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Summary

The application of molecular modelling, and in particular all-atom and coarse grained molecular dynamics simulation, to the study of low molecular weight surfactants with relevance to food systems is reviewed. Two key aspects of surfactant behaviour – their ability to form micelles and their tendency to adsorb to fluid-fluid interfaces (air-water and oil-water) is covered. Since the modelling literature on synthetic amphiphilic surfactants is vast, and much of it not directly relevant to foods, the review concentrates on biosurfactants. Two particular topics are covered in detail: the behaviour of bile salts because of the importance of these in understanding food digestion; the behaviour of novel glycolipid and lipopeptide surfactants derived from microorganisms (bacteria and yeast) due to their increasing importance as functional ingredients in consumer products including foods.

Keywords: molecular simulation; micelle; adsorption; surfactant; bile salt; glycolipid; biosurfactant.

1. Introduction

Surfactants are molecules that have the ability to adsorb at the interfaces between two phases (e.g. air-water, oil-water or liquid-solid), thus lowering the interfacial tension and stabilising the interface. Surfactants are often classified as low molecular weight or polymeric. Polymeric surfactants, such as proteins are out with the scope of this review which will cover only low molecular weight surfactants.

Low molecular weight surfactants are used for a range of applications in the food industry that exploit their surface active and solution properties [1]. These make use of their ability to adsorb to air, oil and solid interfaces to aid in the formation of foams and emulsions or to lubricate solid particles thus altering the flow properties of their solutions, and to form bilayer and micellar structures in foods. Common examples of food grade surfactants are lecithin, mono and diglycerides, sorbitan esters (SPANS) and polyoxyethylene sorbitan esters (Tweens) [1]. The experimental literature on the properties of surfactants is vast. More recently, researchers have made increasing use of molecular simulation methods to complement experiments in an attempt to better relate molecular features to physical properties. Although the literature on simulation of low molecular weight surfactants is extensive, there are few studies that specifically set out to look at surfactant containing model food systems. For this reason, this aspect of the surfactant simulation literature will only be reviewed briefly, with emphasis on indicating where further information can be found. Rather, the review will concentrate on two recent topical areas of study on surfactants that have a relevance to food systems, namely the surface and self-association behaviour of bile salts, and the growing importance of biosurfactants such as glycolipids. Although bile salts are not added directly as food ingredients, they have been studied widely over the past few years due to their importance in food digestion. Microbial biosurfactants are a novel

class of surfactants that are receiving lot of attention due to their “natural” sustainable nature. This means they are an appealing alternative to synthetic surfactants in food systems.

2. Micellization and Adsorption of Surfactants

The solution and adsorption properties of surfactants are an obvious target for simulation studies. The molecules are easy to represent in simplified algorithm form due to their amphiphilic structure and the two-component nature of surfactant solutions is highly amenable to the simulation approach. Low molecular weight surfactants are able to form a number of self-association structures depending on the physicochemical conditions and concentration. Micelles are important in food systems, but not the only structure formed, with bilayers and liquid crystalline phases being the main structures formed by many low molecular weight surfactants in foods. However, for bile salts modelling covered in a later section, the micelle is the most important solution structure, and so emphasis in this section is on the general properties and modelling of surfactant micelles, to serve as an introduction to bile salt micelle modelling in section 3.1.

Surfactant micelles can be characterized using two parameters; the critical micelle concentration (CMC) and the aggregation number. The concentration in solution at which micellar aggregates just start to form defines the CMC. The aggregation number is the average number of surfactant molecules found in the micelle at the CMC. Much of the simulation effort to date has focused on understanding the formation of micelles, characterising the CMC and aggregation number, how this links to micelle structure and relating this back to experimental observation and functional/physicochemical

properties. This work has often focused on surfactants that are not relevant in foods or food grade surfactants in systems not directly related to foods. For this reason, and because the amphiphilic surfactant modelling literature is very extensive it will not be reviewed here. Those interested in these systems are referred to recent reviews of simulation studies of surfactant micellization [2, 3] and simulation of adsorption of surfactants to interfaces that have recently been reviewed elsewhere [4•].

3. Bile Salts

Bile acids are biological surfactants synthesised from cholesterol in the liver and stored in the gall bladder. They are released as their sodium salts in the mixture bile, which is involved in the digestion of triglyceride fats and oils in the gut. There are a number of bile salt structures found in bile. The primary bile acids, cholic acid and chenodeoxycholic acid are synthesized from cholesterol by enzymic oxidation [5]. Secondary bile salts deoxycholic and lithocholic acid are formed when intestinal bacteria dehydroxylate the primary bile acids. Some of these four bile acids are re-adsorbed into the blood stream, and returned to the liver where they can be re-secreted to the gall bladder or conjugated with either the amino acid glycine or the amino sulphonic acid taurine. Bile salt molecules are comprised of a fused five and six membered tetracylic ring steroid core. In cholesterol the sterol ring is flat. In bile salts the presence of a hydroxyl group where two of the six-membered rings fuse, leads to bending of the steroid core due to extra steric strain. The bile salts of the human gut differ in two ways. Firstly, they can be dihydroxy or trihydroxy derivatives, based on how many hydroxyl groups are found on the sterol ring. The position of the attached hydroxyl groups can also vary. Secondly, they can be conjugated to glycine or taurine,

or no conjugation is present. In addition to the hydroxyl substituents methyl groups are also attached to the steroid core. The hydrophobic methyl and hydrophilic hydroxyl groups are oriented such that the two different substituent types are on opposite sides of the steroid rings, thus giving the bile salts a bi-facial amphiphilic structure, with a hydrophilic (OH) and hydrophobic (CH_3) face.

In the human gut the majority of fat is digested in the duodenum, and this is where bile is secreted through the bile duct. The bile salts have an important function in the digestion of fat [6]. As surfactants bile salts are able to emulsify fat, thus increasing the surface area available for digestion, whilst at the same time displacing proteins from the surface of the fat droplets [7$^{\bullet\bullet}$, 8$^{\bullet}$]. The latter aids the adsorption of lipases to the oil-water interface, thus facilitating triglyceride hydrolysis. After fat hydrolysis, the bile salts form so-called dietary mixed micelles (DMMs) with cholesterol and phospholipids [9]. The DMMs act as carriers of free fatty acids formed from the lipolytic digestion of triglycerides in the gut along with other fat soluble molecules. They achieve this by solubilising the products of lipolysis in the hydrophobic core of the micelle, and then transporting these to the intestinal wall where release of the fatty acids and their adsorption into the cells of the intestinal wall occurs [6].

