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Corresponding Author: Professor Anuradha Mishra, PhD

Corresponding Author's Institution: School of Vocational Studies & Applied Sciences, Gautam Buddha University

First Author: Anuradha Mishra, PhD

Order of Authors: Anuradha Mishra, PhD; Divya B Tripathy, Ph.D; James Clark, Ph.D; Thomas Farmer, Ph.D

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Synthesis, Chemistry, Physicochemical Properties and Industrial Applications of Amino acid Surfactants: A Review

Divya Bajpai Tripathy[1], Anuradha Mishra[1]*, James Clark[2] and Thomas Farmer[2]

[1]Department of Applied Chemistry, School of Vocational Studies and Applied Sciences, Gautam Buddha University, Greater Noida-201312, India

[2]Green Chemistry Centre of Excellence, Department of Chemistry, University of York, Heslington, York, YO10 5DD, UK

Abstract

Surfactant use throughout mankind is extensive, from their initial applications as detergents extending into use in medicine, lubricant, cosmetics and even enhanced oil recovery. However, the image of surfactant use has in the past been tarnished by of issues with low biodegradability and their synthesis from non-sustainable resources. Amino acid–based surfact ants are a class of surfactants derived from a hydrophobe source coupled with simple amino acids, mixed amino acids from synthesis or from protein hydrolysates, and as such can be derived solely from renewable resources. There are several pathways for their synthesis and this allows for extensive structural diversity in this class of surfactants, resulting in widespread tuneable functionality in their physiochemical properties. This review includes the details of most of the available routes of synthesis for amino acid surfactants and the impact of the diverse routes on their final physiochemical properties, including solubility, dispersability, toxicity and biodegradability. The diversity offered by the structural variation in amino acids surfactants offers many exciting commercial

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*Corresponding author E-mail: anuradha_mishra@rediffmail.com

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1. Introduction

Surfactants are the group of organic compounds which continue to attain great interest from researchers due to their wide range of applications including as laundry

detergents, emulsifiers, corrosion inhibitors, oil recovery and pharmaceuticals. These are the most representative chemical products to be consumed in major quantities daily and globally and have in the past led to adverse effects on the aquatic environment. Many studies have previously revealed the adverse impact of widespread use of conventional surface-active agents on the environment (Ivanković & Hrenović, 2010). As such non-toxicity, biodegradability and biocompatibility of surfactants have become almost equally important for the consumers as their functional performance.

Biosurfactants are a class of green and sustainable surface active agents naturally synthesized from microorganisms such as bacteria, fungi and yeast or excreted extracellularly. Synthetic equivalents to biosurfactants can therefore be prepared by designing molecules that imitate natural amphiphilic structures such as phospholipids, alkylglucosides and acyl amino acids. Amino acid surfactants (AAS) are one such type of surfactant which can generally be originated from animal or agricultural-derived feedstocks. AAS have been gaining great interest of scientists over the last two decades as novel surfactants as they can be synthesized using renewable sources and their ease of degradability and harmless by-product make them safer for our environment (Kango, 2010).

AAS can be defined as a group of surfactants made up of amino acids comprising of amino acid group (HO_2C-CHR-NH_2) or its residue (HO_2C-CHR-NH_2). These two functional regions of amino acids give the possibility to derive an extensive range of surfactants. There are total of 20 standard proteinogenic amino acids known in nature which are responsible for growth and all physiological reactions of living kingdom. They differ from each other only on the basis of the residue, R (Taubeneck, 1977) (Fig .1). Some are non-polar and hydrophobic, others are polar and hydrophilic, some are basic and some are acidic. As

amino acids are renewable compounds, surfactants synthesized from amino acids also have great potential as sustainable and eco-friendly substances (Infante *et al.*, 1997). Simple and natural structure, low toxicity and fast biodegradation often make them superior over their conventional counterparts. Their production can be via different biotechnological and chemical routes using renewable raw materials such as amino acids and vegetable oils.

Amino acids were first discovered as a substrate for surfactants in the early 20th century (Morán *et al.*, 2004). Primarily they were used as preservatives in pharmaceuticals and cosmetic formulations. Furthermore, they were found to be biologically active against a variety of disease-causing bacteria, tumors, and viruses (Husmann, 2008). In 1988 availability of amino acid surfactants at low cost increased the interest of researchers to study their surface activity (Takehara, 1989). Nowadays, along with the growth of biotechnology, a few amino acids are also able to be synthesized commercially at large scale by yeasts, thus justifying their production to be more environmental friendly (Diniz Rufino *et al.*, 2014).

2. History

The value of the structures of naturally occurring amino acids as raw materials for preparing amphiphiles was predicted as soon as they were discovered early in the 19th century. The first research on amino acid surfactant synthesis was reported in 1909 by Bondi (Bondi, 1909). In this research N-acylglycine and N-acylalamine as hydrophillic moieties of surfactants have been introduced. Subsequent work involved the synthesis of lipo-amino acid using glycine and alanine (Gallot & Hassan,1989). Hentrich and co-workers published a series of findings and filed the first patent on the applications of acylsarcosinate and acylaspartate as surfactants in household cleaning products such as shampoos,

detergents and toothpastes (Hentrich *et al*., 1936). Subsequently the synthesis and physicochemical properties of acyl amino acids have been studied by many researchers (Heitmanr, 1968; Presenz, 1996). To date a significant number of research publications have been published on the synthesis (Heitmanr, 1968; Perez *et al.*, 2009; Lundberg *et al.*, 2011; Seguer *et al.*, 1994a; Seguer *et al.*, 1994b; Piera *et al.*, 1998; Clapes & Infante, 2002), properties (Franklin & Snow 1981; Solans *et al.*, 1989; Kunieda *et al.*, 1992; Fördedal *et al.*, 1993; Wang *et al.*, 2001; Dana *et al.*, 2011), industrial applications (Infante *et al.*, 1984; Infante *et al.*, 1992; Infante *et al.*, 1994) and biodegradability (Piera *et al.*, 1998; Clapes *et al.*, 1999; Burczyk, 2003; Trivedi *et al.*, 2011; Pinazo, 2011) of amino acid surfactants.

3. Structural properties

Non-polar hydrophobic fatty acid chains of amino acid surfactants may vary in their structure, length and number. Structural variety and high surface activities of amino acid surfactants explain their wide compositional diversity and broad range of physicochemical and biological properties. The difference in the amino acid or peptide head group of amino acid surfactants determines the adsorption, aggregation and biological activity of these surfactants. Their types in terms of cationic, anionic, non-ionic and amphoteric, depends on the functional groups present in them. The combination of hydrophilic polar amino acids and non-polar long chain hydrophobic moiety for building up the amphiphilic structure has created molecules with high surface activity (Infante *et al.*, 1997; Rentsch, 2002; Abdullah *et al.*, 2009). In addition asymmetric carbons present in the molecule help in the formation of chiral molecules (Solans *et al.*, 1990; Walther & Netscher, 1996).

4. Chemistry

All peptides and polypeptides are the polymerization product of 20 or so proteinogenic α-amino acids. All 20 α-amino acids are comprised of a carboxylic acid (–COOH) functional group and an amino ($–NH_2$) functional group attached to the same tetrahedral α-carbon atom (Morán *et al.*, 2004). Amino acids differ from each other on the basis of distinct R-groups attached to the α-carbon (except in the case of glycine where the R-group is hydrogen). R groups may differ from each other in structure, size and electric charge (acidity/basicity). These differences also determine the solubility of amino acids in water.

Presence of 4 different substituent attached to α-carbon makes amino acids chiral in nature (except glycine) and hence optically active. Amino acids have two possible configurations; both are non-superimposable mirror images of each other, though in nature the L-stereoisomers are significantly more abundant. Presence of aromatic R-groups in some amino acids (phenylalanine, tyrosine and tryptophan) results in the absorption of ultraviolet light with an absorbance maximum in the range of 280 nm (Solans *et al.*, 1990). Both, the acidic α-COOH and basic α-NH_2 groups present in amino acids are able to ionize and create ionic equilibrium as follows:

$R\text{-}COOH <\!\!\longrightarrow R\text{-}COO^- + H^+$

$R\text{-}NH_3^+ <\!\!\longrightarrow R\text{-}NH_2 + H^+$

Amino acids contain at least two weakly acidic groups; however, the carboxyl group is a far stronger acid than the amino group. At pH 7.4, the carboxyl group is unprotonated and the amino group protonated. An amino acid with no ionizable R-group would be electrically neutral at this pH and form zwitterions (Tamarkin *et al.*, 2014).

5. Classification

Four parameters are taken into consideration to classify amino acid surfactants, these are sequentially addressed below:

5.1 On the basis of origin

On the basis of their origin amino acid surfactants can be categorize into two classes:

5.1.1 Natural (Pathak & Keharia, 2014; Walia & Cameotra, 2015)

Some naturally occurring amino acid containing compounds also possesses the ability to reduce surface and interfacial tension, some even surpassing the efficacy of glycolipids. These types of amino acid surfactants are also known as lipopeptides. Lipopeptides are low molecular weight compounds, which are generally produced by *Bacillus* species. This class of amino acid surfactants are further classified into three subclasses: surfactin, iturin and fengycin (Fig. 2).

The surfactin (Hathout *et al.*, 2000; Arima *et al.*, 1968) family covers the heptapeptide variants of the esperin, pumilacidin, lichenysin and surfactin groups. In such types of surfactants, the peptide moiety is linked with a C_{12}-C_{16}unsaturated linear, iso or anteiso β-hydroxyl fatty acid chain. Surfactins are the macrolactone rings, in which ring closure is catalysed in between the β-hydroxyl fatty acid and the C-terminal peptide.

In the iturin (Peypoux *et al.*, 1999) subclass, there are mainly six variants i.e. iturin A and C, mycosubtilin and bacillomycin D, F and L. In all the cases, the heptapeptide is attached with a varied C_{14}-C_{17} chain of β-amino fatty acids. In Iturins, because of its β-amino nature, an amide bond is formed with the C-terminal group and thus forms amacrolactame structure.

The Fengycins (Winkelmann *et al.*, 1983; Ongena *et al.*, 2007; Kim *et al.*, 2010) subclass encompasses fengycins A and B, which are also known as plipastatins when Tyr9

is D-configured. These decapeptides are also linked with a C_{14}-C_{18}, saturated or unsaturated linear, iso or anteiso-β-hydroxyl fatty acid chain. Structurally, fengycins are also a macrolactone ring that includes a Tyr side chain at third position in the peptide sequence and form an ester bond with the C-terminal residue, thus form an internal ring like structure in many ***Pseudomonas*** lipopeptides.

5.1.2 Synthetic (Xia, 2001)

Amino acid surfactants can also be synthesized experimentally by use of any of the acidic, basic and neutral amino acids. Common amino acids used for their synthesis are glutamic acid, serine, proline, aspartic acid, glucine, arginine, alanine, leucine and protein hydrosylates. Surfactants of this subclass can be prepared chemically, enzymatically and chemoenzymatically however, chemical synthesis has been found more economically feasible for their production. Common examples of this include N-lauroyl-L-glutamic acid, N-palmitoyl-L-glutamic acid.

5.2. On the basis of the substitution of aliphatic chain

On the basis of the substitution of aliphatic chain amino acid based surfactants can be classified into two types (Clapes & Infante, 2002):

5.2.1. On the basis of substitution site

In this type, there are three different categories:

5.2.1.1. N-substituted amino acid surfactants

In N-substituted compounds an amino group is substituted either with a lipophilic moiety or a carboxylic group, resulting in the loss of alkalinity. The simplest examples of N-substituted amino acid surfactants are N-acyl amino acids, which are anionic in nature. N-substituted amino acid surfactants have an amide linkage formed in between the

hydrophobic moiety and the hydrophilic moiety. The ability of amide linkage to form hydrogen bond eases the degradation of such surfactants under acidic environment and hence makes them biodegradable.

5.2.1.2. C-substituted amino acid surfactants

In C-substituted compounds substitution takes place on the carboxylic group through an amide or an ester bond. C-substituted compounds are typically cationic in nature such as esters or amides.

5.2.1.3. N- and C- substituted amino acid surfactants

In second type of the surfactants, both the amino and the carboxylic groups are present as part of the hydrophilic moiety. These types are amphoteric in nature.

