the cavity, and p_i is the number density of solution species. In other words k_s can be represented as a sum of the three terms:

$$k_s = k_\alpha + k_\beta + k_\gamma \quad (1-7)$$

Where k_α is a contribution from the free energy change of cavity formation, k_β is a contribution from the free energy change for the transfer of the solute to the cavity, and k_γ is the particle or number density of the solution, a factor relating to the concentration of the ions around the cavity. The contribution of k_γ is very easy to calculate and can be obtained based on the following equation:

$$k_\gamma = 0.016 - 4.34 \times 10^{-4} \phi_0 \quad (1-8)$$

Where ϕ_0 is the apparent molal volume of the salt at infinite dilution. The contribution from the free energy change of cavity formation k_α to the salting coefficient:

$$\begin{aligned}
 k_\alpha = & 2.15 \times 10^{20}(\sigma_3^3 + \sigma_4^3) - 2.47 \times 10^{-4}\phi_0 \\
 & + \sigma_1[6.45 \times 10^{20}(\sigma_3^2 + \sigma_4^2) + 1.34 \times 10^{28}(\sigma_3^3 + \sigma_4^3) - 4.23 \times 10^4\phi_0] \\
 & + \sigma_1^2[6.45 \times 10^{20}(\sigma_3 + \sigma_4) + 4.01 \times 10^{27}(\sigma_3^2 + \sigma_4^2) + 1.32 \\
 & \times 10^{36}(\sigma_3^3 + \sigma_4^3) - 4.17 \times 10^{12}\phi_0]
 \end{aligned}
 \tag{1-9}$$

Where σ_3 and σ_4 are the diameters of cation and anion and σ_1 is a diameter of the nonelectrolyte molecule. The interaction parameter k_β is the most difficult parameter to calculate and it is very hard to find a satisfying expression for this contribution. Masterton and Lee suggested the following equation for the k_β contribution using polarizabilities of the ions:¹⁰³

$$\begin{aligned}
 k_\beta = & -1.85 \times 10^{14} \left(\frac{\epsilon_1}{k} \right)^{\frac{1}{2}} \left[a_3^{\frac{3}{4}} Z_3^{\frac{1}{4}} \frac{(\sigma_1 + \sigma_3)^3}{\sigma_3^3} + a_4^{\frac{3}{4}} Z_4^{\frac{1}{4}} \frac{(\sigma_1 + \sigma_4)^3}{\sigma_4^3} \right] + 6.26 \\
 & \times 10^{17} \phi_0 \left(\frac{\epsilon_1}{k} \right)^{\frac{1}{2}} (\sigma_1 + \sigma_2)^3 + 4.00 \times 10^{-2} \frac{\phi_0 \alpha_1}{(\sigma_1 + \sigma_2)^3}
 \end{aligned}
 \tag{1-10}$$

Where α_3 and α_4 are the polarizabilities of cation and anion, Z_3 and Z_4 are the numbers of electrons in cation and anion, σ_1 is a diameter of the nonelectrolyte molecule, $\frac{\epsilon_1}{k}$ is an energy parameter of the nonelectrolyte molecule, α_1 is a polarizability of the nonelectrolyte molecule. The implications of k_α , k_β , and k_γ contributions to our experimental data will be further explored in the rest of our work.

1.2.3 Summary of the theories

Despite significant progress that has been made in understanding of specific ion effects, the behavior of ions at hydrophobic interfaces is still poorly understood and remains a subject of intensive research.³ Analysis performed by Pegram and Record on the air-water interface have attributed ion specificity to differential partitioning, while also suggesting that this ion partitioning causes the ion specificity seen at more complex aqueous interfaces.⁹⁰ This model has been illustrated through calculations of surface tension increments, specific for each ion studied.¹⁰⁴ Scaled particle theory considers specific ion effects somewhat differently, by using bulk solution properties to calculate salting coefficients for specific salts. It posits that specific ion effects on the solubility of a non-electrolyte in solution, arise from interplay between three different contributions: the free energy change that is needed to form a cavity in solution (k_α), the change in free energy when the non-electrolyte is solubilized in said cavity (k_β), and the number density of the ions in the solution (k_γ).¹⁰³ Realistically, ion specificity on protein stability is due to numerous contributions and interactions between the solution and protein. These include electrostatic interactions that exist between protein's charged groups and ions in solution, also dispersion forces, Van der Waals interactions, hydrogen bonding and hydrophobic interactions that occur within the protein. However, the most important contribution is arguably the hydrophobic effect, specifically the change in free energy when water molecules are liberated from the interfacial region upon protein folding.¹⁰⁵ To study these specific ion effects we will use micellization of 1, 2 - Hexanediol as a model system which we will discuss in further details in the next section.

1.3 Model system for studying the hydrophobic effect

A reductionist's approach can view a globular protein as a large dielectric sphere having point charges inserted into various locations relative to the surface. The protein surface can then be modeled as the interface between high-dielectric aqueous and low-dielectric protein phases. It is consequently useful to investigate specific ion effects on a model system that offers the best approximation of a low dielectric sphere embedded into water in order to understand salt effects on biological processes that primarily involve changes in the hydrophobic surface area. To model this sphere of low dielectric constant, we have chosen micelles, one of the simplest hydrophobic interactions leading to biologically relevant structures. This model should give a better approximation than the air-water interface of the interactions that occur between water and the low dielectric protein interface. Therefore to determine the specific salt effects on the hydrophobicity we have decided to use the micellization, as it is often used as a model system to study the hydrophobic effect.

1.3.1 Micelles and Micellization

Micellization occurs when surfactant molecules arrange themselves into organized molecular assemblies known as micelles (Figure 1-8). The hydrophobic part of the aggregate forms the core of the micelle, while the polar head groups are located at the micelle-water interface in contact with and hydrated by a number of water molecules. The micelles are stabilized in water by the favourable interaction between the hydrophilic groups and water molecules forming a stable hydrated shell between hydrophobic core and the bulk water.¹⁰⁶

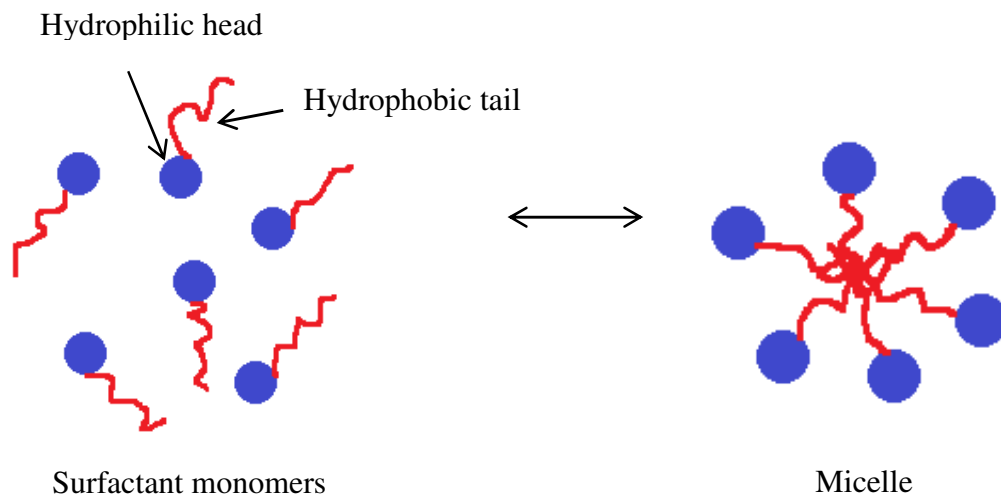
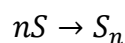


Figure 1-8. Schematic representation of the surfactant molecules and the micelle it forms.

According to the theory once a certain concentration of surfactant is reached in solution, it becomes more favourable for the surfactant molecules to aggregate and form micelles thus concealing hydrophobic tails than orienting these tails out of the aqueous medium.¹⁰⁷ This surfactant concentration is known as the critical micelle concentration (CMC). According to the theory of the formation of micelles, monomeric species and micellar aggregates exist in chemical equilibrium in solution:



Where n is the number of surfactant molecules, S , associating to form a micelle, known as an aggregation number. The aggregation number is one of the most fundamental and important structural parameters of the micelle, characterizing the average number of surfactant molecules per micellar unit. The energy required to transfer one molecule of the monomer from the bulk to the interior of the micelle is known as the Gibbs free energy of the micellization. It has been shown that Gibbs free energy of the micellization for nonionic micelles strongly depends on the aggregation number and CMC and can be obtained based on the either of two following equations:¹⁰⁸

$$\frac{\Delta G_m^0}{RT} = \frac{1}{n} \ln \left[\frac{n^2(2n-1)}{n-2} \right] + \frac{n-1}{n} \ln \left[\frac{n(2n-1)}{(n-1)(2n+2)} \right] + \frac{n-1}{n} \ln CMC \quad (1-11)$$

$$\frac{\Delta G_m^0}{RT} = \frac{(\ln 2 + 2 \ln n)}{n} + \left[\frac{(n-1)}{n} \right] \ln CMC \quad (1-12)$$

Where G_m^0 is the standard Gibbs free energy of micellization change per mole of monomer molecules, R is the universal gas constant, T is the absolute temperature, n is the aggregation number and CMC is the critical micelle concentration.

In cases when n is sufficiently large number (~ 50), first two terms in both equations can be neglected and an approximate expression for the Gibbs free energy of micellization per mole of a neutral surfactant becomes:

$$\Delta G_m = RT \times \ln CMC \quad (1-13)$$

Thermodynamics of the micelle formation can be influenced by the number of factors, including temperature, nature of the surfactant, presence of different salts at different concentrations, etc. In this work we have investigated the effect of different ionic species at different concentrations on the thermodynamics of nonionic micelle formation. Our results shown that when salts are present, the formation and properties of the nonionic micelles altered dramatically by the specific types of ions in solution.

1.3.2 Methods for the CMC determination

Various techniques are employed to determine CMC in aqueous solutions. Most of these techniques are based on the abrupt change of the related physical properties upon formation of micelles. Among most commonly used techniques are conductivity, voltammetry, calorimetry, scattering techniques, surface tension and optical techniques such as UV/Vis and fluorescence spectroscopy.^{109, 110} In most cases, the choice of the

method depends on the availability of various techniques as well as on their reproducibility, accuracy and simplicity.

a) Conductivity

This method can only be used for determination of the CMC of ionic surfactants and based on the finding of the breakpoint on the curves, which describe the relationship between the concentration of the surfactant and measured conductivity. It is well-known, that the conductivity of any solution is directly proportional to the concentration of its ions. The point, where the micelle formation starts, is indicated on the concentration dependence of specific conductivity (K) as a breaking point. The breaking point marks a significant change of the linear slope of the dependence $K = f(c)$. And the CMC value can be obtained as the intercept of two linear functions with mutually different slopes. The break point of conductivity-concentration plots is usually hard to determine and has been reported as a major disadvantage of this technique.^{111, 112}

b) Calorimetry

Isothermal titration calorimetry (ITC) has been widely used as a reliable technique in quantitative thermodynamic studies of micellization. It has been proven to be capable of direct determination of CMC (in the range of $50\mu\text{M}$ to $500\mu\text{M}$) as well as the heat of micellization ΔH_m .^{113, 114, 115, 116} The typical setup for performing the ITC experiment to measure CMC consists of diluting surfactant into water at the fixed temperature and measuring its heat flow. Figure 1-9 shows an experimental set of data one can obtain from performing the ITC experiment. The CMC is determined as an inflection point

based on the plot of enthalpy of dilution vs total surfactant concentration (Figure 1-9C).¹¹⁷

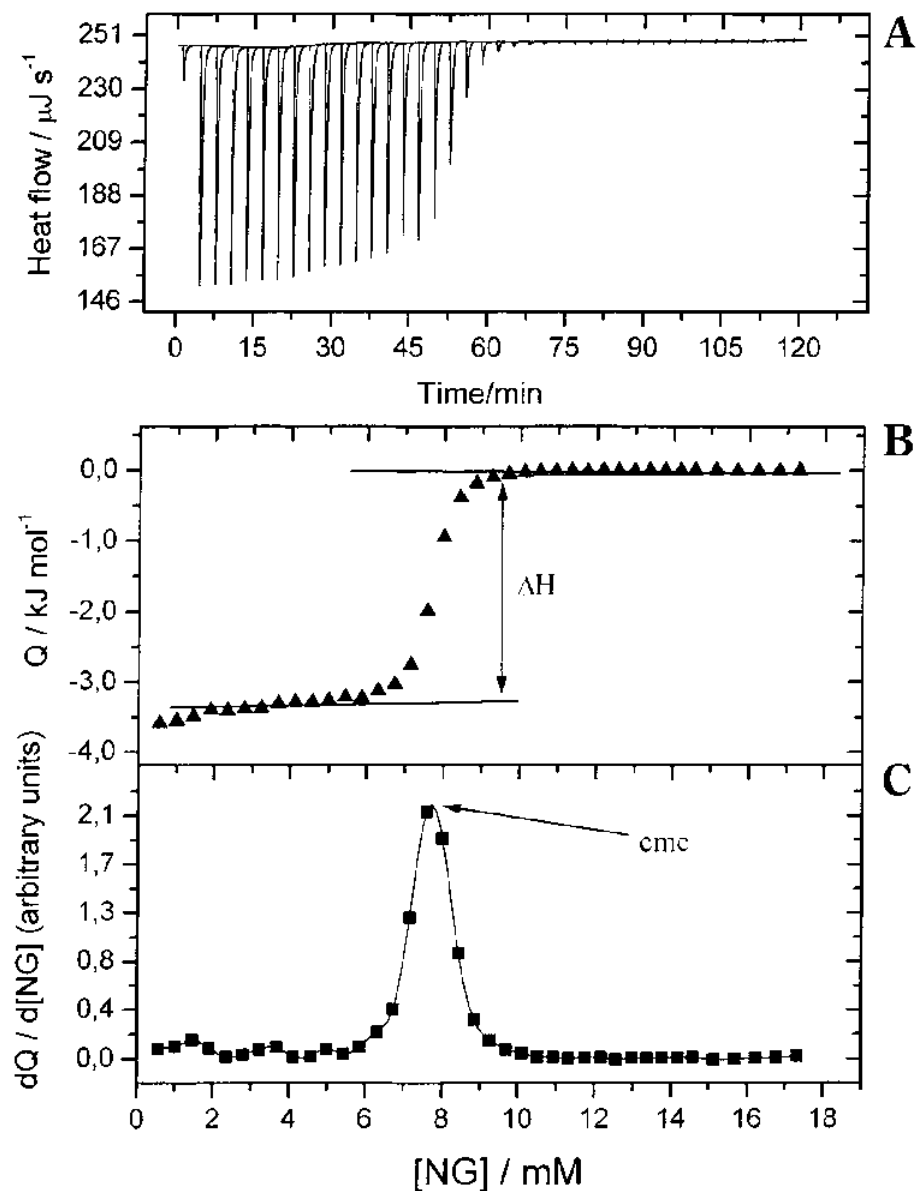


Figure 1-9. Titration of nonylglucoside (NG) surfactant into water in 40 steps with different titration volumes at 30°C. A) Calorimetric traces (heat flow against time); B) Reaction enthalpy vs. concentration of NG in the cell; C) Plot of the first derivative of curve B calculated from interpolated values vs. concentration of NG in the cell. Reprinted with permission from ref 117. Copyright 2001 American Chemical Society.

c) Light Scattering

Dynamic light scattering (DLS) is a technique commonly used to determine particle size in colloidal dispersions, but recently has also been employed to determine CMC of the surfactant solution. Figure 1-10 shows changes in the intensity of the scattered light upon the increase in surfactant concentration. ¹⁰⁹

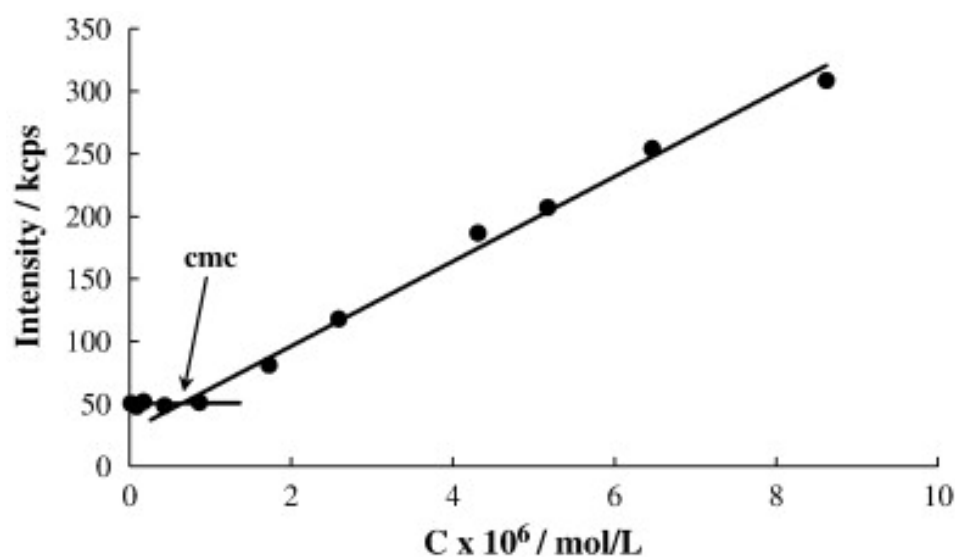


Figure 1-10. Plot of the intensity of scattered light vs. surfactant concentration. Reprinted with permission from ref 109. Copyright 2012 Elsevier B.V.

According to DLS measurements, the intensity values of scattering light detected for surfactant concentrations below the CMC have approximate constant value and similar to that of deionised water. The intensity starts linearly increasing, as the CMC of the surfactant is reached and number of formed micelles keeps rising. The CMC is obtained from the plot of the intensity of scattered light vs surfactant concentration as the intersection point of two lines. ^{118, 119}

d) Surface Tension

The surface tension of aqueous solutions of surfactants decreases very rapidly until the CMC is reached and then stays constant above the CMC (Figure 1-11).¹²⁰

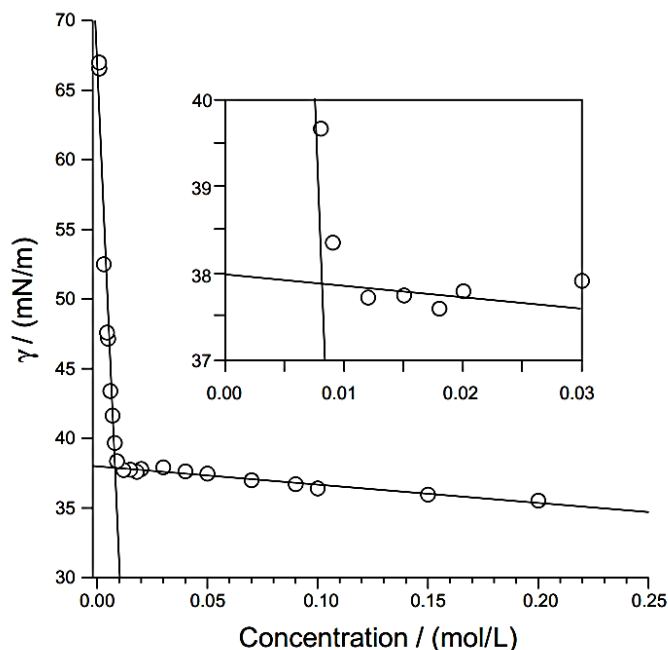


Figure 1-11. Plot of surface tension vs concentration of surfactant. Reprinted with permission from ref 120. Copyright 2001 American Chemical Society.

