Analysis of surfactants by mass spectrometry: coming to grips with their diversity

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TABLE OF CONTENTS:

RUNNING TITLE: Analysis of surfactants by mass spectrometry

Abstract

Surfactants are surface-active agents widely used in numerous applications in our daily lives as personal care products, domestic and industrial detergents. To determine complex mixtures of surfactants and their degradation products, unselective and rather insensitive methods, based on colorimetric and complexometric analyses are no longer employable. Analytical methodologies able to determine low concentration levels of surfactants and closely related compounds in complex matrices are required. The recent introduction of robust, sensitive, and selective mass spectrometry (MS) techniques has led to the rapid expansion of the surfactant research field including complex mixtures of isomers, oligomers and homologues of surfactants as well as their chemically and biodegradation products at trace levels. In this review, emphasis is given to the state-of-the-art MS-based analysis of surfactants and their degradation products with an overview of the current research landscape from traditional methods involving hyphenate techniques (gas chromatography-MS and liquid chromatography-MS) to the most innovative approaches, based on high-resolution mass spectrometry (HRMS). Finally, we outline a detailed explanation on the utilization of MS for mechanistic purposes, such as the study of micelle formation in different solvents.

Keywords: surfactants, gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, high resolution-mass spectrometry, tandem mass spectrometry.

ACRONYMS:

DCI, desorption chemical ionization

PI, phosphatidylinositols

ABS, alkylbenzenesulfonates

AEO, ethoxylated alcohols

AES, alkyl ether sulfates

AFFF, aqueous film-forming foams

AOTNa sodium bis(2-ethylhexyl)sulfosuccinate

AP, alkylphenols

APCI, atmospheric pressure chemical ionization

APEO, alkylphenol polyethoxylates

APEO, alkylphenol polyethoxylates

APG, alkyl polyglycosides

ATR, attenuated total reflectance

BAC, benzalkonium chloride

CAPB, cocamidopropyl betaines

CNPEC, carboxyalkylphenol ethoxycarboxylates

CE, capillary electrophoresis

CES, coceth sulfate

CES, sodium coceth sulfate

CI, chemical ionization

CID, collision induced dissociation

CMC, critical micellar concentration

CS+, cationic surfactants

DP, degree of polymerization

DTAB, dodecyltrimethylammonium bromide

EHCO, ethoxylated hydrogenated castor oil

EI, electron impact

ELSD, evaporative light scattering detector

EO, ethylene oxide

ESI, electrospray ionization

FAB, fast atom bombardment

FD, field desorption

FIA, flow-injection analysis

FTAB, fluorotelomer sulfonamide alkylbetaine

FTIR, Fourier-transform infrared spectroscopy

GC, chromatography

GC×GC, two-dimensional gas chromatography

GCB, graphitized carbon black

HILIC, hydrophilic interaction liquid chromatography

HPLC, high pressure liquid chromatography

HR, high-resolution

IC-CD, ion chromatography with suppressed or non-suppressed conductivity detection

IM, Ion mobility

KMD, Kendrick mass defect

LAE, linear alkyl ethoxylates

LAS, linear alkylbenzenesulfonates

LOD, limits of detection

LPC, lysophosphatidylcholines

MALDI-TOF-MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MS, mass spectrometry

MS/MS, tandem mass spectrometry

NMR, nuclear magnetic resonance spectroscopy

NP, nonylphenol

NPEC, nonylphenol polyethoxycarboxylates

NPEO, nonylphenol polyethoxylate

OP, octylphenol

OPEO, octylphenol polyethoxylate

OTAB, octyl-trimethylammonium bromide

PC, phosphatidylcholines

PE, phosphatidylethanolamines

PEG, poly(ethylene glycol)

PFAS, polyfluorinated alkyl surfactants

PG, phosphatidylglycerols

SDME, single drop microextraction

SFC-MS, supercritical fluid chromatography coupled with mass spectrometry

SIM, selected ion monitoring

SL, sodium dodecylcarboxylate

SM, sphingomyelins

SPE, solid phase extraction

SRM, selected reaction monitoring

TAA, tetraalkylammonium

1. Chemical-physical proprieties of surfactants

Surfactants or surface-active agents are a group of compounds used to modify the interfacial properties of aqueous and non-aqueous solutions. Each surfactant molecule has both hydrophilic and hydrophobic (or lipophilic) moieties (Broze, 1999; Morelli and Szajer, 2001, 2000). Typical structures of surfactant molecules comprise spatially separated polar and apolar parts, elongated shapes, large numbers of conformations and anisotropic surfactant–surfactant interactions. The polar group is localized in a small domain of the molecule and consists of an ionic, zwitterionic or non-ionic hydrophilic group (the so-called surfactant head), whereas the apolar portion is formed by one or more long flexible hydrocarbon or fluorocarbon chains, i.e., the surfactant tail. As summarized in **Table 1**, the polar head group is used to distinguish surfactants into different categories: anionic, cationic, non-ionic.

Due to the competitive interplay of head–head, head–tail and tail–tail interactions, triggered by surrounding molecules, surfactants can self-assemble leading to organized aggregates (see **Figure 1**), with coexistence of polar and apolar domains. When surfactant monomer molecules (**Figure 1A**) are dissolved in highly polar solvents (e.g. water), above a critical concentration, i.e. the critical micellar concentration (CMC), they aggregate as micelles (**Figure 1B**), i.e. supramolecules characterized by an apolar core of surfactant alkyl chains surrounded by an external layer of hydrophilic head groups in contact with solvent molecules (Broze, 1999; Hoffmann, 1994). The main driving force of the surfactant self-assembling is the hydrophobic effect. Yet, reverse micelles can also be formed when surfactants molecules are assembled in apolar media so that the head groups point toward the centre of the aggregate while the alkyl chains are oriented toward the external (**Figure 1C**). This is because, in apolar media, interactions between polar groups dominate, being generally much stronger than those between apolar moieties (Allen et al., 2000). The presence of these aggregates dramatically influences the physico–chemical properties of solutions at the liquid-air interface (Broze, 1999; Morelli and Szajer, 2000). Depending on the nature of the hydrophilic moiety ensuring the water affinity of the molecule, major surfactants can be divided into anionic, cationic, amphoteric, and non-ionic classes. Anionics are historically the earliest and the most common surfactants. Accordingly, they are produced with the highest volumes and most of them are relatively inexpensive. They are especially beneficial for their excellent detersive action and their efficacy to remove particulate soils, a benefit that relies on the fact that many substrates are negatively charged. Depending on the nature of the

184 negatively charged head group, they show a variable resistance to bond cleavages, i.e., C-C, C-S, C-O.
185 Anionics are also enough sensitive to water hardness ions, thus limiting their use in hard waters. Some
186 anionics have also the ability to generate viscous aqueous phases, thereby yielding self-thickened products
187 (Bazel et al., 2014; Scheibel, 2004).

188 Cationics are characterized by very high affinity on various substrates, especially the negatively charged
189 ones, and by the subsequent surface modifications. They are commonly used as conditioning agents in fabric
190 care (i.e., dialkyl or diester quaternary ammonium salts in rinse fabric softeners) and hair care products.
191 Cationics form water-insoluble complexes with anionic surfactants. Some cationics are used as bactericides
192 and fungicides too, such as dodecyl dimethylbenzyl ammonium chloride or cetyltrimethylammonium
193 chloride (Carmona-Ribeiro et al., 2006; Fait et al., 2019; Zakharova et al., 2019). Amphoteric surfactants are
194 usually used in conjunction with other surfactants (anionics or non-ionics) to improve desired properties such
195 as foam or detergency. Since the optimal surface activity and skin compatibility of amphoterics take place
196 around neutral pH, they are suitable for mild detergent products like personal care ones, i.e., shower gels,
197 foam baths, shampoos, etc. (Domsch and Jenni, 2004). Non-ionics are especially useful because are
198 minimally affected by water hardness and pH values. Since they are compatible with charged molecules, they
199 are easily used in mixtures with other ionic surfactants, which often result in beneficial associations. For
200 instance, non-ionics can help to solubilize calcium or magnesium salts of anionic surfactants. These features,
201 together with their low related costs, make non-ionic surfactants widely used in detergent products (Bajpai
202 and Tyagi, 2010; Lindman et al., 2016).

203 Surfactants are not chemically homogeneous being usually a mixture of compounds. For all types of
204 surfactants, there may be a considerable distribution in the nonpolar part, different alkyl chain length, partial
205 unsaturation, and so on, resulting from the fatty raw material used. As an example, coceth sulfate (CES), a
206 commonly used surfactant in liquid cleaning formulations, is formally a blend of homologue molecules with
207 hydrophobic tail C12 and C14, i.e., $C_{12}H_{25}(OCH_2CH_2)_nOSO_3Na$ and $C_{14}H_{29}(OCH_2CH_2)_nOSO_3Na$ (Onzo et
208 al., 2020). Moreover, the number of oxyethylene groups (n) may formally be two but the product will contain
209 species with no oxyethylene units up to at least seven oxyethylene units.

210 The chemical structures of surfactants are very important to provide specific features to the final
211 detergent products with the main aim to fully meet people needs. Degree of polymerization, presence of

unsaturations and length of alkyl chain are examples of structure-related parameters that can be varied to tune the last product properties. For these reasons, knowing surfactant structure is important, in order to obtain more clues on the structure-properties relationship and to take advantage of this information to test new structural motifs (Gaudin et al., 2019). In 2020, the worldwide production of synthetic surfactants was estimated to be larger than 10 million tons; the anionics account for 50% of the total industrial surfactant output, cationics comprise only about 15%, while non-ionics account for 30% (Morelli and Szajer, 2000).

As already mentioned, surfactants are widely used both industrially and domestically being currently ubiquitous into the environment (D. Caniani et al., 2019; Donatella Caniani et al., 2019). Recent works have demonstrated how surfactants, such as alkylphenol polyethoxylates (APEO), and their degradation products possess weakly oestrogenic effects (Gaudin et al., 2019). Apparently, APEO and closely related degradation products are of human health concerns, such as sperm production decrease and sexual reproductive issues (Acquavia et al., 2020; Sharpe and Skakkebaek, 1993). Moreover, halogenated compounds produced by biodegradation of most commonly used surfactants, including APEO and polyfluorinated alkyl surfactants (PFAS) are persistent and accumulate both in groundwater and sludge (Amodeo et al., 2018). Also the contamination of drinking water and foodstuffs is of great concern for human health (Montgomery-Brown and Reinhard, 2003; Trier et al., 2011). Conceivably, data about the total content of surfactants and their degradation products in environmental samples (Caivano et al., 2017b, 2017a) are critical in assessing the environmental impact of these compounds. Thus, information about the exact distribution of individual oligomers in environmental samples is important since it permits the identification of the sources of surfactants and the degree of degradation of the parent compounds. For these reasons, much research is dedicated to developing more sensitive, specific, robust, and efficient analytical methods to identify, structurally characterize and quantify these substances in a wide range of complex samples, also including biological systems where they play important roles comprising human physiology. This is the case of pulmonary surfactants, which are involved in the efficient exchange of gases and the maintenance of the structural integrity of alveoli (Choi et al., 2020). Pulmonary surfactants are molecules of interest for several investigators wishing to understand bio-systems. Thus, sensitive analytical methods for their detection and characterization are needed as well. This review summarises existing literature on the analysis of the different classes of surfactants by mass spectrometry.

