

DSC XXI - Colloid and surfactant studies

Alves F. R., Zaniquelli M. E., Loh W., Castanheira E. M., Real Oliveira M. E. and Feitosa E. (2007) Vesicle-micelle transition in aqueous mixtures of the cationic dioctadecyldimethylammonium and octadecyltrimethylammonium bromide surfactants. *J Colloid Interface Sci* **316**, 132-139.

Abstract: The vesicle-micelle transition in aqueous mixtures of dioctadecyldimethylammonium and octadecyltrimethylammonium bromide (DODAB and C(18)TAB) cationic surfactants, having respectively double and single chain, was investigated by differential scanning calorimetry (DSC), steady-state fluorescence, dynamic light scattering (DLS) and surface tension. The experiments performed at constant total surfactant concentration, up to 1.0 mM, reveal that these homologous surfactants mix together to form mixed vesicles and/or micelles, depending on the relative amount of the surfactants. The melting temperature $T(m)$ of the mixed DODAB-C(18)TAB vesicles is larger than that for the neat DODAB in water owing to the incorporation of C(18)TAB in the vesicle bilayer. The surface tension decreases sigmoidally with C(18)TAB concentration and the inflection point lies around $x(\text{DODAB})$ approximately 0.4, indicating the onset of micelle formation owing to saturation of DODAB vesicles by C(18)TAB molecules. When $x(\text{DODAB}) > 0.5$ C(18)TAB molecules are mainly solubilised by the vesicles, but when $x(\text{DODAB}) < 0.25$ micelles are dominant. Fluorescence data of the Nile Red probe incorporated in the system at different surfactant molar fractions indicate the formation of micelle and vesicle structures. These structures have apparent hydrodynamic radius $R(H)$ of about 180 and 500-800 nm, respectively, as obtained by DLS measurements.

Ambrosi M., Fratini E., Alfredsson V., Ninham B. W., Giorgi R., Lo N. P., and Baglioni P. (2006) Nanotubes from a vitamin C-based bolaamphiphile. *J Am Chem Soc* **128**, 7209-7214.

Abstract: A bolaform surfactant, 1,12-diascorbyl dodecanedioate (BOLA12), with ascorbic acid units as the polar headgroups was synthesized for the first time. Once dispersed in water above 0.5% w/w, BOLA12 forms hollow nanotubes as revealed by cryo-TEM experiments. These nanostructures transform into clear micellar solutions on heating. X-ray diffraction and SAXS experiments were performed both on the pure solid and on its aqueous dispersions. The critical aggregation concentration and the phase behavior were determined by conductivity and DSC experiments. The latter technique provided also the amount of strongly bound, solvating water molecules that surround the polar headgroups. BOLA12 shows the same reducing properties of ascorbic acid, as indicated by the antioxidant activity evaluated with the DPPH method. This feature was used for the reduction of Pd(II) ions on the surface of the nanoassemblies, which lead to the formation of large bundles homogeneously coated with palladium as observed in SEM micrographs.

Antipova A. S., Semenova M. G., Belyakova L. E., and Il'in M. M. (2001) On relationships between molecular structure, interaction and surface behavior in mixture: small-molecule surfactant+protein. *Colloids Surf B Biointerfaces* **21**, 217-230.

Abstract: We report on the effect of distinct in nature small-molecule surfactants (model, a sodium salt of capric acid, Na-caprate; and commercially important, a citric acid ester of monoglyceride, CITREM; a sodium salt of stearyl-lactoyl lactic acid, SSL (Na(+)); polyglycerol ester, PGE (080)) on molecular properties in a bulk and at the air-water interface of globular legumin and random-coiled micellar sodium caseinate. The role of the structure of both proteins and small-molecule surfactants in the effect studied has been elucidated by measurements in a bulk aqueous medium of the enthalpy of their interaction from mixing calorimetry, the change in value of weight average molecular weight of the proteins and the thermodynamics of the pair protein-protein interactions from laser static light scattering as well as, in addition, by measurements of the change in hydrodynamic radius for micellar sodium caseinate from laser dynamic light scattering. The effect of the small-molecule surfactants on the thermodynamics of the protein heat denaturation and thereby on the protein conformational stability has been studied by differential scanning calorimetry in the case of globular legumin. The interrelation between the effects of the small-molecule surfactants on the properties of the proteins in a bulk and at the planar air-water interface has been elucidated by tensiometry. The combined data of mixing calorimetry, differential scanning calorimetry and laser light scattering suggest some complex formation between the small-molecule surfactants and the proteins in a bulk aqueous medium. Predominantly hydrophobic interaction along with electrostatic and hydrogen bonding form the basis of the complex formation. The found effect of the small-

molecule surfactants on the surface activity of their mixtures with proteins is governed primarily by both the extent of the protein association, resulting in specific hydrophobicity/hydrophilicity of the surface of the protein associates, and the specific protein conformational stability, for the globular protein, produced by the interaction between the proteins and the small-molecule surfactants.

Antunes F. E., Brito R. O., Marques E. F., Lindman B. and Miguel M. (2007) Mechanisms behind the faceting of catanionic vesicles by polycations: chain crystallization and segregation. *J Phys. Chem B* **111**, 116-123.

Abstract: Vesicles composed of an anionic and a cationic surfactant, with a net negative charge, associate strongly with a hydrophobically modified polycation (LM200) and with an unmodified polycation with higher charge density (JR400), forming viscoelastic gel-like structures. Calorimetric results show that in these gels, LM200 induces a rise of the chain melting temperature (T_m) of the vesicles, whereas JR400 has the opposite effect. For both polymer-vesicle systems, the shear viscosity exhibits an inflection point at T_m , and for the LM200 system the measured relaxation times are significantly higher below T_m . The neat vesicles and the polycation-bound vesicles have a polygonal-like faceted shape when the surfactant chains in the bilayer are crystallized, as probed by cryo-transmission electron microscopy. Above T_m , the neat and the LM200-bound vesicles regain a spheroidal shape, whereas those in the JR400 system remain with a deformed faceted shape even above T_m . These shape changes are interpreted in terms of different mechanisms for the polymer-vesicle interaction, which seem to be highly dependent on polymer architecture, namely charge density and hydrophobic modification. A crystallization-segregation mechanism is proposed for the LM200-vesicle system, while, for the JR400-vesicle one, charge polarization-lateral segregation effects induced by the polycation in the catanionic bilayer are envisaged.

Arnulphi C., Sot J., Garcia-Pacios M., Arrondo J. L., Alonso A. and Goni F. M. (2007) Triton X-100 partitioning into sphingomyelin bilayers at subsolubilizing detergent concentrations: effect of lipid phase and a comparison with dipalmitoylphosphatidylcholine. *Biophys J* **93**, 3504-3514.

Abstract: We examined the partitioning of the nonionic detergent Triton X-100 at subsolubilizing concentrations into bilayers of either egg sphingomyelin (SM), palmitoyl SM, or dipalmitoylphosphatidylcholine. SM is known to require less detergent than phosphatidylcholine to achieve the same extent of solubilization, and for all three phospholipids solubilization is temperature dependent. In addition, the three lipids exhibit a gel-fluid phase transition in the 38-41 degrees C temperature range. Experiments have been performed at Triton X-100 concentrations well below the critical micellar concentration, so that only detergent monomers have to be considered. Lipid/detergent mol ratios were never <10:1, thus ensuring that the solubilization stage was never reached. Isothermal titration calorimetry, DSC, and infrared, fluorescence, and $(31)P$ -NMR spectroscopies were applied in the 5-55 degrees C temperature range. The results show that, irrespective of the chemical nature of the lipid, ΔG degrees of partitioning remained in the range of -27 kJ/mol lipid in the gel phase and of -30 kJ/mol lipid in the fluid phase. This small difference cannot account for the observed phase-dependent differences in solubilization. Such virtually constant ΔG degrees occurred as a result of the compensation of enthalpic and entropic components, which varied with both temperature and lipid composition. Consequently, the observed different susceptibilities to solubilization cannot be attributed to differential binding but to further events in the solubilization process, e.g., bilayer saturability by detergent or propensity to form lipid-detergent mixed micelles. The data here shed light on the relatively unexplored early stages of membrane solubilization and open new ways to understand the phenomenon of membrane resistance toward detergent solubilization.

Bam N. B., Cleland J. L., Yang J., Manning M. C., Carpenter J. F., Kelley R. F., and Randolph T. W. (1998) Tween protects recombinant human growth hormone against agitation-induced damage via hydrophobic interactions. *J Pharm Sci* **87**, 1554-1559.

Abstract: In the absence of surfactants, recombinant human growth hormone (rhGH) rapidly forms insoluble aggregates during agitation. The nonionic surfactant Tween 20, when present at Tween:protein molar ratios >4, effectively inhibits this aggregation. Differential scanning calorimetry (DSC) of rhGH solutions showed melting transitions that decreased by ca. 2 degrees C in the presence of Tween. Circular dichroism (CD) studies of the same thermal transition showed that the decrease is specific to the relatively high protein concentrations required for DSC. CD studies showed melting transitions that decreased with lower protein concentrations. Tween has an insignificant effect on the melting transition of rhGH at lower protein concentrations (0.18 mg/mL). Injection titration microcalorimetry showed that the interaction of

Tween with rhGH is characterized by a weak enthalpy of binding. For comparison, interferon-g, another protein which has been shown to bind Tween, also shows weak enthalpy of binding. Fluorescent probe binding studies and infrared spectroscopic investigations of rhGH secondary structure support suggestions in the literature (Bam, N. B.; Cleland, J. L., Randolph, T. W. Molten globule intermediate of recombinant human growth hormone: stabilization with surfactants. *Biotechnol. Prog.* 1996. 12, 801-809) that Tween binding is driven by hydrophobic interactions, with little perturbation of protein secondary structure.

Bardavid S. M., Schulz P. C. and Arancibia E. L. (2007) IGC studies of binary cationic surfactant mixtures. *J Colloid Interface Sci* **316**, 114-119.

Abstract: Inverse gas chromatography (IGC) has been used to measure the interaction parameter between two twin-tailed cationic surfactants. Didodecyltrimethylammonium (DDAB) and dioctadecyltrimethylammonium (DODAB) bromides and their mixtures were used as stationary phases. IGC and DSC techniques have been used for the determination of the temperature zone of working. The activity coefficients at infinite dilution (on a mole fraction basis) were calculated for eleven probe solutes on each pure surfactant column. Values of interaction parameter between surfactants obtained at four weight fractions of the mixtures and at five temperatures are positive and suggested that the interactions is more unfavourable with the increment of DODAB concentration in the mixture. The results are interpreted on the basis of partial miscibility between DDAB and DODAB.

Barman S. and Vasudevan S. (2006) Melting of saturated fatty acid zinc soaps. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 22407-22414.

Abstract: The melting of alkyl chains in the saturated fatty acid zinc soaps of different chain lengths, $Zn(C(n)H(2n+1)COO)_2$; $n = 11, 13, 15, \text{ and } 17$, have been investigated by powder X-ray diffraction, differential scanning calorimetry, and vibrational spectroscopy. These compounds have a layer structure with the alkyl chains arranged as tilted bilayers and with all methylene chains adopting a planar, all-trans conformation at room temperature. The saturated fatty acid zinc soaps exhibit a single reversible melting transition with the associated enthalpy change varying linearly with alkyl chain length, but surprisingly, the melting temperature remaining constant. Melting is associated with changes in the conformation of the alkyl chains and in the nature of coordination of the fatty acid to zinc. By monitoring features in the infrared spectra that are characteristic of the global conformation of the alkyl chains, a quantitative relation between conformational disorder and melting is established. It is found that, irrespective of the alkyl chain length, melting occurs when 30% of the chains in the soap are disordered. These results highlight the universal nature of the melting of saturated fatty acid zinc soaps and provide a simple explanation for the observed phenomena.

Barragan-Montero V., Winum J. Y., Moles J. P., Juan E., Clavel C., and Montero J. L. (2005) Synthesis and properties of isocannabinoid and cholesterol derivatized rhamnosurfactants: application to liposomal targeting of keratinocytes and skin. *Eur J Med Chem* **40**, 1022-1029.

Abstract: The usefulness of vesicles to cargo material depends on the design of new ligands able to incorporate easily inside the bilayer and also to direct the vesicles to the targeted site. Therefore, the synthesis of two new rhamnose-bearing surfactants is described. The hydrophobic part consists of cholesterol (in compound 3) and cetylidene phloroglucinol (in compound 6). The ability of these two rhamnolipids to incorporate into a DPPC membrane and to form aggregates is investigated, respectively, by differential scanning calorimetry and by surface tension measurements. Those two new surfactants were incorporated in fluorescent liposomes to study their interactions with keratinocytes and skin sections. Intraliposomal delivery to keratinocytes was observed in both cases, even if the kinetics of delivery were different according to the rhamnosurfactant used. Skin sections were stained by both liposomal formulations, and different interactions between the liposomes and skin cells according to the surfactant used were noted.

Bhattacharya S., Subramanian M., and Hiremath U. S. (1995) Surfactant lipids containing aromatic units produce vesicular membranes with high thermal stability. *Chem Phys Lipids* **78**, 177-188.

Abstract: Six new vesicle-forming, cationic surfactant lipids are synthesized. Four of them contain 'flat' aromatic units at different locations of hydrophobic segments. In order to estimate the influence of aromatic units in the lipid monomer two other surfactant lipids of related structure with n-butyloxy units in the places of aromatic groups were also prepared. Transmission electron microscopy confirmed the vesicular

membrane formation from these newly synthesized lipids. DSC or temperature-dependent keto-enol tautomerism of benzoylacetyl-doped vesicles reveal a remarkable increase in the thermal stability of the membranes formed from aromatic surfactant lipids in contradistinction to their counterparts that contain n-butyloxy units. The enhanced thermal stability originates presumably as a consequence of inter-monomer stacking.

Boonme P., Krauel K., Graf A., Rades T., and Junyaprasert V. B. (2006) Characterization of microemulsion structures in the pseudoternary phase diagram of isopropyl palmitate/water/Brij 97:1-butanol. *AAPS PharmSciTech* **7**, E45.

Abstract: This research was aimed to characterize microemulsion systems of isopropyl palmitate (IPP), water, and 2:1 Brij 97 and 1-butanol by different experimental techniques. A pseudoternary phase diagram was constructed using water titration method. At 45% wt/wt surfactant system, microemulsions containing various ratios of water and IPP were prepared and identified by electrical conductivity, viscosity, differential scanning calorimetry (DSC), cryo-field emission scanning electron microscopy (cryo-FESEM) and nuclear magnetic resonance (NMR). The results from conductivity and viscosity suggested a percolation transition from water-in-oil (water/oil) to oil-in-water (oil/water) microemulsions at 30% wt/wt water. From DSC results, the exothermic peak of water and the endothermic peak of IPP indicated that the transition of water/oil to oil/water microemulsions occurred at 30% wt/wt water. Cryo-FESEM photomicrographs revealed globular structures of microemulsions at higher than 15% wt/wt water. In addition, self-diffusion coefficients determined by NMR reflected that the diffusability of water increased at higher than 35% wt/wt water, while that of IPP was in reverse. Therefore, the results from all techniques are in good agreement and indicate that the water/oil and oil/water transition point occurred in the range of 30% to 35% wt/wt water.

