Research Article

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Comparative analysis of bio-based amino acid surfactants obtained via Diels–Alder reaction of cyclic anhydrides

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Abstract: Current changes in environmental legislation and customer demands set an urge for the development of more sustainable surfactants. Thus, the objective of this work was the development of novel environmentally friendly amino acid surfactants. Combining Diels–Alder cyclization of myrcene with maleic or citraconic anhydride followed by ring opening with amino acids enabled a synthesis route with a principal 100% atom economy. Variation of amino acids resulted in a large structural variety of anionic and amphoteric surfactants. Lysine gave access to either a mono-acylated product bearing a cationic side chain or a bi-acylated gemini surfactant. First, anhydride precursors were synthesized in yields of >90% in a Diels–Alder reaction under microwave radiation and subsequent amino acid coupling in aqueous environment gave fully bio-based surfactants in good yields and purity. Physicochemical characterization showed an enhanced decrease in surface tension upon addition of amino acids to the myrcene–anhydride backbone, resulting in a minimal value of 31 mN·m^{-1} for gemini–lysine. Foamabilitiy and foam stability were significantly increased at skin-friendly pH 5.5 by incorporation of amino acids. The carboxylic groups of surfactants with arginine were esterified with ethanol to access cationic compounds. Comparative analysis revealed moderate antimicrobial effects against yeast, Gram-positive bacteria, and Gram-negative bacteria.

Keywords: green chemistry, bio-based surfactants, amino acids, antimicrobial activity, surface tension

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1 Introduction

In recent years, a general trend towards "greener" products has emerged. Following this trend, research efforts are shifting to the development of new environmentally friendly methods to produce greener chemicals [1,2]. For example, petroleum-derived linear alkylbenzene sulfonates are excellent surfactants but have inherent toxicity to aquatic life [3], while alkylphenol polyethoxylates are reported to cause major environmental problems due to their bioaccumulation and estrogenic effects [4,5]. The use of renewable resources enables the production of more environmentally friendly surfactants with improved biological degradation profiles [6,7]. In addition to sugars such as glucose, amino acids can also be readily used as hydrophilic head group. Amino acid-based surfactants are generally considered to be milder, biocompatible, and ecological substitutes for petrochemical manufactured surfactants [8–10]. Research has shown that amino acid surfactants can be prepared by reductive amination using fatty alcohols or aldehydes, resulting in the formation of *N*-alkyl amino acids. However, the linkage usually relies on the application of reducing agents such as boron hydride or its derivatives to reduce the imine moiety to the more stable amine function [11–13]. In industrial scale synthesis, amino acids or protein hydrolysates are usually converted to *N*-acyl amino acids by acylation with fatty acid chlorides using the Schotten–Baumann method [14,15]. Alongside the major product, a stoichiometric amount of HCl is formed as an unavoidable by-product. The need to neutralize the by-product proves to be the major drawback of the Schotten–Baumann reaction and leads to an overall lower atom economy [16–18]. In recent approaches, the biocatalytic synthesis of acylamino acids with novel aminoacylases has been successfully carried out [19–21].

In the search for alternative environmentally friendly and sustainable routes to new amino acid surfactants, the Diels–Alder reaction of the monoterpene myrcene and anhydrides was identified as a viable method for the conversion of amino acids in a reaction with high atom economy and without stoichiometric additives. First reports of the Diels–Alder reaction by Otto Diels and Kurt Alder date back 90 years and the combination of the terpene myrcene with maleic anhydride (MA) was discovered soon after [22–24]. Myrcene is derived from the turpentine oil obtained from pine trees and has recently been produced by modified microorganisms [25–27]. MA, on the other hand, is regularly produced by oxidation of 1,3-butadiene, but has been shown to be easily obtained from biomass and is well suited as a green and renewable platform substrate [28,29]. Citraconic anhydride (CA), along with its isomer itaconic anhydride, can be accessed by dehydration of citric acid, forming a mixture of both isomers with the thermodynamically more stable CA strongly preferred [30,31]. In recent developments, the reaction of myrcene with MA has been mainly used to produce monomers with unsaturated backbone for the production and further modification of polyesters and polyamides [32,33]. The possibility of application for the synthesis of surfactants by ring-opening addition with organic alcohols, amines, or water has been described only in the patent literature [34,35] and the combination with amino acids as potential new bio-based surfactants has not yet been tested.

In this work, the combination of the Diels–Alder reaction with MA and CA followed by ring-opening coupling to amino acids is presented, which gives fully bio-based products with high atomic economy (Figure 1). The structurally diverse products exhibited surfactant properties, which were analyzed based on their surface tension and foaming capacity. Subsequent esterification of the carboxylic groups resulted in novel cationic lipids with antimicrobial activity.

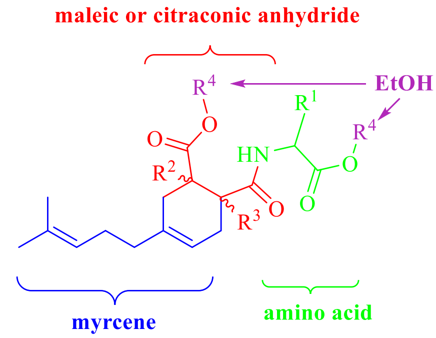

Figure 1: General structure of novel bio-based surfactants obtained from coupling of myrcene (blue) with maleic or CA (red), amino acids (green), and optionally ethanol (purple).

2 Materials and methods

2.1 Materials

Myrcene (90%) was purchased from Acros Organics, MA (≥98%), CA (98%), acetonitrile (99.5%), heptane (98%), formic acid (99%), and citric acid (99%) were purchased from Fisher Scientific. Tetrahydrofuran (THF) (99.5%, not stabilized), EtOH (99.5%), NaOH (99%), and amino acids (≥98.5%) were purchased from CarlRoth. Trimethylsilyl chloride (TMS-Cl) (≥98%) was obtained from Sigma–Aldrich and NMR-solvents ($CDCl_3$, CD_3OD, and D_2O, each 99.9%) were from TCI.

2.2 General microwave assisted Diels–Alder reaction procedure

MA (5,003.0 mg, 50.0 mmol) was dissolved in 10 mL THF and mixed with myrcene (9.52 ml, 7.52 g, 50.0 mmol, 90% purity) in a microwave reaction vessel. The reaction was conducted in a CEM Discover microwave synthesizer at 110°C with P_{max} = 80 W for a reaction time of 15 min. A transparent yellow solution was obtained, from which conversion was determined by GC-FID analysis. For CA, no solvent was added, and the reaction time was prolonged to 30 min.

2.3 Coupling of cyclic anhydride intermediates with amino acids

Condensation reactions with 50 mmol of amino acid in its deprotonated form were carried out in an acetone–water mixture following the Schotten–Baumann approach described by Takehara et al. [14]. Formation of a turbid mixture allowed filtration without the necessity for precipitation by HCl. Solvent was evaporated and the raw product was washed twice with petrol ether. The product was lyophilized and analyzed by HPLC, LC-MS, and NMR as described in supplementary data. Lysine–gemini surfactants were synthesized by application of reduced amount of amino acid (25.0 mmol, 0.5 eq.) and NaOH (0.99 g, 0.25 mmol, 0.5 eq.), while myrcene–anhydride amount was kept at 50 mmol. Selectivity was raised by further prolongation of the anhydride precursor addition time. Investigation of the possible coupling mechanism of lysine and anhydride-precursors was done by application of N_α- or N_ε-*tert*-butyloxycarbonyl (*boc*)-protected lysine as a substrate.

2.4 General esterification protocol for amino acid surfactants

Esterification of carboxylic acid groups was mediated by TMS-Cl using methods according to Takaishi et al. [36].

Prior synthesized amino acid surfactant was used as a substrate (1.0 mmol) and dissolved in EtOH (5 ml, 86.0 mmol) and an excess of TMS-Cl (0.63 ml, 5.0 mmol) was added. The mixture was heated to 50°C for a period of 5 h. After cooling to room temperature, the solvent and volatile by-products were removed in vacuo and the product was obtained as solid.