2. The challenges of surfactant analysis

The continued development of analytical tools and techniques has defined the field of surfactants, focusing on improvements mainly in analytical techniques. Currently, highly selective analytical methods are required to characterize and identify the plethora of surfactants occurring in industrial raw materials, manufactured products, and environmental samples. Because of the existence of many alkyl homologues, lack of chromophores, high polarity, and thermal instability, non-aromatic surfactants are difficult to analyse. Several techniques for their determination have been reported, including titration (Fielden and Claesson, 1998), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy (Jain et al., 2012; Lee et al., 2016), attenuated total reflectance (ATR) with FTIR (Carolei and Gutz, 2005), ion chromatography with suppressed or non-suppressed conductivity detection (IC-CD) (Cataldi et al., 2007; Levine et al., 2000), gas chromatography coupled to mass spectrometry (GC/MS) (Ding and Liao, 2001; Ding and Tsai, 2003), capillary electrophoresis (CE) with UV detection (Liu and Ding, 2004; Piera et al., 1997), high performance liquid chromatography coupled with mass spectrometry (HPLC/MS) or evaporative light scattering detector (HPLC/ELSD) (Crescenzi et al., 1995), and supercritical fluid chromatography coupled with mass spectrometry (SFC/MS) (Ma et al., 2019; Pan et al., 2020). Among the above-mentioned techniques, the chromatographic ones have many advantages for analysing surfactant mixtures. Albeit good resolution can be expected by using IC-CD, this analytical technique can only be applied to ionic compounds (Coviello et al., 2020). GC/MS with direct injection of ionic surfactants is not viable and derivatization or pyrolysis is required for their analysis (Pascale et al., 2018a). Conversely, HPLC is applicable to surfactant mixtures regardless their molecular charge (Morelli and Szajer, 2001, 2000) and can be coupled with different detectors, such as mass spectrometers and UV–Vis diode array, without any derivatization reactions. However, low polymer solubility and increased solution viscosity, with the ensuing issues of sample injection and elution, as well as relatively low chromatographic resolution of individual oligomers, make its use challenging (Pan et al., 2020; Schmitt et al., 1990). As an alternative, stand-alone shotgun MS has been widely used for surfactant analysis, and mass spectrometry provides a wide range of advantages, such as supplying key structural characterization by tandem MS. A huge number of molecules can be quickly

identified, thus making shotgun MS/MS somewhat useful for complex mixtures (Onzo et al., 2020; Pascale et al., 2018c). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been also employed for non-ionic surfactants (Sato et al., 2014, 2001; Treglia et al., 2020). Although it is a convenient and powerful technique, it can be rather cumbersome to identify low-molecular weight oligomers without a proper matrix selection (Calvano et al., 2018). Ion mobility mass spectrometry (IM-MS), which has a short analysis time and minimal solvent usage, has also been used to analyse oligomers (Katzenmeyer et al., 2016; Solak Erdem et al., 2014). Soft ionization techniques such as field desorption (FD), fast atom bombardment (FAB), atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) have been employed for a qualitative characterization of surfactant mixtures in environmental samples (Kühn and Neubert, 2004; Lyon et al., 1984a). Depending on the physico-chemical properties of the surfactants such as polarity and molecular weights, operation in positive or negative ion mode can be selected. In addition, tandem mass (MS/MS) outcomes have greatly increased the accessibility of surfactants to MS analysis; fragmentation patterns allow the isomer distribution examination along with other chemical information, such as location of unsaturations, occurrence of side chains and degree of branching (Onzo et al., 2020). The popularity of high-resolution/accuracy MS (HRMS) has soared in the past decade and suggests that this technique has the potential to provide a significant boost to surfactant analysis. Here, rather than give a complete overview of surfactant MS analysis using uncommon ionization sources (e.g. FAB), we pursued the idea to give enough background of their useful information with a special look at the most popular atmospheric pressure ionization systems.

2.1. Mass spectrometry in surfactant analysis

For several reasons, MS has become the most widely used tool in industrial laboratories to characterize and quantify surfactants and closely related degradation products in the last 20 years. First of all, MS represents a sensitive and highly selective technique for the analysis of complex mixture samples (Morelli and Szajer, 2001, 2000). Secondly, MS has the wide range of sample-related advantages, like the possibility to carried out direct-injection analyses of solution or to analyse directly solid samples without the need to recur to time-consuming sample pre-treatment protocols. Thirdly, the ability to isolate precursor ions by tandem MS (MS/MS) and disclose the relevant fragmentation behaviour (Bianco et al., 2017). Along with

295 the possibility to couple MS with separation techniques, makes this approach the most powerful analytical
296 tool in every field of application, showing unsurpassed levels of specificity and selectivity (Pascale et al.,
297 2021). MS technique has rapidly evolved during the past 30 years invading every discipline within the life
298 and health sciences (Bianco et al., 2017; Pascale et al., 2021; Sun et al., 2020; Zhou et al., 2012), including
299 surfactants field (Agozzino et al., 1986; García et al., 2019; Ma et al., 2019).

300 In this review, a survey of the most common approaches used in surfactant analysis by MS will be
301 illustrated. Initially, a detailed description of the structural analysis by MS will be provided for each class of
302 surfactants (see **Table 1**), including biosurfactants and pulmonary surfactants. Then, the quantification of
303 targeted surfactants will be discussed, focusing the attention on emerging compounds and their
304 environmental concerns. Alternative and innovative approaches related to MS surfactant analysis will be
305 listed to investigate complex mixtures of surfactant. Finally, a detailed explanation on the utilization of this
306 technique for mechanistic purposes, such as the study of micelle formation in different solvents, will be
307 presented.

308 ## 3. Structural analysis of surfactants by MS

309 The structural characterization of surfactants and their degradation products is usually performed by
310 flow-injection analysis with tandem mass spectrometry (FIA-MS/MS) or hyphenate techniques in which MS
311 and MS/MS is coupled to LC or GC. To shed light on the surfactant structure in complex samples, the loss
312 of neutral fragments and the presence of diagnostic product ions in tandem MS experiments are decisive for
313 the qualitative identification of surfactants. Taking into account that surfactants are not commercially
314 available as individual compounds, MS is very useful to obtain their fingerprints, to recognise the chemical
315 composition of commercial products and for traceability purposes as well (Onzo et al., 2020). The fate of
316 surfactants released into the environment along with their degradation products can be attempted by MS
317 detection, thus possibly making the issue less challenging. Furthermore, the possibility to isolate selected
318 product ions (i.e., diagnostic ions) in selected ion monitoring (SIM) and in selected reaction monitoring
319 (SRM) modes, allows to obtain good selectivity and sensitivity, thus demonstrating the suitability of MS for
320 monitoring and quality control routine analyses (Crescenzi et al., 1995; Moody et al., 2001; Pascale et al.,

2020b; Petrović and Barceló, 2000). MS is widely used to characterize all classes of surfactants, *viz*., anionics, cationics, non-ionics, amphoterics and bioderivatives, as discussed below.

3.1 Anionic surfactants

Anionic surfactants comprise the largest class of surfactants and account for ca. 50% of those used in Europe and ca. 60% in the United States. Most of them are high foaming but scarcely tolerant to hard water, thus requiring the addition of substances to complex calcium and magnesium ions, so called detergent builders. Anionic surfactants are more effective than other surfactants in particulate soil removal, especially from natural fabrics. As a rule, they are easily spray-dried and are chosen as detergent powders. Greater sensitivity and selectivity toward anionic surfactants are obtained by MS in negative ionization mode, even though works using positive ionization are also reported (Broze, 1999; Morelli and Szajer, 2001, 2000).

Alkylbenzenesulfonates (ABS) are anionic surfactants made of branched chains, which are only biodegradable with difficulty after long times. Except for specific applications, ABS are not used in developed countries as emulsifiers for agricultural chemicals. To facilitate the chemical and biodegradation under aerobic conditions, linear alkylbenzenesulfonates (LAS) have been introduced. LAS are synthesized by Friedel-Crafts alkylation of benzene using alkylation catalyst and ensuing sulfonation with oleum or sulfur trioxide, producing mainly the para isomers. Albeit LAS are enough sensitive to water hardness, they are plentifully used throughout the world because of their relatively low cost. GC-MS has been successfully applied to identify LAS homologs (Agozzino et al., 1986). The 75-eV EI mass spectrum of dodecylbenzenesulfonate with phenyl substitution in the C1 position (**Figure 2A**) show the tropylium ion, $[C_7H_7]^+$, at *m/z* 91 and the base peak at *m/z* 172 arises from the molecular ion by a benzylic cleavage with hydrogen rearrangement; the peak at *m/z* 171 is formed by a simple benzylic cleavage; both these fragmentations are consistent with unbranched α position. Other cleavages at different positions of the dodecyl chain yield the abundant ions at *m/z* 185, 199, 213, and 227 corresponding to C1, C2, C3, C4, and C5 fragments (the symbol C_n indicates the ions constituted by an alkyl chain of n carbon atoms linked to -C_6H_4-SO_3H). In the low electron beam energy (15 eV) spectrum, reported in **Figure 2B**, the rearrangement ion (*m/z* 172) is always the most abundant fragment, while the simple a cleavage reaction is completely

quenched. This means that in 1-dodecylbenzenesulfonic acid the α cleavage reaction with H rearrangement is the process requiring lower energy.

LAS can be readily distinguished from the branched alkyl chains of tetrapropylene-based ABS by the different fragmentation patterns in tandem MS analysis of their precursor ions. The product ion at *m/z* 183 and chemical formula $[O_3SCH{=}CH_2]^-$ is diagnostic of LAS, while the corresponding ABS product ion $[O_3SC(CH_3){=}CH_2]^-$ is detected at *m/z* 197. Both product ions exhibit the common loss of SO_2 to form species at *m/z* 119 and 133, respectively. Interesting examples of LC-MS analysis with electrospray ionization in the negative ion mode were reported by Gonzàlez-Mazo et al. [51] and Villar et al. (González-Mazo et al., 1997; Villar et al., 2007) with very careful determination of LAS along with their co-products and metabolites in wastewater.

Alkyl sulfates, also called alcohol sulfates, are anionic surfactants formally obtained by sulfuric acid and linear alcohols. Alkyl chain lengths range from C10 to C18, and the properties of the final mixture vary with the alkyl chain length distribution. The alcohol source can be either natural (i.e., usually linear) or synthetic as oxo alcohols with some branching chains. The most used starting materials of fatty alcohols in the United States (ca. 15%) come from saponification of natural oil and fat. They tend to be sensitive to water hardness but are very widely used in cosmetics and detergents; lauryl alcohol sulfate is a major ingredient of hair shampoo (Broze, 1999). Since 1984 alkyl sulfates were structurally characterized by Lyon et al. (Lyon et al., 1984b) in positive and negative-ion mode by fast atom bombardment (FAB)-MS with collision induced dissociation (CID) experiments. A typical positive mass spectrum shows a series of ions with the formula $[R_nNa_{n+1}]^+$ being *n* from 1 to 7, while the negative ion spectrum usually exhibits $[R]^-$ ions. The CID spectra of FAB-generated negative ions allow one to verify that the material is a sulfate and to obtain the lengths of the carbon chains by counting the presence of peaks from *m/z* 96 to the deprotonated molecule. Structural information obtained by the cited work were useful to shed light on the chemical composition of commercial products like sodium oleyl sulfate also named Sipon OS. FAB spectra evidenced that Sipon OS is a mixture of saturated and unsaturated alcohol sulfates; the main component at *m/z* 321 appears to be sodium palmityl sulfate, with lesser amounts of sodium stearyl sulfate at *m/z* 349 and sodium oleyl sulfate at *m/z* 347 jointly with a minor amount of the myristyl sodium sulfate. The tandem MS spectrum of each component was used to confirm the surfactant identity by spectrum matching with authentic samples. The same approach was

376 employed to interpret the composition of sodium dodecylbenzenesulfonate (called Siponate DS-4), known
377 to be a mixture of 17 substituted alkylbenzenesulfonates over a molecular weight range of *m/z* 230-430 (Lyon
378 et al., 1984b). Often the m/z value of the precursor ion is not sufficient to provide an explicit characterization,
379 thus either the LC coupling or tandem MS analysis are very useful for chemical identification (Ogura et al.,
380 1996; Popenoe et al., 1994).

