Simultaneous quantification of poly-dispersed anionic, amphoteric and nonionic surfactants in simulated wastewater samples using C_{18} high-performance liquid chromatography–quadrupole ion-trap mass spectrometry

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Abstract

This paper describes the development of a guantitative method for direct and simultaneous determination of three frequently encountered surfactants, amphoteric (cocoamphoacetate, CAA), anionic (sodium laureth sulfate, SLES), and nonionic (alcohol ethoxylate, AE) using a reversed-phase C_{18} HPLC coupled with an ESI ion-trap mass spectrometer (MS). Chemical composition, ionization characteristics and fragmentation pathways of the surfactants are presented. Positive ESI was effective for all three surfactants in agueous methanol buffered with ammonium acetate. The method enables rapid determinations in small sample volumes containing inorganic salts (up to $3.5\,\mathrm{g\,L^{-1}}$) and multiple classes of surfactants with high specificity by applying surfactant specific tandem mass spectrometric strategies. It has dynamic linear ranges of 2–60, 1.5–40, 0.8–56 mg L^{-1} with R^2 egual or greater than 0.999, 0.98 and 0.999 (10 μL injection) for CAA, SLES, and AE, respectively.
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1. Introduction

Surfactants are the active ingredients in personal hygiene products and detergents for industrial and household cleaning. There are four classes (cationic, anionic, amphoteric and nonionic) based on the ionic charge (if present) of the hydrophilic portion of the surfactant in an agueous solution. The widespread application of these surfactants results in their appearance in wastewaters, and effective removal of surfactants via biodegradation is one of the main objectives in wastewater treatment. Biodegradation of surfactants serves as a key indicator for the efficacy of any treatment systems, and is often studied by monitoring the disappearance of the starting materials and/or the formation of the degradation intermediates or end products (CO_2) of their complete mineralization. Tracing the dynamics of the starting materials would be advantageous over monitoring the CO_2 production in a complex system when the specificity and study of primary degradation kinetics are required. However, the co-existence of multiple classes of surfactants along with other organic contaminants in wastewater poses significant analytical challenges.

Although high-performance liquid chromatography has long demonstrated its usefulness for the analysis of surfactants because of its separation power and direct applicability

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to non-volatile molecules [1–3], there are difficulties associated with the analysis of modern synthetic surfactants. Surfactants are not single well-defined molecules, but rather mixtures of homologous compounds that are difficult to separate chromatographically. Most modern surfactants due to heightened environmental awareness do not possess light absorbing properties and are therefore not amenable to conventional UV–vis detection. Great progress has been made in both separation and detection technologies over the past two decades [3], with a great number of reports on successful quantification of individual class of surfactants; e.g. cationic surfactants [4], anionic surfactants [5,6], nonionic surfactants [7–14]; and amphoteric surfactants [14–17]. Methods for simultaneous determination of surfactants belonging to different classes are less developed. The need for such analysis is increasing given that "modern" cleansing agents often are comprised of more than one type of surfactant to achieve balanced properties (e.g. cleansing power, foaming, low skin irritation, and etc.). The limited number of reports on simultaneous analysis has either focused on separation techniques [18,20] or tailored for unique application [19,21]. For instance, Nakamura and Morikawa [18] successfully demonstrated the separation of four classes of surfactants as well as individual homologues within classes by using two ODS modified silica columns connected in series. They later applied the method, in conjunction with a refractive index detector [19] to analyze a surfactant mixture from shampoos and detergents. The weakness of the method is its reliance on chromatographic retention time for surfactant identification, thus lacking of specificity. Furthermore, its application is limited to commercial products where surfactant concentrations are much higher than in wastewater and environmental samples. Recently, Haefliger developed a two-dimensional HPLC technique utilizing both hydrophobic and hydrophilic properties of surfactants to achieve separation of all four classes of surfactants in one single run [20]. However, the method requires three HPLC pumps, and lacks specificity and sensitivity due to the evaporative light scattering detection (ELSD) employed. An HPLC–mass spectrometery (MS) method developed by Petrovi and Barcelo [21] is by far the most comprehensive approach, allowing quantification of anionic, nonionic surfactants and their degradation intermediates. However, an increasingly important surfactant type (amphoterics) was not evaluated. Most methods for analyzing of environmental samples and industrial water encompass lengthy sample preparation steps (most commonly the solid phase extraction), require large volume of samples (e.g. 1 L) and surfactant (analyte) recovery from such sample clean-up and concentrating procedures is subjective to operators. Therefore, these methods could not meet our need in monitoring the performance of a small-scale laboratory bioreactors. Our objective was to develop a method that allows simultaneous quantification of the most frequently encountered surfactants (anionic, amphoteric and nonionic) without pre-concentrating, fractionation or clean-up steps. This paper presents the development of such a method using a reverse-phase liquid chromatography with gradient elution linked to an electrospray ion-trap mass spectrometer.

2. Experimental

2.1. Chemicals

The industrial products, Miranol C2M Conc-NP from Rhodia Inc. (Cranbury, NJ, USA) and Steol CS-330 from Stepan (Northfield, IL, USA) were used as sources of amphoteric surfactant and anionic surfactant, respectively. According to the manufacture specification, Miranol C2M Conc-NP (lot# W11E089099) contains 38.53% disodium cocoamphodiacetate (cocoamphoacetate, CAA, Fig. 1A), while Steol CS-330 (Lot#119273) contains 28.8% sodium laureth-3 sulfate (SLES, Fig. 1B) as the active ingredient. Polyoxylethylene 10 Lauryl Ether (AE, Fig. 1C) was used as a representation of nonionic surfactant and purchased from Sigma–Aldrich (St. Louis, MO).