2.5 Product purification

Raw product mixtures were purified using a preparative Interchim Inc. PuriFlash 450-LC system, equipped with a preparative Kromasil column (C18, 5 µm, 250 mm × 200 mm). Raw products were dissolved in a 1:1 mixture of acetonitrile and water, chromatographic separation was conducted in a gradient method starting at 20% acetonitrile and ending at 90% acetonitrile with 0.1 vol% formic acid added to both phases. Chromatographic samples were collected and lyophilized.

2.6 Wilhelmy plate and pendent drop surface tension analysis

Surface tension analysis was done with a DCAT tensiometer using a Wilhelmy Plate PT 11 (10 mm × 19.9 mm × 0.2 mm) and analyzed with the DCATS software from DataPhysics as a mean of 50 measured values after the standard deviation was below a threshold of ±0.05 $mN \cdot m^{-1}$. For a concentration series, the measurements were conducted from the lowest to the highest concentration. The critical micelle concentration (CMC) was determined graphically from a plot of the surface tension against the log of the surfactant concentration.

For surface tension determination with the pendant drop method a DataPhysics OCA contact angle system with SCA22 software for analysis was used. The volume of the pendant drop was dosed at a rate of 1 $\mu l \cdot s^{-1}$ to the maximum size in a cuvette partially filled with water to minimize evaporation during the measurement over a period of 10 min. A surfactant concentration of 4 $mmol \cdot l^{-1}$ related to the major active product was used. The pH was set to 5.5 with citric acid or 1 M NaOH solution before the measurements were started. All measurements were done in triplicate.

2.7 Foam measurements

A Krüss DFA100 was used for foam measurements of 4 $mmol \cdot l^{-1}$ solutions at pH 5.5 at 20°C. A total airflow of 0.4 $l \cdot min^{-1}$ was applied through a Krüss FL4551 filter (12–25 µm) for 20 s to induce foaming of the mixture and foam stability was observed measuring at 5 Hz during foaming phase and at 2 Hz in the stability/decay phase.

2.8 Determination of antimicrobial activity

Antimicrobial activity was tested against two Gram-positive bacteria *C. glutamicum* (ATCC 13032) and *Bacillus subtilis* (ATCC 6051) as well as Gram-negative *Escherichia coli* (DSM 102052) and the yeast *Candida viswanathii* (ATCC 20962). Bacteria were cultured on high salt LB media at an incubation temperature of 37°C. The yeast *C. viswanathii* was cultivated on YM media at 30°C.

Samples of overnight cultures were diluted to a final OD_{600} of 0.005 in 3 ml sterile 0.9% NaCl solution and spread on the LB- or YM plates; supernatant liquid was removed and the cultures incubated for 45 min at 37°C and 75 min at 30°C, respectively. After incubation, plates were further prepared by punching holes with the wider end of Pasteur pipettes (d = 5.5 mm). About 40 µl of test substance was placed in four different dilutions (40, 20, 10, and 5 $mmol \cdot l^{-1}$ based on active compound in 100 $mmol \cdot l^{-1}$ Tris-buffer pH 7.5) in the cavities and the plates were incubated for 24 h at 37°C and 30°C. About 100 mM Tris-buffer (pH 7.5) and the water-hydrolyzed Diels–Alder adducts were tested as negative controls. Commercially available cationic *N*-lauroyl arginine ethylester (LAE), ampicillin, and zeocin (*C. viswanathii*) were used as antimicrobial positive control. All tests were run in triplicate setup.

3 Results and discussion

3.1 Diels–Alder catalyzed coupling of myrcene to anhydrides

Starting from the cyclic, unsaturated anhydrides, the Diels–Alder reaction offers an attractive possibility to extend the hydrophobic residue. In particular, the absence of additional coupling reagents, catalysts, and additives leads to a high atom economy of the reaction. The basic lipophilic tail of the surfactants was synthesized following the method of Hornung et al. [32] using microwave irradiation to mediate the [4 + 2]-cycloaddition reaction (Figure 2a). The method was applied to myrcene and MA using THF as a solvent. As described in the literature [32], MA conversion went nearly quantitative (96%) within 5 min (Figure 2b). Furthermore, the reaction was transferred successfully to the fully bio-based CA. The liquid compound enabled a solvent-free synthesis, which makes the overall reaction more sustainable. The cycloaddition reaction with CA required 30 min

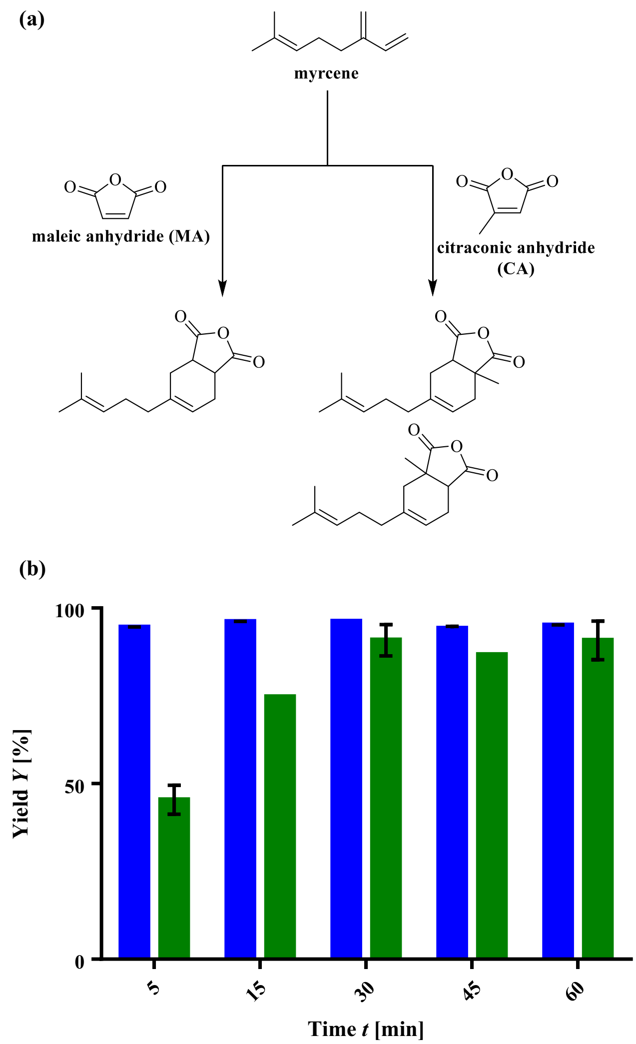

Figure 2: (a) Reaction scheme for conversion of myrcene with cyclic anhydrides MA and CA, (b) time-dependent yield of microwave-assisted synthesis of MA (blue) and CA (green) precursors, (c + d) GC-chromatograms of MSA (c) and CA (d) after 30 min reaction time.

to reach a maximum yield of up to 90%, which may be attributed to the additional steric hindrance of the methylene group or the solvent-free conditions during synthesis. Further investigation employing LC-MS confirmed synthesis of both target molecules in ring-closed anhydride form (Figure 2c and d).?A3B2 tpb 4pt?>

3.2 Synthesis of amino acid-based surfactants by ring-opening condensation

The *N*-terminal ring-opening addition of amino acids to the cyclic anhydrides generates amphiphilic products with great structural variety (Figure 3a). Depending on the charge of the amino acid side chain, either anionic surfactants with two or three acid groups or amphoteric amphiphiles with two carboxyl and one cationic side chain are accessible. The raw anhydride reaction mixture was added slowly to a solution of target amino acid dissolved in water. The pH of the amino acid solution was adjusted to an alkaline pH of >9 to deprotonate the amine group, enabling nucleophilic attack of the anhydride. Similarly, the Schotten–Baumann reaction is conducted for the synthesis of acylamino acids from the corresponding acyl chlorides [14]. Addition of the anhydride to the aqueous amino acid solution gives complete conversion of the anhydride, yielding the desired surfactant molecules in the form of two isomers, which could not be separated by HPLC or

