Atmospheric-Pressure Ionization Mass-Spectrometric Analysis of New Anionic Surfactants: The Alkylpolyglucoside Esters

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ABSTRACT: Atmospheric-pressure ionization mass spectrometry has been successfully applied to characterization of a new class of anionic surfactants, the alkylpolyglucoside esters of sulfosuccinic, citric and tartaric acid. Complex mixtures of final and intermediate products were injected directly into the ion source without prior chromatographic separation. The constituents were identified on the basis of quasi-molecular ions: cationized ions or solute–solute cluster ions in positive-ion mode, and deprotonated ions in negative-ion mode. The mass-spectrometric data show that all three final products contain one nonionic and two different types of anionic surfactants. The "real time," highly sensitive mass-spectrometric approach proposed here is well suited for quality control testing of tensides, to ensure the safety of the final product, and for the validation of the manufacturing process, because it is able to identify the individual components of the mixture.

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KEY WORDS: Anionic surfactants, APG esters, API mass spectrometry, direct characterization, quality control testing strategies, raw materials.

Qualitative analysis of a surfactant mixture is commonly approached by chromatographic methods, mainly by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) and more recently by supercritical-fluid chromatography (SFC), which give rough fingerprints of the several components present in the raw material, and only when hyphenated with infrared (IR), nuclear magnetic resonance (NMR) or mass spectrometry (MS) techniques, they allow characterization of structures. This is important from a toxicological point of view, because surfactant mixtures are frequently contaminated by unreacted intermediates or by-products, which can be responsible for allergic reactions of the skin or irritation of eyes and mucous membranes, even if present in only trace amounts (1).

In recent years, emphasis in our and other laboratories has been placed on the application of mass-spectrometric soft-ionization techniques for direct characterization of surfactant constituents in complex mixtures. In this context, fast atom bombardment mass spectrometry (FAB–MS) in positive and negative-ion modes has been shown to be satisfactory for the identification of different classes of tensides in raw materials and in finished detergent formulations, without any preliminary sample work-up (2–4).

The esters of alkylpolyglucosides (APG) are a new generation of anionic surfactants with interesting technological properties (5–7); they have excellent detergent and lathering properties, are easily rinsed, are not irritating *per se*, and lessen the irritation of the skin and the mucous membrane of the eye caused by other surfactants used in combination with them (8). In addition, they have low environmental impact, because they are highly biodegradable (8).

The aim of this work was to develop a more rapid and versatile mass-spectrometric approach than conventional FAB–MS to be applied to quality control of the intermediates and final products of the manufacturing process of the APG esters of sulfosuccinic, citric and tartaric acids, thus to ensure the safety of the developed products.

With this in mind, in this study we evaluated atmospheric-pressure ionization mass spectrometry (API–MS), based on an ion-spray source, which has been shown in other fields to be the technique of choice for the analysis of polar and thermally labile compounds in complex mixtures (9–12).

EXPERIMENTAL PROCEDURES

All the solvents used were of analytical grade (Merck, Bracco, Milan, Italy).

Reactants (lauryl and myristyl alcohol mixture, glucose, citric acid, tartaric acid), intermediates (APG; alkylpolyglucoside maleates, APGM) and final products, alkylpolyglucoside esters of sulfosuccinic (APGSS), citric (APGC) and tartaric (APGT) acids (purity 90–95%), were from pilot studies carried out by Auschem (Milan, Italy).

Gas-chromatographic determinations of free fatty alcohol fractions were carried out on a Perkin-Elmer Auto System gas chromatograph equipped with a flame-ionization detector, a fused-silica capillary column [SPB5 (30 m × 0.25 mm i.d; film thickness 0.25 μm)] and a PE Nelson model 1020 inte-

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grator (Palo Alto, CA). One-gram samples of APG, APGM, APGSS, APGC and APGT were diluted without shaking to 100 mL with distilled water, and 20 mL aliquots were submitted to solid-phase absorption column chromatography (Extrelut; Merck, Bracco) and eluted with *n*-hexane (100 mL) to recover free fatty alcohols. The *n*-hexane extracts were concentrated *in vacuo* to dryness, and the residues, taken up in 0.5 mL *n*-hexane, were analyzed under the following conditions: column temperature, 200°C; injector and detector temperatures, 280°C; carrier gas, He (20 cm/s^{-1}).