381 Alkyl ether sulfates (AES), also called alcohol ethoxysulfates, are prepared by addition of one to four
382 oxyethylene (-OCH_2CH_2-, 44.05 Da) groups to an organic compound that carries one hydroxyl functional
383 group (−OH), which is then sulfated. Commercial AES consists of a very broad spectrum of compounds,
384 broader than for most other surfactant types. Oxyethylation enhances water solubility and foaming activity
385 over the analogous alcohol sulfate, giving rise to useful products in shampoos and in liquid as well as
386 powdered detergents. The raw material for these products can be either natural fatty alcohols or
387 primary/secondary synthetic alcohols, usually of C12-C18 chain length. However, ether sulfates are not less
388 sensitive to water hardness than other anionic surfactants (Broze, 1999). The analogous alkylphenol ether
389 sulfates are found in industrial applications. Because of their very low volatility, the analysis of alkyl ether
390 sulfates can be fully accomplished by soft ionization sources, both in positive and negative ion modes. The
391 typical two ions observed in ESI(+) for each individual AES component are $[M + Na]^+$ and the "desulfated"
392 species $[M - SO_3 + H]^+$, whereas a single peak due to a desodiated molecule ($[M - Na]^-$) is observed for each
393 compound in ESI(-). APCI(+) produces a protonated "desulfated" ion in the form of $[M - NaSO_3 + 2H]^+$ for
394 each AES species in the mixture under relatively low cone voltage (10 V) conditions. The mass spectral ion
395 intensities of each AES in either ESI(+) or APCI(+) can be used to obtain an estimation of relative abundance
396 in the mixture and an average ethoxylate (EO) number of the sample. The precursor ions produced by positive
397 or negative ESI, when subjected to low-energy (50 eV) collision-induced dissociation, do not fragment to
398 give ions that provide adequate structural information. Conversely, protonated desulfated ions produced by
399 APCI(+) generate fragment ions that disclose structural information of the precursor ions, including alkyl
400 chain length and EO number, under similar conditions. Apparently, APCI(+) is less susceptible to matrix
401 effects for quantitative work compared to ESI(+), thus APCI(+) provides an additional tool for the analysis
402 of anionic surfactants such as AES, especially in complex mixtures where tandem MS is required for the
403 identification of each component (Jewett et al., 1999). The use of ESI was demonstrated for LC-ESI-MS of

alkyl sulfates and ethoxysulfates of C12-C15 alkyl chain length and 0-8 ethoxy groups: partial separation of alkyl chains was reported with quantification of individual homologs accomplished by MS in the negative ion mode (Popenoe et al., 1994). Tandem MS of product ions in positive polarity produced rather uninformative spectra; for instance, whereas MS/MS of $[R(OCH_2CH_2)_nOSO_3Na_2]^+$ gives rise to Na^+ and $[Na_2HSO_4]^+$ ions, the adduct $[R(OCH_2CH_2)_nOH + Na]^+$ yields just sodium ions as the main product ions. In the case of negative polarity, tandem MS of surfactant species with low degree of polymerization of alcohol ethoxysulfates (i.e., $[R(OCH_2CH_2)_nOSO_3]^-$) is also uninformative because the most common product ions $[HSO_4]^-$ at *m/z* 97, SO_3^- at *m/z* 80 and a low intensity peak signal at *m/z* 123 ($[CH_2{=}CHOSO_3]^-$) are reported (Attygalle et al., 2001). Remarkably, tandem mass spectra of AES with a high degree of polymerization are more informative, showing fragmentation of monomeric units, whose product ions can be used to infer the number of EO units along with the alkyl chain (Onzo et al., 2020). CID of the precursor ion $[R(OCH_2CH_2)_nOH + H]^+$, produced by APCI, is more helpful as some important product ions can be obtained, corresponding to the alkyl group loss, namely $[H(OCH_2CH_2)_nOH + H]^+$, from which the alkyl group can be deduced. In addition, a series of ions of the form $[C_2H_4(OCH_2CH_2)_nOH]^+$ with n = 1-4, were observed in the corresponding mass spectra (Jewett et al., 1999). Although largely dismantled in most research and industrial laboratories, FAB-MS/MS was described as structurally informative in negative polarity mode (Lyon et al., 1984a). Fragments of the precursor ion, $[R(OCH_2CH_2)_nOSO_3]^-$, generate several peaks corresponding to many different combinations due to alkyl and ethoxy losses. Very recently, the use of an ESI source in MS was found very effective to unveil the commercial formulas of alkyl ether sulfates. Alkyl chain lengths and degrees of ethoxylation of AES contained in sodium coceth sulfate (CES) solution were inferred from experimental data (Onzo et al., 2020). Single-stage ESI-MS analysis of CES allowed to distinguish two different series whose members differed in molecular mass by 28.05 Da, i.e. the mass of the ethylene (C_2H_4) unit, thus suggesting the presence of C_x and $C_{(x+2)}$ alkyl homologues. For both series, a difference in the molecular mass of 44.05 Da, i.e. the monomeric oxyethylene unit, was ascertained as shown in **Figure 3**, and the formulation fingerprint of CES was qualitatively used to check three commercial detergent formulations.

α-Olefin sulfonates are produced from the reaction between linear C11-C20 α-olefins and SO_3. Whereas compounds with an alkyl chain length below C14 are used in liquid detergents, those above C14 are used in

spray-dried powders. Enhanced water solubility is guaranteed by hydroxy groups, whereas double bonds make the mixture unstable to bleach. One of the most interesting features of α-olefin sulfonates is that they are less sensitive to water hardness than most anionic surfactants and are readily biodegradable (Broze, 1999). EI-MS spectra of methyl derivative of hexadecane sulfonates exhibit the highest *m/z* ion corresponding to the demethoxylated molecular ion $[M - OCH_3]^+$. Other peak signals corresponding to ions $[C_{16}H_{31}]^+$, $[C_{16}H_{29}]^+$, $[SO_2]^+$ and $[CH_3SO_2]^+$ were recognised (Taulli, 1969). The absence of the radical molecular ion peak signals is also common to methyl derivative of 2-hydroxyalkanesulfonate where the main peak at *m/z* 153 corresponds to the following fragment $[CH_3O{=}CHCH_2SO_3CH_3]^+$ (Boyer et al., 1982) along with a peak at *m/z* 121 due to methanol loss. In ESI-MS instead, the deprotonated molecules of olefin sulfonates can be observed (Shi et al., 2014). Taking a look to data reported by FAB-MS/MS in negative polarity mode, there is considerable fragmentation pattern with a recognisable peak due to $[SO_3]^-$ at *m/z* 80, and many peaks corresponding to $[C_nH_{2n-2}SO_3]^-$ (Lyon et al., 1984a).

3.2 Non-ionic surfactants

Non-ionic surfactants are generally more tolerant to water hardness compared to anionic ones. They tend to be more effective than other surfactants for removal of oily soil from synthetic fabrics. Most non-ionics are considered low-foaming products, possess good cold-water solubility and low CMC, making them effective even at low concentration. Their compatibility with cationic fabric softeners makes them preferable to anionics in certain formulations. These features and their low production costs make non-ionic surfactants the most frequently detergent formulations, thus generating a strong environment impact (Bajpai and Tyagi, 2010; Cserhati, 1995).

Among non-ionic surfactants, ethoxylated alcohols (AEO) are largely used as laundry detergents (Nitschke and Huber, 1993; Schmitt et al., 1990). The primary alcohol ethoxylates are the common item of commerce, but secondary alcohol ethoxylates are also available. The most important surfactant properties are controlled by the average EO percent and the average chain length of the starting alcohol (Hanton et al., 2006). Direct EI-MS analysis is handicapped because the ethoxylates of commercial interest are scarcely volatile. After derivatization, however, the identification of AEO can be performed from interpretation of the EI spectrum (Schneider et al., 1983; Vettori et al., 1988). Various types of non-ionic surfactants have been

460 analyzed by mass spectrometry using field desorption (FD), desorption chemical ionization (DCI) and fast
461 atom bombardment (FAB) as ionization techniques. FAB-MS and tandem MS was very useful for direct
462 analysis of mixtures. At relatively low collision activation energies, the precursor ions of AEO give rise to
463 cleavage of the ether bonds with product ions formation corresponding to the loss of 1, 2, 3, and 4 EO groups,
464 respectively (i.e., peak signals at *m/z* of 45, 89, 133, and 177, with others at $[M + H]^+$, $[M + H - 44]^+$, and [M
465 $+ H - 88]^+$). At higher energy, low abundance ions were observed and correspond to the alkyl chain
466 fragmentation. Diagnostically important fragments resulted from the loss of the alkyl chain of the protonated
467 adduct $[M + H]^+$ through olefin displacement, thus giving information on the length of both the alkyl and
468 ethoxy chain lengths. For example, a C12 AEO will give a fragment of $[M + H - 168]^+$ (Ding et al., 1994).
469 FAB-MS/MS of each homologue generates mainly polyethylene glycol peaks ($m/z = [44_n + 1]^+$), almost
470 similar to EI-MS, but other fragments are present, which give information on the alkyl chain composition
471 (Ventura et al., 1991).

472 ESI-MS in the positive ionization mode is largely used in qualitative and quantitative analysis of
473 non-ionic surfactants because the hydroxyl deprotonation in the ionization source is rather negligible (Gomez
474 et al., 2011). So, negative ion mode ESI-MS experiments were conducted successfully on polyethoxylated
475 surfactants, providing useful structural and mechanistic information: MS analysis of each peak provides data
476 on the ethoxylated (EO) chain and identifying unambiguously each compound (Onzo et al., 2020). ESI and
477 MALDI-MS generate protonated adducts $[M + H]^+$ reflecting the true molecular weight distribution (Hanton
478 et al., 2006; Sherrard et al., 1994), which means that most intense peaks are at intervals of 44 Da, with peaks
479 differing by 14 Da. When CID is applied to ions produced by ESI-MS, fragmentation of secondary AEO
480 occurs more easily than primary one; with higher energy CID of secondary AEO compounds, the PEG ions
481 become dominant extending to the higher mass range. With APCI, positive ions of the form $[14n + 44x +$
482 $18]^+$, corresponding to the molecular ions, are observed. Environmental samples will also give ions for PEG,
483 $[44x + 18]^+$, and mono- and di-carboxylated PEG, $[44x + 76]^+$ and $[44x + 134]^+$, respectively (Castillo and
484 Barceló, 1999). APCI in negative ion mode of AEO generates deprotonated molecules.