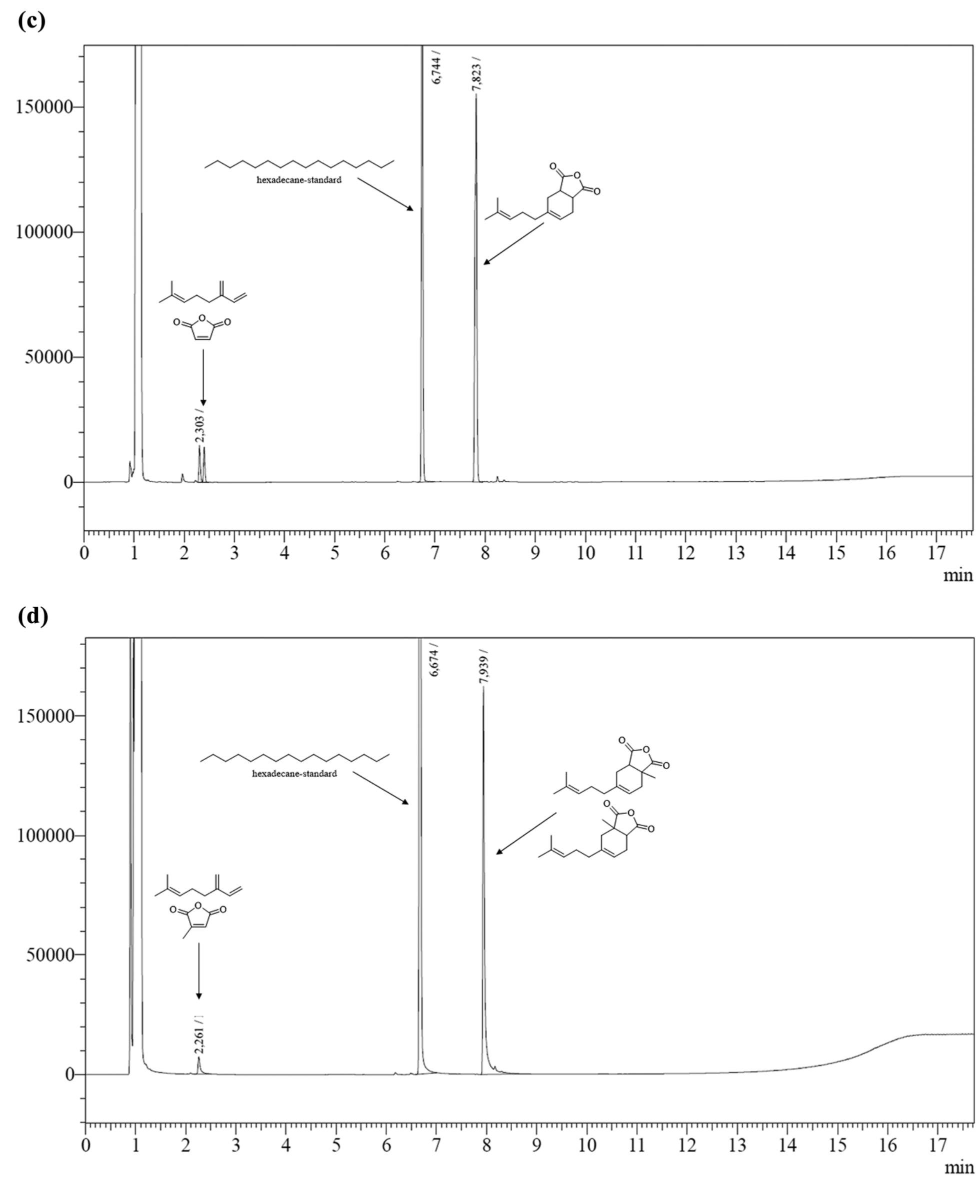

Figure 2: (*Continued*)

GC analysis. Due to the necessity of using aqueous solutions to dissolve the amino acid, the hydrolyzed by-product from ring-opening condensation of the anhydride with water was observed as well (Figure 3b). Success of the amino acid coupling was confirmed by comparing the ^{1}H-NMR spectra of the hydrolyzed and the amino acid-opened product (Figure 3c). Due to the structural and electron-density differences, a chemical shift of one of the former anhydride ring protons is observable. In the hydrolyzed by-product, both protons of the connection between myrcene and anhydride moieties are chemically nearly identical, whereas the protons in the amino acid-opened product lack this equivalency, since one of the acid groups is transformed into an amide function.

Except for the cyclic, secondary amine function of proline, all amino acids were successfully coupled to the anhydride function of the MA intermediate. Best results were obtained for histidine, phenylalanine, and glycine with yields of 85–90% (Table 1). Glutamic acid and aspartic acid could be isolated in 85% and 46% yield, respectively, while the corresponding amides, glutamine and asparagine, performed significantly worse. The reaction was successfully transferred and repeated for selected amino acids, applying CA as a linker molecule. CA possesses an additional methyl group at position 3 of the cyclic anhydride, which results in two different isomeric products after Diels–Alder coupling (Figure 3, structures 2a and b). This leads to the formation of four possible isomeric surfactant products in the

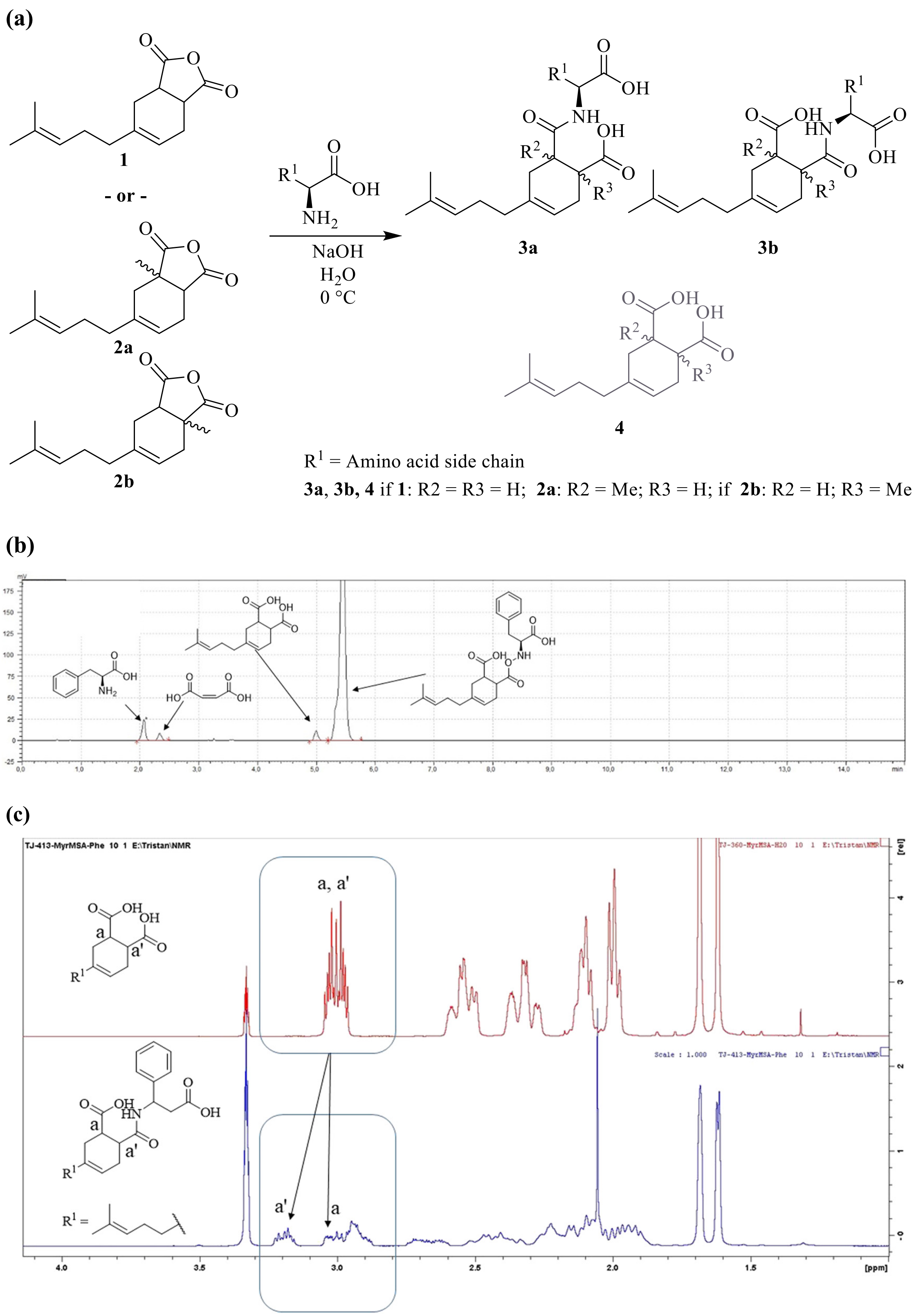

Figure 3: (a) Reaction scheme of Diels–Alder product conversion in condensation reactions with amino acids, (b) evaporative light scatterng detector chromatogram obtained by ring opening of the myrcene–MA intermediate with phenylalanine and (c) comparison of the ^{1}H-NMR spectra of the hydrolysis product (top) and the phenylalanine-coupling product (complete ^{1}H-NMR and ^{1}H–^{1}H-COSY-NMR spectra of the coupling product are shown in Figures S1 and S2).

