**Synthesis, Self-Assembly, Bacterial and Fungal Toxicity,
and Preliminary Biodegradation Studies of a Series of
L-Phenylalanine-Derived Surface Active Ionic Liquids[†]**

*Illia V. Kapitanov^{a,†}, Andrew Jordan^{b,†}, Yevgen Karpichev^a, Marcel Spulak^c, Lourdes Perez^d
Andrew Kellett^b, Klaus Kümmerer^e, Nicholas Gathergood^{d,*}*

^a Department of Chemistry and Biotechnology, Tallinn University of Technology,
Academia tee 15, 12618 Tallinn, Estonia

^b School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland

^c Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Heyrovského 1203,
Hradec Kralove, Czech Republic, CZ500 05

^d Department of Chemistry & Surfactants Technology, CSIC Institute of Advanced Chemistry of
Catalonia (IQAC), Jordi Girona 18-26, ES-08034 Barcelona, Spain

^e Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg,
Scharnhorststraße 1, D-21335 Lüneburg, Germany.

[†]*contributed equally to the publication*

Authors for correspondence:

Prof. Nicholas Gathergood, phone: +372 063 570-8636; fax: +372 062 311-6830;

E-mail: nicholas.gathergood@taltech.ee

[†] Electronic supplementary information (ESI) available. See DOI: _____

Abstract

We report for the first time a comprehensive study on the synthesis (supported by green chemistry metrics), aggregation properties, bacterial/fungal toxicity and preliminary data on biodegradation of a series of 24 L-phenylalanine derived SAILs. The cationic headgroup variation included pyridinium, imidazolium, and cholinium groups and lead to a comprehensive analysis of the effect of the alkyl ester chain (from C₂ to C₁₆) on the synthesis, toxicity, biodegradability, and surfactant properties of the novel SAILs. The evaluation of the SAILs revealed a large variety of properties were strictly dependent of the side chain length, including their bacterial and fungal toxicity (from non-toxic to toxic), and aggregation properties. Addition of the L-phenylalanine moiety which connects the lipophilic side chain to the cationic head group results in that the phenyl group essentially contributes to the self-assembling properties. The interplay of dispersion interactions of phenyl ring and the side chain hydrophobicity allows us to rank the novel SAILs (thus identifying the remarkable ones) as compared to other surfactants. The CMC values for the SAILs reported in this study are significantly (up to 10 times) lower than reported for conventional surfactants with the same length of the side chain. The adsorption and micellization are among the factors affecting toxicity of studied SAILs. Preliminary biodegradation studies have shown that no clear trend was observed when comparing the Closed Bottle Test results of the SAIL C₂ and C₁₀ derivatives. The medium chain length (C₆ to C₈) pyridinium SAILs have been recommended as the most prospective green alternatives for conventional surfactants. These findings can contribute to designing new efficient amphiphiles with optimized antimicrobial activities and to employ them as environmentally benign and potentially mineralisable surfactants.

Over the past decades, the principles of green chemistry have been proposed to develop a new approach for the design and implementation of chemical products and processes¹⁻³. Ionic liquids (ILs) are increasingly seen as an integral part of green chemistry applications⁴⁻⁶ due to their specific properties as solvents⁷, potential for high recyclability⁸, low toxicity⁹⁻¹¹, and synthesis from renewable resources^{12, 13}.

The overlap of surfactants and IL chemistry is one that has been clearly observed from the very beginning of the current upsurge in IL research and development⁴. Indeed, diluted solutions of ILs containing ions with long chains can exhibit self-aggregations similar to that of conventional surfactants. The unique properties of these surface-active ionic liquids (SAILs), in particular, abnormally low melting point and/or low Krafft temperature, gives an obvious advantage to the IL-based surfactants and fosters a growing interest to synthesize and study the self-organization of SAILs.¹⁴⁻¹⁸ This resulted in an increase in studying miscellaneous applications of SAILs, including biomass extraction¹⁹ and micellar catalysis of various organic reactions.²⁰⁻²²

Since the implementation of the EU regulation on detergents,²³ investigations into the ultimate biodegradability of surfactants have been a requirement that has driven a new wave of research on the preparation of biodegradable SAILs.²⁴⁻²⁶ These strategies employed to promote biodegradation are well documented, including many cases utilising hydrolysable ester bonds and cleavable amide links.²⁷ The legislation intends that for a surfactant that does not ultimately undergo biodegradability, the derogation may be applied for depending on the end use application, and the calculated risk to the environment and health by the expected use of a surfactant. Depending on the concentration in the aquatic environment, surfactants (cationic quaternary ammonium salts, in essence²⁸) can be toxic for living (micro)organisms.²⁹ The adsorption of surfactants may cause the disruption of the cellular membranes and result in acute or chronic effects on sensitive organisms through different nonspecific modes of action. The hydrophobic/hydrophilic balance of the molecule and the cationic charge density affect the antimicrobial activity.³⁰

Therefore, an ecotoxicological study of surfactants is essential to establish safe concentrations for the environment and compare with predicted or measured environmental concentrations.³¹ Hence, gathering of additional ecotoxicity data is essential to ascertain the balance of toxicity vs efficiency.³²

An imbalance in the IL research field between the assessment of the environmental impacts of ILs and the impact of manufacturing ILs has recently been pointed out.³³ Some confusion has arisen from the estimation of the ready biodegradability test as an indicator for

ultimate degradation, whereas the life cycle assessment is often considered as a “gold standard method” for determining whether a method or technology is green. Therefore, when designing a new green ILs, undertaking a comprehensive yet feasible green chemistry assessment, still remains a major challenge.³⁴

ILs based on the amino acids can be considered as one of the green alternatives.¹² Synthesis, physicochemical properties and industrial applications of amino acid surfactants demonstrate wide commercial opportunities for this ever-growing class of surfactants.^{35, 36} Special attention should be paid to the plausible synergetic effect of a combination of ILs containing natural biologically active molecules, as compared with conventional ILs³⁷. Development of green ionic liquids demands toxicological studies to facilitate a favourable outcome.³⁸ Toxicity of cationic surfactants towards (micro)organisms is generally considered to be primarily related to the sorption of a surfactant molecule to biological membranes, which is driven by the nonspecific hydrophobic interactions.^{39, 40} Since the free energy of adsorption of cationic surfactants (including SAILs) largely increases with the hydrophobic chain length⁴¹, migration rate of the surfactant to the cell wall interface with the lengthening of the alkyl chain also increases. The longer the alkyl chain, the easier the molecule can insert through the polar head-group region of the membrane bilayer inducing membrane damage and cell death⁴²⁻⁴⁵. At the same time, maximum biological effect at a specific chain length is a result of the combination of various physicochemical parameters (e.g. hydrophobicity, adsorption, CMC value, transport across the membrane, solubility in the aqueous media), complicated by the problem that some of these parameters may show opposite trends.^{41, 46} The antimicrobial activity of cationic amino acid derived SAILs depends on their structures and size (specifically, the amino acid residue and the chain length are key parameters), the molecule hydrophilic/lipophilic balance, and the cationic charge density.³⁰

Recently, we reported a ‘benign by design’⁴⁷ approach for the elaboration of L-phenylalanine ethyl ester platform for designing completely mineralizable ILs^{48, 49}, see Fig.1 (see also Fig. 3 in *rf.*⁴⁹ for IL cations variation). The biodegradation was investigated with a modified Closed Bottle test based on OECD guideline 301D and liquid chromatography method combined with high-resolution mass spectrometry analysis to identify the chemical structures of products resulting from incomplete biodegradation.

In the present work, we undertook to prepare green potentially degradable SAILs by structural modification of the recently reported mineralizable ILs (see Fig. 1). We report for the first time a comprehensive study on the synthesis (supported by green chemistry metrics), aggregation properties, bacterial/fungal toxicity and preliminary data on biodegradation of L-

phenylalanine based SAILs. The cationic headgroup variation included pyridinium, imidazolium, and cholinium to enable a comprehensive analysis of the effect of the alkyl ester chain on synthesis, toxicity, biodegradability, and surfactant properties of the newly synthesized SAILs. For the detailed associated biodegradation study see Kümmerer et.al.⁵⁰

Fig. 1. Structures of L-phenylalanine based ILs studied.

Results and Discussion

The main objective of the study is to determine if green surfactants can be identified by evaluating their synthesis, microbial toxicity and aggregation properties. A preliminary biodegradation study is also included in the assessment. The results and discussion section is therefore split into the aforementioned subsections.

As increased microbial activity is linked to lipophilicity of SAILs²⁸, which is assumed to increase with the extension of the side chain, a comprehensive study is required in which the extent of surface activity of the SAILs is accompanied with their toxicity (bacterial and fungal) and biodegradability evaluation. In essence, which compounds in the series of studied cationic SAILs demonstrate remarkable surfactant properties, while are also considered as not exhibiting high toxicity. The result is that we can identify the greener surfactant chemicals.

Our hypothesis as shown in Fig. 2 is that cleavage of the amide bond vs ester bond in the IL molecule (Fig. 1) will lead eventually to mineralizable transformation products (TPs). This has previously been shown as the breakdown pathway of pyridinium ethyl ester derivatives,^{48, 49}

Fig. 2. Proposed first step of biodegradation of L-Phe derived ILs with pyridinium cation resulting in biodegradable TPs (for $\text{R} = \text{C}_2$ see ^{48, 49})

Synthesis

L-Phenylalanine derived surfactants were synthesised in three stages using methods similar to those reported earlier^{48, 49, 51}, see Scheme 1, Experimental Section, and ESI.

Scheme 1. A synthetic route of the ILs (17-40) via esters (1-8) and alkylating reagents (9-16).

The first step involved acid catalysed esterification of L-Phe with a fatty alcohol using p-toluene sulfonic acid (PTSA) to give esters (1 – 8).⁵² Next was alkylation of the esters using bromoacetyl bromide to form alkylating reagents (9 –16). Last was the alkylation of pyridine,

dimethylethanolamine (DMEA) or 1-methylimidazole to furnish the respective ionic liquid surfactants (**17** – **40**). The molecular structures of the products synthesis are shown in Scheme 1. All SAILs (**17**–**40**) were white solids with melting points in the range 56–120 °C except for **26** which was a liquid at room temperature. It is noted that the isolation of solid SAILs in high purity (as confirmed by ¹HNMR) is easier and more convenient compared to that of high viscosity ILs. Detailed synthesis and characterisation of the products is included in the Experimental part and ESI.

Green Chemistry Metrics

Green chemistry metrics are an important benchmark with which to analyse a synthetic route to a target. A green chemistry metric approach described by Clark et al.⁵³ has been used for calculation of the parameters shown in Table 1 (L-phenylalanine esters and pyridinium SAILs) and Table S2 (alkylating agents; imidazolium and cholinium SAILs).