Organic solvents and other reagents were HPLC grade and analytical reagent grade. Deionized water was further purified by a Barnstead (Dubuque, IA, USA) four module E-pure system, which renders organic-free water with resistivity of greater than $18 \, m\Omega \, cm^{-1}$.

2.2. Qualitative analysis of surfactant mixtures

Both direct infusion and a shallow gradient HPLC/data dependent mass scan experiments were performed to characterize the industrial products investigated in this paper. For direct infusion, Miranol and Steol were diluted in deionized water to reach a concentration $25–50 \, mg \, L^{-1}$ of active surfactants. The solutions were then directly infused into the ESI source via a syringe pump at a flow rate of $5–10 \, \mu L \, min^{-1}$ teed with $0.2 \, mL \, min^{-1}$ HPLC mobile phase containing 70% acetonitrile and 30% ammonia acetate buffer (25 mM).

A Phenomenex (Tottace, CA) Synergi 4μ Hydro-RP 80 A column (2.0 mm × 150 mm) was used to effect the shallow gradient HPLC/data dependent mass scan experiment.
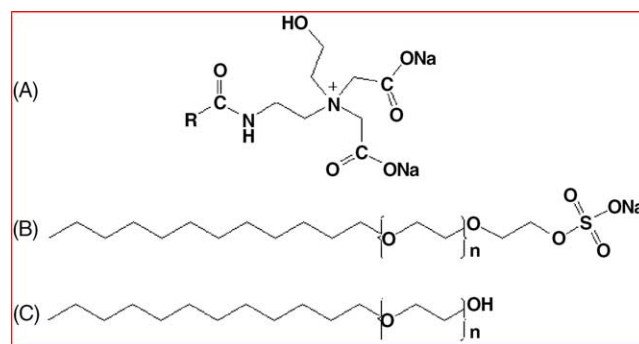

Fig. 1. Chemical structures of representative surfactants (A) cocoamphoacetate; (B) sodium laureth sulfate; (C) polyoxylethylene 10 lauryl ether.

Solvent A and B were 0.5% acetic acid in water and methanol, respectively. Solvent B was linearly increased from 5% to 20% within 5 min and then to 100% within the next 125 min at a flow rate of 0.2 mL min^{-1}. Two microliter of 1/100 diluted Miranol was injected onto the column. Mass spectrometry was operated under positive ESI mode at 3.5 KV spray voltage, 15 and 0 V for capillary and tube lenses. Capillary temperature was maintained at 250 °C, sheath and aux gas (nitrogen) were 60 and 5 arbitrary units, respectively.

2.3. Quantitative analysis of surfactants

A Thermo Separation Product HPLC system coupled with ThermoFinnigan (San Jose, CA, USA) LCQ^{Deca} mass spectrometer was used for this study. Chromatography was performed on an Alltech (Deerfield, IL, USA) Alltima C$_{18}$ column (5 μm particle size, 2.1 mm × 250 mm) preceded by a guard column (4 mm × 4 mm, 5 μm particle) of the same packing using a gradient of ammonium acetate buffer (A) and acetonitrile (B) at a flow rate of 0.2 mL min^{-1}. Solvent A was 30 mM ammonium acetate/acetic acid with pH 3.6. Solvent B was 10% for first 5 min, linearly increased to 70% over 15 min, held at 70% for 30 min, linearly increased to 85% over 5 min and held at 85% for 5 min. Ionization was effected via electrospray in positive ion mode. Mass spectrometer was tuned and optimized for each type of surfactant via direct infusion experiments to achieve efficient ionization, ion transmission and desired fragmentation. The resulting operation parameters and quantification strategies are summarized in Table 1.

Briefly, 10 μL out of 1 mL filtered samples was injected using an autosampler (Model AS 3000), and subjected to chromatographic separation under the gradient program described. LC effluent of the first 5 min was directed to waste via a 6-port switching valve, and that between 5 and 60 min was directed to the electrospray interface of the mass spectrometer. Three specific sets of mass parameters were applied to the chromatographic time windows of 5–26 min, 26–35 min and 35–60 min where CAA, SLES and AE elute. In time segment 1, the m/z 345 [M + H]$^+$ ion of 12C alkyl of CAA underwent collision induced dissociation (CID), and its product ion (m/z 226) was monitored. In time segment 2, source CID was applied. Subsequently the selected ions m/z 512, 556 and 600

corresponding to the ammonium adducts [(M + NH$_4$)−SO$_3$]$^+$ of the desulfonated SLS with 7–9 ethylene oxide (EO) units were monitored. In time segment 3, the m/z 600 [M + NH$_4$]$^+$ ion of the AE with 9 EO units was isolated in the ion trap and underwent successive CID. The total ions resulting were monitored.