As it can be seen from Figure 1-11 above CMC the surface tension of the solution remains constant since only the monomeric form contributes to the reduction of the surface or interfacial tension. For concentrations below, but near the CMC, the slope of the curve is essentially constant, indicating that the surface concentration has reached a constant maximum value. In this range the interface is considered to be saturated with surfactant molecules and the continued reduction in the surface tension is mainly due to the increased activity of the surfactant in the bulk, rather than at the interface. The equilibrium surface tension can be measured by a variety of methods, such as the Wilhelmy plate, du Nouy ring, drop weight or by volume and shape of the drop. The

data are generally plotted against the log of concentration and the transition between a descending line and another one close to the horizontal is taken as the CMC.^{120, 121, 122}

e) Optical techniques

Spectral methods, such as UV/Vis and fluorescence spectroscopy, are using dyes and other compounds as probes for the CMC determination.^{123, 124, 125, 126} Among other techniques, fluorescence probes have been used widely, because of their simplicity, wide scope and extreme sensitivity at very low probe concentrations.¹²⁷ In this work, the major emphasis has been on the techniques using optical probes in the CMC determination and the details of these methods will be given in the Materials and Methods section.

1.3.3 Our model system

The hydrophobic effect is one of the most important factors on protein stability. When a protein transitions from its unfolded state to its folded state, there is a dramatic reduction in the amount of interfacial water needed to solubilize the protein. Hydrophobic residues interact in the core of the protein, while hydrophilic ones orientate to face the solution. Since this effect is so important, considering just this parameter on protein stability is of great value, and much more straightforward. A very simple outlook on the interfacial region of a protein takes into account that a protein protrudes both polar and apolar surfaces, and can be most easily modeled as a sphere of low dielectric constant placed in a solution of high dielectric constant. Hydrophobicity, as mentioned above, plays a vital role in the stability of this low dielectric sphere. Specific ion effects on hydrophobicity can be measured by monitoring the changes in the composition of this model system.

This model should give a better approximation than the air-water interface of the interactions that occur between water and the low dielectric protein interface. To model this sphere of low dielectric constant and to study salt effects on hydrophobicity, micelles of 1, 2 - Hexanediol will be used (Figure 1-12). There are many distinct advantages of using this simple amphiphile over any others. First of all, 1, 2 -Hexanediol is a nonionic surfactant with a very small head group which allows for a sharp change between the high dielectric water and low dielectric surfactant interface, without an undefined middle layer. Additionally, the hydroxide groups are similar to those seen in water, so perturbation and interactions with the solution will be kept to a minimum. And the most importantly 1, 2-Hexanediol does not interact directly with any ionic species.

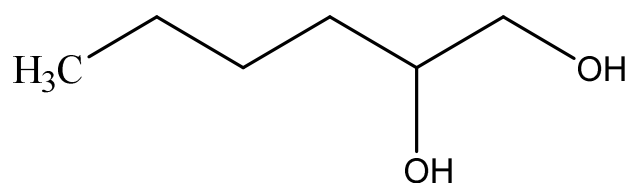


Figure 1-12. Schematic structure of the 1, 2 – Hexanediol molecule.

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Chapter 2

Materials and Methods

1.4 Materials

Potassium chloride, lithium chloride, sodium chloride and tetramethylammonium chloride, sodium hydroxide, sodium diphosphate, sodium nitrate, sodium thiocyanate, sodium bromide, sodium acetate, sodium chloride, sodium formate, sodium chlorate, and sodium perchlorate were purchased from Fisher Scientific (Fair Lawn, NJ); 1,2-Hexanediol, pyrene, benzoylacetone, ANS, dioxane, ammonium chloride, guanidine hydrochloride, tetraethylammonium chloride were purchased from Sigma-Aldrich (St. Louis, MO); cesium chloride was purchased from TCI America (Portland, OR); rubidium chloride was purchased from Alfa Aesar (Ward Hill, MA).

1.5 Methods

1.5.1 Fluorescence spectroscopy

To characterise the fluorescence emission of a molecule, the number of photon emitted is counted by spectrofluorometer. Figure 2-1 shows a schematic diagram of a basic spectrofluorometer. The instrument consists of a source of exciting light (usually xenon based lamp), monochromator (to select both the excitation and emission wavelengths), slits, shutters (are provided to eliminate the exciting light or to close off the emission channel), beam splitter (splits the beam of light in two to provide the reference before light hits the sample), sample holder and detector (typically photomultiplier tube).¹ The intensity of fluorescence is quantified by a computer system with specialized software and converted to give a spectrum. Figure 2-2 shows the actual spectrofluorometer that have been used in this work.

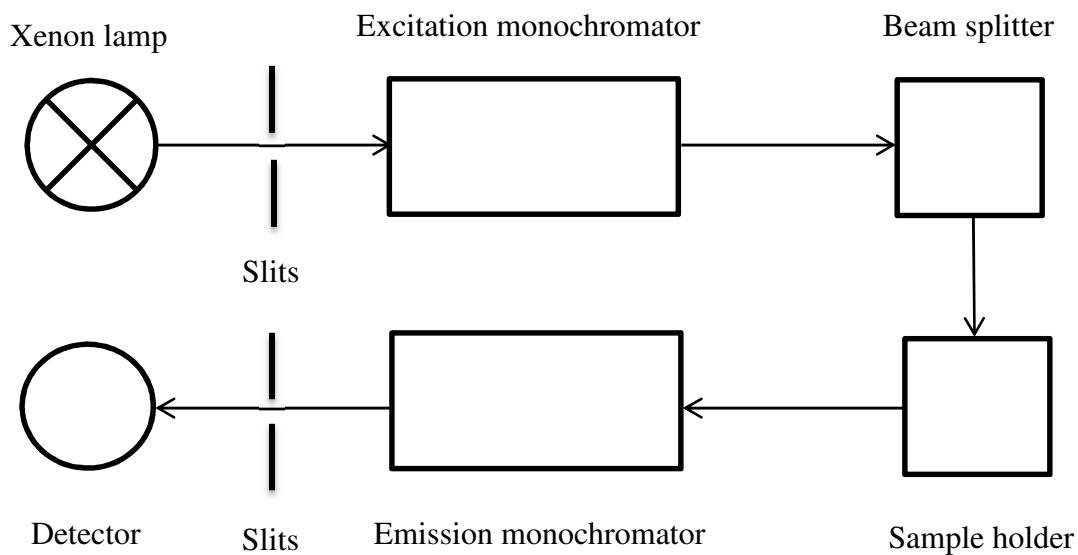


Figure 2-1. Schematic diagram of a spectrofluorometer.

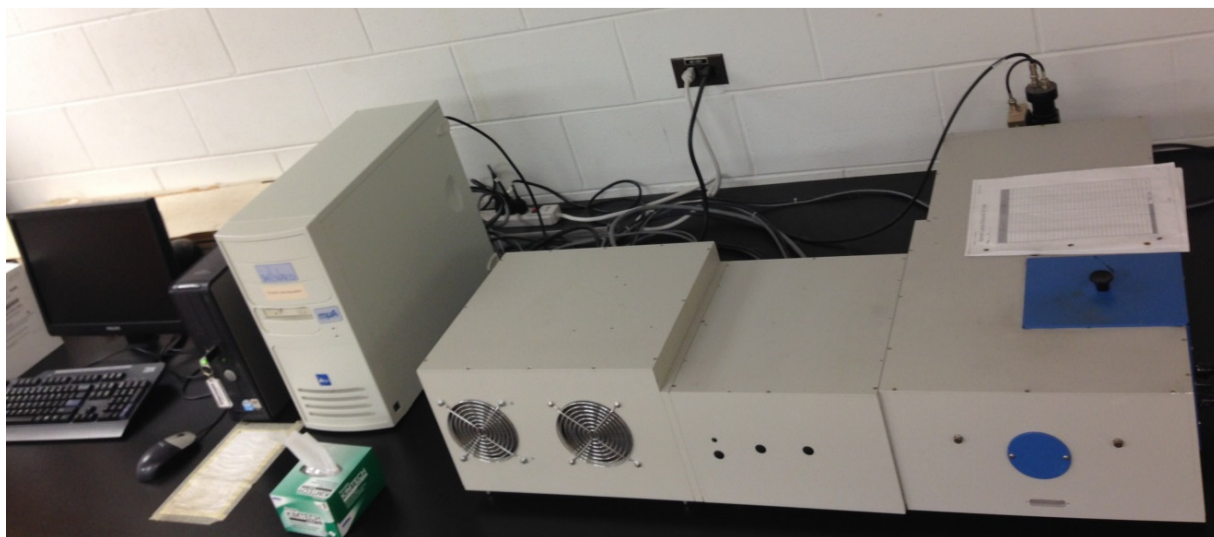


Figure 2-2. Fluorolog-3 Horiba Jobin Yvon Spectrofluorometer.

1.5.2 Fluorescence probes

In this section a brief overview of the various types of fluorescent probes and their main characteristic will be given. Fluorophores can be put in two main categories – intrinsic and extrinsic.¹ Intrinsic fluorophores occur naturally while extrinsic fluorophores are added to the non-fluorescent sample to provide fluorescence. Two of the main characteristics of a fluorophore that define its efficiency are the quantum yield and the fluorescence lifetime. The quantum yield is the ratio of the number of photons emitted to the number of absorbed.¹ Substances with the largest quantum yields display the brightest emission. The lifetime determines the time available for the fluorophore to interact with or diffuse in its environment, and therefore the information available from its emission. Measurements of fluorescence lifetimes can yield valuable information on molecular motion and the environment of the fluorophore.¹ Fluorescence probes have been widely used to measure CMC of the various surfactants. Fluorimetric determination of the CMC has advanced rapidly over the past years, as a result of the intense research in

development of the new fluorescence probes. This technique utilizes a hydrophobic fluorescence dye which exhibits different fluorescence characteristics upon its solubilisation in the micellar interior.² A large number of dyes and specific molecules have been used for precise and accurate determination of CMC. These include pyrene, arcidine orange, coomassie brilliant blue, 8-anilinonaphthalene-1-sulfonic acid amongst others^{3, 4, 5, 6, 7, 8, 9,21} Fluorimetric determination of the CMC with the utilization of these dyes is based on the abrupt change in the fluorescence spectrum upon the formation of the micelles due to the partitioning of the dye molecule in the hydrophobic environment of the micelle. Pyrene and ANS (8-Anilinonaphthalene-1-sulfonic acid) based fluorescent probes were extensively used in this research to measure CMC of the 1, 2 – Hexanediol by the means of fluorescent spectroscopy. The main characteristics of these dyes will be discussed in the next sections.

a) Pyrene Fluorescence

Pyrene is polycyclic aromatic hydrocarbon (PAH). It consists of four fused together benzene rings that result in a flat aromatic system (Figure 2-3).¹⁰

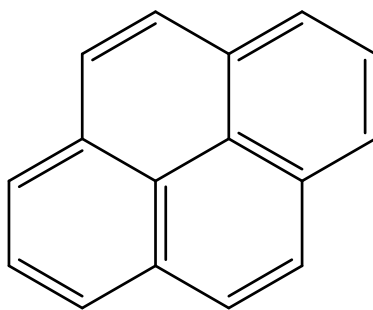


Figure 2-3. Schematic structure of the pyrene.

Pyrene is proven to be one of the most effective fluorescence probes employed in the studies of micellization. It is a non-polar molecule with very high sensitivity to the local

environment. The most important advantage of the pyrene is that as a fluorescent dye it exhibits no effect on the micelle formation: pyrene also does not undergo the change of the dipole moment upon excitation compare to other dyes; therefore pyrene does not perturb the micelles electrostatically.¹² These characteristics makes pyrene a very useful fluorescence probe to study the self-assembly of amphiphilic molecules. The fluorescence of pyrene can be schematically represented by the classical Jablonski diagram, which was first proposed by Alexander Jablonski in 1935 to describe absorption and emission of light.¹ Jablonski diagrams are used to illustrate various molecular processes that can occur in excited states. A simplified Jablonski diagram for the pyrene molecule is shown in Figure 2-4.

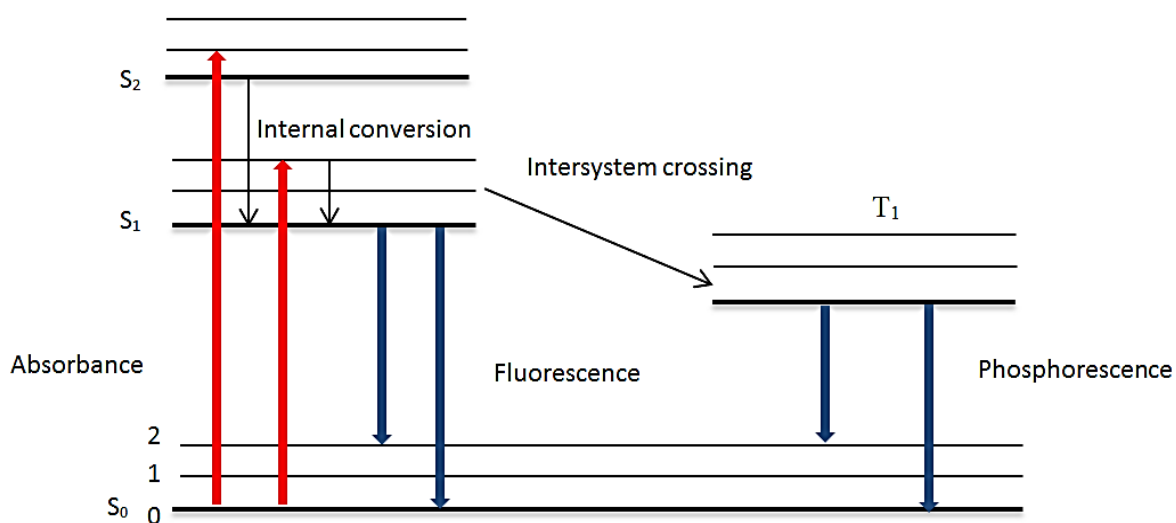


Figure 2-4. Simplified schematic of the different energy levels of a pyrene molecule, known as a Jablonski diagram.

Generally, at each energy level, fluorophores can exist in a number of vibrational energy levels, which are represented by the multiple lines in each electronic state (0, 1, and 2). These vibrational energy levels are due to the vibrations of individual atoms

within a molecule and are much closely spaced than the different electronic energy levels. When a pyrene molecule absorbs light, it is excited to a higher vibrational energy level S_1 . The excitation is followed by the relaxation of excited electrons to the ground state S_0 by emitting a photon, which is the process of fluorescence. Pyrene exhibits significantly fine vibronic structure in its monomer fluorescence spectra in solution phase. Interestingly, this vibronic structure is strongly solvent dependent. First solvent effect investigations on the pyrene vibronic structure are dated back in 1971 and were reported by Nakajima.¹¹ He observed that the intensity of the first vibronic peak with 0-0 transition in the pyrene fluorescence spectrum was enhanced in the presence of the varying polar solvents. He concluded that this behaviour was dictated by perturbations in vibronic coupling due to the solute-solvent interactions (Ham effect). Figure 2-5 shows how the fluorescence intensities of peaks I and III of pyrene change when varying the polarity of the solvent.¹² These earlier studies were extended to cover different types of the possible solvents in order to better understand the details of the probe-solvent interactions.^{13, 14} Since then the intensity ratio between the first and third vibronic bands of the pyrene fluorescence emission spectrum has been widely used as a solvent polarity scale, known as the P_y scale to characterize the hydrophobic regions of the micelles, cell membranes and other complex systems.^{15, 16}

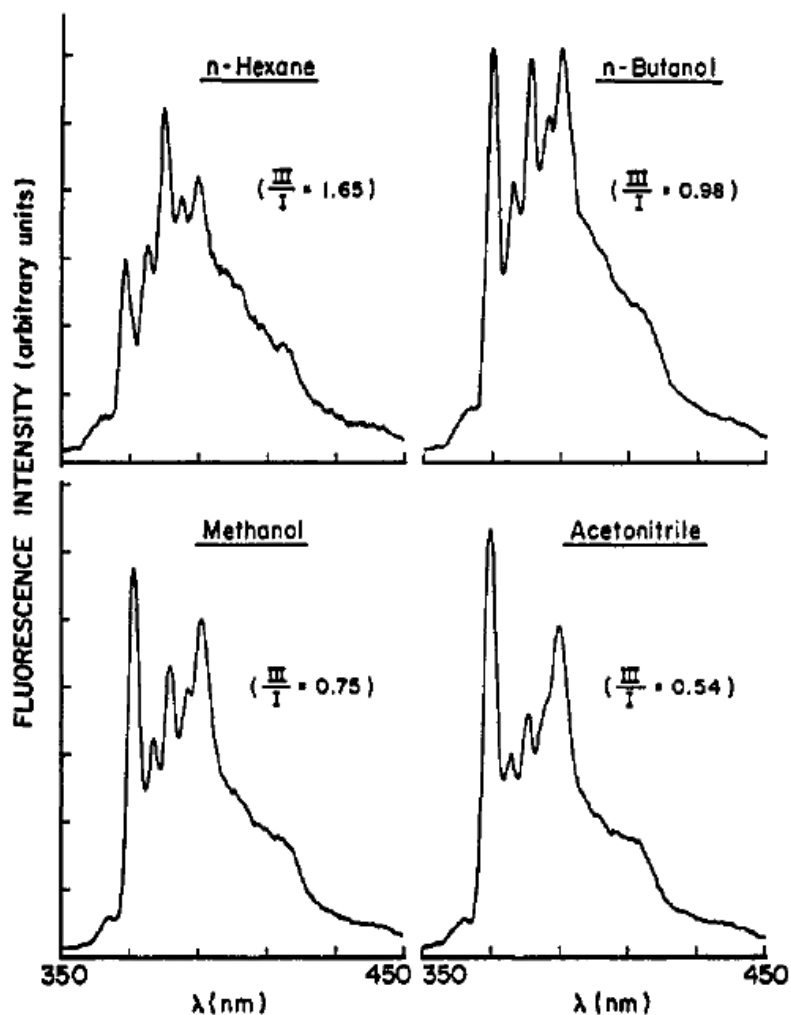


Figure 2-5. Fluorescence emission spectrum ($\lambda_{\text{exc}}=310$ nm) of 2 μM pyrene in different solvents. Reprinted with permission from ref 12. Copyright 1977 Journal of the American Chemical Society.

The principle described above can be used to probe micelle formation. In the absence of micelles (below the CMC) pyrene senses the polar environment of water molecules and the ratio of fluorescence emission intensities corresponding to the peak I and III is high (Figure 2-6a). However, once the CMC of the surfactant is passed and micelles are formed, the pyrene is solubilized in the micelle interior and there is a sharp decrease in this ratio, as can be seen in Figure 2-6b. The change in the peak I to peak III intensity ratio can be used to monitor the formation of the micelles.