Boonme P., Krauel K., Graf A., Rades T., and Junyaprasert V. B. (2006) Characterisation of microstructures formed in isopropyl palmitate/water/Aerosol OT:1-butanol (2:1) system. *Pharmazie* **61**, 927-932.

Abstract: The aim of this work was to determine the type and microstructure of microemulsion samples formed in IPP/water/AerosolOT:1-butanol (2:1) systems as a case study for the investigation of microemulsions. The concentration of the surfactant/cosurfactant mixture was kept constant while the ratio of water to oil was varied. Several techniques were used to investigate the types and phase transitions of the microemulsion formulations. The experimental methods used included visual observation cross-polarized light microscopy (PLM) appearance, conductivity, viscosity, cryo-field emission scanning electron microscopy (cryo-FESEM), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR), and fluorescence resonance energy transfer (FRET). Taken together, the results of the various techniques imply that the systems investigated are undergoing two transitions as a function of water concentration. Between 10-15%w/w of water, the systems change from headgroup hydrated surfactant solutions in oil (or possibly very small reversed micellar systems) to w/o microemulsions. These systems then change to o/w microemulsions between 25-30%w/w of water. The transitions however, appear to be gradual, as for example the DSC data indicates a transition between 15-20%w/w of water. Furthermore, for some methods the changes observed were very weak, and only with supportive data of other techniques can the phase behaviour of the microemulsion systems be interpreted with confidence. Interestingly, no indication of the presence of a bicontinuous intermediate microstructure was found. Liquid crystal formation was detected in samples containing 55%w/w of water.

Brito R. O., Marques E. F., Gomes P., Joao A. M. and Pons R. (2008) Structure/Property Relationships for the Thermotropic Behavior of Lysine-Based Amphiphiles: from Hexagonal to Smectic Phases. *J Phys. Chem B*. (epublication)

Abstract: Amino acid-derived gemini surfactants arise as a potentially good alternative to the more conventional lipid and synthetic cationic systems in view of their enhanced interfacial properties, increased chemical stability, and low toxicity. The presence of an amino acid as the polar headgroup allows toxicity reduction, with the simultaneous increase of biodegradability. For these compounds, the establishment of structure/function relationships from the assessment of their basic aggregation properties is therefore of the utmost interest, e.g., in the design of operative self-assembled systems (e.g., liposomes, nanotubes, etc). In this context, the study of the thermal phase behavior of the dry surfactants is a natural, straightforward first step, the more so as thermotropic liquid crystals are also relevant for practical

applications. In this work, several lysine-based amphiphiles with a gemini-like configuration have been synthesized, with the amino acid side chain as the spacer group. The molecules are either esters (neutral, with C6-C12 even chains) or sodium carboxylates (anionic, with C6-C12 even chains). Upon increasing the temperature, different crystalline (cr) and liquid-crystalline (lc) phases have been detected and the corresponding thermodynamic and structural parameters determined by a combination of differential scanning calorimetry, polarizing light microscopy and small-angle X-ray scattering. The phase behavior of the amphiphiles is highly dependent on both the chain length and the presence of charge on the headgroup, with significant differences occurring within and between each group of molecules. The C6 and C8 esters form reverse hexagonal cr and lc phases, while C10 and C12 self-assemble into smectic cr and lc structures, with C10 showing also a reverse hexagonal lc phase prior to isotropization. All the carboxylate derivatives form smectic lc phases at high enough temperature prior to isotropization. The rationalization of the phase behavior and phase transition energetics of the compounds has been put forth on the basis of the intermolecular interactions at stake (van der Waals, H-bonding, electrostatic, and packing) and the molecular shape of the amphiphile

Canadas O., Guerrero R., Garcia-Canero R., Orellana G., Menendez M., and Casals C. (2004)

Characterization of liposomal tacrolimus in lung surfactant-like phospholipids and evaluation of its immunosuppressive activity. *Biochemistry* **43**, 9926-9938.

Abstract: Tacrolimus (FK506) is a hydrophobic immunosuppressive agent that rapidly penetrates the plasmatic membrane and inhibits the signal transduction cascade of T lymphocytes. The objective of this study was the characterization of liposomal FK506 with surfactant-like phospholipids to be administered intratracheally after lung transplantation or in inflammatory lung diseases. We evaluated the optimal incorporation of FK506 in dipalmitoylphosphatidylcholine (DPPC) and DPPC/1-palmitoyl-2-oleoylphosphatidylglycerol (POPG) monolayers and bilayers and the effects of FK506 on the physical properties of DPPC and DPPC/POPG (8:2 w/w) vesicles. In addition, we assessed the immunosuppressive effects of surfactant-like phospholipid vesicles containing different amounts of FK506 on T-cell proliferation and interleukin 2 production. From surface pressure measurements of FK506/DPPC and FK506/DPPC/POPG mixed monolayers, we determined that FK506 was embedded into these monolayers up to an FK506 concentration of about 0.4 mol %. Beyond this concentration, FK506 was not quantitatively incorporated into the monolayer, suggesting possible concentration-dependent aggregation of tacrolimus. The incorporation of FK506 into DPPC monolayers, at concentrations $\leq 5 \mu\text{M}$, occurred with a partition coefficient of $(3.9 \pm 0.3) \times 10^3$ at the bilayer equivalence pressure. FK506 was incorporated in DPPC bilayers up to an FK506 concentration of about 0.7-1 mol %, which was about double that obtained via the monolayer technique. FK506 hardly affected the transition enthalpy, the T_m , and cooperativity of the phase transition of DPPC and DPPC/POPG vesicles as determined by differential scanning calorimetry and steady-state 1,6-diphenyl-1,3,5-hexatriene anisotropy. Finally, this study provides evidence that liposomal FK506 retains the immunosuppressive efficacy of the drug.

Castile J. D., Taylor K. M., and Buckton G. (1999) A high sensitivity differential scanning calorimetry study of the interaction between poloxamers and dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine liposomes. *Int J Pharm* **182**, 101-110.

Abstract: High sensitivity differential scanning calorimetry (HSDSC) has been used to measure the thermal behaviour of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) liposomes to which poloxamer surfactants P338 or P407 had been added during or after preparation. The phospholipid pre-transition was more sensitive than the main transition to the association of poloxamers with liposomal bilayers. Poloxamers reduced the enthalpy of the pre-transition of liquid-crystalline state DMPC and DPPC MLVs but not that of gel state DPPC MLVs. Freezing and thawing DMPC and DPPC liposomes in the presence of poloxamers was shown to increase their interaction with the liposomal bilayers. Copyright.

Castile J. D., Taylor K. M., and Buckton G. (2001) The influence of incubation temperature and surfactant concentration on the interaction between dimyristoylphosphatidylcholine liposomes and poloxamer surfactants. *Int J Pharm* **221**, 197-209.

Abstract: Differential scanning calorimetry and photon correlation spectroscopy have been used to study the interaction between poloxamers P338 and P407 and dimyristoylphosphatidylcholine (DMPC) liposomes. The extent of the interaction was found to be dependent on the incubation temperature in

addition to the poloxamer concentration. At low poloxamer concentrations (0.1-1.0% w/v) an interaction with the phospholipid bilayer was detected by a reduction of the pre-transition enthalpy of DMPC. At higher concentrations (2.0-5.0% w/v), the main phase transition temperature of the liposomes decreased and the endotherm broadened with a shoulder on the high temperature side, indicative of phase separation. Maximum increases in the diameter of small freeze-thaw extruded liposomes were shown to occur at temperatures close to the poloxamer critical micelle temperatures. At higher temperatures and surfactant concentrations there was evidence of solubilization of phospholipid into mixed micelles.

Chamani J. (2006) Comparison of the conformational stability of the non-native alpha-helical intermediate of thiol-modified beta-lactoglobulin upon interaction with sodium n-alkyl sulfates at two different pH. *J Colloid Interface Sci* **299**, 636-646.

Abstract: Bovine beta-lactoglobulin assumes a dimeric native conformation at neutral pH, while the conformation at pH 2 is monomeric but still native. beta-lactoglobulin has a free thiol at Cys121, which is buried between the beta-barrel and the C-terminal major or alpha-helix. This thiol group was specifically reacted with DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) at pH 7.5 and 2, producing a modified beta-lactoglobulin containing a mixed disulfide bond with 5-thio-2-nitrobenzoic acid (TNB). beta-Lactoglobulin is a predominantly beta-sheet protein, although it has a markedly high intrinsic preference for alpha-helical structure. The formation of non-native alpha-helical intermediate of thiol modified beta-lactoglobulin (TNB-beta-LG) was induced by n-alkyl sulfates including sodium octyl sulfate, SOS; sodium decyl sulfate, SDeS; sodium dodecyl sulfate, SDS; and sodium tetradecyl sulfate, STS at pH 7.5 and 2. The conformation and stability of non-native alpha-helical intermediate (alphaI) state of TNB-beta-LG were studied by circular dichroism (CD), fluorescence and differential scanning calorimetry (DSC) techniques. The effect of n-alkyl sulfates on the structure of alphaI state at both pH was utilized to investigate the contribution of hydrophobic interactions to the stability of alphaI intermediate. The present results suggest that the folding reaction of beta-LG follows a non-hierarchical mechanism and hydrophobic interactions play important roles in stabilizing the native state of beta-LG at pH 2 with more positive charges repulsion than at pH 7.5. Then TNB-beta-LG will become a useful model to analyze the conformation and stability of the intermediate of protein folding.

Chronakis I. S., Fredholm A., Triantafyllou A. O., and Oste R. (2004) Complex formation in aqueous medium of partially hydrolysed oat cereal proteins with sodium stearyl-2 lactylate (SSL) lipid surfactant and implications for bile acids activity. *Colloids Surf B Biointerfaces* **35**, 175-184.

Abstract: Sodium stearyl-2 lactylate (SSL) lipid surfactant molecules specifically bind partially hydrolysed oat proteins in aqueous medium and significantly enhance the dispersion stability of oat cereal preparations. The proposed complexation is composition dependent and a greater understanding of the role of both oat proteins and lipid surfactant in the effect was gained with data from high performance liquid chromatography (HPLC-UV), viscometry and differential scanning micro calorimetry. The effect of the lipid surfactant on the degree of association is primarily governed by the conformational activity of oat protein molecules related to the extent of protein hydrolysed state, as well as protein unfolded and subsequent aggregated structures. SSL does not dissociate oat proteins into subunits or destroy important hydrophobic contacts already stabilising the protein molecules. Although the exact mode of association is unknown, the present study demonstrates that such interactions occur in a specific manner and suggest selectivity of oat proteins for individual fatty acids. The effect of various amounts of bile acids on SSL-oat protein interaction was also investigated, as a first attempt to investigate the role of lipid surfactant molecules in the known cholesterol-lowering action of oat cereal ingredients and to elucidate favourable conditions by which oat cereal can elicit hypocholesterolemic effects.

Collins T., D'Amico S., Georlette D., Marx J. C., Huston A. L., and Feller G. (2006) A nondetergent sulfobetaine prevents protein aggregation in microcalorimetric studies. *Anal Biochem* **352**, 299-301.

Crevelde L. D., Meijberg W., Berendsen H. J., and Pepermans H. A. (2001) DSC studies of *Fusarium solani* pisi cutinase: consequences for stability in the presence of surfactants. *Biophys Chem* **92**, 65-75.

Abstract: The application of cutinase from *Fusarium solani* pisi as a fat-stain removing ingredient in laundry washing is hampered by its lack of stability in the presence of anionic surfactants. We postulate that the stability of cutinase towards anionics can be improved by mutations increasing its temperature stability. Thermal unfolding as measured with DSC, appears to be irreversible, though the thermograms are

more symmetric than predicted by a simple irreversible model. In the presence of taurodeoxycholate (TDOC), the unfolding temperature is lower and the unfolding is reversible. We conclude that an early reversible unfolding intermediate exists in which a number of additional hydrophobic patches are exposed to the solvent, or preferentially are covered with TDOC. Improvement of the stability of cutinase with respect to both surfactants and thermal denaturation, should thus be directed toward the prevention of exposure of hydrophobic patches in the early intermediate.

Custers J. P., Broeke L. J. and Keurentjes J. T. (2007) Phase behavior and micellar properties of carboxylic Acid end group modified pluronic surfactants. *Langmuir* **23**, 12857-12863.

Abstract: The micellar behavior of three different carboxylic acid end standing (CAE) surfactants has been characterized using conductometry, differential scanning calorimetry, isothermal titration calorimetry, and dynamic light scattering. The CAE surfactants are modified high molecular weight Pluronic (PEO-PPO-PEO triblock copolymer) surfactants. The influence of pH and salt additives on the critical micellization temperature (CMT) and the cloud point of the CAE surfactants have been studied. Both the CMT and the cloud points of the CAE surfactants increase as a function of pH and decrease as a function of ionic strength. For the CAE surfactants, the CMT varies by about 5 degrees C, and the cloud point shows a variation in the order of 20-30 degrees C, as compared to the unmodified Pluronics. From the different experimental techniques, it follows that at low pH values (pH < 3.5), the CAE surfactants show the same micellar behavior as the unmodified Pluronic, while at high pH values (pH > 6), the micellar properties of the CAE surfactants are considerably different from those observed for the corresponding Pluronic. It has been demonstrated that the CAE micelles are capable of removing simultaneously divalent ions and phenanthrene. The CAE surfactants are the first known anionic surfactants that show cloud point behavior with the addition of low concentrations of simple salts, such as, for example, NaCl.

Dico A. S., Hancock J., Morrow M. R., Stewart J., Harris S., and Keough K. M. (1997) Pulmonary surfactant protein SP-B interacts similarly with dipalmitoylphosphatidylglycerol and dipalmitoylphosphatidylcholine in phosphatidylcholine/phosphatidylglycerol mixtures. *Biochemistry* **36**, 4172-4177.

Abstract: Porcine pulmonary surfactant-associated protein SP-B was incorporated into bilayers of chain-perdeuterated dipalmitoylphosphatidylglycerol (DPPG-d62) and into bilayers containing 70 mol % dipalmitoylphosphatidylcholine (DPPC) and 30 mol % DPPG-d62 or 70 mol % chain-perdeuterated DPPC (DPPC-d62) and 30 mol % DPPG. The effect of SP-B on the phase behavior, lipid chain order, and dynamics in these bilayers was examined using deuterium nuclear magnetic resonance (²H-NMR). In both DPPG-d62 and the mixed lipid system, SP-B is found to have little effect on chain order in the liquid crystalline phase. With 11% (w/w) SP-B present, both bilayer systems display a continuous change from liquid crystal to gel with no evidence of two-phase coexistence near the transition. Despite its limited effect on chain order in these bilayers, SP-B is found to strongly perturb chain deuteron transverse relaxation in the liquid crystal and gel phases of DPPG-d62 and the DPPC/DPPG (7:3) mixtures. The observation that SP-B associates with the bilayer in a way which substantially alters the slow motions responsible for transverse relaxation without significantly affecting chain order in either the liquid crystal or gel phases may place some constraints on possible models for that association.