The Yield, conversion and selectivity for all the steps of Phe ILs synthesis were high (>89%), and according to Clark et al.⁵³ they can be marked with a green flag. (See ESI) Atom economy (AE), reaction mass efficiency (RME) and optimum efficiency (OE) in case of ester **1-8** and salts **17-40** were no less than 91.5% (AE), 83.3% (RME) and 87.5 % (OE), correspondingly. For synthesis of alkylation agents **9-16**, the parameters of AE, RME and OE were lower than in the case of ester and salt synthesis due to the formation of stoichiometric quantities of HBr (AE 79.5-86.3%; RME 64.0-75.9; OE 80.5-88.6%). Analysis of process mass intensity (PMI) parameters has demonstrated that a major contribution to PMI total parameter is due to PMI workup solvents. Thus, workup solvent use has been identified as the problem of the synthetic procedure which should be tackled first in future studies.

Concerning solvents which have been used for reactions and workup, the most problematic were DCM, petroleum ether and diethyl ether (red flag), then more acceptable, toluene and acetonitrile (amber flag), and finally non-problematic – water (green flag). After synthesis and workup the problematic solvents were recovered with yield in the range 80-85%. Due to the low boiling point temperature of DCM, petroleum ether and diethyl ether, recovery does not have a high energy demand. The substitution of problematic solvents with greener alternatives (e.g. replacing DCM with ethyl acetate for the extraction of ester **1-8** and alkylation agents **9-16**) had a significant negative effect. Previously high product yields decreased to low-moderate (35-60%) for esters and moderate (50-80%) for alkylation agents due to the difficulties separating the water-ethyl acetate phases during the workup step. Formation of a stable emulsion hindered product isolation as well as lose of product into the aqueous phase. Our final optimised synthetic

procedure used less favorable (from a green chemistry point of view) solvents, but incorporation of a solvent recovery step lead to a positive green chemistry metrics result overall, including high yields. In the reactions we also did not use critical elements (with only 5-50 years supply remaining). Energy parameters were marked with a green flag (e.g. reaction run between 0 to 70°C for the synthesis of alkylation agents **9 - 16** and salts **17 - 40**) or amber flag (e.g. reaction run between -20 to 0 °C or 70 to 140°C for the synthesis of esters **1 - 8**).

Improvements in the green chemistry metrics compared to our previous study of ethyl ester derivatives are as follows. The yields are consistently excellent for **1-8** (97-99%) compared to (43-99%) for ethyl derivatives. For the acyl halides the range of yields improved from 54-89% to 93-98% (See Table S2). Also, for the Phe ILs the yield improved from 13-98% to 91-98% (e.g. pyridinium IL **22**, 98% yield, See Table 1). The atom economy for the last step is 100% as the halogen atom is incorporated into the product as the bromide anion. Atom economy for the synthesis of amino esters and acyl halides increases from C₂ to C₁₆ (91.5 to 95.6 and 79.5 to 86.3, respectively). This is due to the increased MW of the product as sidechain increases in length (from C₂ to C₁₆), however the same by-product is formed (water or HBr). In this evaluation we do not consider (based solely on atom economy) that the synthesis of the C₁₆ derivative is greener than C₂, but that they are comparable. Analysis of the PMI data for all compounds prepared shows three distinct groups (Table 1 and S2). In the last step, PMI contribution from reaction and workup components is approximately equal. Future efforts to reduce solvent use should investigate both reaction solvent and work-up solvents requirements. Considering the PMI data for the amino ester synthesis, the work-up solvents value is the dominant contributor, therefore the work-up should be targeted for reducing solvent use. Synthesis of the acyl bromides also has a larger PMI contribution due to work-up solvents albeit not as overriding as observed for the aminoester synthesis. (Table S2)

Table 1. Green Chemistry metrics calculated for pyridinium SAILs (**17-24**) and their precursor L-phenylalanine esters (**1-18**).

Compound s (chain length)	Yield	Conversion	Selectivity	AE	RME	OE	PMI total	PMI Reaction	PMI reactants, reagents, catalyst	PMI reaction solvents	PMI Workup	PMI Workup chemical	PMI workup solvents

1 (C2)	97.0	100.0	97.0	91.5	88.9	97.2	40.0	4.9	2.4	2.5	35.1	1.2	33.8
2 (C4)	97.0	100.0	97.0	92.5	84.5	91.4	47.0	8.9	2.2	6.7	38.1	1.1	36.9
3 (C6)	98.0	100.0	98.0	93.3	85.0	91.1	41.4	8.0	2.1	5.9	33.4	1.0	32.4
4 (C8)	98.0	100.0	98.0	93.9	84.5	90.0	37.4	7.3	2.0	5.3	30.1	0.9	29.2
5 (C10)	99.0	100.0	99.0	94.4	85.1	90.1	33.7	6.7	1.9	4.8	27.0	0.8	26.2
6 (C12)	97.1	100.0	97.1	94.9	83.3	87.8	31.6	6.3	1.9	4.4	25.2	0.7	24.5
7 (C14)	98.9	100.0	98.9	95.3	84.7	88.9	28.7	5.8	1.8	4.0	22.9	0.7	22.2
8 (C16)	97.9	100.0	97.9	95.6	83.7	87.5	27.0	5.6	1.8	3.8	21.4	0.6	20.8

17 (C2)	94.1	100.0	94.1	100.0	94.1	94.1	10.7	4.9	1.1	3.9	5.8	0.0	5.8
18 (C4)	93.0	100.0	93.0	100.0	93.0	93.0	10.2	4.7	1.1	3.6	5.5	0.0	5.5
19 (C6)	97.0	100.0	97.0	100.0	97.0	97.0	9.2	4.3	1.0	3.3	4.9	0.0	4.9
20 (C8)	95.9	100.0	95.9	100.0	95.9	95.9	8.8	4.2	1.0	3.1	4.7	0.0	4.7
21 (C10)	95.9	100.0	95.9	100.0	95.9	95.9	8.4	4.0	1.0	2.9	4.4	0.0	4.4
22 (C12)	98.0	100.0	98.0	100.0	98.0	98.0	10.5	5.1	1.0	4.0	5.5	0.0	5.5
23 (C14)	96.0	100.0	96.0	100.0	95.9	95.9	10.3	5.0	1.0	3.9	5.3	0.0	5.3
24 (C16)	97.0	100.0	97.0	100.0	97.0	97.0	9.7	4.7	1.0	3.7	5.0	0.0	5.0

Microbial Toxicity

Antimicrobial activity denotes a desirable biological activity while toxicity denotes a negative behaviour. The following results report our efforts to identify low/moderate microbial toxicity surfactants in our SAIL series (**17-40**). We also acknowledge that nowadays, given the huge problem with resistant bacteria, it is of great interest to prepare new antimicrobial and antifungal surfactants. As such, one can consider compounds shown to have high microbial toxicity as potential drug discovery 'hit compounds' from an antimicrobial activity perspective. The priority of this work however, is the development of greener (inc. low microbial toxicity) surfactants.

Bacterial toxicity

The *in vitro* antibacterial effect of the linear alkyl ILs (**17-40**) and corresponding Phe esters (**1-8**) were investigated. The results collected in Table 2 and Table S3 demonstrate a broad spectrum of bacterial toxicity for the compounds screened. Phe esters (**1-8**) do not demonstrate toxicity as respect to Gram-negative bacteria. This behaviour is probable due to the cationic charge of these molecules, although these compounds are pH/sensitive, and the cationic charge depends of the pKa of the molecule and the pH of the medium. For the SAILs (**17-40**), a clear trend of increasing toxicity with alkyl chain elongation is observed up to certain chain length, irrespectively to the cation (pyridinium, imidazolium, or cholinium). The Gram-positive strains generally demonstrate higher sensitivity (lower MIC₉₅ values) to the chain length than the Gram-negative strains; i.e. compare *E.coli* vs *S. aureus* on Fig. 3. Decyl chain length SAILs (compounds **21**, **29** and **37**) appear to be in an optimum region to demonstrate the microbial toxicity where lipophilicity, solubility and thus bioavailability are at a maximum, hence the highest levels of bacterial toxicity observed.^{54, 55} Nevertheless, further increasing of the side chain length does not result in the lower MIC values (i.e. in higher toxicity). Such a levelling-off was observed for the derivatives from decyl (compounds **21**, **29** and **37**) to tetradecyl (compounds **23**, **31** and **39**) against Gram-positive up to dodecyl (compounds **22**, **30** and **38**) against Gram-negative bacteria strains scanned. The mode of action of quaternary ammonium compounds (QAC) against bacterial cells is thought⁵⁶ to involve a general perturbation of lipid bilayer membranes as found to constitute the bacterial cytoplasmic membrane and the outer-membrane of Gram-negative bacteria. Such action leads to a generalized and progressive leakage of cytoplasmic materials to the environment. The presence of the outer-membrane makes some Gram-negative species of bacteria relatively insensitive to the biocides. This might be related to a failure of the compounds toxic to Gram-positive strains to penetrate the outer membrane of the Gram-negative strains and to access the cytoplasmic membrane. It is believed for *n*-alkyl SAILs^{57, 58} that in the case of Gram-positive bacteria and

yeast, such activity maximizes with *n*-alkyl chain lengths of *n* = 12 – 14, whilst for Gram-negative bacteria, optimal activity is achieved for compounds with a chain length of *n* = 14 – 16; further side chain extension is considered to make compounds with *n* ≥ 18 virtually inactive. There were mentioned some examples⁵⁸ of imidazolium ILs when Gram-positive was both the most and the least resistant strain, but the toxicity increased with alkyl chain length. In the present work, we report the derivatives with the longest chain (cetyl **24**, **32**, and **40** for Gram-positive, and tetradecyl **23**, **31**, and **39** in addition to it for Gram-negative strains) to exhibit even lower toxicity. This phenomenon called as a “cut-off effect” has been reported for different cationic SAILs, e.g. 1-alkyl-⁴³ and 1-alkoxyl-3-methylimidazolium⁴⁰ salts or 1-alkyl-3-hydroxypyridinium salts,⁵⁹ towards miscellaneous bacteria strains. According to the studies of Ruokonen et al.⁶⁰ on the liposome - IL interactions, the toxic effect of the shortest chain-length ILs which do not rupture cell membrane consists predominantly in affecting cell metabolism at the toxic concentration. The biodegradable ethyl derivatives **17**, **25** and **33** reported earlier^{48, 49}, along with ethyl esters with the chain length ethyl to hexyl (compounds **1** – **3**) fall this category. Most of SAILs reported in this work bearing medium and long length of the *n*-alkyl ester with *n* = 4 to 14 (*n* = 12 in the case of Gram-negative bacteria strains) form a dependence with gradually increasing toxicity caused by partially cell membrane rupturing effect. This tendency is followed by levelling-off the toxicity for the longer chain salts to reach the highest toxic effect (lowest MIC) in the series. The “cut-off” effect turns to the reverse dependence of toxicity on the hydrophobicity for the longest chain length ILs, *n* = 14 (Gram-negative bacteria) or *n* = 16 (Gram-positive bacteria): esters **7** and **8**; cetyl chain length SAILs **24**, **32**, and **40**. This may be connected either to high steric effect affecting ILs interaction with the cell surface or preferable self-aggregation of these salts as compared to their interaction with lipid membrane. The strong correlation of ILs toxicity towards both bacteria and aquatic organisms with their lipophilicity has been widely discussed elsewhere to be an important factor determining environmental application of ILs^{61, 62}. The antimicrobial efficacy of SAILs can be reported relative to their critical micelle concentration (CMC) as one of the reference concentration⁶³ reflecting interplay of the self-aggregation with cut-off effect. As it can be seen from Fig. 3, the CMC values (see discussion below) interfere with the ascending arm of the dependence for some of the strains studied supporting the predominant role of the micellization of the SAILs as a factor decreasing toxicity of these long-chain salts at the concentrations above the CMC.