A Perkin-Elmer Sciex API III triple quadrupole mass spectrometer equipped with an ion-spray source was used. Ion-spray voltage was set at 5000 V, and the orifice voltage at 50 volts. Ultrapure grade N_2 was used at 1.0 mL/min as curtain gas for the API interface. Samples (0.1–1.0 µg/mL diluted with water containing 50% methanol and 2 mM ammonium acetate) were infused directly by a Harvard pump into the ion source (flow rate 5 µL/min). Under these conditions, the liquid, containing the sample to be analyzed, is pumped through a pneumatic nebulizer maintained at a high voltage, so that a mixture of highly charged (positive or negative) droplets is formed. As the droplets evaporate, ions are ejected into the gas phase (without application of heat) by a low-energy process that does not induce decomposition (only quasi-molecular ions are generated, with virtually no fragmentation).

RESULTS AND DISCUSSION

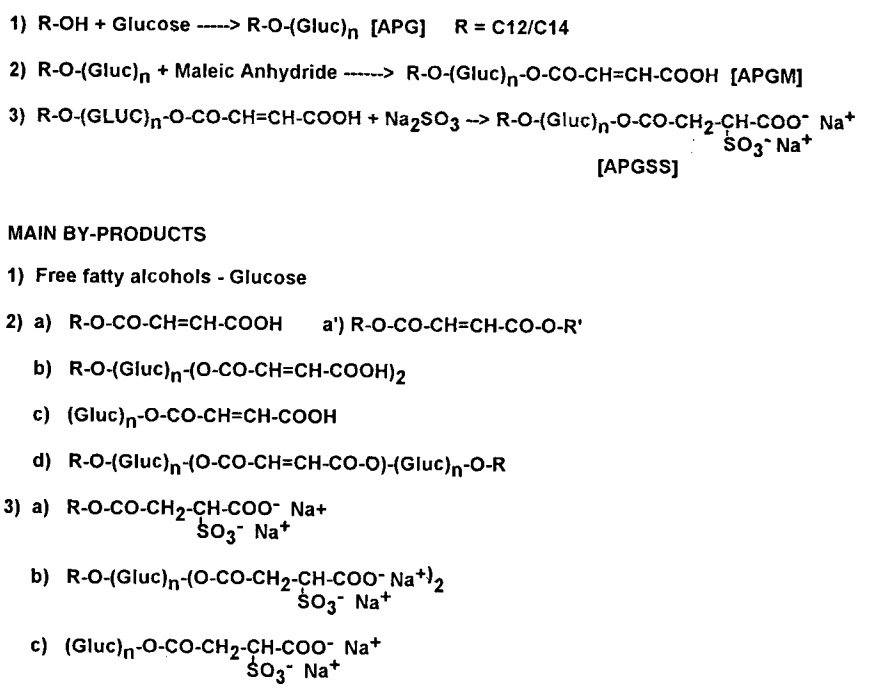
Schemes 1–3 summarize the pathways of the synthesis of the glucoside esters of sulfosuccinic, citric and tartaric acids and highlight the structures of possible by-products. The first step, common to all the compounds, involves the formation of APG by reaction of glucose with a higher alcohol (in this case a mixture of C_{12}/C_{14} fatty alcohols from natural sources) in the presence of an acid catalyst. The half esters of sulfosuccinic acid (Scheme 1) are prepared by reaction of the starting APG with maleic anhydride (second step) to form a half ester APGM. After neutralization, APGM is subjected to subsequent sulfonation (third step) with an aqueous sodium sulfite solution to obtain APGSS.