El Maghraby G. M., Williams A. C., and Barry B. W. (2004) Interactions of surfactants (edge activators) and skin penetration enhancers with liposomes. *Int J Pharm* **276**, 143-161.

Abstract: Incorporating edge activators (surfactants) into liposomes was shown previously to improve estradiol vesicular skin delivery; this phenomenon was concentration dependent with low or high concentrations being less effective. Replacing surfactants with limonene produced similar behaviour, but oleic acid effects were linear with concentration up to 16% (w/w), beyond which it was incompatible with the phospholipid. This present study thus employed high sensitivity differential scanning calorimetry to probe interactions of additives with dipalmitoylphosphatidylcholine (DPPC) membranes to explain such results. Cholesterol was included as an example of a membrane stabiliser that removed the DPPC pre-transition and produced vesicles with a higher transition temperature (T_m). Surfactants also removed the lipid pre-transition but reduced T_m and co-operativity of the main peak. At higher concentrations, surfactants also formed new species, possibly mixed micelles with a lower T_m . The formation of mixed micelles may explain reduced skin delivery from liposomes containing high concentrations of surfactants. Limonene did not remove the pre-transition but reduced T_m and co-operativity of the main peak, apparently

forming new species at high concentrations, again correlating with vesicular delivery of estradiol. Oleic acid obliterated the pre-transition. The T_m and the co-operativity of the main peak were reduced with oleic acid concentrations up to 33.2mol%, above which there was no further change. At higher concentrations, phase separation was evident, confirming previous skin transport findings.

Feitosa E., Bonassi N. M., and Loh W. (2006) Vesicle-micelle transition in mixtures of dioctadecyldimethylammonium chloride and bromide with nonionic and zwitterionic surfactants. *Langmuir* **22**, 4512-4517.

Abstract: We have investigated the effect of mixing spontaneously formed dispersions of the cationic vesicle-forming dioctadecyldimethylammonium chloride and bromide (DODAX, with X being anions Cl- (C) or Br- (B)) with solutions of the micelle-forming nonionic ethylene oxide surfactants penta-, hepta-, and octaethyleneglycol mono-n-dodecyl ether, C12E(n) (n = 5, 7, and 8), and the zwitterionic 3-(N-hexadecyl-N,N-dimethylammonio)propane sulfonate (HPS). We used for this purpose differential scanning calorimetry (DSC), turbidity, and steady-state fluorescence spectroscopy to investigate the vesicle-micelle (V-M) transition yielded by adding C12E(n) and HPS to 1.0 mM vesicle dispersions of DODAC and DODAB. The addition of these surfactants lowers the gel-to-liquid crystalline phase transition temperature ($T(m)$) of DODAC and DODAB, and the transition becomes less cooperative, that is, the thermogram transition peak shifts to lower temperature and broadens to disappear when the V-M transition is complete, the vesicle bilayer becomes less organized, and the $T(m)$ decreases, in agreement with measurements of the fluorescence quantum yield of trans-diphenylpolyene (t-DPO) fluorescence molecules incorporated in the vesicle bilayer. Turbidity data indicate that the V-M transition comes about in three stages: first surfactants are solubilized into the vesicle bilayer; after saturation, the vesicles are ruptured, and, finally, the vesicles are completely solubilized and only mixed micelles are formed. The critical points of bilayer saturation and vesicle solubilization were obtained from the turbidity and fluorescence curves, and are reported in this communication. The solubility of DODAX is stronger for C12E(n) than it is for HPS, meaning that C12E(n) solubilizes DODAX more efficiently than does HPS. The surfactant solubilization depends slightly on the counterion, and varies according to the sequence C12E5 > C12E7 > C12E8 > HPS.

Feitosa E., Jansson J., and Lindman B. (2006) The effect of chain length on the melting temperature and size of dialkyldimethylammonium bromide vesicles. *Chem Phys Lipids* **142**, 128-132.

Abstract: Differential scanning calorimetry (DSC) and dynamic light scattering (DLS) were used to obtain the gel to liquid-crystalline phase transition temperature (T_m) and the apparent hydrodynamic radius (R_h) of spontaneously formed cationic vesicles of dialkyldimethylammonium bromide salts ($C_nH_{2n+1}2(CH_3)_2N^+Br^-$, with varying chain lengths. The preparation of cationic vesicles from aqueous solution of these surfactants, for n=12, 14, 16 and 18 (DDAB, DTDAB, DHDAB and DODAB, respectively), requires the knowledge of the surfactant gel to liquid-crystalline phase transition temperature, or melting temperature (T_m) since below this temperature these surfactants are poorly or not soluble in water. That series of cationic surfactants has been widely investigated as vesicle-forming surfactants, although C12 and C18, DDAB and DODAB are by far the most investigated from this series. The dependence of T_m of these surfactants on the number n of carbons in the surfactant tails is reported. The T_m obtained by DSC increases non-linearly with n, and the vesicle apparent radius R_h is about the same for DHDAB and DODAB, but much smaller for DDAB.

Feitosa E., Alves F. R., Niemiec A., Real Oliveira M. E., Castanheira E. M., and Baptista A. L. (2006) Cationic liposomes in mixed didodecyldimethylammonium bromide and dioctadecyldimethylammonium bromide aqueous dispersions studied by differential scanning calorimetry, Nile red fluorescence, and turbidity. *Langmuir* **22**, 3579-3585.

Abstract: The thermotropic phase behavior of cationic liposomes in mixtures of two of the most investigated liposome-forming double-chain lipids, dioctadecyldimethylammonium bromide (DODAB) and didodecyldimethylammonium bromide (DDAB), was investigated by differential scanning calorimetry (DSC), turbidity, and Nile Red fluorescence. The dispersions were investigated at 1.0 mM total surfactant concentration and varying DODAB and DDAB concentrations. The gel to liquid-crystalline phase transition temperatures (T_m) of neat DDAB and DODAB in aqueous dispersions are around 16 and 43 degrees C, respectively, and we aim to investigate the T_m behavior for mixtures of these cationic lipids. Overall, DDAB reduces the T_m of DODAB, the transition temperature depending on the DDAB content, but the T_m of DDAB is roughly independent of the DODAB concentration. Both DSC and fluorescence

measurements show that, within the mixture, at room temperature (ca. 22 degrees C), the DDAB-rich liposomes are in the liquid-crystalline state, whereas the DODAB-rich liposomes are in the gel state. DSC results point to a higher affinity of DDAB for DODAB liposomes than the reverse, resulting in two populations of mixed DDAB/DODAB liposomes with distinctive phase behavior. Fluorescence measurements also show that the presence of a small amount of DODAB in DDAB-rich liposomes causes a pronounced effect in Nile Red emission, due to the increase in liposome size, as inferred from turbidity results.

Feitosa E. and Alves F. R. (2008) The role of counterion on the thermotropic phase behavior of DODAB and DODAC vesicles. *Chem Phys. Lipids* **156**, 13-16.

Abstract: Dioctadecyldimethylammonium bromide and chloride surfactants (DODAX, X representing Br(-) or Cl(-) counterions) assemble in water, above their melting temperatures ($T(m)$), as cationic unilamellar vesicles at the typical surfactant concentration of 1.0mM. The larger $T(m)$ of DODAC (49 degrees C) relative to DODAB (45 degrees C) has been attributed to the differing affinity and binding specificity of the counterions to the vesicle interfaces. In this communication it is reported differential scanning calorimetry (DSC), conductimetry and dynamic light scattering (DLS) data for mixtures of DODAB and DODAC in water at 1.0mM total surfactant concentration and varying surfactant concentration, to investigate the effect of counterion on the pre-, main- and post-transition temperatures ($T(s)$, $T(m)$ and $T(p)$), and the data compared to the neat surfactants in water. Accordingly, $T(m)$ increases sigmoidally from 45.8 to 48.9 degrees C when DODAC molar fraction ($x(\text{DODAC})$) is varied from 0 to 1. Neat DODAB exhibits in addition to $T(m)$, $T(s)$ and $T(p)$ that are inhibited by DODAC. The main peak width $\Delta T(1/2)$ does not depend on the surfactant molar fraction but the melting enthalpy change ΔH is smaller for DODAB-rich dispersions due to the stronger affinity of Br(-). The conductivity and the apparent hydrodynamic diameter as well do not vary much with $x(\text{DODAB})$, indicating that the surface charge density is similar for DODAB and DODAC, evidencing the role of the counterion binding specificity and affinity on the properties of DODAX vesicles

Feitosa E. (2008) Spontaneous vesicles of sodium dihexadecylphosphate in HEPES buffer. *J Colloid Interface Sci* **320**, 608-610.

Abstract: The formation of spontaneous vesicles of dihexadecylphosphate (DHP) in a HEPES buffered solution at pH 7.4, the size, morphology and melting temperature, obtained by cryogenic transmission electron microscopy (cryo-TEM) and differential scanning calorimetry (DSC), are reported. The vesicles were formed by simply mixing a 5.0 mM lipid-solvent mixture at a temperature (75 degrees C) safely above the higher melting temperature $T(m)=70.4$ degrees C of DHP. The vesicle diameter ranges from 100 to 332 nm and their geometry is spherical, faceted or oblong. $T(m)$ increases from 66.8 to 70.4 as DHP concentration is raised from 0.6 to 5.0 mM

Grabner D., Zhai L., Talmon Y., Schmidt J., Freiburger N., Glatter O., Herzog B. and Hoffmann H. (2008) Phase behavior of aqueous mixtures of 2-phenylbenzimidazole-5-sulfonic acid and cetyltrimethylammonium bromide: hydrogels, vesicles, tubules, and ribbons. *J Phys. Chem B* **112**, 2901-2908.

Abstract: We studied the phase behavior and aggregation in mixed aqueous solutions of the anionic UV-absorber 2-phenylbenzimidazole-5-sulfonic acid sodium salt, PhBSA (Na salt), and the cationic surfactant cetyltrimethylammonium bromide, CTAB. The mixtures of the two components behave similarly to catanionic surfactant mixtures. The samples on the PhBSA-rich side have low viscosity and are turbid. The turbidity, due to uni- and multilamellar vesicles (SUVs and MLVs), increases with the mole ratio of CTAB. The interbilayer distance inside the MLV changes with the mole ratio of the two components from a few 10 nm for the 7:3 (molar ratio of PhBSA, Na salt, to CTAB) system to practically zero for the 5:5 mixture. The latter mixture forms a precipitate within less than 1 h. With the exception of the 5:5 mixture, all samples on the PhBSA-rich side are stable for many days. After that period, within one more day, the turbid vesicle phases are transformed into more or less clear hydrogels. We found that the gelation is due to the formation of very long stiff tubules about 14 nm in diameter, which is independent of the mixing ratio of the samples. The hydrogels and the tubules melt around 45 degrees C. On the CTAB-rich side, the 4:6 sample behaves like the 6:4 sample, whereas at 3:7 a precipitate was found to form shortly after mixing. At still smaller PhBSA (Na salt) to CTAB ratios, only clear, viscoelastic solutions are found that do not change with time. We determined the micellar structures in the samples by cryo-TEM and by SAXS. The rheological

properties of the hydrogels and of the viscoelastic samples were characterized by oscillating rheological measurements. DSC measurements indicated that the tubules are in a semicrystalline state and melt at around 45 degrees C. The semicrystalline bilayer of the tubules seems to have a 1:1 composition of PhBSA to CTAB. The excess PhBSA seems to be adsorbed on the tubules. It is assumed that the stiffness of the bilayer of the vesicles and the stiffness of the tubules are due to the stiffness of the PhBSA molecule

Heerklotz H. (2004) The microcalorimetry of lipid membranes. *J. Phys.: Condens. Matter* **16** R441-R467.

Abstract: Insight into the forces governing a system is essential for understanding its behaviour and function. Calorimetric investigations provide a wealth of information that is not, or is hardly, available by other methods. This paper reviews calorimetric approaches and assays for the study of lipid vesicles (liposomes) and biological membranes. With respect to the instrumentation, differential scanning calorimetry (DSC), pressure perturbation calorimetry (PPC), isothermal titration calorimetry (ITC) and water sorption calorimetry are considered. Applications of these techniques to lipid systems include the measurement of thermodynamic parameters and a detailed characterization of the thermotropic, barotropic, and lyotropic phase behaviour. The membrane binding or partitioning of solutes (proteins, peptides, drugs, surfactants, ions, etc) can also be quantified. Many calorimetric assays are available for studying the effect of proteins and other additives on membranes, characterizing non-ideal mixing, domain formation, stability, curvature strain, permeability, solubilization, and fusion. Studies of membrane proteins in lipid environments elucidate lipid-protein interactions in membranes. The systems are described in terms of enthalpic and entropic forces, equilibrium constants, heat capacities, partial volume changes etc, shedding light also on the stability of structures and the molecular origin and mechanism of structural changes.

Heerklotz H., Tsamaloukas A., Kita-Tokarczyk K., Strunz P., and Gutberlet T. (2004) Structural, Volumetric, and Thermodynamic Characterization of a Micellar Sphere-to-Rod Transition. *J Am Chem Soc* **126**, 16544-16552.

Abstract: The thermotropic sphere-to-rod transition of nonionic surfactants was characterized in terms of a large set of parameters: the transition temperature and width, the partial volume, coefficient of thermal volume expansion, enthalpy, isobaric heat capacity, and structural parameters, such as radius of gyration and hydrodynamic radius. Data were recorded as a function of concentration of surfactants in H(2)O and in D(2)O. To this end, pressure perturbation calorimetry (PPC), small angle neutron scattering (SANS), dynamic light scattering (DLS), differential scanning calorimetry (DSC), and isothermal titration calorimetry (ITC) were applied in a study of aqueous solutions containing myristyl, tridecyl, and lauryl maltoside and heptaethyleneglycoltetradecyl ether (C(14)EO(7)). Small changes in the thermodynamic and volumetric parameters (e.g., the partial volume change is approximately +2 per thousand) are discussed in detail as the result of three effects governing the transition. (i) Reduction of the water accessible hydrophobic surface area (ASA(ap)) drives the transition. (ii) Shrinking in headgroup size by thermal dehydration triggers the transition. (iii) Hypothesized gradual ordering of the chains may control the effect of chain length on the transition.

Heller M. C., Carpenter J. F., and Randolph T. W. (1999) Protein formulation and lyophilization cycle design: prevention of damage due to freeze-concentration induced phase separation. *Biotechnol Bioeng* **63**, 166-174.