Table 1: Isolated yields, purities, and physicochemical data of the myrcene–anhydride–amino acid surfactants (amino acids are outlined in 3-letter code)

Diels–Alder adduct	Amino acid	Y_{isol} (%)	Purity (%)	σ (mN·m^{-1})	h_{Foam} initial (mm)	h_{Foam} at 5 min (mm)
(A) Products leading to dicarboxylic surfactants						
Myr-MA	H_2O^a	99	99	60.9 ± 0.4	110	3
Myr-CA	H_2O^a	99	99	56.9 ± 0.2	96	2
Myr-MA	Ala	69	53	54.0 ± 0.4	116	12
Myr-MA	Asn	46	63	45.7 ± 0.3	134	5
Myr-MA	Cys	99	57	34.0 ± 0.6	120	91
Myr-MA	Gln	85	20	56.4 ± 1.2	130	10
Myr-MA	Gly	91	61	56.4 ± 1.2	117	6
Myr-MA	Ile	32	67	54.0 ± 1.3	105	6
Myr-MA	Leu	37	71	44.3 ± 0.2	125	13
Myr-MA	Met	94	88	53.4 ± 2.6	124	2
Myr-MA	Phe	86	84	43.3 ± 0.5	133	2
Myr-CA	Phe	78	68	44.5 ± 0.8	134	35
Myr-MA	Ser	71	93	40.7 ± 1.1	140	100
Myr-MA	Thr	94	92	37.1 ± 1.0	130	76
Myr-MA	Trp	46	90	38.7 ± 0.8	133	102
Myr-MA	Tyr	95	68	42.4 ± 0.6	138	81
Myr-MA	Val	95	59	45.2 ± 0.5	126	47
(B) Products leading to tricarboxylic surfactants						
Myr-MA	Asp	88	50	53.7 ± 0.4	124	2
Myr-MA	Glu	99	81	52.5 ± 4.4	125	2
Myr-CA	Glu	25	82	56.5 ± 0.4	96	9
Myr-MA	R_2Lys^b	93	93	32.5 ± 0.1	131	98
Myr-CA	R_2Lys^b	99	75	31.5 ± 0.8	122	95
(C) Products leading to surfactants with two carboxylic and one basic group						
Myr-MA	Arg	68	93	38.0 ± 0.3	133	98
Myr-CA	Arg	95	50	31.8 ± 0.2	134	46
Myr-MA	His	91	89	56.7 ± 0.6	128	6
Myr-MA	Lys[c]	89	31/42[a]	36.1 ± 0.1	132	97
Myr-CA	Lys[c]	51	91	33.4 ± 0.8	139	92

The corresponding MS spectra can be found in supplementary data Figures S3–S29. Surface tension was analyzed at a surfactant concentration of 4 mmol·l^{-1} and a pH of 5.5 and foam comparison was done after stirring (h_{Foam} initial) and after 5 min without stirring. a = ring opening of Diels–Alder adduct with water; b = a twofold excess of Diels–Alder precursor was applied to synthesize the bi-acylated product and c = in an equimolar approach, lysine-based surfactants were obtained as a mixture of mono- and bi-acylated product.

consecutive ring-opening reaction, which could not be separated in HPLC analysis. Arginine and phenylalanine were converted in yields up to 95% (Table 1).

Lysine was successfully converted to the desired *N*-acylated product, but HPLC- and LC-MS analyses showed the formation of the bi-acylated product as well (Figure 4). In contrast, the alcohol groups in the side chains of tyrosine, threonine, serine, and the thiol group of cysteine did not act as a nucleophile to catalyze ring opening of the anhydride under the chosen reaction conditions and thus no two-fold acylations were observed in these cases. Therefore, only lysine shows the capability to form a gemini-type surfactant with two hydrophobic chains coupled to a single amino acid group, giving access to interesting surfactants with a larger hydrophobic tail [37,38]. As outlined in Table 1, high yields of 93% and 99% were obtained with the MA and CA, when a two-fold excess of lysine was added to the coupling reaction. Further investigations with *tert*-butyloxycarbonyl (boc) protection groups, starting from *Nα*- and *Nε*-boc-lysine, implicate that only *Nα*-boc-lysine was acylated successfully by the addition of the myrcene–MA precursor at the *Nε* group. This observation indicates that the production of the lysine–gemini surfactant probably proceeds via the initial addition of the anhydride to the epsilon-amine function and a subsequent addition of a second anhydride moiety to the remaining alpha-amine.

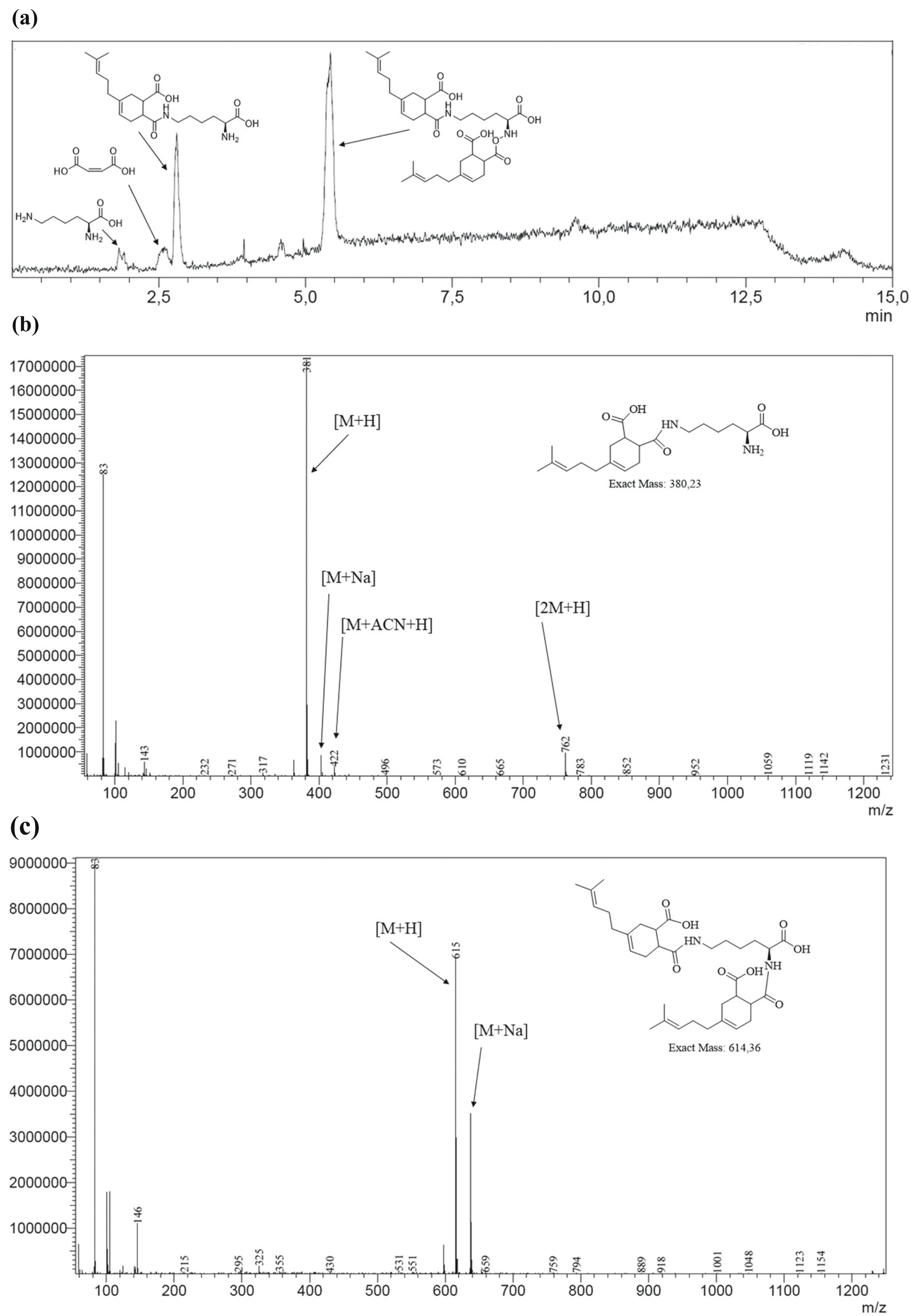

Figure 4: (a) HPLC chromatogram of the product mixture obtained from lysine acylation, (b) + (c) MS spectra of mono-acylated and bi-acylated lysine.