2.4. Monitoring surfactants in membrane bioreactor matrices

A submerged membrane biological reactor (MBR) receiving a spacecraft wastewater analog was used in these studies. The aerobic MBR had a 12 L working volume and a submerged 0.2 μm membrane filter, operated at 48 h hydraulic retention time based on a throughput of 6 L day^{-1} for a two person-crew. The membrane was a pleated hydrophilic PVDF media filter cartridge with 0.2 μm cut-off and 0.7 m^2 membrane area (model FCPV 510S2, US Filter, Tampa, FL, USA). The process liquid was simulated wastewater, replicating the output of a two-person crew, containing an urine analog and two major surfactants (CAA and SLES) found in PertPlus. It contained (g) 1.64 urea; 0.58 KH$_2$PO$_4$; 0.90 NaCl; 0.59 K$_2$SO$_4$; 0.12 NH$_4$Cl; 0.36 (NH$_4$)$_2$SO$_4$; 0.088 CaCl$_2$; 0.071 MgSO$_4$; 0.52 NaHCO$_3$; 0.558 Miranol; and 0.854 Steol per liter, i.e. CAA and SLES concentrations of 215 and 246 mg L^{-1}, respectively. Effluent was continuously withdrawn through a membrane submerged in the reactor liquid at the same rate that simulated wastewater or influent was added to maintain a constant volume.

The influent less surfactants, effluent and deionized water were filter-sterilized, spiked with known amounts of three classes of surfactants combined and subsequently analyzed to examine the effect of co-existing surfactants, inorganics and other organics (e.g. microbial metabolites) on the quantification. Extra attention was given to ensure that foam was subsided before dilution and analysis. Working solutions containing 5, 25 and 50 mg L^{-1} of each surfactant (active component) were prepared by dissolving: (1) individual class of surfactant in DI water; (2) three classes of surfactants combined in DI water; (3) three classes of surfactants combined in MBR influent; (4) three classes of surfactants combined in MBR effluent.

Table 1
Optimized mass spectrometer conditions for quantification

| Surfactants	CAA	SLES	AE
Time window	Segment 1 (5–26 min)	Segment 2 (26–35 min)	Segment 3 (35–60 min)
Target ion (m/z) for instrument tuning	345	592	600
Capillary temperature (°C)	220	170	200
Exit voltage (V)	37	3	41
Ion spray voltage (kV)	5	5	5
Tube lense offset (V)	−15	−10	−15
Shealth Gas (N$_2$; unitless)	60	61	60
Ion polarity	Positive ion	Positive ion	Positive ion
Quantification mode	MS^2 of m/z 345 ⇒ SRM m/z 226	SCID at 15% ⇒ SIM m/z 512, 556 & 600	MS^3 of m/z 600 ⇒ m/z 583 ⇒ TIC [160–610]
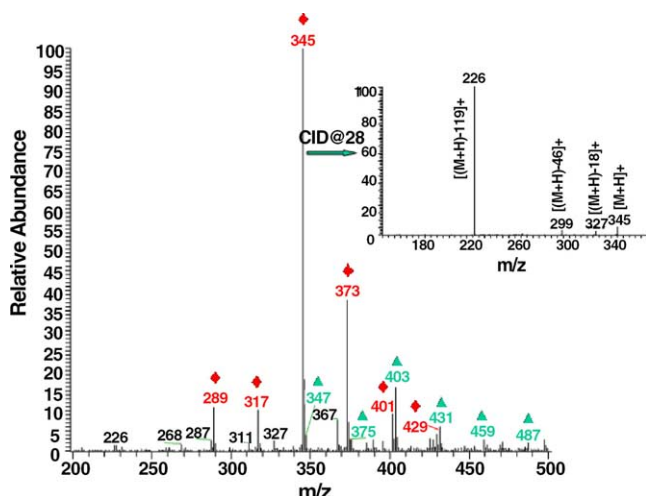
Fig. 2. (+) ESI normal scan mass spectrum of directly infused Miranol containing $25 \, mg \, L^{-1}$ CAA mixed with $0.2 \, mL \, min^{-1}$ LC flow of 70% acetonitrile and 30% 25 mM ammonium acetate buffer pH 3.6, and the collision induced dissociation (CID) spectrum of m/z 345 ion (insert).

3. Results and discussion

3.1. Surfactant constituents in Miranol C2M Conc-NP and their ionization characteristics

One of the prerequisites for an on-line HPLC–MS quantitative method is the clear definition of target ions for the analyte of interest. Target ions must be specific to the analyte, efficiently produced, and reproducible. Surfactants are mixtures of homologous compounds; the selection of a representative target ion is not as straightforward as for a well-defined molecule. The direct infusion experiment was

conducted to gain a rapid understanding of the mixture. The positive ESI mass spectrum of directly infused Miranol containing $25 \, mg \, L^{-1}$ CAA is shown in Fig. 2. Two sets of signals were evident, 28 u apart within set and 58 u apart between the sets. Collision induced dissociation of m/z 289, 317, 345, 373, 401 and 429 ions produced the same fragmentation pattern with predominant product ions $[(M+H)-119]^+$ (Fig. 2. insert) where M is the parent molecule without sodium counterion. The result suggested that these are homologues with 8-, 10-, 12-, 14-, 16- and 18-carbon alkyl chains. The neutral loss (119 u) was *N*-(2-carboxyl ethyl) 2-hydroxyl ethylamine. The most intense signal (m/z 345) corresponds to 12C alkyl amphomonoacetate, while manufacture specification indicates the active ingredient is cocoamphodiacetate that supposedly gives rise to the m/z 403 $[M+H]^+$ ion. Although ESI is a soft ionization technique, producing almost exclusively molecular ions and very little fragmentation, it is unclear if the m/z 345 is a fragment ion of m/z 403 due to the loss of CH_2COO (58 u, Fig. 3) or that from the constitutive corresponding monoacetate. Further clarification via direct infusion MS^n experiments were stymied by the complexity of the mixture, leading to the HPLC/(+)ESI–MS and MS^n experiments described below.