Abstract: Hemoglobin has been previously shown to unfold during freeze drying when lyophilized from formulations that undergo freeze-concentration induced phase separation (Heller et al. 1997. *Biotechnol Prog* 13:590-596). In this report, we show that such damage may be avoided using kinetic strategies to arrest the phase separation. By rapidly cooling samples during liquid nitrogen spray-freeze drying, the time that the formulation spends in temperature regimes (ca. -3 to -23 degrees C) in which phase separation is both thermodynamically favorable and kinetically realizable is minimized. Increased protein damage with decreasing cooling rates and/or longer annealing periods at -7 degrees C is observed by FTIR spectroscopy. Phase separation and concomitant protein damage may also be avoided by addition of mannitol at concentrations sufficient to cause crystallization. Mannitol crystals segregate the freeze concentrated solution into microscopic domains that block propagation and nucleation of phase separating events. Addition of noncrystallizing sugars, such as sucrose and trehalose, or nonionic surfactants, such as Tween

80 and Triton X-100, has little protective effect against phase separation induced damage during freezing drying.

Inoue T., Dong B. and Zheng L. Q. (2007) Phase behavior of binary mixture of 1-dodecyl-3-methylimidazolium bromide and water revealed by differential scanning calorimetry and polarized optical microscopy. *J Colloid Interface Sci* **307**, 578-581.

Abstract: Phase behavior of aqueous mixture of imidazolium ionic liquid, 1-dodecyl-3-methylimidazolium bromide, was investigated by means of differential scanning calorimetry and polarized optical microscopy. The mixture forms two types of lyotropic liquid-crystalline gels, one is composed of lamellar phase and the other is of hexagonal phase. T-X phase diagram of the mixture was constructed, which defines the regions of various phases appearing in this mixture.

Kaper H., Franke D., Smarsly B. M. and Faul C. F. (2007) A pyrrole-containing surfactant as a tecton for nanocomposite SiO₂ films. *Langmuir* **23**, 11273-11280.

Abstract: A surfactant featuring a polymerizable pyrrole head group (dodecyl-dimethyl-(2-pyrrol-1-yl-ethyl)-ammonium bromide, DDPABr) was synthesized. The thermotropic behavior of the surfactant was investigated by differential scanning calorimetry (DSC) and X-ray scattering techniques, with small-angle X-ray scattering (SAXS) analysis revealing a highly ordered lamellar bilayer structure. After full characterization, DDPABr was used in the preparation of mesostructured SiO₂ nanocomposite thin films via evaporation-induced self-assembly (EISA). Resulting thin SiO₂-DDPABr films were studied by 1D and 2D small-angle X-ray scattering (SAXS) techniques, indicating a lamellar nanocomposite structure. Suitable theoretical SAXS models were applied to fit the experimental 1D SAXS data. The surfactant could be chemically polymerized within the lamellar domains.

Katakam M., Bell L. N., and Banga A. K. (1995) Effect of surfactants on the physical stability of recombinant human growth hormone. *J Pharm Sci* **84**, 713-716.

Abstract: The physical stability of a human growth hormone (hGH) formulation upon exposure to air/water interfaces (with vortex mixing) and to nonisothermal stress [determined by differential scanning calorimetry (DSC)] was investigated. The effect of these stresses on the formation of soluble and insoluble aggregates was studied. The aggregates were characterized and quantified by size exclusion-HPLC and UV spectrophotometry. Vortex mixing of hGH solutions (0.5 mg/mL) in phosphate buffer, pH 7.4, for just 1 min caused 67% of the drug to precipitate as insoluble aggregates. These aggregates were noncovalent in nature. Non-ionic surfactants prevented the interfacially induced aggregation at their critical micelle concentration (cmc) for Pluronic F-68 (polyoxyethylene polyoxypropylene block polymer) and Brij 35 (polyoxyethylene alkyl ether) and above the cmc for Tween 80 (polyoxyethylene sorbitan monooleate). However, the same surfactants failed to stabilize hGH against thermal stress in DSC studies. Higher concentrations of surfactants actually destabilized hGH as evidenced by the decrease in the onset temperature for the denaturation endotherm.

Kawashima T., Sasaki A., and Sasaki S. (2006) Transition of nanostructure in DNA-cationic surfactant complexes with the added salt. *Biomacromolecules* **7**, 1942-1950.

Abstract: Nanostructures of complexes of DNA with single-chain surfactant of octadecyltrimethylammonium (OTA) and double-chain surfactant of didodecyltrimethylammonium (DDA) in aqueous NaCl solution at concentration, Cs, from 0 to 500 mM were studied using small-angle-scattering techniques (SAXS). SAXS profiles of the DNA-OTA complex show two SAXS peaks with a spacing ratio of 1:3(1/2) in the solution at Cs below 150 mM and three peaks with a spacing ratio of 1:3(1/2):4(1/2) at Cs above 250 mM. Contents of Na⁺ and Cl⁻ ions in the complexes evaluated from the atomic absorbance for Na⁺ and the potentiometry for Cl⁻ revealed charge molar ratios of OTA/DNA = 1 and DDA/DNA = 1.25. Contents of Na⁺ and Cl⁻ ions per ionic unit of DNA molecule in the DNA-OTA complex equilibrating with the solution at Cs below 100 mM were much less than 0.1, while they increased with NaCl concentration at Cs above 200 mM. The DNA-OTA complex in the solution at Cs above 260 mM exhibited an endothermic peak in the DSC measurements, and the others did not. On the basis of the experimental results, the salt concentration dependent nanostructures are discussed.

Keller M., Kerth A., and Blume A. (1997) Thermodynamics of interaction of octyl glucoside with phosphatidylcholine vesicles: partitioning and solubilization as studied by high sensitivity titration

calorimetry. *Biochim Biophys Acta* **1326**, 178-192.

Abstract: The interaction of the surfactant octyl glucoside (OG) with dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), and soy bean phosphatidylcholine (soy bean PC) was studied using high-sensitivity titration calorimetry. We determined the partition coefficient of OG between water and lipid bilayers and the transfer enthalpy of the surfactant by addition of lipid vesicles to OG monomers or vice versa. Comparison with the micellization enthalpy of the surfactant gives information on differences in the hydrophobic environment of OG in a liquid-crystalline bilayer or a micelle. The average partition coefficient P in mole fraction units for $x(e)$ approximately 0.12-0.2 decreases slightly from 4152 at 27 degrees C to 3479 at 70 degrees C for DMPC and from 4260 to 3879 for soy bean PC, respectively. The transfer enthalpy $\Delta H(T)$ of OG into lipid vesicles is positive at 27 degrees C and negative at 70 degrees C. Its temperature dependence is larger for the incorporation of OG into DMPC than into soy bean PC vesicles. It is concluded that OG in DMPC vesicles is better shielded from water than in soy bean PC vesicles or in micelles. Titration calorimetry was also used to determine the phase boundaries of the coexistence region of mixed vesicles and mixed micelles in the systems OG/DMPC, OG/DPPC, OG/DSPC, and OG/soy bean PC vesicles at 70 degrees C in the liquid-crystalline phase. DMPC and soy bean PC solubilization was also studied at 27 degrees C to investigate the effect of temperature. The effective surfactant to lipid ratios at saturation, $R(e)(sat)$, for all PCs studied are in the range between 1.33-1.72 and the ratios at complete solubilization, $R(e)(sol)$, are between 1.79-3.06. At 70 degrees C, the $R(e)(sat)$ values decrease with increasing chain length of the saturated PC. The ratios depend also slightly on temperature and the degree of unsaturation of the fatty acyl chains. For the OG/soy bean PC system, the coexistence range for mixed vesicles and mixed micelles is larger than for the corresponding PCs with saturated chains.

Kresheck G. C. and Mihelich J. (2003) Observation of complex thermal transitions for mixed micelle solutions containing alkyldimethylphosphine oxides and phospholipids and the accompanying cloud points. *Chem Phys Lipids* **123**, 45-62.

Abstract: The thermal properties of various mixtures of two nonionic surfactants, decyldimethylphosphine oxide (APO10) and dodecyldimethylphosphine oxide (APO12) and two phospholipids, dimyristoylphosphatidyl choline (DMPC) and dipalmitoylphosphatidyl choline (DPPC), were examined by differential scanning calorimetry at various mole fractions. The addition of APO12 to DMPC multilamellar vesicles lowered the temperature of the main transition, produced considerable broadening, and eliminated the pre-transition. Phase separation, as evidenced by the existence of a cloud point, $T(cp)$, occurred when the mole fraction of APO12, with respect to DMPC was 0.58 and above. A small abrupt increase in heat capacity was observed at, or slightly above, the cloud point of APO12 and all mixed micelle solutions. It appeared that mixed micelles coexisted with mixed bilayers when the mole fraction was between 0.58 and 0.75 and perhaps as low as a mole ratio of 0.32. All of the mixtures, except APO12/DMPC, exhibited a clear endotherm below the temperature corresponding to the cloud point, which likely reflects the growth in micellar size. Overlapping chain length dependent endothermic peaks, perhaps resulting from reorganization and/or continued association of the micelles, were observed above the cloud point for all of the mixtures except for APO10/DMPC solutions. However, solutions of mixed micelles consisting of APO10/DMPC with mole fractions of surfactant between 0.81 and 0.93 portrayed a broad unidentified exotherm of about 2 ± 1 kcal/mol, which was centered nearly 10-20 degrees C above the cloud point.

Kresheck G. C. (2006) The temperature dependence of the heat capacity change for micellization of nonionic surfactants. *J Colloid Interface Sci* **298**, 432-440.

Abstract: The thermodynamic parameters that govern micelle formation by four different nonionic surfactants were investigated by ITC and DSC. These included n-dodecyldimethylphosphine oxide (APO12), Triton X-100 (TX-100), n-octyltetraoxyethylene (C8E4), and N,N-dimethyloctylamine-N-oxide (DAO8). All of these surfactants had been previously investigated by solution calorimetry over smaller temperature ranges with conflicting conclusions as to the temperature dependence of the heat capacity change, ΔC_p , for the process. The temperature coefficient of the heat capacity change, B (cal/mol K²), was derived from the enthalpy data that were obtained at small intervals over a broad temperature range. The values obtained for each of the surfactants at 298.2 K for ΔC_p and B were -155 ± 2 and 0.50 ± 0.36 (APO12), -97 ± 3 and -0.24 ± 0.18 (TX-100), -105 ± 2 and 1.0 ± 0.3 (C8E4), and -82 ± 1 and 0.36 ± 0.04 (DAO8), cal/mol K and cal/mol K², respectively. The resulting B -values did not correlate with the cmc, aggregation number, or structure of the monomer in an obvious way, but they were found to reflect

the relative changes in hydration of the polar and nonpolar portions of the surfactant molecule as the micelles are formed. An analysis of the data obtained from DSC scans was used to describe the temperature dependence of the critical micelle concentration, cmc. An abrupt increase in heat capacity was observed for TX-100 and C8E4 solutions of 36.5 ± 0.5 and 21 ± 5 cal/mol K, respectively, as the temperature of the scan passed through the cloud point. This change in heat capacity may reflect the increased monomer concentration of the solutions that accompanies phase separation, although other interpretations of this jump are possible.

Inoue T., Dong B., and Zheng L. Q. (2006) Phase behavior of binary mixture of 1-dodecyl-3-methylimidazolium bromide and water revealed by differential scanning calorimetry and polarized optical microscopy. *J Colloid Interface Sci.*

Abstract: Phase behavior of aqueous mixture of imidazolium ionic liquid, 1-dodecyl-3-methylimidazolium bromide, was investigated by means of differential scanning calorimetry and polarized optical microscopy. The mixture forms two types of lyotropic liquid-crystalline gels, one is composed of lamellar phase and the other is of hexagonal phase. T-X phase diagram of the mixture was constructed, which defines the regions of various phases appearing in this mixture.

Kamlekar R. K. and Swamy M. J. (2006) Studies on the critical micellar concentration and phase transitions of stearyl carnitine. *Biosci Rep* **26**, 387-398.

Abstract: The critical micellar concentration (CMC) of stearyl carnitine was determined at different pH values at room temperature by fluorescence spectroscopy, monitoring the spectral changes of 8-anilino naphthalene-1-sulfonate (ANS). The CMC was found to vary with pH, increasing from about 10 microM at pH 3.0 to ca. 25 microM at pH 7.0, but decreasing slightly with further increase in pH to approximately 19 microM at pH 10.0. Differential scanning calorimetry (DSC) shows that stearyl carnitine dispersed in water at low concentration undergoes a broad thermotropic phase transition at 44.5 degrees C, with a transition enthalpy of 15.0 kcal/mol. The transition temperature (T_t) shifts to ca. 50.5 degrees C in the presence of 1 mM EDTA or when the concentration is increased significantly. The turbidity of aqueous dispersions of stearyl carnitine was found to be considerably high at low temperatures, which decreases quite abruptly over a short temperature range, indicating that a transition occurs from a phase of large aggregates to one of much smaller aggregates, most likely micelles. The phase transition temperature was determined as 29.1 degrees C at pH 3.0, which increased with increasing pH up to a value of 55.3 degrees C at pH 8.6 and remains nearly constant thereafter up to pH 11.2. The pH dependence of CMC and T_t suggest that the pKa of the carboxyl group of long chain acyl carnitines shifts to higher temperatures upon aggregation (micelles or bilayer membranes).

Kresheck G. C. (2006) The temperature dependence of the heat capacity change for micellization of nonionic surfactants. *J Colloid Interface Sci* **298**, 432-440.

Abstract: The thermodynamic parameters that govern micelle formation by four different nonionic surfactants were investigated by ITC and DSC. These included n-dodecyl dimethyl phosphine oxide (APO12), Triton X-100 (TX-100), n-octyl tetraoxyethylene (C8E4), and N,N-dimethyloctylamine-N-oxide (DAO8). All of these surfactants had been previously investigated by solution calorimetry over smaller temperature ranges with conflicting conclusions as to the temperature dependence of the heat capacity change, ΔC_p , for the process. The temperature coefficient of the heat capacity change, B (cal/mol K²), was derived from the enthalpy data that were obtained at small intervals over a broad temperature range. The values obtained for each of the surfactants at 298.2 K for ΔC_p and B were -155 ± 2 and 0.50 ± 0.36 (APO12), -97 ± 3 and -0.24 ± 0.18 (TX-100), -105 ± 2 and 1.0 ± 0.3 (C8E4), and -82 ± 1 and 0.36 ± 0.04 (DAO8), cal/mol K and cal/mol K², respectively. The resulting B-values did not correlate with the cmc, aggregation number, or structure of the monomer in an obvious way, but they were found to reflect the relative changes in hydration of the polar and nonpolar portions of the surfactant molecule as the micelles are formed. An analysis of the data obtained from DSC scans was used to describe the temperature dependence of the critical micelle concentration, cmc. An abrupt increase in heat capacity was observed for TX-100 and C8E4 solutions of 36.5 ± 0.5 and 21 ± 5 cal/mol K, respectively, as the temperature of the scan passed through the cloud point. This change in heat capacity may reflect the increased monomer concentration of the solutions that accompanies phase separation, although other interpretations of this jump are possible.