3.1 Micelle Formation in Bile Salts

The structure of the micelle is important in controlling both solubilisation of fatty acids and facilitating adsorption, but the detailed structure and the mechanism by which micelles form is still under discussion. Bile salt micelles are not considered to be spherical like those of amphiphilic surfactants [10]. The reason for this is believed to be the structure of the bile salt molecules. The bifacial amphilicity that they possess

plays a significant role in the self-assembly of bile salts into micelles and the structure of the micelles. It is more difficult to pack bile salts into a micelle compared to amphiphilic surfactants. This limits the size of bile salt micelles due to restrictions in the number of hydrophobic contacts that the individual molecules can make, and thus the micelles are smaller than the spherical micelles of amphiphilic surfactants. There is much debate over the mechanism of micelle formation and in particular over whether bile salts have a single CMC, show a two-stage self-association process with two CMC's or lack a CMC altogether and have a continuous self-association at all concentrations [11]. Early models of self-association, such as that proposed by Carey & Small [12] put forward the hypothesis that at low concentrations bile salts form small, primary micelles held together by hydrophobic interactions, followed by subsequent self-association through hydrogen bonding of the primary micelles into larger secondary micelles once the concentration exceeds a second CMC (Figure 1). It has proven difficult to rationalise the self-association mechanisms experimentally, but recent simulations have thrown some light on the situation. For some time the favoured mechanism has been the two-stage association process. Evidence for this has been cited as the observation that hydrogen bonds are found in bile salt micelles [13]. All atom-molecular dynamics simulations have been carried out that support this mechanism [14-16]. Partay, Jedlovszky & Sega [14, 16] simulated sodium cholate (NaC) and sodium deoxycholate (NaDC) micelle formation at three concentrations, 30mM (close to the CMC), 90mM and 300mM. The NaDC simulations are in full agreement with the two-stage association model. At low concentration (30mM) only small oligomeric primary aggregates form with these primary aggregates held together by only hydrophobic interactions. At higher concentrations the primary aggregates associate into secondary micelles held together by hydrogen bonds. With NaC, on

the other hand, simulations suggest that hydrogen bonded oligomers as well as hydrophobic oligomers can form at low concentration, and these associate into mixed secondary micelles at higher concentration. The secondary micelles have the same basic structure in both NaC and NaDC systems, but only the NaDC micelles appear to form via the mechanism suggested by Carey & Small [12]. Partay, Sega & Jedlovszky [15] extended this study to look at the detailed morphology of the primary and secondary micelles and the relationship to bile salt structure. The primary aggregates of cholate micelles were found to be disk-like, flat or oblate, whilst NaDC primary micelles were predominantly spherical [15]. This was explained in terms of the differences in amphilicity of the two molecules. The hydroxyl groups are arranged on the edge of the ring in the NaDC molecule thus giving a hydrophilic edge to the tetracyclic ring. In NaC on the other hand these are arranged on one face of the tetracylic ring. This means that face to face conformations of NaDC are less favourable than those of two NaC molecules, and this modifies the primary micelle structures. The NaDC primary micelles have an open structure, and there is sufficient space in the core for a further NaDC to bind [15]. This binding pocket in the primary micelles may have consequences for the binding of other hydrophobic molecules within NaDC micelles or even DMMs. The primary micelles of both cholate and NaDC associate further into secondary micelles at higher concentration that have an irregular structure and varied shapes [15].

The structure and morphology of bile salt micelles has been debated for many years. It is well established that bile salt micelles do not conform to the classical spherical shape seen with amphiphilic surfactants. Three models for bile salt micelle structure have been put forward. Apart from the primary/secondary micelle model proposed by Carey & Small [12] there are also the disc model [10] and the helical model [17-19].

Kawamura et al. [10] carried out stearic acid spin probe immobilization studies using electron spin resonance to deduce the structure of trihydroxy and dihydroxy bile salt micelles. Their results suggested that the micelles adopted the same shape irrespective of bile salt type, although the structure may differ. For dihydroxy bile salts a signal for both strong and weak immobilization of the spin probe is observed, suggesting two types of micelle structure. For trihydroxy bile salts, on the other hand only a single weak binding signal is seen in the spectra. Kawamura et al. [10] interpreted their results as indicating a disk-like structure where the hydrophobic faces of the bile salts are oriented towards the centre of the micelle, with the hydrophilic faces making up the outer surface of the micelle. Giglio and co-workers [17-19] have studied in detail the structure of NaDC micelles. They propose a helical model based on x-ray diffraction data for NaDC crystals and fibres [17] and confirmed with NMR, ESR and SAXS [18, 19]. In this model the NaDC form an extended helix, with the hydrophobic face oriented to the outside of the micelle. The Na^+ cations occupy the central cavity of the micelle, with the whole structure stabilised by electrostatic interactions and hydrogen bonds. A schematic representation of the three model structures is shown in Figure 1.

Two recent comprehensive molecular dynamics simulation studies have attempted to understand better the structure of bile salt micelles, and how this is determined by the specific nature of the bile salt. Warren et al. [20••] simulated systems of six individual bile salts (NaC, glycocholate (NaGC), taurocholate (NaTC), glycochenodeoxycholate (NaGCDC), glycodeoxycholate (NaGDC) and glycolithocholate (NaGLC)) where thirty-one bile salts were modelled in explicit water. All six bile salts exhibited spontaneous self-association within 10ns of simulation time. The NaC, NaGCDC, NaGDC and NaGLC all form micelles of a stable size that undergo rearrangement

after formation. NaGC and NaTC show a more dynamic distribution, with NaGC forming a large single aggregate that is unstable over time, and NaTC showing a higher degree of micelle formation and break up. All micelles form when the bile salts make hydrophobic contact through the steroid rings, with the hydrophilic face in contact with either the solvent or the hydrophilic face of another bile salt. Warren et al. [20••] compared the structural features of their micelles to the structures represented in Figure 1. They noted that rarely was a hydrophobic face in contact with the aqueous phase for any length of time. This ruled out the inverted helix model of Giglio et al. [17-19] where the outside of the micelle is hydrophobic. The simulated micelles [20••] show some structural similarities to the primary/secondary micelle model of Carey & Small [12] and the disk model of Kawamura et al. [10] in that H-bonds appear to be important in the micelle structure. These are buried in the micelle structure and may represent the H-bonds holding together primary micelles to form secondary micelles in the Carey and Small [12] model. However, the representation of the Carey and Small and Kawamura et al. models in Figure 1 may be misleading because the simulations suggest that parallel arrangements of structures must be more disordered and flexible than in Figure 1.

Most simulations of bile salt micellization have been carried out at concentrations well above the CMC since at low concentrations it can take a long time for aggregates to form. This may be problematic when it comes to interpreting the results as the concentration found in the gut is often much lower than found in simulations, and the aggregate size for bile salts is well known to depend on concentration. For example, Small [21] found that all trihydroxy bile salts form small micelles with aggregation number less than 10, whilst dihydroxy bile salts form similar small micelles at the CMC but larger micelles with aggregation number of 12-100 at higher concentrations.

Mustan et al. [22] carried out a set of simulations similar to that of Warren et al. [20••] on six bile salts (NaC, NaDC, NaGC, NaTC, NaGDC & sodium taurodeoxycholate NaTDC), but at a lower physiologically relevant bile salt concentration (10mM, close to the CMC), NaCl concentration (120mM) and temperature (37 ^{o}C). They confirmed that small primary micelles with a radius of about 1nm form in all systems, that the micelles form primarily through hydrophobic association between the steroid rings, forming a hydrophobic core and that the few H-bonds that are observed to form do not affect the stability of the micelle. They also found, however, that the formation of intermolecular H-bonds did speed up the rate of aggregation in the bile salts conjugated to glycine or taurine where more of these form. All bile salts formed irregularly shaped ellipsoidal micelles, with glyco conjugates forming the largest aggregates (hexamers and octamers) and tauro and non-conjugated bile salts forming pentameric micelles.