5.3 On the basis of number of hydrophobic tail

On the basis of number of hydrophobic tail and head groups, amino acid surfactants are classified into four groups (Moran *et al.*, 2004). Single or linear chain amino acid surfactants, dimeric or gemini amino acid surfactants, glycerolipids type amino acid surfactants and bolaamphiphiles amino acid surfactants. Linear chain surfactants are the surfactants, comprised of amino acids with the minimum one hydrophobic tail (Fig. 3). Dimeric amino acid surfactants have two amino acids polar head group and two hydrophobic tails per molecule (Fig. 4). In this class, two linear amino acids surfactants are joined together via spacer and hence also called dimerics. Glycerolipids type amino acid surfactants contain two hydrophobic tails attached to a one common amino acid head group (Fig.5). These surfactants can be considered as an analogue of monoglycerides, diglycerides and phospholipids. Bolaamphiphiles amino acid surfactants are a group of surfactants that has two polar amino acid heads that are joined together via single hydrophobic tails (Fig. 6).

5.4. On the basis of head group type

On the basis of polar head group amino acid based surfactants are further classified into cationic, anionic, zwitterionic and non-ionic amino acid surfactants.

5.4.1 Cationic

In this class of surfactants, the head group bears positive charge. The first commercially available cationic amino acid surfactant is cocoyl arginine ethyl ester (CAE) as a PCA (pyrollidone carboxylic acid) salt. This surfactant has unique and varied properties that make it useful in disinfectants, antimicrobial agents, antistactic agents, hair conditioner and it is also very mild to eyes and skin and easily biodegradable (Xia., 2001). Singare & Mhatre, 2012 have synthesized arginine based cationic amino acid surfactants and evaluated them on the basis of their physicochemical properties. In this study, they claimed the good yield of product using Schotten Baumann reaction conditions. Increase in the surface activity and decrease in the critical micelle concentration (CMC) of surfactants was found with the increase in alkyl chain length and the hydrophobicity. Quaternary acyl protein is another known example of this class which is generally used as conditioner in hair care products (Kim *et al.*, 2010).

5.4.2. Anionic

In anionic surfactants, negative charge is present on the polar head group of surfactants. Sarcosinate surfactants are an example of anionic amino acid surfactants. Sarcosine (CH_3-NH-CH_2-COOH, *N*-methyl glycine) is an amino acid normally found in sea urchins and starfish and is chemically related to glycine (NH_2-CH_2-COOH), a basic amino acid found in the cells of mammals. Lauric acid, myristic acid and oleic acid and their halides and esters are generally used to synthesize sarcosinate surfactants. Sarcosinate are

mild in nature, due to which they are generally used in mouthwashes, shampoos, aerosol shaving lathers, sunscreens, skin cleansers and other cosmetics (Castillo *et al.*, 2001).

Other commercial anionic amino acid surfactants include Amisoft CS-22 and Amilite GCK-12, which are the trade names of Sodium N-Cocoyl-L-Glutamate and Potassium N-Cocoylglycinate, respectively. Amilite is generally used as foaming agent, detergent, solubilizer, emulsifier and as a dispersing agent and has numerous applications in cosmetics as shampoo and bath soap, body wash, toothpaste, face wash, facial soap, contact lens cleaners and household tensides (Aubert & Dussault, 2008). Amisoft is used as a mild skin and hair cleansers and predominantly used in face and body cleansers, synthetic detergent bars, body care hair shampoos and other skin care products (Castillo *et al.*, 2000).

5.4.3. Zwitterionic (Amphoteric)

Amphoteric surfactants contain both acidic and basic sites and thus change charge by varying pH. They behave like anionics in alkaline medium, behave as cationics in acidic environment and amphoterics in neutral medium. Lauroyl lysine (LL) and Alkoxy (2-hydroxyprpyl) arginine are the only known amino acid based amphoteric surfactants. LL is a condensation product of lysine and lauric acid. Due to its amphoteric structure, LL is insoluble in almost all type of solvents except highly alkaline or acidic solvents. Excellent adhesive property towards hydrophilic surfaces and low friction coefficient of LL as organic powder imparts excellent lubricating ability in this surfactant. LL is widely used in skin creams and hair conditioners or as a lubricant (Mohr *et al.*, 2000).

5.4.4. Nonionic

Nonionic surfactants are characterized with polar head groups that carry no formal charge. Sabagh and his co-workers prepared eight novel ethoxylated nonionic surfactants

using oil soluble α-amino acids. In this process, esterification of L-phenylalanine and L-leucine followed by amidation with cetyl alcohol and palamitic acid, respectively yielded two amides and two esters of α-amino acids. The ethylene oxide was then condensed with the amides and esters prepared and produced three different polyethylene oxide units of 40, 60, and 100 as phenylalanine derivatives. These nonionic type amino acid surfactants were found to have good detergency and foaming properties (Sabagh *et al.*, 2009).

6. Synthesis

6.1 Fundamental synthesis paths

In amino acid surfactants, the hydrophobic group may be attached either at the amine moiety, the carboxylic acid moiety, or through the side chain of the amino acid. On the basis of this approach, four fundamental synthesis paths are available to researchers (Kimura *et al.*, 1992) as given in figure 7.

Path 1 shows the production of amphiphilic esteramine through esterification reactions, in which surfactants synthesis is typically achieved by refluxing fatty alcohols and amino acid in the presence of dehydrating agent and acidic catalyst. In some reactions sulphuric acid plays the role of both catalyst and dehydrating agent.

Path 2 shows the synthesis of amphiphillic amidoamine by creating the amide bond through the reaction of an activated amino acid and alkyl amine to create the desired amide bond.

Path 3 shows the production of amidoacid through the reaction of amine group of amino acid with fatty acid.

Path 4 shows the synthesis of long chain alkyl amino acids via the reaction of amine group with alkyl halogen.

Path 5 involves the coupling of the specific function of the side group of the amino acid. In this type of path, generally carboxylic group of aspartic acid and glutamic acid react with fatty alcohol to form anhydride (Fig.8).

6.2. Development in the synthesis/production

6.2.1. Synthesis of single-chain amino acid/peptide surfactants

Synthesis of N-Acyl and O-acyl amino acids (Kimura *et al.*, 1992) or peptides can be achieved by the enzyme-catalysed acylation reaction of amine or alcohol groups with fatty acids. The first description of solvent-free lipase catalyzed synthesis of amino acid amide or methyl ester derivatives was reported by using *Candida antarctica* with the yield in the range of 25-90% depending on the target amino acid (Godtfredsen and Bjoerkling, 1990).In some reactions, ethylmethyl ketone has also been used as solvent. Vonderhagen *et al.*, 1999 also illustrated the lipase and proteases catalyzed N-acylation of amino acids, protein hydrolysates and/or their derivatives using the mixture of water with organic solvents such as dimethylformamide/water and butylmethylketone. In these earlier examples, the major issue with enzymatic synthesis of amino acid surfactants was typically very low yields. Valivety *et al.*, 1997 reported yields of just 2– 10% of N-myristoyl amino acid derivatives even after many days under incubation at 70^{o}C by using different lipases. Problems associated with the low yields of amino acid were also supported by Montet and his co-workers (Montet *et al.*, 1990) during the synthesis of N-acyllysines using fatty acids and vegetable oils. They reported a maximum 19% yield of the product under both the solvent-free conditions as well as under organic solvents. Valivety *et al.*, 1998 also supported the same issue while synthesizing N-Cbz-L-Lysine or N-Cbz- Lysine methyl ester derivatives. In this study, they claimed 80 % yield of 3-O-myristoyl-L-serineusing N-protected serine as

substrate and Novozyme 435 as catalyst under melted solvent free environment. Nagao and Kito studied the results of O-acylation of L-serine, L-homoserine, L-threonine and L-tyrosine using lipases obtained by *Candida cylindracea* and *Rhizopusdelemar* in aqueous buffered medium and reported the acylation of L-homoserine and L-serine upto some extent with low yield whereas no acylation takes place on L-threonine and L-tyrosine (Nagao & Kito, 1989). Various researchers supported the use of cheap and easily available substrate to synthesize cost effective amino acid surfactants. Soo'sresearch group claimed the best results of palm oil based surfactants using immobilized lipoenzyme.In this investigation they stated that although reactions with palm oil fractions required long reaction duration of 6 days but resulted better yields of products (Soo *et al.*, 2003). Gerova and his co-workers studied the synthesis and surface active properties of several optically active and racemic mixtures chiral of *N*-palmitoyl amino acid surfactants based on methionine, proline, leucine, threonine, phenylalanine and phenylglycine (Gerova *et al.*, 2008). Pang & Chu, 2010 described theco-polycondensation of amino acid based monomers and dicarboxylic acid based monomers to synthesize a series of biodegradable functional amino acid-based polyesteramides in solution.

Cantacuzene and Guerreiro reported the esterification of carboxylic acid group of Boc-Ala-OH and Boc-Asp-OH using long chain aliphatic alcohols and diols using dichloromethane and Sepharose 4B as solvent and catalyst, respectively. In this research, good yields (51%)of Boc-Ala-OH was obtained with fatty alcohols up to 16 carbon atoms whereas 6 and 12 carbon atoms were found better and gave 63% of Boc-Asp-OH(Cantacuzene & Guerreiro, 1989).Clapes *et al.* claimed 58–76% yield and 99.9% purity of N-arginine alkyl amide derivatives obtained through the formation of amide and ester

bonds between Cbz-Arg-OMe and various long chain alkyl amines and fatty alcohols in the presence of Papain from Carica papaya latex as catalyst (Clapes *et al.*, 1999).

6.2.2. Synthesis of gemini amino acid/peptide surfactants

Amino acid based gemini surfactants are comprised of two linear amino acid surfactants molecules joined together head to head via a spacer (Yoshimura *et al.*, 2012). Two possible Schemes have been derived for the chemo-enzymatic synthesis of gemini types amino acid based surfactants (Schemes1, 2). In the first Scheme, two amino acid derivatives react with the spacer followed by the introduction of the two hydrophobic groups. In the second Scheme, the two linear structures are joined directly through bifunctional spacer chain (Piera *et al.*, 2000). Valivety and his co-workers (Valivety *et al.*, 1997) were the first to develop the enzymatic synthesis of gemini lipoamino acids. Yoshimura and his co-worker (Yoshimura *et al.*, 2012) studied the synthesis, adsorption, and aggregation properties of an amino acid-based gemini surfactant based on cystine and n-alkyl bromide. Comparisons of the synthesized surfactants have also been performed against their corresponding monomeric counterparts. Faustino *et al.,* 2009 described the synthesis of anionic urea-based monomeric amino acid surfactants and their corresponding geminis based on L-cystine, D-cystine and DL cystine, as well as derived from L-cysteine, L-methionine and L-cysteic acid and their characterization was achieved in terms of electrical conductivity, equilibrium surface tension, and steady-state fluorescence spectroscopy techniques. Comparison studies between monomeric forms and geminis stated that geminis have lower critical micelle concentration (CMC) values.

6.2.3. Synthesis of glycerolipidamino acid/peptide surfactants

Glycerolipidamino acid/peptide surfactants comprises of a novel class of lipoamino acids, which are the structural analogues of mono-diacylglycerides and phospholipids as one or two aliphatic chains and one amino acid joined together via ester bonds in the glycerol backbone. Synthesis of such types of surfactants starts with the preparation of glyceryl esters of amino acid in the presence of an acid catalyst such as BF_3 at elevated temperatures (Valivety *et al.*, 1997). Enzymatic synthesis using hydrolases, proteases and lipases as catalyst was also found to be good alternative (Scheme 3) (Mitin *et al*., 1997). Enzymatic synthesis of dilauroylated arginine glyceride conjugate with papain enzyme has also been reported. Synthesis of diacylglycerolipid conjugates from acetyl-arginine and evaluation of their physicochemical properties have also been reported (Moran *et al.*, 2001; Perez *et al.,* 2004).

6.2.4. Synthesis of bolaamphiphiles amino acid/peptide surfactants

Amino acid-based bolaamphiphiles contain two amino acids connected with a hydrophobic linker. Franceschi *et a.*., 1999 described the synthesis of bolaamphiphiles with two amino acids (D- or L-alanine or L-histidine) and an alkyl chain of varying length and studied their surface active properties. In this research work, they discussed the synthesis and aggregation of novel bolaamphiphiles bearing amino acid moieties using unusual ***β***-amino acids or an alcohol and spacers with C_{12}-C_{20}. The unusual β-amino acids used may be sugar amino acid, an AZT-derived amino acid, a norbornene amino acid, and an AZT-derived amino alcohol (Scheme 4). Polidori *et al*., 2006 has also given synthesis of symmetric bolaamphiphiles derived from tris (hydroxymethyl) aminomethane (Tris) (Scheme 4).