Fungal toxicity

The linear alkyl ILs (**17-40**) along with the esters (**1 - 8**) have also been screened for their fungal toxicity. Results obtained for pyridinium SAILs (**17-24**) are shown in Table 3 with representative strains included in Fig 3a. The fungal toxicity results for Phenylalanine esters (**1-8**) and imidazolium and cholinium SAILs (**25-40**) are provided in Table S4 and Fig. 3b,c. Overall, it can be shown that as alkyl chain length increases so does the antifungal activity. The fungal toxicity for the octyl derivatives is generally moderate, 250-1000 μM for imidazolium and cholinium derivatives (**28** and **36**) whereas pyridinium derivative (**20**) is slightly more toxic, with MIC values ranging from 125-1000 μM . When the alkyl chain lengths were extended to $n=10$, an order of magnitude increase in the toxicity is observed for all three decyl derivatives **21**, **29**, and **37** ranging from 15.62-250 μM . Pyridinium C_{10} SAIL (**21**) is more toxic than its imidazolium (**29**) and cholinium (**37**) counterparts with MIC values reaching as low as 3.9 μM for the *Aspergillus fumigatus* (AF) fungus (Table 3, Fig. 3). As the alkyl chain is extended to dodecyl, a broad spectrum fungal toxicity is observed for all three compounds **22**, **30**, and **38** with MIC values in the region of 3.9 – 125 μM . Further extension of the chain length results in the “cut-off” effect. Some fungi strains (in particular, yeasts *Candida albicans* and *Candida lusitanae*) demonstrate better resistance towards cetyl derivatives **24**, **32**, and **40** whereas other SAILs follow the levelling-off tendency of showing even slightly lower MIC values (e.g. *Trichosporon asahii*). The mechanism of fungal toxicity for linear alkyl quaternary ammonium compounds has been investigated in the literature⁶⁴ and is considered to be similar to that discussed for bacteria. QACs can insert into the plasma membrane of a fungus and cause disruption of the membrane and eventually lysis as well as inhibit spore growth.⁶⁴ Another hypothesis was connected with an assumption that interfacial micelle-like aggregates are formed at the cell surface as a step in the binding process. Even submicellar complexes may initiate the fungicidal effects of cationic amphiphilic compounds as was supposed after studying *Candida albicans*.⁶⁵ Indeed, as it can be seen from Fig. 3, the CMC are far above the MIC values and if the aggregation can be considered among the factor affecting antifungal toxicity, the submicellar (premicellar) aggregates may play role in the interaction with the fungus membrane. For conventional cationic surfactants it was suggested that the mechanism of antifungal action of micelle-forming cationic detergent does not involve fungus cell lysis but rather the change of cell surface charge from negative to positive. Mammalian cells are more fragile regarding CTAB induced cell lysis, being disrupted by cationic amphiphiles over a range of micromolar concentrations.⁶⁶ The fungal toxicity data can be considered as more predictive, as compared to the bacterial toxicity screening data to estimate potential biodegradability.

Fig. 3. Bacterial and fungal toxicity (MIC, μM) as a function of the side chain length of the Phe derived SAILs. a) pyridinium (**17** - **24**), b) imidazolium (**25** - **32**), and c) cholinium (**33**-**40**) head group towards selected Gram-positive (*S. aureus*, SA), Gram-negative (*E. coli*, EC) bacteria and selected fungi (*C. albicans* CA2; *A. corymbifera*, AC) strains.

Table 2. Bacterial toxicity results obtained for the SAILs (17-24).

IL	Side chain length	Time	MIC ₉₅ , μ M							
			Gram Positive				Gram Negative			
			SA	MRSA	SE	EF	EC	KP	KP-E	PA

17	C2 ^{48, 49}	24h	2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
		48h	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
18	C4	24h	500	2000	500	2000	>2000	>2000	>2000	>2000
		48h	1000	2000	500	>2000	>2000	>2000	>2000	>2000
19	C6	24h	62.5	125	31.25	500	500	2000	2000	2000
		48h	62.5	125	31.25	500	500	2000	2000	2000
20	C8	24h	7.81	31.25	31.25	31.25	>500	>500	>500	>500 ^a
		48h	125	250	250	250	>500	>500	>500	>500 ^b
21	C10	24h	1.95	1.95	1.95	1.95	3.9	3.9	3.9	15.62
		48h	1.95	1.95	1.95	1.95	3.9	3.9	3.9	15.62
22	C12	24h	1.95	3.9	1.95	3.9	62.5	62.5	62.5	62.5
		48h	1.95	3.9	1.95	3.9	62.5	62.5	62.5	62.5
23	C14	24h	1.95	1.95	1.95	1.95	>500	>500	>500	>500 ^a
		48h	15.62	31.25	62.5	62.5	>500	>500	>500	>500 ^b
24	C16	24h	3.9	7.81	7.81	7.81	>500	>500	>500	>500 ^a
		48h	31.25	62.5	250	250	>500	>500	>500	>500 ^b

Notes. **SA:** *Staphylococcus aureus* subsp. *Aureus* (ATCC 29213), **MRSA:** *Staphylococcus aureus* subsp. *aureus* methicillin resistant (ATCC 43300), **SE:** *Staphylococcus epidermidis* (HK6966, 112-2016, dialysis tube, University Hospital Hradec Králové, Institute of Clinical Microbiology), **EF:** *Enterococcus faecalis* (ATCC 29212), **EC:** *Escherichia coli* (ATCC 25922), **KP:** *Klebsiella pneumoniae* (HK 11750, 64-2016, urine catheter tube, University Hospital Hradec Králové, Institute of Clinical Microbiology), **KP-E:** *Klebsiella pneumoniae* (ESBL positive, HK 14368, University Hospital Hradec Králové, Institute of Clinical Microbiology), **PA:** *Pseudomonas aeruginosa* (ATCC 27853)

Table 3. Fungal toxicity results obtained for the pyridinium SAILs (**17** – **24**).

N	Side chain length	Time (h)	MIC ₉₅ , µM											
			CA1	CA2	CP	CK1	CK2	CT	CG	CL	TA	AF	AC	TM ^a
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

17	C2 ^{48, 49}	24h	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000 ^a
		48h	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000 ^b
18	C4	24h	>2000	>2000	2000	2000	>2000	2000	>2000	>2000	2000	>2000	>2000	>2000
		48h	2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
19	C6	24h	2000	2000	500	500	500	500	2000	1000	500	2000	2000	500
		48h	1000	2000	500	500	500	1000	2000	1000	500	2000	>2000	1000
20	C8	24h	125	125	31.25	31.25	31.25	250	31.25	62.5	250	250	1000	500
		48h	500	500	62.5	62.5	62.5	500	125	250	250	250	2000	2000 ^b
21	C10	24h	15.62	15.62	15.62	7.81	7.81	7.81	62.5	62.5	125	3.9	31.25	62.5
		48h	15.62	31.25	31.25	31.25	31.25	31.25	62.5	62.5	125	7.81	31.25	62.5
22	C12	24h	3.9	3.9	7.81	7.81	7.81	15.62	7.81	7.81	62.5	7.81	15.62	15.62
		48h	3.9	3.9	7.81	7.81	7.81	15.62	7.81	7.81	62.5	7.81	15.62	15.62
23	C14	24h	1.95	3.9	1.95	1.95	1.95	1.95	0.98	3.9	3.9	3.9	7.81	7.81
		48h	3.9	3.9	1.95	3.9	3.9	1.95	1.95	3.9	3.9	7.81	15.62	15.62
24	C16	24h	7.81	15.62	7.81	7.81	15.62	15.62	7.81	>500	31.25	15.62	15.62	15.62
		48 h	15.62	15.62	7.81	7.81	31.25	15.62	7.81	>500	31.25	31.25	15.62	15.62

Notes: ^a evaluation after 72 h and 120 h, respectively.

CA1: *Candida albicans* (ATCC 24433), CA2: *Candida albicans* (ATCC 90028), CP: *Candida parapsilosis* (ATCC 22019), CK1: *Candida krusei* (ATCC 6258), CK2: *Candida krusei* (E28, University Hospital Hradec Králové, Institute of Clinical Microbiology), CT: *Candida tropicalis* (ATCC 750), CG: *Candida glabrata* (ATCC 90030), CL: *Candida lusitanae* (2446/I, University Hospital Hradec Králové, Institute of Clinical Microbiology), TA: *Trichosporon asahii* (1188, University Hospital Hradec Králové, Institute of Clinical Microbiology), AF: *Aspergillus fumigatus* (ATCC 204305), AC: *Absidia corymbifera* (CCM 8077), TM: *Trichophyton mentagrophytes* (445, University Hospital Hradec Králové, Institute of Clinical Microbiology)

Biodegradation studies

Along with toxicity data, biodegradation studies are an integral part of the green chemistry assessment of a chemical. In 2013, Gathergood and Connon published a combined microbial toxicity, biodegradation, green chemistry metrics and catalyst performance study of a series of ILs.^{67, 68} One of the findings from this work was that the ILs studied containing amide group(s) exhibited very low biodegradation in the CO₂ Headspace test. This was in a remarkable contrast to ILs containing an ester functional group, where facile breakdown to the carboxylic acid and conversion of the alcohol to CO₂ is widely reported.^{9, 26} Fig. 4 shows examples of ILs divided into three categories with respect to their abilities to pass the biodegradability tests, according to the review of Jordan and Gathergood⁹. This demonstrates the importance of the structural features to promote or impeded the biodegradation of ILs and highlights, using corresponding traffic light colours, the goal of attaining a green rating. It is a major challenge, as even in the case of ester containing ILs, the carboxylic acid transformation product has been shown to persist after a 28 day CBT.⁶⁹ Therefore, development of mineralisable ILs remains a demanding task.