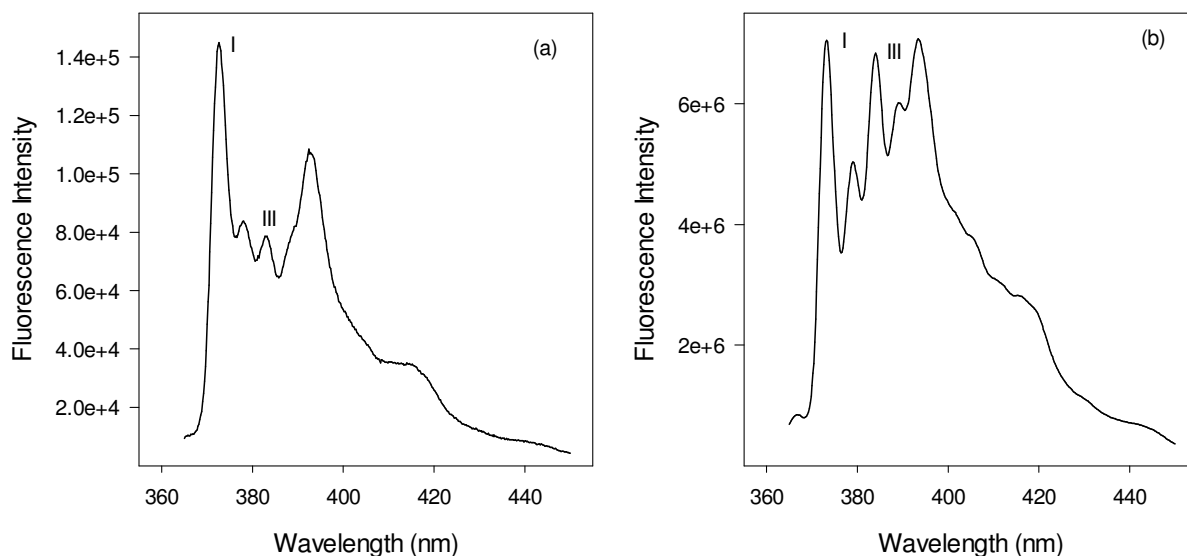


Figure 2-6. Fluorescence emission spectrum ($\lambda_{exc}=334$ nm) of $1\mu M$ pyrene in water (a) and $1.25M$ 1, 2-Hexanediol (b). Maxima at 373 and 384 nm were assigned as peaks I and III respectively. Slits were set to 1nm.

It should be noted that pyrene is capable of forming complexes with itself at high concentrations. As seen above, at low concentrations pyrene gives a highly structured emission spectrum. However, at higher concentrations a visible peak emerges at 480 nm, as can be seen in Figure 2-7.¹ This long wave-emission is due to excimer (excited dimer) formation. So when using a pyrene in CMC determination experiments it is important to retain low concentration, in this work $1\mu M$ pyrene has been used to successfully perform the experiments.

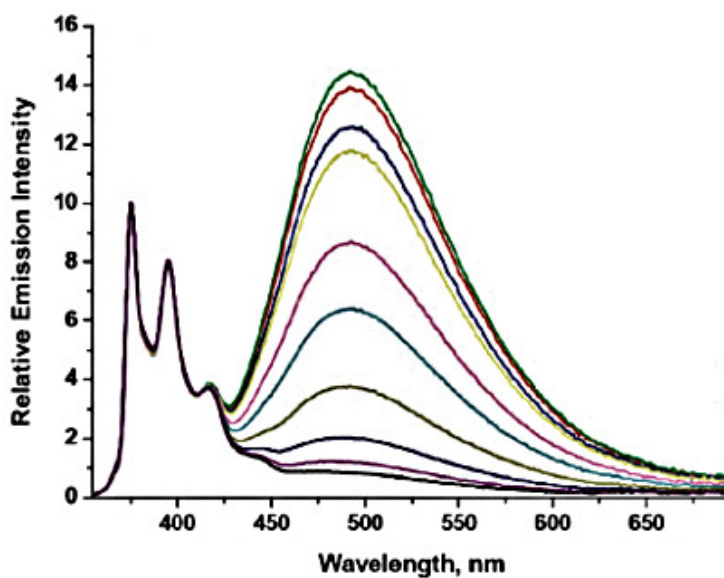


Figure 2-7. Emission spectrum of pyrene and its excimer. Reprinted with permission from ref 24. Copyright 2009 Royal Society of Chemistry.

b) ANS (8-Anilidonaphthalene-1-sulfonic acid) fluorescence

ANS can also be used to determine the CMC of 1, 2 –Hexanediol based on the principles demonstrated in the previous section. ANS is an organic compound containing both a sulfonic acid and an amine group (Figure 2-8).¹⁷

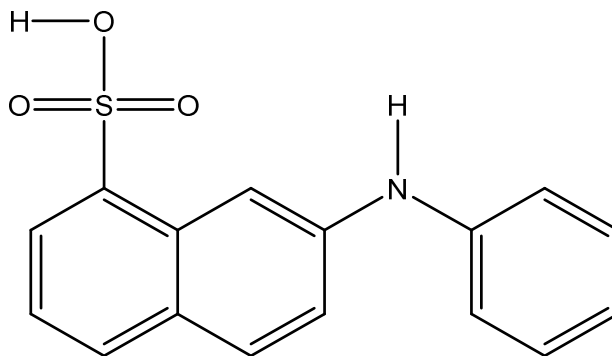


Figure 2-8. Schematic structure of the ANS.

Figure 2-9 shows how the fluorescence intensity of ANS changes with the change of the polarity. The increase in the intensity observed when dye is incorporated into the low

polarity micellar environment. By monitoring the change in the intensity at 521 nm and plotting this change against the surfactant concentrations the CMC can be determined.⁶

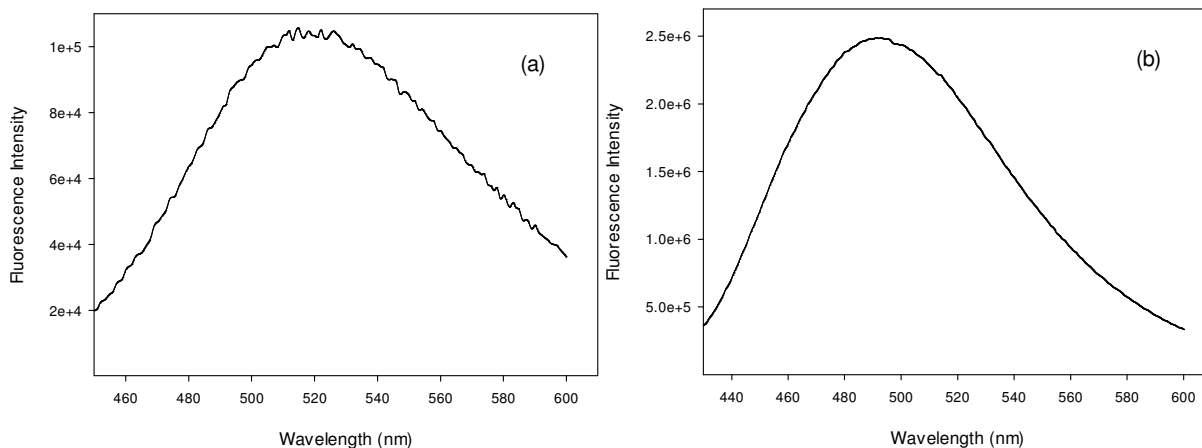


Figure 2-9. Fluorescence emission spectra ($\lambda_{exc} = 370$ nm) of 10 μ M ANS in water (a) and 1.25 M 1, 2-Hexanediol (b). Maxima was assigned at the 521 nm. Slits were set to 5 nm.

1.5.3 UV/Vis spectroscopy

In the cases where the environment of the ionic species quenches both pyrene and ANS fluorescence, UV/Vis spectroscopy can be used. Spectrophotometers measuring the absorbance in the UV/VIS range are mostly either single or double-beam devices. In this work double-beam spectrophotometer has been used (Figure 2-10), the working principle of which will be described below. The basic layout of the double-beam UV/Vis spectrophotometer is shown in Figure 2-11. All spectrophotometers start with the light source. Usually the combination of two lamps is used – a deuterium lamp for the UV part of the spectrum, and a tungsten/halogen lamp for the visible part. The combined output of these two bulbs is focused on to a diffraction grating that splits the light into its component colours. A diffraction grating followed by the slit which allows light of a very narrow range of wavelengths through into the rest of the spectrophotometer. The light

coming from the diffraction grating and slit will hit the rotating disc and split into two beams so that sample and reference cells solutions can be monitored simultaneously. Light then enters the detector that measures the intensity of light transmitted from sample (I), and compares it to the intensity of light reflected from a reference cell (I_0). The output is given as a plot of absorbance against wavelength. By using the Lambert-Beer's law we can determine the concentration of the required solution:¹⁸

$$A = \varepsilon * l * c \quad (2-1)$$

Where ε is a constant called the extinction coefficient, l is the path length (cm) and c is the concentration of the substance.

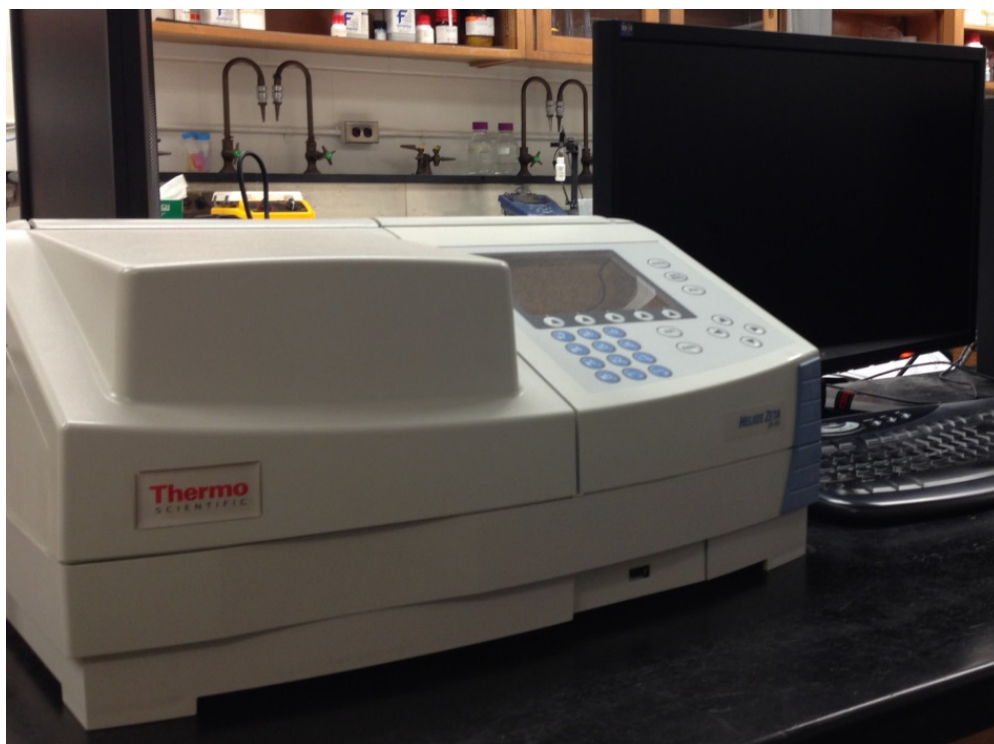


Figure 2-10. Thermo Scientific Helios Zeta UV/VIS spectrophotometer.

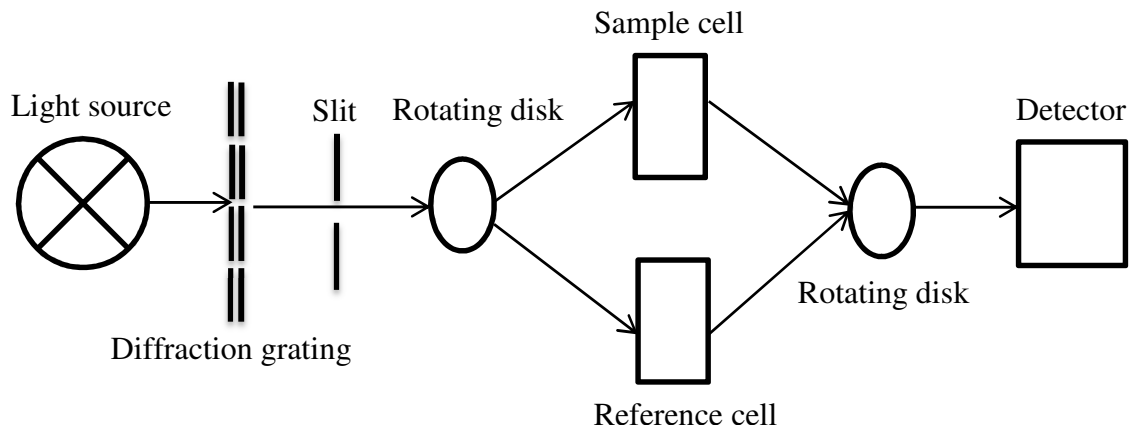
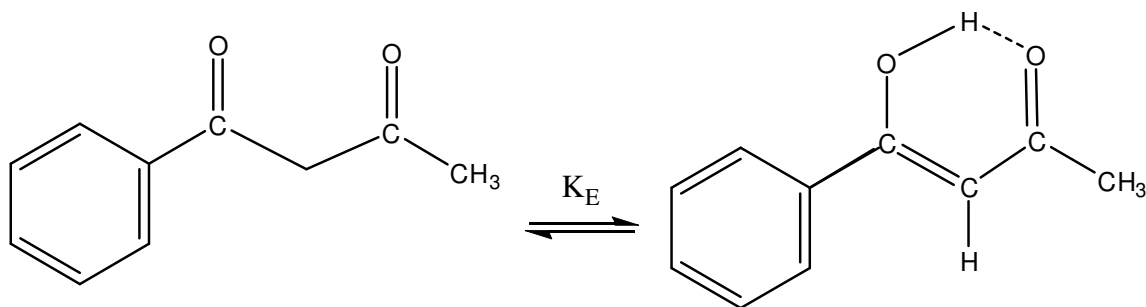


Figure 2-11. Typical arrangement of the basic components of the double-beam UV/VIS spectrophotometer.

a) BZA (benzoylacetone) dye absorbance

The keto-enol tautomerism of benzoylacetone has been used to measure the CMCs of 1, 2-Hexanediol in the presence of cesium chloride. In aqueous solution, BZA enolizes practically completely to the *cis*-enolic form that exists in a cyclic configuration stabilized by intramolecular hydrogen bonding:^{19, 20}



Keto-enol equilibria of BZA are extremely solvent sensitive and the percentage of enolic form is much higher in nonpolar solvents than in water. Upon addition of surfactant to the water solution of BZA, the amount of enol increases abruptly above the CMC because the enolic form is taken up by the micelles, which provide a less polar environment than the aqueous phase.

Figure 2-12 shows the effect of increasing surfactant concentration on the UV-absorption spectrum of aqueous BZA solutions. Below the CMC no changes are observed in the presence of 1, 2 - Hexanediol monomers. As soon as the CMC reached the absorption band centered at 312 nm (due to the enolic form) shows significant increase and the band centred at 250 nm (due to keto form) decreases.

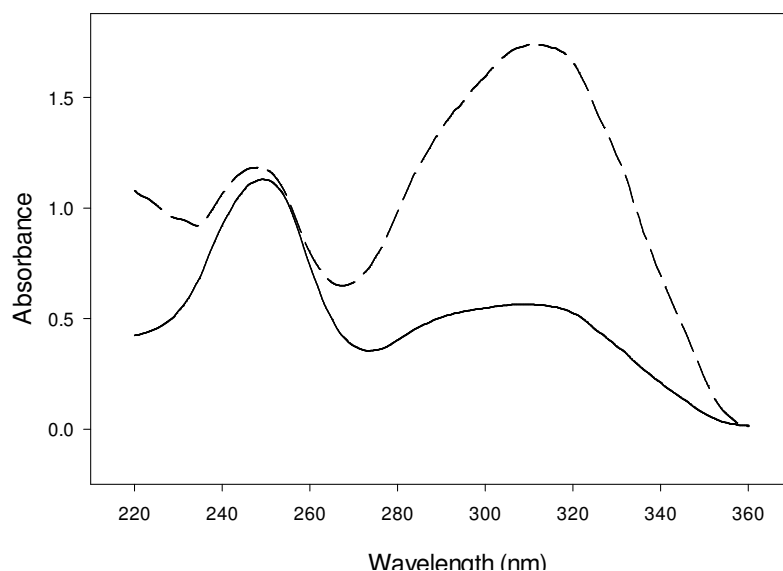


Figure 2-12. Absorption spectrum of benzoylacetone ($70\mu\text{M}$) in water (solid line) and 1.25M 1, 2-Hexanediol (dash line).

1.5.4 CMC determination

All experiments were performed using 4.23M stock solution of 1, 2 – Hexanediol. Stock solution of pyrene was prepared in ethanol and its concentration was determined by UV/Vis spectroscopy. The final concentration of the pyrene in all samples was $1\mu\text{M}$. A series of samples were prepared in the morning and after an overnight incubation, data was collected on the next day. To prepare 1.5 mL of the sample, a concentrated stock solution of 1, 2 – Hexanediol, water, a concentrated salt solution and a concentrated stock of the pyrene solution were added to 1.5 mL microfuge tube. All 1.5 mL samples were

prepared in a triplicate. All measurements were conducted at a room temperature (25°C). In each case fluorescence or absorbance spectrum were recorded. Fluorescence spectra were measured on a Fluorolog-3 Horiba Jobin Yvon spectrofluorometer. The spectrofluorometer parameters were set depending on the fluorescence probe that has been used. For the pyrene - fluorescence emission spectra were recorded from 365 to 450 nm using an excitation wavelength of 334 nm, excitation and emission slits were set to 1 nm band pass resolution. For the ANS - fluorescence emission spectra were recorded from 420 to 600 nm using an excitation wavelength of 370 nm, excitation and emission slits were set to 5 nm band pass resolution. Absorbance spectra were measured on a Thermo Scientific Helios Zeta UV/VIS spectrophotometer. Absorbance spectra were recorded from 200 to 380 nm. Samples were held in semi Micro quartz cuvettes with a 10x4 mm path length.

All collected data were analyzed using Sigma plot (Point Richmond, CA) software. In case of pyrene, the ratio of fluorescence intensities of peaks 373 and 384 nm were plotted as a function of surfactant concentration and fitted to the following sigmoidal equation:⁸

$$y = y_0 + \frac{(a+cx)}{1+e^{\frac{-(x-x_0)}{b}}} \quad (2-2)$$

Where the inflection point is set to be equal to the CMC. Figure 2-13 represents a typical set of data obtained after plotting the experimental values.