7. Physiochemical Properties

Amino acid based surfactants are known to have wide range of desirable properties that increase their applicability in varied applications such as good solubilization, good emulsifying properties, high efficiency, high surface activity and good lime resistance (calcium tolerance).

A variety of surface active properties of amino acids based surfactants like surface tension, CMC, phase behaviour and Krafft temperature have been studied by many scientists and it was concluded that amino acid surfactants have superior surface activity than their conventional counterparts. Properties of amino acid surfactants are discussed below:

7.1. Critical micelle concentration (CMC)

Critical micelle concentration (CMC) is an important phenomenon of surfactants as it governs a number of surface active properties such as solublization, lytic action and their interaction with biological membranes etc. Commonly, increasing in the chain length of hydrocarbon tail (increasing hydrophobicity), resulted in decreased CMC values of surfactant solution and hence increased their surface activity (Mukerjee & Mysels, 1971).When compared to conventional surfactants, amino acid based surfactants generally have lower CMC values (Perez *et al*., 2004).

Infante *et al*., 1984 synthesized three arginine based amino acid surfactants by varying the combination of head group and hydrophobic tail (mono cationic amide, dicationic amide and di cationic amido-ester) and studied their CMC and gamma CMC values (surface tension at the CMC and the results showed a decrease in CMC and Gamma CMC values by increasing the length of hydrophobic tail. In a different study Mhatre & Singare, 2012 also

confirmed the CMC decreases with an increase in the number of carbon atoms in hydrophobic tail of Nα-acyl arginine surfactants [Table1].

Yoshimura *et.al.,* 2012 investigated the CMC values of amino acid based gemini surfactants derived from cysteine and showed the decreasing pattern in CMC values by increasing the carbon length from 10 to 12 within the hydrophobic chain. Further increases in the carbon chain length upto 14 resulted in an increase in CMC values, confirming the low aggregation tendency of gemini surfactants.

Faustino *et al*., 2011 reported the formation of mixed micelles in aqueous solutions of cystine based anionic gemini surfactants. The comparison has also been made with the gemini surfactant with its conventional monomeric counterpart, C_8Cys. CMC values for lipid-surfactant mixtures were reported to be lower than that of pure surfactant. The gemini surfactant and 1,2-diheptanoyl-sn-glycero-3-phosphocholine, a water-soluble, micelle-forming phospholipid was shown to have CMC values in the milimolar range.

Shrestha & Aramaki, 2009 studied the visco-elastic wormlike micelles formation in the aqueous systems of mixed type amino acid-based anionic and non-ionic surfactants in the absence of salt. In this investigation, N-dodecylglutamic acid was found to have higher Krafft temperature; however, when neutralized with alkaline amino acid L-lysine, it generated the micelles and the solution started behaving like a Newtonian fluid at 25^{o}C.

7.2. Good water solubility

Good water solubility of amino acid surfactants is due to presence of additional CO-NH linkage (Moran *et al*., 2004).This ability makes them readily biodegradable and environmentally friendly in comparison to their conventional counterparts. The presence of two carboxylic groups leads to even better solubility of N-acyl-L-glutamic acid in

water.Presence of two ionic arginine groups in one molecule of C*n*$(CA)_2$ also exhibits good water solubility thus resulting in more effective adsorption and diffusion on the cell interface and showing effective antimicrobial action even at lower concentrations.

7.3 Krafft temperature and Krafft point

Krafft temperature can be understood as the unusual solubility behaviour of surfactants and rapid increase in their solubilities above specific temperature (Pilemand, 2002). Ionic surfactants have the affinity to produce solid hydrates through precipitation from the aqueous medium. The solubility of the surfactants will commonly be observed to undergo a sharp and discontinuous increase at some distinctive temperature, which is referred to as the Krafft temperature. Krafft point of ionic surfactants is the Krafft temperature at their critical micelle concentration (Malik & Ali, 2016).

This solubility characteristic is generally observed in ionic type surfactants and can be explained on the basis of limited solubility of non-associated surfactants below Krafft temperature and the solubility increasing gradually until the Krafft point is reached, which was due to micelle formation. To achieve complete solubility it is necessary to prepare surfactant formulations above their Krafft point (Puerto, 2001).

Krafft temperature of amino acids surfactants have been studied by various researchers and comparison were made with the Krafft temperature of conventional synthetic surfactants. Shrestha et. al., 2009 studied the Krafft temperature of arginine based amino acid surfactants which were found to exhibit premicellar aggregation CMC values above 2-5x10^{-6} M followed by the normal micellization at 3-6x10^{-4}. Ohta *et al.*, 2003 synthesized six different types ofN-hexadecanoyl amino acid surfactants, and discussed their relationship between the Krafft temperature and the amino acid residue.

In an experiment, an increase in the Krafft temperature of N-hexadecanoyl amino acid surfactant was found with decrease in the size of the amino acid residue (phenylalanine is an exceptional case). Whereas, an increase in the enthalpy of solution was found endothermic with decrease in the size of the amino acid residue (except for glycine and phenylalanine). From these observations it was concluded that D-L interaction was superior to the L-L interaction in solid state of N-hexadecanoyl amino acid surfactant salt in both the alanine system and the phenylalanine system.

Brito *et al*., 2011 also determined the Krafft temperatures of three series of novel amino acid based surfactants using differential scanning micro calorimetry and found the change from trifluoroacetate ion to iodide which causes a relative large increase (≈6°C) in Krafft temperature from 47 to 53°C. Presence of a cis-double bond and unsaturation present in long chain Ser-derivative significantly decreases the Krafft temperature. N-Dodecylglutamic acid was reported to have higher Krafft temperature. However, neutralization with alkaline amino acid L-lysine resulted in micelles formation in the solution which behaves like a Newtonian fluid at 25°C.

7.4. Surface tension

Surface tension of surfactants increased with increasing chain length of the hydrophobic moiety. Zhang *et al*., 2013 determined the surface tension values of sodium cocoylglycinate by Wilhelmy plate method using a DCAT11 tensiometer at 25°C ± 0.2°C and obtained surface tension values of 33 $mN \cdot m^{-1}$ at the CMC of 0.21 $mmol \cdot L^{-1}$.Yoshimura et.al., 2007 determined the surface tension of some 2C*n*Cys type amino acid based surfactants. The surface tension at CMC were found to lower when increasing the chain

length up to n=8, whereas the trend reversed for surfactants with n=12 or longer chain lengths.

The effect of $CaCl_2$ on surface tension of dicarboxylic amino acids type surfactants has also been studied (Bordes *et al*., 2009). In this investigation addition of $CaCl_2$ in aqueous solutions of three dicarboxylic amino acid-based surfactants ($C_{12}MalNa_2$, $C_{12}AspNa_2$ and $C_{12}GluNa_2$) was carried out. Surface tensions were found to be lower at very low $CaCl_2$ compared compared to that at the plateau value beyond the CMC. This was due to the effect of calcium ions on the packing of the anionic surfactants at the air-water interface. Surface tension of the salts of N-dodecyl aminomalonic acid and N-dodecyl aspartic acid remained almost constant up to a 10 mM concentration of $CaCl_2$. Above 10 mM a rapid increase in surface tension was observed, which was due to the formation of calcium salt precipitate of the surfactant. In case of disodium salt of N-dodecylglutamic acid, a considerable decrease in surface tension was observed by moderate addition of $CaCl_2$ that remained almost constant over a broad range of $CaCl_2$ concentrations.

For the determination of adsorption kinetics at air/water interface for gemini amino acid surfactants, dynamic surface tension measurements were carried out via the maximum bubble pressure method. Results revealed no change in the dynamic surface tension for $2C_{12}$Cysfor the longest measurement time. Reduction in the dynamic surface tension only depends on the length of hydrocarbon tail, number of hydrocarbon tails and the concentration. Faster decay was observed by increasing the concentration and decreasing the chain length and chain numbers of the surfactants. Results obtained at higher concentrations of C_nCys (n=8-12) were found very close to the γ_{cmc} obtained through Wilhelmy surface tension.

In other research (Fan *et al*., 2008) the dynamic surface tension of SDLC (sodium dilauroylcystine) and SDDC (sodium didecaminocystine) was determined using Khan DCA-315 tensiometer through Wilhelmy plate technique and the equilibrium surface tension of their aqueous solution was measured by drop volume method. Further studies on the reaction of the disulfide bond were also performed by other methods. Addition of mercaptoethanol to 0.1 mmol L^{-1} SDLC solution resulted in the rapid increase in surface tension from 34 to 53 mNm^{-1}. As NaClO can oxidize the disulfide groups of SDLC to sulfonic groups, when NaClO (5 mmol L^{-1}) was added into 0.1 mM L^{-1}SDLC solution. Transmission Electron Microscopy (TEM) and Differential Light Scattering (DLS) results showed no aggregate formation in the solution. The surface tension of SDLC was found to increase from 34 to 60 mNm^{-1}over the duration of 20 min.

7.5 Binary surface interaction

The vibrant properties of the mixture of cationic type amino acid surfactants (diacyl-glycerol-arginine based surfactant) with phospholipids at the air-water interface for life sciences have been reported by many research groups and it was finally concluded this non-ideal characteristic as an asset to the pervasiveness of electrostatic interactions (Hines *et al*., 1997; Wu *et al*., 2017).

7.6. Aggregation properties

DLS measurements are commonly used to determine the aggregation properties of amino acid based monomeric and gemini surfactants, at concentrations higher than the CMC and the diffusion coefficient so obtained is converted into the apparent hydrodynamic diameter D_H (=$2R_H$).When compared to the other surfactants,C*n*Cys and 2C*n*Cys formed relatively large aggregates with a wide size distribution. Surfactants other than $2C_{12}$Cys

generally form aggregates of about 10 nm. The micelle size for gemini surfactants was found significantly larger than their monomeric counterparts (Faustino *et al.*, 2009). Increase in the hydrocarbon chain length also results in the increase micellular size (Brito *et al.*, 2009). Ohta *et al.*, 2007 describedthe aggregation properties of aqueous solutions of three different stereoisomers of tetramethyl ammonium N- dodecyl phenyl alanyl phenyl alaninate and revealed that the diastereomers have same critical aggregation concentrations in aqueous solutions. Iwahashi *et al*., 2009 investigated the formation of chiral aggregates of optically active N-lauroyl-L-glutamic acid (L-LGA), N-lauroyl-L-valine (L-LVA) and its methyl ester in different solvents like tetrahydrofuran, acetonitrile,1,4-dioxane and 1,2-dichloroethane through measurement of circular dichroism (CD), NMR chemical shift of N-H proton and vapour pressure osmometry.

7.7. Interfacial adsorption

Amino acids based surfactants have also been characterized in terms of their interfacial adsorption and compared to their conventional counterparts. Dodecyl ester of aromatic amino acids obtained from l-tyrosine (LET) and l-phenylalanine (LEP) were subjected to assess their interfacial adsorption characteristics (Vijay *et al.*, 2008). Results revealed that LET and LEP exhibit lower interfacial area at the air/solution and in water/hexane interface, respectively.

Bordes *et al*., 2009 investigated the solution behaviour and adsorption of three dicarboxylic amino acid-based surfactants, the disodium salts of dodecylglutamic acid, dodecylaspartic acid and dodecylaminomalonic acid with three, two and one carbon atoms, respectively between the carboxyl groups at the air–water interface. It was reported that CMC of dicarboxylic surfactants was 4-5 times higher than mono carboxyl group containing

dodecylglycinate. This was attributed to the presence of hydrogen bonding between adjacent molecules through the amide groups in the dicarboxylic surfactants.

7.8. Phase behaviour

Phase behaviour of the surfactants can be determined using sealed ampoules containing requisite quantities of reagents which were mixed at higher temperatures and get homogenized with a vortex mixer through repeated centrifugation. After mixing properly, the samples are kept in a thermostatically controlled water bath at 25^{o}C for a few days to ensure equilibrium. The equilibrated phases were acknowledged by visual observations using normal and crossed polarizer. At high concentration of surfactant, the isotropic discontinuous cubic phase was observed. A large hydrophilic head group of the surfactant molecule favoured the small and discrete aggregates of positive curvature (Johnsson & Edwards, 2001). Marques *et al*., 2008 studied the phase behaviour of 12 Lys12/12 and 8 Lys8/16 Sersystems and stated that the 12 Lys12/12 Sersystem has phase separation between the micellar and vesicle solution. Small micelles were found to coexist in between the vesicles in the former whereas 8 Lys8/16 Sersystems showed a continuous transition process (where the elongated micelles were found to be present in between the small micelles and vesicles).