Fig. 4. Structural features that promote IL biodegradation.⁹ (courtesy of *Royal Society of Chemistry*)

Our study published previously⁴⁹ provided an insight into the biodegradability of the ethyl ester of cationic phenylalanine-based ILs. The pyridinium IL derivative **7** was found to be the preferred green IL of the studied series (see Fig. 1), based on synthesis, toxicity and biodegradation considerations. The longer alkyl chain ILs studied in the present work demonstrate that the toxicity level increases with increasing alkyl chain length up to C₁₀ – C₁₂ - the chain length for which the “cut-off” effect appears. Initially, we screened the biodegradation of C₁₀ ILs with pyridinium (**21**), imidazolium (**29**) and cholinium (**37**) headgroups. These SAILs were chosen as they contain a side chain length C₁₀, in the middle of our series C₂ to C₁₆. We would expect these compounds to be a suitable

model for the long chain examples, while high microbial toxicity had not been established in both our bacteria and fungus toxicity assessment. Therefore, we would not expect toxicity complications during the CBT with these C₁₀ compounds. The C₁₀ SAILs could primarily breakdown according to two likely pathways: amide hydrolysis and ester hydrolysis. An illustration of these potential breakdown sites are proposed for pyridinium IL (**21**) (Fig. 5), and the biodegradation data from CBT are shown in Table 4.

Fig. 5. Potential sites for the first stage of pyridinium SAIL (**21**) breakdown.

Table 4. Biodegradability and carbon distribution of selected ILs; colour coded according to the traffic light classification.⁶⁷ (Green $\geq 60\%$, Amber $\geq 20\% < 60\%$, Red $< 20\%$)

IL	Chain Length	Head group	Biodegradation %
17	C2	Pyridinium	63 ⁴⁹
21	C10	Pyridinium	36
25	C2	Imidazolium	47 ^{48, 49}
29	C10	Imidazolium	44
33	C2	Cholinium	24 ^{48, 49}
37	C10	Cholinium	27

As can be seen from Table 4, none of the longer chain (as compared to ethyl ester) SAILs passed the CBT. There are three possible reasons for the reduction in biodegradability observed for the C₁₀ chain length SAIL (**21**, **29**, **37**) when compared to the short chain C₂ pyridinium IL (**17**) previously reported to be mineralisable. The SAIL (**21**, **29**, **37**) could undergo slow hydrolysis (compared to C₂) to form *n*-decanol and pyridinium L-phenylalanine carboxylic acid transformation products. The latter may be an unsuitable substrate for further amide cleavage and

therefore be persistent, (see Fig. 5, right). Another reason could be that decyl chain length SAILs undergo amide hydrolysis first (see Fig. 5, left). The second option follows the mechanism confirmed for the ethyl derivative (**21**) to be the favourable route for mineralisation.^{48, 49}

Therefore, upon elongation of the alkyl chains from C₂ to C₁₀, a dramatically reduced biodegradability was observed for the SAILs with all three headgroups (**21**, **29** and **37**). Biodegradation of 36% was observed for pyridinium C₁₀ IL (**21**), 2% less than can be attributed to total ester mineralisation (assuming acid remains persistent in that case); 44% for imidazolium SAIL (**29**), 4% above the theoretical degradation according to total ester degradation; and, finally, cholinium SAIL (**37**) displayed degradation levels 13% less than total ester degradation. These data cannot confirm whether the amide bond of the C₁₀ SAILs underwent hydrolysis. Reduced levels of biodegradability may be attributed to the level of broad spectrum antimicrobial activity of the parent SAILs discussed above (1.96 µM for *S.aureus*, 3.9 µM for *E.coli*, 15.62 µM for *C. albicans* CA2, and 31.25 µM for *A. fumigatus*, Tables 2, 3, S3 and S4). According to Boethling's rules of thumb an extension in alkyl chain length should increase biodegradability.⁷⁰ However, as the toxicity levels have increased with alkyl chain length, the potential increase in biodegradability is offset by the inability of the bacteria to degrade the IL. Indeed, Boethling states that in many cases structural changes made to reduce the toxicity of small molecules can reduce biodegradability.⁷⁰ Although the longer chain pyridinium derivative (**21**) undergoes a remarkably reduced level of biodegradation compared to C₂ compound (**17**), conclusions about their biodegradation pathways and ultimate biodegradability cannot be drawn without a full metabolite analysis and test time extensions. The detail study of the biodegradability of the series of Phe-derived SAILs is presented in the separate publication of Kümmerer et al.⁵⁰

Aggregation behaviour in aqueous solution

The aggregation properties, as well as the adsorption behaviour of the linear alkyl L-phenylalanine IL surfactants **17-40**, have been studied by means of tensiometry and conductivity.

Plots of surface tension of aqueous solutions of SAILs versus the log of their bulk concentration are shown in Fig. 6. The surface tension curves are characteristic of surfactants. At low concentrations, high surface tension values were obtained. On increasing the SAIL concentration, the surface tension values decreased, due to the adsorption of the SAIL molecules at the air/liquid interface, until a plateau attributed to the formation of micelles is observed. Subsequently, the surface tension values do not change with the concentration⁴⁵.

Fig. 6. Surface tension as a function of total surfactant concentration for the L-phenylalanine derived pyridinium SAILs (**17-24**); water, 25° C

For the short and medium-chain homologues in each series, a minimum of surface tension appears before attaining a plateau. The appearance of a minimum in the surface tension isotherm is usually attributed to impurities (which generally can be reduced or eliminated by purification).⁷¹ The repeated purification of studied Phe derived SAILs did not eliminate the minimum in the tensiometry plot, which may support another origin of this phenomenon. Similar observations have been found in literature for the short or medium chain 1-alkyl-3-methylimidazolium salts ($[C_n\text{MIM}]$)⁷², 1-alkylpyridinium⁴⁴, and amide-functionalized pyridinium and imidazolium salts.^{15,73} This behavior has been ascribed to the formation of the surface micelles prior to bulk aggregation. It leads to a minimum in the area per molecule and a maximum in adsorbed film thickness, as well as formation of a surface monolayer at the concentrations higher than the CMC. As expected, a progressive CMC diminution when the alkyl chain increased was observed for the three SAILs families (Table 7). This behaviour is common in all types of surfactants and it is attributed to the higher hydrophobic content of the molecule. The $\log$ CMC value decreases

linearly with the chain length extension, following the empirical Klevens equation⁷⁴: $\log \text{CMC} = A - Bn$, where A and B are constants for a particular homologous series at a constant temperature. The parameter A is related to the contribution of the polar head to micelle formation, and the parameter B indicates the average contribution to the micelle formation of each additional methylene group in the hydrophobic chain. The Klevens dependencies for the SAILs studied in this work are presented on the Fig. 7 separately for the salts with pyridinium (a), imidazolium (b), and cholinium (c) head group, along with selected data on the structurally similar salts reported in the literature. All three series exhibit about the same value of the slope close to -0.30, see Eqs. 1 – 3.

$$\log \text{CMC}_{\text{Py}} = -0.297 \cdot n - 0.157 \quad (1)$$

$$\log \text{CMC}_{\text{Im}} = -0.305 \cdot n + 0.062 \quad (2)$$

$$\log \text{CMC}_{\text{Chol}} = -0.296 \cdot n - 0.178 \quad (3)$$

The slopes ca. -0.30 are similar to those for either conventional ionic surfactants (alkyl pyridinium⁷⁵, $[\text{C}_n\text{mim}]$ ⁷⁶, or alkyl cholinium⁷⁷ salts), or ester-functionalized⁷³ and amide-functionalized⁷⁸ SAILs. They all form a series of nearly parallel plots, as shown on Fig. 7. It is worth noting that these regularities were reported both for various conventional ionic surfactants⁷¹ and SAILs^{45, 79}. Therefore, an extension of the side chain on one more CH_2 group affects similarly the aggregation properties in all these classes of surfactants. Diethylmethylammonium iodides of Phe esters⁸⁰ with side chain from C_{10} to C_{16} reporting slope of the Klevens equation ca. 0.4, which is steeper than in the Eqs (1 – 3).

Fig. 7. Effect of the side alkyl chain on the CMC of Phe-based cationic surface active ILs (○) with pyridinium **17** – **24** (a), imidazolium **26** – **32** (b), and cholinium **34** – **40** (c) headgroup; water, 25°C. Data on CMC values for the series of phenylalanine alkyl esters hydrochlorides⁸¹ and the different surfactants with tetraalkylammonium⁸², pyridinium^{73, 75, 83, 84}, imidazolium^{41, 44, 85-88}, and cholinium^{77, 89} head groups are taken from literature (see Table S1).

Comparing cholinium, pyridinium and imidazolinium polar heads, it can be observed that very similar CMC values were obtained for SAILs with the same alkyl chain. This indicates that the polarity of these three structural groups is similar and consequently its derivatives have the same hydrophobic/hydrophilic balance.

When compared with cholinium, imidazolinium and pyridinium ILs without phenylalanine in the polar head, these SAILs have much lower cmc values. Quantitatively, the CMC values for ILs (**17** – **24**) are similar to the CMC values for alkylpyridinium bromides⁷⁵ (conventional cationic surfactants with pyridinium headgroup) with the side chain, longer by six CH₂ groups (C_{n+6}) and comparable to pyridinium ILs functionalized with ester or amide moiety⁷³, longer by four CH₂ groups (C_{n+4}). This tendency can also be seen in the case of imidazolium SAILs (**26** – **32**) as compared to [C_nmim] salts (C_{n+6}) or imidazolium ILs with ester moiety⁷³ (C_{n+4}), and for and

cholinium SAILs (**34** – **40**) as compared to *n*-alkyl cholinium bromides^{77, 89}. These results show how the introduction of the aromatic moieties into the IL molecule affects the micellization of SAILs.