Lah J., Bester-Roga C. M., Perger T. M., and Vesnaver G. (2006) Energetics in correlation with structural features: the case of micellization. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 23279-23291.

Abstract: Understanding micellization processes at the molecular level has direct relevance for biological self-assembly, folding, and association processes. As such, it requires complete characterization of the micellization thermodynamics, including its correlation with the corresponding structural features. In this context, micellization of a series of model non-ionic surfactants (poly(ethylene glycol) mono-octyl ethers, C(8)E(γ)) was studied by isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). The corresponding structural properties of C(8)E(γ) micelles were investigated by small-angle X-ray scattering (SAXS). The C(8)E(γ) micellization, characterized independently from ITC, DSC, and structural data, reveals that $\Delta H(M)(o) > 0$, $\Delta S(M)(o) > 0$, and $\Delta C(P)(M)(o) < 0$, while the dissection of its energetics shows that it is primarily governed by the transfer of 20-30 C(8) alkyl chains from aqueous solution into the nonpolar core (r approximately 1.3 nm) of the spherical micelle. Moreover, thermodynamic parameters of micellization, estimated from the structural features related to the changes in solvent-accessible surface areas upon micellization, are in a good agreement with the corresponding parameters obtained from the analysis of ITC and DSC data. We have shown that the contributions to $\Delta S(M)(o)$ other than from hydration ($\Delta S(M)(other)(o)$), estimated from experimental data, appear to be small ($\Delta S(M)(other)(o) < 0.1 \Delta S(M)(other)(o)$) and agree well with the theoretical estimates expressed as a sum of the corresponding translational, conformational, and size contributions. These $\Delta S(M)(other)(o)$ contributions are much less unfavorable than those estimated for a rigid-body association, which indicates the dynamic nature of the C(8)E(γ) micellar aggregates. the dynamic nature of the C8EY micellar aggregates.

Lazzara G., Milioto S. and Muratore N. (2008) Solubilization of an organic solute in aqueous solutions of unimeric block copolymers and their mixtures with monomeric surfactant: volume, surface tension, differential scanning calorimetry, viscosity, and fluorescence spectroscopy studies. *J Phys. Chem B* **112**, 5616-5625.

Abstract: The ability of aqueous systems, formed by unimeric copolymers and their mixtures with a monomeric surfactant, in solubilizing large quantities of 1-nitropropane (PrNO₂) was explored. The copolymers are F68 and L64, which differ for the hydrophilicity, and the surfactant is sodium dodecanoate. For a better understanding of the mechanism of solubilization, thermodynamic (volume and differential scanning calorimetry), spectroscopy (steady-state fluorescence), viscosity, and interfacial investigations were carried out. PrNO₂ causes the micellization of the unimeric copolymer, and the required amount of PrNO₂ depends on the composition, the copolymer nature, and the temperature. Large quantities of PrNO₂ form mixed micelles where PrNO₂ experiences an environment similar to its pure liquid state. The presence of the additive allows a decrease of the critical micellar temperature, evidence of which is quantitatively explained through a novel thermodynamic approach. A synergistic effect in solubilizing PrNO₂ was observed when surfactant monomers were added to the unimeric copolymer solutions. The increased amount of PrNO₂ leads to the complete self-assembling of both the copolymer and the surfactant; a process favored by temperature increase. For all of the investigated systems, the presence of PrNO₂ generates a viscosity increase

Lehmler H. J. and Bummer P. M. (2005) Mixing behavior of 10-(perfluorohexyl)-decanol and DPPC. *Colloids Surf B Biointerfaces* **44**, 74-81.

Abstract: Fluorocarbon alcohol such as 10-(perfluorohexyl)-decanol are of interest for novel pulmonary drug delivery approaches. The purpose of this study was to investigate the mixing behavior of 10-(perfluorohexyl)-decanol with dipalmitoylphosphatidylcholine (DPPC), the major component of lung surfactant as an aid in assessing usefulness for this and other biomedical applications. The impact of 10-(perfluorohexyl)-decanol on the phase transitions of DPPC bilayers fully hydrated with a 0.15 M sodium chloride solution were studied using differential scanning calorimetry (DSC). No peak corresponding to excess alcohol was observed. The fluorinated alcohol caused DPPC peak broadening, especially below $X(DPPC) < 0.95$, and elimination of the pretransition of DPPC at $X(DPPC)$ approximately 0.91. The onset of the main phase transition remains constant down to $X(DPPC)$ approximately 0.91, suggesting limited miscibility in the gel phase. Hydration of the 10-(perfluorohexyl)-decanol-DPPC mixtures with calcium chloride (2 mM) in place of sodium chloride did not alter the macroscopic phase behavior. In addition to the thermal properties, the miscibility of 10-(perfluorohexyl)-decanol in DPPC in monolayers at the air

water interface was investigated on water, sodium chloride (0.15 M), calcium chloride (2 mM) or hydrochloric acid (pH 1.9) subphases. The concentration dependence of the onset pressure of the liquid-expanded to liquid condensed phase transition of DPPC showed a slight change with increasing mole fraction on all four subphases. The surface area-mole fraction diagrams of 10-(perfluorohexyl)-decanol and DPPC on water, sodium chloride and calcium chloride showed near ideal behavior with slight negative deviations at higher surface pressure. A more significant negative deviation was observed for the hydrochloric acid subphase. Overall, both the DSC and the monolayer studies suggest that 10-(perfluorohexyl)-decanol and DPPC are partially miscible in biological mono- and bilayers. The macroscopic phase behavior 10-(perfluorohexyl)-decanol-DPPC system is significantly different from the analogous hydrocarbon system, which is attributed to a less favorable packing of the partially fluorinated hydrophobic tails in the mono- and bilayer.

Li J. and Chiappetta D. (2008) An investigation of the thermodynamic miscibility between VeTPGS and polymers. *Int J Pharm* **350**, 212-219.

Abstract: Within the past decade, more than half of the drug candidates generated are poorly water soluble and therefore overcoming the low aqueous solubility of drug candidates becomes critical for product development. Vitamin E TPGS (VeTPGS), a non-ionic surfactant, has been used in both liquid and solid dosage forms to solubilize compounds and improve their bioavailability. To prepare solid dosage forms using VeTPGS, VeTPGS is often mixed with other excipients, mostly polymers. However, there is still a lack of understanding of miscibility between VeTPGS and polymers from a thermodynamic point of view. In this paper, the miscibility of VeTPGS with polymers has been studied in the light of the Flory-Huggins (F-H) theory with an objective to understand the effect of dispersion forces (solubility parameter) and nondispersive interactions on the miscibility between VeTPGS and polymers. A series of polymers with similar solubility parameters and structure similarity were selected. Binary blends of polymers and VeTPGS were prepared using a vapor evaporation technique followed by XRPD, DSC, and SEM characterization. Results suggest that the miscibility between VeTPGS and PMMA is very likely due to a specific interaction between the hydrophobic portion of VeTPGS (Vitamin E) and PMMA.

Liu W. and Guo R. (2008) Effects of Triton X-100 nanoaggregates on dimerization and antioxidant activity of morin. *Mol Pharm.* **5**, 588-597.

Abstract: Dimerization and antioxidant activity of morin in the Triton X-100 micelles were studied by electronic absorption, ATR-FTIR spectra, cyclic voltammetric, DSC, freeze-fracture TEM, molecular modeling and ab initio quantum calculations. Morin can be solubilized in the Triton X-100 micelles and show selective dimerization in Triton X-100 micelles with different structures. In Triton X-100 spherical micelles, morin always exists in the form of dimer, and in Triton X-100 rodlike micelles, it is always in the form of monomer. The solubilization of morin dimer in Triton X-100 spherical micelles changes the micelle morphology from spherical to cubelike, and the size of the single micelle is also increased, while morin monomer links the Triton X-100 rodlike micelles and forms a kind of network micelle structure with the size of the "rod" unchanged. Solubilized and concentrated in Triton X-100 micelles, morin can protect human serum albumin from the damage induced by hydroxyl radicals effectively and even can form a kind of protein complex with human serum albumin showing more thermal stability

Lof D., Niemiec A., Schillen K., Loh W. and Olofsson G. (2007) A calorimetry and light scattering study of the formation and shape transition of mixed micelles of EO20PO68EO20 triblock copolymer (P123) and nonionic surfactant (C12EO6). *J Phys. Chem B* **111**, 5911-5920.

Abstract: The interaction between the nonionic surfactant C12EO6 and the poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer EO20PO68EO20 (P123) has been investigated by means of isothermal titration and differential scanning calorimetry (DSC) as well as static and dynamic light scattering (SLS and DLS). P123 self-assembles in water into spherical micelles at ambient temperatures. At raised temperatures, the DSC data revealed a sphere-to-rod transition of the P123 micelles around 60 degrees C. C12EO6 interacts strongly with P123 micelles in aqueous solution to give mixed micelles with a critical micelle concentration (cmc) well below the cmc for pure C12EO6. The presence of C12EO6 also lowers the critical micelle temperature of P123 so aggregation starts at significantly lower temperatures. A new phenomenon was observed in the P123-C12EO6 system, namely, a well-defined sphere-to-rod transition of the mixed micelles. A visual phase study of mixtures containing 1.00 wt % P123 showed that in a narrow concentration range of C12EO6 both the sphere-to-rod transition

and the liquid-liquid phase separation temperature are strongly depressed compared to the pure P123-water system. The hydrodynamic radius of spherical mixed micelles at a C12EO6/P123 molar ratio of 2.2 was estimated from DLS to be 9.1 nm, whereas it is 24.1 nm for the rodlike micelles. Furthermore, the hydrodynamic length of the rods at a molar ratio of 2.2 is in the range of 100 nm. The retarded kinetics of the shape transition was detected in titration calorimetric experiments at 40 degrees C and further studied by using time-resolved DLS and SLS. The rate of growth, which was slow (>2000 s), was found to increase with the total concentration.

Luo S., Xu J., Zhu Z., Wu C., and Liu S. (2006) Phase transition behavior of unimolecular micelles with thermoresponsive poly(N-isopropylacrylamide) coronas. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 9132-9139.

Abstract: This paper describes the double phase transition behavior of a thermoresponsive poly(N-isopropylacrylamide) (PNIPAM) brush at the surface of a hydrophobic core. Reversible addition-fragmentation transfer (RAFT) polymerization of N-isopropylacrylamide (NIPAM) was conducted by using a hyperbranched polyester (Boltorn H40) based macroRAFT agent. The resultant multiarm star block copolymer (H40-PNIPAM) exists as unimolecular micelles with hydrophobic H40 as the core, densely grafted PNIPAM brush as the shell. A combination of laser light scattering (LLS) and microdifferential scanning calorimetry (micro-DSC) studies of H40-PNIPAM in aqueous solution reveals double phase transitions of the PNIPAM corona, which is in contrast to the fact that free PNIPAM homopolymer in aqueous solution exhibits a lower critical solution temperature (LCST) at approximately 32 degrees C. The first phase transition takes place in the broad temperature range 20-30 degrees C, which can be tentatively ascribed to the n-cluster-induced collapse of the inner region of the PNIPAM brush close to the H40 core; the second phase transition occurs above 30 degrees C, which can be ascribed to the outer region of PNIPAM brush. Employing the RAFT chain extension technique, the inner and outer part of PNIPAM brush were then selectively labeled with pyrene derivatives, respectively; temperature-dependent excimer fluorescence measurements further support the conclusion that the inner part of PNIPAM brush collapses first at lower temperatures, followed by the collapse of the outer part at higher temperatures.

Maiti K., Chakraborty I., Bhattacharya S. C., Panda A. K. and Moulik S. P. (2007) Physicochemical Studies of Octadecyltrimethylammonium Bromide: A Critical Assessment of Its Solution Behavior with Reference to Formation of Micelle, and Microemulsion with n-Butanol and n-Heptane. *J Phys. Chem B* **111**, 14175-14185.

Abstract: Octadecyltrimethylammonium bromide (C18TAB) is a much less studied representative in the alkyltrimethylammonium halide surfactant series. A comprehensive study of its normal and reverse micelle (microemulsion) formation has been herein conducted by the methods of conductometry, tensiometry, fluorimetry, and microcalorimetry. The energetics of its air/liquid interfacial adsorption and self-association in aqueous solution have been examined. The phase behavior of its combinations with water, n-butanol, and n-heptane in the formation of microemulsions have been investigated with identification of a variety of phases. The energetics of formation of water dispersion in oil (w/o) has been evaluated from dilution experiments conducted at different temperatures. From the results, structural parameters of the droplets have been determined at different [water]/[surfactant] mole ratios (ω) and temperatures. The w/o dispersions have evidenced both volume- and temperature-induced conductance percolation. The results have been treated in light of the Scaling equations, and the associated parameters for the process have been determined. The activation energies for the temperature-induced percolation process of the w/o dispersion have been evaluated and assessed.

Marques E. F., Brito R. O., Wang Y., and Silva B. F. (2006) Thermotropic phase behavior of triple-chained catanionic surfactants with varying headgroup chemistry. *J Colloid Interface Sci* **294**, 240-247.

Abstract: Catanionic surfactants result from the pairing of oppositely charged amphiphilic molecules, forming a new class of surfactant molecules with various interesting lyotropic and thermotropic properties. With the aim of probing the role of both headgroup chemical nature/structure and molecular shape, a series of catanionic surfactants were synthesized. The cationic portion of the molecule is kept constant, being the dioctadecyldimethylammonium double chain. Different single-chained surfactants with varying headgroups and chain lengths are used as the anionic pair. The thermotropic behavior has been studied by DSC and the mesophase structural investigated by polarized light microscopy. The results indicate that, for a given chain length, parameters such as headgroup polarity and charge density, as well as volume, influence the

catanionic surfactant behavior. The thermodynamic parameters are qualitatively evaluated, considering the headgroup chemical nature and the overall molecular structure.

Moosavi-Movahedi A. A., Gharanfoli M., Nazari K., Shamsipur M., Chamani J., Hemmateenejad B., Alavi M., Shokrollahi A., Habibi-Rezaei M., Sorenson C., and Sheibani N. (2005) A distinct intermediate of RNase A is induced by sodium dodecyl sulfate at its pK(a). *Colloids Surf B Biointerfaces* **43**, 150-157.

Abstract: The chemical denaturation of RNase A was found to be mediated by sodium dodecyl sulfate (SDS) at various pH. The characterization of the unfolding pathway was investigated by spectrophotometry and differential scanning calorimetry (DSC), and was analyzed by multivariate curve resolution (MCR) as a chemometric method. The spectrophotometric titration curve of RNase A upon interaction with SDS indicated a distinct complex intermediate in glycine buffer at pH 3.3. This was accompanied with the catalytic activation of the enzyme and was concurrent with maximum population of the intermediate, determined by MCR. This was confirmed by the DSC profile of RNase A in the presence of SDS, indicated by two transitions in thermal unfolding. The kinetic data on the unfolding process of RNase A upon addition of SDS showed a two-phase pathway under the same conditions. The intermediate appeared at low pH especially at the pK(a) of SDS (pH 3.3). These results provide strong evidence of the influence of low pH (around the pK(a) of SDS) on the existence of an intermediate upon interaction of RNase A with SDS.