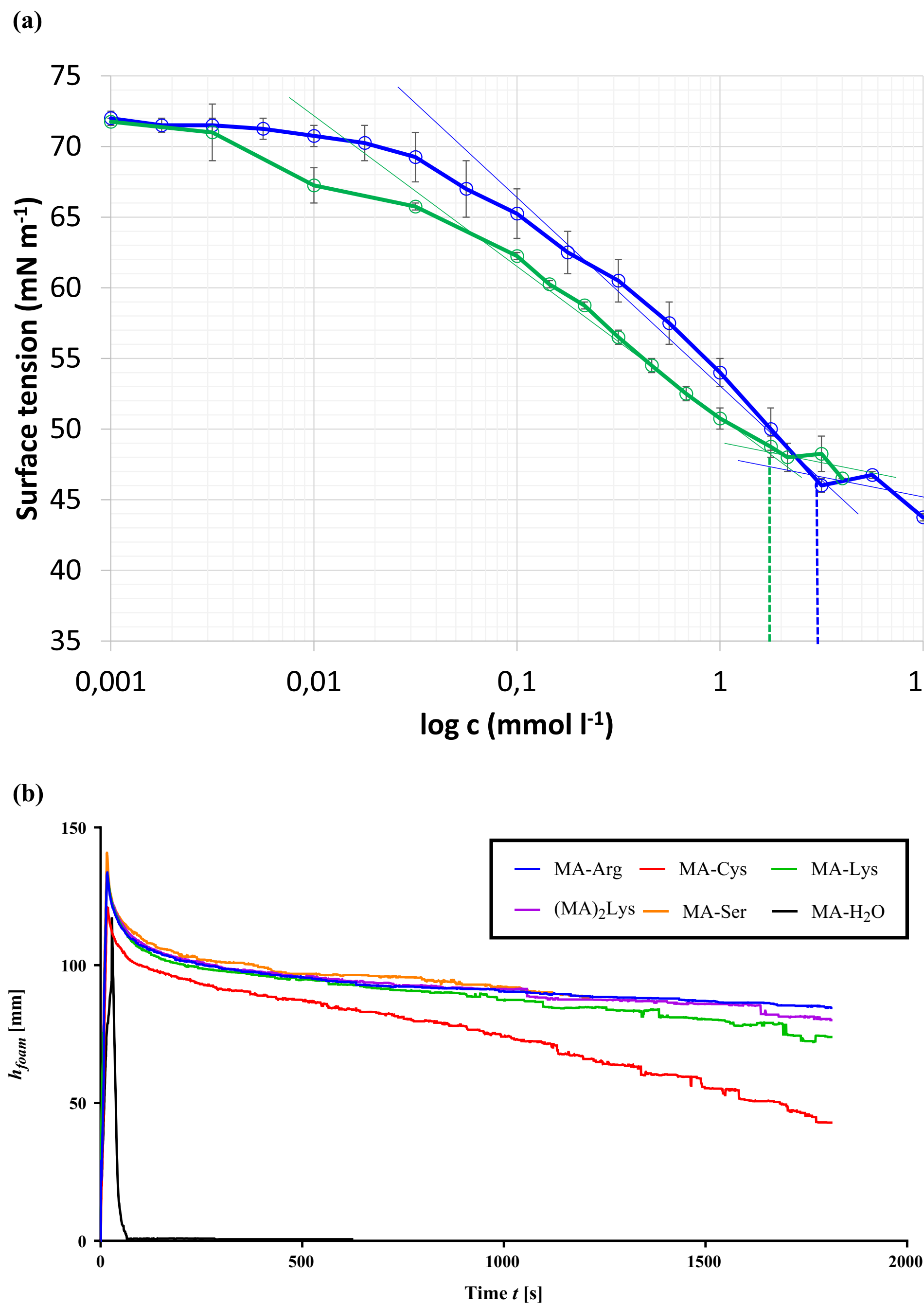

Figure 5: Analysis of surfactant properties of myrcene–MA-based surfactants: (a) Determination of CMC of *N*-(myrcene-MA)-tryptophan (blue) and *N*-(myrcene-MA)-serine (green) with the Wilhelmy plate method and (b) investigation of long-term-foam stability over 30 min of the arginine (Arg), cysteine (Cys), lysine (Lys), and serine (Ser)-based surfactants in comparison to the hydrolysate at 4 mmol^{-1} and pH 5.5.

3.3 Physicochemical properties of the Diels–Alder surfactants

Concentration dependent surface tension measurements were conducted with the Wilhelmy plate method for evaluation of the CMC of *N*-(myrcene-MA)-tryptophan and *N*-(myrcene-MA)-serine at pH 7 (Figure 5a). Similar values of around 2 mmol·l^{-1} for the serine and 3 mmol·l^{-1} for the tryptophan surfactant were obtained, which are close to their maximum solubility. Hence, a surfactant concentration of 4 mmol·l^{-1}, slightly above the CMC, was chosen for surface tension comparison of all amino acid surfactants with the pendant drop method. Analysis of the pH dependence was done at skin-friendly pH 5.5 instead of the