485 Alkylphenol polyethoxylates (APEO) such as octylphenol polyethoxylate (OPEO) and nonylphenol
486 polyethoxylate (NPEO) are commercially important poly(ethylene glycol) (PEG)-type non-ionic surfactants,
487 which have been widely used in industrial, agricultural, and domestic applications such as detergents,

emulsifiers, pesticide additives and so on (Broze, 1999). Upon release into the environment, they undergo slow biodegradation processes and concern has increased about relatively stable degradation products of APEO, alkylphenols (AP) such as nonylphenol (NP) and octylphenol (OP) (**Figure 4**) (Zhang et al., 2009). AP have been classified together with lower ethoxylates (from mono- to tri-), as endocrine-disrupting compounds (EDC), because of their effects on the hormonal system of numerous organisms by competing with oestrogen for binding receptors (Cserhati, 1995). APEO are preferentially detected in positive ion mode, while alkylphenols their self are detected with greatest sensitivity in negative ion mode (Shang et al., 1999). Electrospray MS of an acidic solution of NPEO as protonated adducts, $[M + H]^+$, reflects the true molecular weight distribution, with the main peaks differing by 44 Da, i.e. the monomeric EO unit mass. The presence of sodium and potassium ions lead to the appearance of other peaks corresponding to sodiated and potassiated adducts, $[M + Na]^+$ and $[M + K]^+$ (Sherrard et al., 1994). Application of MS/MS analysis shows product ions corresponding to the neutral loss of CH_2CH_2O (44 Da), $(CH_2CH_2O)_2$ (88 Da), and C_9H_{18} (126 Da). Likewise, tandem MS can be used to identify NPEO present in complex matrices by monitoring the product ion $[PhOCH{=}CH_2 + H]^+$ at *m/z* 121.16 for 1-4 EO units, and $[CH_2{=}CHOCH_2CH_2OCH_2CH_2OH + H]^+$ at *m/z* 133.17 for higher ethoxylates (**Figure 5**) (Plomley et al., 1999).

APEO ions are dissociation resistant to low energy CID, showing very little fragmentation to form protonated PEG species (Sherrard et al., 1994). By increasing the collision energy, the formation of product ions corresponding to the loss of portions of both alkyl and ethoxy chains could be observed (see **Figure 5**). MALDI-ToF MS was applied for APEO characterization, providing interesting information on the fate of these substances in environment samples. For example, *Pseudomonas* sp. biodegradation intermediates of OPEO isolated from a paddy field soil and examined by MALDI–MS showed mass spectrum whereby a hydroxyl end-group and several EO units, distributed mostly from 4 to 17, with maximum intensity peak at 9 EO units. The gradual shortening by biodegradation processes was successfully monitored by MALDI–MS, where the maximum of the ion peak distributions of OPEO decreased from 9 EO units down to 3. Furthermore, among the biodegradation intermediates, the formation of OPEO oligomers with a carboxyl terminal of EO chain was also noted under *m/z* 600. These observations agreed with an exo-scission of EO chain accompanied with oxidation of the hydroxyl terminal side (Sato et al., 2001). Moreover, MALDI-MS

515 alone was used for quantification of NPEO in commercial products too, showing good results by choosing a
516 proper internal standard (Hanton et al., 2006).
517 PEG are polyols used in a wide range of industrial applications including surfactants and precursors
518 for grafted polymers. The characterization of PEG is of significance in correlating compositions and
519 structures with their properties and, for this purpose, the combination of MALDI-ToF MS, ESI MS, and
520 tandem MS demonstrated to be the approach of choice to obtain compositional information required for
521 problem solving. Indeed, Chen et al. (Chen et al., 2001) analysed four EO/PO copolymers to unequivocally
522 demonstrate that one of the samples had a small variation in copolymer composition, thus explaining its
523 abnormal activity. Moreover, two samples, displaying similar properties and activities, were found to be two
524 different PEG blends.
525
526
527 Fatty acid alkanolamides (**Figure 6**), useful for increasing the viscosity of liquid formulations
528 including shampoo (from ca. 0.5 to 5% by weight), analysed by EI-MS, exhibit radical molecular ions $[M]^{+\bullet}$
529 for unsaturated species and protonated adducts $[M + H]^+$ for saturated alkyl compounds. The peak signal
530 intensities due to water loss ($[M - H_2O]^+$) are usually higher in the case of unsaturated compounds. The length
531 of the alkyl chain may be determined by the peak signal values of $[RC{\equiv}O]^+$ and $[RCON(=CH_2)CH_2CH_2OH]^+$.
532 It is worth mentioning that unsaturated homologs give more intense peak signals than the saturated ones. A
533 number of ions are independent of the alkyl chain: McLafferty rearrangement leads to the formation of the
534 $[CH_2=C(OH)N(CH_2CH_2OH)_2]^+$ ion at *m/z* 147. The occurrence of $[HOCH_2CH=NHCH_2CH_2OH]^+$ at *m/z* 104,
535 $[CH_2=NHCH_2CH_2OH]^+$ at *m/z* 74, and $^+O{\equiv}CN(CH=CH_2)CH_2CH_2OH$ at *m/z* 114 is observed too. In the case
536 of monoethanolamides, the radical molecular ions are not observed, but there are low abundance $[M - H_2O]^+$
537 ions. It has been soundly assessed the presence of fragment ions that are independent of the alkyl chain length
538 such as $[CH_2=CHNHCOCH_2CH_3]^+$ at *m/z* 99, being the base peak for unsaturated compounds and an
539 important peak for saturated ones. The ion $[CH_2=C(OH)NH_2CH=CH_2]^+$ at *m/z* 86 is formed following a
540 McLafferty rearrangement, and it is the base peak for saturated compounds and a large peak in the unsaturated
541 homologs (Moldovan et al., 1997).

542 Belonging to the non-ionic surfactant class are the alkyl polyglycosides (see **Figure 7**), obtained by
543 acid-catalyzed condensation of a fatty alcohol with hexoses such as glucose. Alkyl polyglycosides (APG)
544 typically have single alkyl chains with length in the C8-C16 range, with an average degree of polymerization
545 (DP) of the glycoside moiety between 1 and 4. For their natural origin, they are mainly encountered in
546 applications where mildness and good detergency property are important, such as cosmetics and personal
547 care products (Bajpai and Tyagi, 2010; Broze, 1999). EI-MS of underivatized glycosides provides
548 characteristic fragments of the sugar, fragments of the alkyl chain without the molecular ions. Thus, the
549 identity of the alkyl chain cannot be inferred from these spectra alone. Ambient pressure ionization (API)
550 was applied for direct analysis of APG and esters of APG (Facino et al., 1995). LC-ESI-MS of deprotonated
551 APG molecules ($[M - H]^-$) showed an uniform response and good results in single ion monitoring (SIM)
552 mode (Kühn and Neubert, 2004).
553 Amine oxides are synthetised by oxidation of tertiary amines with hydrogen peroxide in aqueous
554 solution. They are useful as foam stabilizers and are used extensively in cosmetics as well as household
555 products. FAB-MS of amine oxides gives positive ions corresponding to protonated molecules and dimer
556 ions (Lyon et al., 1984a). For example, the mass spectrum of cocodimethylamine oxides exhibited intense
557 $[M+H]^+$ ions at *m/z* 230, 258, and 286 for C12, C14, and C16 homologues, respectively and the relative
558 abundances mirror the commercial product composition, i.e., dodecyl (70%), tetradecyl (25%) and hexadecyl
559 (5%). Proton bound dimers of the form $[2M+H]^+$ corresponding to each component appeared at *m/z* 459
560 (20%), 487 (15%) and 515 (6%). Loss of water from the protonated molecule (*m/z* 230) to yield a peak signal
561 at *m/z* 212, occurs upon CID too. As depicted in **Figure 8**, the most abundant fragment ion at *m/z* 58 come
562 from cleavage of the long β alkyl chain to the nitrogen together with the loss of the -OH group from the
563 parent ion. Another fragment at *m/z* 60 is formed by direct cleavage of the C-N bond of the dodecyl moiety
564 with the oxygen remaining attached to the nitrogen (35% relative abundance).
565
566 ***3.3 Cationic surfactants***
567 Cationic surfactants are useful as fabric softeners, corrosion inhibitors, and antimicrobial agents.
568 They are not used in general purpose detergents because they do not provide effective cleaning at neutral pH
569 values. In general, only nitrogen-based compounds are used as cationic surfactants, most of them being

570 quaternary ammonium salts (QAS) (Broze, 1999; Morelli and Szajer, 2000; Zakharova et al., 2019). Under
571 EI or CI conditions, quaternary amines normally decompose to tertiary amines, which can be ionized or
572 protonated for MS detection. Thus, ordinary MS shows only fragments and rearrangement product ions, and
573 its use is rather limited in the analysis of unknown mixture compounds (Tsai and Ding, 2004). FAB tandem
574 mass spectra of aliphatic amines show diagnostic ions useful to infer the alkyl chain length by considering
575 related fragment ions. Both negative and positive ionization modes were successfully performed on
576 quaternary ammonium salts and ethoxylated quaternary amines in order to identify the composition of the
577 commercial product Arquad 12-50 and Ethoquad 18/12 (Lyon et al., 1984a).
578
579 ***3.4 Amphoteric surfactants***
580 Amphoteric surfactants contain both anionic and cationic functional group in the same molecule,
581 therefore they can act either as anionic or cationic surfactants, depending on the solution pH. Their production
582 is more costly than ionic and amphoteric surfactants, representing just 3% of surfactants in Europe and less
583 than 1% in the United States. They are less irritating than other materials and are largely used in personal
584 care products. Bond cleavage amphoteric compounds usually occurs before volatilization in conventional EI
585 or CI sources. Apparently, ESI as a soft ionization source is more suitable for MS analysis (Eichhorn and
586 Knepper, 2001; Lyon et al., 1984a) and their di-functionality can be inferred by the existence of diagnostic
587 ions.
588
589 ***3.5 Biosurfactants***
590 Currently, MS is the most exploited technique to study biosurfactants, which are surface active
591 substances derived from natural sources even though frequently biosurfactant refers specifically to the
592 surfactants produced directly by microorganisms. Biosurfactants represent an eco-friendly and innovative
593 alternative, as they are typically of lower toxicity than synthetic surfactants (Hayes and Smith, 2019). Since
594 biosurfactants boast a lower environmental impact, they have found application in the production of fine
595 chemicals, as surface coatings, additives for environmental remediation and in biological control (Kitamoto
596 et al., 2002; Pacwa-Płociniczak et al., 2011). Biosurfactants are amphiphilic compounds produced in living
597 spaces or excreted as extracellular hydrophobic and hydrophilic moieties that confer on the organism the

ability to accumulate between fluid phases, thus reducing surface and interfacial tension. They are produced by several microorganisms, bacteria, fungi, and yeasts which include *Acinetobacter sp.*, *Bacillus sp*, *Candida antartica*, and *Pseudomonas aeruginosa*. Biosurfactants are glycolipids, lipoamino acids, lipopeptides, lipoproteins, lipopolysaccharides, phospholipids, monoglycerides and diglycerides. During the last few decades, the increasing interest in biological surfactants led to an intensification of research for the cost-efficient production of biosurfactants compared with traditional petrochemical surface-active components. The request of alternative production strains has been also associated with new demands on biosurfactant analysis. MS/MS testing is extensively employed to figure out the biosurfactant chain units. For example, arthrofactin, a biosurfactant produced by *Arthrobacter* species strain MIS38, was analysed by FAB-CID-MS and identified as 3-hydroxydecanoyl-D-leucyl-D-asparagyl-D-threonyl-D-leucyl-D-leucyl-D-seryl-L-leucyl-D-seryl-L-isoleucyl-L-isoleucyl-L-asparagyl lactone (Morikawa et al., 1993) (see **Figure 9**).