Structural changes of the headgroup with non-aromatic moieties does not affect significantly the micellization of SAILs, nicotine-based SAILs containing *N*-methyl pyrrolidine as the substituent⁸⁴ show almost the same CMC values as the ester-containing pyridinium ILs⁷³, Fig. 7a. The plot for a series of imidazolium amino acid-based ILs, histidine surfactants⁸⁵ is intermediate between conventional alkyl imidazolium salts and ester-containing imidazolium ILs^{44, 73}, and the CMCs for novel series of cleavable surfactants, alkyloxycarbonyloxyethyl [mim] ILs⁴¹, eventually coincide with those bearing ester or amide reported by the same group earlier⁷³, see Fig. 7b. Within series we have found in literature, there is also no evident influence of the alkoxy side-chains vs alkyl side-chains. Although ILs with oxy side-chains have been recently demonstrated to have higher polarity relative to those with alkyl side-chains attenuated by intramolecular hydrogen bonding involving the oxygen atom(s) and the relatively acidic imidazolium hydrogens.⁹⁰ An increasing of the hydrophobicity of the head group by an extension of the chain on the position 3 of imidazolium ring (replacing Me by Bu)⁸⁷ also have a minor effect on the CMC values in regard to the imidazolium ILs with ester group^{44, 73} (seen Fig. 8b). This is in spite of the fact that this modification facilitates vesicle formation. A series of cholinium carboxylates (using cholinium ion as a cation and an anion with variable chain length)⁸⁹ demonstrates the Kleven plot just slightly shifted rightwards compared to same chain-length *n*-alkylcholinium bromides with about double CMC values, Fig. 7c.

Therefore, we suggest that a significant factor affecting aggregation properties of the SAILs (**17** – **40**) is the presence of phenyl group of the Phe capable to induce micellization. Noteworthy, a series of cationic surfactants, hydrochlorides of Phe esters, studied by Joondan⁸¹ have similar values of CMC as the SAIL reported in this work while the CMC values of alanine (**19Ala**, **27Ala**, **35Ala**) ILs with the same chain length (C₆), see Fig. 7 and Table 5 are one order of magnitude higher than that of phenylalanine. These results evidently demonstrate the pronounced role of the phenyl ring in micellization of the amino acid-based SAILs.

The literature data indicates that the CMC is significantly influenced by the location of the phenyl ring within the hydrophobic tail segment in the trimethylammonium surfactants⁹¹. The closer the phenyl moiety is located to the head group, the lower is the CMC. The ILs series of benzyl(2-acylaminoethyl)dimethylammonium chlorides,⁸² bearing both a benzyl moiety in the headgroup and an amide group close to the head group, have low CMCs. However, these are still

approximately an order of magnitude higher than the CMC values for ILs reported in the present work, see Fig. 7a. A similar observation has been reported for nonionic surfactants, poly(oxyethylene) glycol alkyl ethers, showing that moving the phenyl group from the terminal region of the hydrophobic chain to neighboring the hydrophilic headgroup, leads to the decreased CMC.⁹² In the case of *N*-arylimidazolium ILs, the authors⁸⁶ observed lower CMC values than those of [C_{*n*}mim]Br, suggesting that the incorporation of the 2,4,6-trimethylphenyl group results in weakening of the electrostatic repulsion between the head groups, and promotes the micelle formation.

Kunitake and co-authors⁹³ have highlighted that the inclusion of aromatic (“mesophase-inducing”) segments, within the hydrophobic tail region of a single-tailed surfactant, can significantly enhance the interactions between the tail segments. This causes such single-tailed surfactants to form vesicular aggregates, which are otherwise seen only in two-tailed or gemini surfactants.

From the surface pressure isotherms, the effectiveness of the surfactant to reduce the water surface tension (π_{CMC}) and the adsorption efficiency (pC₂₀) were also estimated. The π_{CMC} values were determined by the following equation:

$$\pi_{\text{cmc}} = \gamma_0 - \gamma_{\text{cmc}} \quad (4)$$

where γ_0 is the surface tension of pure water and γ_{CMC} is the surface tension observed at the CMC.⁷¹ The effective range for the surface active ILs examined was determined to be not very different from the long chain cationic surfactants and displayed a larger effective range than traditional *n*-alkyl surfactants.

Fig. 8. Effect of the side chain length of the SAILs (17 – 40) on the value of the surface tension observed at the CMC (γ_{CMC}).

The γ_{CMC} values for studied ILs are between 30 to 40 mN/m and are dependent on the chainlength. As plotted on Fig. 8, the γ_{CMC} slightly decreases monotonously with reducing chain length from C_{16} to C_{10} and reach a minimum for the medium-chain ILs (C_6 and C_8). The γ_{CMC} increase again in the case of the shortest chain ILs, C_4 and C_2 . This shape of the plot may be ascribed to the increased viscosity of the solution of the short chain ILs at the concentrations above the aggregation concentration needed for the correct determination of the CMC. Although the short chain ILs are known to form aggregates other than micelles, they are usually considered to behave like short-chain cationic surfactants.⁹⁴ The C_2 and C_4 Phe ILs fall on the Klevens plot for the whole series shown on Fig. 8, which indirect supports that all ILs in the series undergo the same type of aggregates.

The ability of a surfactant to reduce surface tension by 20 mN/m (pC_{20}), was found as:

$$pC_{20} = -\log C_{20}, \quad (5)$$

where C_{20} is the molar concentration required to reduce the surface tension by 20 mN/m.⁷¹

The larger pC_{20} the more efficiently the surfactant is adsorbed at the interface and the more efficiently it reduces surface tension. The pC_{20} values for SAILs (17 – 40) increase gradually with the elongation of the chain length, irrespectively of the head group structure (pyridinium, imidazolium, or cholinium), from ca. 2.5 for C_4 derivatives to ca. 5.2 for C_{16} ILs. The C_6 Ala

derivative SAILs show much lower adsorption efficiency as compared to Phe derivatives, and the values are in the range from 1.51 (**35Ala**) to 1.68 (**27 Ala**) and then 1.74 (**19Ala**). It corresponds to pC₂₀ values of shorter chain length Phe SAILs, between that for C₂ and C₄. The obtained pC₂₀ values also indicate that the adsorption efficiency of these SAILs is superior to that showed by ILs without Phe in their chemical structure.^{75,89,41,45}

The average area per molecule occupied by each surfactant molecule was also determined; using the Gibb's equation:

$$\Gamma_{max} = - \frac{1}{2.30n \times RT} \times \left(\frac{d\gamma}{d\log C} \right)_T \quad (6)$$

Where Γ is the surface excess concentration, $\left(\frac{d\gamma}{d\log C} \right)_T$ is the slope of the dependence of surface tension γ vs. $\log$ surfactant concentration; $R = 8.32 \text{ J/K}\cdot\text{mol}$ and $T = 298.15 \text{ K}$; n is the number of ionic species in solution and a value of two is used for the cation surfactants.

The surface excess concentration Γ is related to the area per surfactant molecule A_{min} using the following equation:

$$A = \frac{1}{N_A \times \Gamma}, \quad (7)$$

Where N_A is Avogadro's constant.

The resulting A_{min} are given in Table 7, although the A_m values must be considered as rough estimates given the minimum obtained in the surface pressure isotherms. However, while acknowledging this issue, A_{min} values should be taken into account as useful estimation data, as some general tendency can be pointed out. As expected, the A_{min} decreases as a function of increasing the length of the alkyl chain due to concomitant closer packing of monomer at the air/water interface. The values for C₄ ILs are about $1.1 - 1.3 \text{ nm}^2$, which is comparable with conventional surfactants C_nTAB⁷⁵. Elongation of the chain length results in lower values of A_{min} to be $0.6 - 0.7 \text{ nm}^2$ for C₈ derivatives that are similar to various pyridinium and imidazolium SAIL reported in literature⁴⁵ or pyridinium and imidazolium pH-responsible functionalized surfactant.⁹⁵

The CMC values of these SAILs were also evaluated by conductivity. The conductivity curves are also characteristic of ionic surfactants. The obtained conductivity values fit into two straight lines of different slopes. The CMC can be determined from the intersection of the two linear dependences of κ against the concentration of SAIL with distinct slopes. The breakpoint is caused by either lower mobility of the micelles that contribute to the charge transport or by loss of ionic charges because of the binding of a fraction of the counterions to the micellar surface.^{15, 45} The

CMC was also evaluated by taking the first and second derivatives of κ plots⁹⁶, see Fig. S1. A derivative curve shows an instantaneous decrease in the CMC region and hence, the center (inflection point) of the reversed sigmoidal curve of the first derivative gives the value of CMC supported by minimum of the function of the second derivative. A comparison of such a plot with that of conventional conductivity has an advantage particularly when the break point in the κ curve is not sharp. The CMC values obtained with this technique are consistent with those obtained by surface tension.

Another important parameter that can be evaluated from conductivity is the degree of counterion binding (β) that corresponds to an average number of counterions per surfactant ion in the micelle and can be estimated by comparing the ratio of the two slopes. The results (Table 5) show that the values of β cover the range of 0.5 - 0.6 and do not dependent remarkably on the chain length as it has been reported elsewhere for other classes of conventional cationic surfactants⁷¹ and SAILs^{45, 51, 79}. This may denote that the micellar surface of all studied ILs have very similar cationic charge.

The standard molar Gibbs energy of micellization, $\Delta G^\circ_{\text{mic}}$, of these SAILs were calculated using the following equation⁸⁹:

$$\Delta G^\circ_{\text{mic}} = (1 + \beta)RT \ln x_{\text{cmc}}, \quad (8)$$

Where β is the previously discussed counter-ion binding parameter (degree of association) and x_{cmc} is the critical micelle concentration expressed as mole fraction. $\Delta G^\circ_{\text{mic}}$ corresponds to the free energy of transfer of one surfactant mole from the aqueous phase to the hydrophobic micellar pseudophase. Results of the investigation into the $\Delta G^\circ_{\text{mic}}$ show that as the alkyl chain length is increased, the energy required to form a micelle is decreased. It occurs due to the increase in hydrophobic repulsion interactions present with longer alkyl chains.

The dependence of the thermodynamic parameters of micelle formation on the surfactant structure shows that a possible interplay of additional dispersion interactions of phenyl ring and possible polar interaction of amide hydrogen places the novel SAILs among the remarkable surface active ILs as compared to other surfactants, e.g. conventional imidazolium, pyridinium, and benzyl (2-acylaminoethyl)dimethylammonium counterparts.⁷⁶ The insertion of the amino acid moiety to link the cationic headgroup and the *n*-alkyl ester chain affects both micellization in the bulk and the adsorption of the SAILs at the interface. The ratio cmc/C_{20} ⁷¹ is a parameter that may shed light on what extent the adsorption is more favoured as compared to the micellization process. The cmc/C_{20} values (see Table 7) are high for short chain (C₄ and C₆) SAILs irrespectively on the headgroup, they decrease significantly for the C₈ derivatives, and do not

change considerably when the side chain is $>C_8$. This behaviour is similar to conventional ILs^{79, 97} and different from that for carbonate-containing SAILs reported⁴¹ to demonstrate augmentation of the cmc/ C_{20} values with the extension of the chain length.