Verde and Frenkel [23•, 24••] have used a different approach to simulating bile salt micellization where they define a coarse-grained structure for the molecules, and a Langevin dynamics algorithm. This method has the advantage of allowing for larger system size and longer simulation times, at the expense of atomic detail in the bile salts. The model was able to reproduce some findings of the all atom molecular dynamics simulations in that dihydroxy bile salt formed larger micelles than trihydroxy ones, and that the aggregation numbers were also reproduced. Two highly significant additional findings resulted from this study. Hydrogen bonds were not included in the simulations and so this suggests that although they may well help to stabilise micelles they do not drive aggregation. In fact, the results showed, unlike the all-atom models, that hydrophobic surface is exposed in the small micelles, and that this would be sufficient to drive further aggregation of primary micelles into secondary micelles at

higher concentrations. The binding of bile salts to the micelle was shown to be weakly cooperative, and the energy required to remove a bile salt from the micelle was only of the order of 2kT [23•, 24••]. This means that the micelles are marginally stable, a feature that will help them rapidly assemble into mixed micelles with other lipid material, but also to disassemble easily when required to release solubilised lipid molecules for adsorption at the gut wall. A second significant finding [24••] is that bile salt micelles assemble by addition of monomers, and not by the initial assembly of small primary micelles which then aggregate further into secondary micelles, thus apparently ruling out the two-stage aggregation mechanism proposed by Carey and Small [12].

Molecular simulations of single bile salts throw some light on the assembly of DMMs, but are not a realistic representation of DMMs. Two models have been proposed for the structure of mixed micelles of phosphatidylcholine lipids (PC) and bile salts, which are often used as model systems for DMMs. These are known to form rod-like micelles in solution. Two models have been put forward for the structure of PC + bile salt mixed micelles. Shankland [25] proposed the stacked-disc model. In this model the overall micelle is formed from smaller disc-shaped mixed micelles comprised of a PC bilayer surrounded at the edge by bile salts. The disc-shaped micelles stack end to end to form a rod like micelle (Figure 2). Both PC and bile salts are arranged parallel to the rod axis. In a second model, the radial shell model [26, 27], the PC molecules radiate outward from the axis of the rod, with the charged phosphatidyl head group on the outer surface. The bile salts sit on the micelle surface between the PC head groups (Figure 2). In this model, there is debate as to whether the bile salts are parallel to the micelle rod axis [27] or partially perpendicular [26]. Marrink and Mark [28] carried out an extensive MD simulation of mixed micelles of palmitoyloleoylphosphatidylcholine

(POPC) and NaC to understand better the structure of mixed micelles in the presence and absence of small amounts of solubilised cholesterol. Mixed micelles formed rapidly within about 10ns, with internal reorganization taking place over timescales an order of magnitude longer. Although the systems used were too small to give extended rod-like micelles, nonetheless the structure of the micelles fitted better the radial shell model, with the bile salts oriented perpendicular to the POPC, a finding that is also supported by experiment [29]. Furthermore, they found that the micelles were capable of solubilizing small amounts of cholesterol without significant changes to the micelle structure. Haustein et al. [30] have simulated dipalmitphosphatidylcholine (DPPC) plus NaC mixed micelles using a coarse-grained model and Brownian dynamics. They did not report micelles that have the radial shell structure, which may be because the model lacks atomic detail. However, they did observe formation of small mixed DPPC/NaC micelles with disc-like structures. These were comprised of one or two DPPC molecules with either oriented bile salts or disordered bile salts arranged around the central DPPC molecules. This may represent the coarse-grained equivalent of the primary phospholipid/bile salt building blocks hypothesised for the stacked disc and radial shell models. Haustein et al. [30] also observed that larger worm-like micelles were able to form under certain conditions. In these the disordered phospholipids appear to form a linear micellar core with bile salts surrounding this oriented with their hydrophobic face towards the lipid chains and hydrophilic face on the outside. This structure seems closer to the stacked-disc [25] or radial disc models [26, 27] rather than the models for pure bile salts [10, 12, 17-19]. The fact that they do not appear to reproduce the stacked-disc or radial disc models more accurately is probably a reflection of the coarse grained nature of the model.

3.2 Adsorption of Bile Salts

In addition to the role bile salts play as micellar carriers of hydrophobic digestion products in the gut, they also have a role as surfactants. In this, they function to adsorb on to the surface of lipid droplets, competing for interface with and displacing proteins and other surface active food components. Lipase enzymes seem to be able to interact with this composite adsorbed layer more easily, thus facilitating digestion of triglycerides [31]. A more complete understanding of digestion requires that we elucidate the adsorption mechanisms of bile salts. This area is also suitable for study by molecular simulation, but to date there have been few studies that have addressed this [7••, 8•]. Two hypotheses have been put forward to explain the conformation bile salts adopt when they adsorb to an oil-water interface. From studies of bile salts adsorbed to phospholipid bilayers, Small [32] believes the bile molecules adsorb perpendicular to the interface with the sterol ring penetrating into the lipid layer and the charged end solvated in the aqueous phase. In this conformation the sterol rings interact via hydrogen bonds to form dimers or tetramers in a form of reverse micelle. This view is consistent with the idea that bile salts in micelles can form hydrogen bonds, and has received experimental support from the studies of Vadnere & Lindenbaum [33] who found that bile salts do appear to form reverse micelles at the octanol-water interface.

The second model for bile salt adsorption hypothesises that they adsorb flat at an oil-water interface with the hydrophilic hydroxyl face in contact with the aqueous phase and the methyl groups of the hydrophobic face in contact with the oil [34]. This model is also supported by experiment in that the area occupied per bile salt molecule at saturation coverage is found to be consistent with a flat conformation [34].

Euston and co-workers [7••, 8•] have used all atom molecular dynamics simulation of NaC and NaDC at the decane-water interface in an attempt to deduce which of these models is more likely. They found that both NaC and NaDC adopt a perpendicular conformation at the decane-water interface (Figure 3), with the sterol ring in the decane phase, although this is not upright but is tilted at an angle of 44-49^{o} to the normal of the interface depending on the surface coverage.

The affinity of each bile salt type for the decane water surface was estimated by elucidating the potential of mean force using umbrella sampling [7••].The free energy of adsorption (ΔG_{ads}) for NaC was approximately 80 kJ/mol compared to 104 kJ/mol for NaDC. This reflects the greater hydrophobicity of the NaDC molecule as it has one less hydroxyl group attached to the sterol ring. The greater affinity for the oil-water interface of NaDC also led to it being more efficient at displacing protein from the oil-water emulsion droplet interface [7••]. Furthermore, both bile salts were observed to form clusters at the interface, which grew larger as the surface coverage increased and were stabilised by the formation of hydrogen bonds. This suggests the bile salt interfacial aggregates are oriented hydrophilic face to hydrophilic face and may take the form of reverse micelles.

