

**Micellization Behaviour of Some Ionic Surfactants in DMSO-Water and
Glycerol-Water Mixed Solvent Systems and Influence of BSA**

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DECLARATION

I declare that thesis entitled "Micellization Behaviour of Some Ionic Surfactants in DMSO-Water and Glycerol-Water Mixed Solvent Systems and Influence of BSA" has been prepared by me under the guidance of Professor (Dr.) Ashish Kumar and Professor (Dr.) Ramesh C. Thakur, Faculty of Technology and Sciences, Lovely Professional University, Phagwara. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

We certify that Mr. Vivek Sharma has prepared his thesis entitled "Micellization Behaviour of Some Ionic Surfactants in DMSO-Water and Glycerol-Water Mixed Solvent Systems and Influence of BSA" for the award of Ph.D. degree of Lovely Professional University, Phagwara under our guidance. He has carried out the research work at the School of Chemical Engineering and Physical Sciences, Lovely Professional University.

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Dedicated to
My Beloved Parents,
My Lovely Wife
And
Little Daughter

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ABSTRACT

Use of surfactants in various industrial and household products and their interactions with proteins under the influence of different solvent environments has made our society dependent upon these applications in our day to day life. The main focus of this thesis is to understand the solution (self-assembly) behaviour of ionic surfactants in presence of different additives (co-solvents and protein) and to see the effect of ionic surfactants on the binding efficiency of protein. The applications of surfactants in various fields generally depend upon the solvent medium in which they are used, so the primary step was to study the micellization and thermodynamic behaviour of ionic surfactants (SDBS and CPC) in aqueous and binary aqueous mixtures of DMSO and glycerol. Thermodynamic stability of industrial products in different solvent environments is another major issue among the researchers of current era, so thermodynamic study of micellization of these industrially important ionic surfactants (SDBS and CPC) was also done in presence of two widely used co-solvents (DMSO and glycerol). The understanding of molecular interactions of proteins such as Bovine serum albumin (BSA) in micellar environments is very important because BSA serves as carrier of various drugs, hydrophobic ligands, and is collectively used with surfactants in different industrial processes. The structure and solution behaviour of both proteins and surfactants are influenced by presence of each other. Moreover, due to the heterogeneity of surfactant binding sites in protein molecule and denaturing effect of the surfactant, various attempts have been made to understand the interaction at molecular level in terms of the conceptual models, which take account of the observed solution behaviour of such systems. In recent years, although the investigations in this field have accelerated with the introduction of new experimental techniques, the classical techniques of physical chemistry still remain relevant.

Electrical conductivity method was employed to study thermodynamics of micellization and effect of different co-solvents (DMSO and glycerol) on the micellization behavior of these ionic surfactants (SDBS and CPC) because of its simplicity and accuracy. Moreover, this technique does not require any additional treatment or addition of foreign species to stabilize solution properties (i.e. pH and ionic strength) during measurement, which may introduce errors in the results. The

study was supported by determining parameters of micellization like critical micellar concentrations (CMC) and degree of counterion dissociation (α). The temperature dependence of CMC values was used to calculate other thermodynamic parameters of micellization i.e., standard free energy of micellization (ΔG_m^0), standard enthalpy of micellization (ΔH_m^0) and standard entropy of micellization (ΔS_m^0). Both the selected surfactants consist of aromatic head groups and this hydrophobicity (aromatic head group) in SDBS and CPC is the main reason for their low CMC (critical micellar concentration). CMC of these surfactants were studied in aqueous and binary aqueous mixtures of DMSO and glycerol at the temperature range of (298.15 to 308.15 K) using William's (classical method) and Carpena method (non-linear fit method). Thermodynamic parameters of micellization were also determined to study the effect of co-solvents on the thermodynamic of micellization of SDBS and CPC. Results of conductometric studies suggested that the micellization of ionic surfactants (SDBS and CPC) was delayed in presence of co-solvents (DMSO and glycerol). Thermodynamic parameters of micellization suggested that micellization was spontaneous and entropically driven process both in aqueous and binary aqueous mixtures of DMSO and glycerol.

The influence of Bovine Serum albumin (BSA) on the micellization characteristics was also studied in both aqueous and binary aqueous mixtures of ionic surfactants (SDBS and CPC) using conductometric technique. Micellization was delayed by the effect of all additives and temperature. BSA showed specific and strong binding with CPC (oppositely charged protein-surfactant system) than SDBS (similarly charged protein-surfactant system). Thermodynamic stability of both the systems decreased with increase in co-solvents in the medium. Hydrophobic and London dispersion forces were the main type of interactions found active in both BSA-CPC and BSA-SDBS systems.

The effect of surfactants on the binding efficiency of BSA was studied employing UV-Visible and Fluorescence Spectroscopic techniques. BSA showed strong binding affinity for oppositely charged surfactant CPC in aqueous medium. Effect of pH was also studied at 3 different pH conditions i.e. pH 4.0 (below isoelectric point), pH 5.4 (at isoelectric point) and pH 7.0 (above isoelectric point). Results reflected that, as the protein changes its charge and structural dynamics the binding preferences of BSA also changes and its binding affinity for oppositely charged

surfactant increases respectively. Fluorescence studies demonstrated the dynamic and spontaneous quenching of BSA in presence of both surfactants and co-solvents.

These experimental studies have been found quite significant in the progress of research in this area with special emphasis on the work of intermolecular interactions between protein and surfactant that have been identified both quantitatively and qualitatively using a variety of experimental and computational techniques. Thanks to major advances in experimental and computational approaches in recent years, some efforts in understanding the interactions and thermodynamics properties of these systems have been made using computational techniques (i.e. molecular docking and molecular dynamic studies) in order to enunciate the findings of experimental research.

Quantum chemical calculations and molecular dynamic (MD) simulations provide important information and represent a new insight about the study of micellization process, allowing us to identify the significant intermolecular characteristics such as aggregation phenomena and behaviour of the hydrophilic heads and the hydrophobic tails of surfactants in a certain environment such as organic solvents (DMSO and glycerol). This helps to establish a better molecular picture of the micellization process. Molecular docking technique is used as an attractive scaffold to understand the protein-surfactant interactions. Docking studies confirmed the presence of 8 binding sites of BSA for SDBS and CPC. It was found that the low energy binding sites of BSA were more preferred by ionic surfactants. In general, the entire experimental observations and computational studies were found to be reasonably consistent with each other. In future work, it will be interesting to study the micellization behaviour of these surfactants in presence of other agrochemical, pharmaceutical and biochemical products in presence of co-solvents for their enhanced applications in pharmaceutical, agrochemical and other industries.

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LIST OF ABBREVIATIONS

| | |
|-----------------------------------|---|
| SDBS | Sodium dodecyl benzene sulfonate |
| CPC | Cetyl pyridinium chloride |
| BSA | Bovine serum albumin |
| DMSO | Dimethyl sulfoxide |
| CMC | Critical micellar concentration |
| X_{CMC} | Mole fraction of CMC |
| M | Molar |
| T | Temperature |
| K | Kelvin |
| ΔG_{m}^0 | Standard Change in Free Energy of micellization |
| ΔH_{m}^0 | Standard Change in Enthalpy of micellization |
| ΔS_{m}^0 | Standard Change in Entropy of micellization |
| $\Delta G_{\text{trans}}^0$ | Standard Change in Free Energy of tail transfer |
| $\Delta G_{\text{binding}}^0$ | Standard Change in Free Energy of binding |
| $\Delta G_{\text{quenching}}^0$ | Standard Change in Free Energy of quenching |
| $\Delta_{\text{m}}C_{\text{p}}^0$ | Standard change in heat capacity of micellization |
| G | Gordon Parameter |
| E_{T} | Reichardt's Parameter |
| S_{P} | Solvophobic Parameter |
| V_{m} | Molar Volume |

Chapter-1

Introduction

Chapter 1

Introduction

Surfactants play a major role in our daily life with the variety of applications in biology, chemistry and pharmaceuticals industries ¹. They are also known as surface active agents. Surfactants are those compounds which lowers the surface tension between two liquids or between liquid and a solid. Surfactants may act as detergents, emulsifier, foaming agents and plays an important role in drug delivery ². In the bulk of a solution, the surfactants can assemble into aggregates called ‘micelles’ and this phenomenon is known as ‘micellization’. Micellization is due to diphillic nature of surfactant molecule having different spatial arrangement in different system ³. The concentration at which the surfactant begins to form micelles is known as critical micelle concentration (CMC), which can be correlated with many other properties referring to solution and thermodynamic behavior of the surfactant.

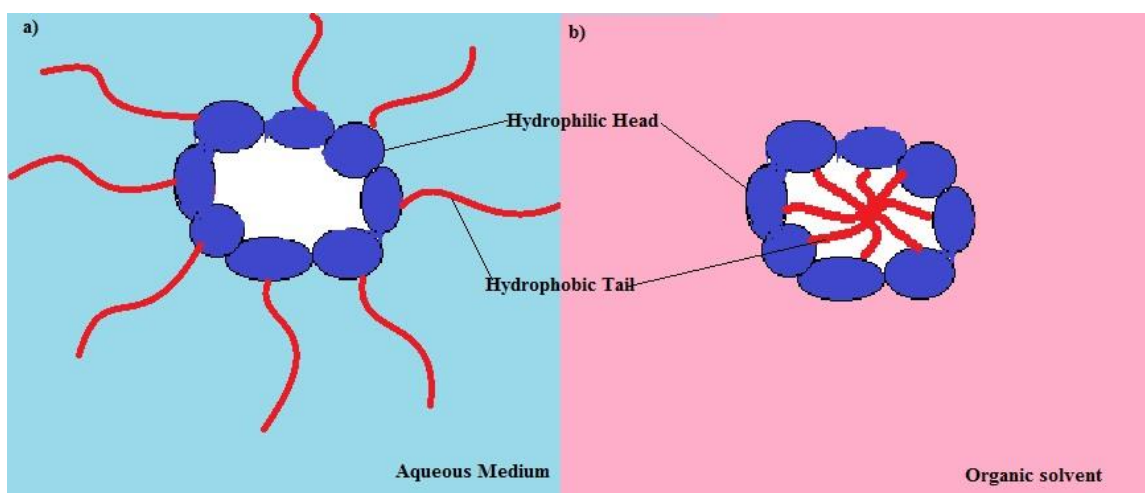


Figure 1.1: Representation of surfactant micelles (a) in aqueous solution (b) in organic solvent

A surfactant molecule is formed by two parts with different affinities for the solvents. One of them has affinity for water (polar solvents) and the other for oil (non-polar solvents)². When micelles are formed in water, their tails form a core there by reducing the unfavorable hydrocarbon – water contacts, and the polar head groups form an outer shell that maintains favorable contact with water (figure 1.1a). During micellization, the local ordering of water molecules i.e., hydrophobic hydration is disrupted and the entropy of the system is increased.

Thus, micellization is entropy driven process. On the other hand, in oil or nonpolar solvents, the head groups are at the core and tails maintain favorable contact with medium; this aggregate is referred to as a 'reverse micelle'. It has been observed that the CMC values for all type of surfactant ranges from 0.5 to 20 mmol/kg.⁴ In spite of hydrophilic and hydrophobic bonding of surfactant other factors such as temperature, pressure also affects the behavior of micellization. The physical and chemical properties of diphilic substance have been determined by Traube's law. The reactivity of diphilic substance will be triple fold by introducing the CH₂ group. As the hydrocarbon chain increases the large amount of energy is gained by the micellization and hence the value of CMC decreases as the length of hydrocarbon increases. The diphilic nature of surfactant molecules is responsible for its electrolytic behavior at low concentration and micellar behavior at high concentrations.

Classification of Surfactants:

Surfactants can be classified based on charged groups present in their head. A non-ionic surfactant does not have any charge groups over its head². If surfactant head carries some net charge on their head and this net charge is negative, it is known as anionic surfactant whereas if this charge is positive then it is known as cationic surfactant. A surfactant containing both oppositely charged groups on its head is known as zwitter-ionic surfactant. The ionic surfactants selected for the present work are Sodium dodecyl benzene sulfonic acid (SDBS) an anionic surfactant and a cationic surfactant, Cetyl pyridinium chloride (CPC). The solution properties of these surfactants have not been critically examined although some reports are available in the literature⁵⁻⁷.

Sodium dodecyl benzene sulfonate

This negatively charged surfactant is widely used in industry as well as in household (Cleaning and hygiene) because of their highly potent detergency and low cost of manufacture^{8,9}. Sodium dodecyl benzene sulfonate is a important surfactant used in tertiary oil recovery process¹⁰. SDBS when dissolved in the aqueous medium at low concentration always show specific or co-operative micellization and at high concentration of surfactant in aqueous medium always causes the nonspecific or non-cooperative micellization. CMC of

SDBS is 1.3 mM.Kg^{-1} ¹¹. It is able to interact with neutral and cationic polymers and also show antifungal properties⁵. It is also known as a good corrosion inhibitor¹².

Cetyl pyridinium chloride (CPC)

This cationic surfactant besides its surface activity show some other advantageous properties like antibacterial activity and is also used as cationic softeners, lubricants, retarding agents, antistatic agents and in some cases in hygiene and cleansing products . It is a common component of mouthwash liquids and dental medicines¹³. CPC is very popular surfactant in agriculture as adjuvant in spray solutions because of its low CMC ($0.96 \text{ mmol.kg}^{-1}$)^{7,14}.

Co-solvency and self-assembly of surfactants:

Solvents play a decisive role in controlling the adsorption and micellization characteristics of surfactants. Mixing of solvents changes solvent properties, particularly polarity, and carrying out adsorption and aggregation studies of surfactants in mixed solvents provides knowledge of fundamental and practical importance. The aggregation behavior of different types of surfactants, i.e. cationic, anionic, non-ionic, zwitterionic and gemini surfactants are affected by properties of solvent such as the fluidity, polarity, hydrogen-bonded structure, hydrophobicity and lipophilicity, which can be varied/controlled in mixed solvents with suitable combinations and compositions^{15,16}. Application of surfactants in various industries and household processes generally depend on their self-aggregating property which largely depends on the solvent medium in which they are used. Hence different additives or co-solvents are added in surfactant formulations to improve their surface properties¹⁷⁻²⁷.

Formation of micelles largely depends upon the hydrophobicity of surfactant as well solvent medium²⁸⁻³⁷. So, it is important to study the role of solvent media to understand the process of micellization³⁸⁻⁴⁰. Previous studies on co-solvent effect on micellization process have been reported in terms of intermolecular interactions between water and co-solvent⁴¹⁻⁴⁴ and results of such studies have been interpreted in terms of water “structure making” or “structure breaking” ability of co-solvents⁴⁵. Micellization behavior of a surfactant varies with respect to temperature and is also influenced by additives^{7,46}. Shape and size of a micelle is purely dependent on the surrounding environment. The interfacial and micelle properties of

surfactant solutions are governed by a delicate balance of solvophobic and solvophilic interactions⁴⁷. The results of some studies on the effect of cosolvent on micellar properties of surfactants are mainly due to changes in water structure. If the CMC of surfactant increases in presence of a cosolvent then it shows decrease in the strength of hydrophobic interactions which is considered as water structure breaker. Similarly decrease in CMC shows increase in hydrophobic interactions and is considered as water structure maker⁴⁸.

Co-solvents are classified into two categories: a) Penetrating and b) Non-penetrating.

Penetrating co-solvents fills the vacancies in water structure and in result ordering of water structure and lowers the energy and iceberg entropy of the system. It generally lowers the CMC of ionic surfactant if present in the system. Non-penetrating co-solvents increase the CMC by lowering the dielectric constant of water and breaking of micelles.^{47,49-51}

The study of interfacial and thermodynamic properties of surfactants in solution and effect of co-solvents can provide extensive information about solute-solute and solute-solvent interactions of the surfactant in solution. Keeping this view and to study kosmotropic (structure maker) and chaotropic (structure breaker) effect of co-solvents on micellization of ionic surfactants and their interactions, two industrially important solvents DMSO and Glycerol were selected.

Dimethyl sulfoxide

DMSO an oxidized product of Dimethyl sulfide and is widely used as commercial solvent. It is polar aprotic solvent and has low toxicity. Due to its high penetrating power it is used as a cryo-protectant and a vehicle for transport of various chemicals through skin⁵². It is also used to extract oxidized amino acids from unoxidized peptide chains. The oxidative property of DMSO is responsible for the changes in amino acid structure and amino acid oxidation is frequently observed in protein hydrolysis⁵³. DMSO is a Non-penetrating cosolvent (i.e. Unable to penetrate surfactant micelle) and it increases the CMC of ionic surfactants⁵⁴⁻⁵⁷. DMSO is a chaotropic cosolvent and destabilizes structure of water as well as surfactant micelles.

Glycerol

Glycerol is a biologically important polar organic solvent. It occurs in intestine as a product of hydrolysis of lipids and also plays an important role in metabolism of glucose in liver ⁵⁸. Glycerol and surfactants are collectively used in many pharmaceutical and cosmetic products ⁵⁹. Due to low toxicity of glycerol it is also preferred in food industry ⁶⁰. Glycerol is also used in production of a variety of chemicals, polymers and fuels. It also plays an important role in everyday life as solvent, antifreeze, as detergent additive, monomer for textiles, as drug delivery vehicle and is an important constituent of fine-chemicals industry ⁶¹. Glycerol is a kosmotropic cosolvent which stabilizes the water structure and increases the solubility of polar solutes ⁶².

Presence of glycerol causes the reduction of the cohesive energy density of the solvent system and results in increased solubility of surfactant due to which its CMC is also increased.

Influence of Proteins

Protein –surfactant interactions have wide range of applications in formulation of detergents, food emulsion, pharmaceuticals, cosmetic products etc.⁶³. As known, 20% of the human body is made up of proteins which play a decisive role in almost every biological process ⁶⁴. The understanding of these interactions at molecular level is very complicated since structure of proteins is very complex and it is very difficult to generalize the consequences of protein behavior in different conditions. Folding of proteins is a physical process and there are many conditions due to which proteins undergo folding process (i.e. High concentrations of solute, extremes of pH, mechanical forces, and the presence of chemical denaturants can do the same). Folding of proteins is a major concern for all researchers of current era because most of the proteins aggregate and give rise to diseases like Alzheimer and Parkinson's. In order to cure these diseases scientists are developing such kind of medicines or systems which are helpful in unfolding of aggregated proteins. Anson ⁶⁵ recognized that proteins were denatured by synthetic surfactants. Many reviews regarding thermodynamic stability and folding-unfolding of proteins have been published in recent past⁶⁶⁻⁶⁸. Instead of extensive study on protein structure many contradictory views exist regarding forces responsible for folding as well as stability of proteins ^{69,70}. So the concept of protein denaturation is not yet completely

understood and remains a subject of extensive study. Thus in order to seek more knowledge about solution behavior of proteins we have selected Bovine serum albumin (BSA) for this study.

Bovine Serum Albumin

It is a serum albumin protein derived from cows. Serum albumins (SAs) are the most abundant plasma proteins in mammals, responsible for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. Bovine serum albumin (BSA) is structurally homologous to HSA and consists of 607 amino acid residues⁷¹. Serum albumins have the interesting properties of binding a variety of hydrophobic ligands such as fatty acids, lysolecithin, bilirubin, warfarin, tryptophan, steroids, anesthetics and several dyes. It is used as a model protein in various biophysical and biochemical studies⁷². Serum albumins serve as carrier of various drugs and other biologically important compounds so it is important to see the effect of surfactants and solvents on the rheology and binding efficiency of proteins^{73,74}. The other reason of studying the properties of surfactants in BSA is because the surfactants inhibit protein aggregation which is essential for long-term storage of pharmaceutical products. Surfactant-induced unfolding/denaturation of proteins improve the surface properties and promote the enzymatic degradation in laundry detergents. In the food industry surfactants act as emulsifying, foaming, antioxidant agents, stabilizers and anti-adhesives⁷⁵⁻⁷⁷.

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Chapter-2

Literature Review

Chapter 2

Literature Review

The study of micellization behaviour of surfactants is important in many processes of industry from mineral processing to formulation of personal care products and foods, to drug delivery systems and to new surfactant remediation technologies. In all these processes, surfactant must usually be present at a concentration higher than the CMC because the greatest effect of the surfactant, whether in interfacial tension lowering¹, emulsification, suspension stabilization, as a delivery vehicle, or in promoting foam stability,² is achieved when a significant concentration of micelles are present. The study of thermodynamic properties of micellization of ionic surfactants like changes in enthalpy and free energy in aqueous and aqueous rich mixtures of organic solvents and influence of protein can provide valuable clues in relation to solution behaviour of surfactants and protein unfolding³.

R. Nagarajan studied the effect of surfactant on polymer in aqueous solution. He also derived thermodynamic parameters of micellization and concluded that the extent of surfactant binding increase with increase in hydrophobicity of polymer and is maximum for a polymer which consists of equal number of hydrophobic and hydrophilic segments⁴.

Rio et al. studied the effect of temperature and alkyl chain length on the micellar properties of surfactant (i.e. cationic surfactants) using conductometric technique. They also derived thermodynamic parameters of micellization and interpreted results according to the same⁵.

Olofsson and Wang studied the micellization behaviour of ionic surfactants in aqueous solutions of neutral polymers using micro calorimetric technique. They concluded the paper suggesting that there is no significant difference in binding of cationic and anionic surfactants with neutral polymers. Micellization of surfactant molecules in uncharged polymers is similar to the process of solubilization of uncharged micro molecules in ionic micelles⁶.

Lee and Huang studied thermodynamics of micellization of SDS in binary aqueous mixtures of ethylene glycol, methanol and glycerol. They observed that the CMC of SDS is increased in presence of organic solvents. As hydrophobicity is lowered on addition of co-solvents due to which enthalpy and entropy of the system is reduced⁷.

Torbjorn Warnheim reviewed the aggregation behaviour of surfactants in non-aqueous polar solvents. He focused on the effect of solvents and co-solvents on the micellar behaviour of surfactants by observing CMC values and reported nature of aggregation i.e. cooperative and non-cooperative ⁸.

D. Zanette *et al.* studied the interaction of PEO and BSA with anionic surfactants (SDS, LDS, CDS and TMADS) using conductometric technique to evaluate the nature of binding and effect of counterion hydrophobicity on the same. They have also calculated the mean aggregation numbers in support of their findings and result shows the nature of binding doesn't solely depend upon the nature of counterion present in the system ⁹.

The Review by M. N. Jones is a milestone for the researchers of current era because it describes all the techniques and progress of 20th century in the field of protein - surfactant interactions. Dr. Jones suggested researchers about the parameters to be studied and what we have to see while studying protein - surfactant interactions. He also made a comparison between other denaturants like urea and guanidinium chloride with surfactants on the basis of concentration required for denaturation of proteins. He suggested that protein surfactant complexes attain stability when free surfactant molecules in the system reach their respective CMC and main point to be considered is protein surfactant complexes are more stable than the micelles formed in the system ¹⁰.

Chen and Dickinson studied interaction of Gelatin and SLES in presence of β -lactoglobulin through flocculation and emulsification experiments. They found that oil soluble surfactants are beneficial for bridging flocculation of β -lactoglobulin stabilized emulsion made after mixing with gelatin and cross linking mechanism containing surfactant micelles should be preferred over neutralization flocculation mechanism for these types of systems ^{11,12}.

Turro and Lei studied spectroscopic aspects of protein surfactant interactions. They studied BSA-SDS system using fluorescence, NMR and ESR spectroscopic techniques. They supported the formation of necklace bead model during denaturation process of BSA by SDS micelles ¹³

D.E. Otzen *et al.* compared the unfolding of endoglucanase Cel45 in surfactant and denaturant using kinetics as a mode of study. They interpret the results in terms of simple hydrophobic-electrostatic interactions. They concluded that different detergent-mediated

unfolding pathways exist and ligands that interact strongly with the denatured state may therefore alter the unfolding process ¹⁴.

Capalbi *et al.* studied electrical conductivity and surface tension of following poly (vinyl-pyrrolidone) and sodium caprylate, or poly (vinyl-pyrrolidone) and tetraethyl ammonium perfluorooctanesulfonate. They studied the effect of short chain surfactants and the findings suggested that polymer surfactant interactions are affected by a combination of hydrophobic and electrostatic forces. Both CAC and CMC values are sensitive to temperature, ionic strength and polymer content in the solvent medium. The mass action model suggests that the width of the interaction region is controlled by the affinity between surfactant and polymer¹⁵.

Shashank Deep and Jagdish C. Ahluwalia investigated the effect of anionic surfactants on rheology and stability of BSA. They used differential scanning calorimetry (DSC), circular dichroism (CD), Fluorescence and UV spectroscopic methods to study these interactions. The results indicate that SDS shows cooperative binding with BSA at low concentration and stabilizes the structure of BSA but at higher concentration of SDS it denatures BSA showing non-specific binding ¹⁶.

Otzen studied the effect of micelle structure, ionic strength, pH, and temperature on protein unfolding by detergents and found that the unfolding of proteins by surfactants is a strongly pH dependent process. Moreover formation of cylindrical micelles in anionic surfactant (SDS) increases unfolding rate as compared to cationic surfactants because of formation of dead end complexes with cationic surfactants ¹⁷.

Bakshi and Sachar published the interaction between oppositely charged polymer surfactant interaction by conductivity and turbidity experiments. They studied the dependence of aggregation on chain length and head group of surfactants. They observed that the cationic surfactants bind cooperatively with anionic polyelectrolytes. They compared the micellar behaviour of conventional and dimeric cationic surfactants and observed that they show less interaction with anionic polyelectrolytes ¹⁸.

P. Somasundaran *et al.* emphasized on the interaction of Zein protein and SDS. The fluorescence study shows that the micelles of SDS –Zein complex is more hydrophobic than the SDS micelles .Unfolding of Zein protein occurs in two stages. In first stage (near SDS CMC) Zein protein forms small micro-domains and in second stage (above CMC) complete unfolding takes place ¹⁹.

Sreelekha K. Singh *et al.* searched thermo-acoustic behaviour of ionic surfactants in solutions of glycine, alanine, valine, leucine, and lysine at 298.15 K. They derived partial molar volumes, apparent molar volumes and hydration numbers for these systems and concluded that with increase in concentration of solute lowers the hydration number which in turn increase solute solvent interactions and reduce electrostriction (electrostatic forces of interaction)²⁰.

Harutyunyan *et al.* studied the effect of diethyl sulfoxide on the micellar properties of SDS and compared the results with effect of dimethyl sulfoxide. The results reveal that DESO inhibits micellization of SDS and has better inhibition than DMSO²¹.

Errico *et al.* observed the effect of Glycerol on CTAB and Briz-58 micellization and reported the increase in CMC of both the surfactants at high glycerol concentrations. They suggested that lowering of dielectric constant of medium by glycerol is responsible for the increased electrostatic interactions²².

R. Lopez-Esparza *et al.* measured diffusion coefficient of PEG and two Surfactants. The Result of diffusion experiments shows that polymer and surfactants form complexes in aqueous solution. PEG and C₁₄DMAO show hydrophobic type of interactions. The polar heads of polymer and surfactant bind and result in interaction of PEG-C₁₂E₅²³.

M.S. Chauhan *et al.* studied thermodynamics of Gelatin –SDS system in presence of DMSO as a co-solvent. The effect of DMSO on water structure is very prominent and due to hydrophobic and hydrogen bonding this effect is enhanced with increase in DMSO concentration. Moreover results show that CMC of SDS increase on increase in Concentration of DMSO²⁴.

Ruiz *et al.* studied the effect of glycerol on micellar behaviour of TTAB at different temperatures and reported that the micellization of the surfactant is less favorable as the presence of the co-solvent increases in the solvent system²⁵.

L. R. Harutyunyan and S. A. Markarian investigated the effect of DMSO and DESO on the micellar properties of SDS and found that the CMC of SDS increases on addition of DMSO and DESO. They emphasized on the effect of cosolvent concentration on water structure as well as CMC of SDS which shows importance of polarization effect²⁶.

Rodriguez *et al.* investigated the effects of polar organic solvents on micellization of different surfactants and reported that organic solvents increases solubility of surfactant

molecules due to which the hydrophobic tail transfer from the bulk phase into the micelles less favorable and, as a consequence, ΔG^0_m increases (becomes less negative), making the aggregation process less spontaneous²⁷.

Johnson *et al.* compared the effect of co-solvents DMSO and Glycerol on water structure and dynamics in presence of peptide and emphasized on the solution properties of peptide in pure water and in presence of co-solvents. Results reveal the stabilizing effect of glycerol and denaturing effect of DMSO on Peptide²⁸.

Andersen *et al.* made a global account of myoglobin - surfactant interaction. They suggested that enthalpy of interaction is exothermic and is indicator of the weak hydrophobic nature of these interactions between myoglobin – SDS. Enthalpy of interaction increases with an increase in protein concentration. Graphs of these interactions represent the non-stoichiometric binding and are signs of enthalpic effect. SDS concentration in the system is responsible for unfolding rates of protein²⁹.

Nielsen *et al.* studied the effect of SDS on β - Sheet proteins .They investigated that β - Sheet proteins are not denatured by SDS. On the basis of mutagenic analysis they suggested that SDS induces α - helix structure in proteins and participate in denaturation process and Unfolding of proteins take place through global approach rather than local unfolding³⁰.

R.G. Shrestha *et al.* represented formation of wormlike viscoelastic micelles in aqueous mixtures of mixed amino acid based nonionic and anionic micelles. They studied Kraft point variation of LAD micelles in NaOH and Lysine. They concluded that Lysine is a better neutralizing agent than NaOH. They studied the effect of temperature on worm like micelles and they tried to find a new way to control stability and rheology of these micro-structures^{31,32}.

M. Y. Khan *et al.* used conductivity and surface tension methods to study the interaction between water soluble polymers and anionic surfactants (SDS and SDBS). Effect of alkali NaOH is also seen in these interactions. In the presence of NaOH surface tension of system first decreases and then increases with increase in concentration of NaOH. The presence of NaOH increases bonding capacity of Polymer - surfactant system and in turn reduces surface tension of the system. Both conductivity and surface tension measurements were in complete agreement with each other³³.

D. E. Otzen *et al.* combined results of spectroscopy, chromatography, calorimetry and small angle X-ray scattering (SAXS) techniques to summarize the denaturation process of α -helical bovine acyl-coenzyme-A-binding protein (ACBP) by SDS micelles³⁴.

Bhadane and Patil investigated the micellization behaviour of non-ionic surfactant in aqueous mixtures of Alanine and Phenylalanine. Thermodynamic parameters of micellization of Brij-58 has been derived using cloud point technique and the results of phase separation of clouding process show the possibility of interaction between other macromolecules and surfactants³⁵.

M. Ruiz-Pena *et al.* investigated the interaction between BSA and non-ionic surfactants (Tween 20 and Tween 80) and the results of fluorescence indicate the effect of surfactant on the tryptophan environment which is a result of modifications in intensity and wavelength of tryptophan. Computational analysis of these systems also helped the authors to explain the results of surface tension and fluorescence measurements. The results reflected clear interaction between surfactant molecules and BSA caused by fluorescence quenching of tryptophan residues³⁶.

A. Ali and N. H. Ansari measured electrical conductivity of SDS in aqueous mixtures of amino acids and peptides. Data shows that CMC of SDS is directly proportional to temperature, whereas change in free energy of the hydrophobic interactions is inversely proportional to temperature and it also depends upon availability of Na⁺ ions. In presence of amino acids and peptides, change in free energy of micellization increased with increase in temperature. The trend of thermodynamic parameters is due to the solubilization of additives in surfactant micelles³⁷.

T. Chakraborty *et al.* studied various methods to calculate CMC of amphiphilic systems. In this paper they emphasized mainly on structure of micelles and surface tension, conductivity and fluorescence techniques for determination of CMC³⁸.

Daniel Otzen and Sven Frokjaer reviewed protein surfactant interaction and gave an overview of history, present and future of protein – surfactant interactions. He emphasized on the role of surfactants and other additives in stability and delivery of therapeutic proteins^{39,40}.

Harutyunyan and Markarian observed the micellization and viscometric properties of cetyl pyridinium bromide in presence of DMSO and DESO as co-solvents and reported that both

the sulfoxides behave as solvent structure breakers, micellization process is less favored on addition of cosolvent in the medium and is enthalpy driven ⁴¹.

M. S. Chauhan *et al.* studied thermodynamics of micellization of SDS in aqueous mixtures of Glycine, Alanine, Valine and Leucine. They used Conductometric method to determine CMC of SDS. They determined the effect of temperature on CMC of SDS. They obtained qualitative data and observed the presence of intermolecular hydrophobic interactions between SDS and amino acid. They also used Lumry–Rajender enthalpy – entropy compensation model to produce compensation temperature (T_c) and the results indicate that it lies between 270 -300 K ⁴².

S. Chauhan and K. Sharma compared the micellization behaviour of SDBS and DTAB in aqueous mixtures of glutamine, histidine and methionine at different temperatures. They also reported entropy- enthalpy compensation effect. Hence CMC is inversely proportional to temperature. Negative values of free energy of micellization indicate the feasibility of the system which increases with an increase in temperature. CMC depends upon the magnitude of hydrophobic and hydrophilic dehydration ⁴³.

A. Chandra *et al.* tried to study acoustic and friccohesity of glycine, L-alanine and L-phenylalanine with aqueous Methyl trioctyl ammonium and Cetyl pyridinium chloride at different temperatures. They calculated apparent molar volume, isentropic compressibility and apparent molar isentropic compressibility from density, viscosity and sound velocity measurements. The variations of data show the presence of strong solute solvent interactions⁴⁴.

Chauhan *et al.* studied the effect of glycine on micellar behaviour of CTAB by means of volumetric and conductance studies. The results show the presence of electrostatic interactions at lower surfactant concentrations and hydrophobic interactions at higher surfactant concentration. CMC of CTAB is temperature dependent and is directly proportional to temperature. CMC Lowers with increase in glycine concentration ⁴⁵.

S. Chauhan *et al.* researched on the effect of organic solvents on micellar behaviour of SDS in presence of ribose, glucose, sucrose and raffinose at different temperatures. They calculated X_{CMC} using conductance measurements and used the values of X_{CMC} for further calculation of thermodynamic parameters of micellization. They confirmed that in presence of organic solvents the binding between sugars and surfactants become specific/non

cooperative. Free energy of system is negative and shows the spontaneity of the system. Presence of enthalpy -entropy compensation is also confirmed by the results of this research⁴⁶.

A. Ali *et al.* studied surfactant-surfactant interactions i.e. Cetrimide –SDS interactions using conductance measurements. Authors first calculated individual CMC for both the surfactants and then they measured CMC for the mixtures. Thermodynamic parameters of micellization show the interactions are favorable and exothermic in nature. Enthalpy-entropy compensation model suggests the destruction of the structured water surrounding the hydrophobic groups of the surfactants when these groups are transferred from the bulk into the interior of the micelle⁴⁷.

A latest review by Nisar Ahmed Malik about amino acid-surfactant and surfactant-surfactant interactions gave an overview of the research area. He emphasized on the solution thermodynamics and solute solvent interactions. As per his review thermodynamic stability of proteins is a major concern and the question is still unanswered. He concluded the paper by telling us about the uses and roles of protein - surfactant/surfactant- surfactant interactions in various industries⁴⁸.

Chauhan *et al.* studied the effect of cosolvent N-Methylacetamide on thermodynamic and micellization behaviour of SDS and CTAB using conductometric technique. The comparison shows that NMA favors micellization of SDS whereas it inhibits micellization of CTAB⁴⁹.

Research Gap

A detailed description presented above highlights the extensive work on effect of solvent medium on the micellization behaviour of ionic surfactants and protein – surfactant systems. These earlier studies have been found quite significant in the progress of research in this area with special emphasis on the work of intermolecular interactions between protein and surfactant that have been identified both quantitatively and qualitatively using a variety of experimental techniques. However, it is found that the contribution due to the classical techniques, such as conductance, UV-Visible spectroscopy and steady state fluorescence technique in the development of understanding the mode of surfactant binding to protein is lacking, although there are abundant evidence available in literature that reflect the vital use of these experimental techniques in the understanding of solution behaviour of electrolytes, polymers, surfactants, proteins etc. Solution behaviour of industrially important ionic surfactants and comparison of their thermodynamic and micellization properties in mixed solvent systems is a less studied topic. Interaction of proteins (especially transport proteins i.e. BSA, HSA etc.) with ionic surfactants and effect of co-solvents (i.e. DMSO and Glycerol) is important from industrial point of view (primarily in terms of drug delivery). The molecular interactions between proteins and ionic surfactants are of two types:

1. Effect of proteins on the micellization and thermodynamics of micellization of ionic surfactants.
2. Effect of ionic surfactants on the binding behaviour/efficiency of proteins.

The simultaneous study covering both types of interactions under influence of different solvent environments is least studied but important topic. Majority of studies of protein surfactant interactions has been carried out in aqueous medium at a single pH and effect of pH on the binding behaviour of proteins (in terms of isoelectric point) was very less studied in the past. Theoretical studies on the solution thermodynamics of ionic surfactants and their interactions with proteins was also lacking in the literature.

Aim and Objectives of the Present Work

Guided by these observations, the main objective of the present work is to gain qualitative understanding of surfactant micellization in different solvent environments because surfactants used in different solvent systems have a wide range of applications. The information drawn from the studies of thermodynamic properties of micellization of ionic surfactants have been related to structural aspect of the solvent in terms of solute – solvent interactions and structure making/breaking capacity of various co-solvents.

Studies of mixed aqueous organic systems highlight the importance of both solvent – solvent interactions and also the interaction between surfactant (solute) and solvent molecules (in terms H – bonding and hydrophobic interactions). Therefore the main objective of the present study is to investigate the thermodynamic properties of micellization of ionic surfactants in water and binary aqueous mixtures of DMSO and Glycerol.

Protein – surfactant interactions is a another highlight of the present investigations so, we organized the present studies by selecting two oppositely charged surfactants, namely SDBS, a negatively charged surfactant and CPC, a positively charged surfactant, and a globular water soluble negatively charged protein, BSA. Thus the interaction behaviour is investigated for SDBS – BSA (a similarly charged protein – surfactant system) and CPC – BSA (a oppositely charged protein – surfactant system). This allows us to make a comparative study of protein – surfactant interactions, and enable us to evaluate the relative contribution of electrostatic and hydrophobic interactions between surfactant and protein molecules.

Another interest in this work is to study the effect of co-solvents on protein – surfactant interactions, because the role of the solvent medium is also important in such studies. To meet this effect, we extend our studies to the aqueous – rich mixtures of DMSO and Glycerol because the structural consequences of intermolecular interactions for these solvent components in these regions are well established in literature.

The main objectives of the present investigations are:

- 1) Study of micellization behaviour of ionic surfactants (SDBS and CPC) in aqueous solution at different temperatures.
- 2) Study of co-solvent (Glycerol and DMSO) effects on micellization of SDBS and CPC at different temperatures.
- 3) Influence of Bovine serum albumin on the aggregation behaviour of ionic surfactants in both aqueous and aqueous rich mixtures of DMSO and Glycerol at different temperatures.
- 4) Determination of different thermodynamic parameters of micellization i.e. ΔH_m^0 , ΔS_m^0 and ΔG_m^0 .
- 5) Confirmation of binding between BSA and Ionic surfactants (SDBS and CPC) in both aqueous and binary aqueous mixtures of Glycerol and DMSO using spectroscopic techniques at different pH.

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Chapter-3

Experimental Section

Chapter 3

Experimental Section

The experimental part of this dissertation has been discussed in detail under the following headings:

Materials

Water:

Ordinary tap water of conductivity in the range $3 - 5 \times 10^{-6} \text{ S cm}^{-1}$ was distilled thrice in the presence of alkaline KMnO_4 . Triple distilled water of conductivity range is $0-2 \times 10^{-7} \text{ S cm}^{-1}$ and pH 6.8-7.0 was used in all the experiments.

Sodium dodecyl benzene sulfonate (SDBS):

SDBS obtained from Sigma Aldrich was recrystallized several times with ethanol (99.9% pure) to get a pure sample of SDBS as suggested in literature¹. 10 g of SDBS was dissolved in about 100 ml A.R. grade ethanol. The suspension formed was filtered and then heated on water bath, in order to reduce the volume of the solution by one fourth of total volume. The solution was left to cool at room temperature for about 2 h. Appearance of white colored needles of pure surfactant was observed soon after the liquid attained the room temperature. The solution was decanted and the crystals were allowed to dry at room temperature. The sample was however, re-crystallized twice from ethanol and finally dried in vacuum oven in the presence of P_2O_5 for 24 h at $\sim 60^\circ\text{C}$. The commercially available samples of SDBS may contain different positional isomers (i.e., $2\phi\text{C}_{12}$, $3\phi\text{C}_{12}$, $4\phi\text{C}_{12}$, $5\phi\text{C}_{12}$ and $6\phi\text{C}_{12}$) and it is very difficult to separate and identify these isomers from their mixture². Micellization properties of SDBS isomers are different from each other and are reported in table 3.1³⁻⁷. The SDBS obtained was of molecular formula $\text{C}_{12}\text{H}_{25}\phi\text{SO}_3^- \text{Na}^+$ and its isomeric composition was not provided by the company. Chemical structure of SDBS has been represented in figure 3.1. We checked the Purity of SDBS by HPLC and found one major component with an indication of one minor component. HPLC of recrystallized SDBS has been reported in Figure 3.2. The value of CMC obtained for SDBS at 298.15 K was $1.29 \times 10^{-3} \text{ mol kg}^{-1}$ which was approximately equal to the value of $3\phi\text{C}_{12}$ isomer as reported in

literature², thus we consider the SDBS studied in this research was essentially 3 ϕ C₁₂ (major component found in HPLC analysis).