With a shallow gradient, resolution among the CAA components was achieved (Fig. 4). Specifically, the compounds generating m/z 345 and 403 ions were chromatographically separated, indicating they were derived from different compounds. In addition to m/z 345 and 403 $[M+H]^+$ ions, there were the respective $[M+Na]^+$ ions, strong indicators of the m/z 345 ion being an $[M+H]^+$ ion and not a fragment ion. The shallow gradient provided high resolution separation, not only between monoacetate alkyl homologues (base peak m/z 289, 317, 345, 373, 401 and 429), but also between monoac-
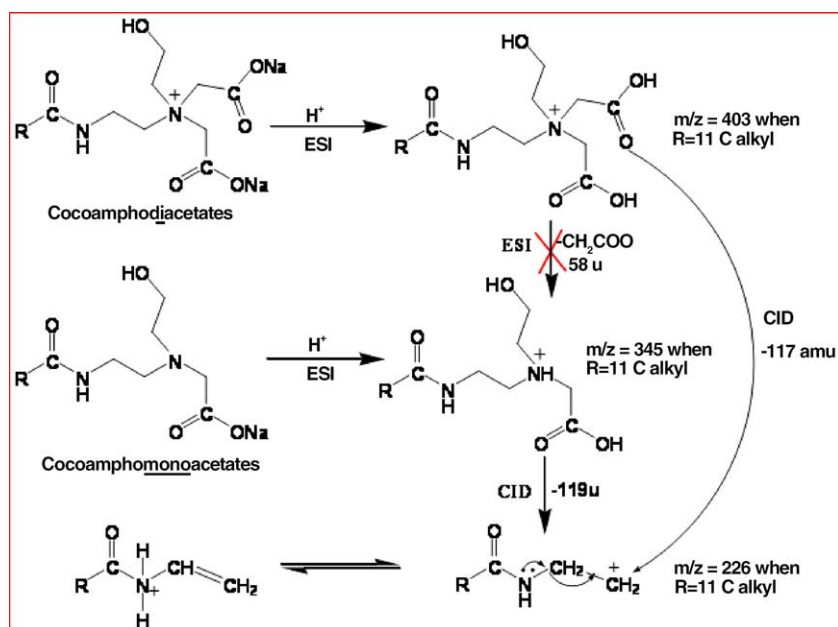

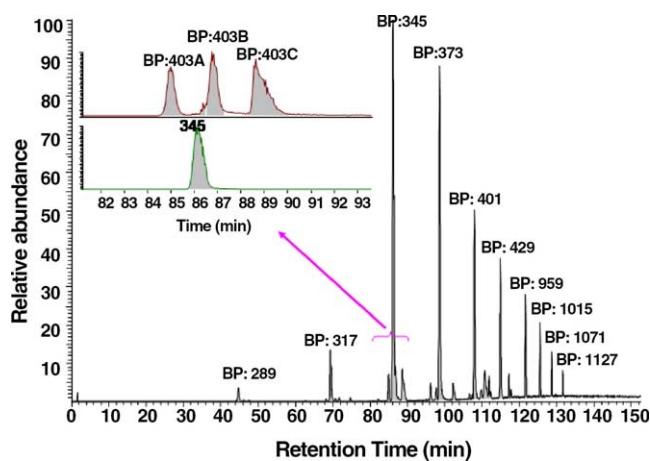Fig. 3. Fragmentation path of cocoamphodiacetate and monoacetate.

Fig. 4. A total ion chromatogram of Miranol obtained under a shallow mobile phase gradient program that enabled the resolution between cocoamphomonoacetate and its diacetate, as well as among diactate isomers. Insert is an enlarged view of a peak cluster of the 12C alkyl components.
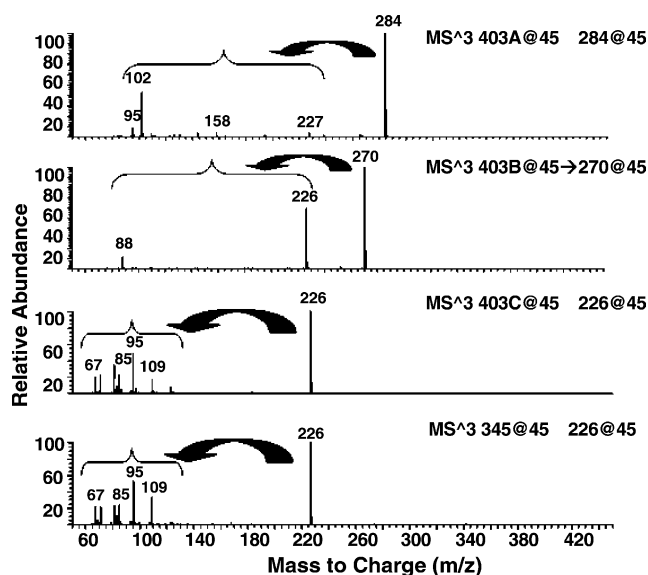

Fig. 5. (+)ESI-CID MS–MS spectra of the m/z 403A, 403B, 403C and 345 ions shown in Fig. 4. This confirmed that the m/z 345 ion was not a product ion of corresponding diacetate m/z 403, but a molecular-type ion of monoacetate. The differences in the MS/MS spectra of the three m/z 403 ions suggest their different structures.