Euston et al. have also used MD simulation to probe the effect of conjugation of bile salts with the amino acid glycine or the amino sulphonic acid taurine on interfacial adsorption and protein displacement [8•]. They followed the adsorption of NaC, NaDC, NaGDC and NaTC to the decane-water interface. Conjugation with the amino acids reduces the overall hydrophobicity of the bile salt and alters the conformation they adopt at the oil-water interface. This manifests itself as a reduction in the ΔG_{ads} as calculated from the potential of mean force using umbrella sampling [8•]. The calculated ΔG_{ads} for NaC was found to be 78.5 kJ/mol and for NaDC 104.2 kJ/mol.

Adding a glycine to NaDC to form NaGDC reduced ΔG_{ads} to 82.3 kJ/mol, and adding taurine to NaC to form NaTC reduced ΔG_{ads} to 57.4 kJ/mol. The orientation of the bile salts was also affected by conjugation. Both the longitudinal (along the axis of the sterol ring) and lateral (across the ring) tilt angles were calculated. For both values of 0 and 180^o indicate a conformation oriented upright at the interface, whereas 90^o indicates a flat conformation. The average longitudinal tilt angle for NaC and NaDC (the angle the molecule makes to the normal of the interface) was 49^o, whilst for NaGDC this was 56^o and for NaTC 54^o, although the range of angles was large i.e. on average bile salts are oriented at an angle approximately half way between upright and flat, but NaGDC and NaTC adopt a slightly flatter conformation at the surface (Figure 3).

The lateral tilt angle increases in the order NaC (73°) > NaGDC (93°) > NaDC (104°) > NaTC (116°). In other words, the average lateral tilt angle also suggests a close to flat conformation at the interface. The distribution of lateral tilt angles, tells a more complex story. Based on average lateral tilt angle NaGDC is the closest to the flat conformation. However, NaGDC adopts a very wide range of flat and inclined conformations. Although the average lateral tilt angle for NaC is less than 90°, the overall distribution of tilt angles is closest to a flat conformation. The average lateral tilt angle for NaDC and NaTC is above 100° with the overall distribution skewed towards non-flat conformations. These simulations suggest that the adsorption of bile salts at interfaces is complex and dynamic and varies from bile salt to bile salt. It appears that the hypothesised models for bile salt conformation that propose they either adsorb upright or flat at the interface may be an oversimplification and that both conformations can occur in a dynamic, changing system. The predominant conformation the bile salt adopts will depend not only on the nature of the bile salt, but

also the specific conditions of the system (ionic strength, pH, bile salt concentration etc.). Indeed, this dynamic state of adsorption may be essential to the function of bile salt as surfactants and micelle formers, and will facilitate both protein displacement from fat droplet surfaces and micelle formation during digestion. It is known that the surface tension lowering ability of bile salts is not consistent with their high ability to displace protein from an oil-water interface [6], especially when compared to amphiphilic surfactants. It has been suggested that this is because they adsorb flat to the interface, unlike amphiphiles that adsorb upright, thus maximising the area they occupy and making them more efficient at competing for interfacial area with adsorbed proteins [6]. The simulation results of Euston et al. [7••, 8•] suggest that this view could be refined to allow for both flat and upright conformations, and that it is the dynamic nature of the bile salts that allows them to occupy a wide range of both upright and flat conformations at the interface that could be the origin of their protein displacing power.

4. Biosurfactants

Biosurfactants are amphiphilic surfactant molecules that are produced by living organisms including bacteria and fungi [35]. They have received much attention over the past few years as potential additives for a wide range of industries. Many surfactants used currently in the food (and other) industries are synthesised from petrochemical feedstock, and thus are perceived by the consumer as being unnatural or synthetic. In addition, oil stocks are finite and so the sustainability of surfactants production is a major consideration. There is also the questionable environmental credentials of some common surfactants to consider. Palm oil is a common source of mono and diglycerides surfactants used in the food industry. The demand for palm oil

has led to significant deforestation of native forests in parts of South East Asia in favour of palm oil plantations [36]. The subsequent detrimental effect on native fauna, and in particular on the orangutan [36] has led to negative publicity in the press. This has led some food manufacturers to re-evaluate their use of mono/di-glycerides. All of these factors feed into consumer sentiment against current surfactant types, and are powerful drivers for the food industry to look for new sustainable, environmentally friendly surfactants. There is hope that biosurfactants will be able to at least partially replace synthetic surfactants in food and other consumer products.

There are several types of biosurfactant produced by microorganisms that have the potential to be produced at commercial scale. Of these, the glycoplipid surfactants and the bacterial cyclic lipopeptide surfactins have received the most attention and some have been produced at a scale sufficient for incorporation in consumer (particularly green cleaning) products. Their appeal is that they are natural, have low or no toxicity and surface activity that is comparable to the synthetic surfactants they replace. The three most commonly studied classes of glycolipids are the rhamnolipids [37], sophorolipids [38], and mannosylerythritol lipids (MELs) [39]. From a structural point of view they differ markedly from the classical amphiphilic surfactants. As their name suggests, they contain a hydrophilic sugar head group attached to a lipid tail. The number of sugar rings and the number and size of lipid chains can vary between the glycolipids. Rhamnolipids can be either mono or di-rhamnolipids with one or two rhamnose rings covalently bonded with one or two β-hydroxyalkanoic acids. Sophorolipids have a sophorose (β-1,2 linked glucose disaccharide) sugar head group covalently linked to a long-chain hydroxylated fatty acid. In the MELs the head group is a mannose-erythritol disaccharide with the mannose ring acetylated with either two short chain (n=2-8) or two long chain (n=10-18) fatty acids. The structure of these

glycolipids and the way they differ from amphiphilic surfactants suggests the structure they will adopt in micelles and how they adsorb to interfaces will also differ.

Surfactin is the generic name given to a class of a cylic peptides [40] consisting of a heptapeptide ring linked to a lipid tail of 13-15 carbons. A common example is the iso-C15 surfactin that has the structure GLU, ASP, LEU, LEU, VAL, LEU, LEU attached to a C15 lipid chain. Several studies have shown that surfactin will form micelles in solution that are either spherical and of size 5-9 nm [41-43] or elliptical with major and minor axes of 19 and 11 nm, respectively [41]. Shen et al. [43] deduced that the micelles were of core-shell type, with the hydrophobic core comprised of the lipid tail and four hydrophobic leucine residues. In addition to its solution properties, surfactin is also known to adsorb to and self-assemble at air-water and oil-water interfaces, and to be able to lower surface tension more efficiently than most synthetic surfactants [44].

4.1 Micellization of Biosurfactants

Glycolipid surfactants show a complex phase behaviour that depends on the number, nature and stereochemistry of the sugar head group, and the number and nature of the attached lipid tails [45]. They can form spherical, disc shaped and rod-like micelles in dilute solution [46], whilst at higher concentrations liquid crystalline phases can occur [47].