Half esters APGC or AGPT are obtained by reaction of APG with citric or tartaric acid (Schemes 2 and 3).

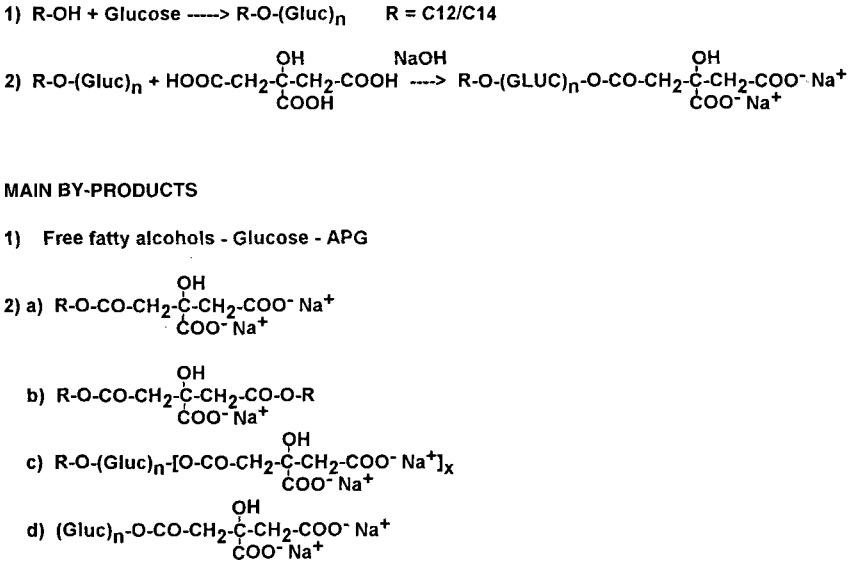
As shown in Scheme 1, both the intermediates (APG, APGM) and the final products of the synthesis of APGSS can be contaminated by starting materials, such as free fatty alcohols and glucose from the first step and/or by-products (from the second step): mono/dialkylmaleates (a,a′), APG–dimaleate (b), glucose–maleate (c) or dialkylpolyglucoside maleate (d), all of which can undergo sulfonation in the final reaction. The same holds true for APGC and APGT, in which citric/tartaric acid can react with free fatty alcohols to give the corresponding mono/diesters as main contaminants, and/or with glucose or with two hydroxyl groups of APG to give APG-dicitrate/ditartrate as minor by-products.

Because of these possibilities, we determined first the C_{12}/C_{14} ratio of alcohols present in the starting material and then quantitated the free fatty alcohol fraction after separation from APG, which is the first intermediate in the synthesis of all these tensides.

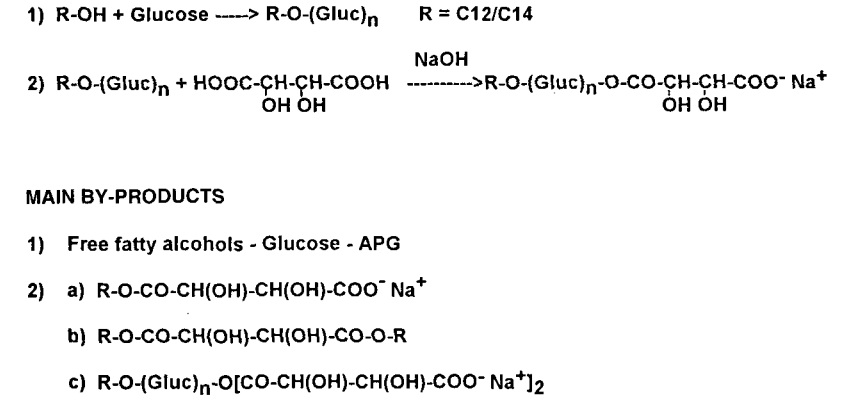
Gas chromatographic (GC) analysis of the alcohol mixture from natural sources shows that lauryl (R.T. = 4.16 min) and myristyl (R.T. = 5.90 min) alcohols are present in a 70:30 ratio; the *n*-hexane extracts from three different batches of APG gave a free fatty alcohol content of 12.3 ± 1.7%.