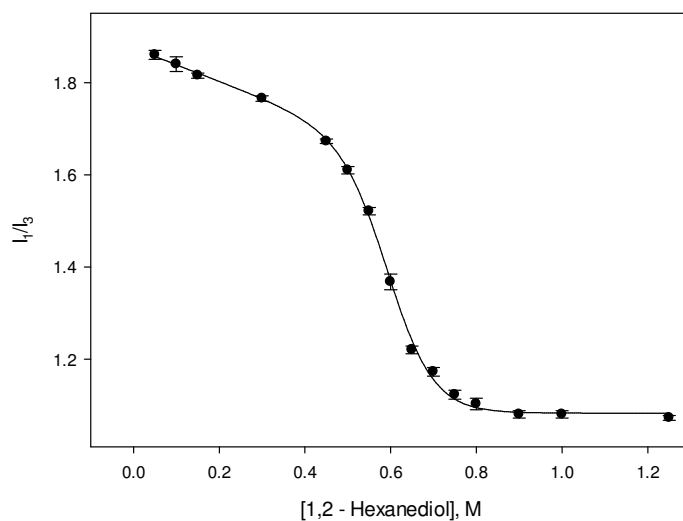


Figure 2-13. Fluorescence intensity ratio I_1/I_3 as a function of surfactant concentration.

In case of ANS, mean values of the fluorescence intensity at 521 nm were plotted against total surfactant concentration and the resulted data were fitted to the Equation 2-2. CMC was taken as an inflection point of the curve.

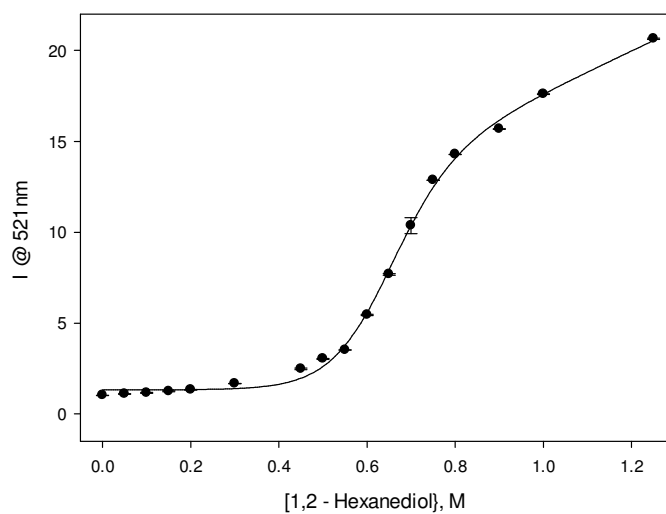


Figure 2-14. Fluorescence intensity at 521 nm as a function of surfactant concentration.

In case of BZA, absorbance ratio of peaks at 250 and 312 nm were plotted against total surfactant concentration. Data were fitted to the Equation 2-2 and CMC was determined as an inflection point of the curve.

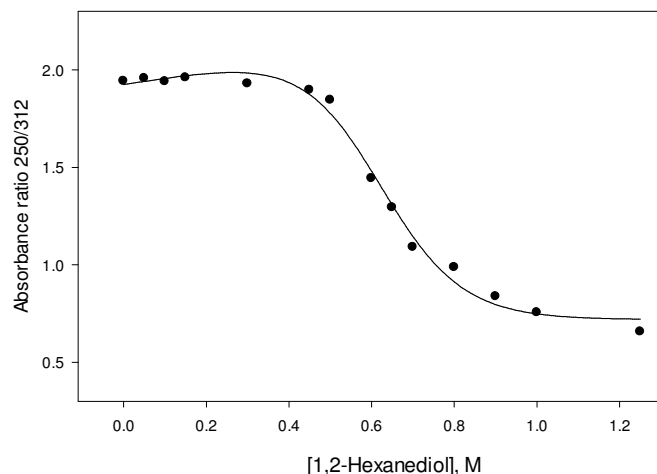


Figure 2-15. The ratio of the BZA absorbance measured at 250 and 320 nm as a function of surfactant concentration.

1.5.5 Aggregation number determination

As defined in the Introduction section, the aggregation number N_{agg} is the average number of surfactant molecules per micelle. This number can be determined via fluorescence quenching through the methodology developed by Turro and Yekta.²² This method involves labelling micelles with a fluorescence probe and measuring fluorescence intensity after addition of various amounts of the quencher. The properties of the quencher are of a great importance, because it has to be almost completely incorporated into micelle when added to surfactant solution in order to deactivate all probe molecules in the same micelle. To determine the aggregation number of 1, 2 – Hexanediol 1-Dodecylpyridinium chloride has been used as a quencher and pyrene as a fluorescence probe. In this case if we measure the fluorescence intensity at various quencher

concentrations with fixed surfactant concentration, the aggregation number can be calculated from the following equation:²³

$$\ln\left(\frac{I_0}{I}\right) = \frac{Q \times N_{agg}}{S - CMC} \quad (2-3)$$

Where I_0 is the fluorescence intensity in the absence of quencher, I is the fluorescence intensity in the presence of quencher, Q is the bulk concentration of quencher, N_{agg} is the aggregation number, S is the surfactant concentration and CMC is the critical micelle concentration of surfactant. Experimental setup in determination of N_{agg} of 1, 2 - Hexanediol was as follows: the stock concentration of the quencher 1-Dodecylpyridinium chloride was 30mM; from this stock various amounts of quencher were added to samples to achieve desired concentrations. Surfactant concentration was kept constant at 1M during all measurements. All samples contained 1 μ M final pyrene concentration. A series of samples were prepared in the morning and the data was collected after two hours. To prepare 1.5 mL of the sample, a 1M solution of 1, 2 – Hexanediol, water, a concentrated salt solution, a concentrated stock of the pyrene solution and various concentrations of quencher were added to 1.5 mL microfuge tube to give the final concentrations determined in advance. All 1.5 mL samples were prepared in a triplicate. All measurements were conducted at a room temperature (25°C). Fluorescence intensities at single wavelength were measured on a Fluorolog-3 Horiba Jobin Yvon spectrofluorometer. The spectrofluorometer parameters were set as following: an excitation wavelength - 337 nm, emission wavelength – 394 nm. Slits were set to 5nm band pass resolution. Samples were held in semi Micro quartz cuvettes with a 10x4 mm path length. All collected data were analyzed using Sigma plot (Point Richmond, CA) software.

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Chapter 3

Results

In this work we have determined the effects of cations and anions on the micellization of 1, 2 – Hexanediol. We have used the probes pyrene, ANS and BZA to determine how various cations and anions affect hydrophobicity as represented by the thermodynamics of micelle formation and obtained the following results.

1.5.6 Cation effects

a) Monovalent alkaline cations

Figure 3-1 demonstrates the effects of the alkaline salts LiCl, NaCl, KCl, and RbCl on the micellization of 1, 2 - Hexanediol. In these figures, the characteristic pyrene Py scale (the ratio of the fluorescence intensities of peaks I_1 and I_3) has been plotted against the total surfactant concentration. The data are well-described by Equation 2-2 and the resulting fits allow the CMC values to be determined easily. These CMC values are tabulated in Table 3-1. It can be observed that the addition of each salt decreases the CMC values. However, it is also clear that each of these cations affect the micellization process to a different degree. Because the chloride salt of each cation has been used in all

experiments, the anion effect is constant; therefore any salt specificity observed must be due to the cation.

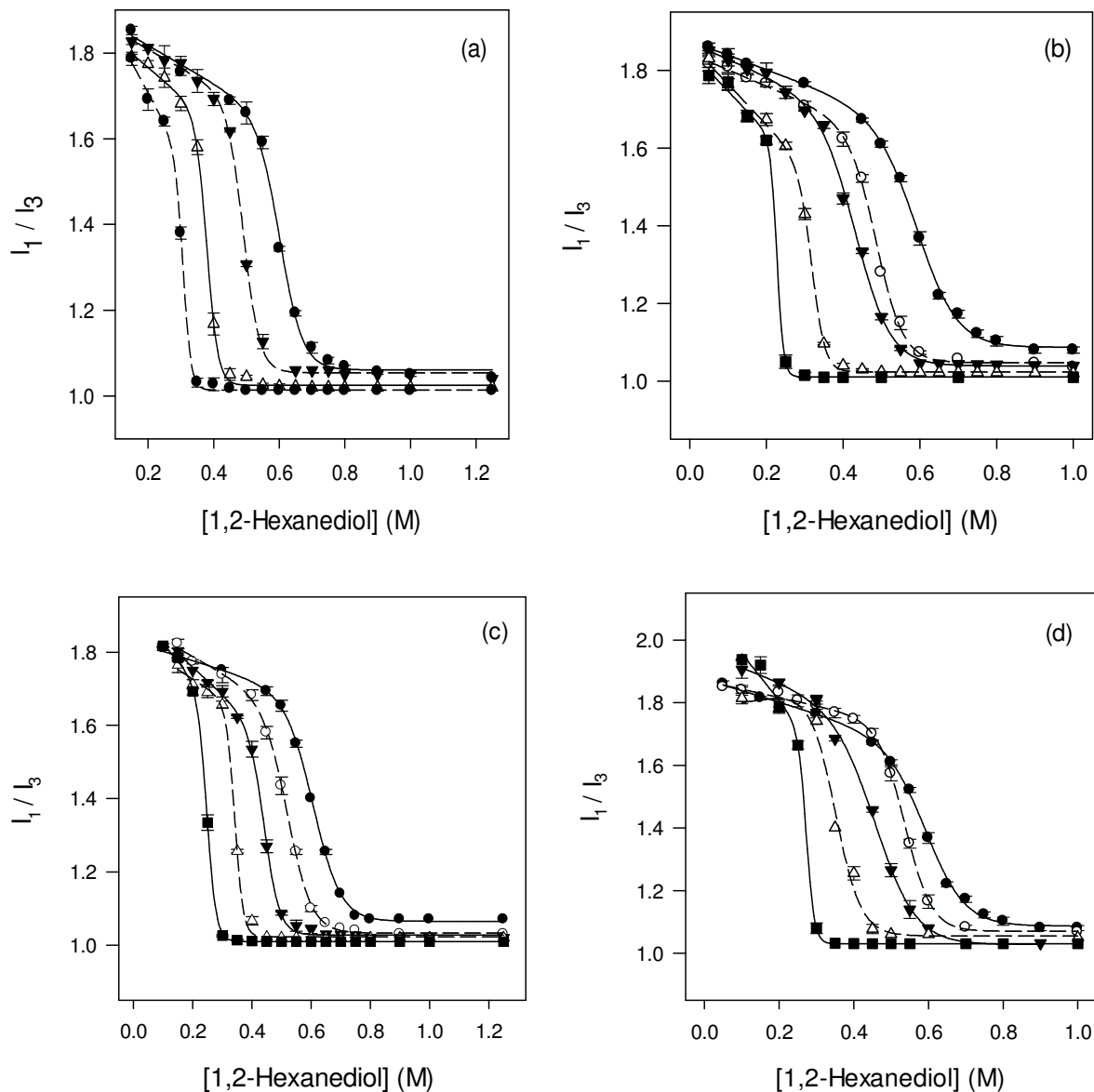


Figure 3-1. Fluorescence intensity ratio of pyrene at $1\mu\text{M}$ formal concentration I_1/I_3 as a function of 1, 2-Hexanediol concentration at various salt concentrations: 0M (filled circles), 0.5M (open circles), 1M (filled triangles) 2M (open triangles) and 3M (filled squares) sodium chloride. The salts are: (a)-LiCl, (b)-NaCl, (c)-KCl, (d)-RbCl.

Determining the effect of Cs^+ on the CMC of 1, 2 - Hexanediol presents a challenge because this cation quenches pyrene fluorescence. In order to determine CMC values,

changes in BZA absorbance have been used. Figure 3-2 shows the effect of increasing CsCl concentration on the micellization of 1, 2 – Hexanediol. In this figure, the absorbance ratio of peaks 250 and 315 (taken from the measured BZA absorbance profiles) has been plotted against total surfactant concentration. The data are fitted to Equation 2-2 and calculated CMC values are presented in Table 3-1. It can be observed that the addition of various concentrations of CsCl reduces CMC of surfactant and its effect is different from that of other chloride salts. Therefore, the observed specificity must be due to the cationic portion of the CsCl salt. To verify the BZA protocol, we have determined CMC of 0M and 1M NaCl using BZA dye and then compared it to the CMC value obtained with the pyrene procedure. The values are 0.61 ± 0.05 (BZA) and 0.60 ± 0.01 (pyrene) for 0M NaCl, 0.40 ± 0.02 (BZA) and 0.44 ± 0.01 (pyrene) for 1M NaCl respectively, which show a very good agreement between these two techniques.

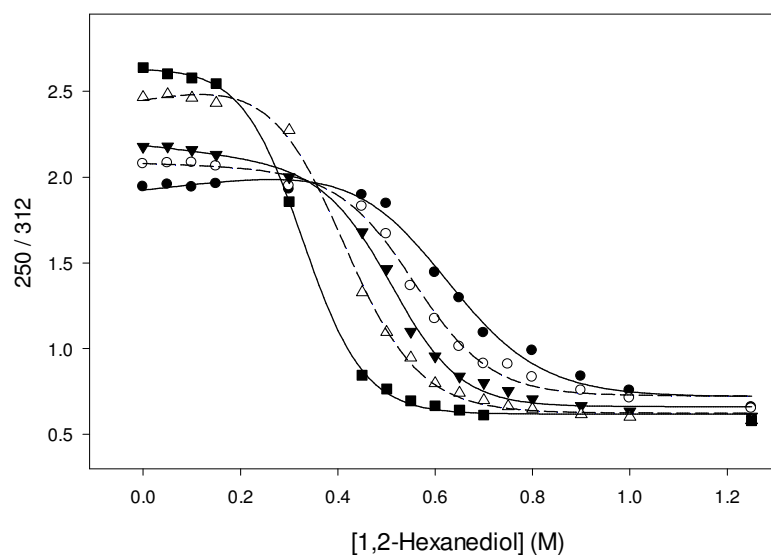


Figure 3-2. The ratio of the BZA absorbance measured at 250 and 320 nm as a function of 1,2-Hexanediol concentration at 0M (filled circles), 0.5M (open circles), 1M (filled triangles), 2M (open triangles) and 3M (filled squares) CsCl.

b) Ammonium derived salts

Figure 3-3 represents the effects of ammonium derived salts a) NH_4Cl , b) TMACl , c) TEACl and d) GdmCl on the micellization of 1, 2 – Hexanediol as defined by the pyrene fluorescence ratio I_1/I_3 . As it can be observed, the data follow Equation 2-2 closely and the resulting fits allow the CMC values to be determined easily. The resulting CMC values are placed in Table 3-1. It can be observed that NH_4Cl causes a decline in CMC values. Therefore the effect of NH_4Cl is very similar to the effect of the alkaline salts. But as the cation becomes bulkier the micellar behavior start to change: in the case of TEACl and GdmCl there is an increase in the CMC values, which indicates that these cations starting to disrupt the micelles. It is also interesting to focus on the first data points in Figures 3-3 b), c) and d), in other words the Py values measured in the absence of surfactant. The data demonstrate that fluorescence intensity ratio I_1/I_3 has increased from 1.8 (0M TMA^+) to 2.3 (3M TMA^+) and from 1.8 (0M TEA^+) to 2.15 (0.75M TEA^+) indicating that the addition of both of these cations causes an increase in the polarity of the aqueous solvent that the pyrene experiences. On the contrary, the intensity ratio I_1/I_3 shows a decline from 1.8 (0M Gdm^+) to 1.6 (3M Gdm^+), indicating that the addition of Gdm^+ causes a decrease in the solvent polarity measured by the pyrene probe. Based on the above observations, it can be assumed that adding these cations may lead to changes in the bulk properties of the water solvent. This hypothesis will be examined further in the discussion section.

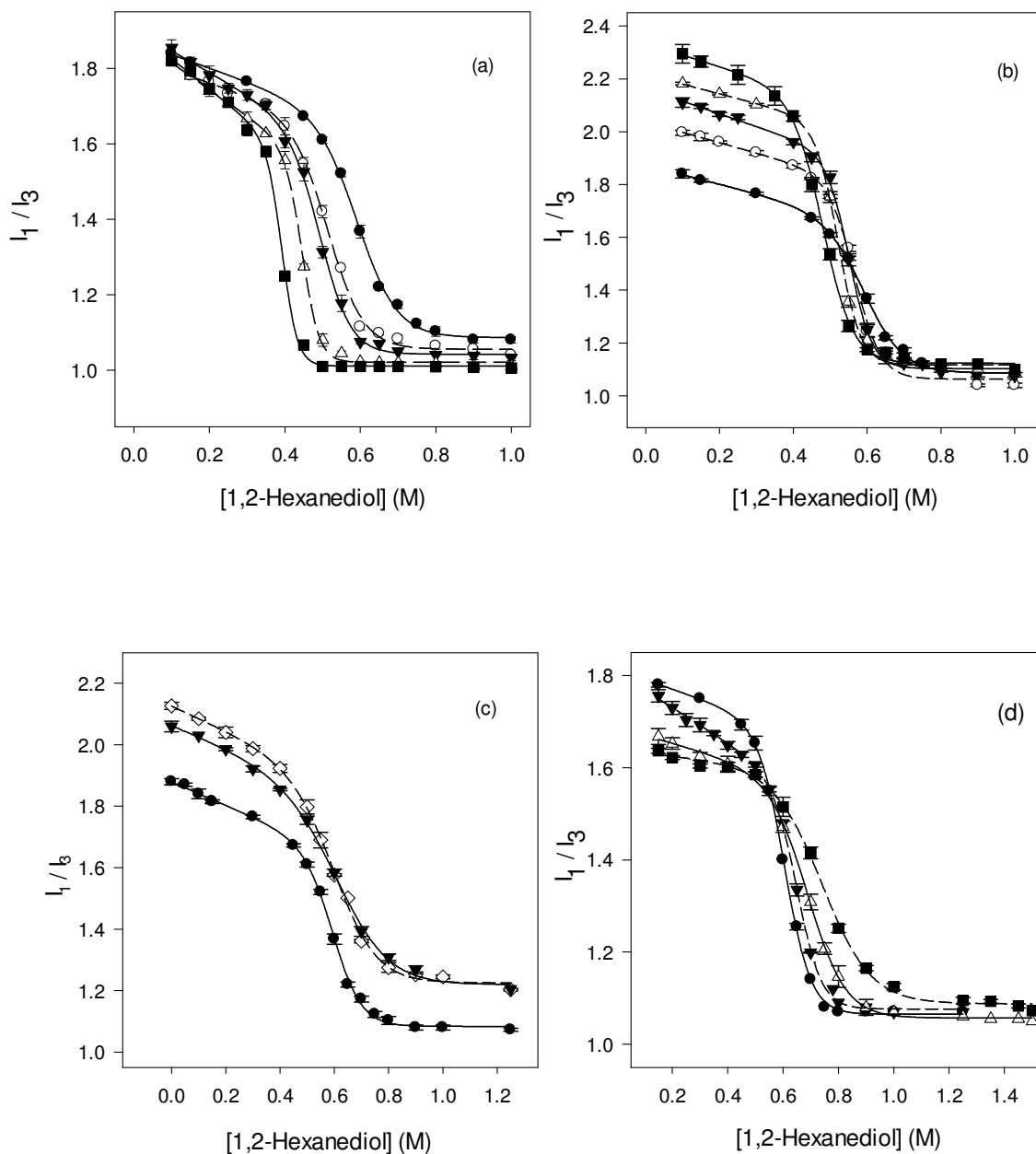


Figure 3-3. Fluorescence intensity ratio of pyrene at $1\mu\text{M}$ formal concentration I_1/I_3 as a function of 1, 2-Hexanediol concentration at various salt concentrations: 0M (filled circles), 0.5M (open circles), 0.75M (open diamonds), 1M (filled triangles), 1.5M (open squares), 2M (open triangles) and 3M (filled squares); the salts are: (a)- NH_4Cl , (b)- TMACl , (c)- TEACl and (d) GdmCl .

a) Divalent chloride cations

The effects of divalent chloride salts CaCl_2 , MgCl_2 , SrCl_2 and BaCl_2 on the micellization of 1, 2 – Hexanediol are shown in Figure 3-4. In this figure, the changes in the pyrene fluorescence ratio I_1/I_3 are also plotted as a function of total surfactant concentration. The data are well described by Equation 2-2 and the resulting fits allow the CMC values of the salts to be obtained easily. The determined CMC values are listed in the Table 3-1. The tabulated data demonstrate a decrease in the CMC values as the concentration of the salts change. Salt specificity is also observed in the divalent cation data. The properties of the solvent also seem to change with the addition of divalent salts: Figures 3-4 a), b) and c) show a decrease in the first data points as characterised by the Py values measured in the absence of surfactant. It can be observed that the fluorescence intensity ratio has decreased from 1.83 (0M salt) to 1.75 (3M salt) for Ca^{2+} , Mg^{2+} and Sr^{2+} , indicating that the addition of all these divalent cations causes a decrease in the polarity of the aqueous solvent that the pyrene experiences. It is important to note that the effect of the divalent chloride cations on the pyrene Py factor seems to be less pronounced than the effect of TMA^+ , TEA^+ and Gdm^+ . On the other hand divalent chloride cations show to have significant effect on the CMC values of the surfactant, disrupting micelles the most compared to the monovalent alkaline cations and ammonium derived salts.