etate and diacetate (e.g. 345 and 403), and different diacetate isomers (Fig. 4). There were at least three compounds that have the same m/z 403 [M + H]$^+$ ion forming a cluster of peaks surrounding m/z 345 (Fig. 4. insert). CID tandem MS^3 spectra of these three ions were distinctively different (Fig. 5). One of them (m/z 403C) had the same spectrum as that of m/z 345, confirming that the two acetate groups and hydroxyl ethylene were attached to the same nitrogen (Fig. 3). The presence of both m/z 284 and 102 in the CID mass spectrum of m/z 403A suggests a non-quaternary amine structure and probable fragmentation path as shown in Fig. 6. The spectrum of m/z 403B had m/z 270 as the predominant product ion along with m/z 226 and 88, also suggesting a non-quaternary amine struc-

ture, but with two acetate groups attached to the same nitrogen atom forming tertiary amine. Similar clusters of peaks were found for other alkyl homologues, i.e. each monoacetate was surrounded by several diacetes with the same alkyl chain length (Fig. 4).

The amphomonoacetates and amphodiacetate may have different molar responses due to the pH-dependencies of the acetate groups. However, under the conditions used here, the
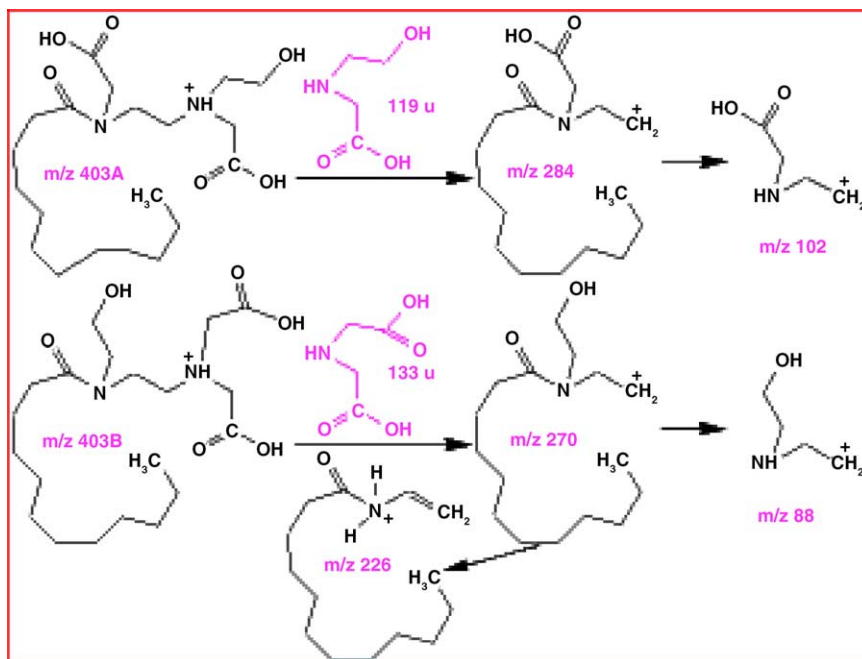

Fig. 6. Probable structures of other diacetates in Miranol and associated fragmentation pathways.

difference is not expected to be large. Thus, based upon the peak size shown in Fig. 4, it is obvious that the major component of the Miranol is the 12C alkyl amphomonoacetate (*m/z* 345) and not the 12C alkyl amphodiacetate. The 12C amphomonoacetate was therefore chosen as the quantifier for the CAA.

3.2. Surfactant constituents in Steol CS-330 and their ionization characteristics

Although other commercial mixture containing SLES has been characterized by ESI and APCI triple quadrupole mass spectrometer (22), it was not in the context of LC mobile phase. As we quickly realized that the mass spectrum of SLES differs significantly depending both on the solvent used and analyte concentration. Both positive and negative ESI of Steol CS-330 were investigated under various conditions (solvent modifier, capillary exit voltage and temperature). There was a sharp contrast between (−)ESI and (+)ESI spectra collected in the presence of acetonitrile:water containing 1% acetic acid (70:30, v/v) and in acetonitrile:25 mM ammonium acetate buffer pH 3.6 (70:30, v/v), respectively (Fig. 7). The (−)ESI resulted in lower ion abundance than the (+)ESI did either in the presence of ammonium acetate or only acetic acid (data not shown). Negative ion mode favored ionization of lower molecular weight homologues (fewer number of ethoxylate unit), while (+)ESI favored ionization of higher mass molecular weight homologues (Fig. 7). There were apparently two ion series in (−)ESI spectrum with 44 u apart within the series and 28 u apart between the series. Negative ESI-MS yielded predominantly [M−H]⁻ ions where M represents the SLES without sodium counterion. Thus, the ion series starting at *m/z* 265 with an increment of 44 could be assigned to the molecular-type [M−H]⁻ ions of acidic SLES with zero to 8 EO units. The second ion series (beginning with *m/z* 293) had a mass 28 u higher than the first series, and probably belong to 14C alkyl homologues.

Positive ESI of SLES in the presence of ammonium acetate favored the formation of [M+NH₄]⁺ instead of the [M+H]⁺ions. Concomitantly "desulfation" occurred, resulting in [(M+NH₄)−SO₃]⁺ ions. Again, the co-existence of 12 carbon alkyl and 14 carbon alkyl homologues is very evident (Fig. 7). The assignment of these ions was confirmed by performing tandem CID on selected ions. Fig. 8 is an example of the MS⁴ spectrum of the *m/z* 680 ion. The loss of 80 and 17 during first two stages of CID were attributable to the loss of SO₃ and NH₃, respectively. Consecutive losses of 44 u (CH₂CH₂O) confirmed the nature of ethoxylation, while signature losses of 168 (C₁₂H₂₄) and 186 (C₁₂H₂₅OH) indicated that the hydrophobe was a 12C alkane with an ether linkage to the hydrophile.