There have been several molecular dynamics studies on glycolipids that have looked at the micellization behaviour, some on general glycolipid structures, and some on the sophorolipids specifically. Chong et al. [48] used all atom MD simulations to compare the micelle properties of the alkyl glycosides n-octyl-α-D-galactopyranoside and n-octyl-α-D-glucopyranoside. Their simulations showed that the arrangement of water

around the head groups was altered due to the differing orientations of the hydroxyl groups. This did not affect greatly the structure of the micelles at low concentration, but did alter the temperatures at which transitions between liquid crystalline phases occurred. Abel et al. [49] have looked at the influence of the anomeric form of the sugar head group on micelle structure of α and β-anomers of N-dodecyl-β-maltoside and found that the β-anomer gave more elliptical micelles. Konidala et al., [50] used MD simulation to follow at spontaneous micellization in n-octyl-β-d-glucopyranoside and suggest that spherical micelles are highly unlikely to form even close to the critical micelle concentration due to geometric constraints of the sugar head group and alkyl chain, and that a prolate ellipsoid was the most likely shape. A detailed MD study of glycolipid micelle structure has been made by Manet et al. [51••] on an acidic sophorolipid. These authors carried out a combination of small-angle X-ray scattering (SAXS), and small-angle neutron scattering (SANS) to deduce acidic sophorolipid micelle structure that was supported by all-atom molecular dynamics simulations. They found that the micelles were ellipsoidal in nature and of the core-shell form, presumably with a hydrophobic core surrounded by a hydrophilic shell (Figure 4). However, unlike micelles formed by other surfactants, the hydrophilic shell was not evenly distributed around the hydrophobic core, and the thickness was found to be almost zero at the ends of the long axis of the ellipse. Manet et al. [51••] were able to use contrast matched SANS to show that the sophorolipid micelle was most likely comprised of three regions: a hydrophobic core; a hydrophilic shell (the sugar head groups); and a mixed region along the long axis of the ellipse (Figure 4). They carried out MD simulations in an attempt to confirm this unusual micelle structure. The results showed that the sophorolipids formed a range of micelles sizes and shapes during the simulation. Small micelles tended to be spherical and the sophorolipids were arranged

in a bent conformations where the sugar and carboxyl groups were and the micelle surface and the alkyl chain in the micelle core (Figure 5), a structure closer to that observed in amphiphilic micelles. As the micelles grew in size, the sophorolipids adopted a wider range of conformations in the micelle, and the overall micelle shape became more ellipitical. In the latter, the distribution of sophorolipids in the micelle became more segregated (Figure 5) and supported the hypothetical structure in Figure 4.

There have been few simulation studies of surfactin micelle formation. She et al. [52] have used MD simulation to try to understand better which parts of the surfactin molecule constitute the hydrophobic and hydrophilic regions in the micelle. They found that prolate ellipsoidal micelles formed of radius about 2.5nm where the cyclic peptide occupied the surface of the micelle with the lipid chains in the core of the micelle. This study was extended by the same authors [53], with the MD results giving slightly smaller (2.2nm radius) but mostly spherical micelles, and confirmed the presence of the peptide rings at the micelle surface. They also found that the four hydrophobic leucine residues where localised at the surface and not in the micellar core, thus not supporting the surfactin micelle model proposed by Shen et al. [43]. A small proportion (about 1%) of hydrogen bonding in β- and γ-turn structure was observed in the peptide rings, which would not appear to be sufficient to be a major driving force for micelle formation or stabilization.

4.3 Adsorption of Glycolipids

The simulation literature relating to glycolipid interaction with model biological membranes is extensive due to the important function that these molecules play in

membrane function and in particular their role in lipid raft formation. This area has been extensively reviewed by Shorthouse et al. [54]. Molecular dynamics simulation has also been used to study the effect of rhamnolipids on the interaction between oil and solid quartz or calcite surfaces [55, 56]. Although these simulations are not directly relevant to foods they do serve to demonstrate the applicability of molecular dynamics and other simulation methods to glycolipids.

Rhamnolipid adsorption has been studied experimentally at the air-water interface using neutron reflectance [57, 58]. In these studies the adsorption and competitive adsorption of mono- and di-rhamnolipids has been followed using the two most common rhamnolipid structures, the L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (mono-rhamnose R1) and L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (di-rhamnose R2). Each, in addition to one or two rhamnose rings, contains two attached C10 fatty acids. Both types of rhamnolipid exhibit Langmuir adsorption type behaviour, which has allowed the area per molecule at the interface to be calculated as 60 (mono) and 75 (di) $Å^2$ [57] with the latter value related to the larger size of the di-rhamnose head group. When R1 and R2 are present in mixtures, the R1 adsorbs preferentially to the interface due to the steric constraints on the R2 sugar headgroup. Experiments where sodium dodecyl benzene sulphonate (SDBS) is also included indicated that the R1 adsorbs ideally with the SDBS, but R2 is outcompeted for interfacial area by the SDBS [58]. Zhu et al. [59] have used dissipative particle dynamics to explore the adsorption of R1 and R2 to the octane-water interface. They confirmed the lower adsorption of R2 to the octane water interface due to the larger size of the hydrophilic di-rhamnose head group of R2 [57-59]. Zhu et al. [59] also studied the coexistence of anionic surfactants (SDS and SDBS) with R1 and R2 at the oil-water interface. Again, the results confirmed the experimental

findings [57-58] and gave some clues as to why this occurs. In addition to the larger head group of R2 leading to a lower ability to compete with anionic surfactants, for R2 there was also a very noticeable phase separation into R2 rich and anionic surfactant rich regions at the interface which was absent in the R1 plus anionic surfactant simulations [59]. This confirmed the view of Chen et al. [57-58] that R1 and SDBS have interfacial mixing behaviour that is closer to ideal than R2 and SDBS. Abbassi et al. [60] have also used all-atom MD simulation to investigate the effect of ionization of the free carboxyl group on one of the C10 chains. The presence of the negative charge at pH above the pK_a (pH 5.9) disrupts the structure of a mono-rhamnolipid mononlayer and reduces the diffusion coefficient of the adsorb rhamnolipid [60]. Similarly, the distribution of dissociated carboxylic acid groups within the interfacial layer are different compared to the non-dissociated form in that the charged form extends further into the water layer [60]. This was reflected in the experimental results in the same study that showed that at pH 7 where 97% of the rhamnolipid is expected to be charged the surface tension as a function of concentration is slightly higher than at pH 4 (97% uncharged). Similarly, the calculated surface area per molecule is slightly higher (63.2 $Å^2$) at pH 4 than at pH 7 (57.2 $Å^2$) due to the interfacial rearrangement of the lipid chain containing the free carboxyl.