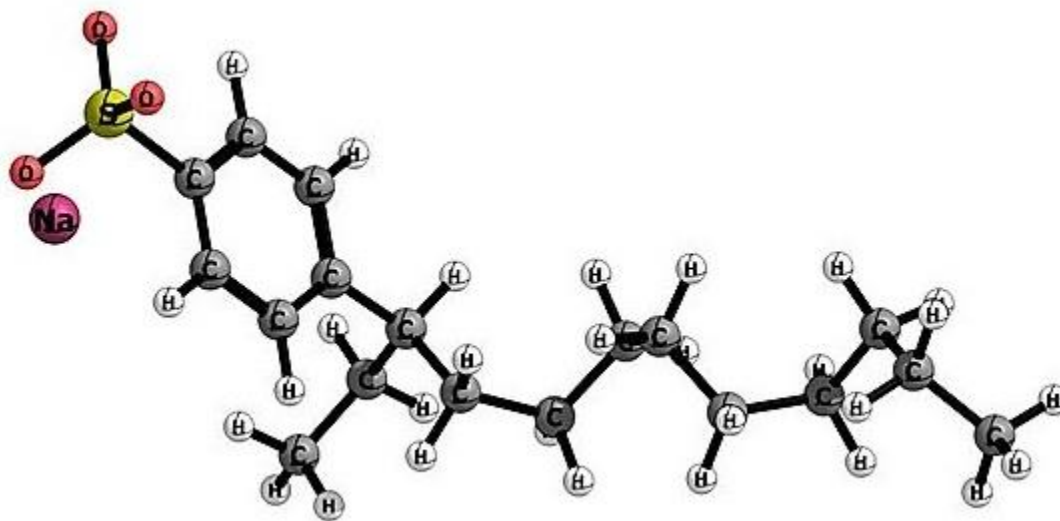


Figure 3.1: Chemical structure of Sodium dodecyl benzene sulfonate (SDBS)

Table 3.1: Reported CMC values for isomeric SDBS

| Isomeric Forms SDBS | T/K | 10 ³ (mol. Kg ⁻¹) CMC | References |
|--------------------------|--------|--|------------|
| 2 ϕ C ₁₂ | 298.15 | 0.10 | 3 |
| 3 ϕ C ₁₂ | 298.15 | 1.30, 1.50 | 3,4 |
| 3 ϕ C ₁₂ | 298.15 | 1.29 | 3 |
| 4 ϕ C ₁₂ | 298.15 | 1.40, 1.70 | 3,4 |
| 5 ϕ C ₁₂ | 298.15 | 2.20, 2.40 | 3,4 |
| 6 ϕ C ₁₂ | 298.15 | 2.25, 2.40, 2.78 | 6 |

Area % Report

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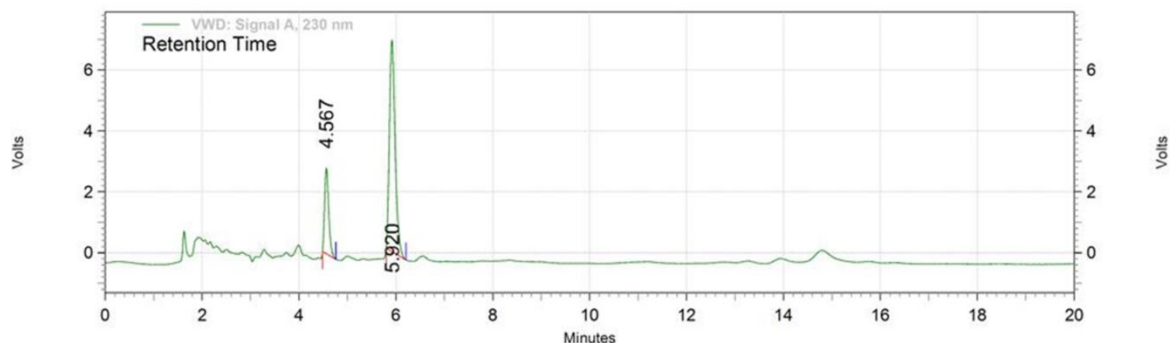


Figure 3.2: HPLC monograph of Sodium dodecyl benzene sulfonate (SDBS)

Cetyl pyridinium chloride (CPC):

CPC (confirming to USP) of 99% purity was obtained from LOBA Chemie Pvt. Ltd. and was used without further purification. Chemical structure of CPC has been represented in figure 3.3.

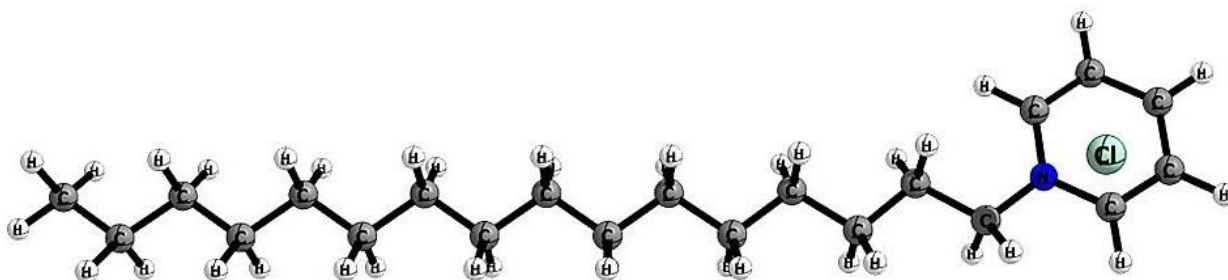


Figure 3.3: Chemical structure of Cetyl pyridinium chloride (CPC)

Dimethyl Sulfoxide (DMSO):

Dimethyl Sulfoxide of 99% purity was supplied by Finar Ltd. and was used without further purification. Figure 3.4 represents the chemical structure of DMSO.

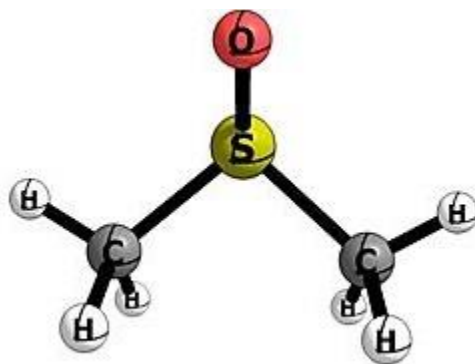


Figure 3.4: Chemical structure of Dimethyl sulfoxide (DMSO)

Glycerol

Glycerol of 99% purity (confirming to IP) was obtained from LOBA Chemie Pvt. Ltd. Chemical structure of glycerol has been provided in figure 3.5.

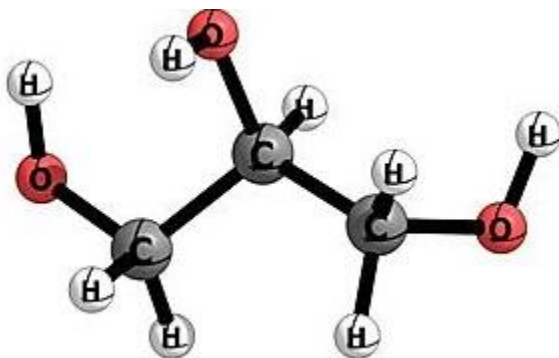


Figure 3.5: Chemical structure of Glycerol

Bovine Serum Albumin (BSA)

BSA of 98% purity was procured from Himedia Ltd. The protein was however stored at $\sim 4 - 5$ °C, and used without giving any additional treatment. X-ray crystallographic structure of BSA has been represented in figure 3.6.

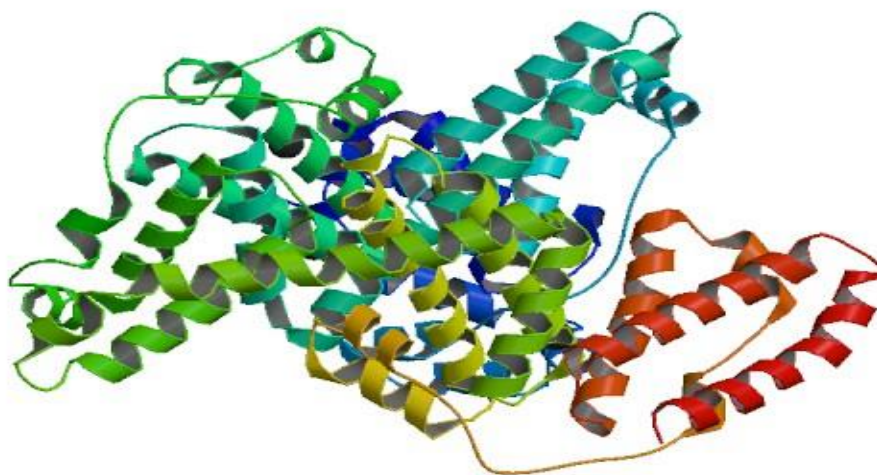


Figure 3.6: Schematic representation of X-ray crystallographic structure of BSA (PDB ID: 3V03)

Potassium Chloride:

Potassium Chloride (KCl) was required for the determination of cell constant of the conductivity cell. It was of AR grade purchased from S. D. Fine Pvt. Ltd. (India), and was recrystallized twice from double distilled water. Initially, the salt was dried at $60 - 80$ °C in vacuum, and then ground and again dried for 2 – 3 days at $280 - 300$ °C in the presence of P_2O_5 . The purified sample was stored in vacuum desiccator in the presence of P_2O_5 and allowed to attain the room temperature before use.

The specifications and mass percentage purity of chemicals used have been provided in table 3.2.

Table 3.2: Specifications and mass fraction purity of chemicals used

| Chemical Name | Source | CAS Registry Number | Purification method | Mass fraction purity |
|---|-----------------------|----------------------------|--------------------------------|-----------------------------|
| Sodium dodecyl benzene sulfonate (SDBS) | Sigma Aldrich. | 25155-30-0 | Recrystallization ^a | > 0.90 ^b |
| Cetyl pyridinium Chloride (CPC) | Loba chemie Pvt. Ltd. | 6004-24-26 | None | 0.99 ^b |
| Glycerol | Loba chemie Pvt. Ltd. | 56-81-5 | None | 0.99 ^b |
| Dimethyl Sulfoxide (DMSO) | Finar Pvt. Ltd. | 67-68-5 | None | 0.99 ^b |
| Bovine Serum Albumin | Himedia | 9048-46-8 | None | > 0.98 ^b |

^a Ref. [1]

^b Declared by supplier

Apparatus and Experimental Procedures

Thermostat:

A high precision water thermostat of capacity ~ 15 L fitted with a digital temperature controlled device as shown in figure 3.7 was used for conductance measurements. It was supplied by Bombay scientific Pvt. Ltd. (India). The temperature of the thermostat was maintained within $\pm 0.05\text{K}$ over the entire temperature range studied i.e., $293.15 - 308.15$ K. However, the temperature of the bath was continuously monitored with the help of a calibrated thermometer.



Figure 3.7: Digital Thermostat Water Bath.

Conductance Measurements:

Conductivity measurements were carried out with a digital conductivity meter (fig. 3.8) operating at 1 KHz, supplied by Labtronics Pvt. Ltd. (India). The cell constant of this conductivity cell was determined at 298.15 K from conductance measurements with aqueous solutions of KCl as described by J. Lind *et al.*⁸ The temperature of the solution was maintained to $\pm 0.05\text{K}$ by circulating water from thermostat through a double walled vessel containing the solution.



Figure 3.8: Electrical Conductivity Meter

pH Measurements

The pH meter was first calibrated with the help of standard pH buffers (4.0, 7.0 and 9.2) and the pH of the purified water was tested using Cyber scan 2500 pH meter (Fig. 3.9). Buffer solutions of pH 4.0, 5.4 and 7.0 were made for investigation of protein surfactant interactions (BSA-SDBS or BSA-CPC) at different pH conditions and the pH of the systems was monitored regularly using this instrument.



Figure 3.9: Digital pH Meter

UV-Visible Measurements

Both the ionic surfactants (SDBS and CPC) selected were UV active and show UV absorbance around $\lambda = (255-265)$ nm. Nature of surfactant binding with protein (BSA) has been studied using UV-Visible spectroscopy. The absorption spectra of Bovine serum albumin (BSA) have been recorded on an UV-Visible spectrophotometer UV-1800, (figure 3.10) supplied by Shimadzu using quartz cuvette with a path length of 1 cm. All measurements were taken at $298.15 \text{ K} \pm 0.1\text{K}$.



Figure 3.10: Shimadzu-1800 UV-Vis. Spectrophotometer

Fluorescence Measurements

The fluorescence spectra of 2×10^{-6} mol/ Kg of BSA were recorded with a 1 cm path length quartz cell and titrated with different concentration of SDBS/CPC in presence and absence of different concentrations of DMSO and glycerol. The excitation wavelength for BSA fluorescence was 280 nm and its emission spectra were scanned at the range of 290- 500 nm. All experiments were performed on Varian Carey Fluorescence spectrophotometer (fig. 3.11) using a slit width (excitation = 10 nm, emission = 10 nm) at a stated excitation. (We are thankful to Dr. Kamaldeep Paul, Thapar University, Patiala, for providing this instrumentation facility)



Figure 3.11: Fluorescence Spectrophotometer

References

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Chapter-4

Results and Discussion

Section- 4.1

Conductometric Studies

Micellization behaviour of ionic surfactants (SDBS and CPC) in aqueous and binary aqueous mixtures of DMSO and glycerol

Aqueous solutions of surfactants (SDBS and CPC) of different molal concentrations in the range (0.3 to 3.0) mmol kg⁻¹ for SDBS and (0.2 to 2.0) mmol kg⁻¹ for CPC were prepared by the addition of small aliquots of concentrated solution of surfactant to 50 ml of water (solvent medium). The solutions obtained were gently stirred using magnetic stirrer before subjecting to conductivity measurements. Similar experiments were repeated in presence of 5 %, 10%, 15% and 20% w/w glycerol and DMSO solutions at 293.15, 298.15, 303.15 and 308.15 K respectively. CMC is the most important parameter used to study the micellization behaviour of amphiphiles. So to analyze the effect of co-solvents on micellization behaviour of ionic surfactants, it was necessary to determine the CMC values of these surfactants (SDBS and CPC) in aqueous medium at different temperatures. CMC values of SDBS and CPC were determined by conductivity experiments employing William's method¹. In this method CMC values are determined from the inflection in the plot between specific conductance (κ) versus surfactant concentration^{2,3}. This inflection/ break point between two straight lines gives the value of CMC. The CMC's of both the surfactants (SDBS and CPC) in aqueous medium were in excellent agreement with the literature values^{4,5}. A Non-Linear fit approach introduced by Carpena *et al.*^{6,7} was successfully applied to elaborate conductivity data obtained on ionic surfactants (SDBS and CPC). In this method the fit of whole data was set to a function (which represented the integral function of a sigmoid) was proposed (equation 4.1)^{8,9}.

$$F(x) = F(0) + A_1x + \Delta x (A_2 - A_1) \ln \left(\frac{1 + e^{\frac{(x-x_0)}{\Delta x}}}{1 + e^{\frac{-x_0}{\Delta x}}} \right) \quad (4.1)$$

In above equation, the transition from two linear regimes at low and high concentration is described. Here $F(0)$ is the initial conductivity of water, A_1 and A_2 are the limiting slopes for low and high concentration respectively, x_0 is the central point of the transition; i.e., the CMC and Δx is the width of the transition. The CMC values obtained from both William's and Carpena method has been represented successfully in tables 4.1 to 4.4.

Table 4.1: CMC values of SDBS and CPC and in aqueous and aqueous rich mixtures of glycerol at different temperatures calculated using William's Method at pressure $p = 100$ kPa.

| Glycerol (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|--------------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0 | 1.26 | 1.29 | 1.35 | 1.41 | 0.90 | 0.96 | 1.00 | 1.04 |
| 5 | 1.27 | 1.32 | 1.36 | 1.42 | 0.92 | 0.98 | 1.02 | 1.05 |
| 10 | 1.30 | 1.33 | 1.37 | 1.44 | 0.98 | 1.00 | 1.08 | 1.10 |
| 15 | 1.35 | 1.44 | 1.46 | 1.47 | 1.08 | 1.14 | 1.16 | 1.22 |
| 20 | 1.41 | 1.45 | 1.47 | 1.49 | 1.12 | 1.18 | 1.20 | 1.24 |

Table 4.2: CMC values of SDBS and CPC in aqueous rich mixtures of DMSO at different temperatures calculated using William's Method at pressure $p = 100$ kPa

| DMSO (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3) mM for CPC</u> | | | |
|----------------|--|---------------|---------------|---------------|---|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 5 | 1.29 | 1.31 | 1.36 | 1.43 | 1.03 | 1.08 | 1.14 | 1.16 |
| 10 | 1.35 | 1.38 | 1.44 | 1.48 | 1.10 | 1.18 | 1.22 | 1.24 |
| 15 | 1.38 | 1.44 | 1.46 | 1.50 | 1.20 | 1.26 | 1.30 | 1.34 |
| 20 | 1.44 | 1.47 | 1.48 | 1.52 | 1.24 | 1.30 | 1.33 | 1.36 |

Table 4.3: CMC values of SDBS and CPC and in aqueous and aqueous rich mixtures of glycerol at different temperatures calculated using Carpena Method (non-linear fit) at pressure $p = 100$ kPa.

| Glycerol (%w/w) | CMC (10^3) mM for SDBS | | | | CMC (10^3) mM for CPC | | | |
|--------------------|----------------------------|--------|--------|--------|---------------------------|--------|--------|--------|
| | T/K | | | | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0 | 1.18 | 1.25 | 1.31 | 1.39 | 0.81 | 0.89 | 1.00 | 1.02 |
| 5 | 1.20 | 1.27 | 1.33 | 1.40 | 0.87 | 0.88 | 0.98 | 1.00 |
| 10 | 1.24 | 1.29 | 1.35 | 1.41 | 0.99 | 1.00 | 1.05 | 1.10 |
| 15 | 1.33 | 1.38 | 1.40 | 1.42 | 1.10 | 1.14 | 1.15 | 1.18 |
| 20 | 1.41 | 1.44 | 1.46 | 1.49 | 1.15 | 1.18 | 1.20 | 1.22 |

Table 4.4: CMC values of SDBS and CPC in aqueous rich mixtures of DMSO at different temperatures calculated using Carpena Method (non-linear fit) at pressure $p = 100$ kPa.

| DMSO (%w/w) | CMC (10^3) mM for SDBS | | | | CMC (10^3) mM for CPC | | | |
|----------------|----------------------------|--------|--------|--------|---------------------------|--------|--------|--------|
| | T/K | | | | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 5 | 1.24 | 1.27 | 1.30 | 1.39 | 1.01 | 1.09 | 1.12 | 1.15 |
| 10 | 1.32 | 1.37 | 1.42 | 1.45 | 1.18 | 1.19 | 1.20 | 1.22 |
| 15 | 1.37 | 1.40 | 1.45 | 1.48 | 1.21 | 1.23 | 1.27 | 1.30 |
| 20 | 1.42 | 1.46 | 1.47 | 1.50 | 1.22 | 1.25 | 1.31 | 1.37 |

The values of critical micellar concentration (CMC) have been converted into their mole fraction unit (X_{CMC}) before determining other thermodynamic parameters of micellization. The variation of κ (specific conductance) of the surfactants was quite linear before and after the inflection. The comparison of monomeric and micellar region of both the surfactants (SDBS and CPC) was made by determining the pre (S_1) and post micellization (S_2) slopes. The slopes of pre micellar regions were always found to be greater than post micellar region. In case of SDBS breakpoints of conductivity data was not as sharp as compared to conductivity data of CPC. It may be due to stepwise micelle formation or insoluble salt formation in case of SDBS on addition of organic solvents^{10,11}. Figure 4.1 depicts the representative plots of κ versus surfactant concentration in pure water at $T = (293.15, 298.15, 303.15, 308.15)$ K. We observed the inhibitory effect of temperature on the micellization of SDBS and CPC. The linear increase of CMC's for both the surfactants with respect to temperature^{12,13} signified the increase in thermal motions of surfactants as well as solvent system. The increased thermal motions inhibited the formation of ordered micelles due to disruption in structure of water.

Effect of solvent system on micellization characteristics

The micellization behaviour of ionic surfactants can be understood on the basis of differences in the properties of glycerol-water and DMSO-water solvent systems. In this case micellization is inhibited by the presence of co-solvents in the medium. Co-solvents like glycerol and DMSO when added to water increase the CMC's of ionic surfactants by decreasing the polarity of water, increasing the solubility of hydrocarbon chain leading to increase in hydrophobic character of the solvent medium¹⁴⁻¹⁶. Increase in $\text{CMC}/X_{\text{CMC}}$ values of SDBS and CPC on addition of co-solvents may be due to different properties of glycerol and DMSO. In case of glycerol the increase in X_{CMC} of surfactants can be attributed in terms of decrease in dielectric constant of the medium, resulting in better solubility of hydrocarbon chain of surfactant in glycerol-water mixed solvent system¹⁷. Glycerol is a kosmotropic co-solvent which is known for its water structure making properties over the latter¹⁸. DMSO is known for its chaotropic effect of water structure and its hydrogen bonding properties with water is responsible for lowering the CMC of ionic surfactants. But in this case former dominates (disruption of water structure) and is considered as a non-penetrating co-solvent

which breaks the micelles, hence CMC/X_{CMC} values of surfactants is directly proportional to concentration of DMSO in the medium^{19,20}. The CMC values of SDBS and CPC showing co-solvent effect have been represented in tables 4.1 to 4.4.

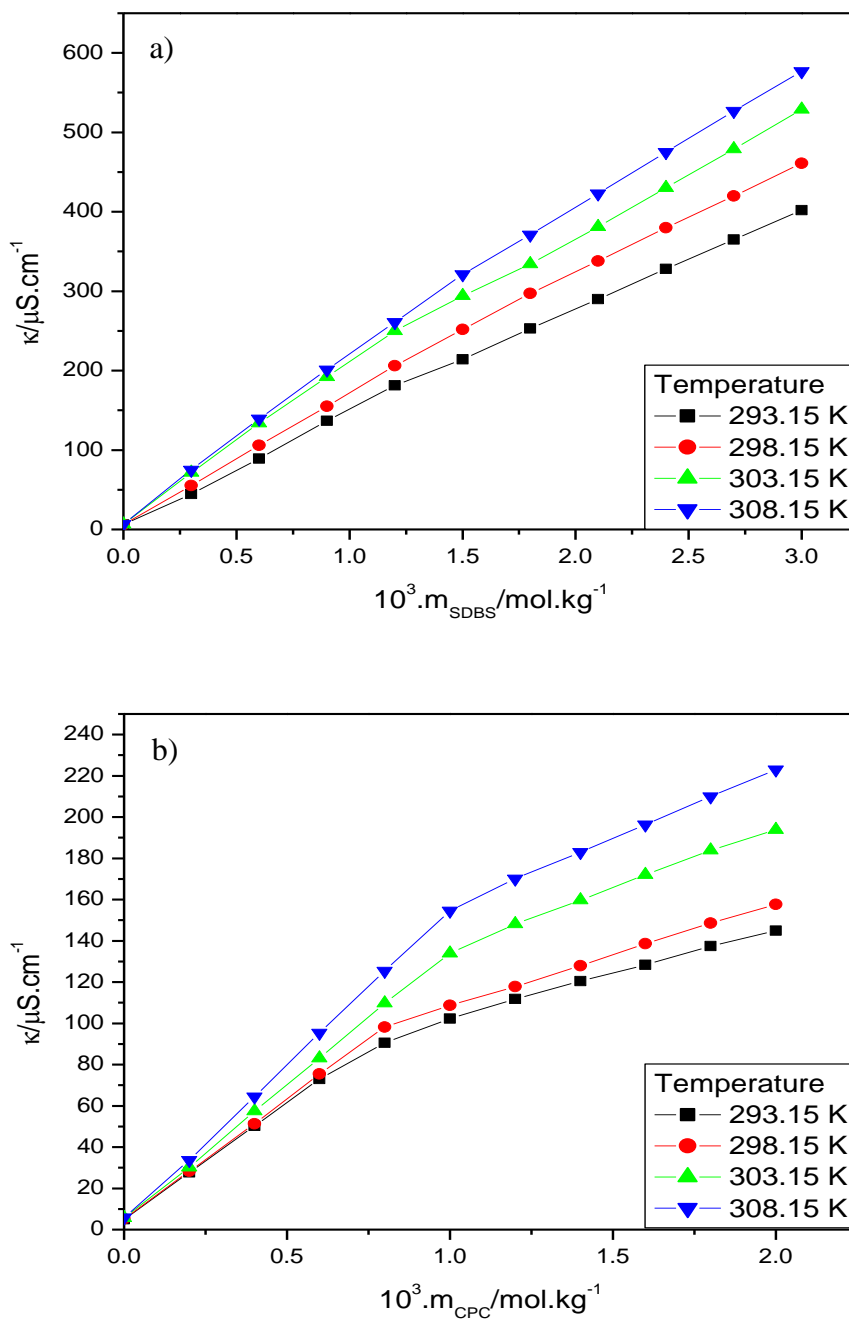


Figure 4.1: Specific conductance vs. concentration plots for aqueous solution of (a) SDBS and (b) CPC at different temperatures.

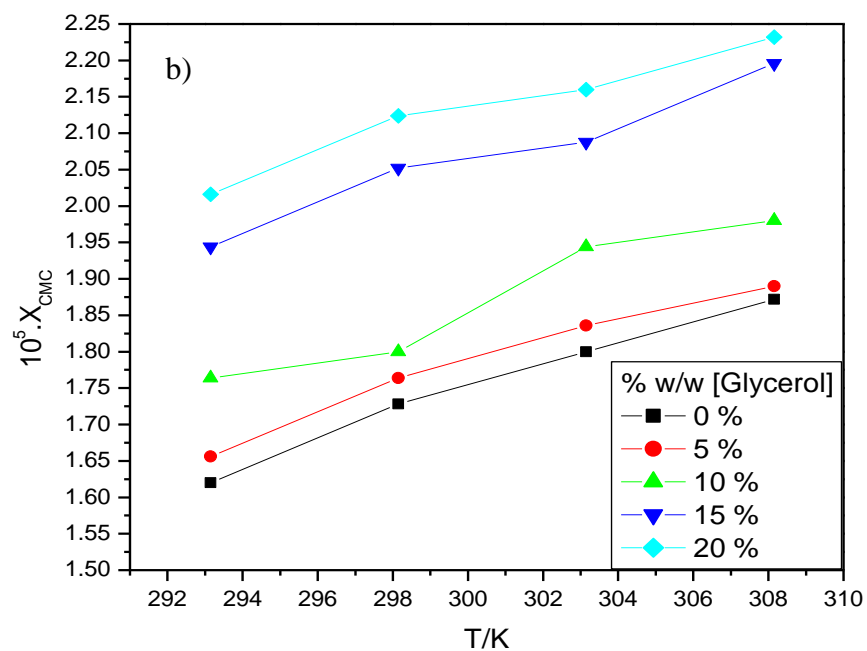
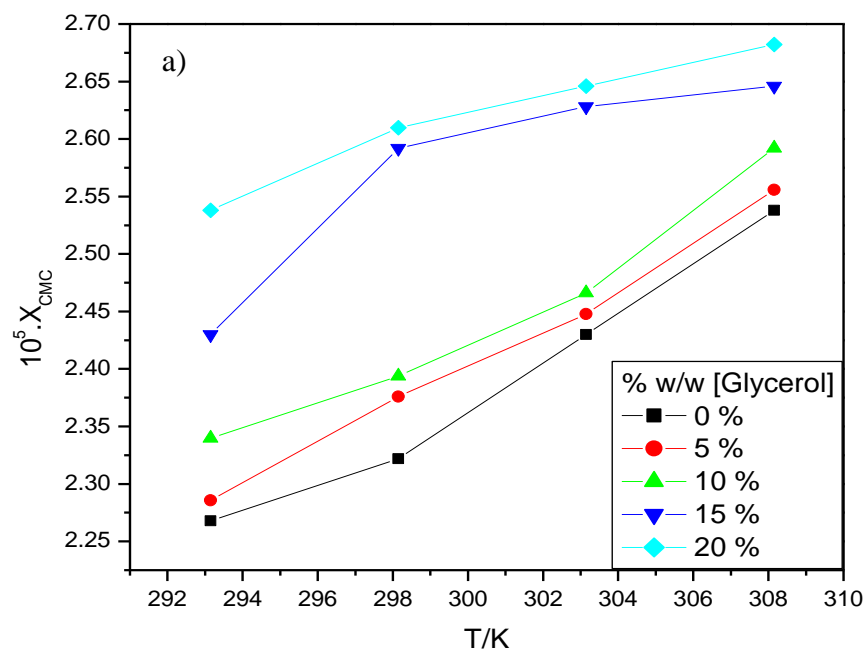


Figure 4.2: Plots of X_{CMC} vs. temperature in aqueous solution for (a) SDBS and (b) CPC containing different concentrations of glycerol.

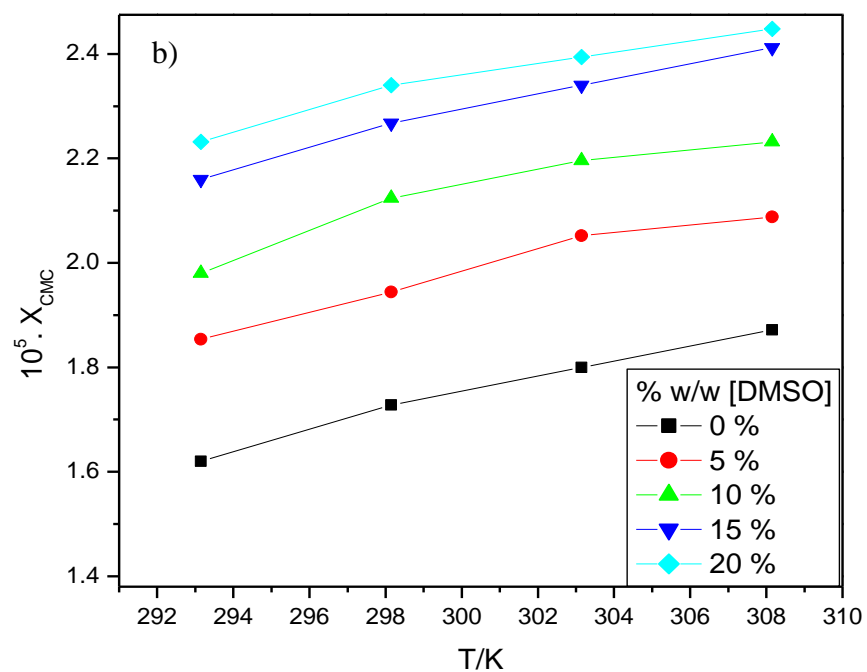
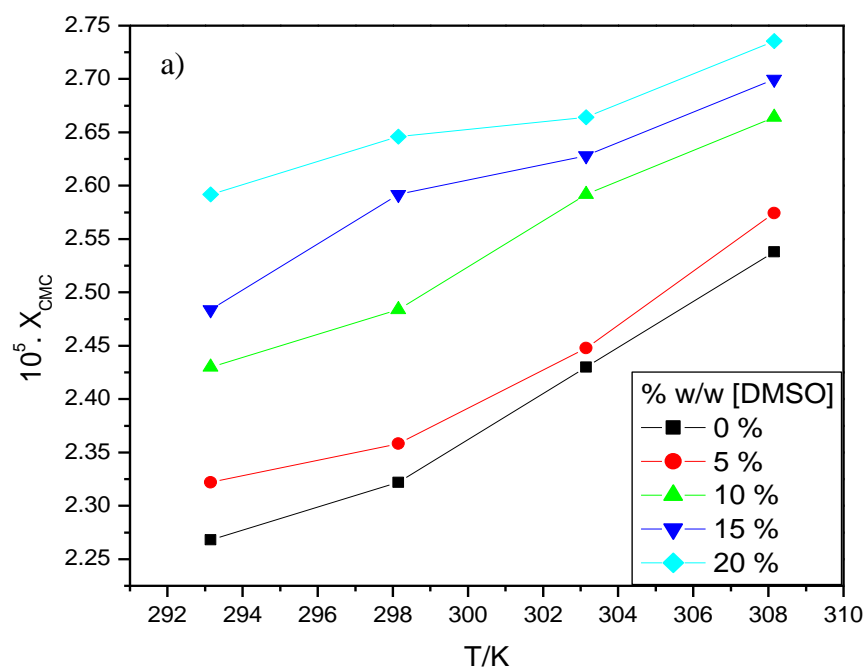


Figure 4.3: Plots of X_{CMC} vs. temperature in aqueous solution for (a) SDBS and (b) CPC containing different concentrations of DMSO.

Thermodynamics of micellization can be interpreted by two widely accepted approaches, phase separation and mass action. Whereas the mass action model is best suited for ionic surfactants, so thermodynamic parameters of micellization for ionic surfactants was deduced from the temperature dependence of X_{CMC} using the mass-action model (figures 4.2 and 4.3)²¹. Thermodynamic parameters of micellization are important in understanding the effect of structural and environmental factors on the process of micellization. For predicting the effect of these factors (i.e. co-solvents) on micellization of SDBS and CPC, the thermodynamic parameters of micellization, ΔG_m^0 , ΔH_m^0 and ΔS_m^0 were calculated in aqueous solution as well as aqueous solution containing organic solvents (glycerol and DMSO). Standard enthalpy of micellization (ΔH_m^0) of the ionic surfactants has been deduced from equation (4.2)²².

$$\Delta H_m^0 = -RT^2(2-\alpha) \frac{d \ln X_{\text{CMC}}}{dT} \quad (4.2)$$

Where R is gas constant, T is temperature in Kelvin and $\frac{d \ln X_{\text{CMC}}}{dT}$ is the slope of straight line obtained by plotting $\ln X_{\text{CMC}}$ against T and α is the degree of the counter ion dissociation and was calculated using relation (4.3)²³.

$$\alpha = \frac{\text{Postmicellizationregion}(S_2)}{\text{Premicellizationregion}(S_1)} \quad (4.3)$$

Where, S_1 and S_2 are the slopes of pre and post micellar region obtained by plotting conductivity data against surfactant concentration. The standard free energy of micellization ΔG_m^0 and standard entropy of micellization ΔS_m^0 were calculated by using the following relations (4.4) and (4.5) respectively.

$$\Delta G_m^0 = (2 - \alpha)RT \ln X_{\text{CMC}} \quad (4.4)$$

$$\Delta S_m^0 = \frac{\Delta H_m^0 - \Delta G_m^0}{T} \quad (4.5)$$

The effect of co-solvents (glycerol and DMSO) on the micellization process of SDBS and CPC can be calculated using free energy of surfactant tail transfer, $\Delta G_{\text{trans}}^0$, is given by equation (4.6)^{24,25}

$$\Delta G_{trans}^0 = (\Delta G_m^0)_{Glycerol/DMSO+water} - (\Delta G_m^0)_{water} \quad (4.6)$$

The values of thermodynamic parameters of micellization for aqueous solutions of SDBS and CPC have been presented in tables 4.5 and 4.6. After analyzing the data, it was found that ΔH_m^0 values for SDBS and CPC in aqueous solution were negative and largely dependent on temperature. This observation reflected the exothermal and spontaneous behaviour of micellization process. ΔH_m^0 values of SDBS and CPC were much less as compared to ΔS_m^0 values in aqueous medium which suggest that the micellization process was entropically favored and on comparing between the ΔH_m^0 values of these surfactants, it was observed that ΔH_m^0 values of SDBS were less negative as compared to CPC, so micellization process in case of SDBS was more entropically driven as compared to CPC in which it was enthalpically driven in pure water. Decrease in values of ΔS_m^0 with increasing temperature indicates that the self-assembly of SDBS and CPC become poorer at high temperatures due to melting of iceberg clusters around the alkyl chains of surfactant molecules and enhanced thermal motions/ randomness of alkyl chains towards the micellar core at high temperatures²⁶⁻²⁸. Negative values of ΔG_m^0 suggest that both SDBS and CPC form micelles spontaneously in water as well as in presence of water + co-solvents (DMSO and glycerol) medium. The values of thermodynamic parameters of micellization (ΔG_m^0 , ΔH_m^0 and ΔS_m^0) are reported in tables 4.5 to 4.8 and were found to be negative, negative and positive respectively in all cases. ΔG_m^0 values were more negative with respect to temperature and became less negative on increasing co-solvent concentration in the medium. This type of results signified that the spontaneity of the system increased with the increase in temperature and was inversely proportional to co- solvent concentration. ΔG_{trans}^0 (tables 4.5 to 4.8) values were positive and showed a gradual increase with increase in concentration of glycerol and DMSO in the water at specific temperatures^{29,30}. This type of results represented the interactions between surfactant tail with co-solvents (glycerol and DMSO) and ionic head groups with water. The positive values of ΔG_{trans}^0 signified the reduction in solvophobic interactions caused by improved solvation of hydrocarbon tail which resulted in reducing the ability of surfactants (SDBS and CPC) to aggregate and promotes the delay in micellization process³¹. The positive values of standard entropy of micellization and negative values of

standard free energy of micellization reflected the dominance of London-dispersion interactions³². Presence of co-solvents inhibited micellization and entropic contribution increases with increase in concentration of co-solvents in the medium² because the co-solvent addition decreases the cohesiveness of solvent medium and reduces the solvophobic effect by solubilizing hydrocarbon tails³³. Inhibitory effect of glycerol dominated over DMSO as the values of ΔH_m^0 in presence of glycerol were more negative than DMSO and the main reason behind this observation was the low polarity of glycerol ($\epsilon = 42.5$) as compared to DMSO ($\epsilon = 48.9$)³⁴. As a result the solubility of hydrocarbon chains of surfactant molecules increase in water + co-solvent mixed medium and it reduces the aggregation tendency of surfactant monomers^{35,36}.