7.9. Emulsification

Kouchi *et al.*, 2001 examined the emulsification potency of N-[3-lauryl-2-hydroxypropyl]-L-arginine, L-glutamate, along with other amino acid surfactants, in terms of interfacial tension, dispersability and viscosity. Comparison of synthesized surfactants has also been made with their conventional non-ionic and amphoteric counterparts and

results revealed that the amino acid surfactants have greater emulsification potency than conventional surfactants.

Baczko *et al.*, 2004 synthesized novel amino acid-based anionic surfactants and investigated their usability as chiral oriented NMR spectroscopy solvents. A series of sulfonated amphiphilic L-Phe or L-Ala derivatives with pentyl to tetradecyl tails were synthesized by reacting the corresponding amino acid esters with o-sulfobenzoic anhydride. Wu *et al*., 2014 synthesized SFAAA (sodium N-fatty acyl amino acid) surfactant using pupa oil and obtained PPH (pupa protein hydrolysates) as a waste by-product of the silk industry. Studies on the emulsifying power of PPH and SFAAA in an oil/water emulsion revealed that these surfactants were found superior with ethyl acetate as the oil phase over n-hexane.

7.10. Lime tolerance

Lime tolerance can be understood as the resistance of surfactant to precipitate as lime soap in the presence of calcium, magnesium like ions present in hard water. Surfactants with high tolerance against water hardness were found useful for detergent formulations and personal care products. Determination of the lime tolerance can be achieved by calculating the solubility and the surface activity change of surfactants in the presence of calcium ions (Bordes *et al*., 2009). Another method used to determine lime tolerance involve the calculation of percentage or the number of grams of surfactant required for the dispersion of lime soap formed from 100 g sodium oleate in water (Holmberg *et al.*, 2004). In hard water areas, high concentrations of calcium and magnesium ions and mineral content can lead to difficulties in some practical applications. Sodium ions are generally used as the counter ions in the synthesis of anionic surfactants. By virtue of being bivalent calcium ions bind to two surfactant molecules resulting in the easier surfactant precipitation from solution and

thus reducing detergency. Studies on lime tolerance of amino acid surfactants showed that 'Acid and lime tolerance' is vividly augmented by additional carboxyl group and they further increase with increase in the length of spacer group between two carboxyl groups increase. The order of acid and lime tolerance found was C_{12}Glycinate<C_{12}Aspartate< C_{12}Glutamate. Comparison of dicarboxylamido linkage surfactants with dicarboxyl amino linkage surfactants revealed that dicarboxyl amino linkage surfactants have wider pH range in which surface activity increases with moderate addition of acid. Dicarboxyl N-alkyl amino acids have a chelating effect in the presence of calcium ions and C_{12}Aspartate forms white gel. C_{12}Glutamate shows high surface activity at high concentration of Ca^{++} and has the potential to get used in seawater desalination (Li, 2012).

7.11. Dispersability

Dispersability is the ability of surfactants to inhibit the agglomeration and settling down of surfactants in solution. Dispersability is an important property of surfactants that make them suitable for detergents, cosmetics and pharmaceuticals (Satyanarayana, 2012). Dispersing agents must contain an ester, ether, amido, or amino linkage between the hydrophobic group and the terminal hydrophilic group, as well as a straight-chain hydrophobic group (Rosen & Kunjappu, 2012). Generally the anionic surfactants like sulphated alkanolamide and zwitterionic surfactants such as amidosulfobetaine are found to be very effective as lime soap dispersing agents (Linfield, 1978; Weil *et al.*, 1970; Parris *et al*., 1973; Parris *et al*., 1976).

Various studies have been made to determine the dispersability of amino acid surfactants, in which N-lauroyllysine was found to have poor compatibility with water and was found difficult to make cosmetic formulations. In this series, N-acyl basic amino acid

was found to have superior dispersability and is being utilized in cosmetic industries to improve formulations (Sagawa *et al.*, 2015).

8. Toxicity

Conventional surfactants, especially cationic surfactants are intensely toxic against aquatic life which includes algae, fish and molluscs. Their acute toxicity is because of their tendency to disrupt the integral membrane which is due to the adsorption-ionic interaction phenomenon of surfactant at the cell water interface. Lowering the CMC of surfactants typically results in greater adsorption of surfactants onto interfaces, typically raising their acute toxicity. Increasing the length of hydrophobic chain of the surfactant molecule also results in the increase of acute toxicity of surfactant. Low or non-toxicity of the majority of amino acid surfactants to human and the environment, specifically to marine organisms, make them suitable as food ingredients, medicines and cosmetics (Ito & Inoue, 1982; Berger & Gacon, 1992; Davila *et al.*, 1997; George *et al*., 1998; Myers, 2005; Obata *et al*., 2008; Sagawa *et al.*, 2015). Various researchers have verified amino acid-based surfactants as mild and non-irritating to the skin (George *et al*., 1998). Arginine based surfactants are known to be less toxic than their conventional counterparts. Brito *et al*., 2009 studied the physicochemical and toxicological properties of amino acid-based amphiphiles and their spontaneously formed cationic vesicles derived using tyrosine (Tyr), hydroxyproline (Hyp), serine (Ser) and lysine (Lys and presented data for their acute toxicity to ***Daphnia magna*** (IC_{50}). In this research work they synthesized cationic vesicles of dodecyltrimethylammonium bromide (DTAB)/Lys-derivative and or Ser-/Lys-derivative mixtures and tested their ecotoxicity and hemolytic potential and stated that all amino acid surfactants along with their vesicle-containing mixtures showed lower ecotoxicity than the

conventionally used surfactant DTAB. Rosa *et al*., 2007 studied the association of DNA and stable cationic amino acid-based vesicles. As compared to conventionally used cationic surfactants that are generally toxic, the interaction of cationic amino acid-based surfactants appeared to be nontoxic. This cationic amino acid surfactant based on arginine, ALA, gives spontaneously stable vesicles with some with anionic surfactants. Corrosion inhibitors based on amino acids were also reported to be non-toxic by various researchers (Morad, 2008; de Souza & Spinelli, 2009; Umoren *et al*., 2009; Singh & Quraishi, 2010; Abiola & James, 2010; Barouni *et al*., 2014).These surfactants were also found to be easy to synthesize with high purities (up to 99%), low in cost, readily biodegradable and were completely soluble in aqueous media. In various studies, sulfur containing amino acids based surfactants were found to be more competent corrosion inhibitors (El-Naby, 1985; Rahim *et al.*, 1997; Abiola, 2005; Morad, 2008; Özcan *et al.*, 2008; Chandra & Tyagi, 2013).

Perinelli *et al*., 2017 in their latest investigation reported the favorable toxicological profile of Rhamnolipids over conventional synthetic surfactants. Rhamnolipids were already known as permeability enhancers. They have also reported the impact of Rhamnolipids on the epithelial permeability of macromoleculer drugs.

9. Antimicrobial activity

Antimicrobial activity of surfactants can be calculated on the basis of minimal inhibitory concentration (MIC) (Infante *et al.*, 1984). Arginine based surfactants have been studied in detail for their antimicrobial properties by many researchers (Franklin & Snow, 1981; Infante & Moses, 1994; Clapes *et al*., 1999; Xia., 2001; Burczyk, 2003; Yoshimura *et al*., 2012). The gram negative bacteria were found to be more resistant to arginine based surfactants than gram positive bacteria. Antimicrobial activity of surfactants typically

increases by the presence of hydroxyl group, cyclopropane or unsaturation within the acyl chain. Castillo *et al.*, 2006 stated that the length of acyl chains and the positive charge that determine the HLB of the molecule do affect their membrane disrupting property. *N*α-acylarginine methyl ester is another important class of cationic surfactants that are known to have broad spectrum antimicrobial activity with easy biodegradability and less or no toxicity (Kamimura, 1973; Piera *et al.*, 1998). Studies on the interaction of Nα-acyl arginine methyl ester based surfactants with 1, 2-dipalmitoyl-*sn*-glycero-3-phosphocoline (DPPC) and 1, 2-dimiristoyl-*sn*-glycero-3-phosphocoline (DMPC), model membranes, as well as with living organisms with or without external barriers also revealed their good antimicrobial activity (Singare & Mhatre, 2012).

10. Biodegradability

Kamimura, 1973, Shida *et al.*, 1973 and 1975, and Kubo *et al*., 1976 extensively studied the biodegradability of amino acid based surfactants and found that N-acylamino acids are easily biodegrade through decomposition into amino acid and fatty acids.

Single hydrophobic chain containing surfactants are comparatively more biodegradable than their branched counterpartssuch as Bis (Args). Typically, the more hydrophobic the surfactant the poorer will be their biodegradability. Akinari *et al*., 2004synthesized the amino acid surfactants based on fatty acids rich amino acid surfactants and studied their physicochemical properties and biodegradability. Biodegradation studies of these surfactants showed their microbial degradation between 57-73% over 14 days. Zhang *et al.*, 2016 prepared supramolecular hydrogels mixtures of the biosurfactants based on sodium deoxycholate (NaDC) and amino acids such as glycine (Gly), alanine (Ala), lysine (Lys) and arginine (Arg) using different buffered solutions and claimed their unique

sensitivity towards multi-stimuli environments, their facile biodegradability and pH-sensitiveness makes them promising and versatile vehicles for dye (or drug) delivery.

11. Haemolytic activity

Nogueira *et al.*, 2011 investigated the five anionic lysine-based amino acid surfactants that differ on the basis of their counter ion and examined their ability to disrupt the cell membrane under varied pH range, concentrations and incubation period. For this purpose they used a standard hemolysis assay as a model for endosomal membranes. Results confirmed the pH sensitive hemolytic activity and better kinetics of these surfactants at the endosomal pH range.

Surfactants are known to have the capability to interact with lipid bilayer of cell membranes. R.B.C. (red blood cell) is one of the most frequently used cellular membranes as reference model to investigate the mechanisms of fundamental surfactant-induced osmotic cell resistance. Pérez *et al*., 2009 studied the mechanisms of surfactant membrane interaction by monitoring the action of three arginine-based cationic amino acid surfactants and five lysine-based anionic amino acid surfactants on hypotonic hemolysis. Results revealed the dissimilar anti-haemolytic behaviours among amino acid-based surfactants, both linked to the maximal protective concentration. Physicochemical and structural properties of these compounds dictated the protection against hypotonic hemolysis. A good correlation was found in between the CMC and the concentrations of cationic surfactants that resulted in maximum protection against hypotonic hemolysis. In contrast, no correlation was observed for the anionic surfactants. Lysine based surfactants differ only in their counterions, this difference being responsible for their anti-hemolytic potency and the hemolytic activities.

Toxicological study revealed that the capability of arginine based monomeric and gemini surfactants to disrupt the erythrocyte membranes depends is size as well as hydrophobicity dependent (Tavano *et al*., 2013).

Pinheiro & Faustino, 2017 discussed erythrocyte interaction with Nα, Nε-dioctyl lysinate salts with different counterions (Li^+, Na^+, K^+, Lys^+, and $Tris^+$). Surfactants interacted with erythrocyte membranes in a biphasic way, at low concentrations through protecting against hypotonic hemolysis and at high concentrations by inducing hemolysis.

12. Rheological Properties

Rheological properties of surfactants play a very important role to decide/predict their applications in different industries such as food, pharmaceuticals, oil extraction and in personal/home care products (Yang, 2002; Lin *et al.,* 2016; Kumar & Mandal, 2017). The relation of viscoelastic characteristics and CMC of amino acid based surfactants have been discussed by many researchers (Shrestha & Aramaki, 2009; Singh *et al.*, 2015; Saavedra *et al*., 2017).

13. Industrial Applications

Special structural characteristics, non-toxicity and biodegradability of AAS make them suitable for various industrial applications.

13.1. In agriculture

Amino acid surfactant can be used as insecticides, herbicides and plant growth inhibitors for agricultural means. Betaine ester surfactants are a class of cationic surfactants that can be used as ‘temporary biocides’ and can be hydrolyzed very easily into harmless components (Shida, *et al*., 1973; Solans *et al.*, 1990; Tamarkin *et al.*, 2014).

A lawn pesticide was also reported in an US patent, that was synthesized using the mixture of refined oils extracted from the plants of *Cupressaceae* family and an amino acid derived surfactant solution, in which amino acid derived surfactant contributed 20 to 50% by weight of the said solution. Herbicidal action of nonionic type amino acid-surfactant has also been reported by various workers (Shida *et al*., 1975).