The CMC value appears to be not the only parameter explaining toxicity of the SAILs, since the antimicrobial effects occur at the concentration far below the CMC (it is worth noting that the CMC has been determined in water, it is expected that the CMC in the medium used to determine the MICs was much lower) and this parameter does not demonstrate sensitivity to the “cut-off” effect in bacterial/fungal toxicities, compare Fig. 3 and Fig. 7. Moreover, the “cut-off” effect (increasing MIC₉₅ values, which correspond to lower toxicities) observed in biological activity of the longest chain length SAILs of the series could be attributed to the aggregation process of molecules in aqueous solution.⁴³ This is because the tendency to form molecular aggregates at lower concentrations limits the rate of diffusion to the cell surface. An elegant attempt to overcome the problems related to using the CMC or the partition coefficient octanol/water K_{ow} (the latter is often difficult to determinate experimentally for the surfactants) as regards to toxicity was proposed by Rosen and co-workers.^{39, 71} They have introduced the parameter $\Delta G^\circ_{ad}/A_{min}$, where ΔG°_{ad} is the standard free energy of adsorption of a surfactant to the air/water interface (tendency of the surfactant to adsorb onto the membrane of (micro)organism and A_{min} as a measure of the ability of the surfactant to penetrate the membrane. This parameter is a hydrophobicity measure, which has been shown³⁹ to correlate well with aquatic toxicity values for algae and rotifers across the investigated cationic, nonionic, and anionic surfactants. It can be calculated experimentally based on surface tension measurements at varying surfactant concentrations up to C_{20} rather than to determine the CMC, Eq. 9⁷¹.

$$\Delta G^\circ_{ad} = RT \ln C_\pi / \omega - \pi A_\pi, \quad (9)$$

where $R = 8.31 \text{ J/mol}\cdot\text{K}$, T is absolute temperature, and C_π is the molar concentration of the surfactant in the aqueous phase at a surface pressure of π , ω is the number of moles of water per liter of water (55.5 mol/L, at 25°C). The values for ΔG°_{ad} and $\Delta G^\circ_{ad}/A_{min}$ found according to Rosen^{39, 98} are collected in Table 5. The $\Delta G^\circ_{ad}/A_{min}$ are linearly correlate to the chain length (C_n) for the whole series of the SAILs, from C_2 to C_{16} (Fig. S2,a) whereas dependence of $\log \text{MIC}_{95}$ vs $\Delta G^\circ_{ad}/A_{min}$ is linear only for the chain length up to C_{14} - C_{16} , see the data for SAILs **17 - 24** against *S. aureus* in Fig. S2, b. One of the possible reasons authors³⁹ point out is that the adsorption data at the air/aqueous solution interface might not be representative of adsorption at the cell membrane. Even considering this factor, the longest chain length member of the series (C_{14} and C_{16}) can be taken with precaution as the green surfactants, in spite of lower microbial toxicity, as

compared to their C₁₀ and C₁₂ counterparts. The high bacterial/fungal toxicity observed for the moderately long chain C₁₀ and C₁₂ SAILs make it difficult to consider them ‘green’. Although, a key finding is that their surface activity (in terms of CMC, in essence) is comparable to that of conventional cationic surfactants with C₁₆-C₁₈ chain length (those with low CMC but high T_K restricting their practical use in sufficient concentrations). Thus, these longer chain SAILs can be considered as greener disinfectants with relatively mild action and a potential to biodegradability. The high microbial toxicity of the C₁₀ SAILs (Table 4) may be the main factor responsible for their low degradability (Table 4). Therefore, the most promising surfactants revealing the sufficient surface activity along with reasonably low toxicity (and designed for desirable high biodegradability⁵⁰) are the medium chain length members of the studied series. The SAILs with hexyl (**19**, **27**, **35**) and octyl (**20**, **28**, **36**) chain length demonstrate CMC values comparable to those for C₁₂TAB⁷⁵ or C₁₂PyBr⁹⁹ (in the case of C₆ SAILs) and C₁₄TABr⁷⁵ (in the case of C₈ SAILs), widely used surfactants. In addition, low to moderate toxicities towards microorganisms for these SAILs may lead to their biodegradability.

Table 5. Aggregation properties for the surface-active ILs (**17** – **40**)

IL	Chain length	CMC ^a (mM)	β	$-\Delta G^{\circ}_{\text{mic}}$ (kJ/mol)	γ_{CMC} (mN/m)	π_{CMC} (mN/m)	pC ₂₀	CMC/ C ₂₀	Γ_{max} (mmol/m ²)	A _{min} (nm ²)	$-\Delta G^{\circ}_{\text{ads}}$ (kJ/mol)	$-\Delta G^{\circ}_{\text{ad}}/A_{\text{min}}$ (mJ/m ²)

17	2	187 (ST)	-	-	42.857	29.14	1.02	2.0	2.14	0.77	25.06	34.00
18	4	57 (ST); 89 (C)	0.55	24.70	32.9	39.1	2.38	13.4	1.49	1.11	36.89	35.30
19	6	11 (ST); 19.9 (C)	0.52	24.10	29.27	42.73	3.06	12.1	1.87	0.89	38.03	51.53
19Ala	6	107 (ST); 92 (C)	0.52	29.15	34.95	37.05	1.74	5.9	2.03	0.82	29.65	40.53
20	8	2.25 (ST); 4.90 (C)	0.58	36.55	29.16	42.84	3.42	5.9	2.76	0.60	36.66	81.34
21	10	0.65 (ST); 1.60 (C)	0.56	40.40	30.70	41.3	3.93	5.5	2.47	0.67	40.42	80.02
22	12	0.19 (ST); 0.50 (C)	0.58	45.50	33.90	38.10	4.39	4.7	2.34	0.71	43.52	81.91
23	14	0.056 (ST); 0.18 (C)	0.58	49.50	38.07	33.93	4.76	3.2	2.39	0.69	45.43	88.90
24	16	0.0125 (ST); 0.037 (C)	0.48	52.15	41.98	30.02	5.21	2.0	2.89	0.58	46.56	114.60

26	4	9.0 (ST); 7.90 (C)	0.47	23.94	33.85	38.15	2.50	28.6	1.24	1.34	40.29	30.19
27	6	23 (ST); 2.50 (C)	0.51	28.79	29.98	42.02	3.09	22.0	1.61	1.03	39.96	44.41
27Ala	6	122 (ST); 122 (C)	0.52	23.05	38.6	33.40	1.68	6.3	1.18	1.41	28.84	43.06

28	8	3.0 (ST); 5.50 (C)	0.59	36.43	29.3	42.70	3.85	21.4	2.24	0.74	40.81	71.70
29	10	1.2 (ST); 1.30 (C)	0.51	39.81	34.3	37.70	3.70	4.8	2.53	0.66	38.94	78.90
30	12	0.20 (ST); 0.44 (C)	0.55	44.97	36.86	35.14	4.20	4.1	2.83	0.59	40.94	96.03
31	14	0.05 (ST); 0.18 (C)	0.55	48.66	38.75	33.25	4.94	4.8	2.75	0.60	45.40	105.02
32	16	0.016 (ST); 0.046 (C)	0.47	50.90	40.5	31.50	5.19	3.1	3.26	0.51	45.66	128.78

34	4	80 (ST); 72 (C)	0.47	24.26	33.85	38.15	2.41	22.9	1.39	1.19	37.95	33.16
35	6	18 (ST); 23 (C)	0.52	29.31	29.98	42.02	3.06	23.1	1.57	1.06	40.12	43.13
35-Ala	6	124 (ST); 117 (C)	0.58	24.12	38.6	33.4	1.51	4.1	1.95	0.85	28.77	36.46
36	8	4.0 (ST);5.70 (C)	0.59	36.16	29.3	42.7	3.54	10.5	2.70	0.62	37.54	81.56
37	10	1.30(ST); 1.40(C)	0.56	41.01	34.3	37.7	3.63	5.1	2.36	0.70	39.08	72.47
38	12	0.28 (ST); 0.40 (C)	0.57	46.06	36.86	35.14	4.23	5.1	2.09	0.79	43.62	71.53
39	14	0.08 (ST); 0.16 (C)	0.50	47.37	38.75	33.25	4.77	3.5	2.53	0.66	45.03	94.05
40	16	0.024 (ST); 0.061 (C);	0.56	52.97	40.50	31.50	5.32	5.0	2.41	0.69	48.58	97.00

^a Obtained from conductivity (C) and surface tension (ST) measurements;

Conclusions

This study presents the first comprehensive study devoted to designing green surface active ionic liquids (SAILs) based on the 'benign-by-design' approach for the preparation of mineralizable pyridinium ILs developed recently by Gathergood and Kummerer.^{48, 49} Since the employment of identified readily biodegradable building blocks is an integral part of the targeted design of green SAILs, a prospective direction consists in the extension of this platform by means of side chain length variation. A series of 24 novel L-phenylalanine derived ionic liquids with variable length of *n*-alkyl ester chain (from C₂ to C₁₆) and quaternary ammonium (pyridinium, imidazolium, and cholinium) head group has been prepared, and the synthetic methods have been assessed using green chemistry metrics. The ILs reported in this paper have been confirmed to be surface active ionic liquids (SAILs), revealing a large variety of properties strictly dependent of the side chain length. This includes their bacterial and fungal toxicity, biodegradation (C₂ vs C₁₀) and aggregation properties.

The short chain length (C₂ and C₄) and medium chain length (C₆ and C₈) SAILs generally show low toxicity against microorganisms screened. The MIC₉₅ decreases with elongation of the chain length to reach the lowest value (respectively, the highest levels of bacterial and fungal toxicity) for the C₁₀ to C₁₄ derivatives, irrespectively of the cationic head group structure. In contrast to the observed low toxicity to microorganisms C₂ ester IL **7** (reported previously to be mineralisable in the case of pyridinium head group IL), the C₁₀ ILs were found to have poor biodegradability in CBT. Depending on the bacteria strain and type (Gram-positive or Gram-negative), the cut-off effect appears when the ester side chain is C₁₄ to C₁₆. This phenomenon is connected to the adsorption and aggregation (CMC) properties of the SAILs studied.

Evaluating the effect of adding the amino acid linker with a lipophilic side group has resulted in the finding that the phenyl moiety of L-phenylalanine contributes to the self-assembling properties. Comprehensive study of colloid properties allows us to conclude that the interplay of dispersion interactions of phenyl ring in addition to the side chain hydrophobicity enables us to rank the novel SAILs as remarkable compared to other surfactants. The CMC values for the SAILs reported in this study are significantly (up to 10 times) lower than for conventional surfactants with the same length of the side chain. Substituting Phe by Ala in the SAIL molecule (i.e. replacing CH₂Ph with CH₃) results in loss of the remarkable self-aggregation properties. At the same time, the linear plots of critical micelle concentration (*log* CMC) of the SAIL (**17** – **40**) demonstrate about the same sensitivity to the ester chain length as conventional cationic surfactant or SAILs with no amino acid fragment, and micellar surface is not significantly dependent on the

chain length. The adsorption is among the main factors affecting the SAILs toxicity with shorter chain whereas micellization is important in the case of the longer chain ILs causing the “cut-off” effect.