Table 4.5: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0), degree of counter ion dissociation (α) and free energy of transfer ($\Delta G_{\text{trans}}^0$) for SDBS in aqueous and binary mixtures of glycerol at different temperatures and at pressure $p = 100$ kPa.

| T/K | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol^{-1} | $\Delta G_{\text{trans}}^0$ kJ mol^{-1} | ΔH_m^0 kJ mol^{-1} | ΔS_m^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|--|----------------------------|----------|--|---|--|--|
| SDBS in <u>aqueous medium</u> | | | | | | |
| 293.15 | 2.268 | 0.810 | -31.027 | - | -6.515 | 83.615 |
| 298.15 | 2.322 | 0.817 | -31.296 | - | -6.698 | 82.502 |
| 303.15 | 2.430 | 0.822 | -31.534 | - | -6.892 | 81.286 |
| 308.15 | 2.538 | 0.823 | -31.897 | - | -7.115 | 80.422 |
| SDBS in <u>5% w/w Glycerol</u> | | | | | | |
| 293.15 | 2.286 | 0.815 | -30.871 | 0.155 | -6.174 | 84.249 |
| 298.15 | 2.376 | 0.827 | -30.961 | 0.336 | -6.32 | 82.645 |
| 303.15 | 2.448 | 0.828 | -31.374 | 0.160 | -6.53 | 81.953 |
| 308.15 | 2.556 | 0.836 | -31.536 | 0.361 | -6.699 | 80.6 |
| SDBS in <u>10% w/w Glycerol</u> | | | | | | |
| 293.15 | 2.340 | 0.828 | -30.449 | 0.578 | -5.642 | 84.622 |
| 298.15 | 2.394 | 0.837 | -30.686 | 0.611 | -5.796 | 83.484 |
| 303.15 | 2.466 | 0.841 | -30.998 | 0.535 | -5.969 | 82.563 |
| 308.15 | 2.592 | 0.845 | -31.260 | 0.637 | -6.148 | 81.493 |
| SDBS in <u>15% w/w Glycerol</u> | | | | | | |
| 293.15 | 2.430 | 0.832 | -30.239 | 0.788 | -4.497 | 87.813 |
| 298.15 | 2.592 | 0.840 | -30.367 | 0.930 | -4.621 | 86.352 |
| 303.15 | 2.628 | 0.846 | -30.684 | 0.850 | -4.754 | 85.536 |
| 308.15 | 2.646 | 0.849 | -31.090 | 0.807 | -4.899 | 84.993 |
| SDBS in <u>20% w/w Glycerol</u> | | | | | | |
| 293.15 | 2.538 | 0.833 | -30.107 | 0.920 | -2.994 | 92.488 |
| 298.15 | 2.610 | 0.846 | -30.190 | 1.106 | -3.062 | 90.988 |
| 303.15 | 2.646 | 0.849 | -30.580 | 0.954 | -3.158 | 90.459 |
| 308.15 | 2.682 | 0.850 | -31.016 | 0.881 | -3.26 | 90.074 |

Table 4.6 Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0), degree of counter ion dissociation (α) and free energy of transfer ($\Delta G_{\text{trans}}^0$) for CPC (C*) in aqueous and binary aqueous mixtures of Glycerol at different temperatures and at pressure $p = 100$ kPa

| T/K | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol^{-1} | $\Delta G_{\text{trans}}^0$ kJ mol^{-1} | ΔH_m^0 kJ mol^{-1} | ΔS_m^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|---------------------------------------|----------------------------|----------|--|---|--|--|
| CPC in <u>aqueous medium</u> | | | | | | |
| 293.15 | 1.620 | 0.406 | -42.853 | - | -10.810 | 109.314 |
| 298.15 | 1.731 | 0.430 | -42.686 | - | -11.010 | 106.228 |
| 303.15 | 1.800 | 0.438 | -43.016 | - | -11.331 | 104.531 |
| 308.15 | 1.870 | 0.440 | -43.501 | - | -11.690 | 103.246 |
| CPC in <u>5% w/w Glycerol</u> | | | | | | |
| 293.15 | 1.656 | 0.414 | -42.555 | 0.298 | -9.893 | 111.418 |
| 298.15 | 1.764 | 0.432 | -42.553 | 0.133 | -10.120 | 108.783 |
| 303.15 | 1.836 | 0.438 | -42.932 | 0.084 | -10.420 | 107.251 |
| 308.15 | 1.890 | 0.442 | -43.403 | 0.099 | -10.740 | 106.011 |
| CPC in <u>10% w/w Glycerol</u> | | | | | | |
| 293.15 | 1.764 | 0.421 | -42.126 | 0.727 | -9.556 | 111.102 |
| 298.15 | 1.800 | 0.438 | -42.310 | 0.377 | -9.780 | 109.105 |
| 303.15 | 1.944 | 0.439 | -42.668 | 0.348 | -10.102 | 107.434 |
| 308.15 | 1.980 | 0.443 | -43.196 | 0.305 | -10.410 | 106.394 |
| CPC in <u>15% w/w Glycerol</u> | | | | | | |
| 293.15 | 1.944 | 0.446 | -41.094 | 1.760 | -8.506 | 111.163 |
| 298.15 | 2.052 | 0.455 | -41.340 | 1.346 | -8.747 | 109.317 |
| 303.15 | 2.088 | 0.460 | -41.819 | 1.197 | -9.011 | 108.225 |
| 308.15 | 2.196 | 0.471 | -42.030 | 1.471 | -9.249 | 106.381 |
| CPC in <u>20% w/w Glycerol</u> | | | | | | |
| 293.15 | 2.016 | 0.448 | -40.899 | 1.955 | -7.154 | 115.111 |
| 298.15 | 2.124 | 0.456 | -41.184 | 1.502 | -7.353 | 113.47 |
| 303.15 | 2.160 | 0.462 | -41.631 | 1.385 | -7.569 | 112.359 |
| 308.15 | 2.232 | 0.479 | -41.735 | 1.766 | -7.778 | 110.196 |

Table 4.7: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0), degree of counter ion dissociation (α) and free energy of transfer ($\Delta G_{\text{trans}}^0$) for SDBS (C) in aqueous mixtures of DMSO at different temperatures and at pressure $p = 100$ kPa.

| T/K | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol^{-1} | $\Delta G_{\text{trans}}^0$ kJ mol^{-1} | ΔH_m^0 kJ mol^{-1} | ΔS_m^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|------------------------------|----------------------------|----------|--|---|--|--|
| SDBS in 5 % w/w DMSO | | | | | | |
| 293.15 | 2.322 | 0.814 | -30.833 | 0.193 | -5.870 | 85.154 |
| 298.15 | 2.358 | 0.817 | -31.244 | 0.053 | -6.059 | 84.471 |
| 303.15 | 2.448 | 0.822 | -31.436 | 0.097 | -6.240 | 83.446 |
| 308.15 | 2.574 | 0.834 | -31.571 | 0.326 | -6.380 | 81.749 |
| SDBS in 10 % w/w DMSO | | | | | | |
| 293.15 | 2.430 | 0.825 | -30.432 | 0.595 | -5.357 | 85.537 |
| 298.15 | 2.484 | 0.828 | -30.814 | 0.482 | -5.528 | 84.809 |
| 303.15 | 2.592 | 0.833 | -31.061 | 0.472 | -5.689 | 83.695 |
| 308.15 | 2.664 | 0.837 | -31.393 | 0.504 | -5.859 | 82.86 |
| SDBS in 15 % w/w DMSO | | | | | | |
| 293.15 | 2.484 | 0.828 | -30.289 | 0.738 | -4.422 | 88.239 |
| 298.15 | 2.592 | 0.832 | -30.577 | 0.720 | -4.558 | 87.268 |
| 303.15 | 2.628 | 0.839 | -30.860 | 0.673 | -4.684 | 86.349 |
| 308.15 | 2.700 | 0.842 | -31.215 | 0.682 | -4.828 | 85.632 |
| SDBS in 20 % w/w DMSO | | | | | | |
| 293.15 | 2.592 | 0.832 | -30.067 | 0.960 | -2.813 | 92.969 |
| 298.15 | 2.646 | 0.837 | -30.373 | 0.923 | -2.895 | 92.160 |
| 303.15 | 2.664 | 0.843 | -30.716 | 0.817 | -2.979 | 91.496 |
| 308.15 | 2.736 | 0.848 | -31.006 | 0.891 | -3.065 | 90.676 |

Table 4.8: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_{m}^0), enthalpy (ΔH_{m}^0), entropy (ΔS_{m}^0), degree of counter ion dissociation (α) and free energy of transfer ($\Delta G_{\text{trans.}}^0$) for CPC (C*) in aqueous mixtures of DMSO at different temperatures and at pressure $p = 100$ kPa

| T/K | X_{CMC} 10^5 | α | ΔG_{m}^0 kJ mol^{-1} | $\Delta G_{\text{trans}}^0$ kJ mol^{-1} | ΔH_{m}^0 kJ mol^{-1} | ΔS_{m}^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|-----------------------------|----------------------------|----------|---|---|---|---|
| CPC in 5 % w/w DMSO | | | | | | |
| 293.15 | 1.854 | 0.425 | -41.831 | 1.022 | -9.244 | 111.175 |
| 298.15 | 1.944 | 0.428 | -42.281 | 0.405 | -9.540 | 109.812 |
| 303.15 | 2.052 | 0.433 | -42.629 | 0.387 | -9.829 | 108.196 |
| 308.15 | 2.088 | 0.437 | -43.167 | 0.334 | -10.130 | 107.199 |
| CPC in 10 % w/w DMSO | | | | | | |
| 293.15 | 1.980 | 0.429 | -41.47 | 1.383 | -8.812 | 111.405 |
| 298.15 | 2.124 | 0.439 | -41.646 | 1.039 | -9.059 | 109.299 |
| 303.15 | 2.196 | 0.441 | -42.159 | 0.857 | -9.353 | 108.217 |
| 308.15 | 2.232 | 0.448 | -42.589 | 0.912 | -9.619 | 106.993 |
| CPC in 15% w/w DMSO | | | | | | |
| 293.15 | 2.160 | 0.463 | -40.245 | 2.608 | -7.962 | 110.125 |
| 298.15 | 2.268 | 0.474 | -40.460 | 2.226 | -8.178 | 108.274 |
| 303.15 | 2.340 | 0.479 | -40.872 | 2.144 | -8.425 | 107.035 |
| 308.15 | 2.412 | 0.480 | -41.407 | 2.094 | -8.700 | 106.139 |
| CPC in 20% w/w DMSO | | | | | | |
| 293.15 | 2.232 | 0.486 | -39.528 | 3.326 | -6.492 | 112.694 |
| 298.15 | 2.340 | 0.491 | -39.880 | 2.806 | -6.691 | 111.317 |
| 303.15 | 2.394 | 0.500 | -40.223 | 2.793 | -6.876 | 110.001 |
| 308.15 | 2.448 | 0.509 | -40.549 | 2.952 | -7.061 | 108.675 |

Temperature dependence of hydrophobic effect can be expressed in terms of heat capacity of micellization ($\Delta_m C_p^0$), which is calculated from the slope of ΔH_m^0 vs. temperature curve and represented as follows (equation 4.7):

$$\Delta_m C_p^0 = \frac{\partial \Delta H_m^0}{\partial T} \quad (4.7)$$

The calculated values of heat capacity of micellization have been represented in Table 4.9. All values of $\Delta_m C_p^0$ were negative and show linear increase with increase in percentage of cosolvent (glycerol/DMSO). These type of results were also observed by Bhattarai *et al*²⁹.

Table 4.9: Values of $\Delta_m C_p^0$ ($J.mol^{-1} K^{-1}$) for SDBS and CPC in aqueous and binary aqueous mixtures of glycerol/DMSO at pressure $p = 100$ kPa

| $\Delta_m C_p^0$ ($J.mol^{-1} K^{-1}$) | | | | |
|--|----------|---------|----------|---------|
| % Cosolvent | CPC | | SDBS | |
| | Glycerol | DMSO | Glycerol | DMSO |
| 0 | -58.947 | | -39.886 | |
| 5 | -57.623 | -58.392 | -35.743 | -34.201 |
| 10 | -56.517 | -54.318 | -33.817 | -33.368 |
| 15 | -49.870 | -49.236 | -26.796 | -26.893 |
| 20 | -41.791 | -37.875 | -17.828 | -16.795 |

Correlation of free energy of micellization (ΔG_m^0) with solvent parameters.

The self-assembly of ionic surfactants is generally controlled by solvophobic effect and is related to solvent cohesiveness which can be expressed in terms of Gordon Parameter (G). It was determined from the equation 4.8¹

$$G = \frac{\gamma_{sol}}{\sqrt[3]{V_m}} \quad (4.8)$$

Where γ_{sol} the surface tension of the solution, V_m is the molar volume of the solution and determined using relation 4.9:

$$V_m = \sum_{i=1}^2 X_i V_i \quad (4.9)$$

Where X_i and V_i are the mole fraction and molar volume of i^{th} component respectively. The plots of Gordon parameters with ΔG_m^0 have been represented in figure 4.4.

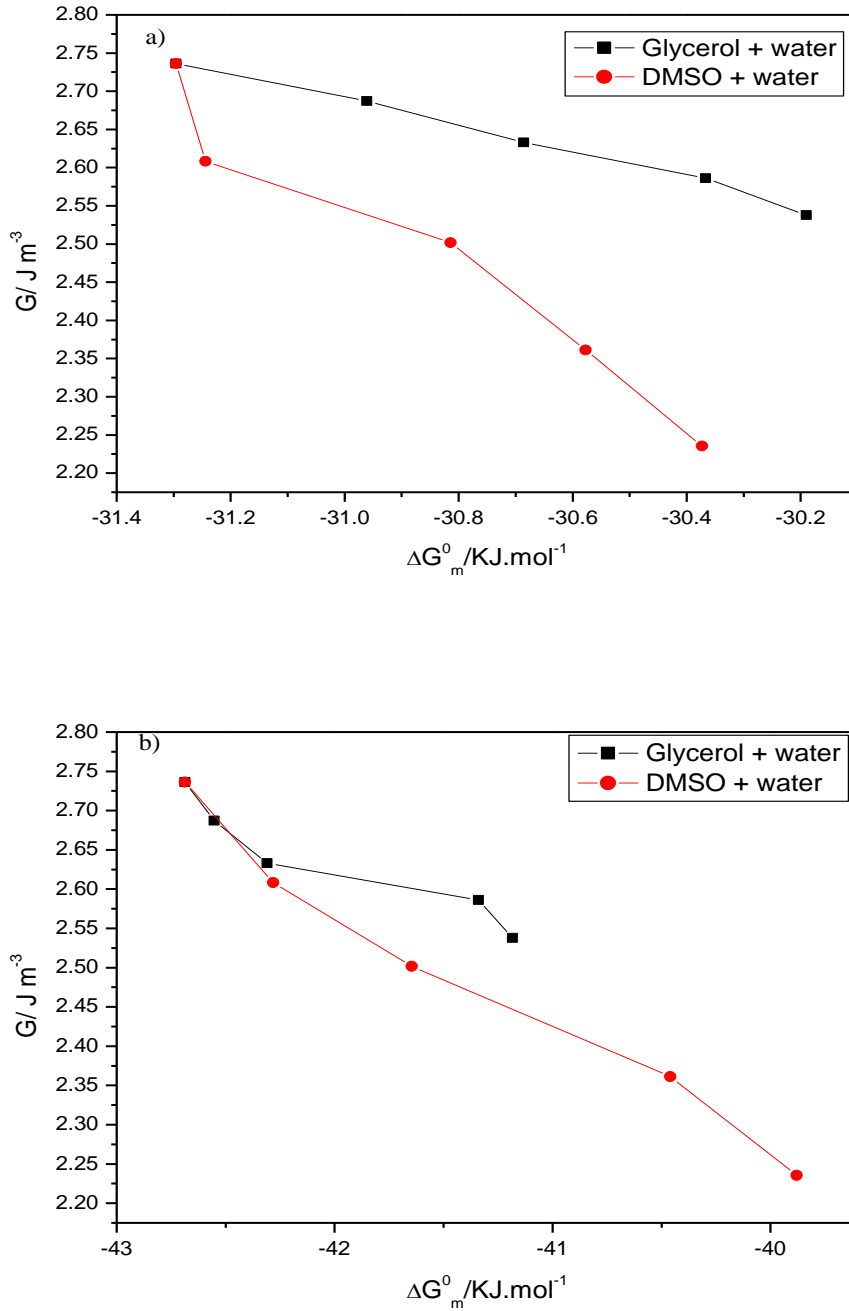
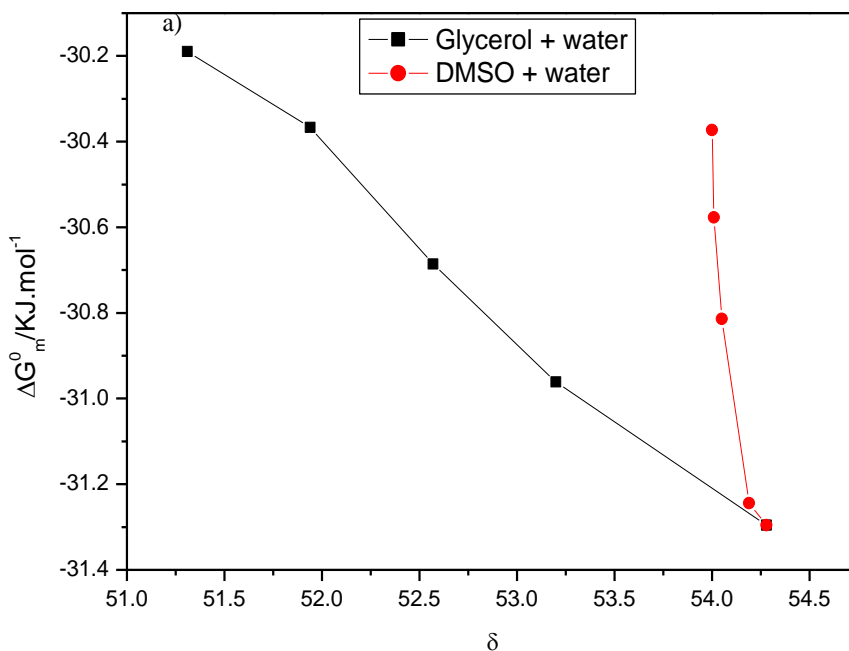


Figure 4.4 Plot of ΔG_m^0 versus G for a) SDBS and b) CPC at 298.15 K.

A curvilinear relationship between ΔG_m^0 and G has been observed (similar results were obtained by bhattarai *et al.*)²⁹. A single solvent property might not be sufficient to explain the process of micellization of SDBS/ CPC in presence of co-solvents glycerol/ DMSO, so we correlated ΔG_m^0 values with different solvent parameters ($E_T(30)$, D , G and δ) of water+ glycerol/ DMSO systems. Correlation is always established with ΔG_m^0 because it is considered to be the best thermodynamic parameter as counter ion binding influences it especially in case of ionic surfactant²⁹ The $E_T(30)$ and D values for glycerol+ water system³⁷ and DMSO+water system were taken from literature. δ values were also determined using relation 4.10^{38,39} and its relationship with ΔG_m^0 have been represented in figure 4.5

$$\delta = 0.45 D + 18.5 \quad (4.10)$$

The calculated physiochemical parameters for binary aqueous mixtures of glycerol and DMSO have been reported in Table 4.10 and 4.11 respectively.



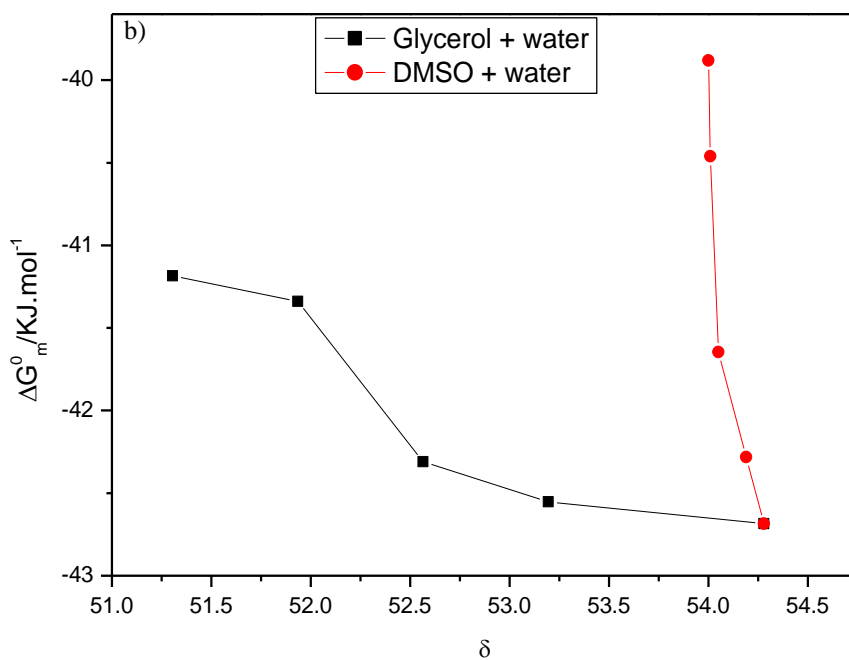


Figure 4.5: Plot of ΔG_m^0 versus δ for a) SDBS and b) CPC at 298.15 K.

Table 4.10: Various physiochemical parameters of mixed solvent (Glycerol+ water) at 298.15 K and pressure $p = 100$ kPa

| % Glycerol (w/w) | Dielectric Constant | Surface Tension $\gamma_0 / (\text{mN.m}^{-1})$ | Molar volume $V_m / (\text{dm}^3, \text{mol}^{-1})$ | Gordon Parameter $G / (\text{J m}^{-3})$ | Reichardt's Parameter $(E_T / \text{KJ. mol}^{-1})$ | δ |
|------------------|---------------------|---|---|--|---|----------|
| 0 | 79.50 | 71.80 | 18.07 | 2.74 | 264.01 | 54.28 |
| 5 | 77.10 | 71.20 | 18.61 | 2.69 | 262.76 | 53.20 |
| 10 | 75.70 | 70.50 | 19.19 | 2.63 | 261.50 | 52.57 |
| 15 | 74.30 | 70.00 | 19.83 | 2.59 | 260.24 | 51.94 |
| 20 | 72.90 | 69.50 | 20.54 | 2.54 | 258.99 | 51.31 |
| 100 | 40.10 | 63.00 | 73.21 | 1.51 | 238.49 | 36.55 |

Table 4.11: Various physiochemical parameters of mixed solvent (DMSO + water) at 298.15 K and pressure $p = 100$ kPa

| % DMSO (w/w) | Dielectric Constant | Surface Tension $\gamma_0 / (\text{mN.m}^{-1})$ | Molar volume $V_m / (\text{dm}^3, \text{mol}^{-1})$ | Gordon Parameter $G / (\text{J m}^{-3})$ | Reichardt's Parameter ($E_T / \text{KJ. mol}^{-1}$) | δ |
|--------------|---------------------|---|---|--|---|----------|
| 0 | 79.50 | 71.80 | 18.07 | 2.74 | 264.01 | 54.28 |
| 5 | 79.30 | 71.65 | 20.73 | 2.61 | 260.22 | 54.19 |
| 10 | 79.00 | 71.54 | 23.39 | 2.50 | 256.44 | 54.05 |
| 15 | 78.90 | 70.00 | 26.05 | 2.36 | 252.65 | 54.01 |
| 20 | 78.90 | 68.45 | 28.71 | 2.24 | 248.86 | 54.00 |
| 100 | 48.40 | 42.80 | 71.30 | 1.03 | 188.28 | 40.28 |

Correlation of free energy of micellization (ΔG_m^0) with Solvophobic Parameter (S_p)

S_p Values for Binary aqueous mixtures of DMSO were adopted from literature²⁹ and S_p values for glycerol water system has not been reported due to unavailability in literature¹⁸. Concentrations investigated in this work are different to those reported in literature, So S_p values for the values of present system were found with the help of correlation method suggested by Wang *et al.*⁴⁰. Linear relationship between DMSO concentration (%w/w) and S_p has been observed for DMSO+ Water systems (figure 4.6). Similarly the plot between (ΔG_m^0) and S_p also show linear relationship (figure 4.7).

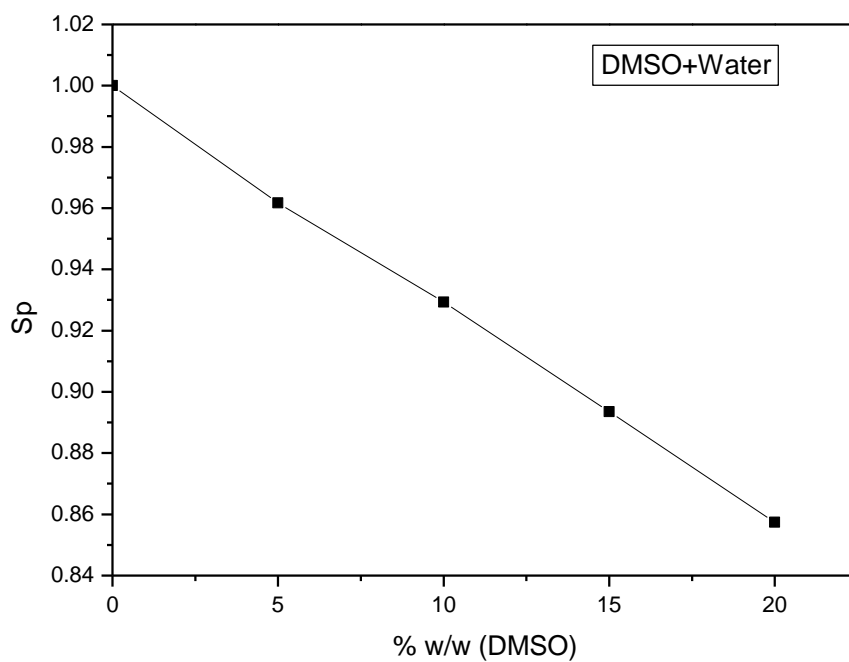


Figure 4.6: Plot of Sp versus [DMSO] at 298.15 K

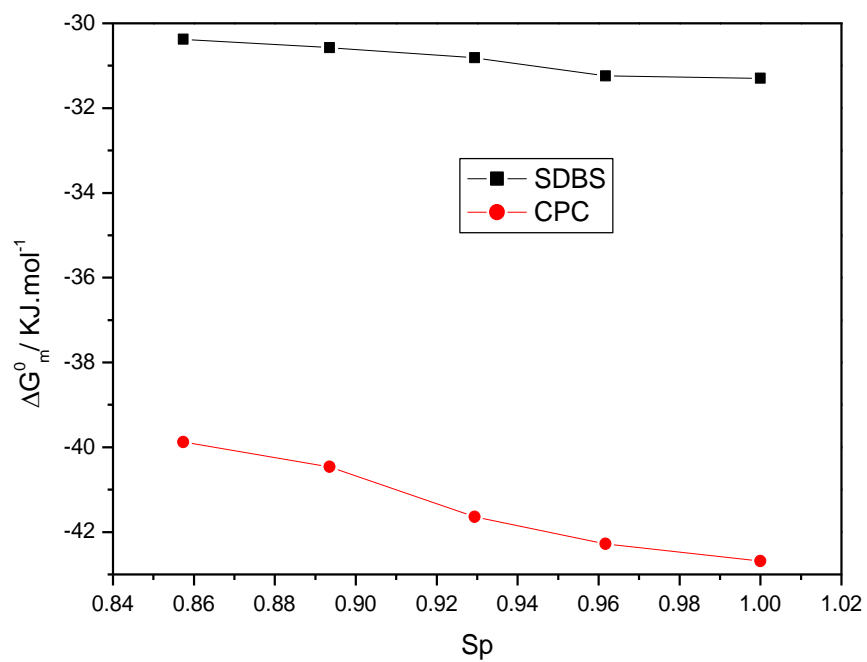


Figure 4.7: Plot of ΔG_m^0 versus Sp for at 298.15 K.

Section 4.1(b)

Influence of Bovine serum albumin (BSA) on the aggregation behaviour of ionic surfactants in aqueous medium

Aqueous solutions of surfactants (SDBS and CPC) of different molal concentrations in the range (0.3 to 3.0) mmol kg⁻¹ for SDBS and (0.2 to 2.0) mmol kg⁻¹ for CPC were prepared by the addition of small aliquots of concentrated solution of surfactant to 50 grams of aqueous solutions of BSA (0.05 % w/w, 0.075 % w/w, 0.1% w/w, 0.25% w/w and 0.5% w/w) at 293.15 to 308.15 K respectively. The solutions obtained were gently stirred using magnetic stirrer before subjecting to conductivity measurements.

William's method¹ was employed to determine CMC's of SDBS and CPC in presence of aqueous mixtures of BSA. The inflection between the plots of specific conductance (κ) versus surfactant concentration gives the value of CMC. The representative plots of κ versus [SDBS] or [CPC] in absence and presence of different concentrations of BSA at 298.15 K has been represented in figure 4.8. The CMC values for SDBS and CPC as reported in Table 4.12 increased with increase in concentration of BSA, indicating greater interactions of protein with ionic surfactants.

A Non-Linear fit approach introduced by Carpena *et al.*^{6,7} was successfully applied to elaborate conductivity data obtained on ionic surfactants (SDBS and CPC). Details of Carpena method has been given in previous section 4.1(a). The CMC values calculated from Carpena method have been reported successfully in Table 4.13. The method can be very useful when applied to ionic surfactants like SDBS which gives smooth curves where classical William's method is having difficulty in finding the inflection point. Temperature also played an inhibitory role in micellization of SDBS and CPC. The general behaviour of temperature (inhibiting micellization of ionic surfactants) can be attributed to two main reasons

- i) Desolvation of ionic head groups of these surfactants,

- ii) Disruption of ordered structures surrounding hydrophobic moieties of both SDBS and CPC done by increased thermal motions of surfactants monomers as well as solvent system⁴¹.

Table 4.12: CMC values of SDBS and CPC in aqueous mixtures of BSA at different temperatures calculated using William's Method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.29 | 1.32 | 1.36 | 1.44 | 0.92 | 1.00 | 1.06 | 1.12 |
| 0.075 | 1.31 | 1.35 | 1.38 | 1.44 | 0.96 | 1.02 | 1.10 | 1.16 |
| 0.100 | 1.38 | 1.41 | 1.44 | 1.47 | 0.98 | 1.06 | 1.14 | 1.18 |
| 0.250 | 1.41 | 1.47 | 1.53 | 1.56 | 1.00 | 1.08 | 1.16 | 1.20 |
| 0.500 | 1.44 | 1.5 | 1.56 | 1.59 | 1.10 | 1.16 | 1.20 | 1.26 |

Table 4.13: CMC values of SDBS and CPC in aqueous mixtures of BSA at different temperatures calculated using Carpena Method (non-linear fit) at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.24 | 1.26 | 1.34 | 1.40 | 0.89 | 0.94 | 1.03 | 1.13 |
| 0.075 | 1.25 | 1.27 | 1.34 | 1.41 | 0.90 | 0.97 | 1.10 | 1.14 |
| 0.100 | 1.33 | 1.38 | 1.45 | 1.47 | 0.93 | 1.04 | 1.13 | 1.18 |
| 0.250 | 1.38 | 1.44 | 1.46 | 1.52 | 0.94 | 1.05 | 1.11 | 1.16 |
| 0.500 | 1.40 | 1.46 | 1.55 | 1.56 | 1.05 | 1.07 | 1.22 | 1.24 |

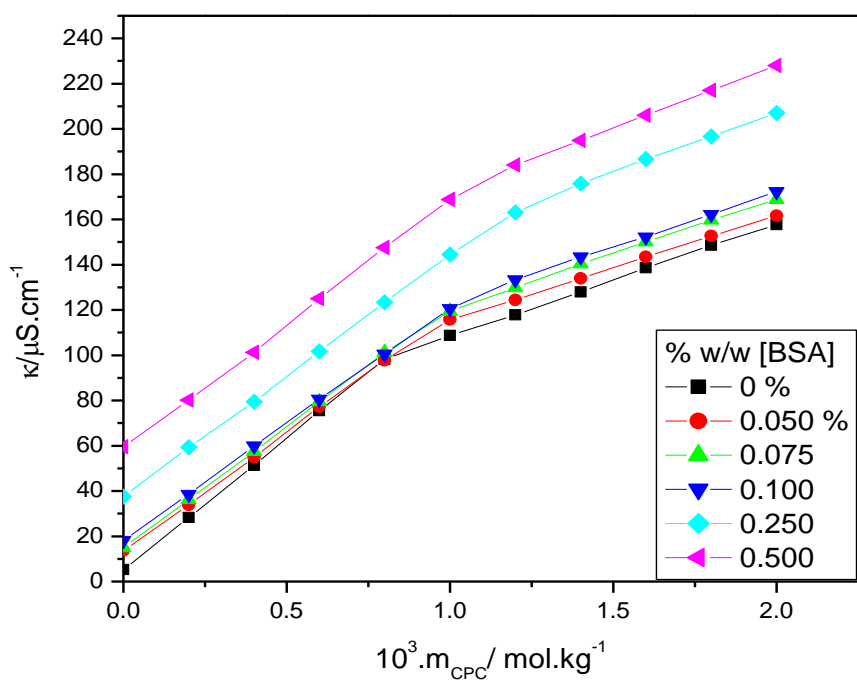
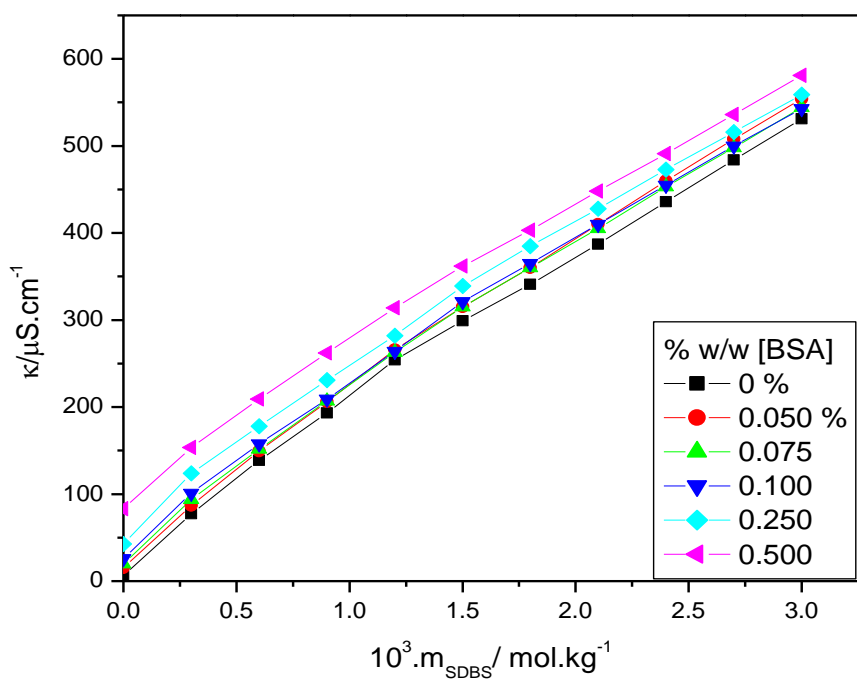


Figure 4.8: Specific conductance vs. concentration plots for (a) SDBS and (b) CPC containing different concentrations of BSA at $T = 298.15 \text{ K}$.

Influence of Bovine serum albumin (BSA) on thermodynamics of micelle formation of SDBS and CPC

Thermodynamic study of micellization was carried out to analyse the effect of BSA on the properties of ionic surfactants that controls their applications. Thermodynamic parameters of micellization provides the necessary information needed to explain how SDBS and CPC behaved in presence of BSA and confirm the type of interactions (i.e. hydrophobic or electrostatic) occurred between these protein-surfactant systems. The values of critical micellar concentration (CMC) were converted to their mole fraction unit (X_{CMC}) before determining other thermodynamic parameters of micellization. The standard thermodynamic parameters of micellization such as degree of counter ion dissociation (α), Standard Gibbs free energy of micellization (ΔG_m^0), Standard enthalpy of micellization (ΔH_m^0) and standard entropy of micellization ΔS_m^0 were calculated from the temperature dependence of X_{CMC} using the mass-action model^{41,42} and employing equations 4.2 to 4.5 (section 4.1(a)). The values of thermodynamic parameters of micellization of SDBS and CPC in presence of BSA have been presented in tables 4.14 and 4.15 respectively. The values of free energy of micellization were less negative in presence of BSA as compared to the values found in aqueous medium. This observation indicates that micellization of SDBS and CPC was less favored in presence of BSA^{43,44}. Temperature dependence of free energy values indicate that the magnitude of hydrophobic effect increases with increase in temperature for SDBS and was independent of temperature in case of CPC^{31,45}. Negative values of enthalpy of micellization for both the surfactants indicated that the micellization process was exothermic in nature. ΔH_m^0 values become more negative with respect to temperature and this behaviour of ΔH_m^0 indicated the formation of new bonds between BSA- surfactant/ surfactant- water/ BSA- water systems which may be due to existence of London- dispersion interaction which increased with increase in temperature of the studied systems⁴⁶. ΔH_m^0 of SDBS become less negative on increasing BSA concentration indicating the presence of electrostatic repulsions between SDBS and BSA due to similarly charged protein-surfactant system⁴⁷. In case of CPC- BSA (oppositely charged protein surfactant) system ΔH_m^0 values were more negative as compared to water as well as SDBS- BSA system which means the presence of electrostatic forces of attraction between oppositely charged species. ΔH_m^0 becomes more

negative up to 0.075 % w/w BSA concentration and suddenly starts decreasing from 0.1 % w/w BSA concentration. Increase in [BSA] in the system was held responsible for the promotion of hydrophobic interactions between solute – solvent/ solvent-cosolvent moieties. ΔS_m^0 values were positive for both surfactants (SDBS and CPC) over the studied temperature range and it decreased as the temperature was increased⁴⁸. This observation revealed the presence of strong hydrophobic interactions at lower temperatures which decreased with increasing temperature due to weakening of intermolecular bonds of the studied systems⁴⁹. Electrostatic repulsions between the similarly charged proteins -surfactant (SDBS- BSA) system led the ΔS_m^0 values to increase with concentration of BSA up to 0.1 w/w % BSA but after that ΔS_m^0 values were stable at higher BSA concentrations due to compensation between the liberation of water by unfolding of BSA and binding of SDBS to specific sites appeared after unfolding of protein structures⁵⁰. Such behaviour makes the micellization process less convenient and leads to increase CMC of SDBS in presence of BSA molecules⁵¹. In case of CPC-BSA system, entropy first decreases at lower BSA concentrations (up to 0.1% w/w) and then started increasing at higher BSA concentrations. This may be due to strong and specific binding of CPC to that of BSA which leads to formation of structured complexes around CPC micelles. At higher BSA concentrations hydrophobic interactions also come into existence which leads to increase in entropy of the system.

Table 4.14: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS (C) in aqueous mixtures of BSA at different temperatures and at pressure $p = 100$ kPa.

| T/K | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol^{-1} | ΔH_m^0 kJ mol^{-1} | ΔS_m^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|--------------------------------|----------------------------|----------|--|--|--|
| SDBS in 0.05 % w/w BSA | | | | | |
| 293.15 | 2.32 | 0.813 | -30.874 | -6.098 | 84.517 |
| 298.15 | 2.38 | 0.818 | -31.191 | -6.281 | 83.549 |
| 303.15 | 2.45 | 0.824 | -31.468 | -6.460 | 82.491 |
| 308.15 | 2.59 | 0.826 | -31.752 | -6.661 | 81.424 |
| SDBS in 0.075 % w/w BSA | | | | | |
| 293.15 | 2.36 | 0.817 | -30.731 | -5.800 | 85.042 |
| 298.15 | 2.43 | 0.820 | -31.090 | -5.984 | 84.203 |
| 303.15 | 2.48 | 0.826 | -31.379 | -6.154 | 83.212 |
| 308.15 | 2.63 | 0.830 | -31.601 | -6.334 | 81.997 |
| SDBS in 0.1 % w/w BSA | | | | | |
| 293.15 | 2.43 | 0.819 | -30.583 | -5.324 | 86.164 |
| 298.15 | 2.54 | 0.825 | -30.825 | -5.481 | 85.006 |
| 303.15 | 2.59 | 0.828 | -31.198 | -5.651 | 84.272 |
| 308.15 | 2.68 | 0.837 | -31.367 | -5.794 | 82.988 |
| SDBS in 0.25 % w/w BSA | | | | | |
| 293.15 | 2.54 | 0.825 | -30.296 | -5.020 | 86.225 |
| 298.15 | 2.65 | 0.827 | -30.634 | -5.183 | 85.362 |
| 303.15 | 2.75 | 0.831 | -30.934 | -5.340 | 84.427 |
| 308.15 | 2.77 | 0.837 | -31.256 | -5.489 | 83.618 |
| SDBS in 0.5 % w/w BSA | | | | | |
| 293.15 | 2.59 | 0.829 | -30.148 | -4.921 | 86.056 |
| 298.15 | 2.70 | 0.831 | -30.479 | -5.079 | 85.191 |
| 303.15 | 2.79 | 0.832 | -30.866 | -5.247 | 84.510 |
| 308.15 | 2.83 | 0.841 | -31.099 | -5.380 | 83.464 |

Table 4.15: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α)) for CPC (C) in aqueous mixtures of BSA at different temperatures and at pressure $p = 100$ kPa.

| T/K | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol^{-1} | ΔH_m^0 kJ mol^{-1} | ΔS_m^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|-------------------------------|----------------------------|----------|--|--|--|
| CPC in 0.05 % w/w BSA | | | | | |
| 293.15 | 1.66 | 0.427 | -42.197 | -14.566 | 94.254 |
| 298.15 | 1.80 | 0.436 | -42.356 | -14.981 | 91.817 |
| 303.15 | 1.91 | 0.466 | -42.023 | -15.195 | 88.499 |
| 308.15 | 2.02 | 0.468 | -42.422 | -15.673 | 86.806 |
| CPC in 0.075 % w/w BSA | | | | | |
| 293.15 | 1.73 | 0.443 | -41.605 | -14.249 | 93.317 |
| 298.15 | 1.84 | 0.448 | -41.955 | -14.696 | 91.425 |
| 303.15 | 1.98 | 0.466 | -41.862 | -15.011 | 88.574 |
| 308.15 | 2.09 | 0.474 | -42.119 | -15.429 | 86.613 |
| CPC in 0.1 % w/w BSA | | | | | |
| 293.15 | 1.76 | 0.464 | -40.981 | -13.915 | 92.330 |
| 298.15 | 1.91 | 0.472 | -41.164 | -14.322 | 90.029 |
| 303.15 | 2.06 | 0.488 | -41.132 | -14.653 | 87.345 |
| 308.15 | 2.12 | 0.491 | -41.612 | -15.109 | 86.008 |
| CPC in 0.25 % w/w BSA | | | | | |
| 293.15 | 1.80 | 0.465 | -40.875 | -13.633 | 92.929 |
| 298.15 | 1.94 | 0.482 | -40.821 | -13.943 | 90.150 |
| 303.15 | 2.09 | 0.489 | -41.039 | -14.35 | 88.037 |
| 308.15 | 2.16 | 0.492 | -41.506 | -14.799 | 86.669 |
| CPC in 0.5 % w/w BSA | | | | | |
| 293.15 | 1.94 | 0.480 | -40.197 | -11.177 | 98.991 |
| 298.15 | 2.09 | 0.491 | -40.300 | -11.474 | 96.683 |
| 303.15 | 2.19 | 0.505 | -40.431 | -11.758 | 94.582 |
| 308.15 | 2.27 | 0.505 | -40.954 | -12.144 | 93.493 |

Section 4.1(c)

Effect of co-solvents (DMSO and Glycerol) on the aggregation behaviour of ionic surfactants (SDBS and CPC) in presence of Bovine serum albumin (BSA)

Aqueous solutions of SDBS (0.3 to 3.0 mmol Kg⁻¹) and CPC (0.2 to 2.0 mmol Kg⁻¹) were prepared and added to aqueous solutions of BSA (0.05 % w/w, 0.075 % w/w, 0.1% w/w, 0.25% w/w and 0.5% w/w) at 293.15 to 308.15 K respectively. The solutions obtained were gently stirred using magnetic stirrer before subjecting to conductivity measurements. Similar experiments were repeated in presence of 5 %, 10%, 15% and 20% w/w glycerol and DMSO solutions.

Self-assembly behaviour of ionic surfactants is expressed in terms of CMC values. Micellization behaviour of SDBS+BSA/ CPC+ BSA systems in presence of co-solvents was studied by the determination of CMC values employing William's method^{2,52}. The inflection between the plots of specific conductance (κ) verses surfactant concentration gives the value of CMC. The CMC values obtained from William's method has been represented successfully in tables 4.16 to 4.23.

It is evident from the CMC data presented in tables 4.16 to 4.23 that micellization of SDBS/CPC was hindered by presence of BSA as well as both the co-solvents. The rise in CMC of both SDBS and CPC in presence of BSA is because of intermolecular interactions which increased with increase in concentration of DMSO and glycerol in the system. Presence of co-solvents (DMSO and glycerol) in the system leads to reduction in hydrophobic effect caused by hydrocarbon chain of ionic surfactants. Co-solvents dissolve the hydrocarbon chain which leads to rise in CMC values of SDBS and CPC⁵³. Rise in temperature also lead to delay in micellization process of ionic surfactants due to increased thermal motions, the organized configuration of water molecules around the hydrophobic moieties of the system (i.e. iceberg clusters) was demolished^{54,55} and hence the CMC of both surfactants SDBS and CPC increased.