Morii H., Uedaira H., Ishimura M., Kidokoro S., Kokubu T., and Ohashi S. (1997) Special folding pathway to tetramer only through the micelle state of the corticotropin-releasing factor. *Biochemistry* **36**, 15538-15545.

Abstract: The ovine corticotropin-releasing factor (CRF), a peptide hormone of 41 residues stimulating the secretion of adrenocorticotrophic hormone, was thermodynamically investigated. By means of size exclusion chromatography and/or ultrafiltration, the CRF solution could be separated into random coil monomers and highly alpha-helical tetramers, which seem to have amphipathic helix bundle structure. Circular dichroism measurements along with diluting or concentrating the CRF solution revealed that there exists the micelle state above the concentration of 0.1 mM, which would be the critical micelle concentration (cmc). The micelle state was also proved by binding ability for 8-anilino-1-naphthalenesulfonate and endothermic change by dilution across the cmc. The tetramer showed the cooperative thermal transition at about 55 degrees C in the buffer solution (pH 7.5), so that it would have native-protein-like folding. On the other hand, the micelle undergoes gradual change to dissociated state by heating, regardless of the similar alpha-helicity to the tetramer. Above the cmc the equilibrium between the tetramer and the micelle takes place as well as that between the monomer and the micelle. Whereas, the direct conversion between the tetramer and the monomer scarcely occurred below the cmc. The titration experiment with 2,2,2-trifluoroethanol (TFE) revealed that the cmc decreases with increasing the concentration of TFE. This tendency is the same as that of general surfactants. Most of experimental results can be well explained by this three-phase model involving the monomer, the tetramer, and the micelle. The lack of the equilibrium between the monomer and the tetramer indicates that the folding pathway of the tetramer is the transformation only through the micelle state and not from the monomer. This pathway resembles the collapse model among the phenomenological models for thermodynamic protein folding. By the mathematical consideration for the dissociation of micelle, we have demonstrated that the expected content of undegradable k-mer is $2/(k + 1)$, which agreed well with the observed tetramer content of CRF (40%).

Nag K., Pao J. S., Harbottle R. R., Possmayer F., Petersen N. O., and Bagatolli L. A. (2002) Segregation of saturated chain lipids in pulmonary surfactant films and bilayers. *Biophys J* **82**, 2041-2051.

Abstract: The physical properties of organized system (bilayers and monolayers at the air water interface) composed of bovine lipid extract surfactant (BLES) were studied using correlated experimental techniques. 6-Dodecanoyl-2-dimethylamino-naphthalene (LAURDAN)-labeled giant unilamellar vesicles (mean diameter approximately 30 microm) composed of BLES were observed at different temperatures using two-photon fluorescence microscopy. As the temperature was decreased, dark domains (gel-like) appeared at physiological temperature (37 degrees C) on the surface of BLES giant unilamellar vesicles. The LAURDAN two-photon fluorescent images show that the gel-like domains span the lipid bilayer. Quantitative analysis of the LAURDAN generalized polarization function suggests the presence of a gel/fluid phase coexistence between 37 degrees C to 20 degrees C with low compositional and energetic differences between the coexisting phases. Interestingly, the microscopic scenario of the phase coexistence

observed below 20 degrees C shows different domain's shape compared with that observed between 37 degrees C to 20 degrees C, suggesting the coexistence of two ordered but differently organized lipid phases on the bilayer. Epifluorescence microscopy studies of BLES monomolecular films doped with small amounts of fluorescent lipids showed the appearance and growth of dark domains (liquid condensed) dispersed in a fluorescent phase (liquid expanded) with shapes and sizes similar to those observed in BLES giant unilamellar vesicles. Our study suggests that bovine surfactant lipids can organize into discrete phases in monolayers or bilayers with equivalent temperature dependencies and may occur at physiological temperatures and surface pressures equivalent to those at the lung interface.

Nagahara Y., Nishida Y., Isoda M., Yamagata Y., Nishikawa N. and Takada K. (2007) Structure and performance of cationic assembly dispersed in amphoteric surfactants solution as a shampoo for hair damaged by coloring. *J Oleo. Sci* **56**, 289-295.

Abstract: In recent years, hair coloring gains popularity as a trend of consumer's hair care. This coloring frequently damages hair. In response to this, a new shampoo-base was developed for repairing hair damaged by coloring. The new shampoo-base was prepared by dispersing cationic assembly in a solution of amphoteric surfactants. The mixture of behenyl trimethyl ammonium chloride (C22TAC) and behenyl alcohol (C22OH) was applied as the cationic assembly, which are dispersed in amido propyl betaine laurate (LPB) solution. LPB, which behaves as an amphoteric surfactant, was used as the wash-base. It was verified from the results on the measurements of DSC, calorimeter polarization, cryo-SEM and X-ray diffraction that the cationic assembly has a crystalline structure in the LPB solution. The new shampoo-base was highly efficient to change the color-damaged hair from hydrophilic to hydrophobic. The friction level of the hair washed with the new shampoo-base recovered to the same state as that of healthy hair. The exfoliation of cuticle was reduced after washing with the new shampoo-base.

Nichifor M., Bastos M., Lopes S. and Lopes A. (2008) Characterization of Aggregates formed by Hydrophobically Modified Cationic Dextran and Sodium Alkyl Sulfates in Salt-Free Aqueous Solutions. *J Phys. Chem B*.(epublication)

Abstract: The interaction between polyelectrolytes based on dextran with pendant N-alkyl- N, N-dimethyl-N-(2-hydroxypropyl) ammonium chloride groups, where n = 2, 4, 8, 12, or 16, and sodium alkyl sulfates, SC n S, with n = 8, 10, 12, 14, and 16, has been studied by conductometry and fluorescence techniques. Comparison of cumulative specific conductivities of the mixtures of polymer-surfactant over a large surfactant concentration range, with those of pure surfactant and NaCl, has clearly shown that the surfactants start to bind to polymer at very low concentrations (10⁻⁶ M), forming mixed aggregates. The steady-state emission fluorescence measured in the presence of pyrene, 1,3,6-diphenylhexatriene (DPH), and 1-pyrenylbutyric acid sodium salt demonstrated the existence of a critical surfactant concentration (CAC S) at which the previously formed mixed aggregates are interconnected due to self-association of surfactant molecules included in different mixed polymer/surfactant aggregates. Above CAC S, the mixed aggregates change dramatically their properties (hydrophobicity, size, DPH solubilization) which depend on both polymer and surfactant hydrophobicities and concentrations. The characterization of the new formed aggregates at different surfactant concentration ranges is derived mainly from their ability to solubilize hydrophobic compounds. The variety of fluorescence techniques used, combined with conductometric measurements and previous calorimetric information allowed us to provide here a comprehensive study and new interpretation of the solution behavior of these polymer-surfactant systems

Olofsson G., and Wang G. (1998) Isothermal Titration and Temperature Scanning Calorimetric Studies of Polymer-Surfactant Systems *in* Polymer-Surfactant Systems. Kwak J.C.T. ed., Marcel Dekker Inc. New York, , pp. 317-356.

Olbrich C., Gessner A., Kayser O., and Muller R. H. (2002) Lipid-drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediacetate. *J Drug Target* **10**, 387-396.

Abstract: The objective of the present study was to incorporate the hydrophilic drug diminazenediacetate at a high loading into lipid nanoparticles by creating nanoparticles from lipid-drug conjugates (LDC). IR and DSC data showed that the antitrypanosomal drug diminazene is able to react with fatty acids to form water-insoluble salts like diminazenedistearate and -dioleate. The salts could be transformed into nanoparticles using high-pressure homogenization technique, established for solid lipid nanoparticles

(SLN). By using polysorbate 80 as surfactant, physically stable LDC nanoparticle dispersions of both salts could be obtained. The mean PCS diameters and polydispersity indices were 364 nm and 0.233 for diminazenedistearate and 442 nm and 0.268 for diminazenedioleate, respectively. Due to the composition of the LDC bulk materials, nanoparticles with a high drug load of 33% (w/w) were obtained even for this highly water-soluble drug diminazenediacetate. The new carrier system of LDC nanoparticles overcomes one limitation of SLN, i.e. the limited loading capacity for hydrophilic drugs. Transforming water-soluble hydrophilic drugs into LDC and formation of nanoparticles allows prolonged drug release and targeting to specific sites by i.v. injection. These results provide a first basis of using LDC-polysorbate 80 nanoparticles for brain delivery of diminazene to treat second stage human African trypanosomiasis (HAT).

Orioni B., Roversi M., La Mesa C., Asaro F., Pellizer G., and D'Errico G. (2006) Polymorphic behavior in protein-surfactant mixtures: the water-bovine serum albumin-sodium taurodeoxycholate system. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 12129-12140.

Abstract: Mixtures containing water, bovine serum albumin (BSA), and sodium taurodeoxycholate (NaTDC), a component of the bile in mammals, have been investigated in a wide range of composition and pH. Depending on the concentration of both solutes and the pH, solutions, precipitates, and gels are formed. Under spontaneous pH conditions, the transport properties in dilute solutions indicate the occurrence of significant interactions between BSA and the surfactant. Conversely, acidic media favor the formation of nonsoluble protein-surfactant complexes, with subsequent precipitation. The nucleation kinetics of the protein-surfactant complexes in solid form and the related precipitation processes can be slow or fast, depending on the overall solute content and the mole ratio. At high concentrations, a gel, extending on both sides of the charge neutralization line, and two-phase regions are observed. Gels shrink in open air and swell in the presence of excess water. Depending on concentration and temperature, the gels transform from an essentially liquidlike behavior to that peculiar to true gels (when $G' > \text{or} = G''$). The thermal gelation threshold, the temperature above which $G' > \text{or} = G''$, depends on BSA and NaTDC content and is concomitant to moderate heat effects, inferred by differential scanning calorimetry (DSC). The above data also indicate that the protein thermal denaturation in the gel is shifted to higher temperatures compared to water. Such a stabilizing effect is presumably related to the occurrence of both electrostatic and hydrophobic interactions with NaTDC. Water self-diffusion in the gels is slightly slower than that in the bulk and poorly sensitive to composition: it is about 65% the value of neat H₂O in a wide concentration range, irrespective of the BSA, or NaTDC, concentration. A peculiar behavior is also observed in ²³Na longitudinal and transverse relaxation rates. The T₁ and T₂ values, measured at 105.75 MHz on BSA-NaTDC gels, indicate that the motions determining the NMR relaxation of the sodium ions in the hydration layer of the protein-surfactant aggregates are not slow, having frequencies comparable with the Larmor one. The above properties, especially the rheological and the spectroscopic ones, are important for understanding the behavior of gels based on protein-surfactant mixtures.

Panyukov Y. V., Nemykh M. A., Dobrov E. N. and Drachev V. A. (2008) Surfactant-induced amorphous aggregation of tobacco mosaic virus coat protein: a physical methods approach. *Macromol. Biosci.* **8**, 199-209.

Abstract: The interactions of non-ionic surfactant Triton X-100 and the coat protein of tobacco mosaic virus, which is an established model for both ordered and non-ordered protein aggregation, were studied using turbidimetry, differential scanning calorimetry, isothermal titration calorimetry, and dynamic light scattering. It was found that at the critical aggregation concentration (equal to critical micelle concentration) of 138×10^{-6} M, Triton X-100 induces partial denaturation of tobacco mosaic virus coat protein molecules followed by protein amorphous aggregation. Protein aggregation has profound ionic strength dependence and proceeds due to hydrophobic sticking of surfactant-protein complexes (start aggregates) with initial radii of 46 nm. It has been suggested that the anionic surfactant sodium dodecyl sulfate forms mixed micelles with Triton X-100 and therefore reverses protein amorphous aggregation with release of protein molecules from the amorphous aggregates. A stoichiometric ratio of 5 was found for Triton X-100-sodium dodecyl sulfate interactions

Pfeiffer H., Klose G., Heremans K., and Glorieux C. (2006) Thermotropic phase behaviour of the pseudobinary mixtures of DPPC/C(12)E(5) and DMPC/C(12)E(5) determined by differential scanning calorimetry and ultrasonic velocimetry. *Chem Phys Lipids* **139**, 54-67.

Abstract: The present paper reports on the phase behaviour of the pseudobinary aqueous mixtures of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)/pentaethylene glycol monododecyl ether (C(12)E(5))

and 1,2-dimyristoyl-sn-glycero-3-phosphocholine monohydrate (DMPC)/C(12)E(5). Both systems exhibit a variety of mesophases, such as lamellar gel, liquid crystalline and micellar phases. The phase diagrams show peritectic and eutectic behaviours. The existence of a compound complex is established. From the phase diagrams, the temperature dependence of the solubilisation parameters is obtained. The phase diagrams, especially with respect to the solubilisation process were qualitatively explained assuming that the packing of the constituents plays a dominating role. Finally, differential scanning calorimetry and ultrasonic velocimetry are compared concerning their potentials to determine characteristics of phase transitions in pseudobinary phospholipid/surfactant mixtures.

Pham Trong L. C., Djabourov M. and Ponton A. (2008) Mechanisms of micellization and rheology of PEO-PPO-PEO triblock copolymers with various architectures. *J Colloid Interface Sci.* (epublication)
Abstract: Micelle formation was followed by micro-DSC and rheology for aqueous solutions of two copolymers of PEO-PPO-PEO, the Pluronic F127 (from BASF) and the EG56 (from PolymerExpert), a branched copolymer built with three chains of F127 type. It is shown that micellization is endothermic and that, for both polymers, the enthalpy of formation/melting is proportional to total concentration. The rheology of the solutions was carefully analyzed, before gelation for F127, and it reveals firstly the progressive changes of solubility of the unimers (decrease of relative solution viscosity), followed by micelle formation over a 10 degrees C range. In this range, the micelle concentration dependence on temperature was deduced from enthalpy measurements and the corresponding volume fractions were derived. Viscosity was interpreted within the framework of well-known theories for hard sphere suspensions (Krieger-Dougherty or Quemada) based on an analogy between micelles and nanosized hairy grain suspensions. The gel state is achieved due to formation of the colloidal crystal. For EG56, the rheology is quite different; as the aggregation increases with temperature, a progression is observed from Newtonian to visco-elastic liquid. The characteristic frequency, defined by the relation $G'(\omega)=G''(\omega)$, for EG56 varies with temperature and the corresponding times increase by two orders of magnitude according to an Arrhenius law. The frequency dependence of $G'(\omega)$ and $G''(\omega)$ at different temperatures can be superposed with a horizontal shift factor and a small amplitude adjustment. There is no elastic solid formation in this case. The "gelation" of these two copolymers is compared to the physical gelation of cold-set gels (gelatin)

Portilla-Arias J. A., Garcia-Alvarez M., Martinez d., I, Holler E., and Munoz-Guerra S. (2006) Nanostructured complexes of poly(beta,L-malate) and cationic surfactants: synthesis, characterization and structural aspects. *Biomacromolecules* **7**, 161-170.