MALDI-ToF MS was used to characterize a biosurfactant produced by a novel marine bacterium *P. korlensis* SBK47 showing significant properties like excellent surface tension reduction, CMC and emulsification activity (Balan et al., 2016). Mass spectra revealed a cluster of three peaks at *m/z* 732.8, 754.8 and 770.8, recognized as protonated, Na^+ and K^+ adducts (**Figure 10a**). The tandem MS analysis of the precursor ion at *m/z* 732.8 revealed the amino acid sequence as Ser-Asp-Val-Ser-Ser (**Figure 10b**). ~~The molecular mass of the fatty acid was 240.3 *m/z*, which is a protonated palmitoyl group as ketene.~~ Based on these data, the structure of the lipopeptide biosurfactant was elucidated as palmitic acid-Ser-Asp-Val-Ser-Ser (**Figure 10c**), which represents a new lipopeptide biosurfactant named *pontifactin*.

HPLC-ESI-MS was used by Costa et al. (Costa et al., 2006) to characterize the rhamnolipids produced by *Pseudomonas aeruginosa* LBI in several Brazilian plants. ~~In detail,~~ Mass spectrometry analysis revealed the presence of two major components detected as the anions of *m/z* 649 and 503, which corresponds to the deprotonated molecules $[M–H]^-$ of the dirhamnolipid ($Rha_2C_{10}C_{10}$) and ~~the~~ monorhamnolipid ($RhaC_{10}C_{10}$), respectively, with the latter (*m/z* 503) being the predominant ion in all samples analyzed. Mass selection of these anions followed by ~~collision induced dissociation~~ CID-MS/MS produced characteristic ~~tandem mass~~ spectra (**Figure 11**) that confirm the structural assignments. For instance, $[RhaC_{10}C_{10}–H]^-$ at *m/z* 503 generated two major product ions by the gas-phase neutral loss of a monounsaturated C10 acyl chain to form $[RhaC_{10}–H]^-$ at *m/z* 333, and *m/z* 169 by the neutral loss of $RhaC_{10}H$ and formation of [unsaturated $C_{10}–H]^-$

(**Figure 11b**). Likewise, $[Rha_2C_{10}C_{10}–H]^-$ of *m/z* 649 dissociates to generate two major product ions at *m/z* 479 and 169 (**Figure 11a**).

In a recent study of Moro et al. (Moro et al., 2018) on bacteria related to the *Bacillus* genus, surfactin isoforms were identified through ultra-high-performance LC coupled with high-resolution MS (UHPLC-HRMS). The surfactins are a mixture of isoforms, in which an amino acid of the cyclic peptide chain of surfactin Glu/Leu/Leu/Val/Asp/Leu/Leu is replaced by another amino acid. These isoforms may be differentiated according to their fragmentation pattern and structural features (**Figure 12A-E**). In detail, the MS/MS spectra of surfactin isoforms present some common fragments of $[M+H]^+$ at *m/z* 685.44, 671.43 and 699.46, which are due to protonated amino acid sequences of Val/Leu/Asp/Val/Leu/Leu, Leu/Leu/Asp/Val/Leu/Leu and Leu/Leu/Asp-OMe/Val/Leu/Leu, respectively. Common fragments of an isoform with primary structure Val/Leu/Asp/Val/Leu/Val was found and proposed in **Figure 12E**, suggesting the occurrence of a new surfactin component (Moro et al., 2018).

3.6 Pulmonary surfactants (PS)

Several MS-based approaches have been exploited ~~in order~~ to characterize pulmonary surfactants (PS). PS are mixtures of lipids and proteins occurring in the alveolar surface of lungs, which form a layer between the aqueous airway liquid and the inspired air (Choi et al., 2020). Due to their essential role in preventing the collapse of lungs, a pulmonary surfactant deficiency could contribute to the etiology of persistent or severe respiratory distress (e.g. newborn respiratory distress syndrome, NRDS) (Akella et al., 2013). In the last years, many natural mixtures of lipids and proteins have been proposed for the pharmacological treatment or prophylaxis of distress syndromes associated with PS deficiency.. The composition of Curosurf, a pulmonary surfactant made up of 99% of polar and neutral lipids and about 1% of low molecular weight hydrophobic apoproteins, has been extensively investigated by Pelizzi et al. (Pelizzi et al., 2002). These authors developed a rapid and selective ESI-MS/MS method without preliminary derivatization or chromatographic separation; the following phospholipids (PL) were qualitatively identified: phosphatidylcholines (PC), lysophosphatidylcholines (LPC), sphingomyelins (SM), phosphatidylethanolamines (PE),

phosphatidylglycerols (PG) and phosphatidylinositols (PI). Tandem MS experiments proved to be very useful for the unequivocal discrimination of PL. ~~In detail, PC, SM and LPC were identified by 59 Da neutral loss (trimethylamine molecule) in ESI (+). PE identification was performed through precursor ion scan experiments for the fragment ion at *m/z* 164, i.e. the sodiated adduct of the ethanolamine phosphoric acid. Instead, in ESI (-), PS components were identified by monitoring the neutral loss of the serine moiety (87 Da); PI through precursor ion scans for cyclic inositol phosphate (*m/z* 241), and PG by precursor ion experiments on the *m/z* 153~~ (**Figure 13**).

Liu et al. (Liu et al., 2008) used nano ESI-MS/MS to characterize the amino acid sequence of bovine surfactant proteins B (SP-B) and C (SP-C), i.e. other natural surfactants used for the treatment of respiratory distress. It should be noted that, to determine the amino acid sequences, SP-B was first digested with trypsin and then analyzed by tandem MS after a preliminary chromatographic separation. From the LC-MS/MS analysis of the protein digest, six unique peptides and some mis-cleaved peptides were detected. By assembling the peptides together and by considering SP-B from other species as templates, the complete sequence of bovine SP-B was obtained, namely FPIPI-PYCWL-CRTLI-KRIQA-VIPKG-VLAMT-VAQVC-HVVPL-LVGGI-CQCLV-ERYSV-ILLDT-LLGRM-LPQLV-CGLVL-RCSS. Thus, seven cysteine residues (C) were identified for the first time in the amino acid sequence.

Porcine pulmonary phospholipids (SP-C) have been widely used as well as natural surfactants. Their native form was investigated by plasma desorption (PD) MS, showing a lipopeptidic structure with two palmitoylated cysteine residues (Curstedt et al., 1990). In addition to PDMS, ESI MS has been used as well for the analysis of porcine SP-C preparation, demonstrating the existence of extended forms of pulmonary SP-C including histidine and methionine as C-terminal residues (Griffiths et al., 1998). In order to obtain structural information, Griffiths et al. (Griffiths et al., 1998) fragmentated $[M+3H]^{3+}$ ion of methionine oxidized C-terminally methylated SP-C, by using CID. In the tandem MS spectrum, three major product ions series were observed consisting of B_{12}^{2+} –B_{32}^{2+}, B_{14}^{3+}–B_{34}^{3+} and B_2^{+}-B_6^{+}, B_{17}^{+}-B_{22}^{+} (**Figure 14**). The sequence ion B_{34}^{3+} allowed to locate the site of methylation on the C-terminal amino acid. Moreover, the masses of sequence ions B_{33}^{3+} and B_{32}^{3+} enabled the

identification of an oxidized methionine residue as amino acid 33. Instead, the B^+ ion series allowed to identify the palmitoylation sites on 5 and 6 cysteine residues.

4. Quantitative analysis of surfactants by using MS

To determinate very low content of targeted compounds such as surfactants in complex mixtures, the MS quantitative analysis needs high sensitivity and selectivity. MS coupled to GC or LC markedly improves its ability to cope with complex matrices (Bianco et al., 2013, 2010; Pascale et al., 2020a) and targeted MS/MS experiments increase even more the selectivity (Pascale et al., 2020a). Both chromatographic step and tandem mass experiments reduce background noise, thus reaching outstanding levels of sensitivity and selectivity as well (Pascale et al., 2020b; Ventura et al., 2019). Most scientific efforts have triggered the development of methods mainly concentrated on specific classes of surfactants, for their toxicity or their occurrence in common commercial products. Among these compounds, the discussion was focused on non-ionic APEO, anionic LAS, polyfluorinated alkyl surfactants (PFAS) and cationic surfactants as quaternary ammonium salts.

4.1 Non-ionic surfactants: the case of alkylphenol ethoxylates (APEO)

Non-ionic APEO deserve special attention because of their massive use and ubiquitous occurrence in the environment. The global production of APEO is well over 300.000 tons/year and have been extensively used in the last 40 years as detergents, emulsifiers, dispersants, anti-foamers and pesticide adjuvants. Approximately 60% of APEO that enter mechanical and biological sewage and sewage sludge treatment are subsequently released into the environment (Cserhati, 1995; Petrovic et al., 2002). Some of these surfactants biodegrade before reaching the environment: primary degradation (chain oxidation) may be 90% complete within a few hours to 1 month under favourable laboratory conditions, and treatment plants often remove more than 95% of the higher ethoxylates through secondary treatments (Ying et al., 2002). Initial degradation proceeds as an attack on the long ethoxy chain to produce short chain nonylphenol ethoxylates (NP_nEO, with n = number of ethoxy units) or carboxylic acids. Apparently, these APEO metabolic intermediates are more toxic than their parent compounds and more bio-accumulative in aquatic environments (Marcomini et al.,

1990; Petrović and Barceló, 2000). Their environment degradation is highly variable with the result that the effluent concentrations of NPEO and metabolites are also highly variable. As expected, a portion of NPEO passes through treatment plants to enter the environment a recent concerns have aroused on APEO as potential endocrine disrupters (Petrović and Barceló, 2000). Considering the toxicity, the large production volumes and likely persistence, APEO and especially NPEO have emerged as a leading issue in Europe where a withdrawal for many uses largely occurred (Ying et al., 2002). All these worries lead to the need to improve and standardize analytical methods for their determination in complex environmental matrices. In this sense, the use of GC-MS was a turning point for APEO and relative derivative quantitation, because of higher sensitivities and specificities obtained in comparison to other techniques used on a routine basis for quality control purposes, like UV/Vis spectroscopy. Indeed, Stephanou et al. (Stephanou and Giger, 1982) successfully identified and quantified NP and NPEO with one and two oxyethylene groups in the effluents of six mechanical-biological sewage treatment plants. In detail, limits of detection in μg/L range were obtained, thus allowing the quantitation of analytes from 36 to 202 μg/L, representing from 0.5% to 2.3% of the total residual dissolved organic carbon. The same approach was exploited by Ding et al. for the targeted analysis of nonylphenol polyethoxycarboxylates (NPEC) and their related degradation products (i.e., carboxyalkylphenol ethoxycarboxylates, CNPEC) in water samples. The method involved an extraction step using graphitized carbon black (GCB) cartridges, and the direct derivatization with tetraalkylammonium (TAA) salts in the GC injection-port by using a large-volume (10–20 mL) direct sample introduction device. The analytes were identified and quantitated, while on-line derivatization technique provided sensitive, fast and reproducible results for NPEC and their metabolites, with good recoveries ranging from 90 to 108% (Ding and Chen, 1999).