13.2. In laundry detergents

Nowadays, the demand for amino acid based detergent formulations is increasing globally. Amino acid surfactants are known to have better cleaning ability, foaming ability and fabric softening properties that make them suitable to be used in house hold detergents, shampoos, body wash etc. An amino acid surfactant derived from aspartic acid is reported as an effective detergent with ampholytic and chelating characteristics. Less skin irritating effects were observed by using detergent compositions made up of N-Alkyl-β-amino-ethoxyacids (Leonard, 1976). Liquid detergent compositions made up of N-coco-β-aminopropionate was claimed as an effective cleaning agent for grease mark on metallic surfaces (Kennedy *et al.*, 1980; Cooper *et al.*, 1988). An aminocarboxylic surfactant, $C_{14}CHOHCH_2NHCH_2COONa$, was also proved to have better detergency and used to clean textiles, carpets, hair, glass etc (Miyamoto *et al.*, 1988). 2-hydroxy-3-aminopropionic acid N,N di acetic acid derivatives were also known to incorporate stability in bleaching agents with good complexing ability.

Detergent composition based on N-(N'-long chain acyl-β-alanyl)-β-alanine were reported to have better ability and stability, foam breakage and good softening properties (Miyamoto *et al.*, 1988). Keigo & Tatsuya, 1996 prepared and patented acyl amino acid based detergent compositions. Kao formulated N-acyl-1-N-hydroxy-β-alanine based

detergent compositions and claimed their low skin irritation property, high water resistance and high detergency (Nozaki, 1995). Ajinomoto company used low toxic and easily degradable, L-glutamic acid, L –arginine and L-Lysine based amino acid surfactants as the principal ingredients in shampoos, detergents and cosmetics (Fig. 9). Ability of enzymatic additives in detergent compositions to remove protein based soilings has also been documented in the literature (Keshwani *et al.*, 2015). Glutamic acid, alanine, methyl glycine, serine and aspartic acid derived N-acyl amino acid surfactants were reported as good liquid cleaning agents in aqueous solutions. These surfactants did not show any increase in viscosity even at very low temperatures and can easily transfer from foamer container, thus providing uniform foam (Moriyama, 1998).

13.3. Lubricants

Amphoteric type amino acid surfactants are generally used as lubricants. Having excellent adhesive property for hydrophilic surfaces and low friction coefficient make them ideally suited to be lubricants. Various researchers prepared and studied the lubricating properties of AAS specifically glutamic acid (Jiang et al., 2005; Jiang et al., 2006; Xia & Jianhua, 2007).

13.4. In medicine

13.4.1. Drug carriers and preparation of functional liposomes

In the recent years, various researchers have claimed the ability of synthetic acyl amino acid/peptide as drug carrier and for preparing functional liposomes with lipopeptide ligands. As compared to the conventional liposomes of lecithin, vesicles of long aliphatic chain *N*α-acyl amino acids also showed encapsulation efficiencies for solutes (Boeckler *et al.*, 1998; Yagi *et al.*, 2000).

13.4.2. Gene therapy and DNA transfection

Gene therapy is a very important technique currently used in life science to safely introduce selected gene into living cells. Gemini surfactants have been found to have potential to be used as a carrier for the transportation of bioactive molecules. Lysine and 2, 4-diaminobutyric acid based polycationic gemini surfactants can be easily synthesized using standard peptide chemistry (Kirby *et al*., 2003).

McGregor *et al*., 2001 prepared a new class of amino acid based gemini surfactants as carrier for gene delivery into cells. Preliminary results revealed that combining these amino acid based gemini surfactants with dioleoylphosphatidylethanolamine (DOPE) allowed the synthesis of liposomes of different sizes and lipid compositions. Investigation revealed that suspensions of DOPE/surfactants mixtures in water, leads to a mixture of lipid vesicles with more complex structures corresponding to particles e500 nm in diameter. Different molar ratios of DOPE and surfactants (50/50, 60/40, and 70/30) were found to effect luciferase expression in CHO cells at comparable levels. The size of colloid and the molecular composition of the gemini surfactants matter in delivering optimum gene expression in living models.

DNA transfection efficiency of cysteine based amino acid surfactants with low molecular weight and its corresponding gemini were evaluated by Pena *et al.*, 2017. These surfactants showed no cytotoxicity and had greater efficacy than their commercially available counterparts to transfect CHO-K1 cells.

13.4.3. Antiviral agents

Lipoamino acids are attracting researchers due to their significant antiviral activity. Some acyl amino acid derivatives have also been reported to inhibit influenza

neuraminidase (Kondoh *et al.*, 2009). Several *N*α-palmitoylated amino acids/peptides when integrated into model membranes, affected the transition temperature between the surface tension at their critical micelle concentration (CMC).

Amino acid surfactants derived cationic surfactants obtained by condensation of fatty acids such as lauric acid and esterified dibasic amino acids such as Arginine may be exploited for the protection against the microorganism and these cationic surfactants were also found to be effective against viral infections. Furthermore, the addition of LAE into the cultures of Herpes virus type 1 Vaccinia virus and bovine parainfluenzae 3 virus leads to almost complete diminution of the virus organisms in these cultures, such effects being observed from 5 to 60 minutes (Bonvila *et al.*, 2006).

13.5. In food processing industry

Amino acid surfactants are widely used as emulsifiers in the food sectors such as in margarine, dairy products, low-calorie spreads and dressings (Flack, 1997). In addition, new insights into eating habits and health aspects need the changes in the food formulations time to time in terms of calories, fat content, vitamins or minerals that lead to a constant demand of optimizing product formulations. These surfactants also include optimal selection of raw materials. Formerly, Industries were giving great attention to the different glycerides, most commonly of monoglyceride derivatives but due to health consciousness, Arginine based amino acid surfactants with antimicrobial activity and wide antibacterial spectra are receiving great interest as a promising alternative of commercially available pure glycerides in food formulations. These surfactants additionally show considerable antimicrobial activity against *Escherichia coli* and *Salmonella*, which are the most common food borne pathogens and create a serious health hazard due to increased drug resistance (Lynde, 2001).

13.6. In cosmetic industry

AAS are used in the formulation of various personal care products. Potassium N-cocoylglycinate was found to be mild to skin and applied and used in face cleansers to remove soil and make-up. N-acyl-L-glutamic acid has two carboxylic groups, which lead to better water solubility. Among these, AAS based upon C_{12} fatty acid are extensively used in face cleansers in order to clean soil and make up. AAS with C_{18} have found to be used as emulsifiers in skin care cosmetics. N-dodecanoylalaninate is known to have ability to create non irritating creamy foam for skin, and therefore employed to formulate baby care products. N-lauroyl based AAS is used in toothpaste shows good detergency similar to soap and strong enzyme-inhibiting efficacy (Rosen & Kunjappu, 2012).

In the past few decades, in the selection of surfactants in cosmetics, personal care products and pharmaceuticals, less toxicity, mildness, gentleness and safety are becoming the major concerns. Consumers of these products are genuinely conscious for the potential irritation, toxicity and environmental factors. Nowadays, amino acids surfactants are used to formulate many shampoos, rinses, and body soaps because of the numerous merits over their conventional counterparts for use in cosmetics and personal care products. Protein based surfactants [PBS] possess desirable properties that a personal care product must have (Linfield, 1978). Some amino acid surfactants are known to have film forming ability whereas others have good foaming ability. Amino acids are important naturally occurring moisturizing factors (NMF) present in the stratum corneum. When epidermal cells die they become a part of the stratum corneum, and the proteins present in the cells gradually degrade into amino acids. These amino acids are then transported further into the stratum corneum, absorb the fat or fatlike substances into horny layer and thus improve the elasticity

of the skin surface. About 50% of the NMF present in the skin is made up of amino acids and pyrrolidone (Cruz & Korchin,1994). Collagen, a common cosmetic ingredient also comprises of amino acids, which keep the skin supple. Various skin problems such as roughness and dark colouration of the skin are largely due to the deficiency of amino acids. A case study revealed that mixing of an amino acid with ointment give relief to skin burn and the affected area returned to the normal state and did not turn in to a keloid (Kiran *et al.*, 2010).

Amino acids are also found very useful for the care of damaged cuticles. Lacklustre and dry hair can indicate a decrease of amino acid concentration in heavily damaged cuticles. Amino acids have the ability to penetrate through the cuticle into hair shaft and drawing in moisture from the skin. This capability of amino acid based surfactants make them useful in shampoos, rinses, hair softeners, hair treatment agents, and the presence of amino acids makes hair less breakable (Franklin & Snow, 1981).

13.7. In microbial enhanced oil recovery

Amino acid surfactants are also found useful in microbial enhanced oil recovery. A bacterium strain, *B. aureum* MSA13 synthesized an amino acid surfactant that has potential for use in microbial oil recovery. This surfactant has octadecanoic acid as a hydrophobic moiety and hydrophillic group comprises of a tetrapeptide, a short sequence of four amino acids pro-leu-gly-gly. Another example involve in microbial enhanced oil recovery in marine surroundings is an actinobacterium, MSA13 produced surfactant (Zhou *et al.*, 2011).

13.8. In nanomaterials

Amino-acid-based polymerizable surfactants were also found to be useful in the synthesis of chiral nanoparticles. Preiss et al., 2016 reported the synthesis of an amino-acid-

based chiral surfactants with polymerizable moieties which were then exploited to prepare nanoparticles with a chiral surface functionality. They also checked their potential as nucleating agents in the enantioselective crystallization of amino acid conglomerate systems, taking a model system of rac-asparagine. Comparison were made in the particles synthesized from chiral surfactants having different tail groups and results revealed that only the chiral nanoparticles made up of polymerizable surfactant were found to have ablity to act efficiently as nucleation agent in the enantioselective crystallization.

13.9. Other applications

Amino acid surfactants are also known for use in forming PEDOT [Poly (3,4 ethylene dioxythiophene)] films (Hernandaz *et al.*, 2011; Moral-Vico *et al.*, 2013; Ahuja *et al.*, 2007; Lowe , 1989; Malhotra *et al.*, 2006; Malhotra *et al.*, 2005; Andreescu & Sadik, 2004; Guimard *et al.*, 2007; Sackmann *et al.*, 1968; Sarfati *et al.*, 2000).Poly PEDOT films through direct anodic oxidation of EDOT (3, 4-ethylenedioxythiophene) in aqueous solution comprisingofthe sodium N-lauroylsarcosinate (SLS), an eco-friendly amino acid surfactant were also prepared. In addition to the above mentioned applications, amino acid surfactants are also used in the optimization of dry-cleaning process with carbon dioxide and as chiral solvents (Sarfati *et al.*, 2001; Weiss-López *et al.*, 2001; Roosmalen *et al.*, 2004; Bordes & Holmberg, 2015)

Roosmalen *et al.*, 2004 have optimized the dry-cleaning process using carbon dioxide along with the amino acid surfactants, and studied the impact of various reaction parameters on the cleaning. The reaction parameters studied were the cleaning time, temperature, and power involve in mechanical action. In addition, the influence of the

quantity of each substance used such as CO_2, isopropyl alcohol, water and surfactant was also investigated.

Self-assembly of amino acid surfactants resulted into micelles with a chiral surface. Such a property of amino acid surfactant micelles of being supra-molecularly chiral makes them suitable for asymmetric organic synthesis (Liu *et al.*, 2006). The chiral micelles can also impart chirality in mesoporous materials prepared through surfactant templating route and make their pores chiral. Dicarboxylic based amino acid surfactants can also behave like surface-active chelating agents. It has been observed that the interaction of dicarboxylic amino acid surfactants with divalent ion like calcium is reliant on the distance between the two carboxyl groups. For example, N-acyl glutamate that has three $–CH_2$ groups between the carboxyl groups does not form an intramolecular chelate with calcium, and is not able to precipitate in waterwith high concentrations of calcium. The binding property of chelating surfactants can also be used in mineral ore flotation. Calcium containing minerals like calcite and apatite can be separated by means of flotation reagent with the right distance between the carboxyl groups. N-alkyl amino acid surfactants are true amphoteric type surfactants that can provide exceptionally low values of surface tension at the CMC, which is due to formation of self-assemblies consisting of alternating anionic and cationic species. This represents the micellization-driven protonation of surfactants.

Amino acid surfactants can also be used to design switchable surfactants. Cysteine derivatives are the best known example of this class that can readily covert into cystine derivatives via reversible processes. For example, long chain N-acylcystine, a gemini surfactant, is highly surface active be transformed into a cysteine derivative. Dithiothreitol,a poor surface active agent, can alsorevert back to thegemini surfactant via an oxidation. The

exchange from one state to the other can also be achieved through electrochemical means (Fiechter, 1992).