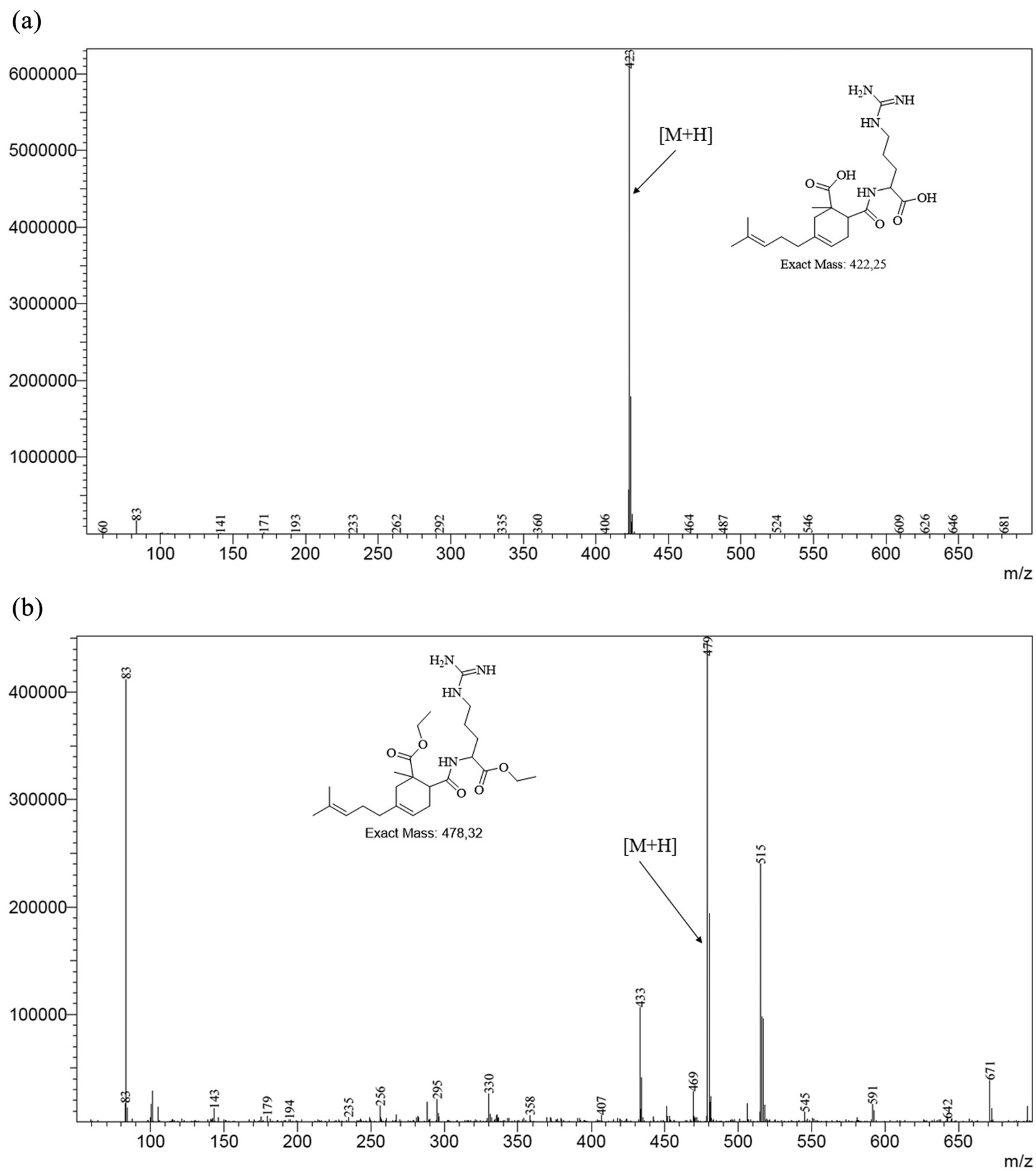

Figure 6: Comparison of MS spectra of (a) *N*-(myrcene-CA)-arginine and (b) *N*-(myrcene-CA)-arginine-OEt after esterification. The spectra for the corresponding MA-based surfactants are shown in Figures S5 and S30.

initially used pH 7 with the pendent drop method over a period of 10 min (Figure 5b). In dependence of the amino acid head group, different effects were observed (Figures S31–S33). While the arginine surfactant was not influenced by pH, surface tension was lower at pH 5.5 for tryptophan, but higher at pH 5.5 for lysine. The lysine-based surfactant gave the lowest surface tension of around 30 $mN{\cdot}m^{-1}$ at pH 7.

Interference of the adjacent carboxylic groups was proven for the water hydrolyzed myrcene–maleic acid product by titration resulting in pK_S values of around 3.8 and 6.8 (Figure S34). Titration of the amino acid surfactants could not resolve the individual pK_S values; nevertheless, a partial protonation at a pH of 5.5 can be expected. The protonation state of the carboxylic groups will result in mixtures of anionic and nonionic surfactant molecules, which influence the surface properties in a pH-dependent manner. The exact ratios of protonated and non-protonated forms and how they influence the surface properties need further investigation.

Table 2: Microbial inhibition of *B. subtilis*, *C. glutamicum*, *E. coli*, and *C. viswanathii* in agar plate tests applying surfactants in a concentration range from 5 to 40 mmol·l^{-1}

Diels–Alder adduct	Amino acid	*B. subtilis*	*C. glutamicum*	*E. coli*	*C. viswanathii*
Myr-MA	Phe	40+/20−	40+/20−	40*X*/20−	nd
Myr-MA	Arg	nd	nd	nd	nd
Myr-MA-OEt	Arg-OEt	40+/20−	40++/20+/10○/5−	40*X*/20−	40+/20○/10−
Myr-CA	Arg	nd	nd	nd	nd
Myr-CA-OEt	Arg-OEt	40+/20−	40++/20+/10+/5○	40+/20−	40+/20−

Note: nd = no inhibition detected at highest concentration, ++ = large inhibition area, + = clearly visible inhibition area, ○ = small inhibition area, *X* = weak inhibition, ambiguous test results and − = no inhibition detected.

For comparison of all MA- and CA-based surfactants at a concentration of 4 mmol·l^{-1}, a pH of 5.5 was chosen, which is a typical value for shampoo and cosmetic preparations (Table 1). A minimal decrease of the surface tensions to final values of 57 and 61 mN·m^{-1} was observed, when the water hydrolyzed Diels–Alder adducts were applied. All amino acid surfactants exhibited at least slightly better surface activities than the water hydrolyzed products and their surface tensions ranged between 31 and 56 mN·m^{-1}. The acidic amino acids, aspartate and glutamate, gave a minimal improvement in comparison to the hydrolysis product, resulting in minimal surface tensions of 52–57 mN·m^{-1}. This leads to the assumption that the hydrophobic tail is too small for a large hydrophilic section bearing a total of three negative charges within the surfactant head group. The finding can be substantiated by the fact that the bi-acylated lysine, which possesses three carboxylic acid groups but a larger hydrophobic moiety due to its gemini-type structure, exhibited the lowest surface tension with values of 31.5 and 32.5 mN·m^{-1} for the CA and MA adducts.

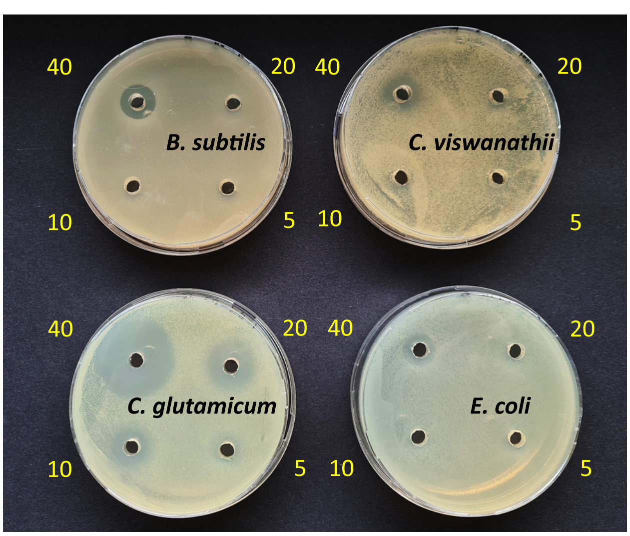

Figure 7: Antimicrobial plate assay exemplarily shown for the fully esterified cationic surfactant *N*-(myrcene-CA)-arginine-OEt in concentrations from 5 to 40 mmol·l^{-1} with *B. subtilis*, *C. glutamicum*, *E. coli*, and *C. viswanathii*.

Application of the non-polar and polar amino acids usually produces surfactants with two negative charges and reach mediocre minimal surface tensions of around 40–50 mN·m^{-1}. Only cysteine and threonine, bearing a thionyl- or a hydroxyl side chain, gave better results of 34.0 and 38.7 mN·m^{-1}. Particularly, the cysteine-based product may be interesting for e.g. cosmetic applications due to its potential antioxidative properties [39]. Upon addition of the basic amino acids, arginine, histidine, and mono-acylated lysine, amphoteric-type surfactants were produced, each with two negative and one positive charge. Good surface activity of 31–38 mN·m^{-1} was obtained for the arginine and lysine derivatives, whereas histidine exhibited a significantly lower surface activity reaching 56.7 mN·m^{-1}. According to Tabohashi et al. [40], comparable amino acid surfactants show similar results with surface tensions around 30 mN·m^{-1}. The slightly lower activity in comparison to lauric acid-based surfactants might be explained by the fact that the Diels–Alder products contain a cyclic moiety, which shortens the length and reduces the flexibility of the hydrophobic tail at comparable carbon count.