The adsorption of surfactin to the air-water interface has been studied by several authors in an attempt to understand how the molecule orients at the air-water interface. Maget-Dana and Ptak [61] measured the adsorption isotherm of surfactin under compression at different pH, temperature and salt content and deduced that the molecule adopted a range of conformations. At low surface coverage, the surfactin sits at the surface in and expanded, flat monolayer, with a change in orientation toward a more upright conformation accompanying an increase in surface compression until

at the end of the plateau in the compression isotherm an upright ring conformation is adopted with the lipid chain in the hydrophobic phase. Ishigami et al. [62] found that adsorbed surfactin layers formed intermolecular β-sheet at the interface. Neutron reflectance studies [43, 63] suggests that surfactin adopts a compact ball-like conformation at the air-water surface. Galle et al. [64] have put forward a model for the molecular conformation of adsorbed surfactin based on surface area measurements and molecular modelling. They propose that the hexapeptide ring sits flat in the plane of the interface with the acidic glutamic and aspartic acid residues in contact with the aqueous phase and the lipid tail looping over the ring into the hydrophobic phase to form a hydrophobic contact with leucine residues. This would explain the ball-like conformations reported by neutron reflectance [43, 63]. Various computer simulation studies have been reported that look at the molecular conformation of the adsorbed surfactin [65-67]. Nicholas [65] used MD simulation to show that surfactin conformation is sensitive to the concentration at the interface and can be highly flexible. Nicholas [65] observed that the peptide ring of surfactin molecules does not appear to adopt a completely flat conformation, but has what is described as a saddle structure where the ring distorts and twists, presumably to allow hydrophobic and hydrophilic amino acid residues to occupy their most preferred environment. Gang et al. [66] have also confirmed using MD simulation that the surfactin adsorbed conformation is highly flexible. The results support the experimental observations of Maget-Dana and Ptak [61] in that the simulated surfactin adopts relatively flat conformations at low surface coverage, which became more tilted towards upright as surface coverage increases, although an upright conformation of the surfactin lipid chain is only rarely seen. In addition, the lipid chain was also observed to fold back to form a hydrophobic contact with a hydrophobic LEU and VAL

residue in the peptide, which is similar to the interaction proposed by Galle et al. [64]. The packing of the peptide rings in the interfacial layer at higher surface concentrations favoured hydrogen bond formation and stabilised β-secondary structure formed between the molecules at the interface. Iglesias-Fernández [67] have also simulated surfactin adsorption at the air-water interface using MD but at different pH. They confirmed experimental observations that surfactin adsorbed conformation is independent of pH [67] and that the peptide ring adsorbs predominantly in a flat conformation, with the lipid chain more flexible and able to adopt a range of conformations in the hydrophobic phase.

Conclusions

Biosurfactants are a growing area of research interest within the food sciences. It is believed that the development of an understanding of the role of bile salts in the digestion and adsorption of complex foods will facilitate the design of foods that have a controlled digestibility, and can contribute towards the production of healthier foods for the consumer. Similarly, the development and use of new, sustainable biosurfactants will be welcomed by the consumer who have a negative view of their synthetic counterparts. Molecular simulation clearly has a role to play in understanding the molecular features that control surfactant behaviours such as micellization and adsorption. This is already well established for amphiphilic surfactants, and as has been shown this can be extended to food relevant biosurfactants. To date, molecular simulation has been limited to simple systems two or three component systems (air-water-surfactant or oil-water-surfactant). The challenge will be in how these methods can be extended and applied to more realistic systems that better capture the unique biological complexity of food systems.

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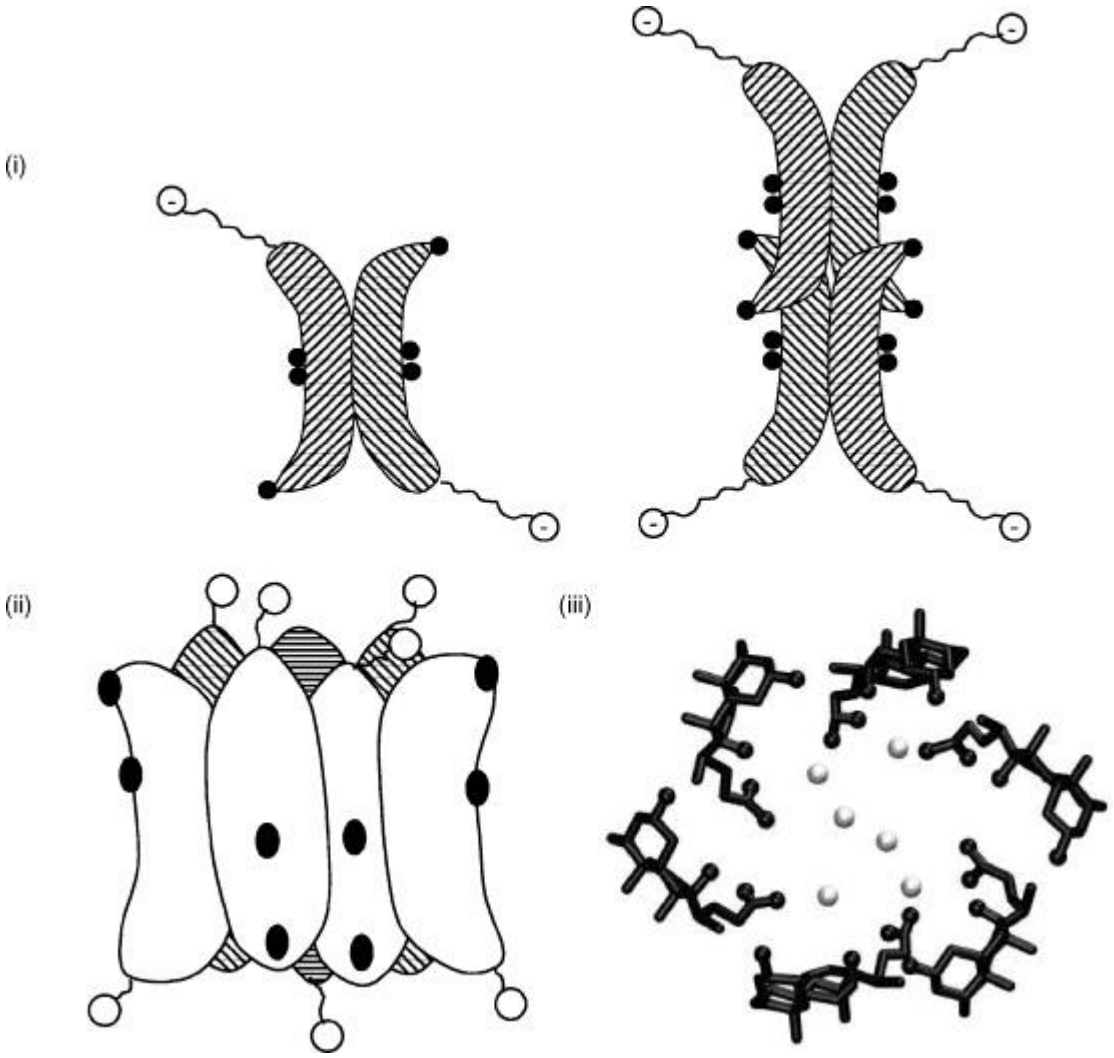

Figure 1. – Schematic diagrams of the three proposed bile salt micelles structures: (i) primary and secondary micelles by Carey and Small (1972); (ii) disk-like model by Kawamura et al. (1989), and (iii) helical shaped micelles by Giglio et al.(Conte, Di Blasi, Giglio, Parretta & Pavel, 1984; Esposito, Zanobi, Giglio, Pavel & Campbell, 1987; Esposito, Giglio, Pavel & Zanobi, 1987)). Reprinted from Colloids Surf A, 280, Warren DB, Chalmers DK, Hutchison K, Dang W, Pouton CW, Molecular dynamics simulations of spontaneous bile salt aggregation, 182–193, Copyright (2006), with permission from Elsevier.