SCHEME 1



SCHEME 2



SCHEME 3

API–MS—APGSS. In the ion-spray mass spectra, all preionized compounds are detected either as protonated $[M + H]^+$ or deprotonated $[M - H]^-$ molecular ions, in accord with their forms in solution, while nonionic species and other less polar compounds are detected as clusters with metals, by alkali attachment in solution. This is well demonstrated by the mass-spectrometric behavior in positive-ion mode of nonionic APG, the first product in the synthesis of APGSS. In the API mass spectrum (Fig. 1), six series of ions relative to homologous compounds that differ by 162 u (a glucose unit, G) were observed: three of them (A, B, C) are the glucosidic derivatives of the C_{12} fatty alcohol cationized with NH_4^+, Na^+ and K^+, respectively, the most abundant ion being the $[M + Na]^+$ species relative to the monoglucosidic term (ion *m/z* 371). The same pattern was also observed for the C_{14} series (D, E, F), the first terms being the ions at *m/z* 394 $[C_{14}G_1 + NH_4]^+$, *m/z* 399 $[C_{14}G_1 + Na]^+$, *m/z* 415 $[C_{14}G_1 + K]^+$ and the last those at *m/z* 880 $[C_{14}G_4 + NH_4]^+$ and at *m/z* 885 $[C_{14}G_4 + Na]^+$. The monoglucosidic derivatives of both C_{12} and C_{14} alcohols were also evident as protonated molecular ions $[M + H]^+$ at *m/z* 349 and 377.

The glucosidation process involves a maximum of four glucose units because the last detectable terms were $C_{12}G_4$ and $C_{14}G_4$, present in the spectrum with low abundance (the species $C_{12}G_1/C_{14}G_1$ and $C_{12}G_2/C_{14}G_2$ were predominant).

In the higher mass range (700–1000 u), the most abundant ions desorbed were due to solute–solute clusters of the type $[2M + Na]^+$, by addition of neutral molecules [M] to cation-