Table 4.16: CMC values of SDBS and CPC in 5 % w/w glycerol containing BSA at different temperatures calculated using William’s Method at pressure p = 100 kPa.

| BSA (%w/w) | <u>CMC (10³) mM for SDBS</u> | | | | <u>CMC (10³)mM for CPC</u> | | | |
|---------------|---|--------|--------|--------|---------------------------------------|--------|--------|--------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.29 | 1.31 | 1.35 | 1.36 | 1.04 | 1.08 | 1.10 | 1.15 |
| 0.075 | 1.33 | 1.34 | 1.36 | 1.39 | 1.05 | 1.08 | 1.12 | 1.16 |
| 0.100 | 1.35 | 1.36 | 1.38 | 1.40 | 1.10 | 1.12 | 1.17 | 1.19 |
| 0.250 | 1.36 | 1.38 | 1.40 | 1.42 | 1.16 | 1.21 | 1.24 | 1.26 |
| 0.500 | 1.38 | 1.39 | 1.42 | 1.43 | 1.22 | 1.24 | 1.26 | 1.30 |

Table 4.17: CMC values of SDBS and CPC in 10 % w/w glycerol containing BSA at different temperatures calculated using William’s Method at pressure p = 100 kPa.

| BSA (%w/w) | <u>CMC (10³) mM for SDBS</u> | | | | <u>CMC (10³)mM for CPC</u> | | | |
|---------------|---|--------|--------|--------|---------------------------------------|--------|--------|--------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.33 | 1.37 | 1.38 | 1.40 | 1.12 | 1.18 | 1.22 | 1.24 |
| 0.075 | 1.35 | 1.39 | 1.40 | 1.42 | 1.14 | 1.20 | 1.23 | 1.26 |
| 0.100 | 1.38 | 1.40 | 1.42 | 1.44 | 1.20 | 1.24 | 1.28 | 1.30 |
| 0.250 | 1.40 | 1.42 | 1.44 | 1.45 | 1.24 | 1.28 | 1.32 | 1.33 |
| 0.500 | 1.42 | 1.44 | 1.46 | 1.48 | 1.28 | 1.32 | 1.34 | 1.36 |

Table 4.18: CMC values of SDBS and CPC in 15 % w/w glycerol containing BSA at different temperatures calculated using William's Method at pressure p = 100 kPa.

| BSA (%w/w) | <u>CMC (10³) mM for SDBS</u> | | | | <u>CMC (10³)mM for CPC</u> | | | |
|---------------|---|--------|--------|--------|---------------------------------------|--------|--------|--------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.39 | 1.44 | 1.47 | 1.48 | 1.24 | 1.29 | 1.34 | 1.36 |
| 0.075 | 1.40 | 1.45 | 1.48 | 1.50 | 1.30 | 1.33 | 1.37 | 1.40 |
| 0.100 | 1.42 | 1.46 | 1.49 | 1.50 | 1.32 | 1.36 | 1.40 | 1.42 |
| 0.250 | 1.44 | 1.46 | 1.50 | 1.51 | 1.36 | 1.40 | 1.43 | 1.46 |
| 0.500 | 1.46 | 1.48 | 1.50 | 1.52 | 1.40 | 1.44 | 1.46 | 1.48 |

Table 4.19: CMC values of SDBS and CPC in 20 % w/w glycerol containing BSA at different temperatures calculated using William's Method at pressure p = 100 kPa.

| BSA (%w/w) | <u>CMC (10³) mM for SDBS</u> | | | | <u>CMC (10³)mM for CPC</u> | | | |
|---------------|---|--------|--------|--------|---------------------------------------|--------|--------|--------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.41 | 1.45 | 1.48 | 1.50 | 1.28 | 1.32 | 1.36 | 1.40 |
| 0.075 | 1.42 | 1.46 | 1.48 | 1.51 | 1.32 | 1.36 | 1.38 | 1.41 |
| 0.100 | 1.44 | 1.47 | 1.49 | 1.52 | 1.34 | 1.38 | 1.40 | 1.44 |
| 0.250 | 1.46 | 1.49 | 1.50 | 1.54 | 1.40 | 1.42 | 1.46 | 1.50 |
| 0.500 | 1.48 | 1.50 | 1.52 | 1.55 | 1.50 | 1.54 | 1.55 | 1.56 |

Table 4.20: CMC values of SDBS and CPC in 5 % w/w DMSO containing BSA at different temperatures calculated using William's method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|--------|----------------------|--------|--|--------|----------------------|--------|
| | 293.15 | 298.15 | <u>T/K</u> 303.15 | 308.15 | 293.15 | 298.15 | <u>T/K</u> 303.15 | 308.15 |
| 0.050 | 1.31 | 1.34 | 1.37 | 1.44 | 1.08 | 1.12 | 1.16 | 1.20 |
| 0.075 | 1.34 | 1.36 | 1.38 | 1.45 | 1.16 | 1.19 | 1.24 | 1.26 |
| 0.100 | 1.35 | 1.38 | 1.46 | 1.48 | 1.24 | 1.26 | 1.30 | 1.35 |
| 0.250 | 1.42 | 1.48 | 1.50 | 1.52 | 1.28 | 1.30 | 1.35 | 1.36 |
| 0.500 | 1.45 | 1.50 | 1.52 | 1.53 | 1.32 | 1.35 | 1.36 | 1.38 |

Table 4.21: CMC values of SDBS and CPC in 10 % w/w DMSO containing BSA at different temperatures calculated using William's method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|--------|----------------------|--------|--|--------|----------------------|--------|
| | 293.15 | 298.15 | <u>T/K</u> 303.15 | 308.15 | 293.15 | 298.15 | <u>T/K</u> 303.15 | 308.15 |
| 0.050 | 1.38 | 1.40 | 1.45 | 1.46 | 1.20 | 1.22 | 1.28 | 1.30 |
| 0.075 | 1.39 | 1.41 | 1.46 | 1.48 | 1.23 | 1.28 | 1.30 | 1.34 |
| 0.100 | 1.40 | 1.42 | 1.47 | 1.50 | 1.32 | 1.36 | 1.39 | 1.42 |
| 0.250 | 1.44 | 1.48 | 1.51 | 1.53 | 1.36 | 1.40 | 1.42 | 1.44 |
| 0.500 | 1.46 | 1.48 | 1.52 | 1.54 | 1.41 | 1.43 | 1.44 | 1.45 |

Table 4.22: CMC values of SDBS and CPC in 15 % w/w DMSO containing BSA at different temperatures calculated using William's method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|--------|------------|--------|--|--------|------------|--------|
| | 293.15 | 298.15 | <u>T/K</u> | | 293.15 | 298.15 | <u>T/K</u> | |
| | | | 303.15 | 308.15 | | | 303.15 | 308.15 |
| 0.050 | 1.45 | 1.48 | 1.49 | 1.52 | 1.28 | 1.32 | 1.36 | 1.39 |
| 0.075 | 1.46 | 1.48 | 1.50 | 1.53 | 1.35 | 1.39 | 1.42 | 1.46 |
| 0.100 | 1.48 | 1.50 | 1.52 | 1.54 | 1.37 | 1.40 | 1.44 | 1.47 |
| 0.250 | 1.50 | 1.52 | 1.53 | 1.55 | 1.40 | 1.42 | 1.44 | 1.48 |
| 0.500 | 1.51 | 1.52 | 1.54 | 1.56 | 1.46 | 1.47 | 1.48 | 1.50 |

Table 4.23: CMC values of SDBS and CPC in 20 % w/w DMSO containing BSA at different temperatures calculated using William's method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|--------|------------|--------|--|--------|------------|--------|
| | 293.15 | 298.15 | <u>T/K</u> | | 293.15 | 298.15 | <u>T/K</u> | |
| | | | 303.15 | 308.15 | | | 303.15 | 308.15 |
| 0.050 | 1.46 | 1.48 | 1.50 | 1.53 | 1.32 | 1.35 | 1.38 | 1.42 |
| 0.075 | 1.48 | 1.49 | 1.51 | 1.54 | 1.40 | 1.42 | 1.46 | 1.48 |
| 0.100 | 1.49 | 1.50 | 1.53 | 1.54 | 1.42 | 1.45 | 1.48 | 1.50 |
| 0.250 | 1.50 | 1.52 | 1.54 | 1.56 | 1.50 | 1.51 | 1.52 | 1.54 |
| 0.500 | 1.52 | 1.53 | 1.55 | 1.60 | 1.53 | 1.54 | 1.55 | 1.56 |

The CMC values for SDBS/ CPC determined by William’s method were utilized in a non-linear fit approach introduced by Carpena *et al.*^{7,56} to elaborate conductivity data obtained for SDBS+ BSA/ CPC+BSA systems in presence of different concentrations of DMSO and Glycerol (5 %, 10 %, 15%, 20% w/w), measured at 4 different temperatures (293.15 K, 298.15 K, 303.15 K and 308.15 K). The CMC values obtained from Carpena method has been represented successfully in tables 4.24 to 4.31.

Table 4.24: CMC values of SDBS and CPC in 5 % w/w glycerol containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure p = 100 kPa.

| BSA (%w/w) | <u>CMC (10³) mM for SDBS</u> | | | | <u>CMC (10³)mM for CPC</u> | | | |
|---------------|---|---------------|---------------|---------------|---------------------------------------|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0.050 | 1.24 | 1.28 | 1.34 | 1.37 | 1.06 | 1.08 | 1.09 | 1.10 |
| 0.075 | 1.27 | 1.32 | 1.34 | 1.38 | 1.05 | 1.06 | 1.10 | 1.13 |
| 0.100 | 1.30 | 1.35 | 1.36 | 1.38 | 1.12 | 1.15 | 1.16 | 1.18 |
| 0.250 | 1.32 | 1.37 | 1.38 | 1.40 | 1.17 | 1.21 | 1.22 | 1.25 |
| 0.500 | 1.35 | 1.37 | 1.39 | 1.41 | 1.20 | 1.24 | 1.26 | 1.28 |

Table 4.25: CMC values of SDBS and CPC in 10 % w/w glycerol containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure p = 100 kPa.

| BSA (%w/w) | <u>CMC (10³) mM for SDBS</u> | | | | <u>CMC (10³)mM for CPC</u> | | | |
|---------------|---|---------------|---------------|---------------|---------------------------------------|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0.050 | 1.27 | 1.32 | 1.36 | 1.37 | 1.10 | 1.17 | 1.20 | 1.22 |
| 0.075 | 1.31 | 1.35 | 1.36 | 1.38 | 1.12 | 1.16 | 1.22 | 1.30 |
| 0.100 | 1.33 | 1.38 | 1.40 | 1.41 | 1.15 | 1.26 | 1.28 | 1.30 |
| 0.250 | 1.38 | 1.40 | 1.41 | 1.44 | 1.24 | 1.26 | 1.30 | 1.32 |
| 0.500 | 1.40 | 1.42 | 1.43 | 1.45 | 1.26 | 1.30 | 1.32 | 1.35 |

Table 4.26: CMC values of SDBS and CPC in 15 % w/w glycerol containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0.050 | 1.35 | 1.38 | 1.42 | 1.43 | 1.25 | 1.30 | 1.35 | 1.36 |
| 0.075 | 1.38 | 1.40 | 1.43 | 1.44 | 1.30 | 1.32 | 1.33 | 1.39 |
| 0.100 | 1.40 | 1.41 | 1.44 | 1.47 | 1.32 | 1.35 | 1.37 | 1.40 |
| 0.250 | 1.41 | 1.45 | 1.47 | 1.49 | 1.39 | 1.40 | 1.42 | 1.45 |
| 0.500 | 1.43 | 1.46 | 1.48 | 1.50 | 1.40 | 1.44 | 1.45 | 1.48 |

Table 4.27: CMC values of SDBS and CPC in 20 % w/w glycerol containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0.050 | 1.39 | 1.42 | 1.44 | 1.47 | 1.30 | 1.32 | 1.34 | 1.35 |
| 0.075 | 1.40 | 1.44 | 1.45 | 1.49 | 1.32 | 1.34 | 1.35 | 1.36 |
| 0.100 | 1.43 | 1.45 | 1.46 | 1.50 | 1.33 | 1.37 | 1.40 | 1.44 |
| 0.250 | 1.45 | 1.48 | 1.47 | 1.50 | 1.37 | 1.39 | 1.45 | 1.49 |
| 0.500 | 1.46 | 1.47 | 1.49 | 1.52 | 1.50 | 1.51 | 1.52 | 1.55 |

Table 4.28: CMC values of SDBS and CPC in 5 % w/w DMSO containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3) mM for CPC</u> | | | |
|---------------|--|--------|--------|--------|---|--------|--------|--------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.28 | 1.29 | 1.33 | 1.35 | 1.12 | 1.15 | 1.16 | 1.18 |
| 0.075 | 1.30 | 1.34 | 1.35 | 1.39 | 1.18 | 1.20 | 1.23 | 1.25 |
| 0.100 | 1.32 | 1.36 | 1.39 | 1.42 | 1.24 | 1.25 | 1.26 | 1.32 |
| 0.250 | 1.38 | 1.42 | 1.46 | 1.48 | 1.30 | 1.32 | 1.33 | 1.35 |
| 0.500 | 1.40 | 1.47 | 1.49 | 1.50 | 1.32 | 1.33 | 1.35 | 1.36 |

Table 4.29: CMC values of SDBS and CPC in 10 % w/w DMSO containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3) mM for CPC</u> | | | |
|---------------|--|--------|--------|--------|---|--------|--------|--------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | 293.15 | 298.15 | 303.15 | 308.15 |
| 0.050 | 1.36 | 1.38 | 1.42 | 1.45 | 1.24 | 1.25 | 1.26 | 1.28 |
| 0.075 | 1.38 | 1.40 | 1.44 | 1.47 | 1.25 | 1.26 | 1.28 | 1.34 |
| 0.100 | 1.39 | 1.40 | 1.45 | 1.49 | 1.34 | 1.36 | 1.39 | 1.40 |
| 0.250 | 1.41 | 1.46 | 1.48 | 1.51 | 1.40 | 1.40 | 1.42 | 1.44 |
| 0.500 | 1.42 | 1.45 | 1.50 | 1.51 | 1.43 | 1.43 | 1.44 | 1.45 |

Table 4.30: CMC values of SDBS and CPC in 15 % w/w DMSO containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0.050 | 1.40 | 1.43 | 1.44 | 1.47 | 1.32 | 1.33 | 1.35 | 1.36 |
| 0.075 | 1.42 | 1.46 | 1.47 | 1.48 | 1.35 | 1.36 | 1.40 | 1.42 |
| 0.100 | 1.44 | 1.45 | 1.48 | 1.49 | 1.37 | 1.40 | 1.42 | 1.45 |
| 0.250 | 1.45 | 1.47 | 1.49 | 1.51 | 1.40 | 1.41 | 1.44 | 1.46 |
| 0.500 | 1.47 | 1.48 | 1.50 | 1.53 | 1.46 | 1.46 | 1.48 | 1.49 |

Table 4.31: CMC values of SDBS and CPC in 20 % w/w DMSO containing BSA at different temperatures calculated using non-linear fit (Carpena) method at pressure $p = 100$ kPa.

| BSA (%w/w) | <u>CMC (10^3) mM for SDBS</u> | | | | <u>CMC (10^3)mM for CPC</u> | | | |
|---------------|--|---------------|---------------|---------------|--|---------------|---------------|---------------|
| | <u>T/K</u> | | | | <u>T/K</u> | | | |
| | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> | <u>293.15</u> | <u>298.15</u> | <u>303.15</u> | <u>308.15</u> |
| 0.050 | 1.43 | 1.47 | 1.48 | 1.50 | 1.30 | 1.32 | 1.36 | 1.4 |
| 0.075 | 1.45 | 1.47 | 1.49 | 1.51 | 1.39 | 1.40 | 1.44 | 1.47 |
| 0.100 | 1.47 | 1.48 | 1.50 | 1.53 | 1.40 | 1.42 | 1.47 | 1.48 |
| 0.250 | 1.48 | 1.51 | 1.52 | 1.54 | 1.47 | 1.49 | 1.50 | 1.52 |
| 0.500 | 1.49 | 1.51 | 1.53 | 1.55 | 1.52 | 1.54 | 1.54 | 1.56 |

Effect of co-solvents on thermodynamics of protein-surfactant interactions

Thermodynamic parameters of micellization for SDBS and CPC in aqueous and aqueous rich mixtures of BSA, DMSO and glycerol were successfully reported by us in previous sections 4.1(a) and 4.1 (b) ^{23,57}. Thermodynamic parameters are determined to get insights of co-solvent effects (structural and environmental) on the protein-surfactant interactions. Thermodynamics of protein surfactant interactions were calculated by employing mass action model. Values of thermodynamic parameters of micellization (viz. α , ΔG_m^0 , ΔH_m^0 and ΔS_m^0) for BSA- CPC and BSA- SDBS systems in presence of different concentrations of DMSO and Glycerol at different temperatures have been reported in tables 4.32 to 4.48. The values of derived thermodynamic parameters (viz. ΔG_m^0 , ΔH_m^0 and ΔS_m^0) for the studied systems signified that the overall trend was thermodynamically stable, spontaneous, entropically driven and dominated by London-dispersion forces of interaction. Degree of counter ion dissociation (α) increased with increase in temperature as well as co-solvent concentration. α was found to be resultant of interaction between protein and surfactant on the micellar surface and these interactions were directly proportional to co-solvent concentration. Rise in temperature also lead to the increase in values of counter ion dissociation due to high thermal agitation of the medium. It is evident from the data given in tables 4.32 to 4.48 that the free energy of micellization (ΔG_m^0) for BSA-CPC/SDBS systems was negative for all the systems studied and becomes less negative on increasing co-solvent concentration suggesting ability of forming micelles is decreased with increase in concentration of glycerol and DMSO in the medium. Such a behaviour of ΔG_m^0 showed that the micellization process was spontaneous and becomes less favorable with increase in co-solvents (DMSO and glycerol concentration)⁵⁸. It also signified that the magnitude of hydrophobic effect was found to be directly proportional to co-solvent concentration⁵⁹. ΔG_m^0 was independent of temperature in presence of glycerol in the medium which signifies that the variation of ΔH_m^0 and ΔS_m^0 compensate each over the temperature range studied in BSA+CPC+ glycerol⁶⁰ system and micellization was favored by temperature on addition of DMSO (become more negative with temperature on addition of DMSO in the system). ΔH_m^0 values were negative for all the systems studied but increase (becomes less negative) with increase in co-solvent concentration which signifies that the non-polar (London dispersion forces) interactions between BSA-CPC increases on increasing co-solvent concentration⁶¹. The ΔH_m^0 values for

BSA+ SDBS system were found to be independent of co-solvent concentration. The presence of large positive values of ΔS_m^0 revealed that the micellization process was entropy dominated / driven and it also signified the presence of London dispersion forces which came under existence on addition of co-solvents (DMSO and glycerol). Dissolution of iceberg clusters takes place on addition of DMSO and glycerol in the system and hydrophobic interactions are reduced as a result of it. In BSA+ SDBS system, presence of glycerol and increase in its concentration lead to decrease in ΔS_m^0 values of the system which signified that glycerol acted as a kosmotropic co-solvent, it stabilizes protein structure and also lead to decrease in electrostatic repulsions between (SDBS-BSA) similarly charged protein surfactant systems. On the other hand DMSO acted as chaotropic co-solvent as it destabilizes protein structure and due to which electrostatic repulsions between protein and surfactant increased. It also behaved as water structure breaker due to which entropy of the system increased at lower DMSO concentrations⁵⁷.

Table 4.32: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 5 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 1.908 | 0.472 | -40.478 | -8.823 | 107.984 |
| 298.15 | 1.944 | 0.520 | -39.802 | -8.839 | 103.852 |
| 303.15 | 1.980 | 0.529 | -40.142 | -9.079 | 102.467 |
| 308.15 | 1.998 | 0.533 | -40.678 | -9.360 | 101.632 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 1.890 | 0.479 | -40.310 | -7.290 | 112.639 |
| 298.15 | 1.944 | 0.523 | -39.726 | -7.326 | 108.669 |
| 303.15 | 2.016 | 0.530 | -40.053 | -7.536 | 107.265 |
| 308.15 | 2.088 | 0.534 | -40.476 | -7.766 | 106.150 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 1.980 | 0.488 | -39.901 | -6.038 | 115.517 |
| 298.15 | 2.016 | 0.524 | -39.551 | -6.097 | 112.207 |
| 303.15 | 2.106 | 0.531 | -39.869 | -6.274 | 110.819 |
| 308.15 | 2.142 | 0.536 | -40.326 | -6.461 | 109.899 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.088 | 0.492 | -39.615 | -5.873 | 115.103 |
| 298.15 | 2.178 | 0.525 | -39.255 | -5.942 | 111.733 |
| 303.15 | 2.232 | 0.536 | -39.527 | -6.098 | 110.232 |
| 308.15 | 2.268 | 0.537 | -40.093 | -6.296 | 109.676 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.196 | 0.497 | -39.29 | -4.435 | 118.899 |
| 298.15 | 2.232 | 0.526 | -39.132 | -4.499 | 116.160 |
| 303.15 | 2.268 | 0.537 | -39.426 | -4.616 | 114.830 |
| 308.15 | 2.340 | 0.539 | -39.912 | -4.764 | 114.061 |

Table 4.33: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0), degree of counter ion dissociation (α) and free energy of transfer ($\Delta G_{\text{trans}}^0$) for SDBS in aqueous mixtures of BSA containing 5 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa.

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.394 | 0.814 | -30.751 | -2.491 | 96.401 |
| 298.15 | 2.412 | 0.814 | -31.248 | -2.576 | 96.164 |
| 303.15 | 2.448 | 0.815 | -31.725 | -2.663 | 95.867 |
| 308.15 | 2.502 | 0.815 | -32.174 | -2.751 | 95.483 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.430 | 0.816 | -30.658 | -2.098 | 97.426 |
| 298.15 | 2.448 | 0.816 | -31.154 | -2.170 | 97.216 |
| 303.15 | 2.484 | 0.816 | -31.630 | -2.242 | 96.941 |
| 308.15 | 2.520 | 0.817 | -32.106 | -2.317 | 96.669 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.430 | 0.817 | -30.645 | -2.097 | 97.384 |
| 298.15 | 2.448 | 0.817 | -31.141 | -2.169 | 97.174 |
| 303.15 | 2.484 | 0.817 | -31.609 | -2.241 | 96.875 |
| 308.15 | 2.520 | 0.818 | -32.078 | -2.315 | 96.5867 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.448 | 0.817 | -30.603 | -2.433 | 96.094 |
| 298.15 | 2.484 | 0.818 | -31.080 | -2.517 | 95.800 |
| 303.15 | 2.520 | 0.818 | -31.550 | -2.601 | 95.492 |
| 308.15 | 2.556 | 0.818 | -32.017 | -2.687 | 95.180 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.484 | 0.818 | -30.546 | -2.170 | 96.794 |
| 298.15 | 2.502 | 0.819 | -31.019 | -2.243 | 96.516 |
| 303.15 | 2.556 | 0.821 | -31.423 | -2.315 | 96.017 |
| 308.15 | 2.574 | 0.822 | -31.906 | -2.391 | 95.781 |

Table 4.34: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 10 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.016 | 0.480 | -40.050 | -7.352 | 111.542 |
| 298.15 | 2.124 | 0.523 | -39.381 | -7.388 | 107.307 |
| 303.15 | 2.196 | 0.530 | -39.735 | -7.603 | 105.996 |
| 308.15 | 2.232 | 0.533 | -40.248 | -7.840 | 105.169 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.052 | 0.483 | -39.921 | -7.047 | 112.140 |
| 298.15 | 2.160 | 0.526 | -39.241 | -7.079 | 107.872 |
| 303.15 | 2.214 | 0.535 | -39.586 | -7.278 | 106.575 |
| 308.15 | 2.268 | 0.536 | -40.118 | -7.514 | 105.805 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.160 | 0.489 | -39.561 | -5.873 | 114.917 |
| 298.15 | 2.232 | 0.531 | -39.010 | -5.908 | 111.025 |
| 303.15 | 2.304 | 0.537 | -39.379 | -6.082 | 109.839 |
| 308.15 | 2.340 | 0.539 | -39.904 | -6.273 | 109.136 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.232 | 0.492 | -39.362 | -5.193 | 116.558 |
| 298.15 | 2.304 | 0.530 | -38.921 | -5.238 | 112.975 |
| 303.15 | 2.376 | 0.537 | -39.266 | -5.389 | 111.751 |
| 308.15 | 2.394 | 0.542 | -39.734 | -5.547 | 110.945 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.304 | 0.502 | -38.98 | -4.216 | 118.587 |
| 298.15 | 2.376 | 0.538 | -38.578 | -4.256 | 115.117 |
| 303.15 | 2.412 | 0.539 | -39.160 | -4.399 | 114.666 |
| 308.15 | 2.448 | 0.547 | -39.519 | -4.519 | 113.581 |

Table 4.35: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0), degree of counter ion dissociation (α) and free energy of transfer ($\Delta G_{\text{trans.}}^0$) for SDBS in aqueous mixtures of BSA containing 10 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.394 | 0.829 | -30.364 | -2.702 | 94.362 |
| 298.15 | 2.466 | 0.829 | -30.793 | -2.795 | 93.907 |
| 303.15 | 2.484 | 0.829 | -31.280 | -2.889 | 93.655 |
| 308.15 | 2.520 | 0.830 | -31.745 | -2.984 | 93.334 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.430 | 0.831 | -30.262 | -2.655 | 94.173 |
| 298.15 | 2.502 | 0.832 | -30.681 | -2.745 | 93.696 |
| 303.15 | 2.520 | 0.832 | -31.166 | -2.837 | 93.446 |
| 308.15 | 2.556 | 0.833 | -31.630 | -2.931 | 93.131 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.484 | 0.832 | -30.174 | -2.361 | 94.875 |
| 298.15 | 2.520 | 0.833 | -30.638 | -2.441 | 94.573 |
| 303.15 | 2.556 | 0.833 | -31.092 | -2.522 | 94.243 |
| 308.15 | 2.592 | 0.834 | -31.555 | -2.606 | 93.945 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.520 | 0.833 | -30.125 | -2.335 | 94.796 |
| 298.15 | 2.556 | 0.833 | -30.587 | -2.415 | 94.491 |
| 303.15 | 2.592 | 0.834 | -31.048 | -2.496 | 94.186 |
| 308.15 | 2.628 | 0.834 | -31.505 | -2.577 | 93.876 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.556 | 0.833 | -30.072 | -2.301 | 94.733 |
| 298.15 | 2.592 | 0.834 | -30.536 | -2.380 | 94.438 |
| 303.15 | 2.628 | 0.834 | -31.002 | -2.460 | 94.154 |
| 308.15 | 2.664 | 0.835 | -31.452 | -2.539 | 93.825 |

Table 4.36: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 15 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.232 | 0.490 | -39.413 | -6.796 | 111.264 |
| 298.15 | 2.322 | 0.527 | -38.966 | -6.859 | 107.686 |
| 303.15 | 2.412 | 0.534 | -39.276 | -7.055 | 106.287 |
| 308.15 | 2.448 | 0.535 | -39.846 | -7.286 | 105.666 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.340 | 0.497 | -39.063 | -5.413 | 114.789 |
| 298.15 | 2.394 | 0.529 | -38.797 | -5.479 | 111.747 |
| 303.15 | 2.466 | 0.536 | -39.137 | -5.636 | 110.512 |
| 308.15 | 2.520 | 0.539 | -39.640 | -5.814 | 109.771 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.376 | 0.498 | -38.987 | -5.324 | 114.831 |
| 298.15 | 2.448 | 0.531 | -38.673 | -5.386 | 111.643 |
| 303.15 | 2.520 | 0.540 | -38.977 | -5.535 | 110.315 |
| 308.15 | 2.556 | 0.540 | -39.545 | -5.716 | 109.782 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.448 | 0.503 | -38.740 | -5.006 | 115.075 |
| 298.15 | 2.520 | 0.536 | -38.423 | -5.063 | 111.888 |
| 303.15 | 2.574 | 0.540 | -38.882 | -5.220 | 111.040 |
| 308.15 | 2.628 | 0.545 | -39.306 | -5.375 | 110.114 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.520 | 0.507 | -38.525 | -3.850 | 118.282 |
| 298.15 | 2.592 | 0.538 | -38.260 | -3.899 | 115.245 |
| 303.15 | 2.628 | 0.544 | -38.696 | -4.015 | 114.402 |
| 308.15 | 2.664 | 0.549 | -39.155 | -4.135 | 113.646 |

Table 4.37: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS in aqueous mixtures of BSA containing 15 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.501 | 0.834 | -30.112 | -3.482 | 90.839 |
| 298.15 | 2.592 | 0.835 | -30.497 | -3.599 | 90.216 |
| 303.15 | 2.646 | 0.846 | -30.656 | -3.686 | 88.966 |
| 308.15 | 2.664 | 0.849 | -31.060 | -3.798 | 88.470 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.520 | 0.838 | -29.988 | -3.769 | 89.438 |
| 298.15 | 2.610 | 0.839 | -30.373 | -3.896 | 88.804 |
| 303.15 | 2.664 | 0.846 | -30.636 | -4.003 | 87.854 |
| 308.15 | 2.700 | 0.850 | -30.994 | -4.122 | 87.204 |
| 0.100 % w/w BSA | | | | | |
| 293.15 | 2.556 | 0.840 | -29.897 | -3.067 | 91.523 |
| 298.15 | 2.628 | 0.842 | -30.274 | -3.167 | 90.918 |
| 303.15 | 2.682 | 0.847 | -30.590 | -3.260 | 90.154 |
| 308.15 | 2.700 | 0.850 | -30.994 | -3.359 | 89.679 |
| 0.250 % w/w BSA | | | | | |
| 293.15 | 2.592 | 0.842 | -29.805 | -2.797 | 92.133 |
| 298.15 | 2.628 | 0.844 | -30.222 | -2.888 | 91.678 |
| 303.15 | 2.700 | 0.848 | -30.544 | -2.975 | 90.941 |
| 308.15 | 2.718 | 0.852 | -30.920 | -3.063 | 90.401 |
| 0.500 % w/w BSA | | | | | |
| 293.15 | 2.628 | 0.844 | -29.715 | -2.222 | 93.785 |
| 298.15 | 2.664 | 0.846 | -30.131 | -2.294 | 93.364 |
| 303.15 | 2.700 | 0.849 | -30.517 | -2.366 | 92.864 |
| 308.15 | 2.736 | 0.854 | -30.847 | -2.434 | 92.205 |

Table 4.38: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 20 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.304 | 0.494 | -39.197 | -6.424 | 111.796 |
| 298.15 | 2.376 | 0.530 | -38.786 | -6.484 | 108.341 |
| 303.15 | 2.448 | 0.537 | -39.157 | -6.674 | 107.151 |
| 308.15 | 2.520 | 0.537 | -39.686 | -6.895 | 106.413 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.376 | 0.500 | -38.939 | -5.092 | 115.458 |
| 298.15 | 2.448 | 0.533 | -38.621 | -4.609 | 114.077 |
| 303.15 | 2.484 | 0.537 | -39.085 | -4.749 | 113.264 |
| 308.15 | 2.538 | 0.539 | -39.613 | -4.903 | 112.641 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.412 | 0.506 | -38.705 | -4.920 | 115.25 |
| 298.15 | 2.484 | 0.534 | -38.529 | -4.995 | 112.476 |
| 303.15 | 2.520 | 0.540 | -38.963 | -5.142 | 111.565 |
| 308.15 | 2.592 | 0.543 | -39.411 | -5.301 | 110.69 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.520 | 0.507 | -38.518 | -4.585 | 115.753 |
| 298.15 | 2.556 | 0.538 | -38.310 | -5.077 | 111.466 |
| 303.15 | 2.628 | 0.541 | -38.781 | -5.239 | 110.644 |
| 308.15 | 2.700 | 0.548 | -39.125 | -5.387 | 109.487 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.700 | 0.518 | -37.998 | -3.177 | 118.783 |
| 298.15 | 2.772 | 0.539 | -38.012 | -2.678 | 118.509 |
| 303.15 | 2.790 | 0.552 | -38.280 | -2.744 | 117.221 |
| 308.15 | 2.808 | 0.554 | -38.830 | -2.831 | 116.822 |

Table 4.39: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS in aqueous mixtures of BSA containing 20 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.538 | 0.834 | -30.071 | -3.432 | 90.871 |
| 298.15 | 2.610 | 0.846 | -30.189 | -3.514 | 89.470 |
| 303.15 | 2.664 | 0.849 | -30.557 | -3.623 | 88.844 |
| 308.15 | 2.700 | 0.850 | -30.994 | -3.740 | 88.441 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.556 | 0.839 | -29.922 | -3.285 | 90.866 |
| 298.15 | 2.628 | 0.847 | -30.143 | -3.374 | 89.783 |
| 303.15 | 2.664 | 0.850 | -30.530 | -3.479 | 89.231 |
| 308.15 | 2.718 | 0.852 | -30.920 | -3.589 | 88.695 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.592 | 0.842 | -29.805 | -2.912 | 91.738 |
| 298.15 | 2.646 | 0.848 | -30.098 | -2.997 | 90.897 |
| 303.15 | 2.682 | 0.851 | -30.484 | -3.090 | 90.362 |
| 308.15 | 2.736 | 0.853 | -30.874 | -3.187 | 89.847 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.628 | 0.844 | -29.715 | -2.750 | 91.982 |
| 298.15 | 2.682 | 0.850 | -30.007 | -2.830 | 91.151 |
| 303.15 | 2.700 | 0.853 | -30.411 | -2.918 | 90.691 |
| 308.15 | 2.772 | 0.853 | -30.836 | -3.015 | 90.282 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.664 | 0.845 | -29.651 | -2.517 | 92.561 |
| 298.15 | 2.700 | 0.860 | -29.727 | -2.570 | 91.086 |
| 303.15 | 2.736 | 0.863 | -30.108 | -2.650 | 90.577 |
| 308.15 | 2.790 | 0.865 | -30.494 | -2.733 | 90.089 |

Table 4.40: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0), degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 5 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 1.94 | 0.440 | -41.234 | -7.822 | 113.976 |
| 298.15 | 2.02 | 0.453 | -41.464 | -8.027 | 112.148 |
| 303.15 | 2.09 | 0.455 | -41.957 | -8.285 | 111.073 |
| 308.15 | 2.16 | 0.466 | -42.220 | -8.502 | 109.422 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.09 | 0.446 | -40.807 | -6.416 | 117.316 |
| 298.15 | 2.14 | 0.461 | -41.027 | -6.576 | 115.548 |
| 303.15 | 2.23 | 0.464 | -41.470 | -6.785 | 114.417 |
| 308.15 | 2.27 | 0.466 | -42.016 | -6.998 | 113.641 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.23 | 0.453 | -40.393 | -6.324 | 116.215 |
| 298.15 | 2.27 | 0.463 | -40.732 | -6.496 | 114.829 |
| 303.15 | 2.34 | 0.472 | -41.060 | -6.677 | 113.418 |
| 308.15 | 2.43 | 0.479 | -41.409 | -6.869 | 112.086 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.30 | 0.490 | -39.295 | -4.736 | 117.891 |
| 298.15 | 2.34 | 0.495 | -39.787 | -4.884 | 117.067 |
| 303.15 | 2.43 | 0.496 | -40.287 | -5.046 | 116.250 |
| 308.15 | 2.45 | 0.498 | -40.858 | -5.206 | 115.698 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.38 | 0.497 | -39.005 | -3.018 | 122.760 |
| 298.15 | 2.43 | 0.499 | -39.540 | -3.118 | 122.160 |
| 303.15 | 2.45 | 0.501 | -40.111 | -3.218 | 121.698 |
| 308.15 | 2.48 | 0.502 | -40.687 | -3.323 | 121.255 |

Table 4.41: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS in aqueous mixtures of BSA containing 5 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.358 | 0.824 | -30.540 | -5.142 | 86.636 |
| 298.15 | 2.412 | 0.827 | -30.915 | -5.306 | 85.896 |
| 303.15 | 2.466 | 0.828 | -31.342 | -5.480 | 85.309 |
| 308.15 | 2.592 | 0.829 | -31.682 | -5.658 | 84.454 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.412 | 0.826 | -30.423 | -4.211 | 89.415 |
| 298.15 | 2.448 | 0.828 | -30.846 | -4.348 | 88.875 |
| 303.15 | 2.484 | 0.829 | -31.294 | -4.491 | 88.412 |
| 308.15 | 2.610 | 0.831 | -31.608 | -4.633 | 87.537 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.430 | 0.828 | -30.350 | -5.560 | 84.563 |
| 298.15 | 2.484 | 0.830 | -30.751 | -5.742 | 83.883 |
| 303.15 | 2.628 | 0.832 | -31.047 | -5.926 | 82.869 |
| 308.15 | 2.664 | 0.833 | -31.492 | -6.117 | 82.345 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.556 | 0.831 | -30.129 | -3.641 | 90.353 |
| 298.15 | 2.664 | 0.833 | -30.470 | -3.760 | 89.585 |
| 303.15 | 2.700 | 0.835 | -30.889 | -3.881 | 89.090 |
| 308.15 | 2.736 | 0.836 | -31.331 | -4.007 | 88.674 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.610 | 0.842 | -29.786 | -2.888 | 91.757 |
| 298.15 | 2.700 | 0.844 | -30.144 | -2.982 | 91.104 |
| 303.15 | 2.736 | 0.846 | -30.558 | -3.077 | 90.651 |
| 308.15 | 2.754 | 0.847 | -31.016 | -3.177 | 90.343 |

Table 4.42: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 10 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.160 | 0.443 | -40.772 | -6.409 | 117.222 |
| 298.15 | 2.196 | 0.454 | -41.108 | -6.582 | 115.801 |
| 303.15 | 2.304 | 0.459 | -41.467 | -6.781 | 114.419 |
| 308.15 | 2.340 | 0.468 | -41.853 | -6.967 | 113.210 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.214 | 0.447 | -40.571 | -6.048 | 117.767 |
| 298.15 | 2.304 | 0.467 | -40.584 | -6.176 | 115.406 |
| 303.15 | 2.340 | 0.469 | -41.132 | -6.373 | 114.658 |
| 308.15 | 2.412 | 0.472 | -41.622 | -6.574 | 113.736 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.376 | 0.456 | -40.078 | -5.319 | 118.573 |
| 298.15 | 2.448 | 0.468 | -40.329 | -5.458 | 116.956 |
| 303.15 | 2.502 | 0.472 | -40.804 | -5.627 | 116.037 |
| 308.15 | 2.556 | 0.480 | -41.181 | -5.784 | 114.869 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.448 | 0.492 | -39.033 | -4.009 | 119.475 |
| 298.15 | 2.520 | 0.496 | -39.469 | -4.134 | 118.513 |
| 303.15 | 2.556 | 0.499 | -39.992 | -4.265 | 117.854 |
| 308.15 | 2.592 | 0.504 | -40.472 | -4.393 | 117.082 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.538 | 0.504 | -38.59 | -1.946 | 125.003 |
| 298.15 | 2.574 | 0.507 | -39.114 | -2.008 | 124.453 |
| 303.15 | 2.592 | 0.509 | -39.688 | -2.074 | 124.079 |
| 308.15 | 2.610 | 0.510 | -40.284 | -2.141 | 123.782 |

Table 4.43: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS in aqueous mixtures of BSA containing 10 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | <i>A</i> | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.484 | 0.828 | -30.287 | -3.416 | 91.662 |
| 298.15 | 2.520 | 0.829 | -30.736 | -3.531 | 91.245 |
| 303.15 | 2.610 | 0.834 | -31.015 | -3.635 | 90.318 |
| 308.15 | 2.628 | 0.837 | -31.424 | -3.746 | 89.821 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.502 | 0.830 | -30.215 | -3.728 | 90.353 |
| 298.15 | 2.538 | 0.832 | -30.636 | -3.850 | 89.842 |
| 303.15 | 2.628 | 0.834 | -30.994 | -3.973 | 89.134 |
| 308.15 | 2.664 | 0.835 | -31.438 | -4.102 | 88.711 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.521 | 0.832 | -30.143 | -4.031 | 89.074 |
| 298.15 | 2.556 | 0.833 | -30.590 | -4.166 | 88.627 |
| 303.15 | 2.646 | 0.835 | -30.948 | -4.299 | 87.905 |
| 308.15 | 2.700 | 0.837 | -31.344 | -4.435 | 87.326 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.592 | 0.836 | -29.960 | -3.352 | 90.766 |
| 298.15 | 2.664 | 0.838 | -30.340 | -3.461 | 90.152 |
| 303.15 | 2.718 | 0.839 | -30.763 | -3.575 | 89.686 |
| 308.15 | 2.754 | 0.840 | -31.205 | -3.691 | 89.289 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.628 | 0.848 | -29.612 | -3.0536 | 90.596 |
| 298.15 | 2.664 | 0.849 | -30.0524 | -3.156 | 90.211 |
| 303.15 | 2.736 | 0.851 | -30.4263 | -3.257 | 89.623 |
| 308.15 | 2.771 | 0.852 | -30.8632 | -3.362 | 89.245 |

Table 4.44: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 15 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.304 | 0.446 | -40.448 | -6.152 | 116.993 |
| 298.15 | 2.376 | 0.46 | -40.656 | -6.307 | 115.206 |
| 303.15 | 2.448 | 0.463 | -41.126 | -6.505 | 114.203 |
| 308.15 | 2.502 | 0.471 | -41.505 | -6.687 | 112.991 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.430 | 0.451 | -40.100 | -5.676 | 117.429 |
| 298.15 | 2.502 | 0.472 | -40.142 | -5.794 | 115.201 |
| 303.15 | 2.556 | 0.475 | -40.653 | -5.979 | 114.379 |
| 308.15 | 2.628 | 0.480 | -41.076 | -6.157 | 113.318 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.466 | 0.461 | -39.802 | -5.267 | 117.805 |
| 298.15 | 2.520 | 0.476 | -39.990 | -5.394 | 116.037 |
| 303.15 | 2.592 | 0.479 | -40.487 | -5.567 | 115.190 |
| 308.15 | 2.646 | 0.480 | -41.039 | -5.747 | 114.529 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.520 | 0.495 | -38.852 | -3.883 | 119.287 |
| 298.15 | 2.556 | 0.500 | -39.312 | -4.001 | 118.432 |
| 303.15 | 2.592 | 0.506 | -39.778 | -4.122 | 117.616 |
| 308.15 | 2.664 | 0.508 | -40.267 | -4.253 | 116.872 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.628 | 0.503 | -38.492 | -1.883 | 124.882 |
| 298.15 | 2.646 | 0.505 | -39.072 | -1.945 | 124.524 |
| 303.15 | 2.664 | 0.507 | -39.629 | -2.007 | 124.102 |
| 308.15 | 2.700 | 0.515 | -40.03 | -2.064 | 123.206 |