Figure 2

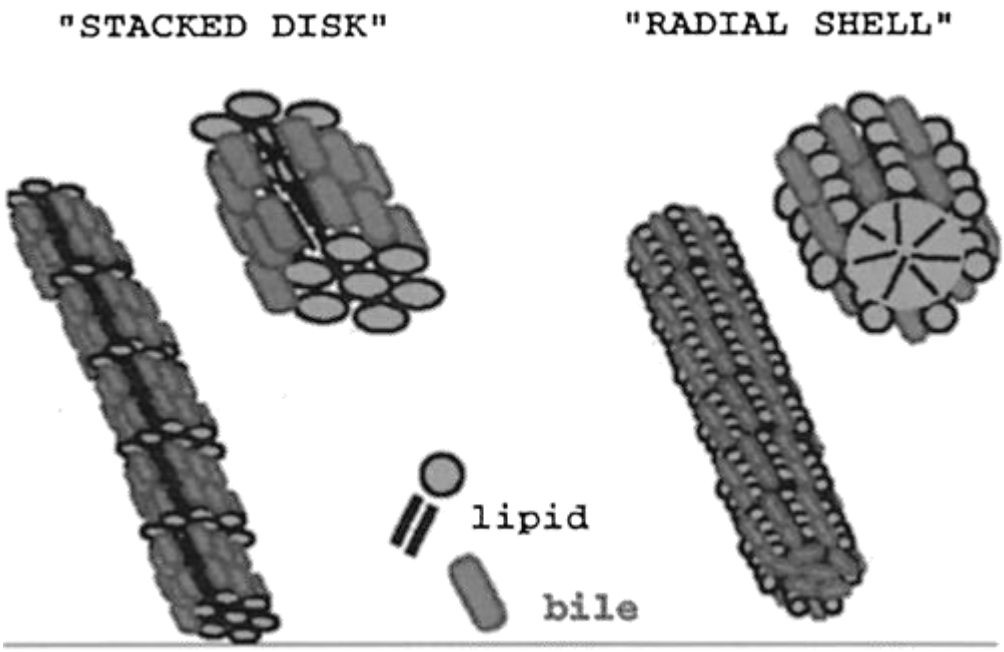

Figure 2 - Schematic picture of the two models for the structure of bile/salt lipid mixed micelles. In the stacked disk model a parallel orientation of the phospholipids is proposed. The radial shell model proposes a radial orientation. The hypothesized mechanisms by which the micelles can further associate into rodlike aggregates is also shown. Reprinted with permission from Marrink SJ, Mark AE. Molecular dynamics simulations of mixed micelles modeling human bile. Biochemistry 2002: 41: 5375-5382. Copyright 2002 American Chemical Society.

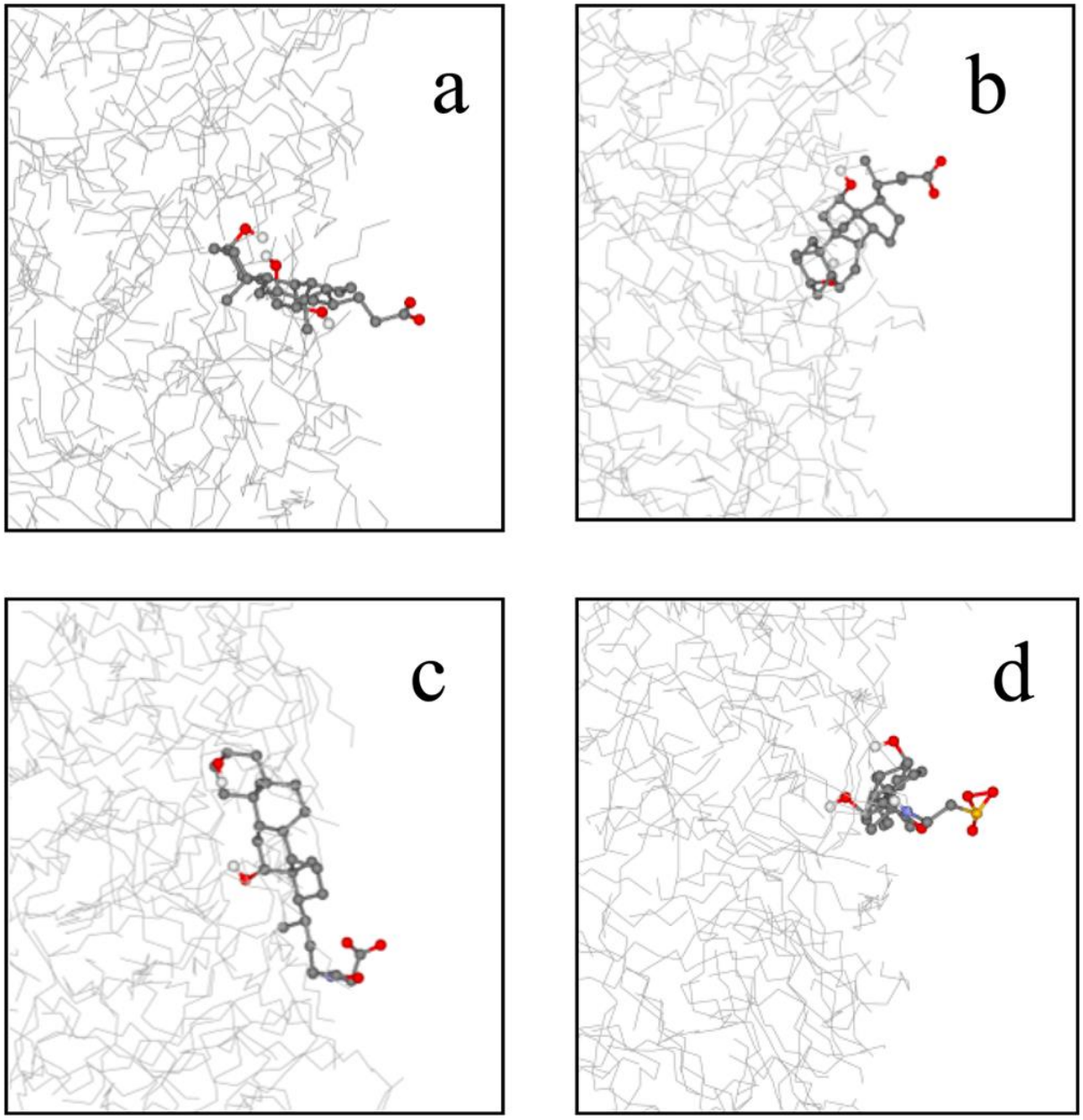

Figure 3 - Adsorbed molecular dynamics simulation conformations of four bile salts at the decane−water interface: (a) NaC, (b) NaDC, (c) NaTC, and (d) NaGDC. The range of conformations that the different bile salts can adopt from upright to flat can be seen in these snapshots. Reprinted with permission from Euston SR, Baird WJ, Campbell L, Kuhns M. Competitive adsorption of dihydroxy and trihydroxy bile salts with whey protein and casein in oil-in-water emulsions. Biomacromolecules 2013: 14: 1850-1858. Copyright 2013 American Chemical Society.