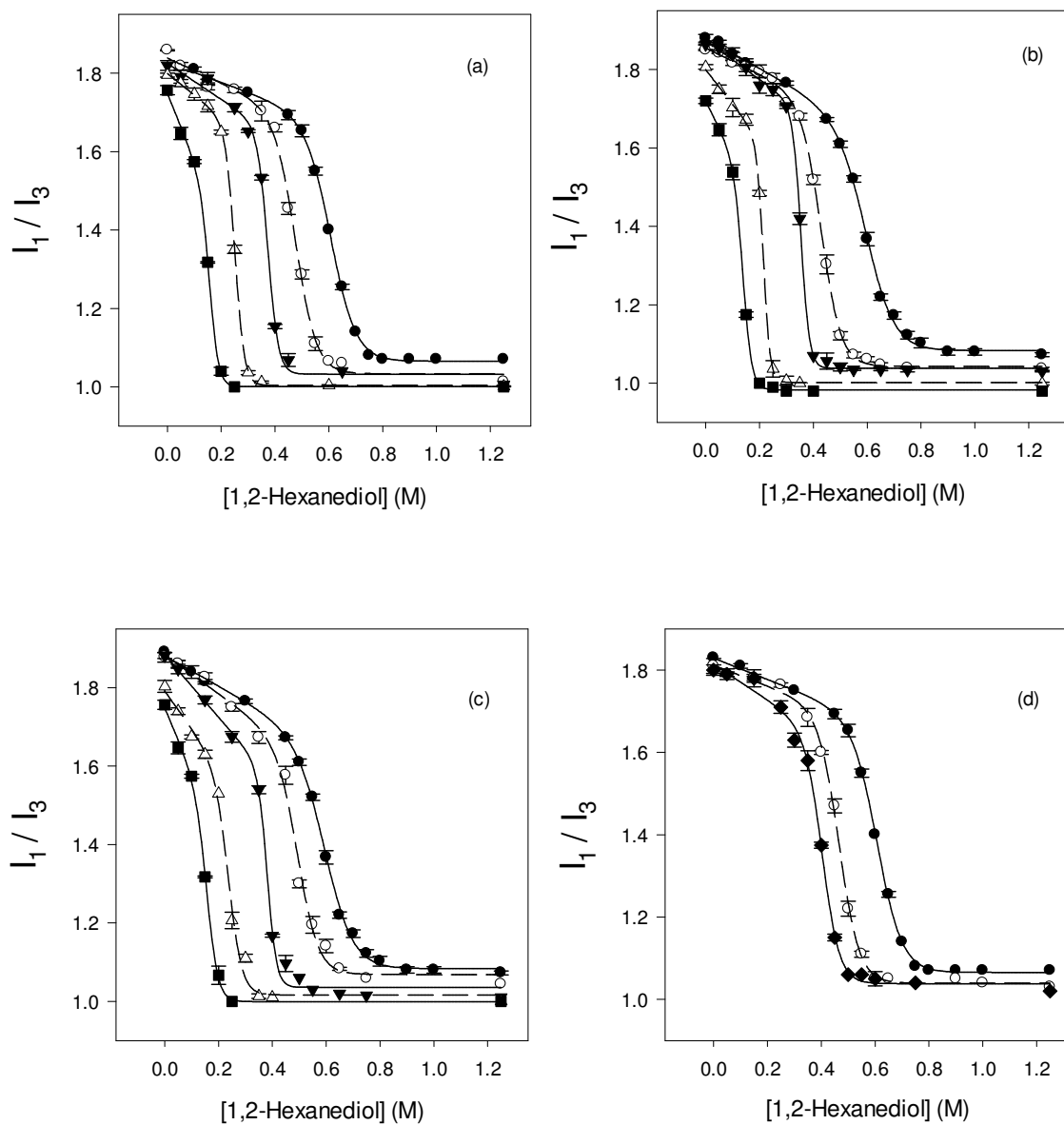


Figure 3-4. Fluorescence intensity ratio of pyrene at $1\mu\text{M}$ formal concentration I_1/I_3 as a function of 1, 2-Hexanediol concentration at various salt concentrations: 0M (filled circles), 0.5M (open circles), 0.75M (filled diamonds), 1M (filled triangles) 2M (open triangles) and 3M (filled squares); the salts are: (a)- MgCl_2 , (b)- CaCl_2 , (c)- SrCl_2 and (d)- BaCl_2 .

Table 3-1. Calculated CMC's for chloride salts. CMC's were calculated using Eq. 2-2.

	0M	0.5M	0.75M	1M	2M	3M
LiCl	0.60±0.02	-	-	0.49±0.01	0.38±0.01	0.31±0.01
NaCl	0.60±0.01	0.49±0.01	-	0.44±0.01	0.32±0.01	0.23±0.01
				/0.40±0.02*		
KCl	0.61±0.01	0.53±0.01	-	0.45±0.01	0.34±0.01	0.25±0.01
RbCl	0.60±0.01	0.54±0.01	-	0.46±0.02	0.35±0.05	0.27±0.02
CsCl	0.61±0.05	0.56±0.05	-	0.52±0.04	0.40±0.04	0.33±0.03
NH ₄ Cl	0.60±0.01	0.52±0.02	-	0.50±0.01	0.45±0.01	0.39±0.01
TMACl	0.60±0.01	0.57±0.03	-	0.55±0.02	0.52±0.02	0.48±0.02
TEACl	0.60±0.01	-	0.61±0.02	0.63±0.02	-	-
GdmCl	0.61±0.01	-	-	0.66±0.01	0.70±0.01	0.75±0.01
MgCl ₂	0.61±0.03	0.48±0.03	-	0.38±0.03	0.25±0.02	0.16±0.01
CaCl ₂	0.60±0.02	0.46±0.01	-	0.36±0.02	0.23±0.01	0.14±0.01
SrCl ₂	0.60±0.01	0.50±0.01	-	0.38±0.01	0.24±0.01	0.16±0.01
BaCl ₂	0.61±0.03	0.47±0.03	0.41±0.02	-	-	-

*CMC of 1M NaCl was also measured by UV/Vis spectroscopy to verify the procedure for CsCl UV/Vis CMC's determination.

1.5.7 Anion effects

Along with cationic chloride salts, we have also investigated the effect of eight monovalent anions on the micellization of 1, 2 – Hexanediol in order to study anionic effect. In all cases the sodium salt of the anions is used; thus, any salt specificity observed is due to the anion. The results are divided into monatomic and polyatomic anions.

a) Monatomic anions

Figure 3-5 show how the addition of NaF, NaCl and NaBr influence the micellization process. In these figures, the characteristic pyrene Py scale (the ratio of the fluorescence intensities of peaks I_1 and I_3) has been plotted against the total surfactant concentration. The data are described by Equation 2-2 and the resulting fits allow for the CMC values to be determined. The obtained CMC values are given in Table 3-2. It can be noted that all these salts decrease the CMC of 1, 2 – Hexanediol, with the lowest CMC found at the highest salt concentration. It can be observed for the sodium bromide data that there is a shift in the first data point as characterized by the Py values in the absence of the surfactant: the fluorescence intensity ratio I_1/I_3 has decreased from 1.8 (0M NaBr) to 1.6 (3M NaBr). In contrast, the first data points of F^- and Cl^- solutions do not show any concentration-dependant Py value change, as can be observed from Figure 3-5 a) and c). The above observations indicate that the addition of Br^- decreases the polarity of the aqueous solvent experienced by the pyrene. On the contrary, F^- and Cl^- seem to have minimal effect on the microenvironment of pyrene.

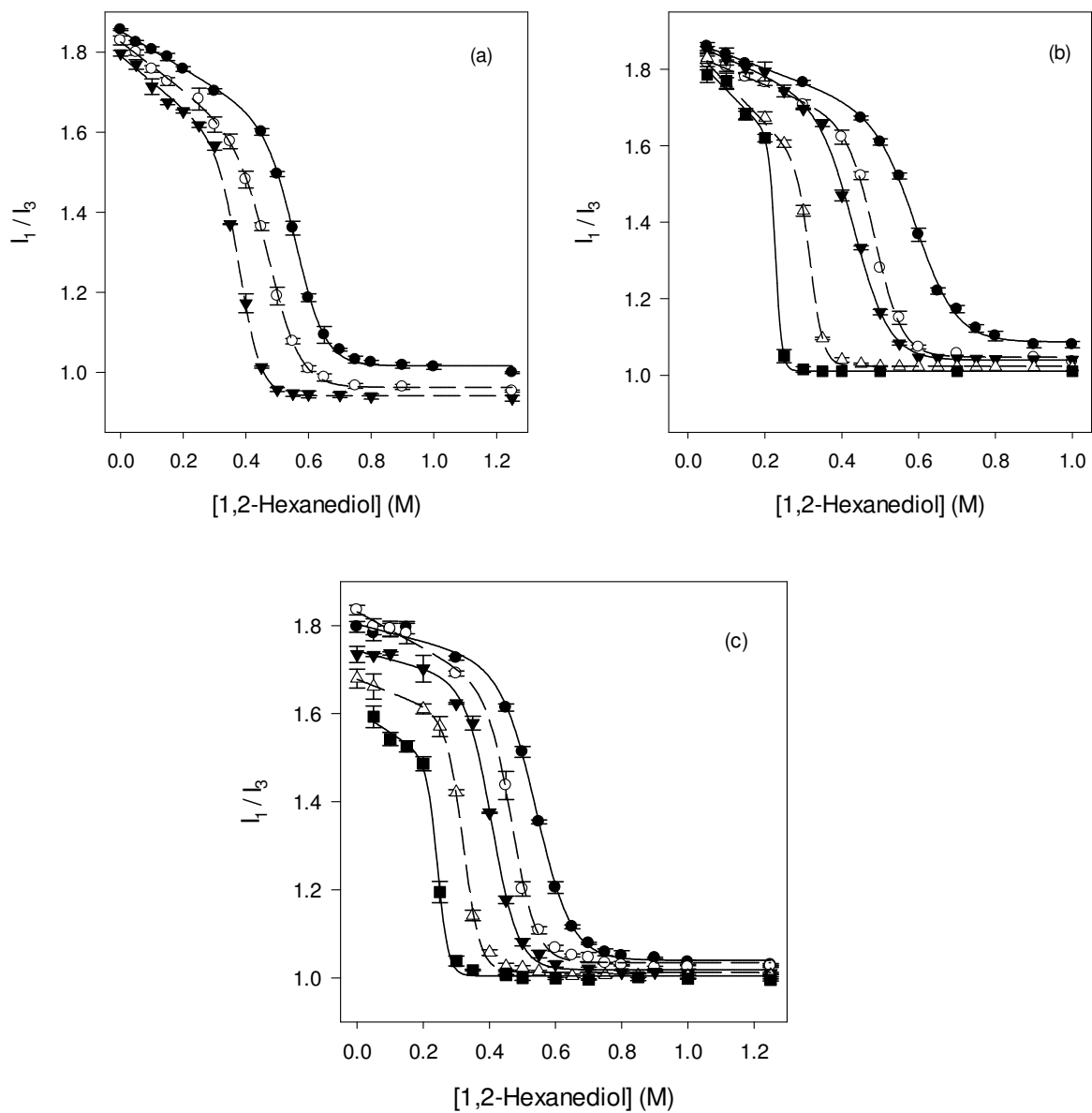


Figure 3-5. Fluorescence intensity ratio of pyrene at $1\mu\text{M}$ formal concentration I_1/I_3 as a function of 1, 2-Hexanediol concentration at various salt concentrations: (a) 0M (filled circles), 0.25M (open circles), 0.5M (filled triangles); (b) and (c) 0M (filled circles), 0.5M (open circles), 1M (filled triangles) 2M (open triangles) and 3M (filled squares) sodium chloride. The salts are: (a)-NaF, (b)-NaCl, (c)-NaBr.

The effect of I^- on the micellization properties of 1, 2 – Hexanediol has been measured by the use of an alternative dye - ANS, as the iodide anion quenches pyrene fluorescence. Figure 3-6 demonstrates the effect of NaI on CMC properties of surfactant. In this figure, mean fluorescence intensity values measured at 521 nm were plotted against total surfactant concentration. The data are fitted to the Equation 2-2 and the obtained CMC values are listed in Table 3-2. It can be observed from the tabulated data that NaI decreases the CMC values of the surfactant with the lowest CMC found at the highest salt concentration.

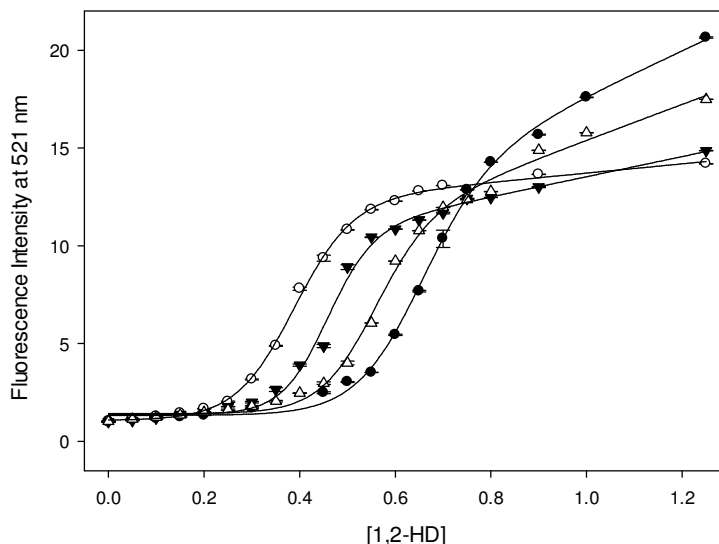


Figure 3-6. Fluorescence intensity ratio of ANS at $10\mu\text{M}$ formal concentration I_1/I_3 as a function of 1, 2-Hexanediol concentration at various NaI concentrations: 0M (filled circles), 1M (open triangles), 2M (filled triangles) and 3M (open circles).

b) Polyatomic anions

The effects of the polyatomic anions NO_3^- , ClO_4^- , SCN^- and $\text{C}_2\text{H}_3\text{O}_2^-$ on the micelle formation of 1, 2 – Hexanediol are displayed in Figure 3-7. This figure represents the plot of fluorescence intensity ratios I_1/I_3 against total surfactant concentration.

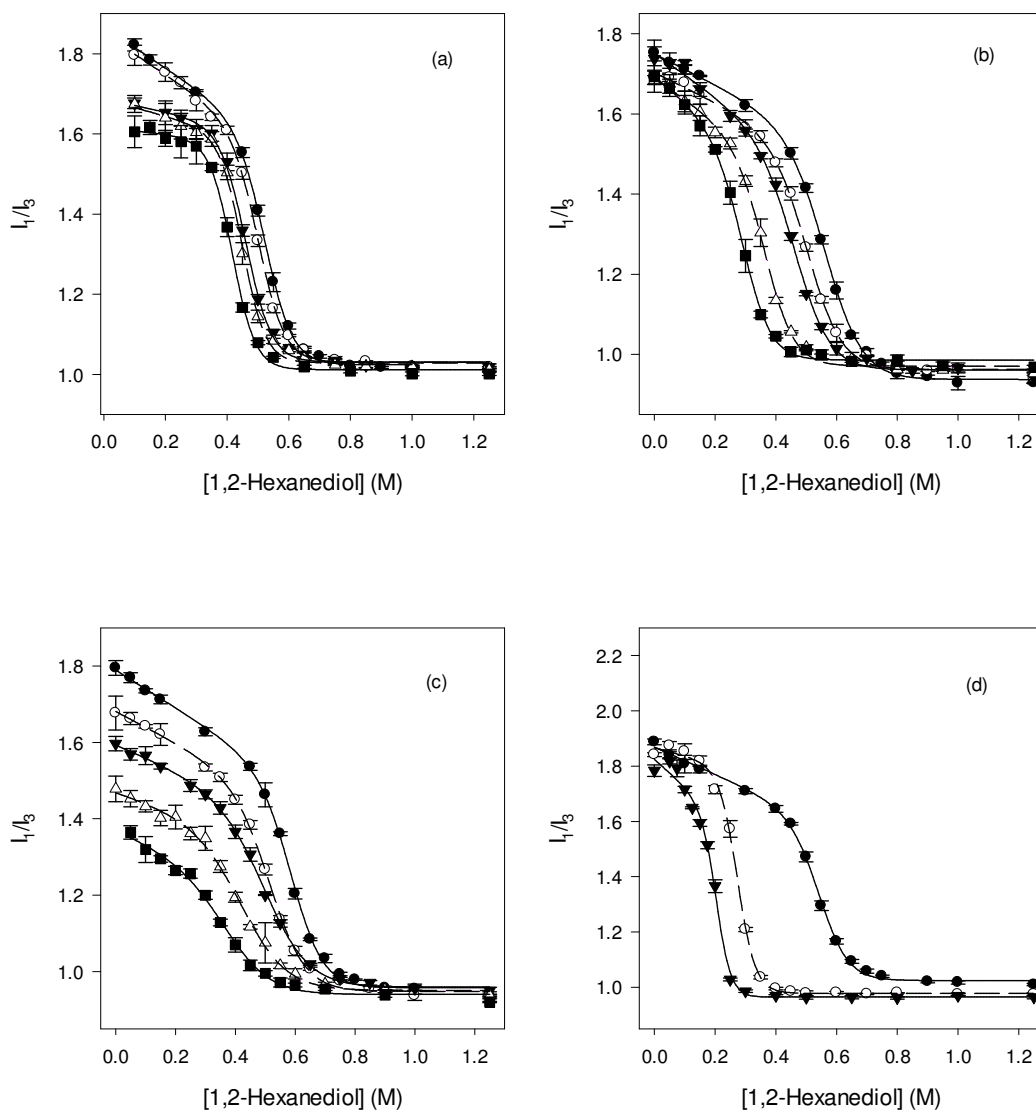


Figure 3-7. Fluorescence intensity ratio of pyrene at $1\mu\text{M}$ formal concentration I_1/I_3 as a function of 1, 2-Hexanediol concentration at various salt concentrations: (a) 0M (filled circles), 0.25M (open circles), 0.5M (filled triangles) 0.75M (open triangles); (b), (c) and (d) 0M (filled circles), 0.5M (open circles), 1M (filled triangles) 2M (open triangles) and 3M (filled squares) sodium chloride. The salts are: (a)- NaNO_3 , (b)- NaClO_4 , (c)- NaSCN , (d)- $\text{NaC}_2\text{H}_3\text{O}_2$.