Table 4.45: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS in aqueous mixtures of BSA containing 15 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol^{-1} | ΔH_m^0 kJ mol^{-1} | ΔS_m^0 $\text{JK}^{-1} \text{mol}^{-1}$ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.610 | 0.831 | -30.069 | -2.481 | 94.110 |
| 298.15 | 2.664 | 0.831 | -30.522 | -2.566 | 93.766 |
| 303.15 | 2.682 | 0.832 | -30.988 | -2.650 | 93.475 |
| 308.15 | 2.736 | 0.834 | -31.385 | -2.734 | 92.978 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.628 | 0.835 | -29.946 | -2.547 | 93.464 |
| 298.15 | 2.664 | 0.836 | -30.392 | -2.632 | 93.106 |
| 303.15 | 2.700 | 0.837 | -30.836 | -2.719 | 92.747 |
| 308.15 | 2.753 | 0.838 | -31.259 | -2.807 | 92.330 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.664 | 0.836 | -29.882 | -2.196 | 94.445 |
| 298.15 | 2.700 | 0.837 | -30.326 | -2.269 | 94.106 |
| 303.15 | 2.736 | 0.838 | -30.770 | -2.344 | 93.769 |
| 308.15 | 2.771 | 0.839 | -31.213 | -2.420 | 93.438 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.700 | 0.840 | -29.741 | -1.716 | 95.602 |
| 298.15 | 2.736 | 0.842 | -30.158 | -1.772 | 95.210 |
| 303.15 | 2.753 | 0.845 | -30.566 | -1.827 | 94.803 |
| 308.15 | 2.789 | 0.846 | -31.006 | -1.886 | 94.498 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.718 | 0.848 | -29.518 | -1.803 | 94.543 |
| 298.15 | 2.736 | 0.849 | -29.976 | -1.863 | 94.292 |
| 303.15 | 2.771 | 0.851 | -30.389 | -1.923 | 93.901 |
| 308.15 | 2.807 | 0.853 | -30.799 | -1.983 | 93.512 |

Table 4.46: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for CPC in aqueous mixtures of BSA containing 20 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.05 % w/w BSA | | | | | |
| 293.15 | 2.376 | 0.489 | -39.205 | -5.203 | 115.990 |
| 298.15 | 2.430 | 0.493 | -39.702 | -5.370 | 115.151 |
| 303.15 | 2.484 | 0.503 | -40.008 | -5.513 | 113.787 |
| 308.15 | 2.556 | 0.512 | -40.310 | -5.662 | 112.440 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.520 | 0.493 | -38.890 | -4.188 | 118.374 |
| 298.15 | 2.556 | 0.496 | -39.412 | -4.323 | 117.690 |
| 303.15 | 2.628 | 0.507 | -39.691 | -4.438 | 116.288 |
| 308.15 | 2.664 | 0.516 | -40.056 | -4.558 | 115.194 |
| 0.1 % w/w BSA | | | | | |
| 293.15 | 2.556 | 0.496 | -38.754 | -3.975 | 118.639 |
| 298.15 | 2.610 | 0.498 | -39.287 | -4.107 | 117.996 |
| 303.15 | 2.664 | 0.507 | -39.624 | -4.219 | 116.788 |
| 308.15 | 2.700 | 0.520 | -39.897 | -4.324 | 115.440 |
| 0.25 % w/w BSA | | | | | |
| 293.15 | 2.700 | 0.503 | -38.386 | -1.829 | 124.704 |
| 298.15 | 2.718 | 0.503 | -39.015 | -1.892 | 124.511 |
| 303.15 | 2.736 | 0.508 | -39.520 | -1.950 | 123.933 |
| 308.15 | 2.772 | 0.521 | -39.771 | -1.997 | 122.584 |
| 0.5 % w/w BSA | | | | | |
| 293.15 | 2.754 | 0.507 | -38.219 | -1.377 | 125.679 |
| 298.15 | 2.772 | 0.511 | -38.731 | -1.420 | 125.141 |
| 303.15 | 2.790 | 0.513 | -39.300 | -1.466 | 124.806 |
| 308.15 | 2.808 | 0.519 | -39.753 | -1.508 | 124.112 |

Table 4.47: Mole fraction of CMC (X_{CMC}) values, standard thermodynamic parameters of micellization (free energy (ΔG_m^0), enthalpy (ΔH_m^0), entropy (ΔS_m^0) and degree of counter ion dissociation (α) for SDBS in aqueous mixtures of BSA containing 20 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| <i>Temp</i> (/K) | X_{CMC} 10^5 | α | ΔG_m^0 kJ mol ⁻¹ | ΔH_m^0 kJ mol ⁻¹ | ΔS_m^0 JK ⁻¹ mol ⁻¹ |
|------------------------|----------------------------|----------|--|--|--|
| 0.050 % w/w BSA | | | | | |
| 293.15 | 2.629 | 0.832 | -30.023 | -2.545 | 93.732 |
| 298.15 | 2.664 | 0.834 | -30.444 | -2.628 | 93.293 |
| 303.15 | 2.700 | 0.835 | -30.889 | -2.715 | 92.936 |
| 308.15 | 2.754 | 0.836 | -31.313 | -2.802 | 92.519 |
| 0.075 % w/w BSA | | | | | |
| 293.15 | 2.664 | 0.835 | -29.907 | -2.181 | 94.581 |
| 298.15 | 2.682 | 0.836 | -30.372 | -2.254 | 94.309 |
| 303.15 | 2.718 | 0.838 | -30.790 | -2.326 | 93.893 |
| 308.15 | 2.771 | 0.839 | -31.213 | -2.401 | 93.497 |
| 0.100 % w/w BSA | | | | | |
| 293.15 | 2.682 | 0.838 | -29.811 | -1.951 | 95.038 |
| 298.15 | 2.699 | 0.839 | -30.275 | -2.016 | 94.779 |
| 303.15 | 2.753 | 0.839 | -30.725 | -2.085 | 94.476 |
| 308.15 | 2.771 | 0.841 | -31.159 | -2.150 | 94.138 |
| 0.250 % w/w BSA | | | | | |
| 293.15 | 2.700 | 0.841 | -29.716 | -2.145 | 94.051 |
| 298.15 | 2.736 | 0.842 | -30.159 | -2.217 | 93.719 |
| 303.15 | 2.772 | 0.845 | -30.548 | -2.286 | 93.227 |
| 308.15 | 2.807 | 0.847 | -30.960 | -2.358 | 92.819 |
| 0.500 % w/w BSA | | | | | |
| 293.15 | 2.736 | 0.843 | -29.628 | -2.761 | 91.648 |
| 298.15 | 2.753 | 0.850 | -29.932 | -2.839 | 90.871 |
| 303.15 | 2.789 | 0.851 | -30.370 | -2.932 | 90.510 |
| 308.15 | 2.880 | 0.855 | -30.670 | -3.019 | 89.731 |

Section-4.2

Spectroscopic Studies

Confirmation of binding between Bovine serum albumin (BSA) and Ionic surfactants (SDBS and CPC) using Absorbance Spectroscopy

Nature of surfactant binding with protein (BSA) has been studied using UV-Vis. Spectroscopy. Both the ionic surfactants (SDBS and CPC) selected were UV active and show UV absorbance around $\lambda = (255-265)$ nm. The absorption spectra of Bovine serum albumin (BSA) have been recorded on an UV-Visible Spectrophotometer (UV-1800 SHIMADZU) using quartz cuvette with a path length of 1 cm. The absorption spectrum of pure BSA ($2 \mu\text{M. kg}^{-1}$) and in the presence of various concentrations of SDBS and CPC has been recorded at 298.15 K in the range of wavelength $\lambda = (200-500)$ nm). Concentration of SDBS and CPC was varied from 0 to $100 \mu\text{M kg}^{-1}$. Figure 4.9 represents the absorbance spectra of BSA in presence and absence of surfactants (SDBS and CPC) in aqueous medium. Absorption of BSA, SDBS and CPC at 278 nm, 261 nm and 259 nm corresponds to $\pi-\pi^*$ transitions respectively. The absorption band of SDBS and CPC at 261 nm and 259 nm were used to study the interactions between BSA and SDBS/ CPC systems. Increase in absorbance of BSA was observed on addition of both similarly charged (SDBS) and oppositely charged (CPC) surfactants but rise in absorbance of BSA on addition of CPC was more as compared to SDBS because the formation of protein surfactant complex takes place in the oppositely charged protein-surfactant (BSA-CPC) system which was dissolved on addition of extra surfactant into the protein (BSA) solution. No such complex was formed in the case of BSA-SDBS systems because of the presence of electrostatic force of repulsion between similarly charged protein-surfactant systems.

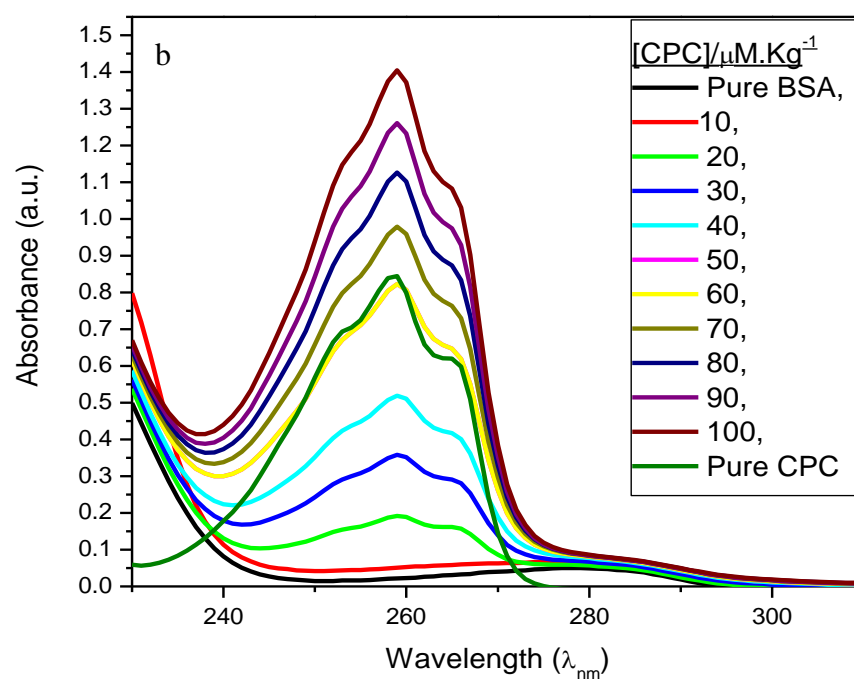
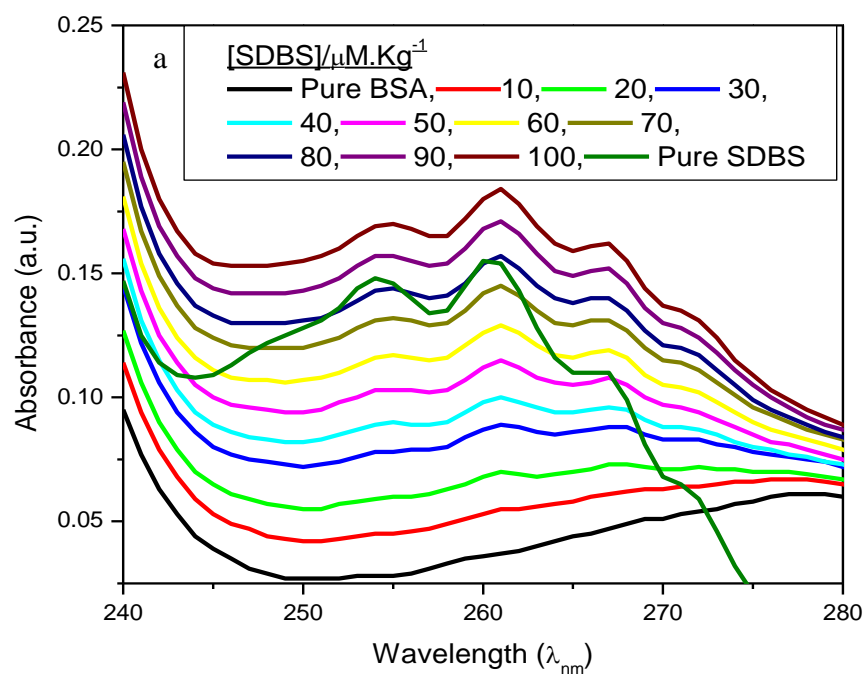


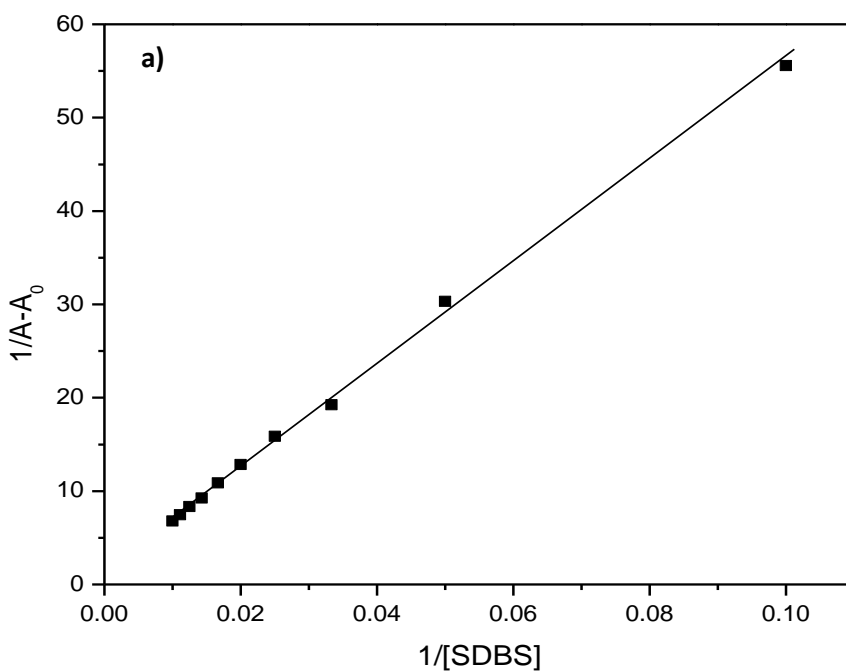
Figure 4.9: Absorption spectra of BSA at various concentrations of (a) SDBS (b) CPC in aqueous medium at 298.15 K.

Determination of Binding Constant (K):

Binding studies of ionic surfactants with proteins are required for an understanding of how specific chemical moieties may affect the binding efficiency of the protein. So determination of binding ability and interaction behaviour of BSA to that of ionic surfactants, ground state binding constant (K) was determined using Benesi-Hildebrand equation (Equation 4.11)^{62,63}.

$$\frac{1}{A - A_0} = \frac{1}{K(A_{max} - A_0)[surfactant]^n} + \frac{1}{(A_{max} - A_0)} \quad (4.11)$$

Where A_0 is the absorbance of BSA, A is the absorbance obtained with SDBS/CPC, A_{max} is the absorbance obtained with excess amount of SDBS/CPC, K is the binding constant, and $[surfactant]$ is the concentration of SDBS/CPC added (M). Ratio of intercept to the slope of the plot between $1/A - A_0$ versus $1/[surfactant]$ gives the value of binding constant (K). The binding study of ionic surfactants (SDBS and CPC) with BSA under different pH conditions was investigated to monitor both electrostatic and hydrophobic interactions and the energetics of the interaction was also measured by monitoring the binding constants.



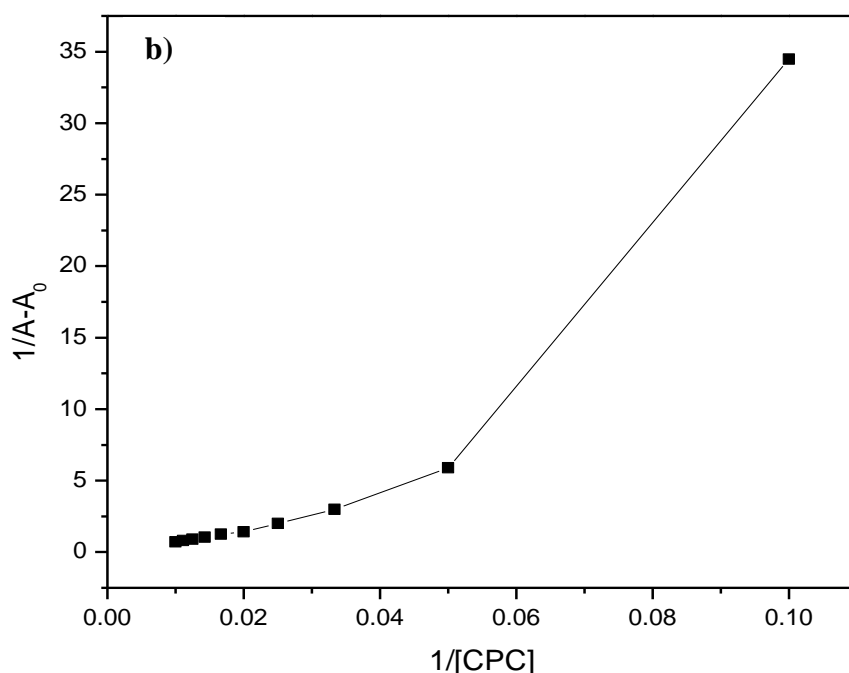


Figure 4.10: B-H plots for the calculation of K (a) BSA/SDBS and (b) BSA/CPC systems in aqueous medium.

Effect of pH on the structure and binding efficiency of BSA:

Each protein has an isoelectric pH above which its effective charge is negative and below isoelectric pH it is positive. Protein structure also changes with change of pH. In aqueous medium the physiological pH of BSA was 7.4 and it was higher to its isoelectric pH (5.4)⁶⁴. In aqueous medium BSA is negatively charged⁶⁵, so the electrostatic repulsion was felt by similarly charged protein surfactant (SDBS-BSA) system and only hydrophobic interactions were taking place, as a result poor binding of SDBS to BSA takes place in aqueous medium. In case of oppositely charged protein surfactant (CPC- BSA) system, presence of both electrostatic and hydrophobic interactions made the binding very strong. Benesi-Hildebrand (B-H) plots for the calculation of binding constant (K) in aqueous medium has been represented in figure 4.10. In order to study structural variations of BSA with respect to surfactant pH of the system was varied i.e. pH 4 (below isoelectric point) and pH 5.4 (at isoelectric point). The pH of BSA was maintained by mixed phosphate buffers of pH 4, 5.4 and 7 for the studied systems. The binding constant at these pH conditions were also

determined and reported in table 4.48. Free energy of binding was calculated by employing equation 4.12.

$$\Delta G_{binding} = RT \ln K \quad (4.12)$$

Results (Table 4.48) showed that at a pH near isoelectric point where protein is neutral, electrostatic binding between the protein surfactant systems is lacking, so poor binding was observed in both the cases. Also at pH below isoelectric point (4.0) where protein is positively charged, strong electrostatic binding has been observed with anionic surfactant (SDBS). Binding efficiency of BSA-CPC system is more than that of BSA-SDBS system because CPC promotes unfolding and aggregation of BSA, whereas the binding of the SDBS only promotes protein unfolding.⁵⁶ Free energy of binding was found negative at all the pH conditions which mean that the protein surfactant binding was a spontaneous process.

Table 4.48: Binding constants (K) and standard free energy of binding ($\Delta G_{binding}$) for SDBS and CPC at different pH.

| pH | K for SDBS | $\Delta G_{binding}$ | Net Charge | K for CPC | $\Delta G_{binding}$ |
|-----------|---|--|------------------------|---|--|
| | $10^3 \mu\text{M/Kg}$ | kJ mol^{-1} | On BSA | $10^3 \mu\text{M/Kg}$ | kJ mol^{-1} |
| 4.00 | 3.80 | -31.23 | Cationic (Positive) | 2.18 | -32.31 |
| 5.40 | 0.66 | -35.27 | Neutral | 4.55 | -30.49 |
| 7.00 | 3.16 | j-31.39 | Anionic (Negative) | 14.01 | -27.70 |

Confirmation of binding between Bovine serum albumin (BSA) and ionic surfactants (SDBS and CPC) in both aqueous and binary aqueous mixtures of DMSO and Glycerol using fluorescence spectroscopy

The fluorescence spectra of 2×10^{-6} mol/ Kg of BSA were recorded with a 1 cm path length quartz cell and titrated with different concentrations of SDBS and CPC in aqueous and binary aqueous mixtures of DMSO and glycerol. The excitation wavelength for BSA fluorescence was 280 nm and its emission spectra were scanned at the range of 290- 500 nm. All experiments were performed on Varian Carey Fluorescence spectrophotometer using a slit width (excitation = 10 nm, emission = 10 nm) at a stated excitation.

Fluorescence quenching of BSA by ionic surfactants (SDBS and CPC)

Intrinsic fluorescence of BSA is due to the fluorophores (Tryptophan, Tyrosine and Phenylalanine) present in its structure. Quantum yield of Phenylalanine is very low so in majority of cases it does not contribute in fluorescence of BSA. Fluorescence emission of BSA is quenched on addition of SDBS or CPC (figure 4.11). The dip in fluorescence of BSA on addition of ionic surfactants was resultant of conformational changes in BSA and these changes in protein structure lead to the exposure of intrinsic fluorophores to more hydrophobic environment^{66,67}. The energy transfer between BSA and ionic surfactants during this quenching phenomenon leads to interaction between protein and surfactant⁶⁸. The fluorophores quenching is divided into two categories:

- a) Dynamic quenching (due to collisions between fluorophores and quenchers which leads to formation of excited state protein –ligand complex).⁶⁹
- b) Static quenching (formation of ground state complex takes place between fluorophore and quencher).

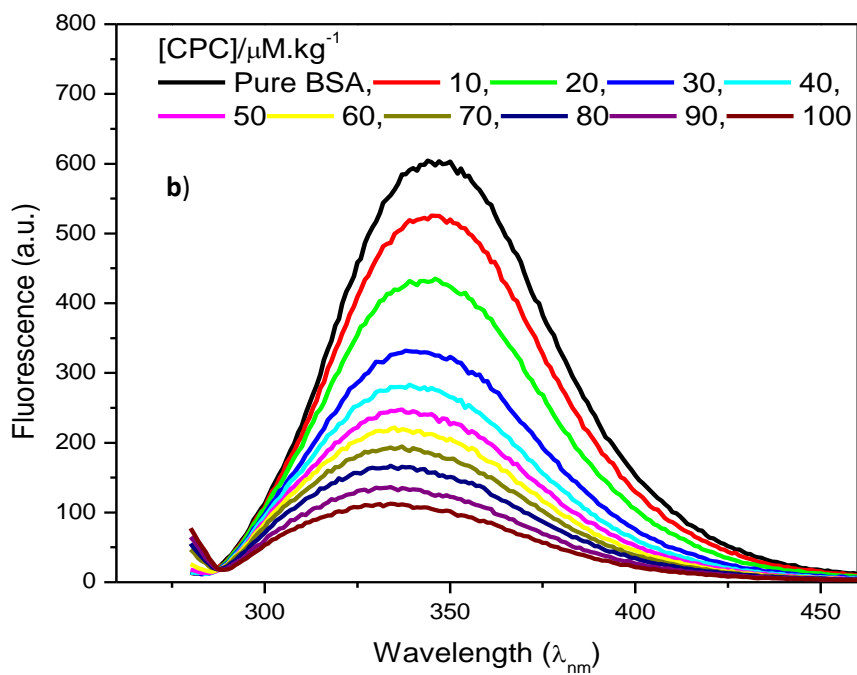
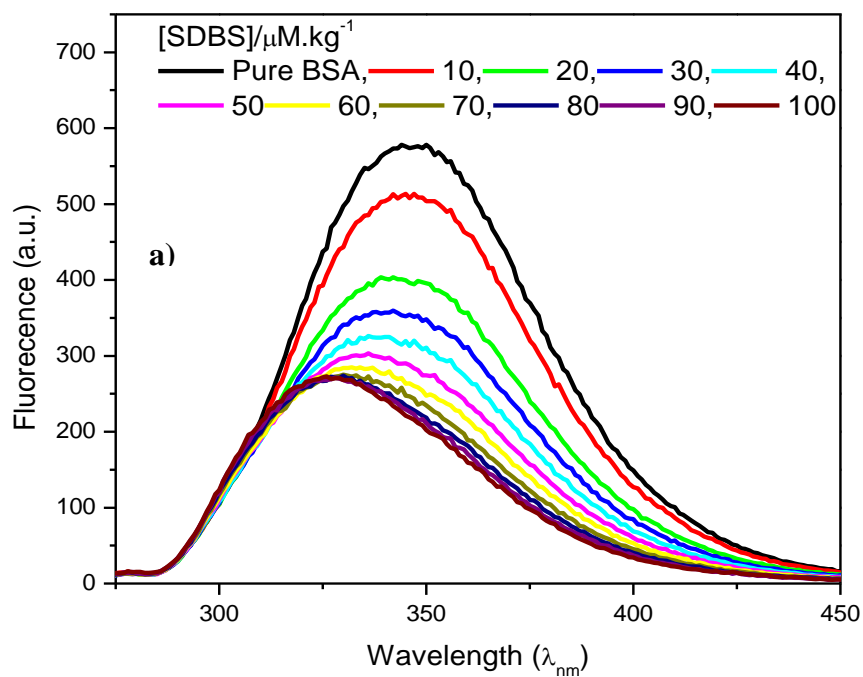


Figure 4.11: Fluorescence quenching spectra of BSA in Presence of different concentrations of a) SDBS and b) CPC at 298.15 K

Dynamic quenching usually takes place in case of protein (BSA) and ionic surfactants⁷⁰. The dynamic quenching for protein surfactant interactions is best described in terms of Stern-Volmer equation⁷¹.

$$\frac{f_0}{f} = 1 + K_{sv}[Q] \quad (4.13)$$

Where f_0 and f are the fluorescence intensities in absence and presence of quencher, Q is quencher concentration (SDBS/CPC) and K_{sv} is the Stern-Volmer quenching constant obtained by linear Stern-Volmer plot between f_0/f versus $[Q]$ (equation 4.13) at 298.15 K. The linear relationship of Stern-Volmer plot shows that the quenching is dynamic in nature. The process of quenching is also affected by the presence of co-solvents (DMSO and glycerol) in the medium. Effect of Co-solvents on fluorescence quenching of BSA in presence of ionic surfactants has been represented in figures 4.12 and 4.13. As BSA- CPC system is oppositely charged protein surfactant system so both electrostatic and hydrophobic interactions are present in this system. BSA-SDBS system is similarly charged protein surfactant system so both electrostatic (repulsions) and hydrophobic interactions are present in this system. Presence of co-solvents also affects the hydrogen bonding and Vander Waal's forces present in the studied systems. To make better understanding of quenching process free energy of quenching was also calculated using (equation 4.14)⁷².

$$\Delta G_q = -RT \ln K_{sv} \quad (4.14)$$

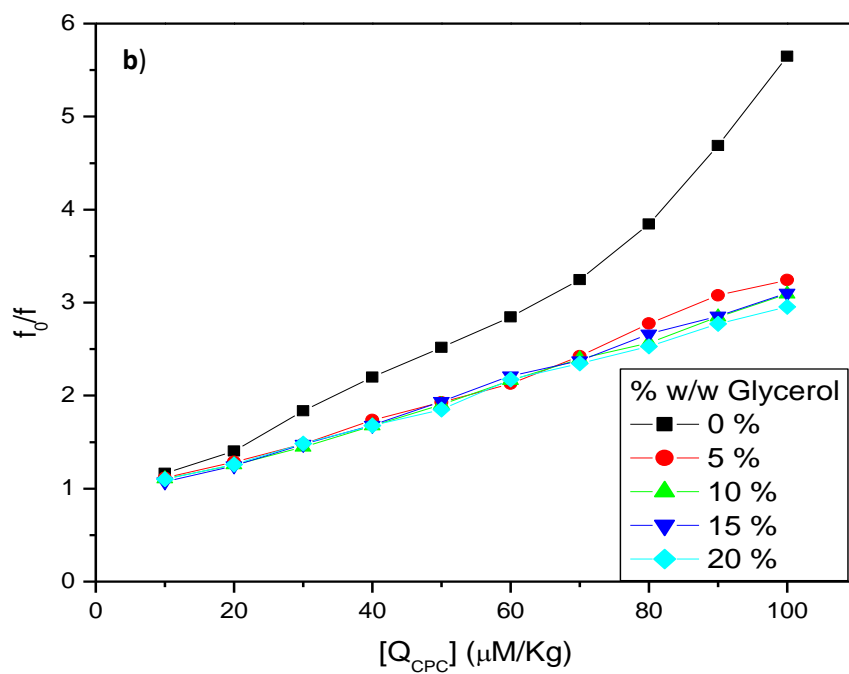
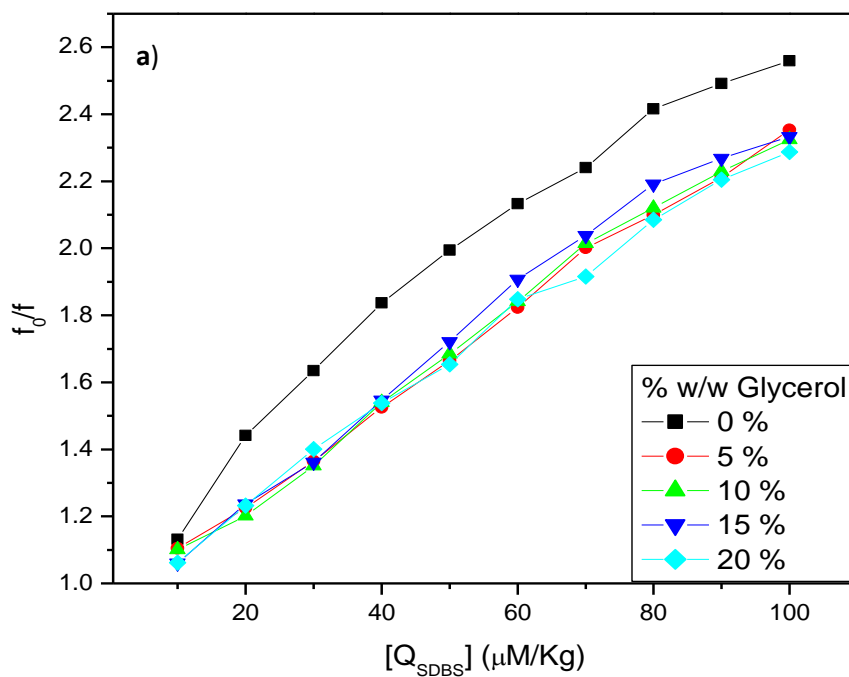


Figure 4.12: Stern Volmer plots for (a) BSA-SDBS (b) BSA-CPC in presence of different concentrations of glycerol at 298.15 K

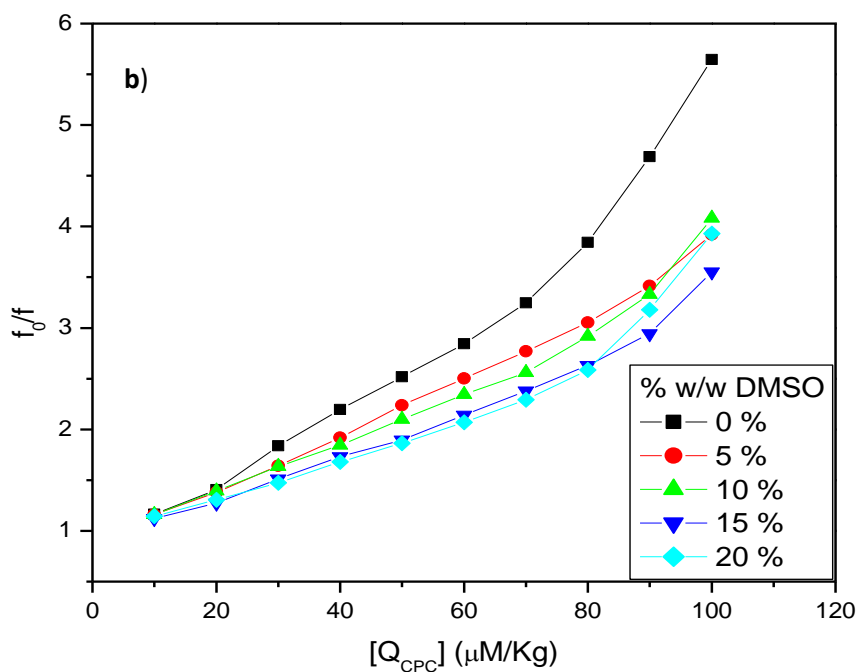
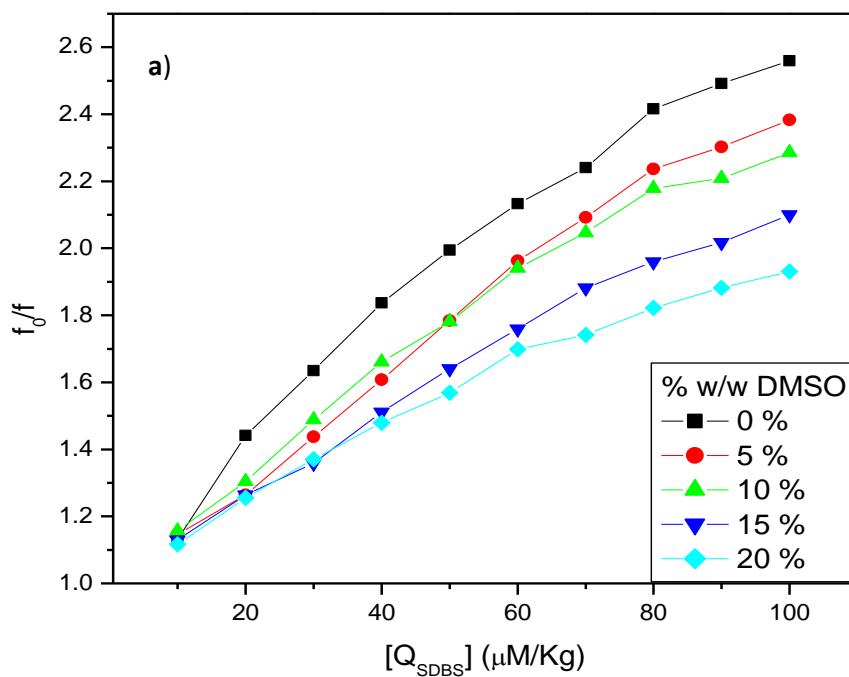


Figure 4.13: Stern Volmer plots for (a) BSA-SDBS (b) BSA-CPC in presence of different concentrations of DMSO at 298.15 K

Association Constant and Binding efficiency

The apparent association constant (K_a) for BSA-CPC system was calculated using modified Stern-Volmer equation (equation 4.15)

$$\log \left[\frac{(f_0 - f)}{f} \right] = \log K_a + n \log [Q] \quad (4.15)$$

Here, n is the number of binding sites or binding efficiency of BSA. Both K_a and n were obtained by plotting $\log \left[\frac{(f_0 - f)}{f} \right]$ vs. $\log [Q]$. The data obtained from the above said equations (Eq. 4.13, Eq. 4.14 and Eq. 4.15) in absence and presence of DMSO and glycerol have been represented in Tables 4.49 to 4.50 respectively. On analyzing the data provided in tables 4.49 and 4.50 it was observed that binding of CPC to BSA was a spontaneous process but the spontaneity of the system was decreased on addition of co-solvents. Binding of SDDBS to BSA was a spontaneous process but the spontaneity of the system was decreased on addition of DMSO and free energy of quenching was found to be independent of glycerol concentration. Values of association constant (k_a) signified that DMSO behaved as chaotropic cosolvent which destabilizes protein structure and lead to decrease in binding efficiency of BSA to SDDBS. Whereas, glycerol acted as a protein structure stabilizer and it promoted the binding of BSA with SDDBS monomers.

Table 4.49: Quenching and Binding parameters of BSA-SDBS system in presence and absence of different concentrations of co-solvents (DMSO and glycerol) at 298.15 K.

| % w/w Co-solvent | K_{sv} | Δ G | K_a | n |
|-------------------------|-----------------------|------------|----------------------|----------|
| Aqueous (0%) | 0.015 | -23.906 | 0.021 | 1.140 |
| 5 % DMSO | 0.015 | -23.761 | 0.019 | 1.025 |
| 10 % DMSO | 0.013 | -23.458 | 0.008 | 0.934 |
| 15 % DMSO | 0.011 | -23.081 | 0.007 | 0.945 |
| 20 % DMSO | 0.009 | -22.574 | 0.004 | 1.091 |
| 5 % Glycerol | 0.014 | -23.698 | 0.047 | 1.129 |
| 10 % Glycerol | 0.014 | -23.722 | 0.067 | 1.165 |
| 15 % Glycerol | 0.015 | -23.809 | 0.295 | 1.314 |
| 20 % Glycerol | 0.014 | -23.610 | 0.166 | 1.261 |

Table 4.50: Quenching and Binding parameters of BSA-CPC system in presence and absence of different concentrations of co-solvents (DMSO and glycerol) at 298.15 K.

| % w/w Co-solvent | K_{sv} | Δ G | K_a | n |
|-------------------------|-----------------------|------------|----------------------|----------|
| Aqueous (0%) | 0.047 | -26.644 | 1.749 | 1.410 |
| 5 % DMSO | 0.030 | -25.526 | 0.253 | 1.240 |
| 10 % DMSO | 0.029 | -25.512 | 0.184 | 1.211 |
| 15 % DMSO | 0.025 | -25.107 | 0.293 | 1.278 |
| 20 % DMSO | 0.028 | -25.361 | 0.209 | 1.241 |
| 5 % Glycerol | 0.024 | -25.052 | 0.350 | 1.295 |
| 10 % Glycerol | 0.022 | -24.837 | 0.388 | 1.313 |
| 15 % Glycerol | 0.022 | -24.834 | 1.386 | 1.440 |
| 20 % Glycerol | 0.021 | -24.683 | 0.303 | 1.289 |

Effect of pH on fluorescence quenching of BSA by ionic surfactants (SDBS and CPC)

The effect of pH on the quenching and binding parameters of BSA was studied primarily using mixed phosphate buffers of pH 4.0, 5.4 and 7.0 and the results has been represented in table 4.51 signified that the binding of ionic surfactants were spontaneous and thermodynamically stable with BSA. Values of n (aggregation number) demonstrates the tendency of protein (BSA) to form complex with the surfactant monomers and in case of BSA-SDBS system, the aggregation number decreased with rise in pH of the system, whereas it was found directly proportional to pH in case of BSA-CPC system. All other quenching and binding parameters behaved in the similar way and such results for the effect of pH on the binding efficiency of BSA with ionic surfactants complimented the previously reported results of (Section 4.2(a)) Absorbance Spectroscopy. Values of association constant signified strong binding of BSA-CPC system at pH 7.0 (oppositely charged protein-surfactant system). BSA changes its charge and becomes cationic at a pH 4.0 (below isoelectronic point) due which its binding with oppositely charged surfactant SDBS increases whereas it decreases to minimum for similarly charged surfactant CPC.

Table 4.51: Quenching and Binding parameters of BSA- SDBS and BSA- CPC system at different pH and at 298.15 K.

| pH | K _{sv} | ΔG | K _a | n |
|-------------|-----------------|---------|----------------|-------|
| SDBS | | | | |
| 4.0 | 0.0226 | -24.848 | 0.541 | 1.342 |
| 5.4 | 0.0196 | -24.503 | 0.330 | 1.310 |
| 7.0 | 0.0154 | -23.906 | 0.020 | 1.140 |
| CPC | | | | |
| 4.0 | 0.0225 | -24.843 | 0.043 | 1.090 |
| 5.4 | 0.0186 | -24.366 | 0.101 | 1.190 |
| 7.0 | 0.0465 | -26.644 | 1.749 | 1.410 |

Section-4.3

Computational Studies

Computational studies on the effect of co-solvents (DMSO and Glycerol) on the micellization behaviour of ionic surfactants (SDBS and CPC)

In this chapter, quantum chemical calculations and molecular dynamic (MD) simulations provided important information and represented a new insight about the study of micellization process, identifying the significant intermolecular characteristics such as aggregation phenomena and behaviour of the hydrophilic heads and the hydrophobic tails of surfactants in a certain environment such as organic solvents. This helps to establish a better molecular picture of the micellization process.

Quantum chemical calculations

The final geometry for all species involved was found using molecular dynamics simulation. Subsequent full structural optimizations and frequency calculations for the low-lying structures were carried out using B3LYP functional and 6-31G** basis set. Non-covalent interactions (NCI) were performed using the Multiwfn program⁷³.

Non-covalent interaction index (NCI)

To reveal the possible non-covalent interactions, such as hydrogen bonds, steric repulsion, Van der Waals interactions, non-covalent interaction index (NCI) was performed. NCI is based on the reduced density gradient (s) at low density regions (r). This analysis provides a graphical index, which allows the characterization of the interactions mentioned before. In this framework, the reduced density gradient is given by equation (4.16)^{74,75}

$$s = \frac{1}{2(3\pi^2)^{1/3}} \frac{\nabla\rho}{\rho^{4/3}} \quad (4.16)$$

The reduced density gradient at low density regions verifies the presence of non-covalent interactions. To distinguish between attractive and repulsive interactions, one must consider

accumulation or depletion of density in the plane perpendicular to the interaction. This is mainly characterized by the second eigenvalue, λ , of the electron-density Hessian (second derivative) matrix. The values of λ give information about the type of binding force: attractive forces, such as hydrogen bonds ($\lambda < 0$), weak interactions ($\lambda = 0$) or repulsive forces ($\lambda > 0$).