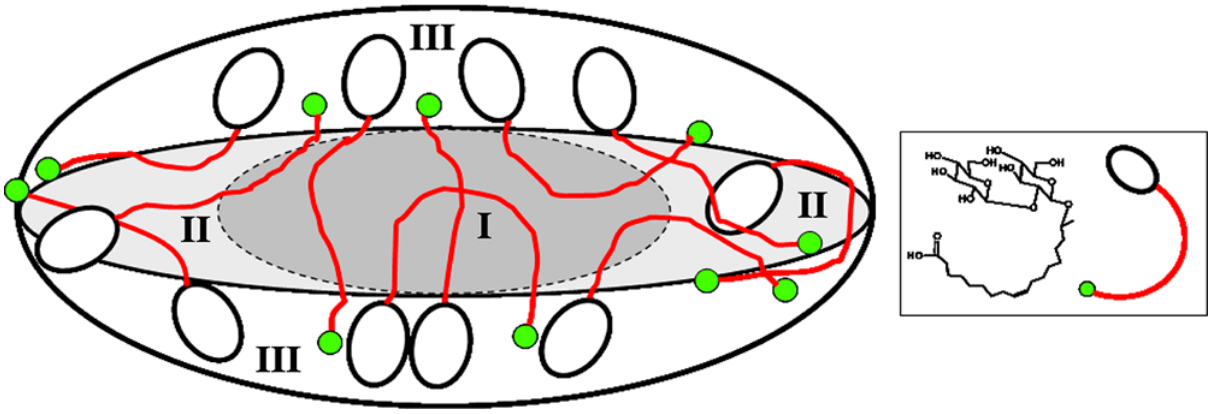

Figure 4 - Hypothetical model structure of an acidic sophorolipid micelle at its equilibrium pH (<5). Region I is composed of the lipid chains; Region II is a mixed region composed of sophorose sugar head group/water/carboxyl group/lipid chains; Region III is a mixed region composed of sophorose sugar head group/water/carboxyl group. At 5 < pH < 7, the boundaries between the three regions are probably less defined, and the COO^- groups occupy a broader volume in the hydrophilic region. Reprinted with permission from Manet S, Cuvier A-S, Valotteau C, Fadda GC, Perez J, Karakas E, Abel S, Baccile N. Structure of bolaamphiphile sophorolipid micelles characterized with SAXS, SANS and MD simulations. J Phys Chem B 2015: 119: 13113−13133. Copyright 2015 American Chemical Society.

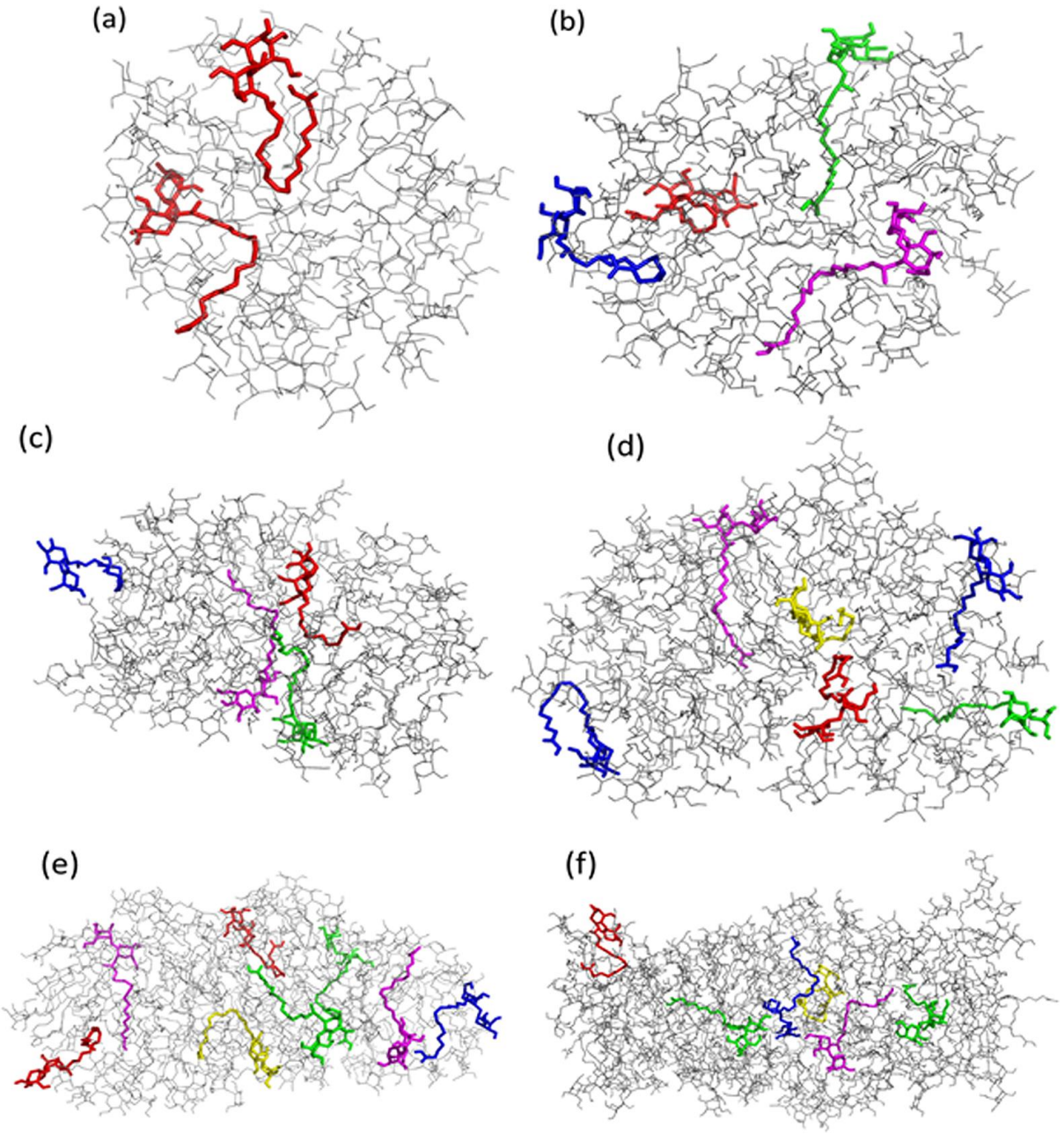

Figure 5 - Representative molecular dynamics simulation conformations of sophorolipids in micelles with differing numbers of molecules (a): 28, (b) 37, (c) 56, (d) 68 and (e) 80, (f) 112 monomers. Red, blue, green, and yellow colors corresponds to the surfactant conformations where the sophorose and the COOH headgroups are located at the micelle surface with the rest of the surfactant in the hydrophobic core (red), the sophorolipid reside entirely at the micelle surface (blue), alkyl chain is extended and cross the hydrophobic core and with the sophorose and headgroups at the surface (magenta), where COOH in the hydrophobic core (green), where the overall sophorolipid are deeply buried in the hydrophobic core (yellow). Reprinted with permission from Manet S, Cuvier A-S, Valotteau C, Fadda GC, Perez J, Karakas E, Abel S, Baccile N. Structure of bolaamphiphile sophorolipid micelles characterized with SAXS, SANS and MD simulations. J Phys Chem B 2015: 119: 13113−13133. Copyright 2015 American Chemical Society.