All data were fitted to the Equation 2-2 and the calculated CMC values are summarized in Table 3-2. It can be observed in Figures 3-7 a), b) and c) that as NO_3^- , ClO_4^- and SCN^- are added to the solution there is a decrease in the first data points as characterised by the Py values measured in the absence of surfactant: the fluorescence intensity ratio I_1/I_3 has decreased from 1.8 (0M NaNO_3) to 1.6 (1M NaNO_3), from 1.8 (0M NaClO_4) to 1.7 (3M

NaClO₄) and from 1.8 (0M NaSCN) to 1.4 (3M NaSCN), indicating that the addition of all these polyatomic anions causes a decrease in the polarity of the aqueous solvent that the pyrene experiences.

Table 3-2. Calculated CMC's for sodium salts. CMC's were calculated using Eq. 2-2.

	0M	0.25M	0.5M	0.75M	1M	2M	3M
NaF	0.57±0.01	0.48±0.01	0.424±0.02	-	-	-	-
NaCl	0.58±0.01	-	0.50±0.02	-	0.41±0.01	0.30±0.01	0.21±0.01
NaBr*	0.55±0.01	-	0.47±0.01	-	0.41±0.01	0.32±0.01	0.24±0.01
NaI	0.64±0.03	-	-	-	0.55±0.03	0.45±0.04	0.39±0.02
NaNO ₃ *	0.53±0.01	0.51±0.01	0.48±0.01	0.45±0.02	0.42±0.01	-	-
NaClO ₄ *	0.57±0.01	-	0.51±0.01	-	0.47±0.02	0.37±0.01	0.30±0.01
NaSCN*	0.59±0.01	-	0.52±0.01	-	0.51±0.01	0.43±0.01	0.39±0.01
NaC ₂ H ₃ O ₂ *	0.55±0.01	-	-	-	-	0.28±0.02	0.21±0.03

1.5.8 The Aggregation number of 1, 2 –Hexanediol micelles

To determine the aggregation number of 1, 2 – Hexanediol, the fluorescence quenching method with 1-Dodecylpyridinium chloride as the quenching species was used. A typical set of data is presented in Figure 3-8. In this figure $\ln\left(\frac{I_0}{I}\right)$ is plotted against increasing quencher concentrations. The data were fitted to Equation 2-3 and the slopes of the straight lines were used to calculate the aggregation numbers of 1, 2 – Hexanediol in 0M and 1M NaCl. The calculated values are $N_{agg}^0=138$ for 0M NaCl and $N_{agg}^1=151$ for 1M NaCl. In the case of other cationic and anionic salts, calculated aggregation numbers were always significantly greater than 50. The obtained aggregation numbers allow us to characterise the thermodynamics of micelle formation using the simplified analytical form (Equation 1-13), which was discussed in detail in the Introduction chapter of this thesis.

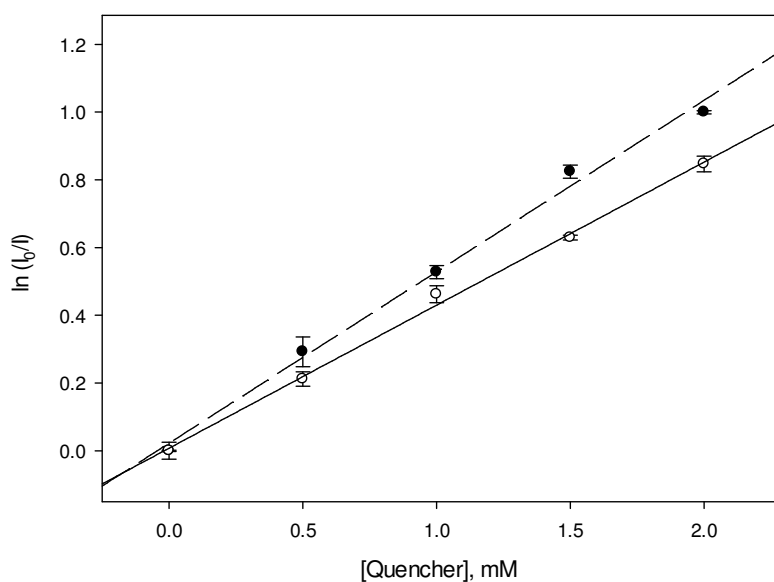


Figure 3-8. The influence of quencher concentration on the pyrene fluorescence intensity in micellar solution. Filled circles – 0M NaCl, open circles – 1M NaCl.

1.5.9 Thermodynamics of 1, 2 –Hexanediol micellization

In order to quantify the salt effect on the micellization of 1, 2 - Hexanediol, Gibbs free energy of micelle formation has been calculated for each salt at various concentrations based on the following equation:

$$\Delta G_m = RT \ln(CMC) \quad (3-1)$$

Where ΔG_m is a molar Gibbs energy of micellization.

Furthermore we have calculated $\Delta\Delta G_m$ which is an indicative of how the free energy changes with regards to the free energy of micelle formation in aqueous solution:

$$\Delta\Delta G_m = \Delta G_0 - \Delta G_m \quad (3-2)$$

Where ΔG_0 is Gibbs free energy of micelle formation in aqueous solution without any salt added, and ΔG_m is Gibbs free energy of micelle formation at various salt concentrations. Subsequently $\Delta\Delta G$ was plotted against salt concentration to obtain micellization free energy increments values, known as MFI values. MFI values are characterized by the slopes of the straight line obtained from the equation $y = ax$. Figures 3-9 and 3-10 represent the plots of MFI's against salt concentrations for both sets of salts: cationic and anionic respectively. MFI values demonstrate how a specific salt affects the hydrophobicity of the system, and obtained values along with their errors for the sodium and chloride salts are summarized in Tables 3-4 and 3-5. Based on the data presented in Table 3-4 it can be observed that cationic salts contribute to the micellization of 1, 2 – Hexanediol differently for each salt producing varying MFI values. Since for cations chloride was kept constant for all examined salts, any changes in the system are due to cation variation. The same trend is observed in anions and the slopes for all of the sodium salts studied give different MFI values. For anions sodium was kept constant therefore

any changes observed are due to the anion variation. For cations, we obtain a combination of positive and negative MFI values; while for anions, only positive values are observed. Positive MFI's indicate that a given salt will promote micellization of 1, 2 – Hexanediol, whereas negative values show the disruption of micelle formation.

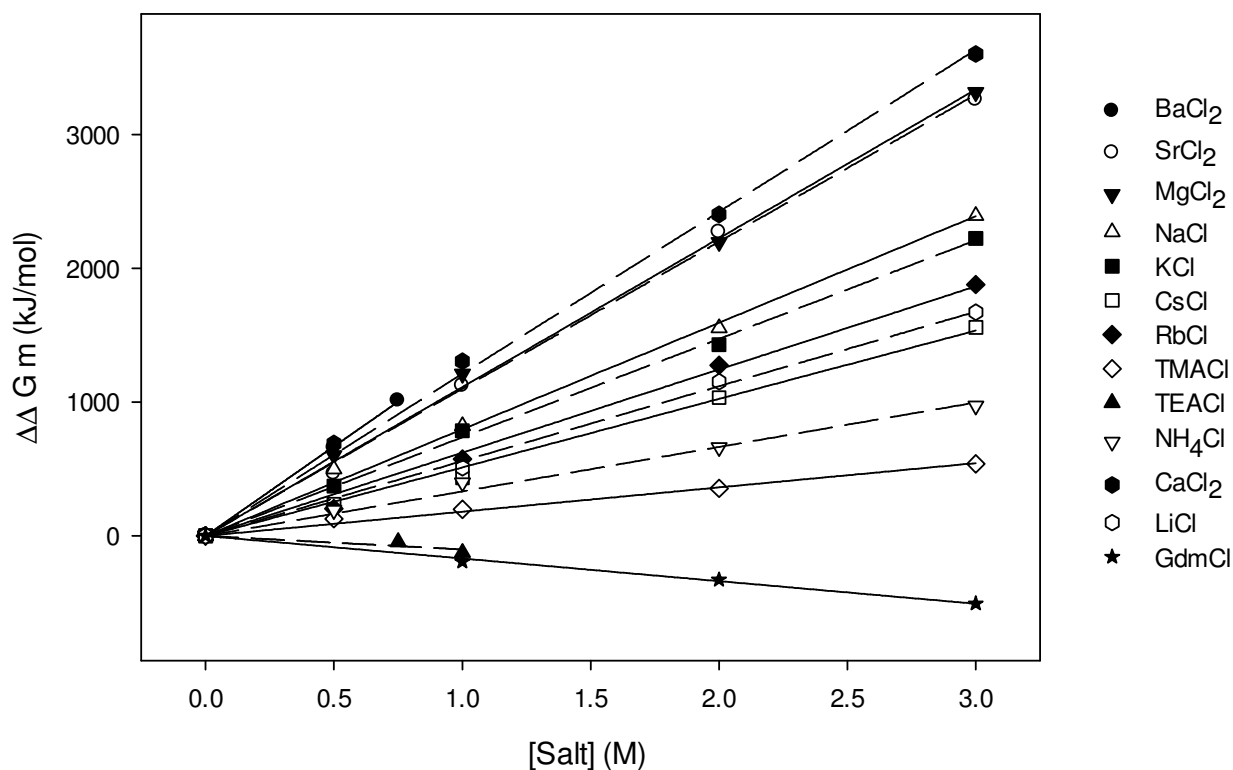


Figure 3-9. Concentration dependence of the change in the Gibbs free energy of micelle formation ($\Delta\Delta G_m$) measured in the presence of cationic salts. The salts are: filled circles - BaCl_2 , open circles - SrCl_2 , filled triangles down - MgCl_2 , open triangles up - NaCl , filled squares - KCl , open squares - CsCl , filled diamonds - RbCl , open diamonds - TMACl , filled triangles up - TEACl , open triangles down - NH_4Cl , filled hexes - CaCl_2 , open hexes - LiCl and filled stars - GdmCl .

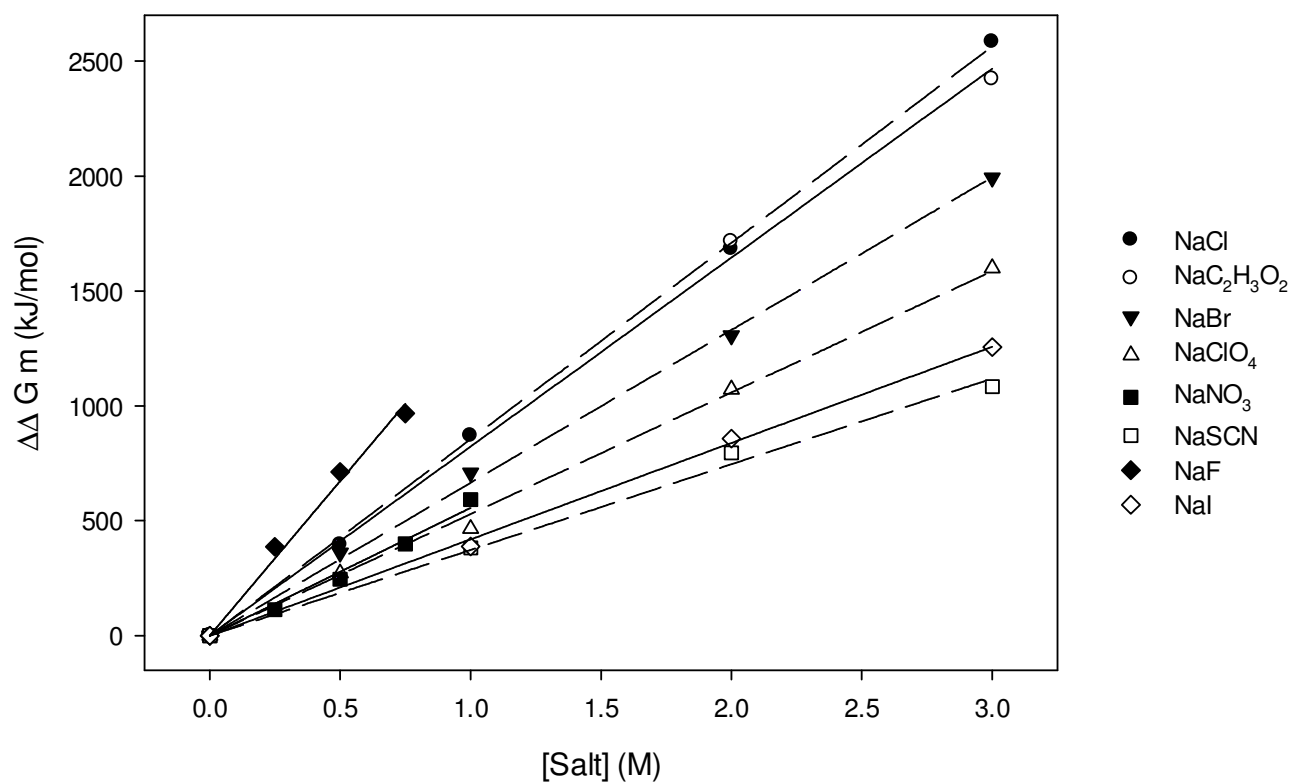


Figure 3-10. Concentration dependence of the change in the Gibbs free energy of micelle formation ($\Delta\Delta G_m$) measured in the presence of anionic salts. The salts are: filled circles – NaCl, open circles – NaC₂H₃O₂, filled triangles down – NaBr, open triangles up – NaClO₄, filled squares – NaNO₃, open squares – NaSCN, filled diamonds – NaF and open diamonds – NaI.

Table 3-4. Parameters associated with equation $y = ax$; result from fitting the data depicted in Figure 3-7.

	a	y_0	r^2
NaCl	796 ± 14	0 ± 0	0.9966
KCl	736 ± 9	0 ± 0	0.9986
RbCl	621 ± 16	0 ± 0	0.9939
CsCl	511 ± 11	0 ± 0	0.9954
TMACl	181 ± 5	0 ± 0	0.9902
TEACl	-101 ± 28	0 ± 0	0.8283
LiCl	558 ± 10	0 ± 0	0.9976
NH ₄ Cl	332 ± 10	0 ± 0	0.9903
GdmCl	-168 ± 14	0 ± 0	0.9954
CaCl ₂	1211 ± 18	0 ± 0	0.9978
MgCl ₂	1111 ± 15	0 ± 0	0.9980
SrCl ₂	1099 ± 16	0 ± 0	0.9981
BaCl ₂	1337 ± 10	0 ± 0	0.9997

Table 3-5. Parameters associated with equation $y = ax$; result from fitting the data depicted in Figure 3-8.

	a	y_0	r^2
NaF	1346 ± 44	0 ± 0	0.9944
NaCl	855 ± 25	0 ± 0	0.9994
NaBr	665 ± 28	0 ± 0	0.9987
NaI	419 ± 21	0 ± 0	0.9985
NaNO ₃	556 ± 29	0 ± 0	0.9923
NaClO ₄	529 ± 33	0 ± 0	0.9973
NaSCN	373 ± 35	0 ± 0	0.9945
NaC ₂ H ₃ O ₂	822 ± 59	0 ± 0	0.9977

Chapter 4

Discussion

The salt specific effects observed on the micellization process can now be examined in light of the theories outlined in the Introduction section. In order to establish if the observed specific ion effects on the hydrophobicity are due to differential partitioning, it is necessary to find a relationship between our measured effects on the micellization (quantified by MFI values) and the specific effects seen on surface tension at the air-water interface and hydrocarbon solubility. Pegram and Record postulate that salt specific changes in hydrocarbon solubility, as described by Setchenow constants, can be used to define a local concentration around the hydrocarbon molecule similar to that defined by STI values.^{1, 2} Thus, salt effects on the solubility of a model compound using solute partitioning model can be described based on the following equation:³

$$SFEI = \frac{d \ln S}{dm_2} = - \left(\frac{1}{RT} \right) \frac{dG_{obs}^0}{dm_2} = - \frac{[v_+(K_{p,+}-1)+v_-(K_{p,-}-1)]b_1(1+\epsilon_{\pm})}{55.5} \quad (4-1)$$

Because the thermodynamics of micellization process is similar to that of solubilisation, Equation 4-1 can be applied to the MFI values for defining the local salt concentration around the hydrophobic portion of the amphiphile molecule. Pegram and Record suggest

that the partitioning of ions in the hydrocarbon-water interface is similar to that of the air-water interface and they obtain a linear correlation between STI and SFEI values for their available cationic data.³

In Figure 4-1 our experimental MFI values have been plotted as a function of STI values for the cationic species. For the monovalent cations, it can be easily observed that a linear correlation exists between MFI and STI values of the NaCl, KCl, RbCl, LiCl, CsCl species. However, as the cations increase in size (NH₄Cl, TMAcI, GdmCl and TEAcI) they significantly deviate from the linear correlation. In the case of divalent cations there is no relationship observed between MFI and STI values. In the case of anions, as seen on Figure 4-2, there is no general correlation between their MFI and STI values. This was also observed by Pegram and Record.⁴ Based on these results it can be concluded that differential ionic partitioning model is not capable of describing the salt effects on the micellization very well. Therefore, we can conclude that the effects of salts on the air-water interface are not similar to their effect on the solubility of the hydrocarbon. In fact, the effects of salts on the available solubility data show much better correlation with our measured MFI values than with STI numbers. This is demonstrated in Figures 4-3 and 4-4: a reasonable linear relationship is obtained for both cations and anions. This correlation is remarkable given that the solubility data for the cationic and anionic species were collected from different sources. Thus, the lack of correlation observed between the STI and MFI values is not due to the properties of the amphiphile. Instead, it may either be a result of the fact that ions interact very differently with hydrocarbon-water interfaces (as compared with air-water interfaces) or that there is an

inherent flaw in describing salt effects on hydrophobicity in terms of a two phase interfacial model.