The L-phenylalanine derived SAILs with medium chain length (C_6 to C_8) can be recommended as the most green in the series with CMC values comparable to those for C_{12} - and C_{14} - quaternary ammonium surfactants, respectively. A crucial difference is that the medium chain SAILs are not very toxic towards studied bacteria and fungi strains and are potentially biodegradable (especially pyridinium derivatives). Therefore, SAILs **19-20**, **27-28** and **35-36** can be considered as a favourable group of green alternatives for conventional cationic surfactants. These findings can contribute to designing new efficient amphiphiles with optimized antimicrobial activities and to employ them as potentially environmentally benign mineralisable surfactants.

Experimental Section

Materials.

All chemicals and solvents were purchased from Sigma Aldrich, Alfa Aesar, or TCI Europe and used without further purification. Deionized water from a Milli-Q system was used in all sample preparation. Silica gel 60 F254 plates were used for TLC.

Characterization of products. Melting points were determined with Stuart SMP40 apparatus with parameters for the melting point analysis set at 2°C per minute ramp; values are expressed in °C. The HRMS identification of compounds was performed on an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS G6540A Mass Spectrometer. IR spectra were collected with Tensor 27 FT-IR spectrometer. Optical rotations were measured using Anton Paar MCP 500 polarimeter in methanol or chloroform at 20°C and values are expressed in degrees. The NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer operating at 400 MHz for ^1H -NMR and 101 MHz for ^{13}C -NMR. Samples were run in deuterated chloroform (CDCl_3) or deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) where appropriate.

Conductivity measurements. Conductivity was measured using a Lab 875 pH set equipped with LF413T conductivity measuring cell at 25°C maintained with cooling thermostat Huber Ministat 125. Cell constant was calibrated with NaCl solutions of known conductivities and was used for calculating the conductivity of the surfactant solution. The conductivity of water was subtracted from the measured conductivity of each sample. Measurements carried out at increasing concentrations to minimise errors from possible contamination of the electrode.

Surface tension measurements. Surface tension was measured by the du Noüy ring detachment method Sigma Force 702 tensiometer with T106 platinum ring at 25°C maintained with cooling

thermostat Huber Ministat 125. Surfactant concentration was varied by adding concentrated surfactant solution, and the readings were noted after thorough mixing and temperature equilibration. Glass containers and plate were cleaned and rinsed thoroughly with deionized water. The ring was flame dried before each measurement. Measurement were repeated at least three times of until the measured value is consistent within the experimental error of ± 0.01 mN/m.

The CMC values were determined from surface tension γ versus $\log$ of the total surfactant concentration profiles.

Screening for bacterial and fungal toxicity. The bacterial toxicity (determination of MIC in μM) was evaluated by microdilution broth method according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) instructions.¹⁰⁰ The fungal toxicity (determination of MIC in μM) was evaluated by microdilution broth method according to the EUCAST instructions.^{101, 102} The details of the procedure are described in ESI.

Biodegradation was studied using the methods described in the previous papers.^{48, 49}

Synthesis of compounds (**1 - 40**) and their characterisation data are presented in ESI.

Conflicts of Interest

There are no conflicts of interest to declare.

Acknowledgements

The authors would like to thank European Union's 7th Framework Programme for research, technological development and demonstration under grant agreement No. 621364 (TUTIC-Green) and Estonian Research Council grant PUT 1656. The antibacterial and antifungal screening was supported by the Czech Science Foundation (project No. 18-17868S). NG, AJ and AK thank the EPA in Ireland for financial support under grant [2012-WRM-PhD-6]. NG, LP and AJ also thank COST Actions CM1206 and TD1203 for their financial contributions (STSMs) to the study. LP thanks MINECO of Spain (CTQ2017-88948-P) for funding. Authors thank Maxime Naudé for surface tension measurements.

References

1. P. T. Anastas, *Green Chemistry: Theory and Practice*, ed. P. T. Anastas and J. C. Warner, Oxford University Press, 1998.

2. P. Anastas and N. Eghbali, *Chem. Soc. Rev.*, 2010, **39**, 301-312.
3. H. C. Erythropel, J. B. Zimmerman, T. M. de Winter, L. Petitjean, F. Melnikov, C. H. Lam, A. W. Lounsbury, K. E. Mellor, N. Z. Jankovic, Q. S. Tu, L. N. Pincus, M. M. Falinski, W. B. Shi, P. Coish, D. L. Plata and P. T. Anastas, *Green Chem.*, 2018, **20**, 1929-1961.
4. N. V. Plechkova and K. R. Seddon, *Chem. Soc. Rev.*, 2008, **37**, 123-150.
5. J. D. Holbrey, M. B. Turner and R. D. Rogers, *Ionic Liquids as Green Solvents: Progress and Prospects*, 2003, **856**, 2-12.
6. *Catalysis in Ionic Liquids: from Catalyst Synthesis to Application*, ed. Ch. Hardacre and V. Parvulescu, Royal Society of Chemistry, 2014, 620 p.
7. T. Welton, *Proc. Royal Soc. A*, 2015, **471**, 26.
8. B. Wu, W. Liu, Y. Zhang and H. Wang, *Chem. Eur. J.*, 2009, **15**, 1804-1810.
9. A. Jordan and N. Gathergood, *Chem. Soc. Rev.*, 2015, **44**, 8200-8237.
10. K. S. Egorova and V. P. Ananikov, *ChemSusChem*, 2014, **7**, 336-360.
11. M. M. Seitkalieva, A. S. Kashin, K. S. Egorova and V. P. Ananikov, *ACS Sustain. Chem. Eng.*, 2018, **6**, 719-726.
12. S. Kirchhecker and D. Esposito, *Curr. Opin. Green Sustain. Chem.*, 2016, **2**, 28-33.
13. J. Hulsbosch, D. E. De Vos, K. Binnemans and R. Ameloot, *ACS Sustain. Chem. Eng.*, 2016, **4**, 2917-2931.
14. *Ionic Liquid-Based Surfactant Science: Formulation, Characterization, and Applications*, John Wiley & Sons, Inc., Hoboken, NJ, 2015.
15. M. Teresa Garcia, I. Ribosa, L. Perez, A. Manresa and F. Comelles, *Coll. Surfaces B - Biointerfaces*, 2014, **123**, 318-325.
16. Q. Q. Baltazar, J. Chandawalla, K. Sawyer and J. L. Anderson, *Coll. Surfaces A, Physicochem. Eng. Aspects*, 2007, **302**, 150-156.
17. H. Y. Wang, H. P. Li, G. K. Cui, Z. Y. Li and J. J. Wang, *Acta Physico-Chimica Sinica*, 2016, **32**, 249-260.
18. F. A. Vicente, I. S. Cardoso, T. E. Sintra, J. Lemus, E. F. Marques, S. P. M. Ventura and J. A. P. Coutinho, *J. Phys. Chem. B*, 2017, **121**, 8742-8755.
19. E. L. P. de Faria, S. V. Shabudin, A. F. M. Claudio, M. Valega, F. M. J. Domingues, C. S. R. Freire, A. J. D. Silvestre and M. G. Freire, *ASC Sustain. Chem. Eng.*, 2017, **5**, 7344-7351.
20. K. Bica, P. Gartner, P. J. Gritsch, A. K. Ressmann, C. Schroder and R. Zirbs, *Chem. Commun.*, 2012, **48**, 5013-5015.
21. A. Cognigni, P. Gaertner, R. Zirbs, H. Peterlik, K. Prochazka, C. Schroder and K. Bica, *Phys. Chem. Chem. Phys.*, 2016, **18**, 13375-13384.
22. M. Taskin, A. Cognigni, R. Zirbs, E. Reimhult and K. Bica, *Rsc Advances*, 2017, **7**, 41144-41151.
23. Regulation (EC) No 648/2004 of the European Parliament and the Council of 31 March 2004 on detergents, 2004, **648/2004**, 1-35.
24. M. T. Garcia, N. Gathergood and P. J. Scammells, *Green Chem.*, 2005, **7**, 9-14.
25. N. Gathergood, M. T. Garcia and P. J. Scammells, *Green Chem.*, 2004, **6**, 166-175.
26. N. Gathergood, P. J. Scammells and M. T. Garcia, *Green Chem.*, 2006, **8**, 156-160.
27. S. Stolte, S. Steudte, A. Igartua and P. Stepnowski, *Curr. Org. Chem.*, 2011, **15**, 1946-1973.
28. M. T. Garcia, I. Ribosa, T. Guindulain, J. Sanchez-Leal and J. Vives-Rego, *Environ. Pollution*, 2001, **111**, 169-175.