Molecular dynamic calculations

MD calculations were performed for the binary aqueous mixtures of DMSO and glycerol, with the presence of the Sodium dodecyl benzene sulfonate (SDBS) and Cetyl pyridinium chloride (CPC). The co-solvents and ionic surfactants were parameterized by analogy using the ParamChem service and implementing the CHARMM General Force Field for organic molecules⁷⁶⁻⁷⁹

The simulations were carried out using the CHARMM force field in an explicit solvent with the TIP3P water model within the NAMD software⁷⁹. Starting configurations were generated in cubic boxes with lateral dimensions of 70 Å. The systems were prepared by randomly placing co-solvents, ionic surfactants and water molecules in the simulation box using a packing molecule in defined regions of space called Packmol^{80,81}.

Firstly, each system was minimized (20,000 steps) and equilibrated (1000 ps). Then, 20 ns long production MD simulations were performed on each system. During the MD simulations, the equations of motion were integrated with a 1 fs time step in the NPT ensemble at a pressure of 1 atm. The SHAKE algorithm was applied for all hydrogen atoms and the Van der Waals (VDW) cutoff was set to 12 Å. The temperature was maintained at 298.15 K, employing the Nosée-Hoover thermostat method with a relaxation time of 1 ps. The Nosée-Hoover Langevin piston was used to control the pressure at 1 atm. Long range electrostatic interactions were considered by means of the Particle Mesh Ewald (PME) approach. Data were collected every 1 ps during the MD runs. Molecular visualization of the systems and MD trajectory analysis were carried out with the VMD software package⁸². We have measured the radial distribution functions (RDF) using VMD.

MD simulation and Quantum chemical results

Experimental data confirms that the micellization process is affected by the use of different co-solvents such as glycerol and DMSO. From the theoretical point of view, we selected two types of system, a system undisturbed by the action of the co-solvent and the other in the presence of the co-solvent as shown in figure 4.14. In the first two cases (figure 4.14 (a,d), where only the surfactant is an aqueous medium, the formation of molecular aggregates that lead to the formation of micelles, is clearly observed. In these cases, when co-solvents like glycerol and DMSO are added to water, they decrease the driving force required for the aggregation of surfactant monomer. Similar behaviour was observed in the experimental results. This suggests a modification of the water structure due to the interaction between glycerol/DMSO, with the water and ionic surfactants. In case of glycerol, a more pronounced breakdown of the micellar structure is observed (figure 4.14). This behaviour can be mainly attributed to 1) Hydrogen bonds between hydroxyl groups belonging to glycerol and the water molecules that are in contact with the hydrophilic regions (figure 4.15). 2) Van der Waals forces that occur as a result of a fluctuating electrical charge between the hydrocarbon chains of ionic surfactant and the hydrocarbon part of the glycerol (figure 4.16). This induces a temporary charge on the neighboring of the carbon atoms by attracting or repelling the electrons associated with it. This allows a better solubility of hydrocarbon chain of surfactant in the glycerol-water mixed solvent system such as was mentioned before. Therefore, it demonstrates the action of glycerol as water structure maker. In the other case, DMSO has the ability to disrupt the hydrogen bonding network of water by chaotropic effect, through the strongest hydrogen bonds interactions between the DMSO oxygen atom and a water-hydrogen atom, being more stable than the hydrogen bond of the water. We also observe that oxygen atom of a water molecule is in close proximity to a hydrogen atom of the DMSO methyl group. In the same way, DMSO methyl group has a weak interaction (Van der Waals forces) with the hydrocarbon chain in the ionic surfactant (figure 4.17). In both cases, the Van der Waals forces are more intense when the co-solvent is added (figure 4.15), and the intermolecular hydrogen bridges in the structural water network are interrupted. Therefore, it is clear that the supramolecular assembly is inhibited by the presence glycerol and DMSO, because hydrophobic regions in the solvent media increased with increasing concentration of

the non-aqueous solvents imparting co-solvency and promotes the decrease of the amphiphilic assembly.

This analysis can be complemented through the radial distribution functions (RDFs) as shown in figure 4.18 and 4.19, respectively, where the terminal carbon of each tail of the surfactant were taken as reference. These figures show an intense peak located at (3–4) Å, which corresponds to the first sphere of solvation. Here it is possible to observe, that there is a lower probability of finding water molecules near the tail of the surfactant in the micellar system (SDBS+water and CPC+water). A higher probability for the case of DMSO and glycerol was observed, being higher in the case of the glycerol. This is in agreement with the experimental results previously discussed.

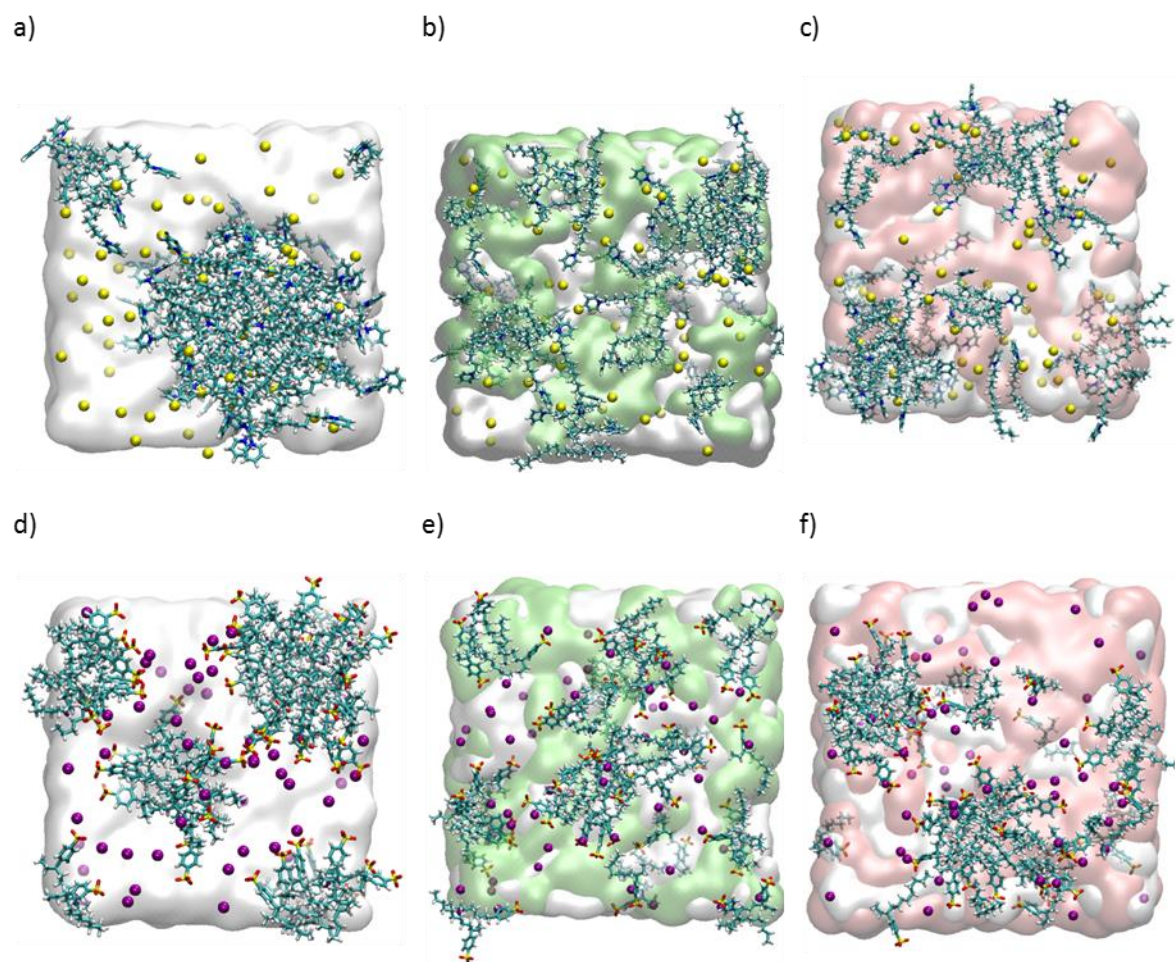


Figure 4.14: Schematic representations at the end (20 ns) of their respective production run for a bulk simulation with the different molecules being shown in different colors and (a-f) several mixture of solution models: (a) A mixture of water and ionic surfactant CPC. (b) Water and co-solvent glycerol with ionic surfactant CPC in the interface. (c) Water and co-solvent DMSO with ionic surfactant CPC in the interface. (d) A mixture of water and ionic surfactant SDBS. (e) Water and co-solvent glycerol with ionic surfactant SDBS in the interface. (f) Water and co-solvent DMSO with ionic surfactant SDBS in the interface. CPC and SDBS are in licorice representation. Water (white), glycerol (lime) and DMSO (pink) are in quicksurf representation.

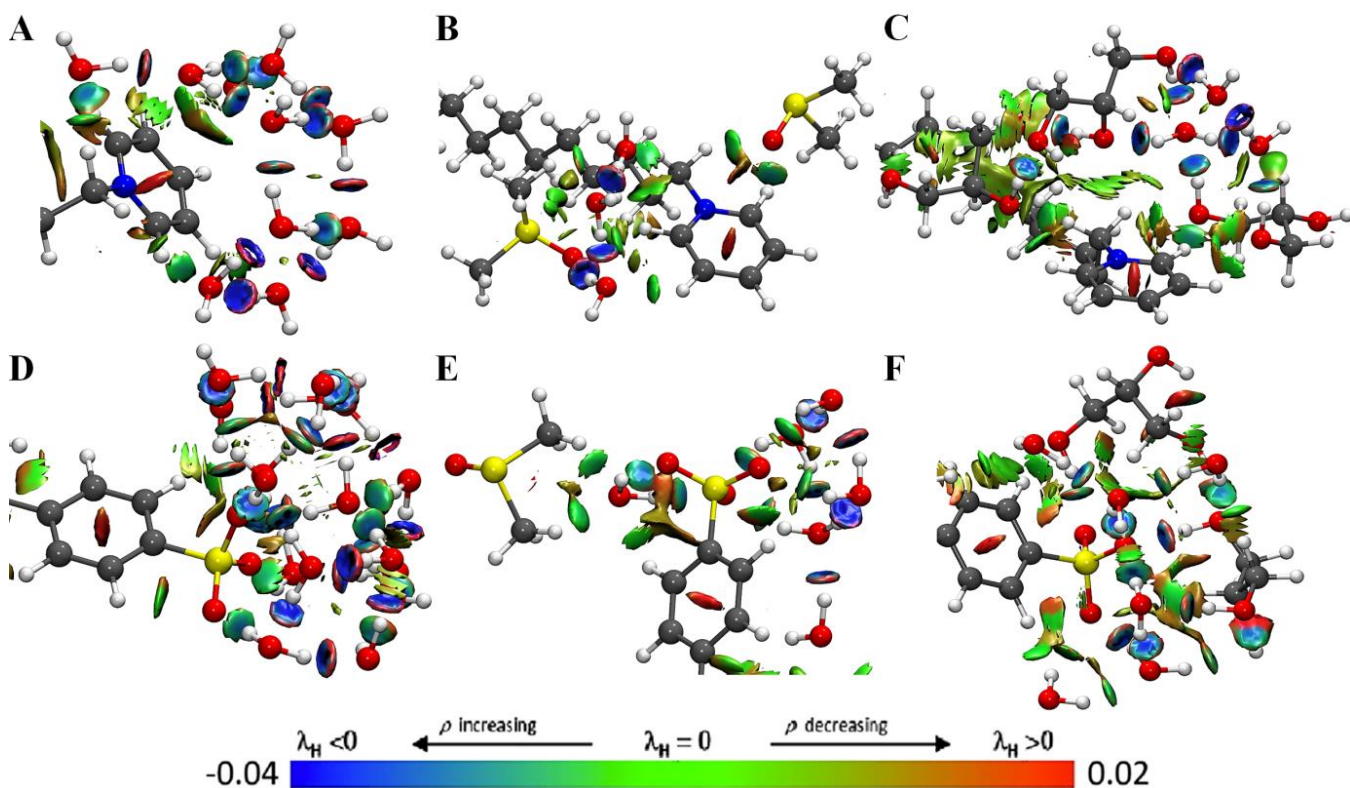


Figure 4.15: Bottom of the NCIPLoT gradient isosurface (0.6 a.u.) for four geometries structures (A = Water with ionic surfactant CPC. B = Water and co-solvent DMSO with ionic surfactant CPC in the interface. C = Water and co-solvent glycerol with ionic surfactant CPC in the interface. D = Water with ionic surfactant SDBS. E = Water and co-solvent DMSO with ionic surfactant SDBS in the interface. F = Water and co-solvent glycerol with ionic surfactant SDBS in the interface, all unit's kcal/mol-1). The surfaces are colored on a blue-green-red scale according to the strength and type (attractive or repulsive) of interaction. Blue indicates strong attractive interactions, green indicate weak VDW interactions, and red indicates strong non-bonded overlap. These calculations were done at the B3LYP/6-31G** level.

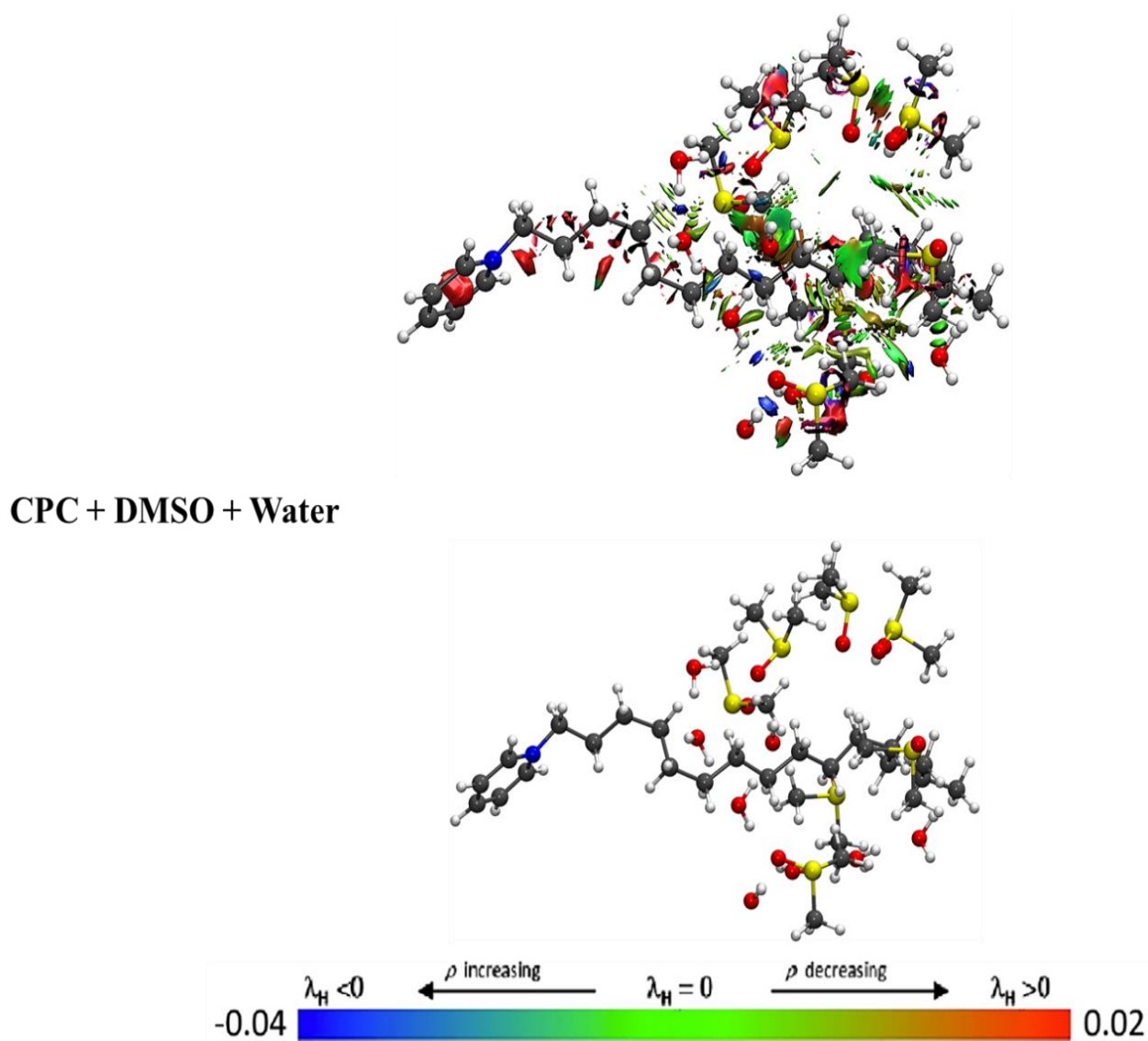


Figure 4.16. NCIPlot gradient isosurface (0.6 a.u.) for Water and co-solvent DMSO with ionic surfactant CPC. The surfaces are colored on a blue-green-red scale according to the strength and type (attractive or repulsive) of interaction. Blue indicates strong attractive interactions, green indicate weak VDW interactions, and red indicates strong nonbonded overlap. These calculations were done at the B3LYP/6-31G** level.

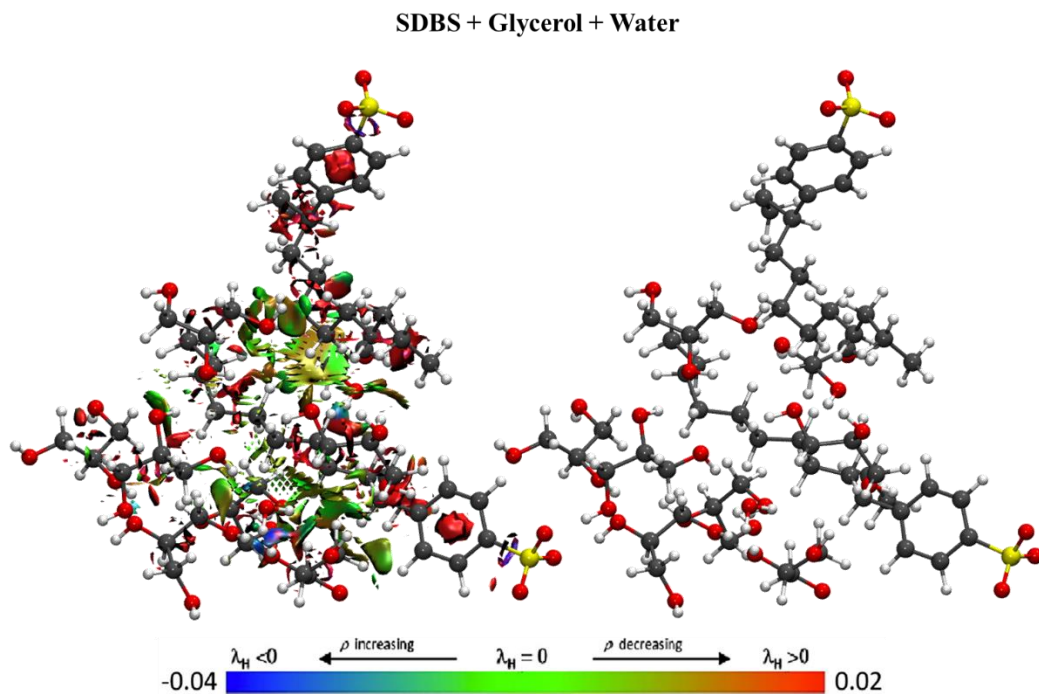


Figure 4.17. NCIPlot gradient isosurface (0.6 a.u.) for Water and co-solvent glycerol with ionic surfactant SDBS. The surfaces are colored on a blue-green-red scale according to the strength and type (attractive or repulsive) of interaction. Blue indicates strong attractive interactions, green indicate weak VDW interactions, and red indicates strong non-bonded overlap. These calculations were done at the B3LYP/6-31G** level.

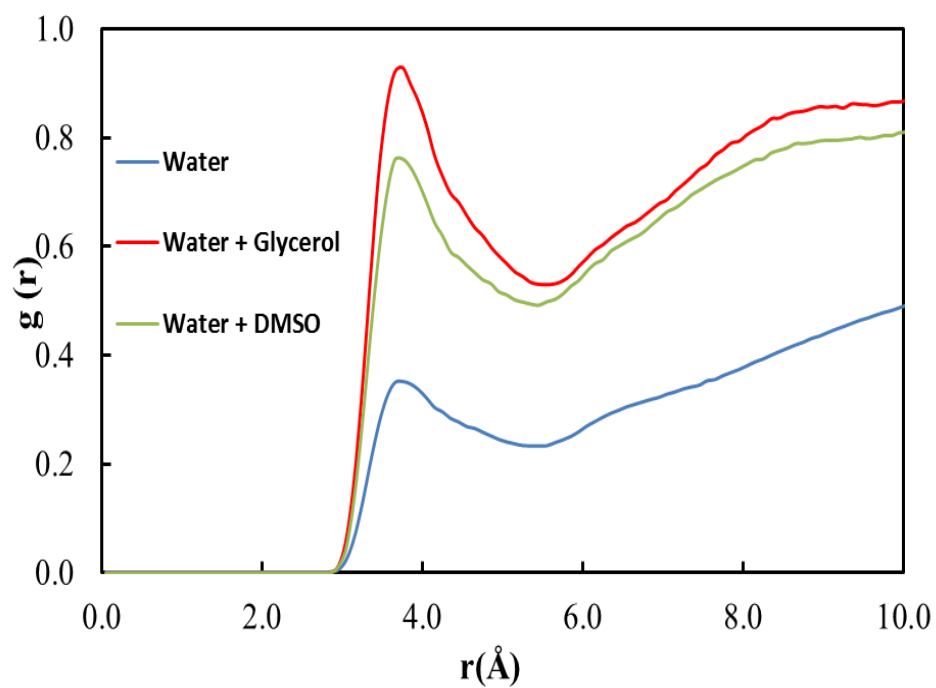


Figure 4.18: Radial distribution function between the terminal carbon of tail of SDBS and the oxygen atom in the water molecule

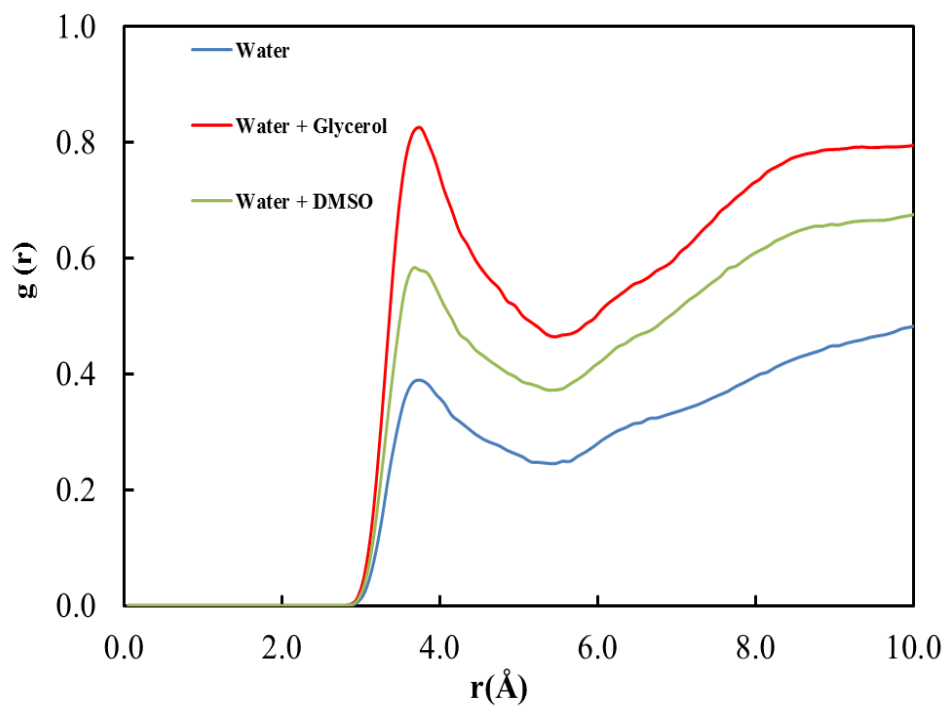


Figure 4.19: Radial distribution function between the terminal carbon of tail of CPC and the oxygen atom in the water molecule.

Theoretical studies on the molecular interactions between Bovine serum albumin (BSA) and ionic surfactants (SDBS and CPC)

The understanding of molecular interactions of proteins such as Bovine serum albumin (BSA) in micellar environments is very important because BSA serves as carrier of various drugs, hydrophobic ligands, and is collectively used with surfactants in different industrial processes. This section reports the interactions of Bovine serum albumin (BSA) with Sodium dodecyl benzene sulfonate (SDBS) and Cetyl pyridinium chloride (CPC) using various computational techniques.

Binding pockets determination.

Binding pockets of the target protein BSA was determined by metaPocket 2.0 (<http://projects.biotech.tu-dresden.de/metapocket/>). It is a consensus method in which eight methods-(LIGSITECS, PASS, Q-Site Finder, SURFNET, Fpocket, GHECOM, Con-Cavity and POCASA) are combined to improve the prediction success rate. There are three steps in the metaPocket 2.0 procedure: calling-based methods, generating meta-pocket sites and mapping ligand-binding residues. Then, only the top 1 pocket sites in each method are taken into further consideration.

Molecular Docking

AutoDock (v 4.2.1) and AutoDock Vina⁸³ (v 1.0.2) were used for all dockings in the present study. The three-dimensional coordinates of ionic surfactants structures were obtained from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov>). The geometry optimizations of the SDBS and CPC were carried out by applying semi-empirical PM7 method, using MOPAC⁸⁴ software with RMS gradient of 0.001 kcal/mol. When ionic surfactants structures were not available from PubChem, they were drawn using Discovery Studio⁸⁵ 3.1 (Accelrys, CA). The ligand files were prepared using the AutoDock Tools package⁸⁶ (<http://autodock.scripps.edu>) provided by AutoDock by accepting all rotatable bonds. The crystal structure of BSA (PDB ID: 3V03) was downloaded from Protein Data Bank. The protein BSA treated with the

protein preparation wizard by Maestro (Schrodinger NY); polar hydrogen atoms were added, nonpolar hydrogen atoms were merged, and charges were assigned. Docking was treated as rigid and carried out using the empirical free energy function and the Lamarckian Genetic Algorithm provided by AutoDock Vina. The grid map dimensions were 10 by 10 by 10 Å³, with 0.375 Å spacing between the grid points, making the binding pockets of BSA the center of the cube. All other parameters were set as the default defined by AutoDock Vina. Dockings were repeated 20 times with 20 conformations. The two best interaction energy of binding (kJ/mol) was selected for evaluation.

Molecular Docking Results

The experimental results were complemented with molecular docking in which SDBS and CPC was docked to BSA, to determine the preferred binding site and the binding mode. There are a total of 8 pocket sites for BSA including the binding site II, which were clustered using a simple hierarchical clustering algorithm, according to their spatial similarity (distance based) as shown in figure 4.20.

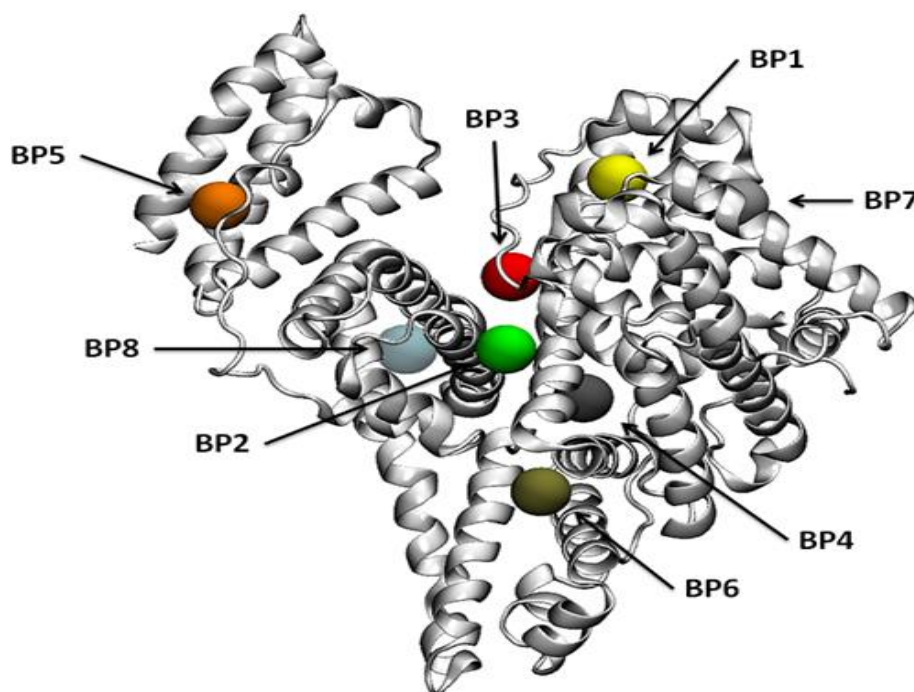


Figure 4.20: Schematic representation of X-ray crystallographic structure of BSA (PDB ID: 3V03) indicating the binding pockets (BP) as determined by metaPocket server. The binding pockets were displayed with spheres different colours.

The description of the 3-D structure of crystalline albumin have revealed that BSA is made up of three homologous domains (I, II, and III): I (residues 1-183), II (184-376), III (377-583), each containing two subdomains that assemble to form heart-shaped molecule^{87,88}, which are divided into nine loops by 17 disulphide bonds^{89,90}, each one formed by six helices, and its secondary structure is dominated by α -helix. The pocket of subdomain IIa corresponds to the binding site I, which is very well-known to be a binding site for several drugs such as warfarin^{90,91}. The subdomain IIIa is known to be drug site II, which is similar to drug site I. Thyroxin, octanoate, and some other drugs binds to this site^{92,93}. For long chain fatty acids, three binding sites with entirely different structural environments have been reported⁹⁴. In this study the three domains mentioned above were evaluated and 8 pocket sites of BSA were found (Figure 4.26), each site was docked with CPC and SDBS into the 3D structure of BSA using AutoDock Vina. These models provide the most probable binding sites and pose in the protein that has not resulted in structural alteration of BSA. The lowest energies obtained are shown in Table 4.52.

Table 4.52: ΔG° of binding CPC-BSA and SDBS-BSA ($\text{kJ}\cdot\text{mol}^{-1}$) for docking positions.^a

| Complex | Binding Pockets | | | | | | | |
|----------|-----------------|--------|--------|--------|--------|--------|--------|--------|
| | BP1 | BP2 | BP3 | BP4 | BP5 | BP6 | BP7 | BP8 |
| CPC-BSA | -28.03 | -23.85 | -23.85 | -21.76 | -10.46 | -28.03 | -22.18 | -25.52 |
| SDBS-BSA | -29.71 | -19.66 | -26.78 | -20.92 | -10.88 | -28.45 | -25.94 | -28.03 |

^a In each site, the energy was calculated to see which site had the highest degree of union with the ligand.

From the data given in table 4.52, it can be observed that the values for ΔG° are negative and are in excellent agreement with the values experimentally obtained in table 4.48 (Section 4.2(a)), which continues to confirm the spontaneity of the binding process. In order of decreasing energy, the binding sites can be ordered in the following way: BP1 > BP 6 > BP 8 > BP 3 > BP 7 > BP 2 > BP 4 > BP 5. Where it is visible that BP1 and BP 6 represent the best binding sites for surfactants. Based on this energetic order, a physicochemical analysis of the BP 1 and BP 6 was carried out. From this analysis it can be argued that BP 1 consists of 6 hydrophobic residues, 3 charged, 1 acidic and 1 basic, with a Solvent-Accessible Surface Area (SASA) of 1927.50 \AA^2 ; while for PB6 there are 7 hydrophobic, 5 charged, 2 acidic and 3 basic residues, with a SASA of 2285.13 \AA^2 . The locations of these sites in the 3D structure

of BSA are shown in figure 4.21 and figure 4.22. It is encouraging to note that the binding sites in BSA are similar to some of the known binding sites as mentioned above, although no bias was introduced in defining the binding sites in BSA. The results obtained from the docking show that for the PB1 site, the CPC-BSA and SDBS-BSA complexes are located in the hydrophobic cavity, near the hydrophobic residues of opposite positive charge such as Leu115, Pro117, Leu122, Phe133, Lys136, Tyr137, Glu140, Ile141, Tyr160, Ile181 and Arg185, suggesting the existence of hydrophobic interaction between them. While for the BP 6 site, the CPC-BSA and SDBS-BSA complexes are surrounded in a pocket that has more character of Van der Waals interactions. Besides hydrophobic and polar interactions with the surrounding Arg208, Ala209, Lys211, Val215, Phe227, Asp323, Leu326, Gly327, Leu346, Ala349, Lys350, Glu353 and Leu480. Therefore, it can be concluded that the interaction between the complex CPC-BSA and SDBS-BSA was dominated by hydrophobic forces as well as hydrogen bonds.

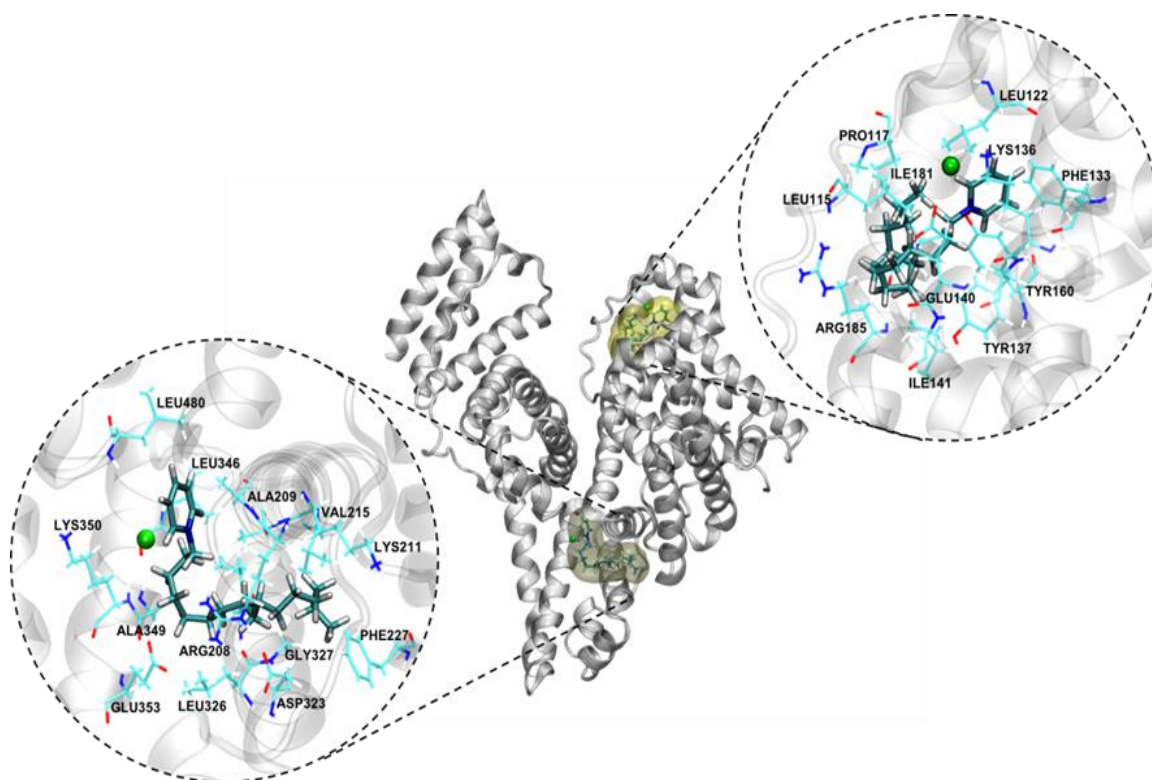


Figure 4.21: Molecular docked model of CPC (Licorice representation) located within the pockets BP1 (Left) and BP6 (Right) of BSA. The surrounding amino acid residues of BSA within 3Å from CPC (cyan colored).

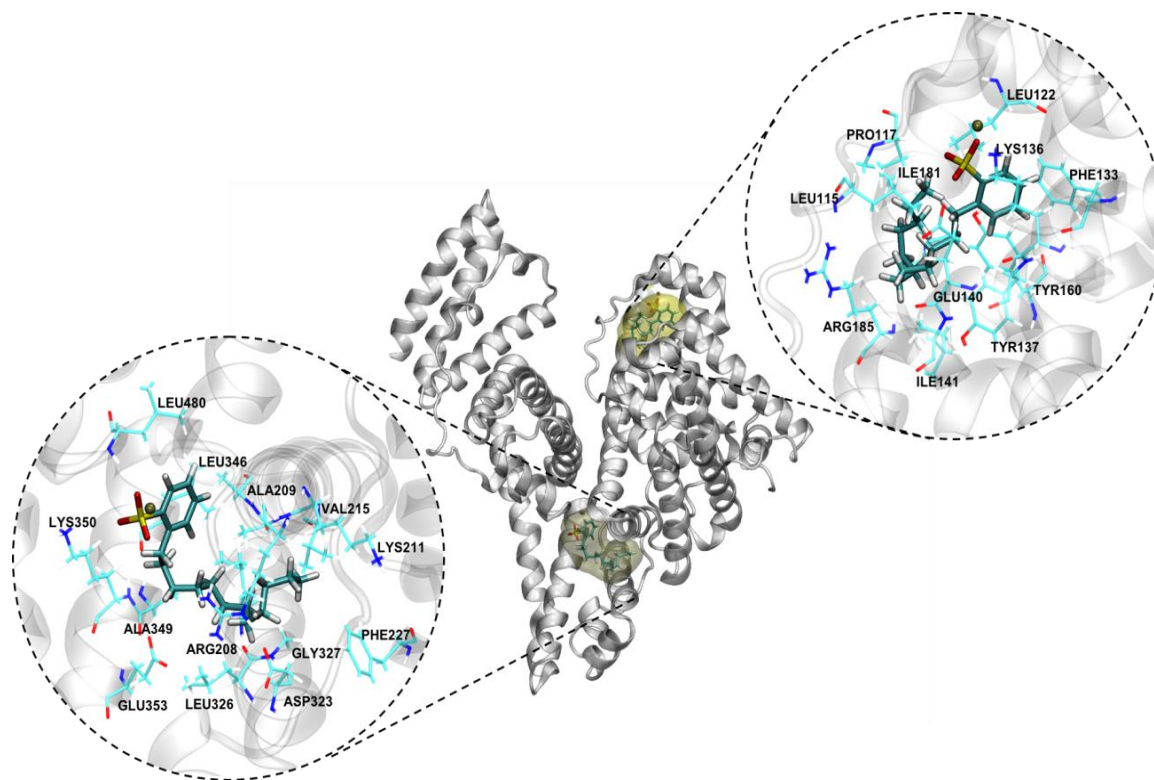


Figure 4.22: Molecular docked model of SDBS (Licorice representation) located within the pockets BP1 (Left) and BP6 (Right) of BSA. The surrounding amino acid residues of BSA within 3Å from SDBS (cyan coloured).

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Chapter-5

Summary

and

Conclusions

Summary and Conclusions

The Present research work involves the study of micellization behaviour, thermodynamic behaviour of micellization and molecular interactions of ionic surfactants (SDBS and CPC) in different solvent environment (i.e. both aqueous and binary aqueous mixtures of DMSO and glycerol). Myriad experimental and theoretical techniques like conductometric, UV-visible spectroscopy, fluorescence spectroscopy and computational methods (i.e. molecular stimulation and molecular docking) have been used to fulfill these objectives.

A brief account of applications of micellization of surfactants (SDBS and CPC), co-solvents (DMSO and glycerol), folding and unfolding of protein (BSA) and protein-surfactant interactions have been provided in Chapter-1. A literature review including micellization behaviour of ionic surfactants in different solvent systems and influence of proteins involving a wide variety of experimental techniques has been presented in Chapter-2. Available literatures however, record very limited studies on this topic using, such experimental techniques as conductance, UV-visible spectroscopy, fluorescence spectroscopy and computational studies. The aim and the objectives of the present work have also been elaborated (on the basis of research gap) in Chapter-2.

The above mentioned experimental techniques have been employed to achieve the goal of the present work have been described in detail in Chapter – 3, the purification of chemicals, calibration of equipment's and experimental procedure for measurements of various parameters have also been described in this chapter-3.

The significant conclusions of the results obtained (Chapter 4) from various techniques have been given below:

Section 4.1- Conductometric studies

Section 4.1 (a) represents the micellization behaviour of SDBS and CPC in aqueous and binary aqueous mixtures of DMSO and glycerol using conductometric technique. This study helped to understand the micellization process of SDBS and CPC in mixed aquo-organic solvents. Micellization of both the surfactants was inhibited by the co-solvents. Temperature

also played an inhibitory role in micellization of ionic surfactants (SDBS and CPC). On analyzing the thermodynamic parameters, it was found that the micellization of both the surfactants were entropy driven and spontaneous in nature. Comparison between micellization behaviour of SDBS and CPC exhibited the importance of aromatic head groups in self-assembly of these surfactants. Effect of solvent parameters on the micellization of ionic surfactants has also been emphasized in this section and which suggested that the inhibitory effect of glycerol dominated over DMSO.

Section 4.1 (b) shows the influence of BSA on the micellization behaviour of ionic surfactants (SDBS and CPC) using Conductometric technique. Micellization of both SDBS and CPC was delayed in presence of BSA. Thermodynamic parameters suggested that the micellization behaviour was entropically driven as well as spontaneous in nature. Presence of strong hydrophobic interactions at lower temperatures was observed for both similarly charged and oppositely charged protein surfactant systems. The decrease in dominance of hydrophobic interactions in both the cases i.e. BSA-SDBS or BSA-CPC with respect to increase in temperature was observed due to weakening of intermolecular bonds.

Section 4.1 (c) represents the effect of co-solvents (DMSO and glycerol) on the micellization behaviour of SDBS and CPC. Micelle formation of both SDBS and CPC was delayed in presence of additives. Thermodynamic stability of both the system decreased with increase in co-solvent concentration. BSA- CPC (oppositely charged protein-surfactant) system showed strong and more stable molecular interactions as compared to BSA-SDBS (similarly charged protein-surfactant) system.