14. Current Challenges for Amino acid Surfactants

The economic feasibility of large scale production of amino acid surfactants is a major issue to be surmounted. Biotechnological processes involved in their production are not easily cost effective especially for the processes where bulk use of surfactants is required such as petroleum and environmental application. Purification of substances is another problem linked with these surfactants which is a needed for pharmaceutical, cosmetic and food applications.Along with these disadvantages, denaturation and dissociation of amino acid surfactants into their subunits and their activity are largely affected by salt solutions.

Various researchers have suggested remedies to overcome these problems. The overall cost cutting in their production can be achieved through the utilization of waste substrate after combating their polluting effect. Development of efficient bioprocesses and their successful optimization is also required, which include the optimization of the culture conditions and cost-effective recovery processes for the maximum production and recovery (Mohamed *et al*. 2017; Wang & Dado, 2017; Vecino *et al*., 2017).

15. Conclusions

Although conceptually simple the process of combining amino acids with a hydrophobic tail can by its very nature offer a variety of synthetic routes, this in turn leading to many potential structures. Since the first studies on the synthesis of simple amino acid surfactants in 1909 research has extended into the production of cationic, anionic, non-ionic and amphoteric molecules, with detailed characterisation and assessment of physiochemical

properties performed throughout. Amino acid surfactants have been shown to have wide reaching applications over different industrial sectors, and the diversity in properties borne out of their potential for structural variety will in the future allow for this range of applications to grow further. Additionally, facile biodegradability and non-toxicity of amino acid surfactants can make them superior to their conventional synthetic counterparts when selected for the right application. Examples even exist whereby the chirality of amino acid surfactants has been exploited in use, such as imparting chirality into the surface of micelle template mesoporous materials. Amino acid surfactants have been reported to have a wide range of applications in different industrial sectors. Facile biodegradability and non-toxicity of amino acid surfactants can make them superior to their conventional synthetic counterparts when selected for the right application. The major challenges associated with amino acid surfactants are their high production cost and their challenging isolation to high purity.Various researchers are working to minimize these problems through selecting suitable renewable alternatives as substrate along with designing cost effective and scalable procedures. Amino acids surfactants have the potential to be widely accepted commercially in various industrial sectors in near future, especially so when the diversity of their structure and physiochemical properties are considered.

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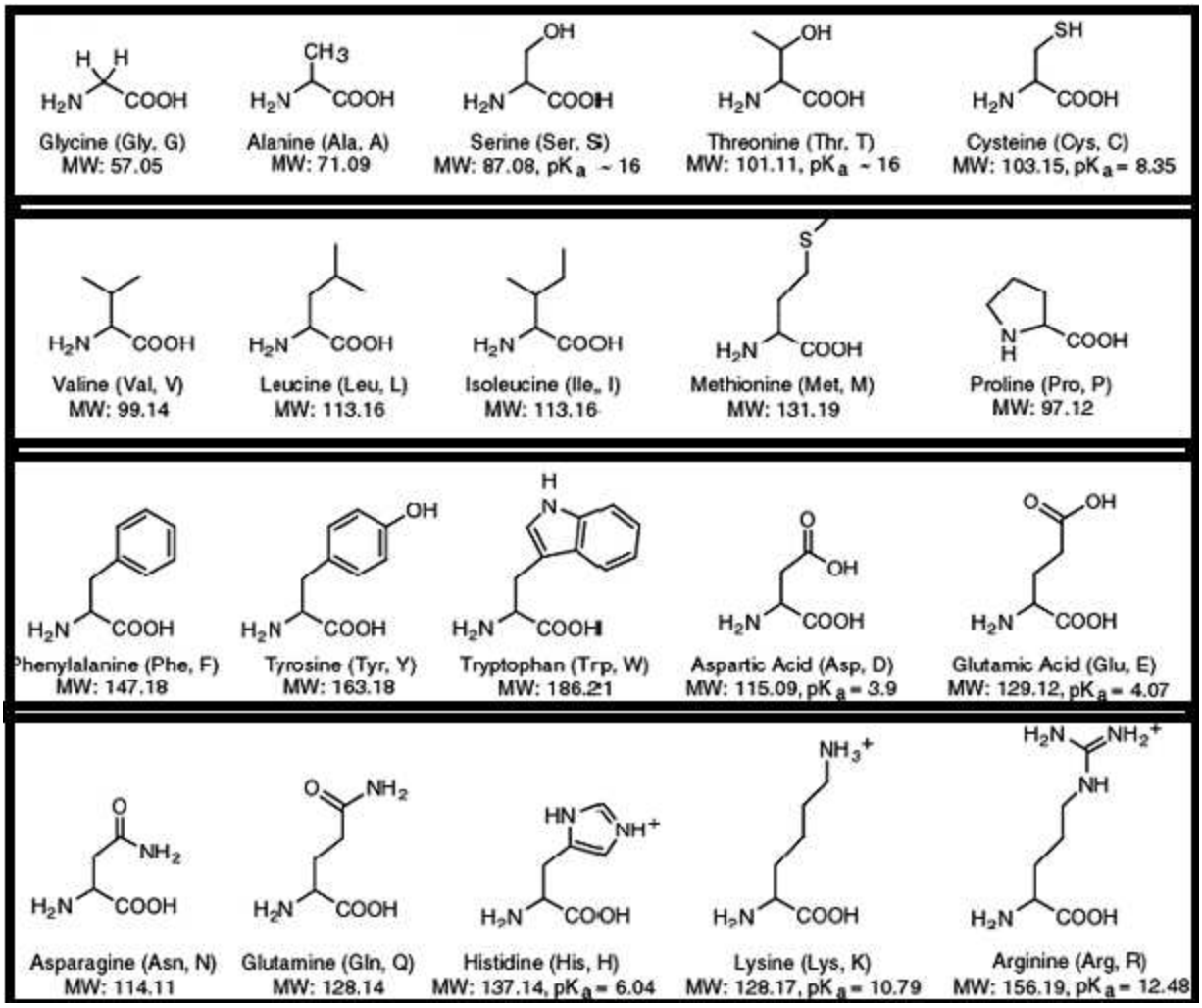
	Reviewers' Comments	Response
1	AAS applications (regarding several technological fields) are dependent on their toxicological profile. Authors should address in a more extensive and careful way, the toxicity issue and its relationship with the physicochemical properties of AAS. Recent papers like Perinelli et al, 2016 (Eur J Pharm Biopharm) could be added to the references list.	Section 8, Toxicity has been rewritten and incorporated all the suggestions and references given by the learned reviewer.
2	The same applies to the antimicrobial activity. There is currently a very broad database on AAS biomedical applications. It is mandatory to incorporate more up-to-date information. Examples of more recent papers are Tavano et al, 2013 (Soft Matter), Pinazo et al, 2016 (ACIS), and Coronel-Léon et al, 2017 (CSBB).	Section 9, Antimicrobial activity has been rewritten and all the suggestions and references given by the learned reviewer have been incorporated.
3	Did the authors considered the inclusion of a rheological appreciation of AAS? Besides Shrestha, R. G., &Aramaki, K. (2009), recent papers dealing with this subject seem quite appealing, since thickening, viscosity consistency and foaming are technological challenges related to many AAS industrial applications.	Rheological properties of AAS have been incorporated as a separate section (Section 12).
4	In terms of application in Medicine concerning drug delivery, the authors did not address the fact that AAS can be promising novel biomaterials in nano-particulate systems. Why?	It has been included in the manuscript in Section 12.8.
5	- Page 33, 12.4.2.: DNA transfection deserves also a more full approach. Literature reports, others than Claffe et al, 2000, are available. Furthermore, gene therapy could be included in DNA transfection item. Considering DNA transfection and Gene therapy, authors should rewrite the corresponding text in a more careful way, accurately relating the scientific results (e.g. what was the main achievement, in terms of transfection efficiency, after combining the Gemini AAS with DOPE?)	Gene therapy and DNA transfection have clubbed and the section 12.4.2. is dedicated to it.

	5. The following corrections and suggestions should be taken into account:	
	- Abstract, line −3: the semicolon punctuation should be substituted by a comma. - The last sentence of Abstract should be globally rewritten.	It has been rewritten
	- Page 4, 7.3: "Critical micelle concentration (CMC)" and not vice versa.	Done
	- Page 10, 5.2: the reference cited here is really Clapés & Infante, 2001? Or 2002??	Correction has been made
	- Page 10and TOC (page 3), 5.2.1 and 5.2.2: other designations will be better and chemically more correct options.	Correction has been made
	- Page 11: the acronym CMC appears for the first time on page 11. Therefore, its meaning should appear here, not on page 17.	Correction has been made
	- Page 11, line +11: Fig 7 (not Fig 6).	Correction has been made
	- Page 18, line −2: "gamma CMC values" instead of Gama CMC values". Gamma CMC should also be defined (surface tension at the CMC), as it appears here for the first time.	Correction has been made
	- Page 19, line +2: Nα-acyl (not Nα-Acyl).	Correction has been made
	- Page 19, line +8: rewrite (briefly) Faustino et al, 2011 and Shrestha&Arakami, 2009 contributions, in terms of CMC.	Correction has been made
	- Page 19, lines −3 and −4: Krafft point and Krafft temperature are not exactly the same (although commonly used as synonyms). The micellar range comprises the interval between Krafft point and Krafft temperature (see Malik&Ali, 2016, J MolLiq).	Correction has been made
	- Page 22, 7.5: considering that the paper cited by the authors was published in 1997, the part "...as new achievement.....interactions" should be rewritten.	Correction has been made
	- Page 23, line +6: reformulate the sentence "The micelle	Correction has been made

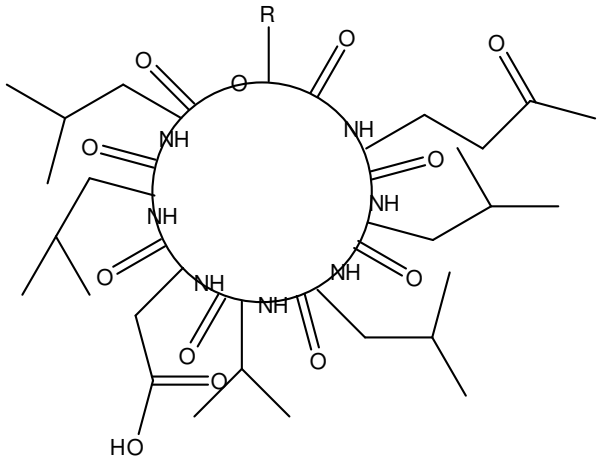
	size for gemini surfactants was significantly larger than their monomeric counterparts." Any citation?	
	- Page 23, line -3: rephrase "In an another study (Bordes et al.,2009) investigated the solution behavior and adsorption of three dicarboxylic amino acid-based surfactants……..."	Correction has been made
	- Page 24, 7.8: More details (although briefly) and references are needed for this part.	Correction has been made
	- Page 29, 10: Change the order to: "Kamimura, 1973, Shida et al., 1973 & 1975 and Kubo et al., 1976 extensively……"	Correction has been made
	- Page 30, line -12.: the reference "145" is not in the same style of referencing as the others.	Correction has been made
	- Page 30, 11: a more comprehensible explanation is needed for the interaction surfactant-erythrocyte membrane.	Explanation has been added as per reviewer suggestions
	- Page 33, 12.4.2.: DNA transfection deserves also a more full approach. Literature reports, others than Claffe et al, 2000, are available.	Corrections have been made accordingly
	- Figure 1: a more careful use of capital letters must be taken into account (an example is the caption of Figure 1). - Figure 1: the information about the meaning of this pKa is missing. pKa presented here is the value of side chain.	Corrections have been made accordingly
	- Figure 3: should be presented in a more careful manner.	Figure 3 has been deleted
	Figures 4, 5, 6 and 7: better structures representations are needed.	Corrections have been made
	- Figure 7: authors should use always the same notation throughout the text (e.g. "bolaamphiphiles" or "bola amphiphiles".	Corrections have been made
	- Figure 10 should be better presented, in order to address the corresponding caption.	Figure 10, which is now Figure 9 has been redrawn.