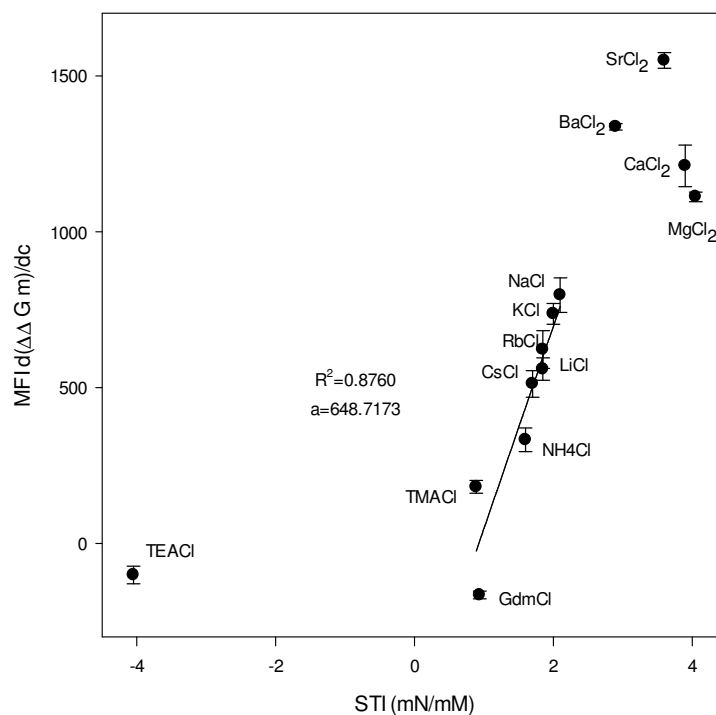


Figure 4-1. MFI values for cations compared to the STI values calculated by Marcus.¹⁴

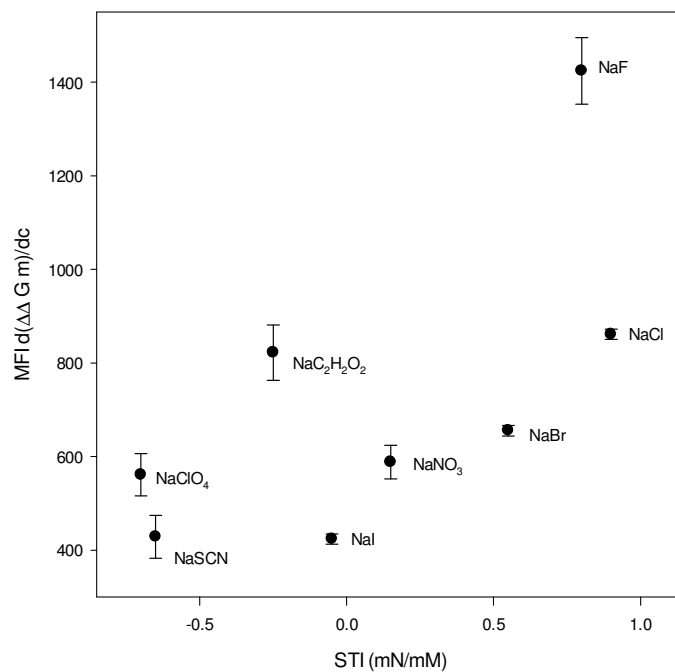


Figure 4-2. MFI values for anions compared to the STI values calculated by Marcus.¹⁴

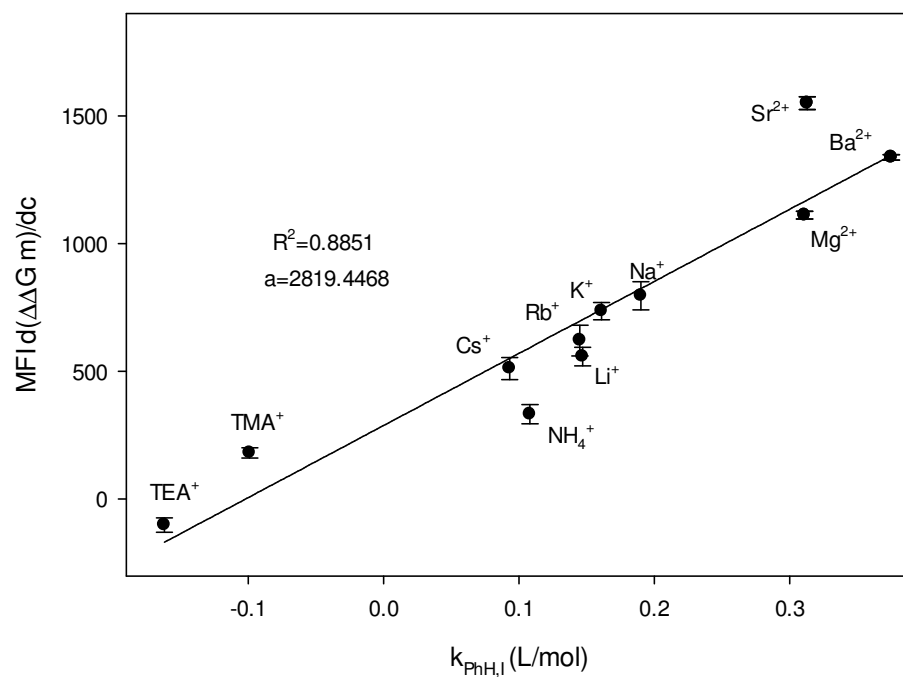


Figure 4-3. MFI values for cations as a function of the solubility constants.

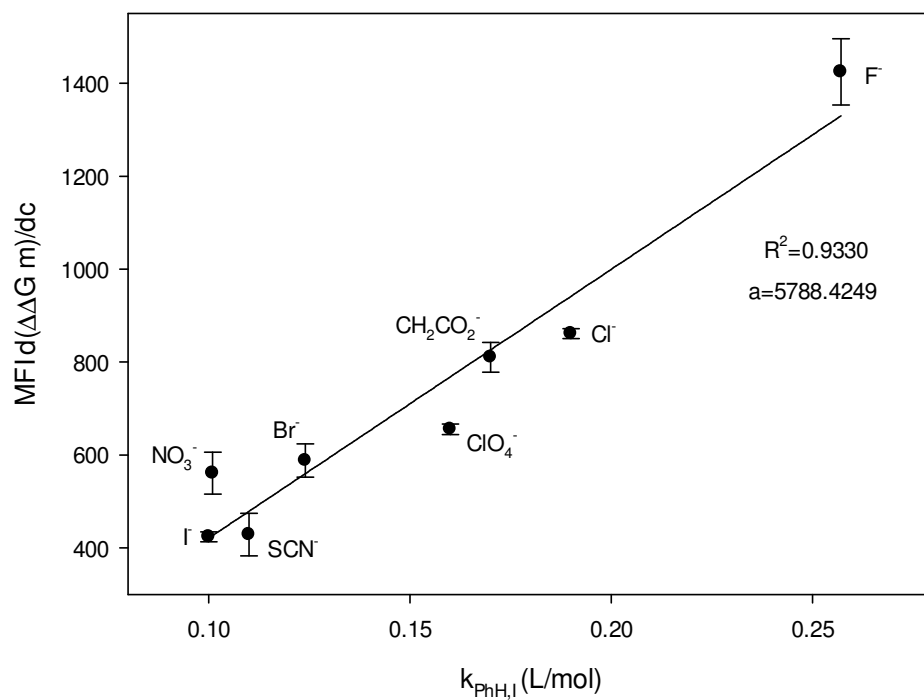


Figure 4-4. MFI values for anions as a function of the solubility constants.

In the light of preceding discussion of the salts effects on hydrophobicity, it is evident that this phenomenon cannot be fully explained within solute partitioning model. Therefore the next concept which will be used to discuss our hydrophobicity data is the sticky ion effect which was introduced by Collins.⁵ According to his approach, salts specific effects seen on hydrophobicity arise from the difference in ion's hydration. Collins demonstrated that if ion is weakly hydrated it will stick to the hydrophobic surface; where in case of strongly hydrated ions, repulsion between hydrophobic surface and ionic system is observed. It has been shown that the transition from strong to weak hydration is different for cations compare to that of anions. For monovalent cations it occurs at a radius of 1.06Å and in case of monovalent anions it is about 1.78Å, which indicates that anions are more strongly hydrated than cations.⁵ Based on Collin's results, ions with small ionic radius will enhance hydrophobicity in order to reduce the surface area that repels ions. In contrast, ions that have large ionic radius will tend to weaken hydrophobicity because a large surface area provides more interaction sites with the anions. This stickiness concept has been applied to our experimental data to see if observed ions specific effects on the micellization (as characterized by MFI values) come from difference in the ion's hydration. To this extent, our experimental MFI values for cations and anions were plotted as a function of ionic radius. The resulting fits are represented in Figures 4-5 and 4-6 for cations and anions respectively. It can be observed that there is a rough size correlation obtained in both cases. It is important to note, that according to Collins data, anions show a lot more stickiness than cations, but based on our experimental data the cation and anion effects appear to be very similar. For example, it can be easily observed in Figure 1-4 (Introduction chapter) there is a huge difference in

stickiness between I^- and Cl^- compare to that between Na^+ and Cs^+ . Looking at our experimental data, the difference in the MFI values between I^- and Cl^- and Na^+ and Cs^+ is almost the same. Thus Collins model of ions stickiness does not necessarily describe the salt specificity on our hydrophobic system.

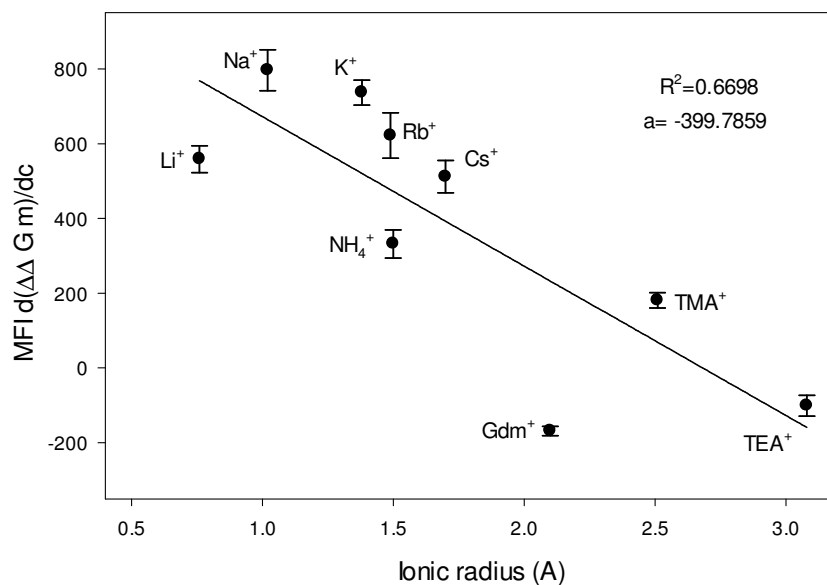


Figure 4-5. Relationship between ionic radius and MFI values for cations. Ionic radius values for cations were taken from the ref. ¹⁴.

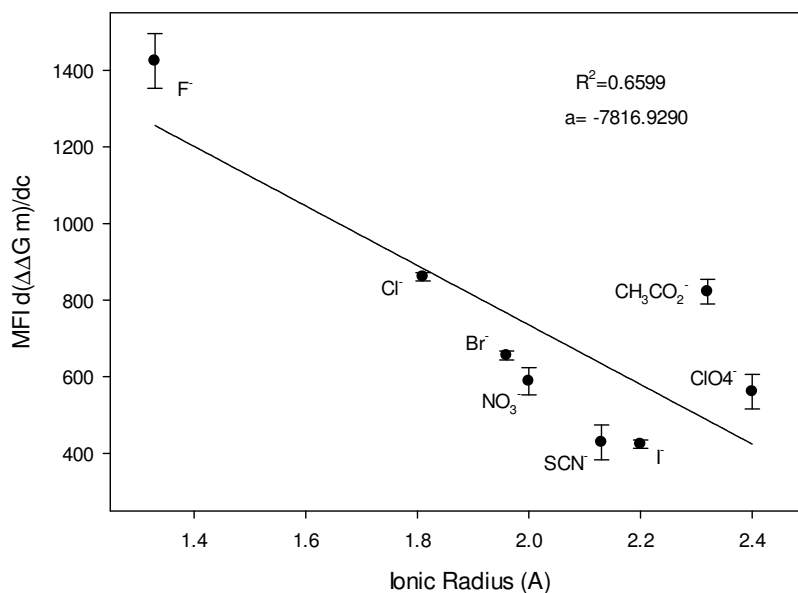


Figure 4-6. Relationship between ionic radius and MFI values for anions. Ionic radius values for anions were taken from the ref. ¹⁴ and ¹⁵.

Given the limitations of our previous analysis, we now interpret our data within the framework of bulk solvent effects. Electrostriction theory predicts that specific salt effects on hydrophobicity result from the contraction of the solvent in response to the ionic electric field. Therefore, measured MFI values should correlate with the electrostriction ΔV : the difference between the partial molar volume of the salt measured at infinite dilution $\tilde{V}$, and the intrinsic volume of the component ions of the salt V_{int} .⁶ In order to determine if the observed salts specific effects on hydrophobicity in our system are due to salt-induced solvent contraction, experimental MFI values were plotted as a function of the calculated ΔV values (Figures 4-7 and 4-8). Figure 4-7 plots MFI versus ΔV values for monovalent and divalent cations. It can be observed that a rough correlation exists between the two parameters (Gdm^+ is an exception). But this correlation is no better than that observed with cationic radii. The anion data of Figure 4-8 show an even poorer correlation with ΔV values: the data points are randomly scattered showing no relationship between their experimental MFI values and calculated ΔV values. These results demonstrate that the theory of electrostriction as described by McDevit and Long⁷ does not provide a workable framework for quantifying specific salts effects on hydrophobic interactions.

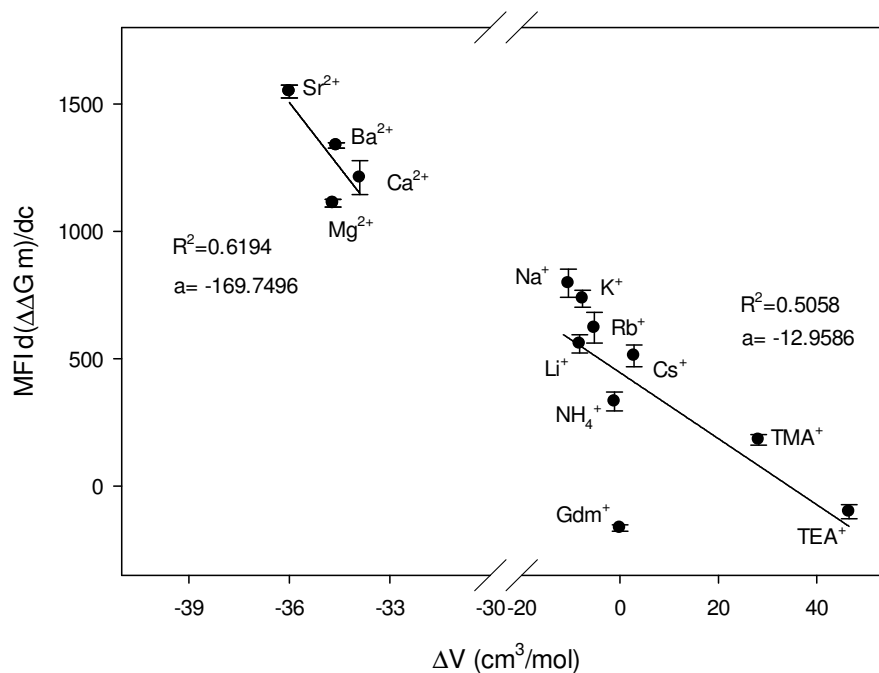


Figure 4-7. MFI cationic values plotted against the difference between the cationic partial molar volume and the cationic intrinsic volume. ΔV cationic values were calculated based on the ref.6 data.

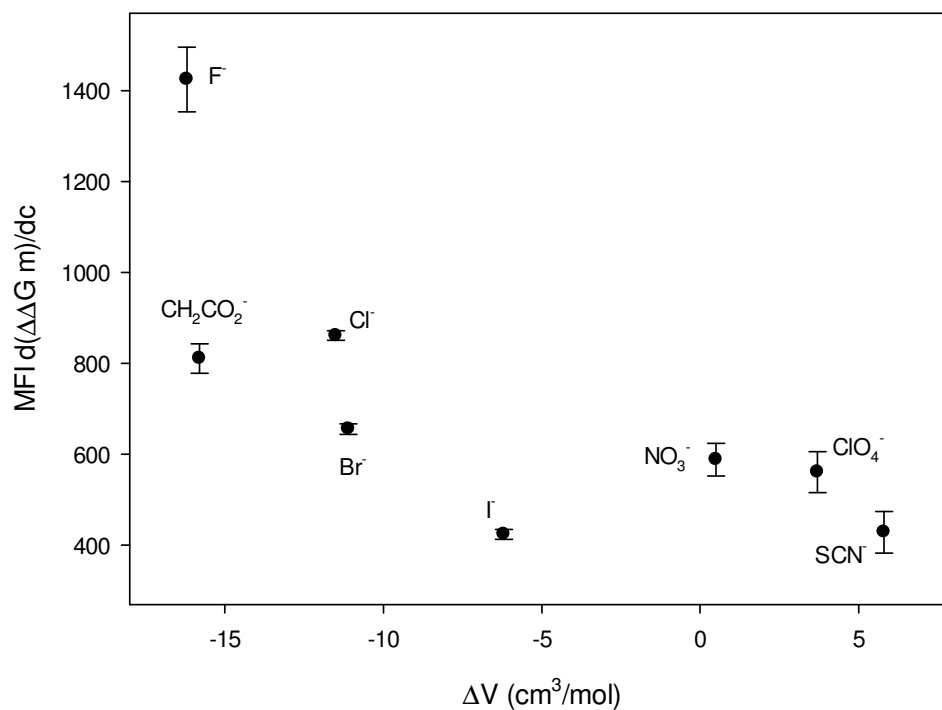


Figure 4-8. MFI anionic values plotted against the difference between the anionic partial molar volume and the anionic intrinsic volume. ΔV anionic values were calculated based on the ref.6 data.