Abstract: Ionic complexes of microbially produced poly(beta,L-malic acid) and alkyltrimethylammonium surfactants with linear alkyl chains containing even numbers of carbon atoms from 14 up to 22, were investigated. Complexes with a stoichiometric or nearly stoichiometric composition were prepared by precipitation from equimolar mixtures of aqueous solutions of the two components. All complexes were found to adopt supramolecular stratified structures made of alternating layers of poly(beta,L-malate) and surfactant with a periodicity on the length scale of 3-5 nm, which increased proportionally to the length of the polymethylene chain. In these complexes, alkyl side chains with more than 16 carbon atoms were partially crystallized showing reversible melting at temperatures between 40 and 70 degrees C. After melting, a smectic LC phase that isotropized at approximately 100 degrees C was observed for all of the complexes. Conformational and dimensional changes taking place in the complexes by effect of heating were analyzed by ¹³C CP-MAS NMR and powder X-ray diffraction.

Richards C., Tiddy G. J. and Casey S. (2007) Lateral phase separation gives multiple lamellar phases in a "binary" surfactant/water system: the phase behavior of sodium alkyl benzene sulfonate/water mixtures. *Langmuir* **23**, 467-474.

Abstract: We have examined the structure of the lamellar phase (Lalpha) that coexists with a micellar solution (L1) for a commercial sodium alkyl benzene sulfonate (LAS) mixed with water. The surfactant is a mixture containing C10-C13 alkyl chains, having all positional isomers of the benzene sulfonate group present except the 1-isomer. Unusually for ionic surfactants, the difference in compositions between the coexisting L1 and Lalpha phases is large (L1 = approximately 20 wt % LAS; Lalpha = approximately 65 wt %). The main technique employed was X-ray diffraction, supplemented by optical microscopy and differential scanning calorimetry (DSC). At ambient temperatures, the lamellar phase gives a single diffraction pattern with the main reflection (d) at approximately 32.5 A, whatever the composition. However, above 40 degrees C, the diffraction peak becomes broader and moves to higher d values. At

higher temperatures still, several distinct and different diffraction peaks are observed, differing in detail according to composition. The largest d values (approximately 42-4 Å) are observed for the lowest LAS concentrations, while the largest number of separate reflections (five) occurs for samples with approximately 44-50% LAS, both at the highest temperatures. Although there are some differences in the data between heating and cooling cycles, the d values return to the original value at low temperature. There are no observable transitions in DSC, nor is there any heterogeneity in the lamellar phase observable by microscopy. The data clearly indicate that there is some lateral separation of the different LAS isomers within the bilayers, which results in the formation of local lamellar regions having different surfactant compositions. This lateral phase separation may arise from the presence of an (electrostatic) attractive interaction, which gives rise to an upper consolute loop within the lamellar phase region of a pure LAS isomer. Similar mechanisms may occur in biological membranes and could be responsible for the occurrence of membrane lipid patches.

Rickert P. G., Antonio M. R., Firestone M. A., Kubatko K. A., Szreder T., Wishart J. F. and Dietz M. L. (2007) Tetraalkylphosphonium polyoxometalate ionic liquids: novel, organic-inorganic hybrid materials. *J Phys. Chem B* **111**, 4685-4692.

Abstract: Pairing of a Keggin or Lindqvist polyoxometalate (POM) anion with an appropriate tetraalkylphosphonium cation is shown to yield the first members of a new family of ionic liquids (ILs). Detailed characterization of one of them, an ambient-temperature "liquid POM" comprising the Lindqvist salt of the trihexyl(tetradecyl) phosphonium cation, by voltammetry, viscometry, conductivity, and thermal analysis indicates that it exhibits conductivity and viscosity comparable to those of the one previously described inorganic-organic POM-IL hybrid but with substantially improved thermal stability.

Rosa M., Dias R., da Graca M. M., and Lindman B. (2005) DNA-cationic surfactant interactions are different for double- and single-stranded DNA. *Biomacromolecules* **6**, 2164-2171.

Abstract: The stability of DNA in solution and the phase behavior in mixtures with dodecyltrimethylammonium bromide (DTAB) were investigated. By means of circular dichroism, UV absorption, and differential scanning calorimetry, we found that for dilute solutions of DNA with no addition of salt the DNA molecules are in the single-stranded conformation, whereas the addition of a small amount of NaBr, 1 mM, is sufficient to stabilize the DNA double-helix. Furthermore, at higher DNA concentrations, native DNA becomes the most stable structure, which is due to a self-screening effect. By phase diagram determinations of the DNA-surfactant system, we found that the effect of salt on phase behavior mainly relates to a difference in interaction of the amphiphile between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). The difference in association between ss and dsDNA with surfactants of different chain lengths can be interpreted in terms of an interplay between hydrophobic and electrostatic interactions, the latter being influenced by polymer flexibility. In this way, a nonmonotonic variation can be rationalized. A crossing of the phase separation lines with DNA concentration can be rationalized in terms of a change in relative stability of ss and dsDNA. The fact that ssDNA phase separates earlier than dsDNA in association with DTAB, may serve as a basis for a method of easily separating dsDNA from ssDNA by the addition of surfactant; this is verified as monitored by circular dichroism measurements.

Rozycka-Roszak B., Przychyna A., and Pernak A. (2004) A study on the interaction between 1-decyloxymethyl-3-carbamoylpyridinium salts and model membranes--the effect of counterions. *Biophys Chem* **109**, 271-279.

Abstract: The interaction between 1-decyloxymethyl-3-carbamoylpyridinium salts (PS-X) and two types of vesicles (multilamellar vesicle and sonicated vesicle) was investigated. Vesicles were formed from two classes of phospholipids: 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine (DPPE). The PS-X salts used had nitrate, perchlorate, tetrafluoroborate and halides as counterions. Measurements were carried out using differential scanning calorimetry and ¹H NMR. All studied compounds decreased the main phase transition temperatures of both DPPC and DPPE bilayers. All of them also decreased the transition enthalpy of DPPC bilayers, however they had a dual effect on the transition enthalpy of DPPE. Namely, at low concentrations the PS-X salts studied significantly increased the main transition enthalpy of DPPE (perchlorate and tetrafluoroborate the least among them) and decreased it at higher concentrations. We have suggested that surfactant rich and pure domains form on the DPPE bilayer in the presence of PS-CIO₄, PS-BF₄ and PS-NO₃, whereas they

form on DPPC bilayer only in the presence of PS-ClO₄. Results are discussed in terms of counterion molecular geometry and the ability of amide group to form hydrogen bonds with lipids.

Rozycka-Roszak B. and Pruchnik H. (2001) Influence of dodecyltrimethylammonium halides on interaction of phenyltin compounds with model membranes. *Z Naturforsch [C]* **56**, 623-628.

Abstract: The effects were studied of dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB) and dodecyltrimethylammonium iodide (DTAI) on thermotropic phase behaviour of phosphatidylcholine bilayers, as well as on ¹H NMR and ³¹P NMR spectra, in the presence of diphenyltin dichloride (DPhT) and triphenyltin chloride (TPhT). The obtained results indicate that in the presence of the surfactant studied the interaction of phenyltin compounds with model membranes was changed and the changes depended on the kind of the counterion. The surfactants studied (especially DTAC) decrease the ability of phenyltin compounds to induce structural changes in the bilayer. It is suggested that DTAB, and especially DTAC, prevent DPhT induced interdigitated phase formation as well as formation of an inverted hexagonal phase (H(II)) in the case of TPhT/DPPC liposomes.

Rozycka-Roszak B. and Przychyna A. (2003) Interaction between N-dodecyl-N,N-dimethyl-N-benzylammonium halides and phosphatidylcholine bilayers-the effect of counterions. *Chem Phys Lipids* **123**, 209-221.

Abstract: The interaction of N-dodecyl-N,N-dimethyl-N-benzylammonium halides (DBeAX) with two types of phospholipid vesicles (MLV and SUV) was investigated using DSC and ¹H NMR. It was suggested that the benzyl group like the micellisation process (J. Colloid Interface Sci. 218 (1999) 529) changes its position when interacting with phosphatidylcholine bilayers and incorporates into the bilayer. In order to enhance counterion-water interactions, the surfactants were added either to the water phase or directly to the lipid phase (a mixed film was formed). It follows from the obtained results that for both types of liposomes and both manners in which the surfactant was added, the interaction of DBeAX with liposomes and consequent changes in the phospholipid bilayer organisation depend on the kind of counterion. Results are discussed in terms of counterion ability to modify water structure.

Saily V. M., Ryhanen S. J., Lankinen H., Luciani P., Mancini G., Parry M. J., and Kinnunen P. K. (2006) Impact of reductive cleavage of an intramolecular disulfide bond containing cationic gemini surfactant in monolayers and bilayers. *Langmuir* **22**, 956-962.

Abstract: The properties of a novel disulfide-bond-containing gemini surfactant bis[N,N-dimethyl-N-hexadecyl-N-(2-mercaptoethyl)ammonium bromide] disulfide (DSP) were studied using a Langmuir balance, supported monolayers, differential scanning calorimetry, giant vesicles, and LUVs. In 150 mM NaCl the cmc for DSP was 7.5 microM whereas that of the monomer N,N-dimethyl-N-hexadecyl-N-(2-mercaptoethyl)ammonium bromide (MSP) was 12.1 microM. Both surfactants exhibited single endotherms upon DSC, with peak temperatures T_m at 21.7 and 20.1 degrees C for DSP and MSP, respectively. The endotherm for MSP was significantly broader indicating less cooperative melting. Both in monolayers and in vesicles reductive cleavage of the disulfide bond of DSP could be obtained by glutathione (GSH). For Langmuir films of DSP the addition of GSH into the subphase led to a decrease in surface pressure pi as well as surface dipole potential psi. Although the cleavage by GSH was significantly slower in the presence of a charge saturating concentration of DNA, it did not prevent the reaction. The resulting monomers detached from supported monolayers, leading to loss of affinity of the surface for DNA. Disruption of giant vesicles containing DSP within approximately 30 s following a local injection of GSH was observed, revealing membrane destabilization.

Sanchez M., Teruel J. A., Espuny M. J., Marques A., Aranda F. J., Manresa A., and Ortiz A. (2006) Modulation of the physical properties of dielaidoylphosphatidylethanolamine membranes by a dirhamnolipid biosurfactant produced by *Pseudomonas aeruginosa*. *Chem Phys Lipids* **142**, 118-127.

Abstract: Rhamnolipids are bacterial biosurfactants produced by *Pseudomonas* spp. These compounds have been shown to present several interesting biological activities, restricting the growth of *Bacillus subtilis* and showing zoosporicidal activity on zoosporic phytopathogens. It has been suggested that the interaction with the membrane could be the ultimate responsible for these actions. Therefore, it is of great interest to get insight into the molecular mechanism of the interaction of purified rhamnolipids with the various phospholipid components of biological membranes. In this paper we report on the phase behaviour

of mixtures of dielaidoylphosphatidylethanolamine (DEPE) with a purified dirhamnolipid (DiRL) fraction from *Pseudomonas aeruginosa*, as studied by a number of physical techniques such as differential scanning calorimetry, FTIR, small angle X-ray (SAX) diffraction and dynamic light scattering. Our data indicate that the presence of DiRL counteracts the tendency of DEPE to form vesicular aggregates of large size, forming vesicles of smaller diameter which most probably have a lower lamellarity index. The partial phase diagram obtained from calorimetric data shows a complex behaviour with a solid-phase immiscibility. X-ray diffraction shows that DiRL has a bilayer stabilizing effect, impeding formation of the inverted hexagonal-HII phase of DEPE. The presented data are discussed focussing into how DiRL/DEPE interactions could help to explain the membrane perturbing activities of this biosurfactant.

Savva M., Aljaberi A., Feig J., and Stolz D. B. (2005) Correlation of the physicochemical properties of symmetric 1,3-dialkylamidopropane-based cationic lipids containing single primary and tertiary amine polar head groups with in vitro transfection activity. *Colloids Surf B Biointerfaces* **43**, 43-56.

Abstract: The physicochemical properties of a novel series of symmetric 1,3-dialkylamidopropane-based cationic amphiphiles [M. Sheikh, J. Feig, B. Gee, S. Li, M. Savva, In vitro lipofection with novel series of symmetric 1,3-dialkylamidopropane-based cationic surfactants containing single primary and tertiary amine polar head groups, *Chem. Phys. Lipids* 124 (2003) 49-61] were studied by several techniques, in an effort to correlate cationic lipid structure with transfection efficacy. It was found that only the unsubstituted amine and tertiary amine dioleoyl derivatives 1,3lmp5 and 1,3lmt5, respectively, mediated in vitro transfection activity in the absence of helper lipids. This activity pattern was consistent with ethidium bromide fluorescence quenching studies, which indicated that only these two derivatives bound to and efficiently condense plasmid DNA at physiological pH. Dynamic light scattering indicated that lipoplexes made by these two cationic lipids were relatively small particles below 1µm, in sharp contrast to lipoplexes bigger than 3µm composed of saturated cationic derivatives. Transmission electron microscopy studies clearly indicated that cationic lipid dispersions made by saturated derivatives form multilamellar tubules at physiological pH. Calorimetric studies showed that cationic amphiphiles with saturated acyl chains longer than 12 carbons exhibit solid-to-liquid crystalline phase transitions above 37 degrees C. In agreement with the microscopy and calorimetry studies, Langmuir film balance experiments indicated that saturated derivatives with hydrophobic chains longer than 12 carbons are not well hydrated and exist at a chain-ordered state at ambient temperature. Calculation of compressibility moduli from monolayer compression isotherms at 23 degrees C suggested that monolayers made by cationic lipids bearing saturated acyl chains are less compressible relative to those of the dioleoyl derivatives 1,3lmp5 and 1,3lmt5. In conclusion, high hydration, increased fluidity and high elasticity of cationic lipid assemblies in isolation, all correlate with high in vitro transfection activity.

Scarzello M., Chupin V., Wagenaar A., Stuart M.C., Engberts J.B., and Hulst R. (2005) Polymorphism of pyridinium amphiphiles for gene delivery: influence of ionic strength, helper lipid content, and plasmid DNA complexation. *Biophys J.* **88**, 2104-13.