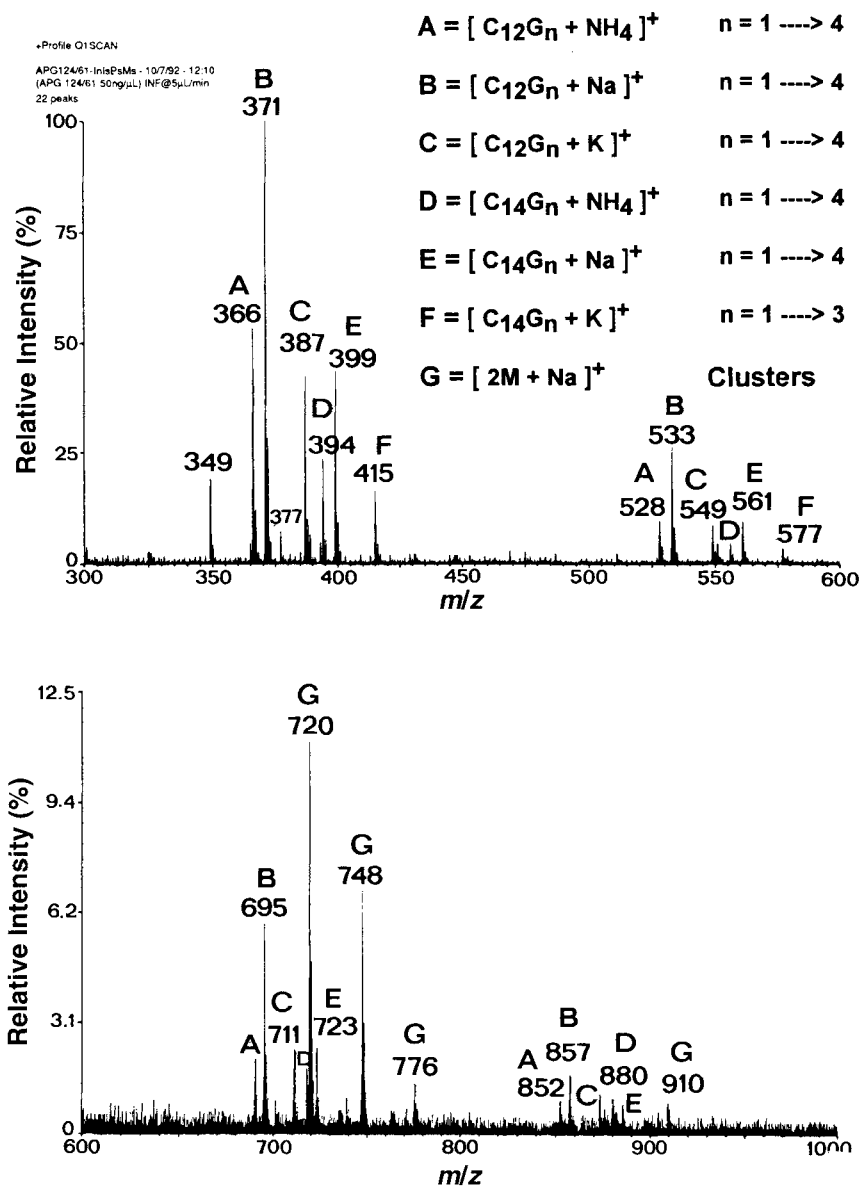


FIG. 1. Atmospheric-pressure ionization ion-spray mass spectrum (positive-ion mode) of alkylpolyglucosides.

ized species $[M + Na]^+$: the ions at *m/z* 720 and 776 arose by cluster formation between the same molecular species, $C_{12}G_1$ (720) or $C_{14}G_1$ (776). The ions at *m/z* 748 $[C_{12}G_1/C_{14}G_1 + Na]^+$ and *m/z* 910 $[C_{12}G_2/C_{14}G_1 + Na]^+$ or $[C_{12}G_1/C_{14}G_2 + Na]^+$are mixed clusters.

The positive-ion mode ion-spray mass spectrum of the mixture from reaction of APG with maleic anhydride had, as expected, a more complex pattern (Fig. 2, top panel). The esterification reaction was not quantitative because starting APG is still well detectable with the following C_{12} series:

$$[C_{12}G_n + NH_4]^+ (n = 1 \rightarrow 3), \text{ ions at } m/z \text{ 366, 528, 690;} \quad [1]$$

$$[C_{12}G_n + Na]^+ (n = 1 \rightarrow 3), \text{ ions at } m/z \text{ 371, 533, 695} \quad [2]$$

Among the ions of the corresponding C_{14} series, only the monoglucosidic terms at *m/z* 394 $[C_{14}G_1 + NH_4]^+$and at *m/z* 399 $[C_{14}G_1 + Na]^+$ can be observed.

The reaction products APGM relative to the C_{12} species were evidenced by two series of cations:

$$A = [C_{12}G_n M + NH_4]^+ (n = 1 \rightarrow 3), \text{ with ions at } m/z \text{ 464, 626, 788;} \quad [3]$$

$$B = [C_{12}G_n M + Na]^+ (n = 1 \rightarrow 3), \text{ with ions at } m/z \text{ 469, 631, 793} \quad [4]$$

The ions of the C (*m/z* 492, 654) and D (*m/z* 497, 659) series, which differ from those of the A and B series by 28 mass units, were the corresponding C_{14} mono- and diglucosidated derivatives.