Foamability was analyzed with a Krüss DFA100 foam analyzer and foam stability was observed over time after an initial foaming induced by aeration (Table 1). Initial foam build-up was detectable with the water hydrolyzed Diels–Alder adducts, but after stopping the air feed, the foam immediately began to collapse, resulting in an almost complete degradation within 30 s. Coupling of an amino acid alters the steric properties of surfactants and charge of the head group resulting in an increase in surface activity and foamability of the products. The addition of an amino acid to the myrcene–anhydride motive increases the initial foamability by around 30%, yielding initial foam heights between 100 and 140 mm. The amino acid surfactant foam showed better stability in comparison to the hydrolysis product, resulting in good stability over a period of 5 min, especially for the polar amino acids. For arginine, serine, and tryptophan as well as the mono- and bi-acylated lysine foam heights of around 120 mm were detected, while threonine and tyrosine had a residual foam height of 80 mm after 5 min, respectively. Among the non-polar amino acids, only valine shows a moderate foam stability resulting in 40 mm after 5 min. Prolonged foam stability tests for some promising candidates were conducted over a period of 30 min (Figure 5b). Hardly any foam decrease from 10 min onwards was observed for the arginine, lysine, and serine derivatives making them interesting product candidates for applications in shampoo formulations, where foam is a desired feature [41]. As expected from their similar structure, the citraconic-anhydride-based derivatives gave similar foaming results. In all cases low surface tension values correlated with good foam stability.

3.4 Synthesis of cationic surfactants and evaluation of antimicrobial properties

Surfactants with the cationic amino acid arginine are known to exhibit antimicrobial activity and LAE is applied in industrially relevant scale [42,43]. Since the Diels–Alder-based products exhibit similar structural motives, esterification was performed with ethanol and TMS-Cl as a catalyst to obtain the fully esterified cationic surfactants. After evaporation of ethanol and trimethyl silyl derivatives, mass spectrometric analyses revealed the successful transformation as outlined in Figure 6 for *N*-(myrcene-CA)-arginine and its ethyl ester. Following a greener pathway, future syntheses may be conducted by application of esterified amino acids or by esterification of the surfactant product following the protocol of Turhanen et al. for both approaches [44].

Antimicrobial activity was analyzed against the yeast *C. viswanathii, E. coli* as Gram-negative and *C. glutamicum* and *B. subtilis* as Gram-positive bacteria. *C. glutamicum* was chosen, because its cell wall structure differs significantly from that of *B. subtilis* containing a mycolate outer membrane similar to the pathogenic bacterium *Mycobacterium tuberculosis* [45]. The series of MA-based surfactants shown in Table 1 was tested in preliminary assays against the bacterial cultures. Here, only *N*-(myrcene-MA)-phenylalanine exhibited some antimicrobial activity (Table 2). Similarly, a weak inhibition of several microorganisms was observed with the structurally related lauroyl-phenylalanine [46]. As expected from literature results [43,44,46], the cationic compounds *N*-(myrcene-CA)-arginine-OEt and *N*-(myrcene-MA)-arginine-OEt exhibited a better antimicrobial activity than the non-esterified counterparts (Figure 7 and Table 2). In comparison, the positive control LAE showed stronger antimicrobial effects, which may be attributed to the chain length differences of the surfactants. Tailoring of the hydrophobic chain, either by exchanging the diene component or choosing a different alcohol for esterification, may enhance the antimicrobial properties of the Diels–Alder-based surfactants. In accordance with Joondan et al. [47], the esterified cationic compounds were more active against Gram-positive, especially against *C. glutamicum* than Gram-negative *E. coli*. Interestingly, the cationic esters also showed activity against *C. viswanathii* and upon structural optimization, this class of compound might be utilized as antifungal agents.

4 Conclusions

Novel bio-based amino acid surfactants were synthesized in good yields and with high atom efficiency in a two- or three-step approach combining Diels–Alder cyclization, ring-opening condensation with amino acids, and esterification of the remaining carboxylic groups. Variation of the renewable diene, anhydride intermediate, amino acid, and alcohol moiety opens up a large structural space for specific tailoring of surfactant properties. In this study, the β-pinene-derived monoterpene myrcene was selected as an example diene for the synthesis of a variety of anionic, amphoteric, and cationic surfactants. In particular, arginine-, lysine-, and cysteine-based products showed promising surfactant behavior, and the cationic arginine derivatives exhibited antimicrobial properties. In summary, this new class of surfactant has the potential to expand the spectrum of mild and environmentally benign amino acid surfactants for skin and hair care applications. Focusing future research on renewable dienes could lead to more hydrophobic surfactants with potentially interesting application profiles.

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References

[1] Le Guenic S, Chaveriat L, Lequart V, Joly N, Martin P. Renewable surfactants for biochemical applications and nanotechnology. J Surfactants Deterg. 2019;22:5–21. doi: 10.1002/jsde.12216.

[2] Bhadani A, Kafle A, Ogura T, Akamatsu M, Sakai K, Sakai H, et al. Current perspective of sustainable surfactants based of renewable building blocks. Curr Opin Colloid Interface Sci. 2020;45:124–35. doi: 10.1016/j.cocis.2020.01.002.

[3] Hampel M, Moreno-Garrido I, Sobrino C, Lubián LM, Blasco J. Acute toxicity of LAS homologues in marine microalgae: Esterase activity and inhibition growth as endpoints of toxicity. Ecotoxicol Environ Saf. 2001;48:284–92. doi: 10.1006/eesa.200.2028.

[4] Staples CA, Klecka GM, Naylor CG, Losey BS. C8- and C9-Alkylphenols and Ethoxylates: I. Identity, Physical Characterization, and Biodegradation Pathways Analysis. Hum. Ecol Risk Assess Int J. 2008;14:1007–24. doi: 10.1080/10807030802387705.

[5] Routledge EJ, Sumpter JP. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Envrion Toxicol Chem. 1996;15:241–8. doi: 10.1002/etc.5620150303.

[6] Schörken U, Barbe S, Hahn T, Zibek S. Biotechnological routes towards bio-based surfactants: State of the art and future challenges. SOFW-J. 2017;05:18–30, https://www.sofw.com/en/shop/books/product/319-sofw-journal-05-2017-english-print.

[7] Willing A, Messinger H, Aulmann W. Ecology and toxicology of alkyl polyglycosides. In: Zoller U, editor. Handbook of Detergents, Part B Environmental Impact. New York: Marcel Dekker; 2004. p. 487–521.

[8] Patel DV, Patel MN, Dholakia MS, Suhagia BN. Green synthesis and properties of arginine derived complexes for assorted drug delivery systems: A review. Sustain Chem Pharm. 2021;21:100441. doi: 10.1016/J.scp.2021.100441.

[9] Wang N, Yao K, Wang Y, Ti J, Tan J, Liu C, et al. Green synthesis, characterization, and properties of acyl lysine, serine, threonine, and methionine derived from three types of natural oils. J Surfactants Deterg. 2020;23:239–50. doi: 10.1002/jsde.12365.

[10] Wang C, Zhang P, Chen Z, Liu Y, Zhao L, Wang N, et al. Effects of fatty acyl chains on the interfacial rheological behaviors of amino acid surfactants. J Mol Liq. 2021;325:114823. doi: 10.1016/j.molliq.2020.114823.

[11] Yan T, Feringa BL, Barta K. Direct *N*-alkylation of unprotected amino acids with alcohols. Sci Adv. 2017;3:1–8. doi: 10.1126/sciadv.aao6494.

[12] Pérez L, Pinazo A, Pons R, Infante M. Gemini surfactants from natural amino acids. Adv Colloid Inerface Sci. 2014;205:134–55. doi: 10.1016.j.cis.2013.10.020.

[13] Morán MC, Pinazo A, Pérez, Clapés P, Angelet M, García MT, et al. "Green" amino acid-based surfactants. Green Chem. 2004;6:233–40. doi: 10.1039/BH400293H.

[14] Takehara M, Yosihimura I, Takizawa K, Yoshida R. Surface active *N*-acylglutamate: I. Preparation of long chain *N*-acylglutamic acid. J Am Oil Chem Soc. 1972;49:157–61. doi: 10.1007/BF02633785.

[15] Sander A, Eilers E, Heilemann A, von Kries E. Herstellung und Anwendungsmöglichkeiten von Eiweiß-Fettsäurekondensaten. Fett/Lipid. 1997;99:115–20. doi: 10.1002/lipi.19970990404.