The loss of SO₃ (80 u) during initial ionization in ESI dramatically reduced the abundance of molecular-type ions and is undesirable for quantification. Attempts were made to reduce or eliminate the fragmentation or decomposition at this stage by lowering the capillary exit voltage and temperature. Lowering the capillary voltage reduced the fragmentation, but also reduced the overall yield of molecular-type ions. Therefore, there was not an overall gain in [M+NH₄]⁺ ion signal. Lowering the capillary temperature also reduced the desulfation to some extent, but could not eliminate this fragmentation while maintaining a significant level of ion
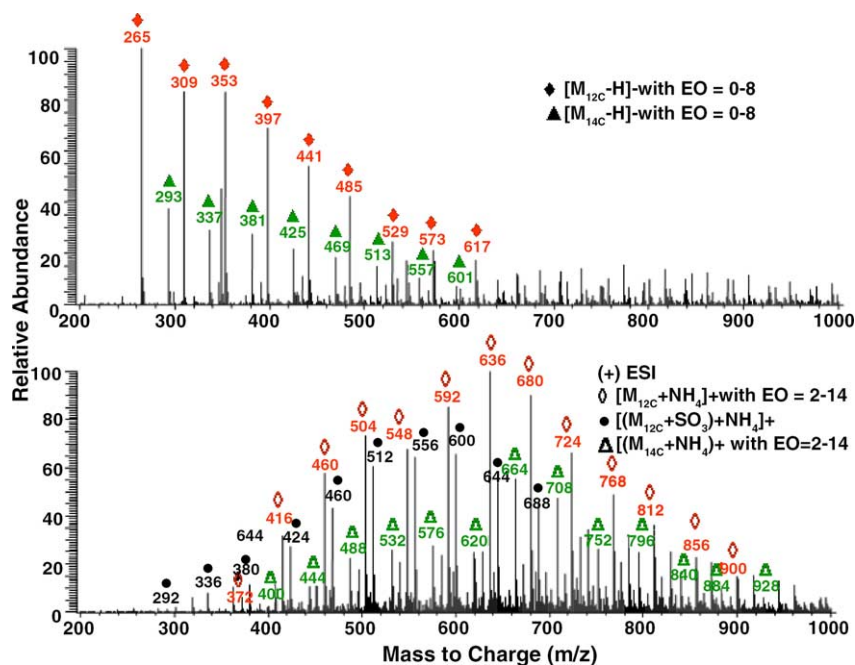

Fig. 7. Negative (top) and positive (bottom) ESI normal scanmass spectra of Steol CS-330 obtained by directly infusing a 25 mg L⁻¹ SLES (active ingredient only) into the 0.2 mL min⁻¹ HPLC flow (without column).
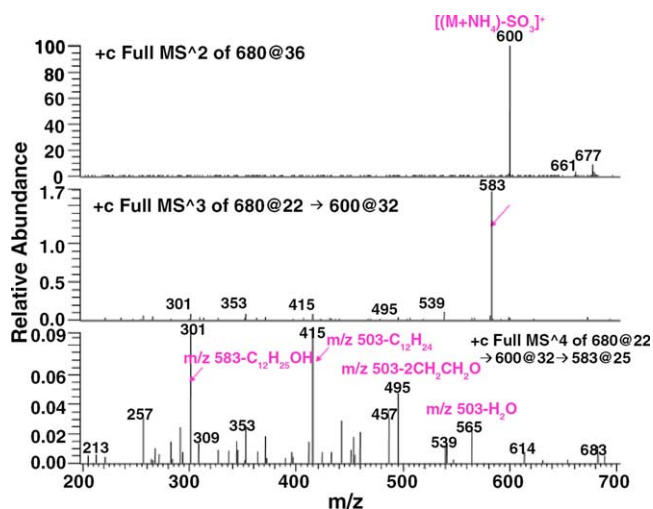
Fig. 8. The (+)ESI-CID tandem mass spectra of the m/z 680 ion in Fig. 7B. The consecutive loss of 80 u (SO_3) and 17 u (NH_3) from the m/z 680 ion confirmed that the parent ion was an ammonium adduct of acidic SLES (12C alkyl).

intensity. Instead of trying to reduce the loss of SO_3, as the loss of SO_3 seemed inevitable, conversion of the $[M + NH_4]^+$ ions to $[(M + NH_4) - SO_3]^+$ ions was optimized by increasing the CID occurring during ion injection, i.e. source CID. For the 12C alkyl homologue ions, a source CID at 15 V was found to be most effective. The resulting m/z 512, 556, and 600 $[(M + NH_4) - SO_3]^+$ ions were chosen to be monitored for the quantification of SLES.

3.3. Chemical composition and ionization characteristics of polyoxyethylene 10 lauryl ether

Polyoxyethylene 10 lauryl ether (an alcohol ethyoxylate, AE) was previously characterized [14]. Like SLES, AE had greater tendency to form the ammonium adduct ions $[M + NH_4]^+$ than the protonated molecules $[M + H]^+$ ions. The m/z 600 $[M + NH_4]^+$ ion of a homologue with 9 EO units was isolated and subjected to CID-MS/MS/MS. The sum of all of the resulting fragment ions was used for quantification (Table 1), while the MS^3 spectrum and chromatographic retention time were used for identification.