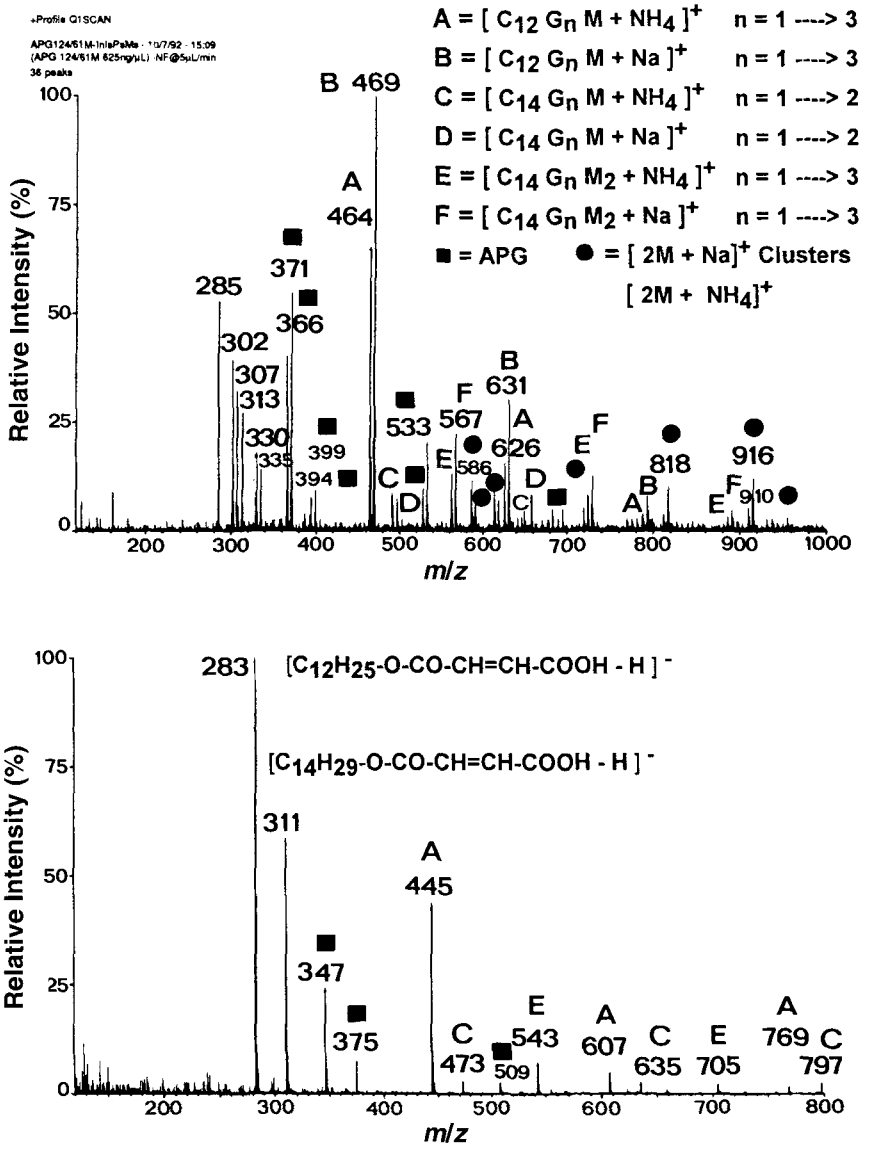


FIG. 2. Atmospheric-pressure ionization ion-spray mass spectra of alkylpolyglucoside maleates.