29. G. G. Ying, *Environ. Int.*, 2006, **32**, 417-431.
30. A. Pinazo, M. A. Manresa, A. M. Marques, M. Bustelo, M. J. Espuny and L. Pérez, *Adv. Colloid Interface Sci.*, 2016, **228**, 17-39.
31. F. Rios, A. Fernandez-Arteaga, M. Lechuga, M. Fernandez-Serrano, in *Toxicity and Biodegradation Testing*, eds. E. D. Bidoia and R. N. Montagnolli, Humana Press Inc, Totowa, 2018, pp. 311-330.
32. K. S. Egorova and V. P. Ananikov, *Biophys. Rev.*, 2018.
33. P. G. Jessop, *Faraday Discus.*, 2018, **206**, 587-601.
34. B. Subramaniam and D. Allen, *ACS Sustain. Chem. Eng.*, 2018, **6**, 4422-4422.
35. D. B. Tripathy, A. Mishra, J. Clark and T. Farmer, *Comptes Rendus Chimie*, 2018, **21**, 112-130.
36. R. Bordes and K. Holmberg, *Adv. Colloid Interface Sci.*, 2015, **222**, 79-91.
37. K. S. Egorova, M. M. Seitkalieva, A. V. Posvyatenko and V. P. Ananikov, *Toxicol. Res.*, 2015, **4**, 152-159.
38. D. Zhao, Y. Liao and Z. Zhang, *CLEAN – Soil, Air, Water*, 2007, **35**, 42-48.
39. M. J. Rosen, F. Li, S. W. Morrall and D. J. Versteeg, *Environ. Sci. Technol.*, 2001, **35**, 954-959.
40. R. Dolezal, O. Soukup, D. Malinak, R. M. L. Savedra, J. Marek, M. Dolezalova, M. Pasdiorova, S. Salajkova, J. Korabecny, J. Honegr, T. C. Ramalho and K. Kuca, *Eur. J. Med. Chem.*, 2016, **121**, 699-711.
41. M. T. Garcia, I. Ribosa, L. Perez, A. Manresa and F. Comelles, *Langmuir*, 2017, **33**, 6511-6520.
42. K. O. Evans, *Coll. Surfaces A: Physicochem. Eng. Aspects*, 2006, **274**, 11-17.
43. J. Luczak, C. Jungnickel, I. Lacka, S. Stolle and J. Hupka, *Green Chem.*, 2010, **12**, 593-601.
44. A. Cornellas, L. Perez, F. Comelles, I. Ribosa, A. Manresa and M. Teresa Garcia, *J. Colloid Interface Sci.*, 2011, **355**, 164-171.
45. M. T. Garcia, I. Ribosa, L. Perez, A. Manresa and F. Comelles, *Langmuir*, 2013, **29**, 2536-2545.
46. S. Kumar, H. A. Scheidt, N. Kaur, A. Kaur, T. S. Kang, D. Huster and V. S. Mithu, *J. Phys. Chem. B*, 2018, **122**, 6763-6770.
47. K. Kümmerer, *Green Chem.*, 2007, **9**, 899-907.
48. A. Haiss, A. Jordan, J. Westphal, E. Logunova, N. Gathergood and K. Kümmerer, *Green Chem.*, 2016, **18**, 4361-4373.
49. A. Jordan, A. Haiss, M. Spulak, Y. Karpichev, K. Kümmerer and N. Gathergood, *Green Chem.*, 2016, **18**, 4374-4392.
50. A. Haiß, J. Westphal, G. Raba, I. Kapitanov, Y. Karpichev, N. Gathergood and K. Kümmerer, *in preparation*, 2019.
51. D. Coleman, M. Spulak, M. T. Garcia and N. Gathergood, *Green Chem.*, 2012, **14**, 1350-1356.
52. K. Baczko, C. Larpent and P. Lesot, *Tetrahedron-Asymmetry*, 2004, **15**, 971-982.
53. C. R. McElroy, A. Constantinou, L. C. Jones, L. Summerton and J. H. Clark, *Green Chem.*, 2015, **17**, 3111-3121.
54. L. Carson, P. K. W. Chau, M. J. Earle, M. A. Gilea, B. F. Gilmore, S. P. Gorman, M. T. McCann and K. R. Seddon, *Green Chem.*, 2009, **11**, 492-497.

55. J. Pernak, I. Goc and I. Mirska, *Green Chem.*, 2004, **6**, 323-329.
56. S. E. Moore, M. Mohareb, S. A. Moore and R. A. Palepu, *J. Colloid Interface Sci.*, 2006, **304**, 491-496.
57. P. Gilbert and A. Altaae, *Lett. Appl. Microbiol.*, 1985, **1**, 101-104.
58. M. Petkovic, K. R. Seddon, L. P. N. Rebelo and C. S. Pereira, *Chem. Soc. Rev.*, 2011, **40**, 1383-1403.
59. J. Pernak, K. Sobaszekiewicz and I. Mirska, *Green Chem.*, 2003, **5**, 52-56.
60. S.-K. Ruokonen, C. Sanwald, A. Robciuc, S. Hietala, A. H. Rantamaki, J. Witos, A. W. T. King, M. Laemmerhofer and S. K. Wiedmer, *Chem. Eur. J.*, 2018, **24**, 2669-2680.
61. C. Zhang, F. Cui, G. M. Zeng, M. Jiang, Z. Z. Yang, Z. G. Yu, M. Y. Zhu and L. Q. Shen, *Sci. Total Environ.*, 2015, **518**, 352-362.
62. M. Amde, J.-F. Liu and L. Pang, *Environ. Sci. Technol.*, 2015, **49**, 12611-12627.
63. A. S. Inacio, N. S. Domingues, A. Nunes, P. T. Martins, M. J. Moreno, L. M. Estronca, R. Fernandes, A. J. M. Moreno, M. J. Borrego, J. P. Gomes, W. L. C. Vaz, O. V. Vieira, *J. Antimicrob. Chemother.*, 2016, **71**, 641-654.
64. G. McDonnell and A. D. Russell, *Clin. Microbiol. Rev.*, 1999, **12**, 147-179.
65. B. Ahlstrom, M. ChelminskaBertilsson, R. A. Thompson and L. Edebo, *Antimicrob. Agents Chemother.*, 1997, **41**, 544-550.
66. D. B. Vieira and A. M. Carmona-Ribeiro, *J. Antimicrob. Chemother.*, 2006, **58**, 760-767.
67. L. Myles, R. G. Gore, N. Gathergood and S. J. Connon, *Green Chem.*, 2013, **15**, 2740-2746.
68. R. G. Gore, L. Myles, M. Spulak, I. Beadham, M. T. Garcia, S. J. Connon and N. Gathergood, *Green Chem.*, 2013, **15**, 2747-2760.
69. Y. Deng, I. Beadham, M. Ghavre, M. F. C. Gomes, N. Gathergood, P. Husson, B. Legeret, B. Quilty, M. Sancelme and P. Besse-Hoggan, *Green Chem.*, 2015, **17**, 1479-1491.
70. R. S. Boethling, E. Sommer and D. DiFiore, *Chem. Rev.*, 2007, **107**, 2207-2227.
71. M. J. Rosen and J. T. Kunjappu, *Surfactants and Interfacial Phenomena, 4th Edition*, Blackwell Science Publ, Oxford, 2012.
72. J. Bowers, C. P. Butts, P. J. Martin, M. C. Vergara-Gutierrez and R. K. Heenan, *Langmuir*, 2004, **20**, 2191-2198.
73. M. Teresa Garcia, I. Ribosa, L. Perez, A. Manresa and F. Comelles, *Langmuir*, 2013, **29**, 2536-2545.
74. H. B. Klevens, *J. Am. Oil Chem. Soc.*, 1953, **30**, 74-80.
75. G. B. Ray, I. Chakraborty, S. Ghosh, S. P. Moulik and R. Palepu, *Langmuir*, 2005, **21**, 10958-10967.
76. P. D. Galgano and O. A. El Seoud, *J. Colloid Interface Sci.*, 2011, **361**, 186-194.
77. Z. Fan, W. Tong, Q. Zheng, Q. Lei and W. Fang, *J. Chem. Eng. Data*, 2013, **58**, 334-342.
78. M. T. Garcia, I. Ribosa, L. Perez, A. Manresa and F. Comelles, *Colloids Surf. B*, 2014, **123**, 318-325.
79. T. Inoue, H. Ebina, D. Bin and L. Zheng, *J. Colloid Interface Sci.*, 2007, **314**, 236-241.
80. N. Joondan, P. Caumul, M. Akerman and S. Jhaumeer-Laulloo, *Bioorg. Chem.*, 2015, **58**, 117-129.
81. N. Joondan, S. Jhaumeer-Laulloo and P. Caumul, *Microbiol. Res.*, 2014, **169**, 675-685.
82. S. Shimizu and O. A. El Seoud, *Langmuir*, 2003, **19**, 238-243.
83. S. Shimizu, P. A. R. Pires and O. A. El Seoud, *Langmuir*, 2004, **20**, 9551-9559.

84. G. Singh, R. Kamboj, V. S. Mithu, V. Chauhan, T. Kaur, G. Kaur, S. Singh and T. S. Kang, *J. Colloid Interface Sci.*, 2017, **496**, 278-289.91.
85. M. Bustelo, A. Pinazo, M. A. Manresa, M. Mitjans, M. P. Vinardell and L. Pérez, *Colloids Surf. A, Physicochem. Eng. Aspects*, 2017, **532**, 501-509.
86. L. Shi, N. Li and L. Zheng, *J. Phys. Chem. C*, 2011, **115**, 18295-18301.
87. S. Chabba, R. Vashishat, T. S. Kang and R. K. Mahajan, *ChemistrySelect*, 2016, **1**, 2458-2470.
88. B. Dong, N. Li, L. Zheng, L. Yu and T. Inoue, *Langmuir*, 2007, **23**, 4178-4182.
89. R. Klein, D. Touraud and W. Kunz, *Green Chem.*, 2008, **10**, 433-435.
90. J. C. de Jesus, P. A. R. Pires, M. Scharf and O. A. El Seoud, *Fluid Phase Equilibria*, 2017, **451**, 48-56.
91. S. De, V. K. Aswal and S. Ramakrishnan, *Langmuir*, 2010, **26**, 17882-17889.
92. Y. J. Li, J. Reeve, Y. L. Wang, R. K. Thomas, J. B. Wang and H. K. Yan, *J. Phys. Chem. B*, 2005, **109**, 16070-16074.
93. T. Kunitake, Y. Okahata, M. Shimomura, S. I. Yasunami and K. Takarabe, *J. Am. Chem. Soc.*, 1981, **103**, 5401-5413.
94. T. Singh and A. Kumar, *J. Phys. Chem. B*, 2007, **111**, 7843-7851.
95. I. V. Kapitanov, A. B. Mirgorodskaya, F. G. Valeeva, N. Gathergood, K. Kuca, L. Y. Zakharova and Y. Karpichev, *Colloids Surf. A, Physicochem. Eng. Aspects*, 2017, **524**, 143-159.
96. M. S. Bakshi, J. Singh, K. Singh and G. Kaur, *Colloids Surf. A, Physicochem. Eng. Aspects*, 2004, **234**, 77-84.
97. B. Dong, X. Y. Zhao, L. Q. Zheng, J. Zhang, N. Li and T. Inoue, *Colloids Surf. A, Physicochem. Eng. Aspects*, 2008, **317**, 666-672.
98. M. J. Rosen, L. Fei, Y.-P. Zhu and S. W. Morrall, *J. Surfact. Detergents*, 1999, **2**, 343-347.
99. J. Skerjanc, K. Kogej and J. Cerar, *Langmuir*, 1999, **15**, 5023-5028.
100. EUCAST DISCUSSION DOCUMENT E.Dis 5.1, Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clin. Microbiol. Infect.*, 2003, **9**, 1-7
101. EUCAST DEFINITIVE DOCUMENT EDEF 7.3.1, Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. The European Committee on Antimicrobial Susceptibility Testing, <http://www.eucast.org>, 2017, 1-21.
102. EUCAST DEFINITIVE DOCUMENT EDEF 9.3.1, Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds. The European Committee on Antimicrobial Susceptibility Testing, <http://www.eucast.org>, 2017, 1-23.