[16] Bordes R, Tropsch J, Holmberg K. Counterion specificity of surfactants based on dicarboxylic amino acids. J Colloid Interface Sci. 2009;338:529–36. doi: 10.1016/j.cis.2009.06.032.

[17] Joondan N, Jhaumeer-Laulloo S, Caumul P, Akerman M. Synthesis, physicochemical, and biological activities of novel *N*-acyl tyrosine monomeric and Gemini surfactants in single and SDS/CTAB–mixed micellar system. J Phys Org Chem. 2017;30:1–13. doi: 10.1002/poc.3675.

[18] Moldes AB, Rodríguez-López L, Rincón-Fontán M, López-Prieto A, Vecino X, Cruz JM. Synthetic and bio-derived surfactants versus microbial biosurfactants in the cosmetic industry: An overview. Int J Mol Sci. 2021;22:2371. doi: 10.3390/ijms22052371.

[19] Takakura Y, Asano Y. Purification, characterization, and gene cloning of a novel aminoacylase from *Burkholderia sp*. Strain LP5_18B that efficiently catalyzes the synthesis of *N*-lauroyl-L-amino acids. Biosci Biotechnol Biochem. 2019;83:1964–73. doi: 10.1080/09168451.2019.1630255.

[20] Haeger G, Wirges J, Tanzmann N, Oyen S, Jolmes T, Jaeger K-E, et al. Chaperone assisted recombinant expression of a mycobacterial aminoacylase in *Vibrio natriegens* and *Escherichia coli* capable of *N*-lauroyl-L-amino acid synthesis. Microb Cell Fact. 2023;22:77. doi: 10.1186/s12934-023-02079-1.

[21] Haeger G, Jolmes T, Oyen S, Jaeger K-E, Bongaerts J, Schörken U, et al. Novel recombinant aminoacylase from *Paraburkholderia monticola* capable of *N*-acyl-amino acid synthesis. Appl Microbiol Biotechnol. 2024;108:93.

[22] Diels O, Alder K. Synthesen in der hydroaromatischen Reihe. Liebigs Ann Chem. 1927;31:98–122. doi: 10.1002/jlac.19284600106.

[23] Diels O, Alder K. Synthesen in der hydroaromatischen Reihe. XII. Mitteilung. ("Dien-Synthesen" sauerstoffhaltiger Heteroringe. 2. Dien-Synthesen des Furans.). Liebigs Ann Chem. 1928;490:243–57. doi: 10.1002/jlac-19314900110.

[24] Littmann ER. Terpene-maleic anhydride resins. Ind Eng Chem. 1936;28:1150–2. doi: 10.1021/ie50322a005.

[25] Kim EM, Eom JH, Um Y, Kim Y, Woo HM. Microbial synthesis of myrcene by metabolically engineered *Escherichia coli*. J Agric Food Chem. 2015;63:4606–12. doi: 10.1021/acs.jafc.5b01334.

[26] Marmulla R, Harder J. Microbial monoterpene transformations-a review. Front Microbiol. 2014;5:1–14. doi: 10.3389/fmicb.2014.00346.

[27] Behr A, Johnen L. Myrcene as a natural base chemical in sustainable chemistry: A critical review. ChemSusChem. 2009;2:1072–95. doi: 10.1002/cssc.200900186.

[28] Wojcieszak R, Santarelli F, Paul S, Dumeignil F, Cavani F, Goncales RV. Recent developments in maleic acid synthesis from bio-based chemicals. Sustain Chem Process. 2015;3:1–11. doi: 10.1186/s40508-015-0034-5.

[29] Cucciniello R, Cespi D, Riccardi M, Neri E, Passarini F, Pulselli FM. Maleic anhydride from bio-based 1-butanol and furfural: A life cycle assessment at the pilot scale. Green Chem. 2023;25:5922–35. doi: 10.1039/d2gc03707f.

[30] Crowell J. Prod Itaconic Citraconic Anhydrides. 1941. US2258947.

[31] Galanti MC, Galanti AV. Kinetic study of the isomerization of itaconic anhydride to citraconic anhydride. J Org Chem. 1982;47:1572–4. doi: 10.1021/jo00347a041.

[32] Hornung CH, Álvarez-Diéguez MÁ, Kohl TM, Tsanaktsidis J. Diels–Alder reactions of myrcene using intensified continuous-flow reactors. Beilstein J Org Chem. 2017;13:120–6. doi: 10.3762/bjoc.13.15.

[33] Yang X, Li S, Xia J, Song J, Huang K, Li M. Novel renewable resource-based UV-curable copolymers derived from myrcene and tung oil: Preparation, characterization and properties. Ind Crop Prod. 2015;63:17–25. doi: 10.1016/j.indcrop.2014.10.024.
[34] Tabor R, Bernhardt RJ, Luxem FJ, Yao C, Wallace GJ. Surfactants Solvents containing Diels–Alder Adducts. 2012. WO2013/148842 A1.
[35] Walker D, Robert E. Unsatur Surfactants. 2004. WO 2004/096965 A1.
[36] Takaishi T, Izumi M, Ota R, Inoue C, Kiyota H, Fukase K. Product Selectivity of Esterification of L-Aspartic Acid and L-Glutamic Acid Using Chlorotrimethylsilane. Nat Prod Comm. 2017;12(2):247–9. doi: 10.1177/1934578X1701200227.
[37] Bordes R, Holmberg K. Amino acid-based surfactants - Do they deserve more attention? Adv Colloid Interface Sci. 2015;222:79–91. doi: 10.1016/j.cis.2014.10.013.
[38] Branco MA, Pinheiro L, Faustino C. Amino acid-based cationic gemini surfactant-protein interactions. Colloids Surf A Physicochem Eng Asp. 2015;480:105–12. doi: 10.1016/j.colsurfa.2015.12.022.
[39] Ohta A, Hossain F, Asakawa H, Asakawa T. Study of the antioxidative properties of several amino acid-type surfactants and their synergistic effect in mixed micelle. J Surfactants Deterg. 2020;23:99–108. doi: 10.1002/jsde.12355.
[40] Tabohashi T, Kazuhiko T, Kazutami S, Kouchi J, Yokoyama S, Sakai H, et al. Solution properties of amino acid-type new surfactant. Colloids Surf B Biointerfaces. 2001;20:79–86. doi: 10.1016/S0927-7765(00)00170-3.
[41] Bureiko A, Trybala A, Kovalchuk N, Starov V. Current applications of foams formed from mixed surfactant–polymer solutions. Adv Colloid Interface Sci. 2015;222:670–7. doi: 10.1016/j.cis.2014.10.001.
[42] Molinero J, Julia MR, Erra P, Robert M, Infante MR. Synthesis and properties of Nα-lauroyl-L-arginine dipeptides from collagen. J Am Oil Chem Soc. 1988;65:975–8. doi: 10.1007/BF02544523.
[43] Czakaj A, Jarek E, Krzan M, Warszynski P. Ethyl lauroyl arginate, an inherently multicomponent surfactant system. Molecules. 2021;26:5894. doi: 10.3390/molecules26195894.
[44] Turhanen PA, Leppänen J, Vepsäläinen JJ. Green and efficient esterification method using dried dowex H^{+}/NaI approach. ACS Omega. 2019;4:8974–84. doi: 10.1021/acsomega.9b00790.
[45] Marchand CH, Salmeron C, Raad RB, Méniche X, Chami M, Masi M. Biochemical disclosure of the mycolate outer membrane of *Corynebacterium glutamicum*. J Bacteriol. 2012;194(3):587–97. doi: 10.1128/jb.06138-11.
[46] Pérez L, García MT, Pinazo A, Pérez-Matas E, Hafidi Z, Bautista E. Cationic surfactants based on arginine-phenylalanine and arginine-tryptophan: Synthesis, aggregation behavior, antimicrobial activity, and biodegradation. Pharmaceutics. 2022;14(12):2602. doi: 10.3390/pharmaceutics14122602.
[47] Joondan N, Jhaumeer-Laulloo S, Caumul P. A study of the antibacterial activity of L-Phenylalanine and L-Tyrosine esters in relation to their CMCs and their interactions with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC as model membrane. Microbiol Res. 2014;169(9–10):675–85. doi: 10.1016/j.micres.2014.02.010.