Section 4.2- Spectroscopic studies

Section 4.2(a) represents the study of binding of BSA with ionic surfactants (SDBS and CPC) at different pH using absorbance spectroscopy. Binding studies reflected strong electrostatic binding between BSA-CPC systems as compared to BSA-SDBS system in aqueous medium. Combination of both hydrophobic and electrostatic forces of attraction was observed in BSA-CPC system which leads to specific and strong binding in this oppositely charged protein surfactant system. Changes in protein confirmation and binding abilities with respect to pH was confirmed by values of binding constant at different pH conditions

which reflected that the binding was strong in case of oppositely charged protein surfactant systems for every pH condition studied in this section.

Section 4.2(b) represents the study of binding of BSA with ionic surfactants (SDBS and CPC) at different pH using fluorescence spectroscopy. Effect of co-solvents (DMSO and glycerol) has also been reported in this section. Fluorescence quenching method was employed to study protein-surfactant interactions. Dynamic quenching was observed for both the studied system (BSA-CPC and BSA-SDBS). Presence of co-solvents (DMSO and glycerol) affected the hydrogen bonding, Vander Waal's forces present in the studied systems. Binding of BSA to ionic surfactants was a spontaneous process and spontaneity of the studied systems was reduced on increasing co-solvents concentration.

Section 4.3- Computational studies

Section 4.3(a):- Computational studies on the effect of co-solvents (DMSO and glycerol) on the micellization behaviour of ionic surfactants (SDBS and CPC) have been reported in section 4.3(a). From a theoretical point of view, it was found that the balance in interaction between the solvent and the hydrophobic and hydrophilic segments of the surfactants (SDBS and CPC) determined their solubility in mixed aquo-organic solvents. Surfactant aggregation in solution depends on hydrophobic, hydrophilic, and counterion interactions. Further the comparison between the effect of kosmotropic (glycerol) and chaotropic (DMSO) co-solvents on the aggregation of SDBS and CPC manifested the role of such systems as agents for their potential use in many industrial and household processes. The findings of this section enunciate the findings of section 4.1(a).

Section 4.3(b):- Similarly to support the findings of Section 4.1 (b) the molecular interaction of protein-surfactant systems have been studied theoretically and it was found that in the BSA, there are a total of 8 pocket sites (BP_n,n=1-8), which were ordered energetically in the following way: BP1 > BP 6 > BP 8 > BP 3 > BP 7 > BP 2 > BP 4 > BP 5. It was visible that PB1 and PB6 represent the best binding sites for surfactants. Finally, the molecular docking study confirms the interaction between the CPC-BSA and SDBS-BSA. The main interaction forces were hydrogen bonding and van der Waals forces for BSA-surfactant systems.

List of Publications

1. Sharma V, Cantero-López P, Yañez-Osses O, Kumar A. Effect of Cosolvents DMSO and Glycerol on the Self-Assembly Behavior of SDBS and CPC: An Experimental and Theoretical Approach. *Journal of Chemical & Engineering Data* 2018;63:3083-3096.
2. Sharma V, Cantero-López P, Yañez-Osses O, Rojas-Fuentes C, Kumar A. Influence of BSA on micelle formation of SDBS and CPC: An experimental–theoretical approach of its binding properties. *Journal of Molecular Liquids* 2018;271:443-451.

List of Conferences attended

1. Participated in the conference and presented a poster on the topic “ Effect of pH on the binding efficiency of BSA-SDBS/CPC” (3rd – Himachal Pradesh Science Congress) at IIT Mandi and got **Best poster award** on October 2018.
2. Participated in the conference and presented a poster on the topic “ Effect of Cosolvents on the binding efficiency of BSA-SDBS Similarly charged protein surfactant system” (Indian Science Congress) on January 2019.

Table 1: Specific conductance (κ) values for CPC in aqueous mixtures of BSA at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|----------------------|-------------|--------|--------|--------|----------------------|------------|--------|--------|--------|-------------------|------------|--------|--------|--------|
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| | κ | | | | | κ | | | | | κ | | | |
| 0 | 5 | 5.3 | 5.7 | 5.8 | 0 | 12.9 | 15 | 18.1 | 21.4 | 0 | 25.7 | 37.5 | 45.3 | 47.8 |
| 0.2 | 27.7 | 28.3 | 30.2 | 33.7 | 0.2 | 33.9 | 36.2 | 45.5 | 47.3 | 0.2 | 42.6 | 59.4 | 67.5 | 68.8 |
| 0.4 | 50.3 | 51.3 | 57.4 | 64.5 | 0.4 | 54.9 | 57.5 | 69.9 | 74.5 | 0.4 | 61.9 | 79.4 | 88.9 | 92.5 |
| 0.6 | 73 | 75.5 | 83.1 | 95.4 | 0.6 | 75.2 | 79.4 | 93.6 | 102.2 | 0.6 | 83.1 | 101.7 | 111.8 | 115.4 |
| 0.8 | 90.6 | 98.2 | 109.8 | 125.5 | 0.8 | 93.7 | 101.2 | 118.3 | 128.6 | 0.8 | 102.5 | 123.4 | 133.8 | 138.5 |
| 1 | 102.3 | 108.8 | 134 | 154.6 | 1 | 109.5 | 119.4 | 141.6 | 154.5 | 1 | 117.9 | 144.5 | 156.8 | 163.7 |
| 1.2 | 111.8 | 117.8 | 148.1 | 170.2 | 1.2 | 117.7 | 129.9 | 154.5 | 175.9 | 1.2 | 127.4 | 163.1 | 178.8 | 188.5 |
| 1.4 | 120.4 | 127.9 | 159.7 | 183.1 | 1.4 | 126.4 | 140.4 | 169.2 | 190.2 | 1.4 | 136.5 | 175.9 | 193.4 | 207 |
| 1.6 | 128.4 | 138.7 | 172.1 | 196.4 | 1.6 | 135.5 | 150.1 | 179.3 | 202 | 1.6 | 145.8 | 186.7 | 204.6 | 219 |
| 1.8 | 137.5 | 148.6 | 183.9 | 210 | 1.8 | 143.9 | 159.7 | 190.1 | 215 | 1.8 | 155.2 | 196.7 | 215 | 230 |
| 2 | 145 | 157.7 | 193.9 | 223 | 2 | 153.2 | 168.8 | 203 | 227 | 2 | 164.5 | 207 | 226 | 242 |
| | BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | |
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| | κ | | | | | κ | | | | | κ | | | |
| 0 | 11.6 | 13.6 | 15.2 | 18.4 | 0 | 17.8 | 18.1 | 24.3 | 28.1 | 0 | 51.4 | 59.5 | 80.6 | 83.1 |
| 0.2 | 31.2 | 33.8 | 37.7 | 42.8 | 0.2 | 36.7 | 38.5 | 46.8 | 51.8 | 0.2 | 68.5 | 80.1 | 93.2 | 99.5 |
| 0.4 | 52.6 | 54.9 | 62.9 | 73 | 0.4 | 56.4 | 59.9 | 68.5 | 77.6 | 0.4 | 85.9 | 101.2 | 113.8 | 120.6 |
| 0.6 | 73.1 | 77.2 | 90.1 | 99.8 | 0.6 | 77.2 | 80.6 | 94.2 | 104.7 | 0.6 | 105.7 | 125 | 136.5 | 143.9 |
| 0.8 | 91.9 | 97.7 | 115.5 | 127.9 | 0.8 | 94.7 | 100.5 | 117.4 | 130.6 | 0.8 | 123.8 | 147.5 | 156.7 | 167.8 |
| 1 | 105.8 | 115.8 | 139.6 | 152.6 | 1 | 109.9 | 120.6 | 138.5 | 155 | 1 | 139.4 | 168.8 | 177.8 | 189.9 |
| 1.2 | 115.3 | 124.4 | 154.1 | 173.8 | 1.2 | 119.4 | 133.4 | 156.8 | 178.8 | 1.2 | 150.9 | 184.1 | 196.3 | 213 |
| 1.4 | 124.4 | 134 | 165.2 | 188.9 | 1.4 | 129 | 143.4 | 171.2 | 192.5 | 1.4 | 160.1 | 194.9 | 209 | 229 |
| 1.6 | 133.5 | 143.5 | 176.6 | 201 | 1.6 | 138.5 | 152.3 | 184.9 | 206 | 1.6 | 169.8 | 206 | 219 | 244 |
| 1.8 | 141.8 | 152.7 | 188.9 | 214 | 1.8 | 146.1 | 162.2 | 197.9 | 218 | 1.8 | 178 | 217 | 230 | 255 |
| 2 | 148.6 | 161.7 | 201 | 225 | 2 | 155.1 | 172.3 | 209 | 229 | 2 | 186.1 | 228 | 241 | 267 |

Table 2: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 5% w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|------------------------------------|--------|--------|--------|--------|------------------------------------|--------|--------|--------|--------|------------------------------------|--------|--------|--------|--------|
| CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 5.7 | 7 | 7.6 | 7.7 | 0 | 12.4 | 13.5 | 15.7 | 17.5 | 0 | 22.1 | 27 | 29.7 | 32.6 |
| 0.2 | 21.7 | 24.1 | 25.1 | 29.2 | 0.2 | 28.3 | 29.5 | 35 | 39.3 | 0.2 | 37.7 | 41.6 | 45.5 | 52.5 |
| 0.4 | 40.1 | 41.6 | 45.6 | 51.5 | 0.4 | 41.1 | 41.6 | 50.5 | 56.4 | 0.4 | 52.3 | 56.4 | 62.4 | 72.3 |
| 0.6 | 58.4 | 59 | 65.4 | 76.2 | 0.6 | 58.2 | 59.4 | 71.3 | 80.2 | 0.6 | 68 | 74.3 | 80.2 | 95.1 |
| 0.8 | 76.4 | 76.3 | 85.1 | 100.1 | 0.8 | 76.5 | 77.2 | 89.1 | 106 | 0.8 | 83.1 | 91.1 | 101 | 117.9 |
| 1 | 87.2 | 92.5 | 105.8 | 123.8 | 1 | 92.8 | 98.1 | 109 | 127.8 | 1 | 98.2 | 107 | 120.9 | 139.7 |
| 1.2 | 94.2 | 102.1 | 117.4 | 140.7 | 1.2 | 101.7 | 104 | 118.9 | 143 | 1.2 | 115.8 | 124.8 | 139.7 | 164.5 |
| 1.4 | 101.7 | 109.4 | 126.8 | 154.6 | 1.4 | 113.3 | 115.9 | 136.7 | 163.5 | 1.4 | 129.9 | 142.7 | 159.5 | 179.3 |
| 1.6 | 109.3 | 117 | 135.5 | 164.7 | 1.6 | 121.4 | 124.8 | 146.8 | 175.4 | 1.6 | 137.5 | 153.7 | 176.6 | 195.4 |
| 1.8 | 116.8 | 124.5 | 144.6 | 175.4 | 1.8 | 129 | 133.7 | 156.1 | 187.2 | 1.8 | 145.1 | 162.1 | 187.2 | 208 |
| 2 | 124.4 | 132.1 | 153.1 | 186.1 | 2 | 136.5 | 142 | 166.4 | 199 | 2 | 152.3 | 170.9 | 196.2 | 219 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 9.7 | 10.4 | 11.9 | 13.6 | 0 | 14.9 | 17.5 | 18.5 | 21.6 | 0 | 49.2 | 55.1 | 68 | 70.6 |
| 0.2 | 26.4 | 27 | 32.8 | 36.8 | 0.2 | 29.6 | 32.9 | 36.7 | 40.4 | 0.2 | 63.5 | 70.3 | 83.2 | 90 |
| 0.4 | 40.3 | 41.6 | 47.5 | 55.4 | 0.4 | 42.8 | 44.5 | 53.5 | 58.4 | 0.4 | 78 | 85.2 | 95.1 | 107 |
| 0.6 | 57.9 | 59.4 | 68.3 | 79.2 | 0.6 | 59.9 | 65.4 | 74.3 | 83.2 | 0.6 | 93.6 | 100.1 | 107 | 125.8 |
| 0.8 | 76 | 77.2 | 89.1 | 103 | 0.8 | 76.8 | 80.2 | 92.1 | 107 | 0.8 | 110.2 | 114.9 | 124.8 | 143.9 |
| 1 | 88.1 | 92.1 | 109.9 | 123.8 | 1 | 89.1 | 95.1 | 107 | 127.8 | 1 | 124.4 | 129.8 | 139.7 | 163 |
| 1.2 | 101.2 | 104 | 121.8 | 141.6 | 1.2 | 102.5 | 109.9 | 121.8 | 143.7 | 1.2 | 141.5 | 146.6 | 157.5 | 182.8 |
| 1.4 | 110.2 | 113.9 | 133.7 | 162.5 | 1.4 | 116.8 | 124.2 | 139.7 | 166.5 | 1.4 | 152.6 | 162.5 | 175.3 | 205.1 |
| 1.6 | 118.4 | 120.6 | 145.6 | 178.3 | 1.6 | 123.9 | 132.8 | 149.1 | 178.3 | 1.6 | 163.1 | 173 | 193.2 | 221 |
| 1.8 | 125.9 | 130.1 | 154.5 | 189.2 | 1.8 | 131.2 | 140.6 | 158.5 | 190.2 | 1.8 | 170.8 | 181.2 | 205 | 231 |
| 2 | 133.5 | 139.2 | 165 | 198.1 | 2 | 139 | 149.1 | 168.4 | 201.1 | 2 | 178.4 | 188.9 | 219 | 241 |

Table 3: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 10 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|------------------------------------|---------------|---------------|---------------|---------------|------------------------------------|---------------|---------------|---------------|---------------|------------------------------------|---------------|---------------|---------------|---------------|
| CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 7.5 | 7.8 | 8.3 | 8.4 | 0 | 11.7 | 12.7 | 15.4 | 16.3 | 0 | 22.5 | 32.6 | 32.7 | 35.6 |
| 0.2 | 21.5 | 23.1 | 23.8 | 31.9 | 0.2 | 26.3 | 28.2 | 32.7 | 35.5 | 0.2 | 35.6 | 38.6 | 50.5 | 53.5 |
| 0.4 | 35.1 | 38.5 | 43.2 | 54.4 | 0.4 | 39.2 | 41.6 | 50.8 | 58.4 | 0.4 | 47.5 | 50.5 | 68.4 | 71.3 |
| 0.6 | 49 | 54.5 | 64.3 | 77.2 | 0.6 | 53.5 | 56.4 | 70.2 | 83.2 | 0.6 | 62.4 | 65.4 | 85.4 | 92.2 |
| 0.8 | 63.2 | 71 | 84.4 | 100 | 0.8 | 66.4 | 73.3 | 87.3 | 104 | 0.8 | 77.3 | 80.2 | 104.6 | 112.9 |
| 1 | 75.8 | 86.8 | 104.8 | 120.8 | 1 | 78.2 | 86.8 | 104.2 | 122.9 | 1 | 90.8 | 95.1 | 121.4 | 132.8 |
| 1.2 | 83.2 | 95.9 | 115.6 | 136.7 | 1.2 | 89.2 | 99.1 | 118.8 | 139.7 | 1.2 | 105.7 | 112.9 | 136.8 | 152.5 |
| 1.4 | 89.1 | 102.8 | 124.8 | 148.6 | 1.4 | 97.1 | 109.9 | 130.7 | 153.6 | 1.4 | 116.9 | 124.8 | 152.6 | 170.4 |
| 1.6 | 95.1 | 109.9 | 133.7 | 158.5 | 1.6 | 104 | 118.9 | 141.8 | 166.6 | 1.6 | 126.5 | 136.7 | 165.5 | 187.3 |
| 1.8 | 101 | 116.9 | 142.6 | 168.4 | 1.8 | 110.2 | 125.8 | 151.1 | 178.3 | 1.8 | 133.7 | 148.6 | 175.3 | 199.1 |
| 2 | 106.5 | 123.8 | 151.6 | 178.3 | 2 | 116.5 | 133.7 | 160.5 | 189.2 | 2 | 140.2 | 157.5 | 184.3 | 209 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 9.7 | 10.5 | 12.1 | 12.8 | 0 | 14.4 | 15.4 | 17.4 | 20.1 | 0 | 50.5 | 51.5 | 58.4 | 67.3 |
| 0.2 | 22.4 | 27 | 31.7 | 33.7 | 0.2 | 27.9 | 30.5 | 34.5 | 38.6 | 0.2 | 62.4 | 62.4 | 77.2 | 83.2 |
| 0.4 | 35.9 | 41.6 | 49.5 | 56.4 | 0.4 | 41.6 | 44.2 | 50.5 | 59.4 | 0.4 | 74.3 | 75.2 | 92.1 | 101 |
| 0.6 | 48.6 | 56.4 | 68.3 | 80.2 | 0.6 | 55.5 | 59.4 | 71.3 | 86.2 | 0.6 | 87.2 | 90.1 | 109 | 115.9 |
| 0.8 | 62.4 | 71.3 | 86.2 | 103.1 | 0.8 | 68.3 | 75.3 | 89.2 | 106.3 | 0.8 | 102 | 106 | 124.9 | 133.7 |
| 1 | 75.3 | 84.6 | 104 | 125.9 | 1 | 80.7 | 89.2 | 107 | 123.9 | 1 | 114 | 120.6 | 140.7 | 151.5 |
| 1.2 | 86.2 | 97.8 | 118.9 | 143.2 | 1.2 | 92.1 | 103.1 | 119.6 | 140.5 | 1.2 | 125.8 | 136.8 | 157.6 | 172.9 |
| 1.4 | 94.7 | 107 | 129.8 | 152.6 | 1.4 | 101.6 | 113.2 | 131.8 | 154.6 | 1.4 | 139.7 | 150.6 | 173.4 | 190.8 |
| 1.6 | 101 | 115.9 | 139.7 | 163.4 | 1.6 | 107.9 | 121.1 | 143.6 | 166.5 | 1.6 | 148.6 | 165.5 | 186.4 | 210 |
| 1.8 | 107.8 | 123.2 | 149.6 | 175.3 | 1.8 | 114.6 | 129.3 | 152.8 | 181.3 | 1.8 | 155.5 | 173.3 | 196.1 | 220 |
| 2 | 113.5 | 130.4 | 158.7 | 187.2 | 2 | 121.1 | 136.7 | 162.4 | 193.3 | 2 | 161.4 | 181 | 204 | 229 |

Table 4: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 15 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 7 | 7.2 | 8.3 | 8.8 | 0 | 10.5 | 12.5 | 12.6 | 14.1 | 0 | 27.1 | 30.3 | 31.2 | 35.5 |
| 0.2 | 18.5 | 22.4 | 26.2 | 31.5 | 0.2 | 23.3 | 27.3 | 31.3 | 35.4 | 0.2 | 37.9 | 38.6 | 41.6 | 47.5 |
| 0.4 | 30.8 | 36.8 | 42.7 | 49.1 | 0.4 | 35.5 | 40.6 | 48.5 | 53.2 | 0.4 | 47.5 | 50.5 | 56.4 | 62.4 |
| 0.6 | 43.5 | 52.1 | 59.4 | 68.3 | 0.6 | 48.5 | 53.4 | 65.4 | 72.5 | 0.6 | 58.4 | 65.4 | 71.3 | 80.2 |
| 0.8 | 56.5 | 66.4 | 76.6 | 88.1 | 0.8 | 60.1 | 68.3 | 82.2 | 93.7 | 0.8 | 68.3 | 80.2 | 88.2 | 98.1 |
| 1 | 68.8 | 80.9 | 93.1 | 107 | 1 | 72.3 | 80.2 | 98.5 | 112.6 | 1 | 80.2 | 94.1 | 103 | 116.9 |
| 1.2 | 79.4 | 89.3 | 104 | 123.1 | 1.2 | 81.4 | 91.5 | 111.4 | 129.3 | 1.2 | 93.1 | 107 | 117.9 | 134.8 |
| 1.4 | 86.3 | 95.1 | 111.9 | 133.7 | 1.4 | 90.2 | 104 | 120.9 | 142.8 | 1.4 | 106.2 | 117.9 | 132.8 | 153.6 |
| 1.6 | 91.8 | 101.5 | 119.5 | 142.6 | 1.6 | 97.1 | 111.5 | 127.8 | 151.6 | 1.6 | 113.2 | 129.4 | 144.1 | 168.8 |
| 1.8 | 97.7 | 108.2 | 127.1 | 151.6 | 1.8 | 103 | 118.6 | 136.7 | 161.5 | 1.8 | 118.9 | 136.7 | 152.6 | 178.3 |
| 2 | 103.1 | 115.1 | 135.1 | 160.5 | 2 | 109 | 125.5 | 145.6 | 172.4 | 2 | 124.1 | 143.6 | 160.1 | 187.2 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.1 | 12 | 12.5 | 14 | 0 | 13.5 | 16.4 | 16.8 | 18.7 | 0 | 42.5 | 50.5 | 54.4 | 62.5 |
| 0.2 | 22.4 | 26.5 | 29.4 | 34.5 | 0.2 | 25.4 | 29.5 | 33.4 | 36.8 | 0.2 | 53.5 | 62.4 | 68.3 | 77.2 |
| 0.4 | 32.7 | 39.6 | 45.9 | 50.5 | 0.4 | 36.6 | 43.6 | 49.5 | 55.5 | 0.4 | 65.4 | 72.3 | 80.2 | 92.1 |
| 0.6 | 44.5 | 54.5 | 63.6 | 71.3 | 0.6 | 47.5 | 58.4 | 65.4 | 74.3 | 0.6 | 76.3 | 83.2 | 95.1 | 107 |
| 0.8 | 56.4 | 69.2 | 82.3 | 92.1 | 0.8 | 59.4 | 72.3 | 83.5 | 95.1 | 0.8 | 87.2 | 95.1 | 107 | 123.8 |
| 1 | 68.3 | 82.2 | 96.7 | 109.1 | 1 | 71.3 | 85.2 | 99.1 | 113.5 | 1 | 98.8 | 107 | 121.8 | 140.7 |
| 1.2 | 79.2 | 96.1 | 109.2 | 124.8 | 1.2 | 82.2 | 98.1 | 113.9 | 129.7 | 1.2 | 109 | 118.9 | 136.7 | 157.5 |
| 1.4 | 89.1 | 104.2 | 118.9 | 139.7 | 1.4 | 91.7 | 109 | 124.8 | 144.9 | 1.4 | 120.9 | 132.7 | 152.6 | 176.4 |
| 1.6 | 95.7 | 110 | 126.3 | 150.4 | 1.6 | 98.1 | 116.6 | 131 | 154.4 | 1.6 | 130.8 | 144.4 | 165.3 | 190.6 |
| 1.8 | 101 | 116.9 | 135.5 | 160.5 | 1.8 | 104 | 124 | 139.7 | 164.5 | 1.8 | 136.2 | 150.5 | 173.3 | 199 |
| 2 | 107 | 124.8 | 143.9 | 170.4 | 2 | 109.5 | 131.2 | 148.6 | 174.7 | 2 | 142.1 | 156.6 | 180.1 | 208 |

Table 5: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 20 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 9 | 10 | 11 | 12 | 0 | 10.5 | 12 | 13 | 14 | 0 | 24 | 27.5 | 29 | 33 |
| 0.2 | 18.8 | 22.2 | 29 | 31.9 | 0.2 | 22.3 | 27 | 28 | 34.5 | 0.2 | 33.7 | 38.7 | 42.3 | 44.6 |
| 0.4 | 29.7 | 35.9 | 43.6 | 48.3 | 0.4 | 32.6 | 40 | 42.5 | 51.5 | 0.4 | 42.6 | 48.5 | 52.5 | 59.4 |
| 0.6 | 40.5 | 49.5 | 58.4 | 65.3 | 0.6 | 43.6 | 53.5 | 57.5 | 71.3 | 0.6 | 52.4 | 59.4 | 65.4 | 77.2 |
| 0.8 | 51.7 | 63.3 | 71.9 | 82.7 | 0.8 | 55.5 | 68.3 | 73.2 | 89.2 | 0.8 | 62.2 | 71.3 | 77.2 | 92.1 |
| 1 | 63.5 | 77.6 | 86.1 | 99.8 | 1 | 66.4 | 80.2 | 88.5 | 107.1 | 1 | 74.3 | 83.2 | 92.1 | 107 |
| 1.2 | 73.2 | 88.8 | 96.5 | 114.9 | 1.2 | 75.3 | 92.4 | 103.1 | 120.2 | 1.2 | 84.2 | 95.1 | 104 | 121.8 |
| 1.4 | 79.2 | 95.1 | 102 | 124.8 | 1.4 | 83.2 | 100.1 | 112.2 | 131.8 | 1.4 | 95.1 | 108 | 114.9 | 136.7 |
| 1.6 | 84.2 | 101 | 108.5 | 134.7 | 1.6 | 89.2 | 107.5 | 117.1 | 139.2 | 1.6 | 103.9 | 117.9 | 126.9 | 149.1 |
| 1.8 | 89.1 | 107.6 | 115 | 142.8 | 1.8 | 95.1 | 114.5 | 124.3 | 148.6 | 1.8 | 109 | 123.8 | 133.7 | 157.5 |
| 2 | 94.2 | 113.9 | 121.8 | 150.9 | 2 | 100.1 | 121.8 | 133.3 | 158.5 | 2 | 114.1 | 130 | 140.4 | 165.4 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 9.2 | 10.2 | 11.4 | 12.2 | 0 | 11.3 | 14 | 14.8 | 15 | 0 | 36.6 | 44 | 50.5 | 55.4 |
| 0.2 | 21.1 | 25.5 | 29.8 | 33 | 0.2 | 23.2 | 28 | 31.4 | 35 | 0.2 | 46.5 | 53.5 | 62.4 | 68.3 |
| 0.4 | 33.1 | 38.6 | 43.6 | 47.5 | 0.4 | 35.4 | 41.6 | 44.6 | 53.5 | 0.4 | 56.4 | 65.4 | 74.3 | 80.2 |
| 0.6 | 44.6 | 52.5 | 59.4 | 68.3 | 0.6 | 45.5 | 56.5 | 60.4 | 74.6 | 0.6 | 65.4 | 77.2 | 85.2 | 95.1 |
| 0.8 | 55.5 | 65.4 | 72.6 | 86.2 | 0.8 | 56.4 | 71.4 | 76.3 | 92.2 | 0.8 | 76.3 | 88.2 | 98.1 | 107 |
| 1 | 65.4 | 77.2 | 88.6 | 104 | 1 | 68.4 | 83.3 | 90.5 | 109.9 | 1 | 86.2 | 98.1 | 109.9 | 121.8 |
| 1.2 | 73.3 | 88.2 | 99.9 | 118.8 | 1.2 | 78.3 | 93.9 | 103.1 | 124.6 | 1.2 | 98.1 | 109.9 | 121.8 | 136.7 |
| 1.4 | 80.3 | 97.1 | 110 | 130.8 | 1.4 | 86.2 | 103 | 112.9 | 133.6 | 1.4 | 109.9 | 121.8 | 136.7 | 150.6 |
| 1.6 | 87.4 | 105.3 | 116 | 139.7 | 1.6 | 92.1 | 109.8 | 119.7 | 142.7 | 1.6 | 118.9 | 136.5 | 153.9 | 167.9 |
| 1.8 | 93.1 | 112.9 | 123.8 | 148.6 | 1.8 | 98.1 | 116.9 | 127.8 | 151.6 | 1.8 | 123.8 | 142.6 | 160.5 | 175.3 |
| 2 | 98.1 | 119.1 | 131.8 | 158.9 | 2 | 103.4 | 124.3 | 135.7 | 160.5 | 2 | 129.4 | 148.5 | 167.3 | 183 |

Table 6: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 5 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|------------------------------------|--------|--------|--------|--------|------------------------------------|--------|--------|--------|--------|------------------------------------|--------|--------|--------|--------|
| CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 6 | 6.5 | 6.8 | 7 | 0 | 12 | 13 | 15 | 16.5 | 0 | 28 | 30 | 33 | 35.6 |
| 0.2 | 20.9 | 23.9 | 24.1 | 25.8 | 0.2 | 27 | 30.1 | 32.7 | 35.8 | 0.2 | 41.5 | 44.6 | 47.5 | 53.5 |
| 0.4 | 36.9 | 41.8 | 43.6 | 47.5 | 0.4 | 43.5 | 47.5 | 50.5 | 53.5 | 0.4 | 54.4 | 58.4 | 62.4 | 71.3 |
| 0.6 | 53.5 | 60.4 | 65.4 | 71.3 | 0.6 | 60.4 | 65.4 | 71.3 | 77.2 | 0.6 | 68.4 | 74.3 | 80.2 | 92.1 |
| 0.8 | 71.4 | 77.6 | 86.2 | 94.1 | 0.8 | 77.2 | 84.2 | 92.1 | 98.1 | 0.8 | 82.1 | 89.1 | 98.1 | 112.9 |
| 1 | 85.3 | 94.5 | 105.9 | 115.9 | 1 | 91.1 | 102.2 | 109.9 | 120 | 1 | 96.1 | 104 | 115.9 | 133.7 |
| 1.2 | 94.5 | 107.5 | 124.1 | 136.5 | 1.2 | 106 | 115.9 | 127.8 | 138.6 | 1.2 | 109 | 117.9 | 133.7 | 158.5 |
| 1.4 | 101.6 | 116.9 | 136.7 | 148.6 | 1.4 | 114.5 | 125.8 | 142.7 | 154.6 | 1.4 | 124.8 | 134.8 | 157.6 | 178.3 |
| 1.8 | 115.8 | 131.7 | 154.5 | 168.4 | 1.8 | 128.8 | 142.6 | 163.4 | 173.3 | 1.8 | 142.2 | 157.5 | 180.3 | 205 |
| 2 | 123.1 | 139.6 | 163.5 | 178.3 | 2 | 136.1 | 150.3 | 171.3 | 182.7 | 2 | 149.1 | 164.3 | 188.3 | 214 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | | CPC [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10 | 10.5 | 12 | 13 | 0 | 16.5 | 18 | 20 | 22 | 0 | 48 | 54.6 | 59.5 | 78 |
| 0.2 | 26.4 | 28.5 | 30.5 | 34 | 0.2 | 31.3 | 33.9 | 35.3 | 39.2 | 0.2 | 63.3 | 69.3 | 77.2 | 92.4 |
| 0.4 | 41.6 | 44.6 | 47.5 | 52.5 | 0.4 | 48.5 | 49.5 | 54.5 | 58.5 | 0.4 | 76.2 | 83.2 | 92.1 | 108.9 |
| 0.6 | 58.4 | 62.4 | 68.3 | 74.3 | 0.6 | 65.4 | 68.4 | 74.2 | 80.2 | 0.6 | 89.1 | 98.1 | 106 | 125.8 |
| 0.8 | 74.2 | 80.2 | 89.2 | 95 | 0.8 | 79.2 | 86.2 | 95 | 104 | 0.8 | 101 | 109.9 | 122.9 | 143.6 |
| 1 | 88.1 | 97.1 | 109 | 118.8 | 1 | 92.1 | 103 | 112.8 | 124.8 | 1 | 112.9 | 124.8 | 138.5 | 163.5 |
| 1.2 | 101 | 110.9 | 125.8 | 141.7 | 1.2 | 107 | 118.8 | 127.8 | 144.7 | 1.2 | 127.8 | 139.7 | 155.5 | 186.3 |
| 1.4 | 112.9 | 120.7 | 140.7 | 156.9 | 1.4 | 115.9 | 128.8 | 144.6 | 164.5 | 1.4 | 144.7 | 160.5 | 175.3 | 205 |
| 1.6 | 119.9 | 128.8 | 149.6 | 167.4 | 1.6 | 125.8 | 136.7 | 156.8 | 181 | 1.6 | 157.6 | 175.5 | 188.2 | 217 |
| 1.8 | 126.8 | 136.7 | 158.5 | 176.3 | 1.8 | 132.6 | 144.6 | 166.4 | 191.2 | 1.8 | 163.4 | 182.3 | 197 | 226 |
| 2 | 133.7 | 144.2 | 167.3 | 186.5 | 2 | 139.5 | 152.5 | 174.3 | 201 | 2 | 170.5 | 189.5 | 204 | 235 |

Table 7: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 10 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 6.5 | 6.9 | 7 | 7.2 | 0 | 12.5 | 13 | 15.1 | 16.5 | 0 | 25.1 | 28 | 30 | 33 |
| 0.2 | 19.4 | 20.1 | 23.1 | 23.4 | 0.2 | 25.7 | 28.5 | 32.4 | 34.9 | 0.2 | 35.7 | 38.6 | 44.5 | 50.5 |
| 0.4 | 33.9 | 36.3 | 40.6 | 44.5 | 0.4 | 38.6 | 41.6 | 48.5 | 52.5 | 0.4 | 47.5 | 53.5 | 59.4 | 65.4 |
| 0.6 | 48.7 | 53.5 | 59.4 | 65.4 | 0.6 | 54.6 | 59.5 | 65.4 | 74.3 | 0.6 | 61.4 | 68.3 | 74.3 | 83.2 |
| 0.8 | 63.3 | 70.9 | 77.4 | 88.5 | 0.8 | 70.3 | 76.3 | 83.2 | 95.1 | 0.8 | 74.3 | 83.2 | 92.1 | 101 |
| 1 | 78.5 | 88.2 | 96.7 | 111.2 | 1 | 85.2 | 92.1 | 104 | 117.9 | 1 | 86.2 | 98.1 | 109.9 | 118.8 |
| 1.2 | 95.2 | 105.6 | 117.1 | 133.5 | 1.2 | 100.1 | 107 | 121.8 | 135.8 | 1.2 | 100.2 | 112.9 | 127.8 | 139.6 |
| 1.4 | 104 | 114.8 | 129.1 | 144.5 | 1.4 | 109.9 | 121.2 | 136.5 | 154.6 | 1.4 | 118.9 | 133.8 | 149.6 | 158.5 |
| 1.6 | 110.1 | 122.1 | 137.6 | 153.5 | 1.6 | 117.8 | 131.8 | 151.6 | 170.1 | 1.6 | 128 | 145.3 | 163.9 | 177.5 |
| 1.8 | 116.7 | 129.5 | 145.7 | 163.4 | 1.8 | 124.8 | 139.7 | 160.5 | 179.3 | 1.8 | 133.7 | 152.6 | 172.3 | 186 |
| 2 | 122.9 | 137.3 | 153.6 | 173.3 | 2 | 131 | 146.6 | 168.3 | 189.2 | 2 | 140.4 | 159.6 | 180.2 | 195 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.2 | 10.5 | 12.1 | 13 | 0 | 15.5 | 17 | 19 | 21 | 0 | 47.2 | 50 | 60.4 | 62.3 |
| 0.2 | 24 | 26.5 | 30.1 | 33 | 0.2 | 27.2 | 31 | 34.5 | 36.1 | 0.2 | 60.4 | 65 | 75.2 | 80.2 |
| 0.4 | 36.7 | 39.6 | 45.6 | 50.2 | 0.4 | 40.6 | 44.6 | 50.6 | 53.5 | 0.4 | 71.3 | 80.2 | 89.1 | 95.1 |
| 0.6 | 50.5 | 57.5 | 62.4 | 71.3 | 0.6 | 55.5 | 60.4 | 68.1 | 77.2 | 0.6 | 82.2 | 95.1 | 104 | 109.9 |
| 0.8 | 68.3 | 74.3 | 80.3 | 91.1 | 0.8 | 71.4 | 77.3 | 86.1 | 98.1 | 0.8 | 92.1 | 107 | 115.9 | 127.8 |
| 1 | 83.2 | 89.2 | 101 | 112.9 | 1 | 86.1 | 95.3 | 107 | 118.8 | 1 | 104 | 120.8 | 130.7 | 142.6 |
| 1.2 | 95.1 | 106.2 | 118.9 | 130.6 | 1.2 | 98.1 | 112.9 | 124.9 | 133.7 | 1.2 | 115.9 | 133.7 | 145.6 | 163.5 |
| 1.4 | 107 | 118.8 | 133.6 | 149.6 | 1.4 | 113.2 | 125.5 | 141.8 | 151.6 | 1.4 | 130.1 | 150.6 | 163.5 | 181.3 |
| 1.6 | 115.9 | 126.1 | 146.3 | 163.7 | 1.6 | 121.8 | 134.7 | 155.6 | 172.5 | 1.6 | 145.8 | 167.9 | 185.7 | 202.2 |
| 1.8 | 121.8 | 133.7 | 154.5 | 173.3 | 1.8 | 128.8 | 142.6 | 163.5 | 181.3 | 1.8 | 151.6 | 175.3 | 193.2 | 211 |
| 2 | 128.7 | 140.6 | 162.6 | 182.2 | 2 | 134.7 | 149.7 | 172.4 | 191 | 2 | 157.1 | 182 | 200 | 219 |

Table 8: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 15 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|----------------------|--------|--------|--------|--------|
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 7 | 7.4 | 7.5 | 7.6 | 0 | 11 | 12.5 | 13.5 | 15 | 0 | 24 | 27 | 27 | 32.1 |
| 0.2 | 15.5 | 16.1 | 16.8 | 19.2 | 0.2 | 23 | 25.5 | 30 | 35.5 | 0.2 | 35.3 | 40 | 41.5 | 47.5 |
| 0.4 | 30 | 31.7 | 36.8 | 40.6 | 0.4 | 35.7 | 39.6 | 43.2 | 50.5 | 0.4 | 45.6 | 53.5 | 56.5 | 62.4 |
| 0.6 | 44.9 | 48.1 | 55.3 | 62.4 | 0.6 | 50.5 | 53.5 | 62.4 | 68.4 | 0.6 | 56.5 | 68.3 | 71.3 | 77.2 |
| 0.8 | 60.1 | 64.9 | 75.2 | 86.2 | 0.8 | 65.4 | 71.2 | 77.2 | 89.1 | 0.8 | 68.4 | 83.2 | 89.2 | 95.1 |
| 1 | 75.3 | 82.1 | 95.1 | 108 | 1 | 77.2 | 86.2 | 98.3 | 109.9 | 1 | 80.2 | 98.1 | 107 | 115.9 |
| 1.2 | 91.2 | 98.5 | 115.9 | 130.8 | 1.2 | 92.1 | 101 | 115.9 | 127.6 | 1.2 | 93.1 | 109.9 | 122.9 | 130.9 |
| 1.4 | 101 | 110.6 | 129.1 | 144.2 | 1.4 | 107 | 115.9 | 133.6 | 150.3 | 1.4 | 110.2 | 124.8 | 138.7 | 150.6 |
| 1.6 | 108.1 | 120.8 | 138.6 | 154.5 | 1.6 | 115.9 | 126.8 | 146.8 | 167.2 | 1.6 | 122.8 | 135.9 | 155.9 | 172.5 |
| 1.8 | 115 | 128.7 | 148.6 | 165.3 | 1.8 | 121.8 | 133.7 | 155 | 176.1 | 1.8 | 128.8 | 142.6 | 163.5 | 180.3 |
| 2 | 121.8 | 136.5 | 157.5 | 176 | 2 | 128.2 | 140.9 | 163 | 185.2 | 2 | 134.1 | 150 | 172.2 | 189.4 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | | CPC [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10 | 11 | 12 | 13.5 | 0 | 14.5 | 17 | 18 | 19.5 | 0 | 44.1 | 49.4 | 58.4 | 60.1 |
| 0.2 | 22.5 | 24.5 | 28.4 | 32.7 | 0.2 | 26.2 | 29.2 | 32.3 | 36.2 | 0.2 | 53.5 | 65.3 | 72.3 | 75.2 |
| 0.4 | 35.9 | 35.7 | 40.8 | 41.6 | 0.4 | 39.6 | 43.6 | 46.6 | 51 | 0.4 | 62.4 | 82.2 | 86.2 | 90.1 |
| 0.6 | 48.5 | 50.5 | 59.4 | 65.4 | 0.6 | 52.1 | 56.4 | 65.3 | 71.3 | 0.6 | 74.3 | 95 | 98.2 | 104 |
| 0.8 | 62.4 | 68.3 | 76.2 | 86.2 | 0.8 | 66.7 | 74.2 | 80.1 | 92.1 | 0.8 | 85.6 | 108 | 109.9 | 118.3 |
| 1 | 74.3 | 83.2 | 95.1 | 107 | 1 | 79.3 | 89.1 | 101 | 112.9 | 1 | 95.2 | 119 | 124.8 | 133.7 |
| 1.2 | 89.1 | 100.1 | 112.9 | 124.8 | 1.2 | 93.6 | 104 | 118.9 | 129.6 | 1.2 | 107 | 130.8 | 139.7 | 148.6 |
| 1.4 | 104 | 114.8 | 130.8 | 142.6 | 1.4 | 109.9 | 119.9 | 136.5 | 148.2 | 1.4 | 113.9 | 145.6 | 155.6 | 173.5 |
| 1.6 | 113.6 | 126.5 | 144.7 | 157.5 | 1.6 | 118.8 | 131 | 150 | 167.1 | 1.6 | 126.8 | 156.7 | 176.7 | 193.9 |
| 1.8 | 119.9 | 133.7 | 152.5 | 171.2 | 1.8 | 124.8 | 137.7 | 158.4 | 176 | 1.8 | 132.7 | 163 | 183.2 | 202 |
| 2 | 125.3 | 140.2 | 160.3 | 184.3 | 2 | 131 | 145 | 166.2 | 185.1 | 2 | 137.4 | 170.3 | 190.2 | 209 |