3.4. Chromatographic separation

Complete resolution between homologous components of a surfactant and among different classes of surfactants is not necessary when a mass spectrometer is used for detection. As a matter of fact, direct flow-injection has been attempted for quantification of surfactant mixtures, although it is more often used for characterization of the mixture [22,23]. One of the pitfalls for the direct flow-injection of environmental samples is that inorganic salts, and other non-volatiles in the sample matrix may clog the heated capillary of the atmospheric pressure interface. In addition, other matrix components can dramatically affect the ionization, e.g. formation of $[M + Na]^+$ ions, in an irreproducible manner. Since there is little or no retention of inorganic salts on the C_{18} column with a high percentage of water mobile phase, a reverse-phase HPLC column could serve to separate inorganic salts. Thus,
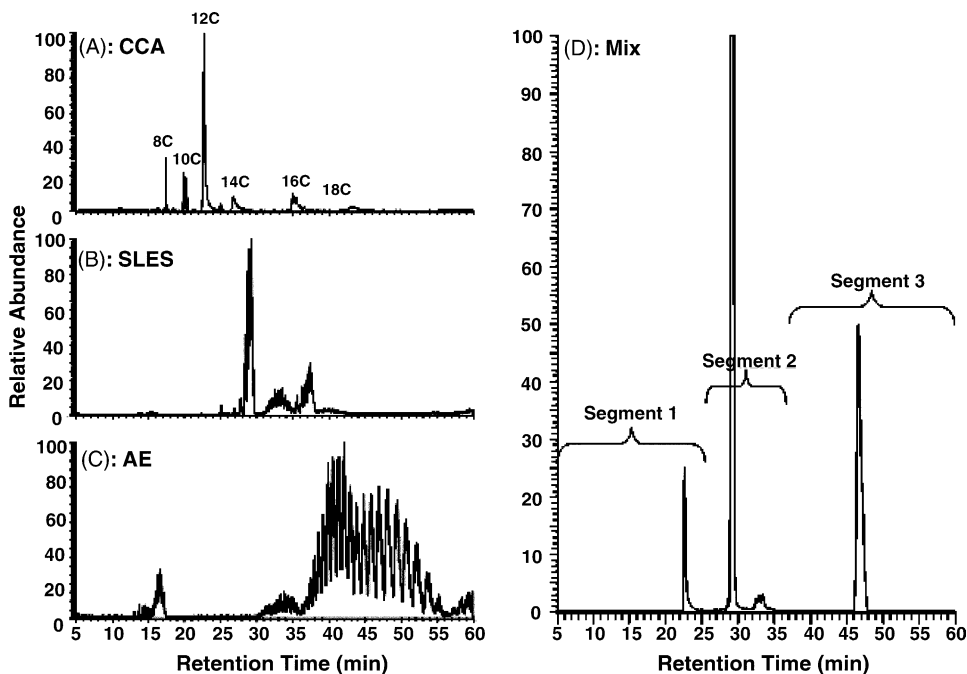

Fig. 9. HPLC-(+)ESI-MS normal scan mass chromatograms of individual class of surfactant, CAA(A), SLES(B), and AE(C); and mass chromatogram of three classes of surfactants (25 mg L^{-1} each) under the optimized quantification conditions (D). Segments 1, 2 and 3 are time windows when specific sets of MS parameters shown in Table 1 were applied.

the initial step of the HPLC gradient was a 5 min hold at 90% aqueous and the effluent from the column during this step was directed to the waste through a 6-port switching valve.

The HPLC column was used to provide on-line separation prior to the mass spectrometer for two other reasons. First, mass spectrometry could not differentiate the desulfated SLES from AE. Secondly, amphoteric, anionic and nonionic surfactants exhibit different ionization characteristics within ion sources, and subsequently formed ions have their own optimal conditions for transmission and fragmentation. HPLC separation would enhance the specificity and offer the opportunity to apply specific MS conditions to individual types of surfactant as they are eluted from the column.

Acetic acid and ammonium acetate were chosen to modify mobile phase pH to the range between 3.6 and 5.8. This pH range and mobile phase gradient defined in Section 2 provided adequate retention of ionic species ($k > 5$ for earliest eluting component of amphoteric surfactant) and separation among three classes of surfactants (Fig. 9). Amphoteric surfactants eluted first, followed by anionic, then nonionic surfactants. These conditions also resulted in the separation of amphoteric surfactants with varied alkyl chain length, and of nonionic surfactants with differing ethylene oxide units. Finally, the same m/z ions (e.g. m/z 600) originated from SLES and AE under the mass spectrometric conditions used here were well separated chromatographically.