As it was mentioned in the Introduction chapter of this thesis, scaled particle theory is an alternative method to consider the origin of the salt specific effects on hydrophobicity, which takes into account bulk properties of the solution, compared to interfacial theories.⁸ If the contributions described by scale particle theory are responsible for the observed ion specificity on the micellization, then our experimental MFI values should be related to the parameters required to obtain salting coefficients. The expression for calculating the salting coefficient was outlined in the Introduction (Equation 1-7). In this equation k_γ and k_α are the parameters that can be relatively easy calculated. On the other hand, calculating k_β contribution presents a challenge because it is hard to find an expression that contains all interaction contributions. The existent definition of interaction parameter k_β by Masterton and Lee appears to be oversimplified (Equation 1-10).⁸ This equation only takes into account polarizabilities of the ions and dipole-dipole interactions; the structure of water and the effects of ions on the hydrogen bonding properties of water are completely neglected. However, Collin's empirically obtained sticky ion data demonstrate that the interactions of cations with molecular surfaces do not show large variation (Figure 1-4 Introduction chapter), especially in comparison with the anion data.⁵ Thus, for a first approximation it can be assumed that the interaction contribution to salting coefficients, the parameter k_β , is not going to vary significantly between cations. Therefore, in this case, any observed specific cation effects on the salting coefficients will be due to the effect each cation species has on the free energy of cavity formation, as quantified by k_α , and the effect it will have on the number density of the solution, as represented by k_γ . We have investigated this assumption by plotting cation experimental MFI values against calculated $k_\alpha + k_\gamma$ parameters in Figure 4-9. It can be observed that

there is a relatively good linear correlation obtained for both monovalent and divalent cation data.

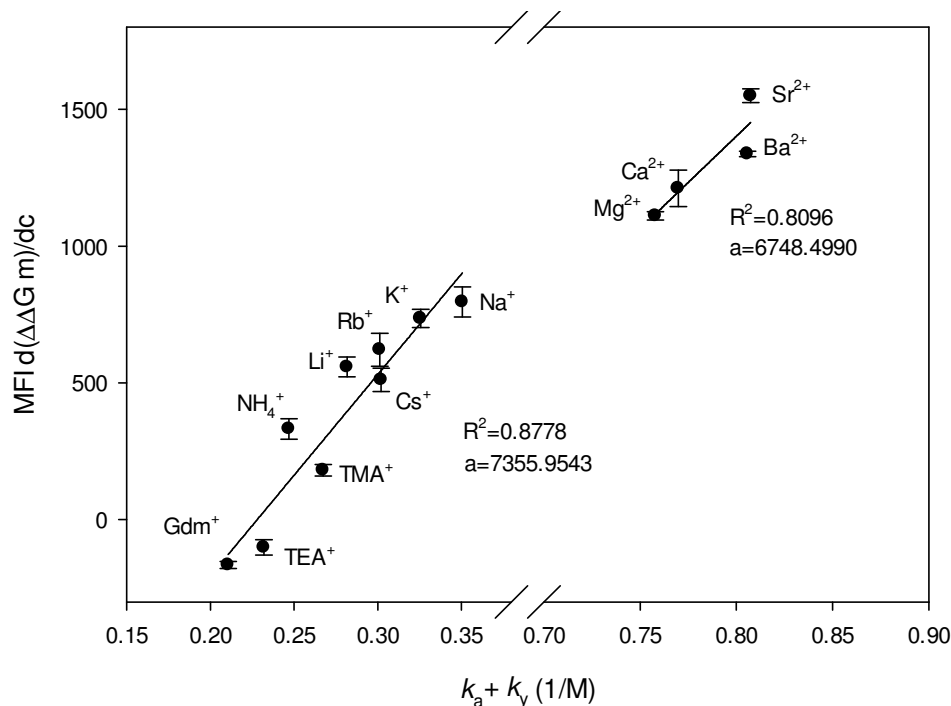


Figure 4-9. Cations specific effects on micellization compared to the cavity formation contribution towards the salting coefficient.

This linear correlation is better than anything obtained from previous models. In addition, the fact that a linear correlation is obtained demonstrates that our assumption that parameter k_β undergoes small variation is valid. Let us now take a closer look at the subcomponents of the k_α parameter, as represented by the Equation 1-9 (Introduction chapter). The first term of this equation is due to the internal pressure, which is the third order parameter and provides a minor constant contribution:⁹

$$k_\alpha = \underbrace{2.15 \times 10^{20}(\sigma_3^3 + \sigma_4^3) - 2.47 \times 10^{-4}\phi_0}_{k_{a1}} + \dots \quad (4-2)$$

The second term is related to the microscopic curvature and strongly dependent on the geometry of the system and provides a smaller contribution than the third term:⁹

$$k_{\alpha} = \cdots + \underbrace{\sigma_1[6.45 \times 10^{20}(\sigma_3^2 + \sigma_4^2) + 1.34 \times 10^{28}(\sigma_3^3 + \sigma_4^3) - 4.23 \times 10^4 \phi_0]}_{k_{a2}} + \cdots \quad (4-3)$$

And the third term of the Equation 1-9 provides the largest contribution to the parameter k_{α} and has been defined as the microscopic surface tension:⁹

$$k_{\alpha} = \cdots + \underbrace{\sigma_1^2[6.45 \times 10^{20}(\sigma_3 + \sigma_4) + 4.01 \times 10^{27}(\sigma_3^2 + \sigma_4^2) + 1.32 \times 10^{36}(\sigma_3^3 + \sigma_4^3) - 4.17 \times 10^{12} \phi_0]}_{k_{a3}} \quad (4-4)$$

Figure 4-10 depicts the plot of the sum of the microscopic surface tension and number density of the solution $k_{a3}+k_y$ against our experimental MFI values for cations. As it can be clearly observed, this parameter significantly improves the quality of the linear correlation. Scaled particle theory therefore can provide an explanation of the effect of cations on hydrophobic interactions such as micellization: these specific cation effects are result from how cation addition influences the cavity formation free energy and the number density of the aqueous solvent. Generally speaking cation effects on hydrophobicity seem to be the result of how cation addition influences the bulk properties of solvent water and are independent of how they are individually hydrated. It is of interest to focus on the Gdm⁺ MFI value. Removing this ion causes further improvement to the correlation as demonstrated by the solid line in Figure4-9. This indicates that guanidinium ion disrupts hydrophobic interactions in a manner consistent with a much larger cation. This is to be expected because the guanidinium ion can specifically interact with hydrophobic moieties through Van der Waals interactions.^{10, 11}

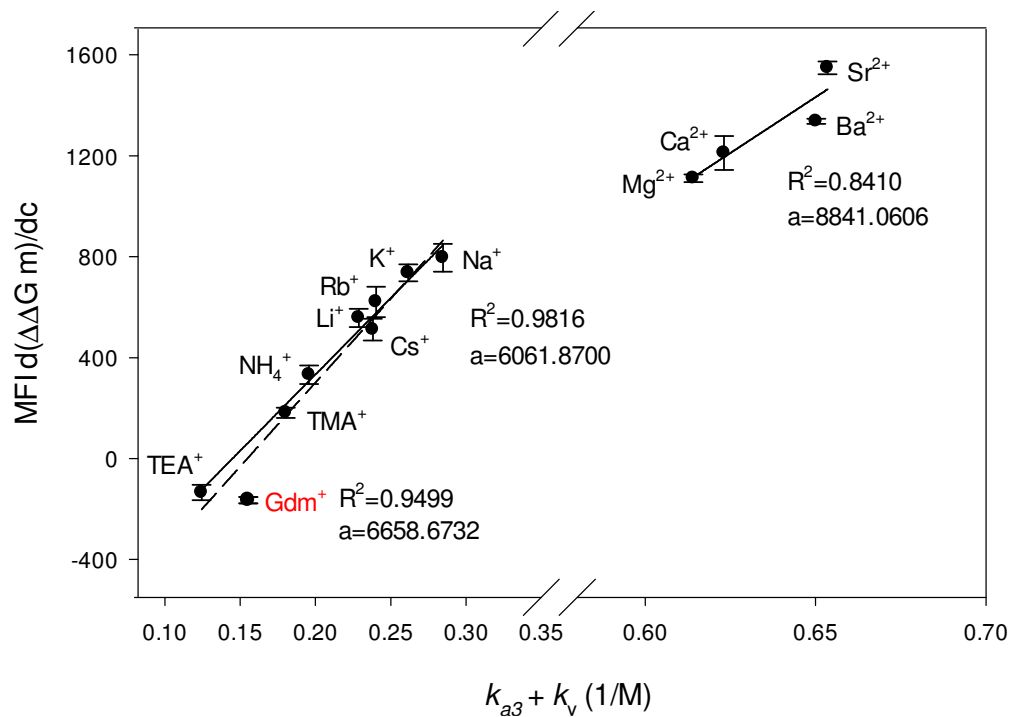


Figure 4-10. Cationic MFI values plotted against the sum of microscopic surface tension and number density. Solid line plots monovalent cations with the exception of Gdm^+ . Short dash line represents the fit obtained for all monovalent cations including Gdm^+ .

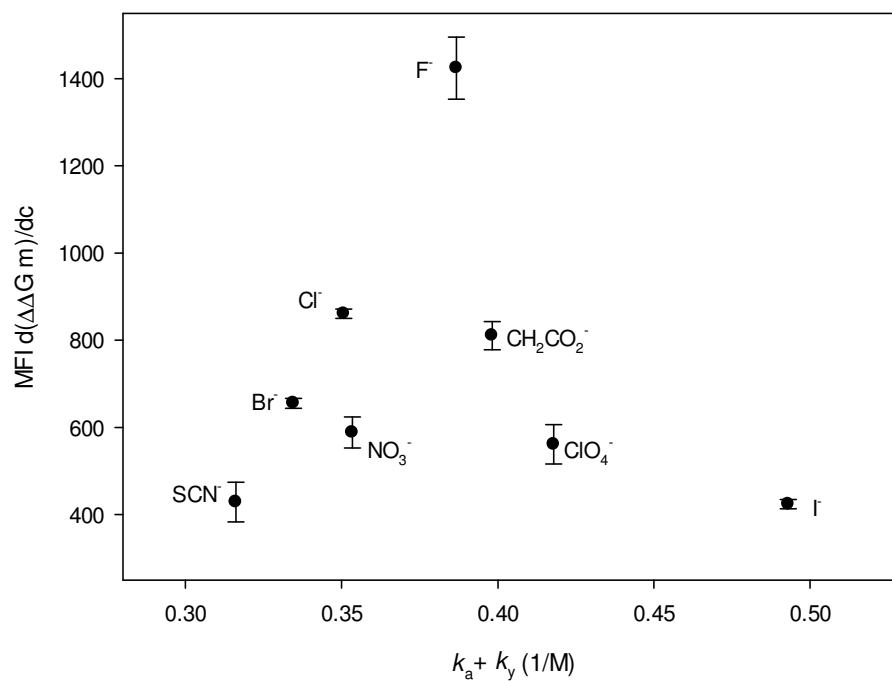


Figure 4-11. Anions specific effects on micellization compared to the cavity formation contribution towards the salting coefficient.

On the contrary, anions do not show any correlation between their MFI and scaled particle $k_\alpha + k_\gamma$ values as can be easily observed in Figure 4-10. These results demonstrate that anions specific effects on hydrophobicity are not due to ion-induced changes in the cavity formation energy. However, the analysis of available interfacial anionic data can be used to characterize anions specific effects observed in our system. As it was mentioned in the Introduction chapter, vibrational sum frequency spectroscopy has been proven to be very powerful technique to study the molecular level details of the interfaces. Measurements performed in a particular region of this vibrational spectrum 3200 cm^{-1} , dependent on the collection of a particular anion at the interface, were linearly related to the surface potential differences caused by anion adsorption into the interface (Figure 1-7 of Introduction chapter).¹² These measurements demonstrate that larger anions tend to partition favorably into the water-air interface and cause the reorientation of water molecules at the interface. The specificity of the anions partitioning into the interface appear to follow the Hofmeister series, indicating that adsorption to the interface plays an important role in anion specific effects. This observed anion partitioning can potentially have several reasons. First of all, according to the Collin's sticky idea, anions tend to lose their hydration very easily and adhere to hydrophobic surfaces.⁵ In addition to that, anions have a soft electron cloud which gives them the ability to modify their spatial charge distribution. This allows anions to expose a fraction of their volume to the interface, reducing the energy needed to dehydrate some of the anion, while still keeping most of its charge hydrated.¹³ Most cations are incapable of doing this and are thus forced to stay hydrated in the bulk solution as dehydration of their charge is extremely unfavourable.¹³

According to scaled particle theory, if the specific anion effects on hydrophobicity in our system are not caused predominantly by cavitation, then the major contributor must be due to interactions between the anions and the interface. To this extent, our experimental MFI values have been plotted against surface potential difference measurements for anions to see if anion adsorption and interactions at the interface is the major contributor towards the specific anions effects on micellization. As it can be clearly observed in Figure 4-11 there is a much better correlation following linearity obtained here than with any previous parameters. Thus this result shows that anions specific effects on micellization of 1, 2 –Hexanediol seem to be dominated by their interactions with the interface, along with their ability to unevenly distribute their charge.

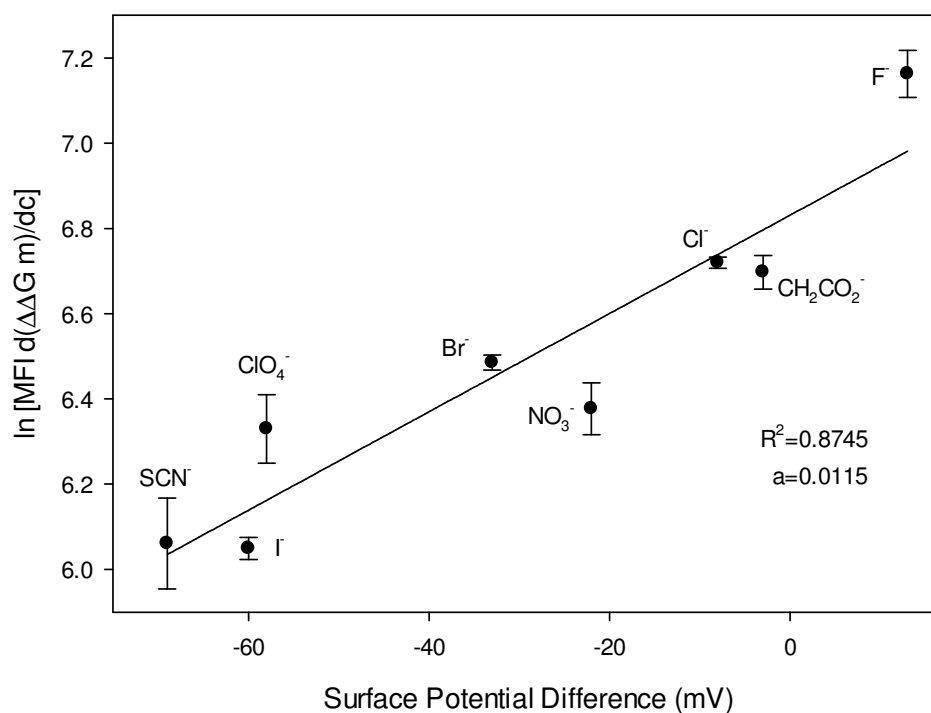


Figure 4-12. Anion specific effects on micellization compared to surface potential difference measurements. Surface potential difference values were taken from ref.8

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Chapter 5

Conclusion

The work presented in this thesis has investigated the specific ions effects on hydrophobicity, using micellization of 1, 2 – Hexanediol as a model system. We have used various optical probes (pyrene, ANS and BZA) to measure the thermodynamics of micelle formation, as represented by the critical micelle concentration. Pyrene fluorescence spectrums were collected to determine the CMC values of our surfactant for both sets of cationic and anionic salts (salts concentration range was from 0 to 3M) with the exception of cesium chloride and sodium iodide (fluorescence quenchers). In the case of cesium chloride, changes in the BZA absorbance were monitored to measure CMC values. As for the sodium iodide we have used another fluorescence probe ANS for the CMC determination. In regards to the CMC values, it was observed that all of the sodium and chloride salts decrease the concentration at which micelles start to form, with the lowest CMC value found at the highest salt concentration. It was concluded that examined salts encouraged the micellization of 1, 2 – Hexanediol with the exception of GdmCl and TEACl, whose CMC values are negative, indicating that these salts disrupt the micelles.

Based on the obtained CMC values we have calculated the micellization free energy increments (MFI), which demonstrate how specific salt affects the hydrophobicity of our system. In addition to the CMC measurements, we have determined the aggregation numbers of 1, 2 – Hexanediol in the presence of the anionic and cationic salts. We have used the fluorescence quenching method with 1-Dodecylpyridinium chloride as the quenching species. The obtained aggregation numbers allowed us to characterise the thermodynamics of micelle formation using the simplified analytical form to calculate Gibbs free energy of micellization.

We then analyzed our data in the light of discussed interfacial and bulk theories. In the case of cations, scaled particle theory provided a good explanation for the observed specific effects on hydrophobicity of our system: these specific cations effects result from how cation addition influences the cavity formation free energy and the number density of the aqueous solvent. Interestingly, the contribution of cavity formation to the salting coefficient did not seem to be the reason for the observed anions specific effects on hydrophobicity. Rather, it is the ability of the polarizable anions to interact with hydrophobic interfaces by distributing their charge unevenly around the atom that seems to be the major contributor towards these effects. Based on the work completed, we have shown that cations and anions have different effects on the hydrophobic interactions, which cannot be treated in the same way.

As it was mentioned in the Introduction chapter, proteins in solution are stabilized by various interactions, including Van der Waals interactions, electrostatic interactions, hydrophobic interactions, hydrogen bonding interactions etc. Thermodynamic measurements show that the most important factor in protein stability is the hydrophobic

effect. In our work we have demonstrated how hydrophobic effect will be affected by different ionic species. Therefore, we expect that the portion of protein stabilization energy that comes from the hydrophobic interactions in proteins to be affected by salts in the same way as our micelle system. And our results might be useful in the explanation of salt effects on the protein stability.

Understanding how salts affect protein solubility has important implications in the field of protein crystallography. Empirically it is known that different salts added in different concentrations to the solution of proteins can increase the supersaturation of that solution. However, some salts can have the opposite effect on the supersaturation and cause the proteins to aggregate together. We think that the effects of salts on hydrophobicity might have similar effects on supersaturation of proteins in solution. And perhaps, this is one of the reasons why NaCl is not a good salt for crystallography. This salt has a high effect on the hydrophobicity due to the electrostatic interactions and causes the proteins to aggregate together. On the other hand $(\text{NH}_4)_2\text{SO}_4$ has been shown to increase supersaturation of the protein solutions by screening the electrostatic interactions and preventing protein aggregation which will allow for the protein crystallization.

Another important phenomenon that can be highly affected by the presence of different ionic species is protein-protein aggregation or protein-DNA aggregation. It is generally observed that proteins tend to aggregate during the process of unfolding when the denaturant is being removed. And this aggregation occurs mainly through the exposed hydrophobic surfaces. It is been shown that currently this problem is being dealt with empirically by the addition of various salts. We think that our data on specific ion effects

on hydrophobic interactions can be helpful in the understanding of how different salts affect exposed hydrophobic residues within the unfolded protein to prevent protein-protein aggregation. Finally, as for the future directions, one can look at how thermodynamics of the protein folding are affected by our salt specific series. And then investigate if the effect of salts observed in our micellar system will be the same for the protein system.