Figure



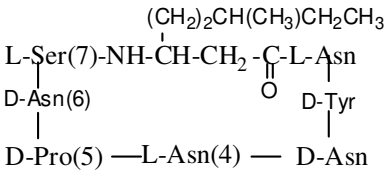
pK_a is the negative base-10 logarithm of the acid dissociation constant (K_a) of a solution. pKa = $-\log_{10}K_a$

Figure 1: 20 standard aminoacids

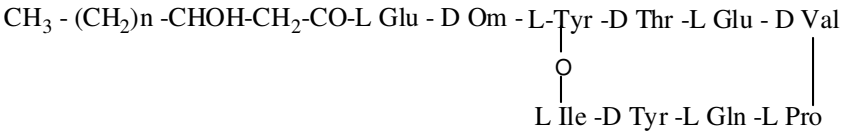


a

$C_{49}H_{76}N_{12}O_{14}$



b



c

Figure 2: a=surfactin, b=iturin A3 and c=fengycin B

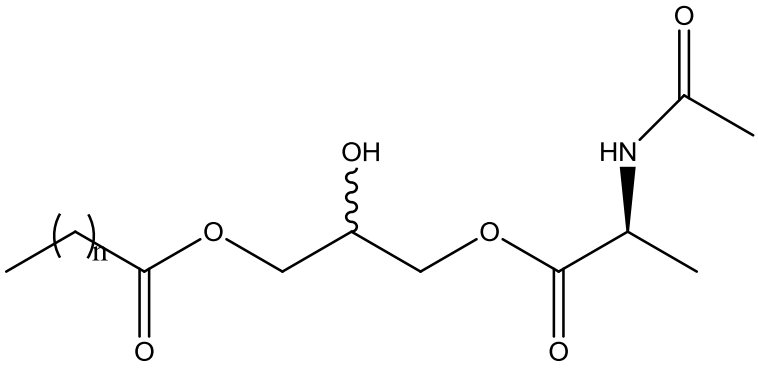


Figure 3: Example of linear AAS

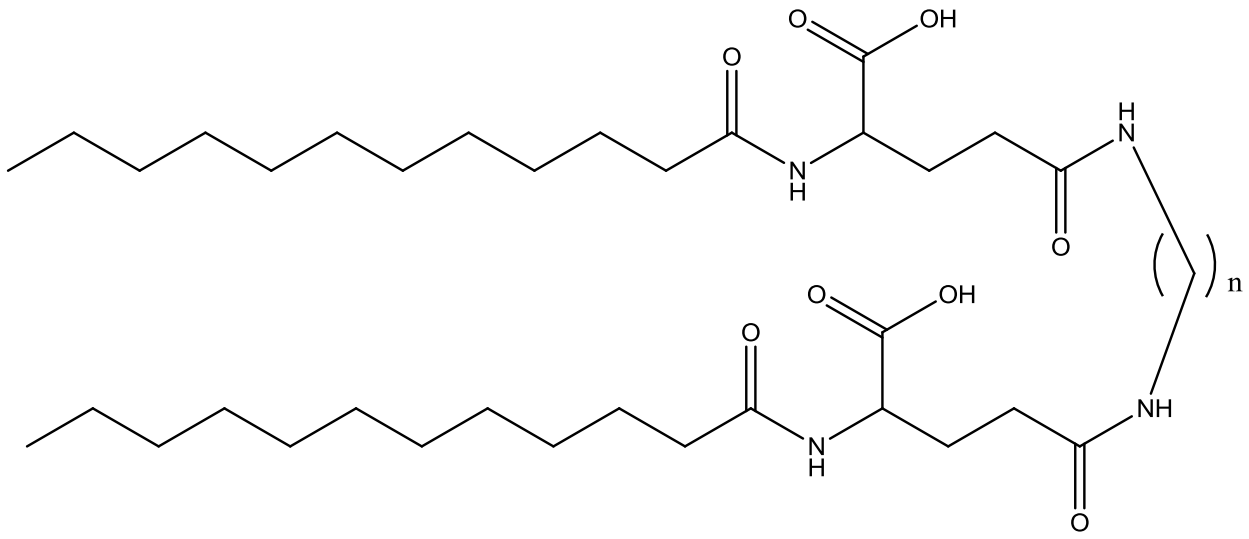


Figure 4: Example of gemini AAS (s = 2, 5, and 8).