Table 9: Specific conductance (κ) values for CPC in aqueous mixtures of BSA containing 20 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|------------------------------------|---------------|---------------|---------------|---------------|------------------------------------|---------------|---------------|---------------|---------------|------------------------------------|---------------|---------------|---------------|---------------|
| CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 8 | 8.2 | 8.5 | 8.6 | 0 | 11.4 | 12.8 | 14 | 15.2 | 0 | 24.2 | 26.6 | 27.5 | 30 |
| 0.2 | 14.1 | 15.4 | 17.2 | 18.8 | 0.2 | 22.3 | 23.8 | 27.2 | 32.2 | 0.2 | 31.3 | 38.1 | 41.5 | 44.5 |
| 0.4 | 27.2 | 29.5 | 33 | 36.9 | 0.4 | 31.9 | 35.7 | 42.6 | 47.6 | 0.4 | 39.7 | 50.5 | 53.5 | 59.5 |
| 0.6 | 41.1 | 44.5 | 49.1 | 55.4 | 0.6 | 43.6 | 50.5 | 59.9 | 65.6 | 0.6 | 50.5 | 65.1 | 69.2 | 74.3 |
| 0.8 | 55.5 | 59.4 | 66.4 | 75.3 | 0.8 | 55.5 | 65.7 | 75.3 | 86.3 | 0.8 | 62.4 | 77.2 | 83.1 | 89.9 |
| 1 | 68.8 | 75.3 | 83.2 | 94.1 | 1 | 68.3 | 80.3 | 95.9 | 107 | 1 | 74.3 | 89.2 | 105 | 108.6 |
| 1.2 | 82.1 | 90.4 | 99.8 | 114.5 | 1.2 | 83.6 | 95.8 | 112.9 | 121 | 1.2 | 88.2 | 104 | 121.9 | 127.6 |
| 1.4 | 92.5 | 106.1 | 118.2 | 137.7 | 1.4 | 95.7 | 114.9 | 127.5 | 139.7 | 1.4 | 100.3 | 119.9 | 138.7 | 145.6 |
| 1.6 | 100.8 | 116.9 | 130.1 | 150.6 | 1.6 | 106.7 | 126.4 | 143.6 | 160.3 | 1.6 | 111.7 | 132.7 | 151.1 | 168.1 |
| 1.8 | 107.4 | 124.3 | 138.6 | 160.4 | 1.8 | 112.9 | 133.6 | 151.5 | 169.6 | 1.8 | 116.9 | 140.3 | 158.6 | 177.3 |
| 2 | 114.1 | 131.7 | 146.7 | 170.1 | 2 | 118.4 | 140.3 | 160.5 | 179 | 2 | 122.5 | 146 | 167.4 | 185.3 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 | CPC [$\mu\text{M}/\text{kg}$] | T/K 293.15 | T/K 298.15 | T/K 303.15 | T/K 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.5 | 11.5 | 12.3 | 13.8 | 0 | 14.8 | 17.5 | 18.2 | 19.6 | 0 | 43 | 48 | 56 | 60 |
| 0.2 | 20.7 | 22.7 | 26.1 | 29.1 | 0.2 | 23.8 | 27.7 | 29.9 | 33.7 | 0.2 | 50.5 | 60.4 | 71.2 | 74.3 |
| 0.4 | 31.5 | 31.5 | 37.6 | 42.6 | 0.4 | 32.7 | 40.6 | 43.7 | 48.5 | 0.4 | 59.4 | 72.3 | 83.2 | 86.2 |
| 0.6 | 42.6 | 47.5 | 53.5 | 62.5 | 0.6 | 44.6 | 53.5 | 62.3 | 69.6 | 0.6 | 68.3 | 83.2 | 95.1 | 98.1 |
| 0.8 | 53.5 | 62.6 | 71.3 | 83.3 | 0.8 | 58.5 | 68.9 | 77.3 | 88.6 | 0.8 | 77.2 | 92.1 | 107 | 112.8 |
| 1 | 65.4 | 77.2 | 89.9 | 104 | 1 | 71.4 | 86.2 | 98.3 | 107.9 | 1 | 90.2 | 104 | 118.9 | 124.8 |
| 1.2 | 80.2 | 92.7 | 107 | 118.9 | 1.2 | 87.3 | 100.2 | 115.9 | 124.7 | 1.2 | 100.1 | 112.9 | 130.8 | 139.7 |
| 1.4 | 94.7 | 107 | 124.7 | 136.6 | 1.4 | 100.2 | 112.9 | 132.5 | 143.6 | 1.4 | 108 | 124.8 | 142.6 | 160.5 |
| 1.6 | 103.8 | 121.6 | 134.7 | 152.8 | 1.6 | 108.1 | 126.1 | 145.3 | 160.7 | 1.6 | 117.6 | 136.5 | 160.1 | 177.9 |
| 1.8 | 109.9 | 130.6 | 142.6 | 162.7 | 1.8 | 113.8 | 133.7 | 153.6 | 170.6 | 1.8 | 122.8 | 141.6 | 166.4 | 185.2 |
| 2 | 115 | 139.1 | 150.7 | 171.3 | 2 | 120.1 | 140.1 | 162.1 | 179.4 | 2 | 127.3 | 147.5 | 172.6 | 192 |

Table 10: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 5.3 | 6.2 | 6.3 | 6.9 | 0 | 19 | 20 | 20.5 | 23 | 0 | 37.7 | 42.7 | 50.3 | 57.9 |
| 0.3 | 65.9 | 77.5 | 89.6 | 105.6 | 0.3 | 78 | 93.9 | 104.2 | 114.5 | 0.3 | 101 | 124 | 126 | 149.3 |
| 0.6 | 117.8 | 138.5 | 165.1 | 178.8 | 0.6 | 133.5 | 151.8 | 171.1 | 184.6 | 0.6 | 151.1 | 178 | 191 | 213 |
| 0.9 | 171.1 | 193 | 225 | 246 | 0.9 | 185 | 207 | 235 | 249 | 0.9 | 199 | 231 | 254 | 272 |
| 1.2 | 226 | 254 | 297 | 318 | 1.2 | 238 | 263 | 298 | 315 | 1.2 | 245 | 282 | 317 | 335 |
| 1.5 | 267 | 299 | 352 | 379 | 1.5 | 281 | 315 | 358 | 378 | 1.5 | 291 | 339 | 380 | 397 |
| 1.8 | 305 | 341 | 394 | 428 | 1.8 | 315 | 360 | 396 | 430 | 1.8 | 332 | 385 | 435 | 454 |
| 2.1 | 347 | 387 | 447 | 483 | 2.1 | 355 | 405 | 451 | 488 | 2.1 | 371 | 428 | 489 | 506 |
| 2.4 | 391 | 436 | 503 | 541 | 2.4 | 399 | 453 | 503 | 541 | 2.4 | 410 | 473 | 541 | 557 |
| 2.7 | 435 | 484 | 562 | 601 | 2.7 | 443 | 498 | 558 | 597 | 2.7 | 451 | 516 | 594 | 609 |
| 3 | 477 | 531 | 617 | 659 | 3 | 485 | 544 | 609 | 652 | 3 | 490 | 559 | 647 | 661 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 14.1 | 15.2 | 19 | 19.3 | 0 | 20.2 | 25.9 | 26.1 | 28.3 | 0 | 70 | 83.1 | 93.2 | 103.2 |
| 0.3 | 80.5 | 86.9 | 95.7 | 108.8 | 0.3 | 84.5 | 100.8 | 110 | 115.3 | 0.3 | 136 | 153.6 | 161.1 | 178.8 |
| 0.6 | 133 | 149.2 | 168.7 | 186.6 | 0.6 | 133.5 | 157.8 | 167.7 | 186.4 | 0.6 | 183.9 | 209 | 219 | 244 |
| 0.9 | 183 | 206 | 234 | 258 | 0.9 | 184.5 | 209 | 231 | 254 | 0.9 | 229 | 262 | 277 | 306 |
| 1.2 | 234 | 266 | 290 | 329 | 1.2 | 236 | 264 | 291 | 319 | 1.2 | 273 | 314 | 340 | 367 |
| 1.5 | 274 | 315 | 352 | 384 | 1.5 | 281 | 321 | 351 | 387 | 1.5 | 315 | 362 | 398 | 430 |
| 1.8 | 314 | 360 | 403 | 433 | 1.8 | 320 | 365 | 403 | 437 | 1.8 | 350 | 403 | 453 | 488 |
| 2.1 | 355 | 410 | 455 | 496 | 2.1 | 363 | 410 | 455 | 491 | 2.1 | 387 | 448 | 501 | 547 |
| 2.4 | 396 | 460 | 511 | 558 | 2.4 | 402 | 455 | 508 | 551 | 2.4 | 425 | 491 | 551 | 600 |
| 2.7 | 438 | 508 | 559 | 617 | 2.7 | 443 | 500 | 555 | 609 | 2.7 | 463 | 536 | 602 | 652 |
| 3 | 480 | 554 | 609 | 675 | 3 | 487 | 543 | 604 | 662 | 3 | 501 | 581 | 650 | 705 |

Table 11: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 5 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 6.7 | 7.2 | 7.4 | 7.6 | 0 | 12.9 | 14.8 | 15.8 | 18.5 | 0 | 33.5 | 29.7 | 32.7 | 38.6 |
| 0.3 | 44.6 | 60.6 | 71.8 | 75.4 | 0.3 | 56.4 | 65.4 | 71.3 | 80.2 | 0.3 | 77.2 | 86.2 | 98.1 | 109.9 |
| 0.6 | 94.1 | 113.7 | 128.7 | 137.8 | 0.6 | 104 | 118.9 | 136.7 | 149.6 | 0.6 | 124.8 | 148.6 | 163.5 | 181.3 |
| 0.9 | 140.7 | 166.9 | 183.5 | 203 | 0.9 | 151.6 | 169.4 | 196.2 | 215 | 0.9 | 169.4 | 202 | 219 | 234 |
| 1.2 | 187 | 217 | 238 | 267 | 1.2 | 199.1 | 225 | 243 | 274 | 1.2 | 214 | 246 | 258 | 294 |
| 1.5 | 230 | 264 | 295 | 322 | 1.5 | 239 | 264 | 298 | 330 | 1.5 | 249 | 282 | 315 | 347 |
| 1.8 | 267 | 306 | 344 | 374 | 1.8 | 275 | 311 | 349 | 396 | 1.8 | 285 | 327 | 368 | 407 |
| 2.1 | 305 | 347 | 389 | 428 | 2.1 | 309 | 353 | 392 | 441 | 2.1 | 321 | 371 | 413 | 460 |
| 2.4 | 343 | 391 | 434 | 481 | 2.4 | 349 | 395 | 439 | 497 | 2.4 | 356 | 413 | 457 | 514 |
| 2.7 | 383 | 435 | 481 | 535 | 2.7 | 380 | 431 | 481 | 547 | 2.7 | 392 | 457 | 505 | 561 |
| 3 | 421 | 478 | 527 | 588 | 3 | 423 | 475 | 532 | 601 | 3 | 428 | 496 | 552 | 615 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.4 | 11.6 | 12.2 | 13.5 | 0 | 16 | 17.7 | 20.2 | 21.3 | 0 | 56.4 | 62.4 | 68.3 | 70.2 |
| 0.3 | 53.5 | 62.4 | 68.3 | 74.3 | 0.3 | 62.4 | 68.3 | 77.2 | 89.1 | 0.3 | 104 | 118.9 | 133.7 | 148.6 |
| 0.6 | 98.1 | 115.9 | 127.8 | 148.6 | 0.6 | 107 | 124.8 | 139.9 | 158.6 | 0.6 | 151.6 | 178.3 | 196.2 | 205 |
| 0.9 | 145.6 | 175.4 | 196.2 | 214 | 0.9 | 157.5 | 174.2 | 202 | 222 | 0.9 | 205 | 222 | 243 | 263 |
| 1.2 | 199.1 | 225 | 243 | 274 | 1.2 | 205 | 228 | 249 | 279 | 1.2 | 237 | 261 | 297 | 321 |
| 1.5 | 237 | 264 | 299 | 327 | 1.5 | 243 | 267 | 300 | 335 | 1.5 | 276 | 309 | 353 | 371 |
| 1.8 | 273 | 312 | 348 | 386 | 1.8 | 279 | 314 | 353 | 398 | 1.8 | 312 | 347 | 401 | 428 |
| 2.1 | 309 | 353 | 392 | 439 | 2.1 | 318 | 356 | 395 | 450 | 2.1 | 350 | 392 | 445 | 472 |
| 2.4 | 347 | 395 | 439 | 496 | 2.4 | 353 | 398 | 442 | 502 | 2.4 | 383 | 434 | 490 | 529 |
| 2.7 | 383 | 436 | 484 | 546 | 2.7 | 389 | 434 | 487 | 555 | 2.7 | 419 | 472 | 535 | 573 |
| 3 | 419 | 475 | 532 | 597 | 3 | 425 | 478 | 536 | 606 | 3 | 457 | 511 | 579 | 627 |

Table 12: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 10 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|---------------|--------|--------|-------------------------------------|--------|---------------|--------|--------|-------------------------------------|--------|---------------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | 293.15 | T/K 298.15 | 303.15 | 308.15 | SDBS [$\mu\text{M}/\text{kg}$] | 293.15 | T/K 298.15 | 303.15 | 308.15 | SDBS [$\mu\text{M}/\text{kg}$] | 293.15 | T/K 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 7.5 | 8 | 8.2 | 8.5 | 0 | 12.9 | 13.9 | 15.5 | 17.5 | 0 | 31.2 | 34.9 | 35.7 | 36.2 |
| 0.3 | 38.6 | 45.5 | 52.5 | 58.4 | 0.3 | 47.5 | 56.4 | 65.4 | 71.3 | 0.3 | 65.4 | 77.2 | 86.2 | 98.1 |
| 0.6 | 80.2 | 95.1 | 105.2 | 119.8 | 0.6 | 85.6 | 101 | 115.9 | 130.7 | 0.6 | 107 | 124.8 | 133.7 | 157.5 |
| 0.9 | 120.8 | 141.6 | 157.8 | 181.2 | 0.9 | 130.7 | 151.6 | 169.4 | 196.2 | 0.9 | 145.6 | 175.3 | 190.2 | 217 |
| 1.2 | 164.3 | 186.2 | 209 | 240 | 1.2 | 183.2 | 202 | 222 | 248 | 1.2 | 193.2 | 219 | 231 | 267 |
| 1.5 | 204 | 228 | 255 | 289 | 1.5 | 218 | 243 | 261 | 300 | 1.5 | 228 | 252 | 273 | 318 |
| 1.8 | 237 | 261 | 294 | 337 | 1.8 | 249 | 276 | 306 | 353 | 1.8 | 258 | 291 | 315 | 365 |
| 2.1 | 271 | 300 | 338 | 387 | 2.1 | 283 | 312 | 347 | 401 | 2.1 | 292 | 327 | 356 | 416 |
| 2.4 | 307 | 340 | 380 | 439 | 2.4 | 310 | 353 | 389 | 454 | 2.4 | 328 | 365 | 395 | 413 |
| 2.7 | 341 | 380 | 425 | 491 | 2.7 | 343 | 386 | 430 | 499 | 2.7 | 357 | 401 | 434 | 514 |
| 3 | 375 | 417 | 470 | 541 | 3 | 380 | 422 | 470 | 546 | 3 | 387 | 436 | 475 | 561 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | 293.15 | T/K 298.15 | 303.15 | 308.15 | SDBS [$\mu\text{M}/\text{kg}$] | 293.15 | T/K 298.15 | 303.15 | 308.15 | SDBS [$\mu\text{M}/\text{kg}$] | 293.15 | T/K 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.5 | 11 | 12.2 | 14 | 0 | 15.9 | 16.9 | 17.5 | 20.3 | 0 | 50.5 | 59.4 | 62.4 | 70.3 |
| 0.3 | 41.6 | 50.5 | 62.4 | 68.3 | 0.3 | 50.4 | 59.5 | 68.4 | 74.3 | 0.3 | 86.2 | 101 | 115.9 | 130.8 |
| 0.6 | 82.1 | 98.5 | 109.9 | 124.8 | 0.6 | 91 | 104 | 118.9 | 136.7 | 0.6 | 130.8 | 154.5 | 172.4 | 199.1 |
| 0.9 | 126.7 | 148.6 | 166.4 | 193.2 | 0.9 | 133.6 | 154.6 | 172.3 | 199.1 | 0.9 | 172.3 | 200 | 222 | 246 |
| 1.2 | 180.3 | 199.2 | 219 | 246 | 1.2 | 186.2 | 205 | 225 | 252 | 1.2 | 211 | 237 | 264 | 300 |
| 1.5 | 215 | 240 | 258 | 297.7 | 1.5 | 222 | 246 | 264 | 303 | 1.5 | 237 | 273 | 300 | 347 |
| 1.8 | 246 | 273 | 303 | 351 | 1.8 | 250 | 279 | 309 | 344 | 1.8 | 273 | 306 | 344 | 398 |
| 2.1 | 280 | 306 | 341 | 398 | 2.1 | 287 | 318 | 350 | 404 | 2.1 | 303 | 347 | 383 | 448 |
| 2.4 | 308 | 350 | 383 | 448 | 2.4 | 314 | 356 | 393 | 456 | 2.4 | 338 | 383 | 422 | 496 |
| 2.7 | 340 | 383 | 428 | 493 | 2.7 | 346 | 389 | 431 | 502 | 2.7 | 368 | 419 | 463 | 541 |
| 3 | 375 | 419 | 460 | 540 | 3 | 383 | 425 | 472 | 548 | 3 | 401 | 448 | 502 | 585 |

Table 13: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 15 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 7.1 | 8 | 8.5 | 10 | 0 | 10.5 | 12 | 12.9 | 14.5 | 0 | 28.5 | 30.5 | 36 | 38 |
| 0.3 | 32.7 | 42.6 | 51.2 | 55.5 | 0.3 | 44.5 | 50.5 | 56.4 | 65.4 | 0.3 | 56.4 | 65.3 | 74.3 | 83.2 |
| 0.6 | 74.3 | 85.2 | 97.1 | 109 | 0.6 | 83.2 | 95.1 | 107 | 118.9 | 0.6 | 92.1 | 109.9 | 121.8 | 139.7 |
| 0.9 | 112.9 | 124.8 | 141.5 | 161.4 | 0.9 | 121.8 | 136.7 | 154.5 | 175.3 | 0.9 | 127.8 | 151.5 | 172.4 | 199.1 |
| 1.2 | 151.6 | 167.5 | 185 | 214 | 1.2 | 160.5 | 181.3 | 205 | 219 | 1.2 | 166.4 | 199.1 | 214 | 243 |
| 1.5 | 185.6 | 206 | 231 | 258 | 1.5 | 199.1 | 217 | 243 | 264 | 1.5 | 209 | 225 | 249 | 288 |
| 1.8 | 214 | 236 | 265 | 293 | 1.8 | 228 | 249 | 282 | 312 | 1.8 | 235 | 255 | 288 | 335 |
| 2.1 | 246 | 271 | 303 | 338 | 2.1 | 258 | 282 | 318 | 353 | 2.1 | 266 | 288 | 327 | 383 |
| 2.4 | 281 | 306 | 341 | 383 | 2.4 | 288 | 315 | 356 | 398 | 2.4 | 300 | 321 | 365 | 431 |
| 2.7 | 313 | 341 | 378 | 428 | 2.7 | 318 | 347 | 392 | 439 | 2.7 | 330 | 353 | 401 | 478 |
| 3 | 345 | 375 | 416 | 472 | 3 | 347 | 377 | 428 | 478 | 3 | 362 | 386 | 436 | 523 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10 | 11 | 12.5 | 14 | 0 | 14 | 15.8 | 17.2 | 19 | 0 | 44.5 | 50.5 | 56.4 | 62 |
| 0.3 | 38.6 | 47.5 | 53.5 | 59.4 | 0.3 | 47.5 | 53.5 | 62.4 | 68.3 | 0.3 | 80.2 | 95.1 | 104 | 118.9 |
| 0.6 | 77.2 | 89.1 | 101 | 112.9 | 0.6 | 86.2 | 98.1 | 112.9 | 121.8 | 0.6 | 115.9 | 139.7 | 151.6 | 178.3 |
| 0.9 | 118.9 | 127.8 | 148.6 | 169.4 | 0.9 | 124.8 | 139.7 | 157.5 | 178.3 | 0.9 | 154.5 | 187.2 | 199 | 225 |
| 1.2 | 157.5 | 175.3 | 202 | 217 | 1.2 | 163.5 | 187.2 | 208 | 222 | 1.2 | 187.2 | 219 | 237 | 270 |
| 1.5 | 196.2 | 211 | 237 | 261 | 1.5 | 202 | 219 | 246 | 267 | 1.5 | 217 | 252 | 276 | 318 |
| 1.8 | 225 | 243 | 273 | 306 | 1.8 | 231 | 252 | 285 | 315 | 1.8 | 243 | 285 | 315 | 359 |
| 2.1 | 255 | 276 | 312 | 350 | 2.1 | 261 | 285 | 324 | 356 | 2.1 | 273 | 321 | 353 | 407 |
| 2.4 | 285 | 309 | 350 | 395 | 2.4 | 291 | 318 | 362 | 401 | 2.4 | 303 | 353 | 389 | 451 |
| 2.7 | 315 | 341 | 386 | 436 | 2.7 | 321 | 350 | 398 | 443 | 2.7 | 332 | 383 | 425 | 493 |
| 3 | 344 | 371 | 425 | 475 | 3 | 350 | 380 | 434 | 481 | 3 | 365 | 413 | 460 | 535 |

Table 14: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 20 % w/w glycerol at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-----------------------|--------|--------|--------|--------|-----------------------|--------|--------|--------|--------|-----------------------|--------|--------|--------|--------|
| SDBS [μ M/kg] | T/K | | | | SDBS [μ M/kg] | T/K | | | | SDBS [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.5 | 11 | 13 | 15 | 0 | 11.5 | 13 | 14 | 16 | 0 | 27 | 29 | 32 | 37.5 |
| 0.3 | 30.5 | 36.7 | 47.5 | 50.5 | 0.3 | 38.6 | 41.6 | 47.5 | 56.4 | 0.3 | 53.5 | 59.4 | 65.4 | 77.2 |
| 0.6 | 65.4 | 71.3 | 88.5 | 98.1 | 0.6 | 77.2 | 83.2 | 92.1 | 104 | 0.6 | 89.1 | 95.1 | 109.9 | 124.8 |
| 0.9 | 98.1 | 105.1 | 126.8 | 148.6 | 0.9 | 109.9 | 121.8 | 133.7 | 157.5 | 0.9 | 124.8 | 130.7 | 154.5 | 175.3 |
| 1.2 | 132.9 | 139.7 | 165.5 | 198 | 1.2 | 142.6 | 162.4 | 180.3 | 208 | 1.2 | 154.5 | 172.4 | 196.2 | 219 |
| 1.5 | 172.4 | 180.1 | 208 | 243 | 1.5 | 181.3 | 196.1 | 219 | 246 | 1.5 | 187.2 | 205 | 228 | 256 |
| 1.8 | 202 | 211 | 237 | 277 | 1.8 | 214 | 225 | 248 | 291 | 1.8 | 219 | 234 | 261 | 300 |
| 2.1 | 231 | 239 | 270 | 318 | 2.1 | 240 | 249 | 281 | 332 | 2.1 | 247 | 261 | 297 | 341 |
| 2.4 | 259 | 268 | 303 | 359 | 2.4 | 267 | 276 | 316 | 371 | 2.4 | 271 | 291 | 329 | 376 |
| 2.7 | 288 | 297 | 337 | 401 | 2.7 | 293 | 306 | 348 | 410 | 2.7 | 298 | 318 | 362 | 416 |
| 3 | 315 | 327 | 370 | 445 | 3 | 322 | 332 | 384 | 451 | 3 | 325 | 347 | 395 | 457 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [μ M/kg] | T/K | | | | SDBS [μ M/kg] | T/K | | | | SDBS [μ M/kg] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 11 | 11.5 | 13.5 | 15.5 | 0 | 12 | 13.5 | 14.5 | 17 | 0 | 41.5 | 47 | 56 | 62.4 |
| 0.3 | 35.6 | 38.6 | 44.5 | 53.5 | 0.3 | 41.6 | 44.5 | 50.5 | 62.4 | 0.3 | 74.3 | 80.2 | 101 | 107 |
| 0.6 | 71.3 | 80.2 | 89.1 | 101 | 0.6 | 80.3 | 86.2 | 95.1 | 112.9 | 0.6 | 107 | 118.9 | 142.6 | 157.5 |
| 0.9 | 107 | 115.9 | 130.8 | 154.5 | 0.9 | 112.9 | 124.8 | 136.7 | 166.4 | 0.9 | 139.7 | 157.5 | 178.3 | 202 |
| 1.2 | 139.7 | 145.6 | 178.3 | 205 | 1.2 | 145.6 | 166.4 | 184.3 | 214 | 1.2 | 175.3 | 193.2 | 214 | 240 |
| 1.5 | 180.3 | 189.2 | 217 | 243 | 1.5 | 182.3 | 202 | 222 | 255 | 1.5 | 202 | 219 | 246 | 280 |
| 1.8 | 211 | 220 | 246 | 288 | 1.8 | 216 | 231 | 252 | 297 | 1.8 | 228 | 246 | 279 | 321 |
| 2.1 | 237 | 243 | 279 | 327 | 2.1 | 242 | 258 | 288 | 338 | 2.1 | 249 | 273 | 315 | 356 |
| 2.4 | 264 | 270 | 312 | 368 | 2.4 | 269 | 288 | 324 | 373 | 2.4 | 276 | 300 | 344 | 395 |
| 2.7 | 291 | 297 | 344 | 404 | 2.7 | 295 | 315 | 356 | 412 | 2.7 | 303 | 329 | 377 | 434 |
| 3 | 318 | 327 | 374 | 442 | 3 | 324 | 341 | 392 | 453 | 3 | 329 | 353 | 410 | 469 |

Table 15: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 5 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 6 | 6.2 | 6.3 | 6.8 | 0 | 12 | 14 | 17 | 18.2 | 0 | 29.1 | 32.7 | 40 | 43.5 |
| 0.3 | 44.5 | 55.1 | 71.5 | 75.2 | 0.3 | 59.4 | 62.4 | 77.2 | 80.2 | 0.3 | 74.2 | 80.2 | 101 | 109.9 |
| 0.6 | 89.1 | 106 | 133.7 | 139.6 | 0.6 | 107 | 118.9 | 142.6 | 154.5 | 0.6 | 121.8 | 133.7 | 163.5 | 181.3 |
| 0.9 | 136.7 | 155.1 | 192.2 | 201 | 0.9 | 154.5 | 175.3 | 196.2 | 222 | 0.9 | 169.4 | 190.2 | 217 | 234 |
| 1.2 | 181.3 | 206 | 250 | 261 | 1.2 | 208 | 220 | 252 | 273 | 1.2 | 214 | 234 | 261 | 289 |
| 1.5 | 214 | 252 | 294 | 321 | 1.5 | 240 | 258 | 303 | 329 | 1.5 | 249 | 279 | 306 | 347 |
| 1.8 | 253 | 297 | 334 | 371 | 1.8 | 283 | 306 | 350 | 389 | 1.8 | 291 | 309 | 356 | 398 |
| 2.1 | 290 | 338 | 381 | 423 | 2.1 | 311 | 344 | 395 | 445 | 2.1 | 324 | 349 | 405 | 451 |
| 2.4 | 328 | 380 | 430 | 475 | 2.4 | 352 | 386 | 442 | 493 | 2.4 | 359 | 393 | 449 | 508 |
| 2.7 | 365 | 420 | 479 | 527 | 2.7 | 390 | 425 | 484 | 544 | 2.7 | 396 | 431 | 491 | 562 |
| 3 | 402 | 461 | 529 | 577 | 3 | 420 | 466 | 529 | 594 | 3 | 426 | 470 | 536 | 615 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10 | 12 | 13 | 15 | 0 | 18 | 19.5 | 22 | 25 | 0 | 55.4 | 62 | 80 | 80.5 |
| 0.3 | 50.5 | 59.4 | 74.3 | 78.3 | 0.3 | 62.4 | 65.4 | 83.2 | 89.9 | 0.3 | 104 | 115.9 | 142.6 | 154.5 |
| 0.6 | 101 | 115.9 | 139.7 | 142.7 | 0.6 | 109.9 | 122.8 | 145.6 | 155.6 | 0.6 | 154.5 | 169.4 | 199.1 | 218 |
| 0.9 | 142.6 | 166.4 | 193.2 | 208 | 0.9 | 157.5 | 178.4 | 199.1 | 225 | 0.9 | 202 | 217 | 249 | 267 |
| 1.2 | 190.2 | 217 | 251 | 264 | 1.2 | 211 | 222 | 254 | 279 | 1.2 | 237 | 252 | 303 | 329 |
| 1.5 | 231 | 255 | 300 | 318 | 1.5 | 246 | 261 | 306 | 332 | 1.5 | 273 | 297 | 344 | 377 |
| 1.8 | 264 | 303 | 347 | 374 | 1.8 | 285 | 309 | 353 | 392 | 1.8 | 312 | 332 | 389 | 431 |
| 2.1 | 300 | 341 | 392 | 431 | 2.1 | 320 | 347 | 400 | 448 | 2.1 | 347 | 374 | 431 | 481 |
| 2.4 | 338 | 383 | 439 | 481 | 2.4 | 355 | 389 | 445 | 505 | 2.4 | 383 | 413 | 475 | 535 |
| 2.7 | 374 | 422 | 481 | 535 | 2.7 | 393 | 428 | 487 | 558 | 2.7 | 419 | 450 | 520 | 585 |
| 3 | 410 | 460 | 526 | 585 | 3 | 423 | 466 | 532 | 609 | 3 | 454 | 487 | 555 | 633 |

Table 16: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 10 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 6.5 | 7 | 7.1 | 7.2 | 0 | 13 | 16 | 17.5 | 20 | 0 | 28 | 29.7 | 30 | 33 |
| 0.3 | 41.6 | 47.5 | 59.4 | 71.8 | 0.3 | 50.5 | 59.4 | 68.3 | 77.2 | 0.3 | 71.3 | 74.3 | 92.1 | 98.1 |
| 0.6 | 88.1 | 97.6 | 115.9 | 133.7 | 0.6 | 98.1 | 115.9 | 127.8 | 142.6 | 0.6 | 115.9 | 130.7 | 148.6 | 160.5 |
| 0.9 | 132.7 | 147.2 | 171.3 | 193.1 | 0.9 | 139.7 | 163.5 | 190.2 | 211 | 0.9 | 160.5 | 175.6 | 199.1 | 222 |
| 1.2 | 180.3 | 196.1 | 225 | 252 | 1.2 | 184.3 | 211 | 237 | 264 | 1.2 | 202 | 222 | 243 | 273 |
| 1.5 | 213 | 235 | 276 | 301 | 1.5 | 222 | 255 | 285 | 321 | 1.5 | 231 | 264 | 294 | 332 |
| 1.8 | 246 | 271 | 322 | 344 | 1.8 | 258 | 297 | 335 | 374 | 1.8 | 264 | 306 | 346 | 383 |
| 2.1 | 283 | 312 | 368 | 395 | 2.1 | 291 | 335 | 383 | 425 | 2.1 | 304 | 347 | 395 | 434 |
| 2.4 | 320 | 353 | 413 | 446 | 2.4 | 327 | 380 | 431 | 475 | 2.4 | 337 | 389 | 439 | 487 |
| 2.7 | 359 | 394 | 460 | 495 | 2.7 | 365 | 422 | 478 | 526 | 2.7 | 369 | 427 | 487 | 536 |
| 3 | 398 | 435 | 506 | 545 | 3 | 402 | 458 | 523 | 576 | 3 | 410 | 466 | 535 | 588 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 11 | 12 | 13 | 14.5 | 0 | 15 | 18 | 20 | 23.5 | 0 | 50.5 | 56.4 | 60.4 | 65.3 |
| 0.3 | 47.5 | 56.4 | 65.4 | 71.3 | 0.3 | 56.4 | 62.4 | 77.2 | 83.2 | 0.3 | 98.1 | 109.9 | 121.8 | 136.7 |
| 0.6 | 92.1 | 104 | 121.8 | 136.7 | 0.6 | 101 | 118.9 | 136.7 | 151.2 | 0.6 | 139.7 | 157.5 | 184.3 | 199.1 |
| 0.9 | 136.7 | 154.6 | 184.3 | 205 | 0.9 | 142.6 | 172.4 | 196.2 | 217 | 0.9 | 187.2 | 208 | 231 | 243 |
| 1.2 | 181.3 | 202 | 231 | 258 | 1.2 | 190.2 | 219 | 240 | 267 | 1.2 | 222 | 243 | 279 | 294 |
| 1.5 | 219 | 243 | 279 | 312 | 1.5 | 228 | 257 | 291 | 327 | 1.5 | 252 | 285 | 321 | 344 |
| 1.8 | 255 | 282 | 326 | 359 | 1.8 | 261 | 303 | 341 | 377 | 1.8 | 288 | 324 | 362 | 392 |
| 2.1 | 288 | 327 | 377 | 418 | 2.1 | 300 | 341 | 392 | 431 | 2.1 | 324 | 362 | 407 | 440 |
| 2.4 | 324 | 371 | 425 | 469 | 2.4 | 335 | 383 | 436 | 481 | 2.4 | 356 | 401 | 448 | 491 |
| 2.7 | 362 | 410 | 469 | 517 | 2.7 | 368 | 425 | 481 | 532 | 2.7 | 389 | 439 | 493 | 540 |
| 3 | 398 | 451 | 511 | 561 | 3 | 406 | 460 | 529 | 582 | 3 | 422 | 478 | 540 | 596 |

Table 17: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 15 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|------------------------------|--------|--------|--------|--------|------------------------------|--------|--------|--------|--------|------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M/kg}$] | T/K | | | | SDBS [$\mu\text{M/kg}$] | T/K | | | | SDBS [$\mu\text{M/kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 7.2 | 7.5 | 7.6 | 7.8 | 0 | 12 | 15 | 16.5 | 16.7 | 0 | 31.4 | 35 | 35.6 | 38.6 |
| 0.3 | 40.2 | 46.9 | 58.5 | 65.3 | 0.3 | 47.5 | 56.4 | 65.4 | 71.3 | 0.3 | 68.3 | 74.3 | 86.2 | 98.1 |
| 0.6 | 80.2 | 90.2 | 105.9 | 122.8 | 0.6 | 89.4 | 98.1 | 115.9 | 133.7 | 0.6 | 101 | 118.9 | 133.7 | 151.5 |
| 0.9 | 118.9 | 131.1 | 151.6 | 180.3 | 0.9 | 130.8 | 145.6 | 163.5 | 187.2 | 0.9 | 139.6 | 157.5 | 175.3 | 205 |
| 1.2 | 157.5 | 173.1 | 199 | 237 | 1.2 | 169.4 | 193.2 | 214 | 243 | 1.2 | 181.3 | 202 | 222 | 251 |
| 1.5 | 208 | 220 | 245 | 287 | 1.5 | 212 | 232 | 252 | 294 | 1.5 | 222 | 240 | 261 | 306 |
| 1.8 | 246 | 255 | 285 | 326 | 1.8 | 253 | 267 | 294 | 344 | 1.8 | 259 | 273 | 303 | 255 |
| 2.1 | 276 | 291 | 324 | 374 | 2.1 | 279 | 303 | 335 | 389 | 2.1 | 285 | 315 | 345 | 404 |
| 2.4 | 309 | 327 | 362 | 424 | 2.4 | 315 | 341 | 374 | 434 | 2.4 | 321 | 348 | 385 | 451 |
| 2.7 | 338 | 360 | 402 | 472 | 2.7 | 344 | 380 | 413 | 484 | 2.7 | 355 | 387 | 423 | 499 |
| 3 | 374 | 395 | 442 | 518 | 3 | 386 | 410 | 457 | 531 | 3 | 395 | 421 | 467 | 544 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M/kg}$] | T/K | | | | SDBS [$\mu\text{M/kg}$] | T/K | | | | SDBS [$\mu\text{M/kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 11.5 | 13 | 13.8 | 15 | 0 | 17.5 | 18 | 20 | 22 | 0 | 50.5 | 56.4 | 65.3 | 70.2 |
| 0.3 | 44.6 | 50.5 | 59.4 | 68.3 | 0.3 | 53.5 | 59.4 | 71.3 | 77.2 | 0.3 | 92.1 | 101 | 175.9 | 121.8 |
| 0.6 | 86.1 | 95.1 | 109.9 | 130.8 | 0.6 | 92.1 | 104 | 121.8 | 140.2 | 0.6 | 127.8 | 142.6 | 157.5 | 172.4 |
| 0.9 | 124.8 | 142.6 | 157.5 | 184.3 | 0.9 | 136.7 | 148.6 | 166.4 | 190.3 | 0.9 | 169.4 | 190.2 | 202 | 222 |
| 1.2 | 163.5 | 190.2 | 205 | 237 | 1.2 | 178.3 | 196.2 | 218 | 246 | 1.2 | 205 | 228 | 243 | 270 |
| 1.5 | 210 | 230 | 247 | 282 | 1.5 | 218 | 234 | 258 | 300 | 1.5 | 234 | 264 | 279 | 315 |
| 1.8 | 250 | 264 | 289 | 331 | 1.8 | 256 | 270 | 300 | 350 | 1.8 | 264 | 297 | 318 | 362 |
| 2.1 | 277 | 297 | 329 | 379 | 2.1 | 282 | 309 | 341 | 398 | 2.1 | 294 | 329 | 356 | 409 |
| 2.4 | 312 | 332 | 368 | 429 | 2.4 | 318 | 344 | 380 | 445 | 2.4 | 327 | 365 | 395 | 456 |
| 2.7 | 341 | 365 | 410 | 478 | 2.7 | 350 | 385 | 419 | 493 | 2.7 | 359 | 401 | 431 | 502 |
| 3 | 380 | 401 | 451 | 522 | 3 | 389 | 416 | 463 | 538 | 3 | 401 | 436 | 475 | 551 |

Table 18: Specific conductance (κ) values for SDBS in aqueous mixtures of BSA containing 20 % w/w DMSO at different temperatures and at pressure $p = 100$ kPa

| BSA = 0% | | | | | BSA = 0.075% | | | | | BSA = 0.25% | | | | |
|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|-------------------------------------|--------|--------|--------|--------|
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 8 | 8.2 | 8.3 | 8.6 | 0 | 11.5 | 13 | 14 | 15.3 | 0 | 29 | 34.5 | 37.5 | 38 |
| 0.3 | 36.7 | 38.6 | 48.1 | 51.2 | 0.3 | 41.6 | 47.5 | 62.3 | 68.6 | 0.3 | 62.3 | 68.6 | 72.6 | 79.6 |
| 0.6 | 69.8 | 80.5 | 92.7 | 99.8 | 0.6 | 80.4 | 86.9 | 94.9 | 109.9 | 0.6 | 95.9 | 101 | 104 | 119.6 |
| 0.9 | 103.7 | 123.7 | 138.7 | 147.6 | 0.9 | 114.6 | 130.6 | 146.5 | 157.6 | 0.9 | 127.6 | 139.5 | 157.6 | 167.4 |
| 1.2 | 137.6 | 165.5 | 185.8 | 196.1 | 1.2 | 150.9 | 166.7 | 195.6 | 206 | 1.2 | 162.5 | 176.6 | 204 | 215 |
| 1.5 | 175.3 | 208 | 229 | 243 | 1.5 | 186 | 214 | 239 | 252 | 1.5 | 196.3 | 222 | 248 | 262 |
| 1.8 | 207 | 239 | 264 | 287 | 1.8 | 220 | 256 | 272 | 291 | 1.8 | 229 | 265 | 281 | 301 |
| 2.1 | 235 | 275 | 303 | 328 | 2.1 | 250 | 280 | 313 | 335 | 2.1 | 262 | 292 | 323 | 345 |
| 2.4 | 263 | 311 | 341 | 368 | 2.4 | 278 | 319 | 352 | 378 | 2.4 | 291 | 328 | 362 | 391 |
| 2.7 | 291 | 346 | 380 | 409 | 2.7 | 304 | 357 | 395 | 423 | 2.7 | 316 | 365 | 402 | 434 |
| 3 | 319 | 381 | 419 | 450 | 3 | 330 | 391 | 430 | 465 | 3 | 340 | 399 | 440 | 478 |
| BSA = 0.05% | | | | | BSA = 0.1% | | | | | BSA = 0.5% | | | | |
| SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | | SDBS [$\mu\text{M}/\text{kg}$] | T/K | | | |
| | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 | | 293.15 | 298.15 | 303.15 | 308.15 |
| κ | | | | | κ | | | | | κ | | | | |
| 0 | 10.6 | 11.5 | 12.5 | 13.8 | 0 | 14.8 | 17.5 | 18.2 | 19.6 | 0 | 48 | 61.2 | 63.5 | 65.4 |
| 0.3 | 37.6 | 44.6 | 50.5 | 58.7 | 0.3 | 44.7 | 50.5 | 68.6 | 71.7 | 0.3 | 83.2 | 92.1 | 101 | 115.9 |
| 0.6 | 72.4 | 86.2 | 92.7 | 107 | 0.6 | 83.6 | 89.9 | 97.8 | 112.8 | 0.6 | 115.9 | 133.7 | 151.6 | 160.5 |
| 0.9 | 107.9 | 130.6 | 139.9 | 154.5 | 0.9 | 118.5 | 136.7 | 150.4 | 161.5 | 0.9 | 148.6 | 175.5 | 196.2 | 211 |
| 1.2 | 144.6 | 166.7 | 191.3 | 202.7 | 1.2 | 153.9 | 171.6 | 199.5 | 209 | 1.2 | 184.3 | 208 | 231 | 246 |
| 1.5 | 179.2 | 211 | 231 | 246 | 1.5 | 189.7 | 219 | 242 | 255 | 1.5 | 217 | 240 | 268 | 285 |
| 1.8 | 212 | 244 | 268 | 287 | 1.8 | 224 | 261 | 276 | 295 | 1.8 | 240 | 273 | 303 | 327 |
| 2.1 | 242 | 277 | 309 | 330 | 2.1 | 255 | 287 | 318 | 339 | 2.1 | 267 | 303 | 338 | 368 |
| 2.4 | 268 | 318 | 346 | 372 | 2.4 | 284 | 323 | 356 | 383 | 2.4 | 294 | 338 | 374 | 410 |
| 2.7 | 294 | 348 | 386 | 419 | 2.7 | 309 | 361 | 399 | 427 | 2.7 | 324 | 371 | 407 | 448 |
| 3 | 321 | 384 | 424 | 460 | 3 | 337 | 395 | 434 | 470 | 3 | 350 | 404 | 445 | 490 |