Thus, for non-ionic surfactants, the employment of GC-MS either with EI or chemical ionization (CI) has proved to be useful for the analysis of these compounds with a low number of polyethoxylation degree. However, several drawbacks are associated with GC-MS, which is not suitable for the analysis of compounds with high degree of polymerization and high polarity, because of their low volatilities. Adding derivatization steps during the sample preparation process is a good solution in few cases and without accounting the completeness of reactions, the analysis is labour and time demanding, making the GC-MS of surfactant not suitable for routine work (Tsai and Ding, 2004).

735 Compared with GC-MS, HPLC with ESI-MS detection offers excellent sensitivity for ionic and polar
736 molecules, making it an ideal candidate for the quantitative determination of low volatile APEO metabolites.
737 For example, a very sensitive and specific analytical procedure for determining non-ionic polyethoxylate
738 surfactants, such as aliphatic ethoxylate alcohols (AEO) and nonylphenol polyethoxylates (NPEO) in
739 aqueous environmental samples using LC-ESI-MS was developed by Crescenzi et al (Crescenzi et al., 1995).
740 The limits of detections (LOD) were 0.6, 0.02, 0.002, and 0.0002 μg/L for NPEOs and each AEO homologue
741 in the influents and effluents of sewage treatment plants, river water, and drinking water, respectively.
742 Recoveries of the analytes ranged between 85 and 97% and the liquid chromatographic conditions were
743 adjusted for eluting all the oligomers of NPEO and of the various AEO homologues as single peaks to
744 enhance detection levels and simplify quantification. LC-MS was also used for the analysis of APEO in solid
745 samples, sediments, and sludges, with LODs values in the low ppb level. Pivotal for the analysis of complex
746 solid matrices was the use of SPE cartridges during sample preparation step, which turned out to be the
747 perfect solution for the matrix effect, one of the major LC-MS related problems (Pascale et al., 2018b).
748 Finally, aquatic organism samples were analysed to find APEO and related derivatives, thus shedding some
749 light on the fate of these substances once released into the environment.
750
751 ***4.2 Anionic surfactants: the case of linear alkyl sulfates (LAS) and polyfluorinated alkyl surfactants***
752 ***(PFAS)***
753 Among anionic surfactants of environmental interest, linear alkyl sulfates (LAS) and polyfluorinated
754 alkyl surfactants (PFAS) represent the emerging contaminants due to their occurrence in humans and wildlife
755 and their bio-accumulative, nondegradative, and toxic properties (Giesy and Kannan, 2001; Grandjean and
756 Clapp, 2015). GC-MS was successfully employed for LAS analysis. In detail, these compounds were found
757 and quantified in anaerobically and aerobically stabilized sewage sludges. Sulfonyl chloride species were
758 properly formed and separated by high-resolution gas chromatography (HRGC) coupled with either flame
759 ionization detection or EIMS and CIMS modes (McEvoy and Giger, 1986). The total LAS concentrations
760 ranged from 0.3 to 1.2% of dry sludge with only minor variations in the relative composition of LAS
761 homologues and isomers. These concentrations were considerably larger than those of other pollutants that
762 have been reported in sewage sludges. Although LAS are soluble in water, the sorption of these amphiphilic

763 chemicals onto suspended particles in sewage seems to be significant. In contrast to their biodegradability in
764 the aqueous phase, LAS are apparently enough resistant to biodegradation during sludge treatment.
765 LC-MS was applied for the polyfluorinated alkyl surfactants (PFAS) analysis. Fluorinated alkyl
766 surfactants as well as hydrocarbon surfactants are used as "active ingredients" in aqueous film-forming foams
767 (AFFF), which are used to fight hydrocarbon-fuelled fires. The fluorinated alkyl substances present in AFFF
768 decrease the surface tension, thus smothering the flames, preventing air from reaching flammable materials,
769 and therefore, suppressing re-ignition of the fire (Moody et al., 2003). Formulations of AFFF contain
770 mixtures of fluorosurfactants produced by either electrochemical fluorination or fluorotelomerization as well
771 as hydrocarbon surfactants, co-solvents, and solvents. Although AFFF compose only a small percent of the
772 total fluorosurfactant production, repeated applications of AFFF during fire training activities conducted at
773 military bases have led to groundwater contamination by AFFF chemicals, unspent fuels, and solvents. Due
774 to this wide spreading of fluorinated surfactant, LC-MS/MS was employed in various investigations to
775 analyse perfluoroalkyl carboxylates and sulfonates in human sera (Olsen et al., 2003), animal tissues (Kannan
776 et al., 2002) and surface water (Moody et al., 2001) showing low LOD (in the μg/L and ng/g ranges,
777 respectively) and recoveries higher than 90%.
778
779 ***4.3 Cationic surfactants: the case of quaternary ammonium salts***
780 Fewer papers were dedicated to the quantitative analysis of cationic surfactants, mostly because of
781 the lower frequency of use in the everyday life as detergents. For quaternary ammonium salts, hyphenated
782 MS techniques are able to reach high sensitivity in the μ- and ng/L dimensions for water samples and in the
783 low-ppb one for solid ones, with recoveries ranging from 70 to 90% (Ferrer and Furlong, 2001; Ford et al.,
784 2002; Radke et al., 1999; Sütterlin et al., 2008; Tsai and Ding, 2004). For example, the determination of alkyl
785 (C12, C14, and C16) dimethylbenzylammonium chloride (benzalkonium chloride or BAC) in water samples
786 was performed by the identification of the main ions at *m/z* of 304, 332, and 360, corresponding to the
787 molecular ions of the C12, C14, and C16 alkyl BAC, respectively (Ferrer and Furlong, 2001). The
788 unequivocal structural identification of these compounds in water samples was performed by LC-MS/MS
789 after the isolation and the subsequent fragmentation of each quaternary ammonium ion. The main product
790 ion observed of all the three different homologues corresponded to the loss of the toluyl group in the chemical

structure, which led to the fragment ions at *m/z* 212, 240, and 268 and a tropylium ion, characteristic of all homologues, at *m/z* 91.

An interesting example of cationic surfactant examined by MALDI-MS was reported by Shrivas et al. (Shrivas and Wu, 2007). A rapid, simple, sensitive, and effective quantitative method for the simultaneous determination of cationic surfactants (CS^+) from river and municipal wastewater was successfully developed by combining single-drop microextraction (SDME), with atmospheric pressure (AP)-MALDI mass spectrometry, without any separation step. SDME uses a microdrop (about 1–3 μL) of organic solvent suspended at the tip of a microsyringe for the extraction of analytes from the aqueous phase, thus allowing the suppression of matrix ions (background interferences) and, consequently, a signal-to-noise increase. This method was found to yield a linear calibration curve in the concentration range from 50 to 1500 μg/L CS^+ with LOD of 10 μg/L. The relative recoveries in river and municipal wastewater were found to be 93.8–103.6% and 91.0–98.7%, respectively.

5. Innovative approaches of surfactant analysis by MS

The most modern advances in MS instrumentation provide the possibility to distinguish isomeric and even isobaric *m/z* values of surfactant complex mixtures by high resolution/accuracy MS (HRMS) along with ion mobility-mass spectrometry (IM-MS) coupled to two-dimensional (2D) separations or supercritical fluid chromatography (SFC).

5.1 High resolution mass spectrometry

The high resolving power (RP) of HRMS instruments reduces interferences and the high mass accuracy allows the prediction of molecular formulas from the *m/z* signals (Bianco et al., 2018; Kind and Fiehn, 2007; Pascale et al., 2016). Other important advances include fast full-scanning rates (i.e. >1 Hz), which allows for on-line coupling to chromatography, and high sensitivity, thereby allowing the detection of trace contaminants at femtogram levels in the full-scan operation mode. Commercially available HRMS apparatuses include various time-of-flight instruments (TOF-MS, RP ≥ 20,000, mass accuracy ≤3 ppm, scan rate up to 200 Hz), Fourier-transform orbital trap (Orbitrap-FTMS, ≥240,000, ≤2 ppm; up to 40 Hz) and

818 Fourier-transform ion cyclotron resonance (FTICR-MS, ≥1,000,000, ≤1 ppm, ≤1 Hz) (Pascale et al., 2018c;
819 Santarsiero et al., 2020).
820 Moe et al. (Moe et al., 2012) proposed a HPLC-ESI-QTOF HRMS method for the structural
821 characterization of a commercial detergent named Forafac®1157, composed of novel fluorinated surfactants
822 developed by the DuPont company. The combined information from the ^{1}H, ^{13}C, ^{19}F, ^{1}H–^{1}H correlation
823 spectroscopy, NMR spectra and *m/z* values allowed the chemical formula assignment of the observed ion at
824 *m/z* 571.09373 in positive ion mode, i.e. $[C_{15}H_{20}F_{13}N_2O_4S]^+$ (Δm=1.1 ppm) to a 6:2 fluorotelomer
825 sulfonamide alkylbetaine (FTAB). The same findings were experienced in negative ion mode where the *m/z*
826 569.073 was isolated and fragmented as reported in plot of **Figure 15**. The same approach was employed for
827 its major metabolites extracted from turbot and mussel, both exposed to the commercial product treated with
828 simulated UV light. The tandem MS spectrum of the precursor ion at *m/*z 511.079 is illustrated in plot b
829 (**Figure 15**), which is 58.00543 Da lower than the parent compound suggesting the neutral loss of oxiran-2-
830 one ($C_2H_2O_2$, 58.00548) from the betaine moiety and the generation of a secondary *N*,*N*-dimethylamine.
831 Photolysis also yielded one by-product whose $[M–H]^-$ ion was observed at *m/z* 425.983 (**Figure 13c**; Δm =
832 2.5 ppm), which corresponds to the 6:2 fluorotelomer sulphonamide.
833 Chiesa *et al*. (Chiesa et al., 2018) developed and validated a sensitive method by HPLC-HRMS to
834 monitor the occurrence of 16 PFAS in eels from the Gard Lake (Italy). The limits of detection were in the
835 order of pg/g and the recoveries were between 80 and 101%. MS/MS targeted analysis was used to improve
836 analyte identification by chemical formula calculation of precursor and product ions.
837 Despite these great advantages, HRMS produces a huge amount of data, which require an effective
838 data processing strategy. Kendrick mass defect (KMD) analysis is one of the most common approach, used
839 for the identification of homologous series, i.e. a series of MS signals differing only by a number of
840 previously chosen base units, such as those present in surfactants, demonstrating to be fully suitable for the
841 by-products identification of unknown surfactants (Fouquet and Sato, 2017; Hughey et al., 2001; Tziotis et
842 al., 2011). Thurman et al. (Thurman et al., 2014) used LC-QTOF-MS to perform studies of ethoxylated
843 surfactants in groundwater and surface water. Since this class of surfactants contains long chains of EO units,
844 the KMD analysis was very useful. A mixture of polyethylene glycols (PEG) with EO number, n_{EO}, from 3
845 up to 33 and linear alkyl ethoxylates (LAE) C9-C15 with n_{EO} = 3−28 were identified in hydraulic fracturing

flowback and produced water. It was noted that upon changing the chain length from n_{EO}=7 to n_{EO}=9, the predominant adduct of PEG changed from Na^+ to NH_4^+ with the protonated adduct also existing (see **Figure 16**). Whereas all these adducts were investigated by tandem MS, in **Figure 17** is shown the ammonium adduct at *m/z* 468. As expected, the accurate mass differences between the ammoniated/sodiated and protonated adducts were 17.0267 *u* are 21.9821 *u*, confirming the designation of *m/z* 451.3629 as $[M+H]^+$.