Abstract: Two double-tailed pyridinium cationic amphiphiles, differing only in the degree of unsaturation of the alkyl chains, have been selected for a detailed study of their aggregation behavior, under conditions employed for transfection experiments. The transfection efficiencies of the two molecules are remarkably different, especially when combined with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) as helper lipid. The phase behavior of the cationic amphiphile/DOPE mixtures have been studied using (31)P- and (2)H-NMR (on deuterated cationic amphiphiles) as main techniques, to monitor independently the behavior of the two components. In water, the lamellar organization is dominant for both the surfactants in their mixtures with the helper lipid. In HEPES saline buffer (HBS), the mixtures of the unsaturated surfactant form inverted phases and, in particular, stable H(II) phases for DOPE contents > or =30 mol %. By contrast, the saturated surfactant does not form homogeneously mixed inverted phases in mixtures with DOPE at room temperature. However, mixed inverted phases are observed for this system at higher temperatures and, after mixing has been achieved by heating, the metastable mixed phases remain present for several hours at 5 degrees C. At 35 degrees C the dominant phase is the cubic phase. The lipoplex composed of equimolar mixtures of the unsaturated surfactant with DOPE and plasmid DNA was found to be organized in highly curved bilayers.

Singh S. K. and Kishore N. (2006) Thermodynamic insights into the binding of Triton X-100 to globular proteins: a calorimetric and spectroscopic investigation. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 9728-9737.

Abstract: The interaction of the nonionic surfactant Triton X-100 (TX-100) with two proteins (bovine serum albumin (BSA) and alpha-lactalbumin (alpha-LA)) has been investigated by using a combination of differential scanning calorimetry, isothermal titration calorimetry, and fluorescence and circular dichroism spectroscopies. All of the calorimetric transitions in BSA were partially reversible, while being two-state and reversible in the case of alpha-LA. TX-100 molecules do not reduce the thermal stability of the protein in the monomeric form. However, in the micellar form the protein might become thermally destabilized by the micelles depending upon the nature of the protein. Isothermal titration calorimetry has been used to demonstrate that TX-100 binds to BSA at two sets of sites with 4:1 stoichiometry in each case. The van't Hoff enthalpy calculated from the temperature dependence of the binding constant did not match with the calorimetric enthalpy indicating conformational change in the protein upon surfactant binding. The surfactant binds to alpha-LA with one class of binding site, and the thermal unfolding results indicate it to be a stronger destabilizer than BSA. The fluorescence, circular dichroism, and differential scanning calorimetric results corroborate well with each other. The effect of ionic strength on the binding parameters suggests that TX-100 can bind to the protein surface via both hydrophobic and polar interactions depending upon the nature of the protein. The physical chemistry underlying the interactions between TX-100 and proteins has been presented. The mode of interaction of TX-100 with proteins is via direct binding, which has been discussed quantitatively in this work.

Skita V., Chester D. W., Oliver C. J., Turcotte J. G., and Notter R. H. (1995) Bilayer characteristics of a diether phosphonolipid analog of the major lung surfactant glycerophospholipid dipalmitoyl phosphatidylcholine. *J Lipid Res* **36**, 1116-1127.

Abstract: Thermal and lyotropic phase behavior was studied by X-ray diffraction and differential scanning calorimetry for a diether phosphonolipid analog (DEPN-8) of the major lung surfactant glycerophospholipid dipalmitoyl phosphatidylcholine (DPPC). DEPN-8 differs in an ether, rather than an ester, bond at the acyl chain-backbone linkage and a headgroup phosphonate (isosteric methylene substitution) versus phosphate constituent. Analysis of lamellar diffraction maxima demonstrated that at high relative humidity (98%) and temperatures below the liquid crystal phase transition (approximately 45 degrees C), DEPN-8 formed interdigitated bilayers with a characteristic periodicity of 41.9-46.5 Å. At low humidity the gel phase DEPN-8 bilayers were characteristic of a normal L beta phase with a periodicity equivalent to DPPC (57-59 Å). Above the liquid crystal thermal phase transition, bilayer spacing for both DEPN-8 and DPPC was 51-52 Å, characteristic of the L alpha phase. Complete assessments of both lamellar and in-plane X-ray scattering used to construct electron density profiles and structure-factor plots for DEPN-8 defined more fully the interdigitated bilayer state at high humidity and low temperature. Compared to DPPC, it is energetically favorable for DEPN-8 to form interdigitated bilayers under conditions of excess water and low temperature. The flexible character of the ether bonds in DEPN-8 allows increased hydrophobic interactions between acyl chains, without generating a steric penalty from the increased packing density of the molecules. Additionally, the ether bond and the phosphonate moiety may allow for more energetically favorable interactions between the choline portion of the headgroup and water. The DEPN-8 ether linkage may also contribute to the improved adsorption and film respreading found previously for this phosphonolipid compared to DPPC.

Tsuchiya K., Ishikake J., Kim T. S., Ohkubo T., Sakai H. and Abe M. (2007) Phase behavior of mixed solution of a glycerin-modified cationic surfactant and an anionic surfactant. *J Colloid Interface Sci* **312**, 139-145.

Abstract: The phase behavior of mixed solution of newly synthesized monoglycerylcetyltrimethylammonium chloride (MGCA) and sodium octyl sulfate (SOS) in water was investigated by cryo-transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS), differential scanning calorimetry (DSC), and fluorescence polarizing for evaluation of the microviscosity of bilayers. No precipitate was observed in the mixed solution except at concentrations below 20 mM over all mixing ratios, and stable vesicles were formed in a considerably wide range of mixing ratio, even at the equimolar ratio. Vesicles formed in aqueous 1/1 MGCA/SOS mixture were found to exhibit no phase transition, and fluorescence polarizing measurements showed that the vesicle bilayers have a high fluidity. This flexibility allows the bilayers to have a spontaneous curvature, and thus vesicles rather than flat

lamellae can be stabilized in the mixture even at the equimolar ratio. In addition, because the glycerin group of MGCA interacts strongly with water, the hydration repulsion contributes to prevent the bilayers consisting of MGCA and SOS from adhering and flocculating even though the charge neutralization between MGCA and SOS occurs at the equimolar ratio.

Vidanovic D., Milic A. J., Stankovic M., and Poprzen V. (2003) Effects of nonionic surfactants on the physical stability of immunoglobulin G in aqueous solution during mechanical agitation. *Pharmazie* **58**, 399-404.

Abstract: The objective of this study was to evaluate the influence of nonionic surfactants in the presence of glycine and sodium chloride on the physical stability of immunoglobulin G (IgG) in aqueous solution. Among surfactants suitable for parenteral preparation, Polysorbate 80 (Tween 80) and Polyoxyl 35 Castor Oil (Cremophor EL) were selected. The physical stability of IgG in the absence and in the presence of excipients was investigated in aqueous solution during mechanical agitation (concentration of IgG 15%; pH 7.1; temperature 6 +/- 2 degrees C). Suitable concentrations of Tween 80 and Cremophor EL were experimentally determined by surface tension measurements at 6 +/- 2 degrees C. Glycine and sodium chloride were used in different concentrations. The influence of the excipients on the physical stability of IgG in solution has been examined by surface tension measurements, protein content assay (Kjeldahl and HPLC) and differential scanning calorimetry (DSC). Based on the results of the investigations, it was found that Tween 80 and Cremophor EL, used in experimentally determined critical micelle concentration (cmc), decreased the physical stability of IgG in solution. Tween 80 and Cremophor EL in the presence of glycine (1.5 g/l) could stabilize the IgG in solution during mechanical agitation. The comparison of the effects of Tween 80 and Cremophor EL on the physical stability of IgG, showed that Tween 80 had better stabilization effects on IgG in solution under the experimental conditions selected.

Wang Y. and Marques E. F. (2006) Thermotropic phase behavior of cationic gemini surfactants and their equicharge mixtures with sodium dodecyl sulfate. *J Phys Chem B Condens Matter Mater Surf Interfaces Biophys* **110**, 1151-1157.

Abstract: The lyotropic phase behavior for the neat cationic gemini surfactants alkanediyl- α,ω -bis(alkyldimethylammonium bromide), designated here as m-s-m, has been investigated previously in several works, but the thermotropic behavior has not been well characterized. Only for 15-s-15 and 14-s-12 have thermotropic liquid crystals (Lc) been reported. In this work, for the first time and in contrast to previous reports, we observe thermotropic Lc formation for m-2-m geminis with m = 12, 14, 16, and 18, by means of polarizing microscopy and differential scanning calorimetry (DSC). Furthermore, we investigate mixtures of m-2-m and SDS, m-2-m Br2.2SDS, which exhibit crystal-to-crystal phase transitions at lower temperature and, at high temperature, smectic Lc phases. The transition temperatures and enthalpies for Lc phases, obtained by DSC, present clear trends upon increase of the chain lengths. Combining Langmuir film experiments, possible lamellar arrangements for the different phases are tentatively discussed.

Yamaoka T., Tamura T., Seto Y., Tada T., Kunugi S., and Tirrell D. A. (2003) Mechanism for the phase transition of a genetically engineered elastin model peptide (VPGIG)₄₀ in aqueous solution. *Biomacromolecules* **4**, 1680-1685.

Abstract: The concentration dependence of the pressure- and temperature-induced cloud point transition (P_c and T_c, respectively) of aqueous solutions of an elastin-like polypeptide with a repeating pentapeptide Val-Pro-Gly-Ile-Gly sequence (MGLDGSMG(VPGIG)₄₀VPLE) was investigated by using apparent light scattering, differential scanning calorimetry, and circular dichroism methods. In addition, the effects of salts and surfactants on these properties were investigated. The P_c and T_c of the present peptide in aqueous solution were strongly concentration dependent. The calorimetric measurements showed that the enthalpy of transitions was 300-400 kJ/mol, i.e., 7-10 kJ/mol per VPGIG pentamer. The T_c of the (VPGIG)₄₀ solution was highly affected by the addition of inert salts or SDS. The effects of salts were consistent with those observed in the lyotropic series or Hoffmeister series. The CD spectrum at low peptide concentrations indicated that the present peptide forms type II beta-turn-like structure(s) at higher temperatures, but the temperature dependence of random coil diminishment (195 nm) and beta-turn formation (210 nm) were not exactly coincident. A hypothetical mechanism of the (VPGIG)₄₀ phase transition that could account for these observations was postulated. Observations suggest that the temperature-responsive properties of the elastin model peptides occur via a mechanism involving conformational change-association-aggregation and that the first two are strongly interactive.

Yamasaki M., Yamashita T., Yano H., Tatsumi K., and Aoki K. (1996) Differential scanning calorimetric studies on bovine serum albumin. IV. Effect of anionic surfactants with various lengths of hydrocarbon chain. *Int J Biol Macromol* **19**, 241-246.

Abstract: Using defatted and SH-blocked bovine serum albumin (BSA), measurements of differential scanning calorimetry (DSC) have been made at pH 7 on the complexes of BSA and a series of sodium alkyl sulfates used were sodium decyl sulfate (SDeS), sodium octyl sulfate (SOS), sodium hexyl sulfate (SHS) and sodium ethyl sulfate (SES). Results obtained were compared with those on the system BSA-sodium dodecyl sulfate (SDS) studied previously. Two peaks P1 and P2 existed in the DSC curve of BSA. These peaks originate in the heat-induced transition of BSA. The pattern of DSC curve changed with the amount of the ligand added, i.e. with the molar mixing ratio ligand/BSA (1). The change for systems BSA-SDeS, BSA-SOS and BSA-SHS was qualitatively the same as that for the system BSA-SDS (2). Interestingly, SES, which is not a surfactant, interacts with BSA. The change for the system BSA-SES was qualitatively the same as that for the system BSA-Na₂SO₄. All alkyl sulfates suppressed the heat-induced transition at lower concentrations. A linear relationship was obtained for the plots of $\log(D/A)$ versus $\log CMC$, where (D/A) is the molar mixing ratio of anionic surfactant (D) to BSA (A) at which the most heat-stable complex is formed. This suggests that the hydrophobic force has a serious effect on the formation of heat-stable complexes.

Yin S., Li W., Wang J. and Wu L. (2008) Mesomorphic structures of protonated surfactant-encapsulated polyoxometalate complexes. *J Phys. Chem B* **112**, 3983-3988.

Abstract: Keggin-type heteropolyanions, H₃PW₁₂O₄₀ (HPW), Na₃PW₁₂O₄₀ (NaPW), H₄SiW₁₂O₄₀ (HSiW) and K₄SiW₁₂O₄₀ (KSiW), were encapsulated by a cationic surfactant, di[12-(4'-octyloxy-4-azophenyl)dodecyloxy]dimethylammonium bromide (L), through the replacement of counterions. The resulting surfactant-encapsulated polyoxometalate complexes were characterized by UV-vis, Raman, and NMR spectra in detail. The measurement results indicated that some azobenzene groups of the surfactant were protonated in the complexes HL/HPW (HL is the abbreviation of the protonated surfactant), HL/NaPW, and HL/HSiW during the process of encapsulation, whereas the protonation was not observed in L/KSiW. The thermotropic liquid crystal properties of these complexes were investigated by differential scanning calorimetry, polarized optical microscopy and variable-temperature X-ray diffraction. Interestingly, different smectic mesophases were observed between the protonated HL/HSiW and the non-protonated L/KSiW, which suggests that the protonation of azobenzene groups in HL/HSiW plays a key role in the liquid crystalline organization. However, protonated HL/HPW and HL/NaPW exhibit a similar smectic B phase to that of the de-protonated one, L/HPW. A competitive balance between the phase separation and the volume minimization of surfactants was proposed to explain the self-organized liquid crystal structures of these protonated and non-protonated complexes. To the best of our knowledge, the present investigation provides a specific example for protonated hybrid materials with stable liquid crystal properties

Zhang C., Ding Y., Ping Q., and Yu L. L. (2006) Novel chitosan-derived nanomaterials and their micelle-forming properties. *J Agric Food Chem* **54**, 8409-8416.

Abstract: Six novel N-alkyl-N-dimethyl and N-alkyl-N-trimethyl chitosan derivatives were chemically synthesized and characterized using FT-IR, ¹H NMR, ¹³C NMR, differential scanning calorimetry (DSC), and X-ray diffraction spectrometry (XRD). The alkyl groups included octyl (C₈H₁₇-), decanyl (C₁₀H₂₁-), and lauryl (C₁₂H₂₅-). These chitosan derivatives were also evaluated for their micelle-forming properties using dynamic light scattering (DLS) and transmission electron microscopy (TEM) techniques. All six chitosan derivatives were capable of forming polymeric micelles in water with an average particle diameter ranging from 36 to 218 nm. Both N-octyl-N-dimethyl and N-octyl-N-trimethyl chitosan derivatives formed nanomicelles under the experimental conditions, with an average particle diameter of 36.0 and 52.5, respectively. Both the length of alkyl group and the N-trimethylation degree of the chitosan derivatives altered the size of their polymeric micelles. To further understand the effect of N-alkyl substitution degree of chitosan derivatives on size of their micelles, additional five N-octyl-N-trimethyl chitosan derivatives with N-alkyl substitution degree ranging from 8 to 58% were prepared and their micelle sizes were determined. The results showed that the diameter of the nanomicelles was proportional to the degree of N-octyl substitution. These data suggest that novel N-alkyl-N-dimethyl and N-alkyl-N-trimethyl chitosan derivatives may form nanomicelles. Additional research is required to further investigate the potential

value-added utilization of these chitosan derivatives in controlled release and targeted delivery of hydrophobic bioactive food factors.