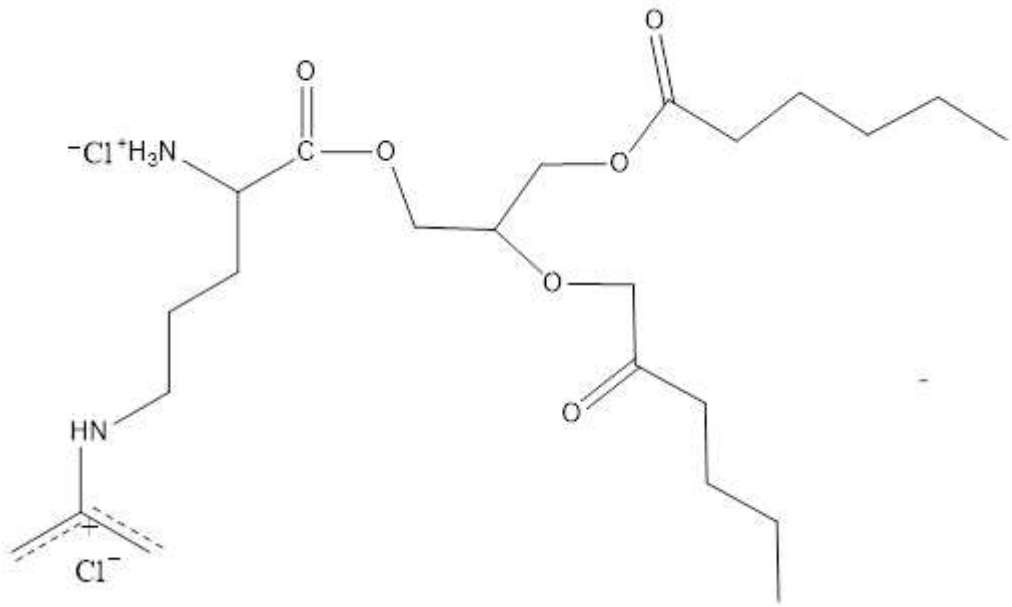


Figure 5: Example of glycerolipid type AAS

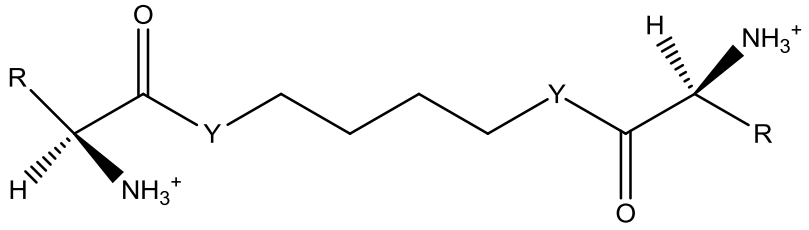


Figure6: Example of bolaamphiphiles AAS

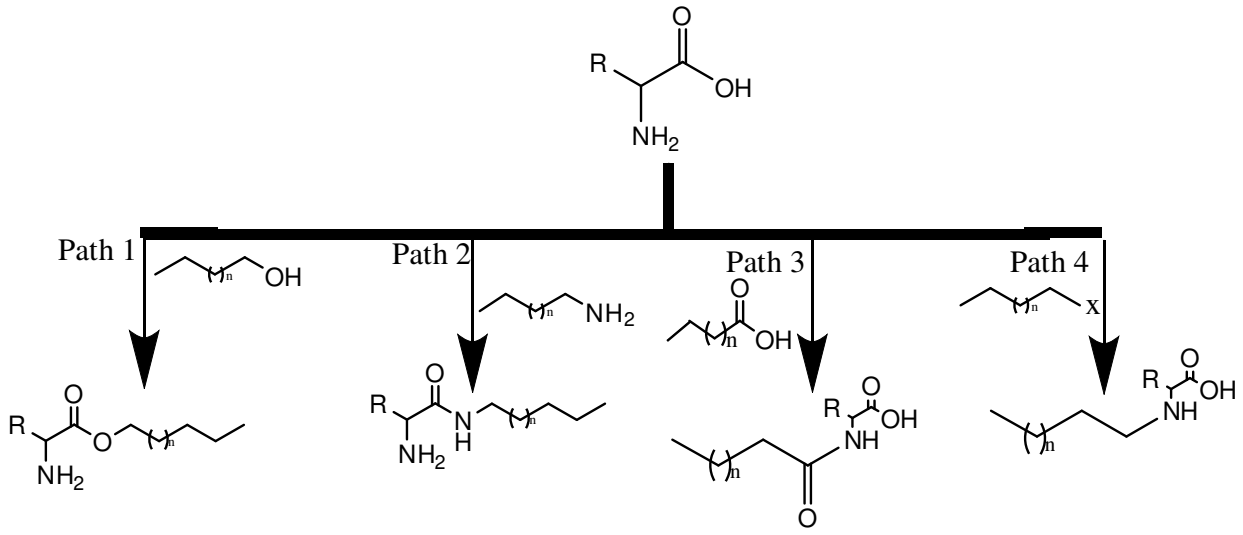


Figure 7: Fundamental synthesis paths of aminoacid based surfactants

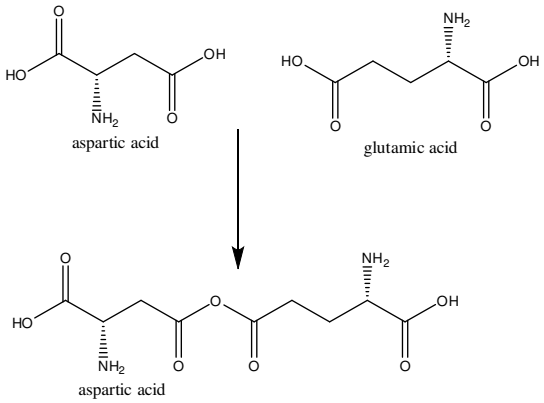


Figure8: Path 5 for synthesis of AAS

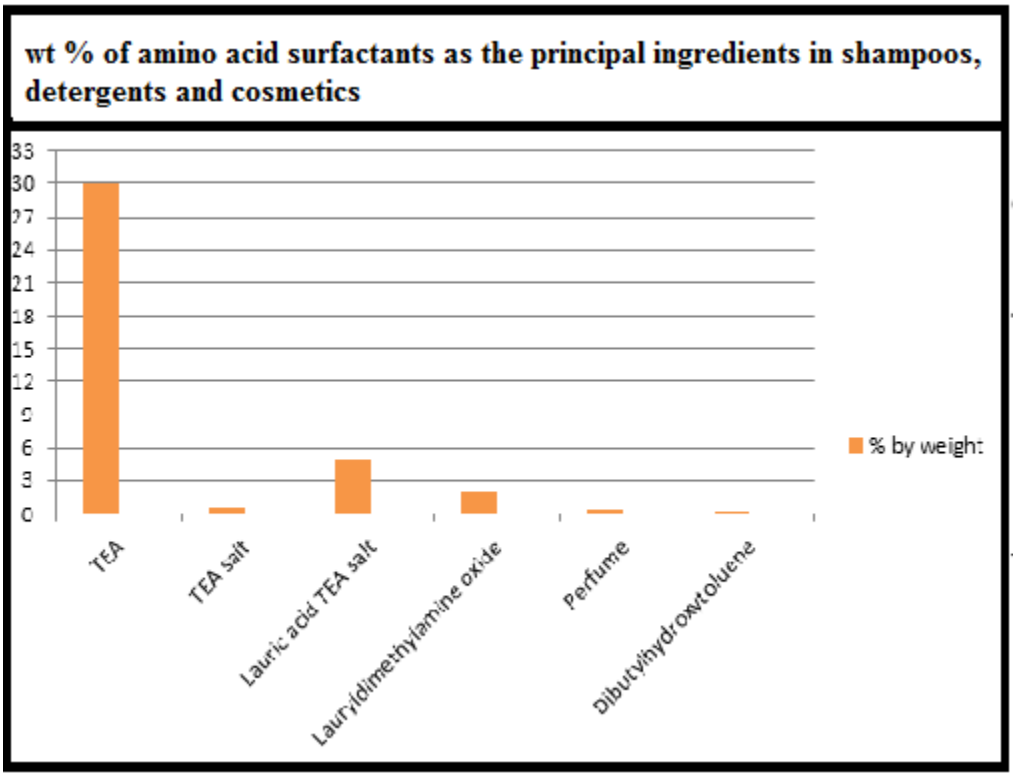
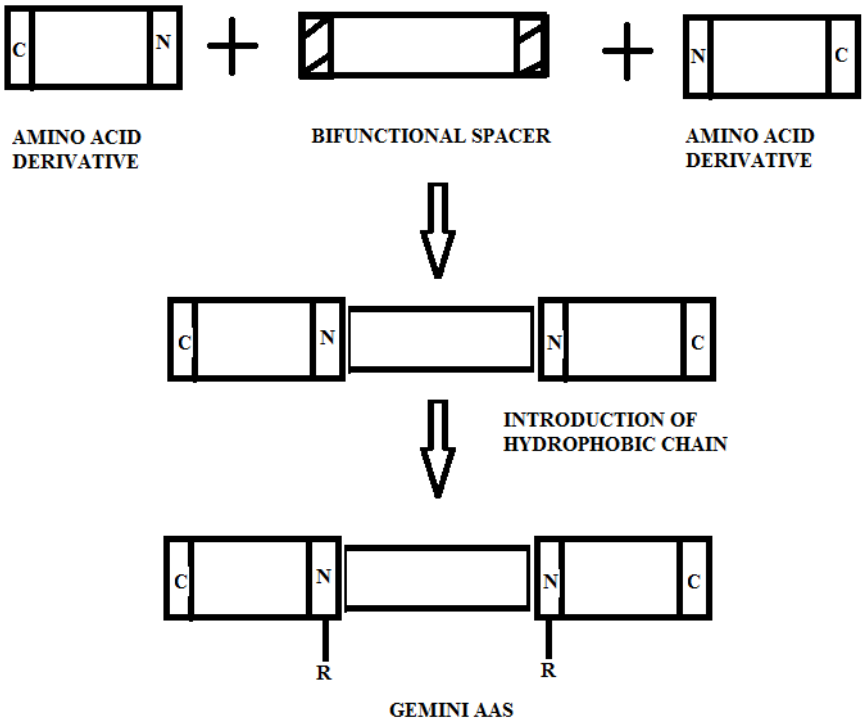
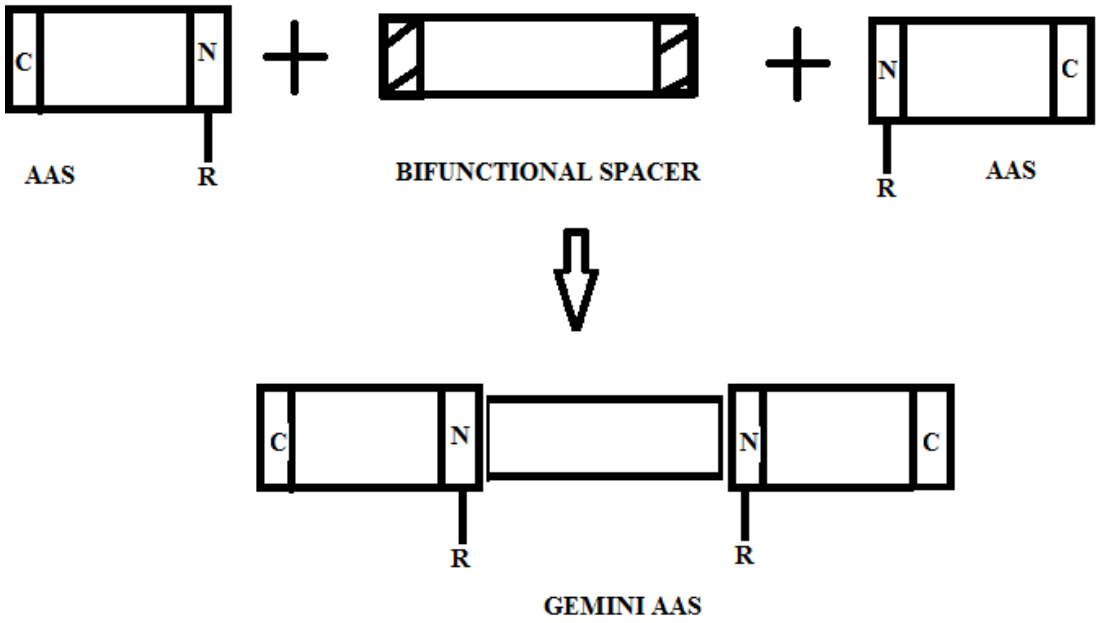


Figure 9: wt % of amino acid surfactants as the principal ingredients in shampoos, detergents and cosmetics

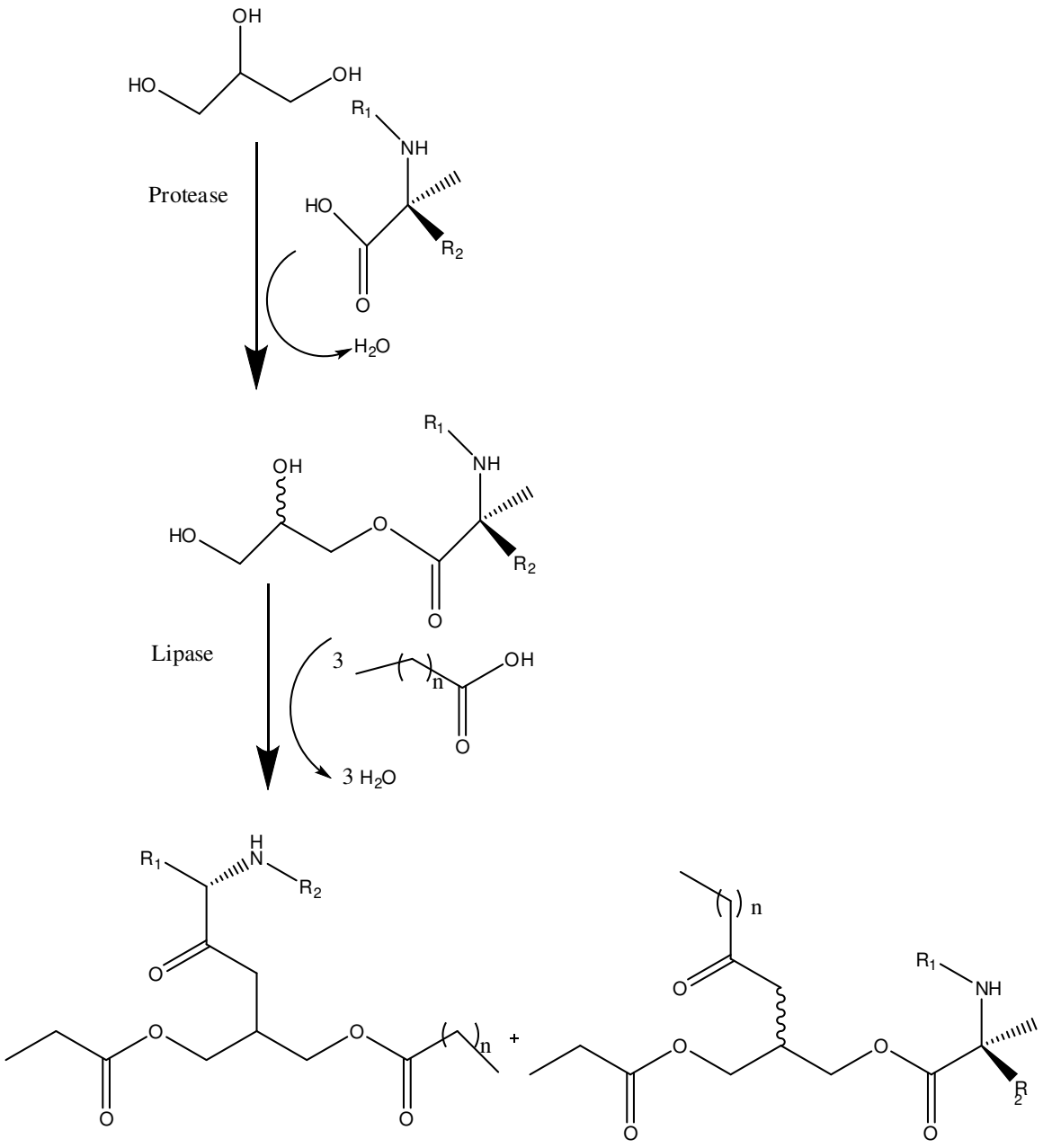
Figure



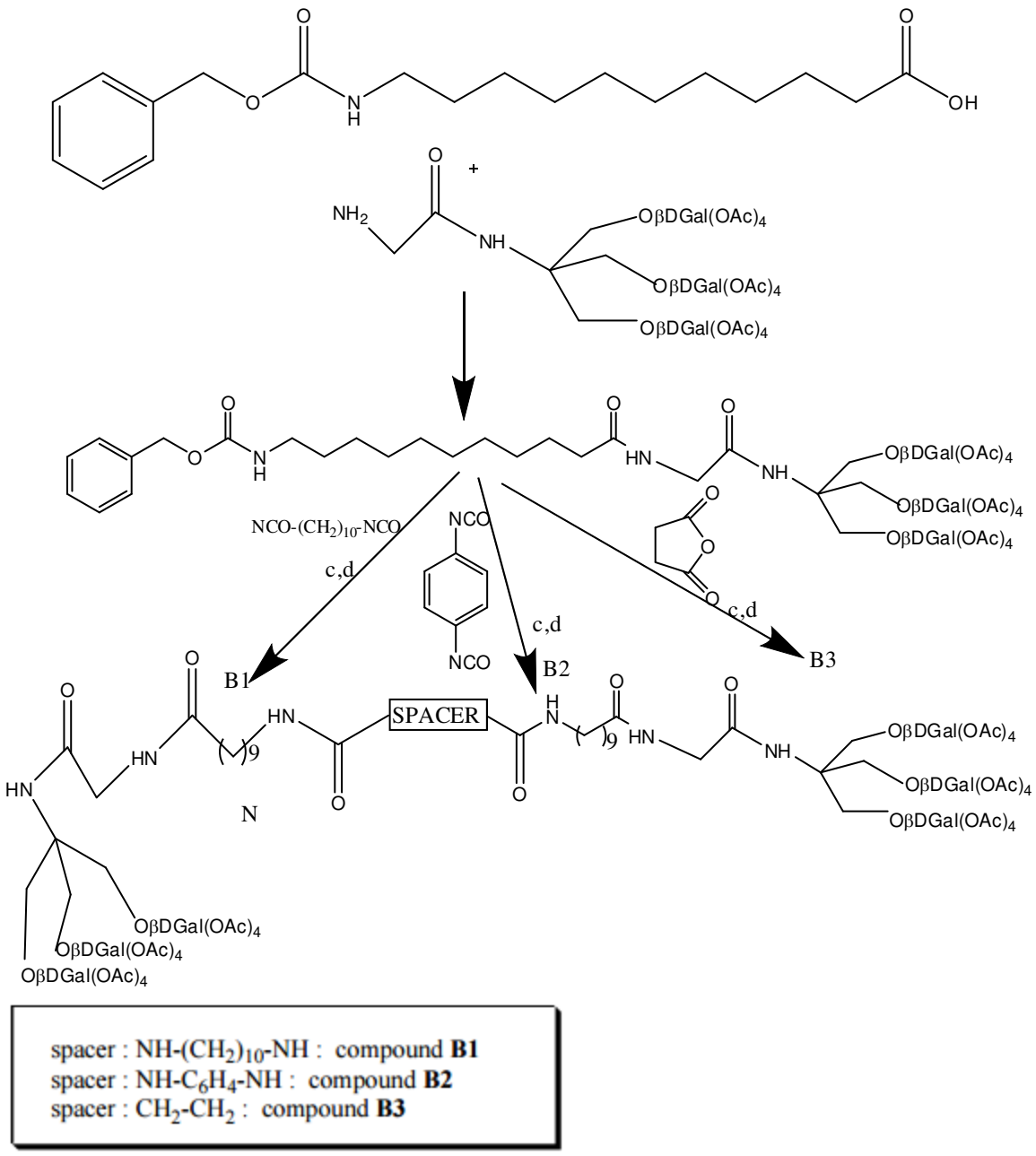
Scheme 1: Synthesis of Gemini AAS using AA derivatives and spacer, followed by insertion of hydrophobic group



Scheme 2: Synthesis of Gemini amino acid surfactants using bifunctional spacer and AAS



Scheme 3: Synthesis of mono and diacyl glycerol amino acid conjugates



Scheme 4: Synthesis of symmetric bolaamphiphiles derived from tris(hydroxymethyl)aminomethane (Tris)

tableau / Table

	CAE	NAE	LAE	MAE
CMC (mg/L)	>1500	820±50	410±10	350±30
γ (mN/m)	27.0±0.5	26.1±0.5	25.5±0.5	24.0±0.5

Table 1: CMC and surface tension of Nα-Acyl arginine surfactants

CAE= N-α-Octanoyl-L-Arginine ethyl ester, NAE= N-α-Nonanoyl-L-Arginine ethyl ester, LAE= Ethyl-N-α- lauroyl-L-arginate, MAE= N-α-Myristoyl-L-Arginine ethyl ester