A major purpose of the study was the identification of the ethylene oxide (EO) surfactants and the construction of a database with accurate masses and retention times to solve the mass spectral complexity of surfactant mixtures employed in hydraulic fracturing fluids. Over 500 accurate mass assignments were used as a fingerprint chromatogram of the water samples. The same approach was applied to a series of flowback and produced water samples to demonstrate the usefulness of ethoxylate "fingerprinting" to monitor water quality that results from fluids used in hydraulic fracturing. Interestingly, KMD analysis was also used in conjunction to LC-MS/MS to fully characterize the unknown fluorinated pollutants of fire-fighting foams in environmental samples (Chiesa et al., 2018; D'Agostino and Mabury, 2014; García et al., 2019). Despite the great advantages of using KMD plots for HRMS data processing, there are still some shortcomings such as the low isotopic resolution and the poor distinction of some homologous series. The finite resolution and mass accuracy of the MS analysis and the intrinsically low variations of the KMD values account for a fuzzy appearance and a limited resolving power of the KMD plot, respectively. To overcome this pitfall, the concept of a fractional base unit was introduced; instead of using a molecule or part of a molecule as the base unit for the calculation of Kendrick masses, a fraction of the repeat unit, i.e. (repeat unit)/X with X being an integer, was proposed. Albeit this base unit has no chemical meaning, it is still mathematically acceptable. This mathematical trick significantly amplifies the variation of KMD values and improves the resolution of the KMD plots without interfering with the basics of a KMD analysis such as the point alignments of homologue ions (horizontal or oblique) (T. Fouquet et al., 2017).

This new high resolution KMD (HRKMD) analysis was used, together with MALDI-HRMS, to characterize non-ionic surfactants made of a PEO core capped by esters of fatty acids (Thierry Fouquet et al., 2017). More specifically, a PEO monostearate surfactant was first analysed as a proof of principle of the HRKMD analysis conducted with a fraction of EO as the base unit (EO/X with X being an integer). KMD plots allowed the formula assignment in an easier and faster way. Moreover, HRKMD plots permitted to

observe the presence of more homologous series, which are hardly identified in MALDI full spectrum because of low intensities. Data visualization was greatly enhanced, and the distributions detected in the MALDI mass spectrum were successfully assigned to a pristine (H, OH)-PEO as well as mono- and di-esterified PEO chains with palmitate and stearate end-groups in HRKMD plots computed with EO/45. The MALDI-HRMS/HRKMD analysis was successfully applied to the more complex case of ethoxylated hydrogenated castor oil (EHCO), found to contain many hydrogenated ricinoleate moieties (up to 14) in its HRKMD plot computed with EO/43, departing from the expected triglyceride structure.

5.2 Surfactant analysis by ion mobility-mass spectrometry (IM-MS)

Due to complementary separation capability and elevated peak capacity, LC in conjunction with IM-MS is becoming increasingly popular for complex mixture analysis. IM-MS is a gas phase electrophoretic technique that allows ionized target molecules to be separated relying on their mobility in the presence of an inert carrier gas and under the influence of an electric field of ~200 V/cm (Kanu et al., 2008). The LC-IM-MS enables the initial separation of components by LC, the transfer and dispersion of the generated ions according to their differing mobility and *m/z* registration. Hence, a two-dimensional (2D) separation can be accomplished in a reasonably short time. Particularly, a HILIC column was chosen using a porous silica stationary phase and acetonitrile–water gradient elution, which is readily compatible with ESI, obtaining the separation of APEO oligomers on the basis of their ethoxy chain lengths (Ma et al., 2012). The IM-MS employed a hybrid quadrupole ion mobility TOF mass spectrometer coupled with an orthogonal separation of APEO that is based on size, shape, and electric charge, and lasting roughly 13.0 ms. The high-throughput IM approach demonstrates a promising alternative to traditional chromatographic separation ones for removing the effects of isobaric interferences. The same method was applied to analyse other types of non-ionic surfactants such as complex mixtures of PEO conjugated with a glucose core and esterified with stearic acid, and to better resolve the composition of commercial surfactant products like polysorbate 85 (Katzenmeyer et al., 2016; Solak Erdem et al., 2014).

5.3 Two-dimensional (2D) chromatographic separation techniques

Among hyphenated MS technique, the coupling of MS with 2D chromatographic separation techniques has led to a marked boost of chromatographic peak resolutions (Gilar et al., 2005; Guiochon et al., 2008; Phillips and Beens, 1999). Different conjunctions were explored for surfactant analysis, each of them demonstrating superior capability in comparison to common 1D separation counterparts. The high peak capacity of 2D gas chromatography (GC×GC) clearly reduces co-elution, so that time- and cost-intensive sample preparation largely become less important (Phillips and Beens, 1999). GC×GC coupled with time-of-flight MS allowed the qualitative and quantitative determination of AG, FAEO, FAS, FAES and cocamidopropyl betaines (CAPB) in shower gel and cleaning agents (Hübner et al., 2007). 2D chromatograms were obtained from the analysis of standard solutions and used as fingerprints to identify and quantify surfactants in commercial samples. This method allowed the simultaneous determination of all GC-accessible components including those present at low concentrations, with no undesirable matrix effects. However, sample preparation issues related to GC-MS analysis of surfactants such as derivatization are still present, thus leading to an unavoidable increase of the analysis time.

5.4 The supercritical fluid chromatography (SFC)

Instead of LC and GC, some applications of surfactant analysis by supercritical fluid chromatography (SFC) coupled to MS also exist. SFC has the advantages of low-cost, green, fast separations, high resolution, and low viscosity (Hofstetter et al., 2019). As compared with LC, hydrophobic moieties of compounds favour the fast elution in SFC. Whist different series can be separated by LC, homologue compounds of the same series can be better separated by SFC, which coupled with MS provided very useful structural information (Pan et al., 2020). The SFC-MS was used to analyse alkyl polyglyceryl ethers (Fan et al., 2015), offering further understanding on the product surfactant composition after etherification and it was also developed for the fast determination of alkylphenol ethoxylates in leafy vegetables (Jiang et al., 2017), thus demonstrating its usefulness for anionic surfactant analysis. Takahashi et al. (Takahashi et al., 2013) optimized the SFC-MS experimental parameters of a synthetic mixture of non-ionic surfactant poly(ethylene glycol) nonylphenyl ethers and Pan et al., (Pan et al., 2016; Takahashi et al., 2013) reported the characterization of commercial polysorbate 80 by ultra-high performance SFC and quadrupole time-of-flight MS.

An interesting example of SFC-IM-MS was reported by Ma et al. (Ma et al., 2019) for non-ionic surfactants, including APEO, e.g., octylphenol ethoxylates and FAE, e.g., lauryl alcohol ethoxylates. A column packed with sub-2-μm diol particles was used for the chromatographic separation prior to ESI as first-dimensional SFC exploiting the differences in ethoxy chain, and IM as the second one. A significant enhancement in peak capacity was achieved by SFC-IM-MS, namely more than 40- and 160-fold than those of SFC and IM-MS alone, respectively.

6. Mechanistic study on surfactants

In addition to qualitative and quantitative studies, MS techniques are useful to investigate micelle formation from aggregation processes to aggregate structure and their reactivity/stability (Ceraulo et al., 2011). Despite the possibility of studying surfactant aggregates with MS, an answer to the question of whether aggregates produced in the gas phase mirror those formed in solution is not obvious. Soft ionization sources (e.g. FAB, ESI and MALDI) are important, being able to produce charged aggregates of surfactants, with a survival life long enough to be studied. For example, octyl-trimethylammonium bromide (OTAB) with chemical formula $C_8H_{17}N(CH_3)_3Br$ was examined by ESI-MS to evaluate the molecular weight of micelles formed in water alone, without any additive. From the measurement of the *m/z* of the doubly or triply charged ions, it was revealed that the micelle of OTAB exhibited an almost symmetrical and polydisperse distribution about the mean molecular weight of 5000–5500 Da (Nohara et al., 1998).

Useful hints on the binding affinity of surfactants with metallic ions were also reported. Using sodium dodecylcarboxylate (SL) and dodecyltrimethylammonium bromide (DTAB) in the presence of different salts, Vlachy et al. (Vlachy et al., 2008) showed how the binding tendency of Li^+ was greater than $Na^+ > K^+ > Cs^+$. This binding tendency affects the types and dimensions of formed micelles, and consequently their behaviours into aqueous solutions. Bongiorno et al. (Bongiorno et al., 2005) studied the structure of sodium bis(2-ethylhexyl)sulfosuccinate (AOTNa) aggregates in the gas phase by ESI-MS and MALDI-MS. Large surfactant clusters with an aggregation number close to that found in apolar media were observed either as positive or negative ions. Moreover, the marked predominance of singly charged species as well as preliminary theoretical calculations strongly suggests an aggregate structure characterized by an internal hydrophilic core hosting the extra charge surrounded by an apolar shell constituted by the surfactant alkyl

chains. This structure is like that of the more familiar reversed micelles formed when an appropriate surfactant is solubilized in apolar solvents, which together with the idea that gas phase can be considered an apolar environment, strongly indicates that self-assembling of the surfactant molecules occurs during the evaporation step (Bongiorno et al., 2011).

Conclusions and outlook

Mass spectrometry–based surfactant analysis, including shotgun approaches, has reached a high level of maturity with respect to sample processing, data acquisition and data analysis. The literature screening demonstrated MS is an indispensable tool for the identification and quantification of surfactants. Emphasis was placed on the description and value of existing methods as well as on the most recent innovation of MS. Traditional hyphenates techniques are widely used to resolve complex surfactants mixture; HPLC/MS has been widely used for surfactant analysis, without any derivatization steps, compared to GC/MS. In addition, tandem MS instruments have greatly increased the accessibility of surfactants to MS analysis: fragmentation patterns allow the determination of isomer distribution and other chemical information, such as location of unsaturation, location of side chains, and degree of branching. However, several significant challenges remain. They are primarily related to the complexity of surfactants, which has so far precluded true surfactant analyses (that is, the analysis of all the components of a sample) and generated partially overlapping datasets from identical samples, suggesting poor reproducibility of the technology. Secondarily, these challenges are related to the analysis of surfactants and degradation products in environmental samples. In combination, these problems have created the impression that published surfactant data are at times of dubious quality. Overall, mature MS technologies are available today for surfactants analysis. The most significant improvements, however, will come from HRMS, ion mobility MS, 2D- and superfluid chromatographic approaches. Stand-alone and coupled MS techniques are reliable, highly reproducible and can analyse hundreds to thousands of samples in a short period of time. MS, HRMS and tandem MS are not labour intensive and time consuming, being highly accurate and can discover the presence of impurities, including truncated chains that are often overlooked when using less sensitive and selective approaches. Great strides have been made and we can anticipate continued improvements and cost reductions in the future.