3.5. Quantification and sample matrix effect

The quantification strategy was derived from the chromatographic separation and mass spectrometric characteristics of surfactants discussed above. Optimized conditions were detailed in Section 2 and Table 1. Briefly, the LC effluent between 5 and 60 min was directed to electrospray interface of the mass spectrometer. Positive ESI was effective for all three types of surfactants. MS operating conditions (Table 1) specific and optimized for each class of surfactant were applied within the time window when the surfactant eluted from the column (Fig. 9D). During time segment 1, the m/z 345 $[M + H]^+$ ion of the 12C alkyl CAA homologue was selected and subjected to collision induced dissociation. A predominant m/z 226 ion of the fragmentation products was in turn monitored. As SLES eluted from the column (time segment 2), source CID was applied to maximize the formation of $[(M + NH_4) - SO_3]^+$ ions, m/z 512, 556 and 600 representing 12C alkyl homologues with 7, 8 and 9 ethylene oxide units respectively were monitored. For AE (time segment 3), $[M + NH_4]^+$ with m/z 600 representing an AE homologue containing 9 EO units was selected and subjected to CID, resulting in m/z 583 ion. This ion was further subjected to CID and all of the resulting product ions were monitored for quantification (total ion count) and identification (spectrum) purposes. This time-event program resulted in a much simplified peak profile (Fig. 9D), allowing easy and accurate integration of peak area. The method demonstrated linear correlation between peak area and

Table 2
Effect of sample matrices on simultaneous surfactant quantification

	DI H$_2$O	MBR influent	MBR effluent
Approximately 5 mg L^{-1}			
CAA	111 ± 2.8[a]	104 ± 1.4	92 ± 9.2
SLES	100 ± 1.7	91 ± 2.0	93 ± 3.0
AE	107 ± 4.3	108 ± 3.0	107 ± 3.2
Approximately 25 mg L^{-1}			
CAA	97 ± 1.8	94 ± 1.5	101 ± 4.2
SLES	100 ± 1.3	95 ± 0.9	95 ± 0.7
AE	104 ± 0.9	102 ± 1.3	101 ± 2.0
Approximately 50 mg L^{-1}			
CAA	97 ± 0.8	91 ± 0.3	99 ± 0.9
SLES	99 ± 0.8	94 ± 1.0	95 ± 1.5
AE	108 ± 0.3	108 ± 0.8	108 ± 0.9

[a] Values are normalized to the response of individual surfactant in DI water, and ± indicates the standard deviation of three replicate analyses.

concentration in the concentration range of 2–60, 1.5–40 and 2–60 mg L^{-1} for CAA, SLES and AE with a 10 µL injection, respectively. The regressions and correlation coefficients for CAA, SLES and AE were $Y = (2 \times 10^7)X - 8 \times 10^6$ ($R^2 \geq 0.999$); $Y = (3 \times 10^8)X + 3 \times 10^9$ ($R^2 \geq 0.98$); $Y = (8 \times 10^7)X + 6 \times 10^7$ ($R^2 \geq 0.999$); based on the result from analyses of surfactant mixtures of four concentration levels (three replicates/level). Excellent reproducibility was indicated by the low standard deviation of three analyses (Table 2).

The three classes of surfactants combined either in deionized water, MBR influent and effluent were analyzed and compared to the recovery of respective single surfactant in deionized water to determine the effect of sample matrices on the accuracy of determination (Table 2). First column revealed the impact of other co-occurring surfactants on the determination, while second column revealed that of inorganics. The difference between influent and effluent is the presence of other small organics in the latter originated from microbial metabolism. In spite of the high concentration (approximately total 30 mM) of non-volatile anions and cations (as described in Section 2) in the MBR influent and co-existence of other types of surfactants, no negative effect was observed on the recovery of AE. Instead, a slight increase in signal was observed at all three surfactant concentration levels. There was minor suppression of CAA and SLES signals. This suppression was most likely due to the competitive ionic interaction between surfactants and divalent cations (e.g. Ca^{2+} and Mg^{2+}), resulting in reduced target molecular-type ions. Since AE does not possess net charge, divalent cations have no impact on AE molecules. The results implied that the standard materials be dissolved in deionized water and used for calibration without significant error.

4. Conclusions

A shallow gradient HPLC tandem MS experiment proved to be more useful than direct infusion to provide insight

into chemical composition and structure of a complex surfactant mixture. A simple reversed-phase liquid chromatographic separation using a C_{18} column and mobile phase gradient of acetonitrile and ammonium acetate enabled the removal of inorganic ions prior to mass analysis, the separation of different classes of surfactants, and application of different quantification strategies for specific surfactant classes. The resulting direct determination of different surfactant classes in the $2\,mg\,L^{-1}$ range without any sample preparation is an improvement over existing chromatography/mass spectrometry-based methods which require sample enrichment and clean-up prior to instrumental analysis. Specific advantages of this approach include: (1) elimination of the use of toxic chemicals normally required for sample enrichment or derivatization, and thus, elimination of hazardous waste production; (2) minimization of analyte loss during sample preparation, which is of special concern due to the tendency of surfactants to adhere to surfaces; (3) reduced sample processing time, and (4) small sample volume requirement (i.e., $1\,mL$ instead of $1\,L$). These advantages outweigh the reduced sensitivity for most applications except those interested in lower surfactant concentrations (i.e., $<2\,mg\,L^{-1}$).

Although only three surfactants (one from each class) were presented here, the same strategy may be extended to analyze other surfactants. For instance, cocamidopropylbetaine (CAPB) another widely used amphoteric surfactant having five alkyl homologues, elutes in the similar time frame as CAA. The characteristic molecular ion (m/z 343 for 12C alkyl homologue) and fragmentation pattern $[(M+H)-103]^+$ [14] permitted differentiation from CAA and quantification under conditions optimized for $[(M+H)-103]^+$.

The method is applicable to laboratory scale studies where sample volume is limited, and rapid assessment of responses to experimental conditions is required rather than low detection limit. It can be also used for process control in surfactant industry.

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