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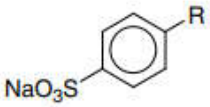
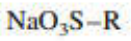
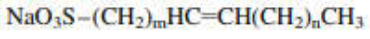
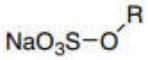
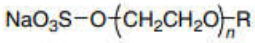
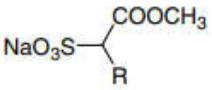
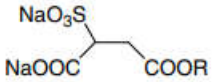
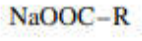
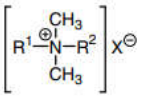
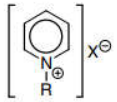
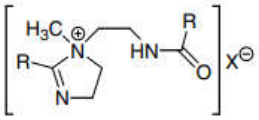
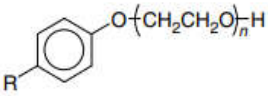
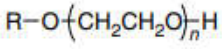
1460 **Table 1.** Classification of surfactants in terms of class, chemical structure, and formula.

Classes	Type	Structure	Formula
Anionic	Linear alkylbenzene sulfonates (LAS)		R= C_{10} –C_{13}
	Alkylsulfonates		R= C_{11} –C_{17}
	α-Olefine sulfonates		m + n = 9-15
	Alkylsulfates		R= C_{11} –C_{17}
	Fatty alcohol ether sulfates		R= C_{12} –C_{14}; n=1-4
	α-Sulfo fatty acid methyl esters		R= C_{14} –C_{16}
	Sulfo succinate esters		R= C_{12}
	Soaps		R= C_{12} –C_{16}
Cationic	Tetraalkylammonium salts		R^1, R^2 = C_1, C_{16} –C_{18} R^1, R^2 = C_{16} –C_{18} R^1 = C_8 –C_{18}, R^2=$CH_2C_6H_5$
	Alkylpyridinium salts		R= C_{16} –C_{18}
	Imidazolium quaternary ammonium salts		R= C_{16} –C_{18}
Non-ionic	Alkylphenolethoxylates (APEO)		R= C_8 –C_{12}; n = 3–40
	Alcoholethoxylates		R= C_9 –C_{18}; n = 1–40

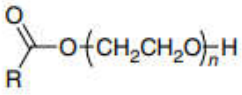
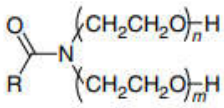
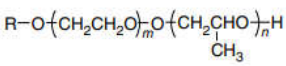
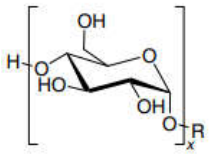
(AEO)		
Fatty acid ethoxylates		R= C_{12} –C_{18}; n = 4
Fatty acid alkanolamide ethoxylates		R= C_{11} –C_{17}; n = 1, 2; m = 0,1
Fatty alcohol polyglycol ethers		R= C_8 –C_{18}; n = 3-6; m = 3-6
Alkylpolyglucosides (APG)		R= C_8 –C_{16}; x = 1-4

Figure Captions

Figure 1 Schematic illustration of the reversible monomer-micelle thermodynamic equilibrium. The red circle represents the surfactant heads (hydrophilic moieties) and the blue curved lines represent the surfactant tails (hydrophobic moieties). When there is a large concentration of surfactants mixed in liquid phase, there may not be enough area at the phase surface for all the surfactant molecules to gather. In these cases, the surfactant monomers (A) will begin to gather in clumps, called normal micelles (B) in polar media and reverse micelles (C) in apolar ones.

Figure 2. EI mass spectra of dodecylbenzenesulfonate at 75 eV (**A**) and 15 eV (**B**). Reproduced with the permission of Elsevier (Agozzino et al., 1986).

Figure 3. ESI(-)-MS spectrum obtained by direct injection of a methanolic solution of coceth sulfate. Inset: the general structure of CES whereby *n* indicates the number of oxyethylene units, between 0 and 7, and *m* (0 or 1) refers to the number of ethylene groups (CH_2CH_2, 28 Da) occurring in the alkyl chain of both series of oligomeric species, i.e. $[C_{12}H_{25}(OCH_2CH_2)_nOSO_3]^-$ and $[C_{14}H_{29}(OCH_2CH_2)_nOSO_3]^-$. Reproduced with the permission of Elsevier (Onzo et al., 2020).

Figure 4. Representative APEO chemical structures and their metabolites nonylphenol (NP) and octylphenol (OP).

Figure 5. Proposed fragmentation pathways for the production of (a) *m/z* 165.21, (b) *m/z* 133.17 and (c) *m/z* 121.16, representative of the formation of product ions of general formula $[NPE_x + H]^+$. Reproduced with the permission of Elsevier (Plomley et al., 1999).

Figure 6. General formula of fatty acid alkanolamides wherein R represents a C10-C18 alkyl group and *n* a number from 1 to 3.

Figure 7. General formula of alkyl polyglycosides whereby DP is the degree of polymerization.

Figure 8. Protonated dodecyl (i.e. coco or lauryl) dimethylamine oxide and the schematic formation of product ions at *m/z* 58 and 60.

Figure 9. Schematic structure of arthrofactin.

Figure 10. MALDI-TOF-MS spectrum of the purified lipopeptide biosurfactant from *Pontibacter korlensis* SBK-47 (b) Tandem MS spectrum of the protonated precursor ion $[M+H]^+$ at *m/z* 732.8 and (c) Deduced structure of the purified lipopeptide biosurfactant from *P. korlensis* SBK-47. Reproduced with the permission of Elsevier (Balan et al., 2016).

Figure 11. ESI tandem mass spectra of: (a) $[Rha_2C_{10}C_{10}–H]^-$ of *m/z* 649 and (b) $[RhaC_{10}C_{10}–H]^-$ of *m/z* 503. Reproduced with the permission of Elsevier (Costa et al., 2006).

Figure 12. Fragmentation pattern for surfactin isoforms. (A) Surfactin A, fragment ions with *m/z* 685.449 and 554.355. (B) Surfactin B, fragments ions with *m/z* 671.434 and 554.355. (C) Surfactin monomethyl ester, fragments ions with *m/z* 699.465 and 568.370. (D) [Leu4] surfactin, fragments ions with *m/z* 699.465 and 568.370. (E) Isoform surfactin, fragments ions with *m/z* 657.418 and 540.339. Groups R1: $(CH_2)_5$-11$CH(CH_3)_2$. Reproduced under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original open access work is properly cited (Moro et al., 2018).

Figure 13. Structures of the main Curosurf phospholipid classes and description of the MS/MS experiments used for their selective monitoring. Reproduced with the permission of Wiley (Pelizzi et al., 2002).

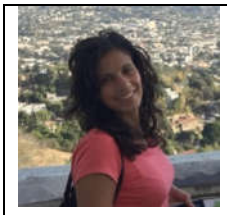
Figure 14. Fragmentation scheme of SP-C The start and end of B-type fragment ion series are indicated. Reproduced with the permission of Wiley (Griffiths et al., 1998).

Figure 15. Mass spectra of deprotonated (a) 6:2 FTAB; (b) the major metabolite from mussel and turbot, and the major photolytic transformation product; (c) the 6:2 fluortelomersulfonamide by-product was produced by turbot, mussel, and photolysis. All spectra were recorded at CV 35 V. Reproduced with the permission of Elsevier (Moe et al., 2012)

Figure 16. Mass spectra of putative structures for PEG-EO7, -EO8, and -EO9 with calculated exact masses showing accuracies of 0.3−0.5 ppm for the $[M+H]^+$. Overall, the mass accuracies are less than 1 ppm. Reproduced with the permission of Elsevier (Thurman et al., 2014).

Figure 17. Tandem MS spectrum at *m/z* 468 with a putative identification of C12 and n_{EO}=6 $[M+NH_4]^+$. Mass accuracies are 0.2 ppm for NH_4^+ adduct and 1.5 ppm for the major fragment ion at *m/z* 283.1746. Reproduced with the permission of Elsevier (Thurman et al., 2014).

SHORT BIBLIOGRAPHY OF AUTHORS



Raffaella Pascale

She obtained a master's degree in Chemical Sciences at the University of Basilicata and she is a Doctor of Philosophy (PhD) in Engineering for Innovation and Sustainable Development (XXXI Cycle) at the University of Basilicata. Her research work concerns, mainly, the validation of GC-MS and HPLC-MS analytical methods according to the ICH, EURACHEM and AOAC guidelines. In addition to academic experience, she worked in a environmental laboratory for the analysis of organic and inorganic pollutants in environmental matrices, applying the EPA and ISO standardized methods. She is currently an IPC-QC Analyst for the quality control at an Italian pharmaceutical industry.

Maria Assunta Acquavia

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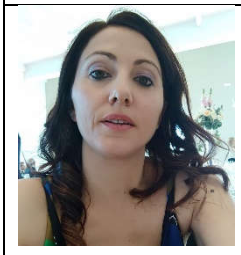
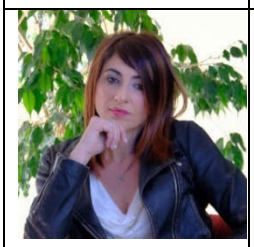
Alberto Onzo

He hold a master's degree in Chemical Sciences at the University of Salerno (Italy) and he is currently a Doctor of Philosophy (Ph. D.) in Analytical Chemistry (XXXIII cycle). His research work is related to the targeted and untargeted metabolomic analysis of food products by means of hyphenated Mass Spectrometry techniques and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and the development of open source software for Mass Spectrometry data elaboration and visualization.

	Nowadays, he is working as a QC analyst in a private italian laboratory, dealing with the analysis of environmental and food matrices.
	**Tommaso R.I. Cataldi** Full Professor at University of Bari (Department of Chemistry). His research work is focused on the use of LC-MS to identify and quantify relevant metabolites with a special focus on the lipidome of biological fluids, bacteria, vegetables and seafood. The main aim is to investigate and understand the fundamental mechanisms whereby lipids (e.g., phospholipids, sulfolipids, etc) are involved in the regulation of metabolic processes, including abiotic stress of bacteria, nutraceutical properties of farmed and wild fishes, and human neurodegenerative disorders.
	**Cosima Damiana Calvano** Assistant Professor of Analytical Chemistry at University of Bari in the Master's Degree Course in Pharmacy and teacher of Applied Mass Spectrometry in the Master's Degree Course in Chemical Sciences. Her current scientific interests relate to the use of mass spectrometry for proteomics and lipidomics applied to clinical, food and cultural heritage fields. Another important research activity concerns the development of new matrices for MALDI MS (second generation ionic liquids, nanostructured materials, binary matrices, strong organic bases, newly synthesized matrices derived from conventional ones).
	**Giuliana Bianco** Professor at University of Basilicata of Analytical Chemistry in the Master's Degree Course in Pharmacy and of Analytical Methods for Environmental Analysis in the Master's Degree Course in Chemical Sciences. The research field concerns mainly the development of new analytical methodologies based on the use of separative techniques as liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE), the most modern

	spectrometry techniques mass (MS) as well as combined techniques (LC-MS, GC-MS, CE-MS) for the determination of compounds of interest in biotechnology, biomedical, agro-food, environmental and microbiological, etc.

1565