Again, the typical adduct of general formula $[2M + Na]^+$, in which M is the C_{12} monoglucosyl maleate $C_{12}G_1M$, was found at *m/z* 916, while the ions at *m/z* 818 and 910 can be interpreted as the following cluster structures:

$$m/z\ 818 = [C_{12}G_1 / C_{12}G_1M + Na]^+; \quad [5]$$

$$m/z\ 910 = [2(C_{12}G_1\ M) + NH_4]^+ \text{ or } [C_{12}G_2/C_{14}G_1 + Na]^+ \text{ or } [C_{12}G_1/C_{14}G_2 + Na]^+ \quad [6]$$

The two additional series of homologous compounds (E and F), which differ from the A/B series by 98 u (a maleate unit), corresponded to the by-product APG-dimaleate (C_{12} derivatives only):

$$E = [C_{12}G_n M_2 + NH_4]^+ (n = 1 \rightarrow 3), \text{ ions at } m/z \text{ 562, 724, 886;} \quad [7]$$

$$F = [C_{12}G_n M_2 + Na]^+ (n = 1 \rightarrow 3), \text{ ions at } m/z \text{ 567, 729, 891} \quad [8]$$

In the lower mass range of the spectrum, the abundant ions at *m/z* 285 for $[C_{12}H_{25}$-O-CO-CH=CH-COOH + H$]^+$ and 313 $[C_{14}H_{29}$-O-CO-CH=CH-COOH + H$]^+$ were indicative of the expected main by-products, the monoalkylmaleates (by reaction of maleic anhydride with starting free fatty alcohols; Scheme 1). The ions at *m/z* 302, 307 and those at *m/z* 330, 335 were the corresponding cationized species with NH_4^+ and Na^+, respectively.

These compounds also form $[2M + X]^+$solute–solute clusters, clearly detected at:

m/z 586 = [2 ($C_{12}H_{25}$-O-CO-CH=CH-COOH + NH_4]$^+$; [9]

m/z 591 = [2 ($C_{12}H_{25}$-O-CO-CH=CH-COOH + Na]$^+$; [10]

m/z 614 = [$C_{12}H_{25}$-O-CO-CH=CH-COOH/
$C_{14}H_{29}$-O-CO-CH=CH-COOH + NH_4]$^+$; [11]

m/z 619 = [$C_{12}H_{25}$-O-CO-CH=CH-COOH/
$C_{14}H_{29}$-O-CO-CH=CH-COOH + Na]$^+$ [12]

The structure attributed to all these species was confirmed by the analysis of the reaction mixture in negative-ion mode. The mass spectrum (Fig. 2, bottom panel) was less complex because only deprotonated molecular ions [M − H]$^-$ were generated: the ions at m/z 445, 607, 769 (A series) and at m/z 473, 635, 797 (C series) are the intermediates of C_{12} and C_{14} APGM; the ions at m/z 543 and 705 (E series) are the C_{12} mono and diglucosyl maleates ($APGM_2$).

Unreacted APG is clearly detected in the negative-ion mode too, although with lower intensity, at m/z 347 [$C_{12}G_1$ − H]$^-$, m/z 509 [$C_{12}G_2$ − H]$^-$, m/z 375 [$C_{14}G_1$ − H]$^-$, while the ions at m/z 283 and m/z 311, corresponding to lauryl- and myristyl-maleates, were the most prominent.

Hence, API–MS provided direct information about the presence in the mixture of APG and of reaction by-products (alkylmaleates).

The positive-ion API mass spectrum of the final products APGSS (Fig. 3, top panel) showed the most abundant ions to be those relative to starting glucosyl compounds at m/z 371 [$C_{12}G_1$ + Na]$^+$, m/z 399 [$C_{14}G_1$ + Na]$^+$, m/z 533 [$C_{12}G_2$ + Na]$^+$, m/z 561 [$C_{14}G_2$ + Na]$^+$ and m/z 695 [$C_{12}G_3$ + Na]$^+$, whose presence was further confirmed by the typical clusters at m/z 720 and 748 (see Fig. 1).

The sulfonation reaction of APGM intermediates is quantitative, because their diagnostic ions at m/z 464/469 (see Fig.

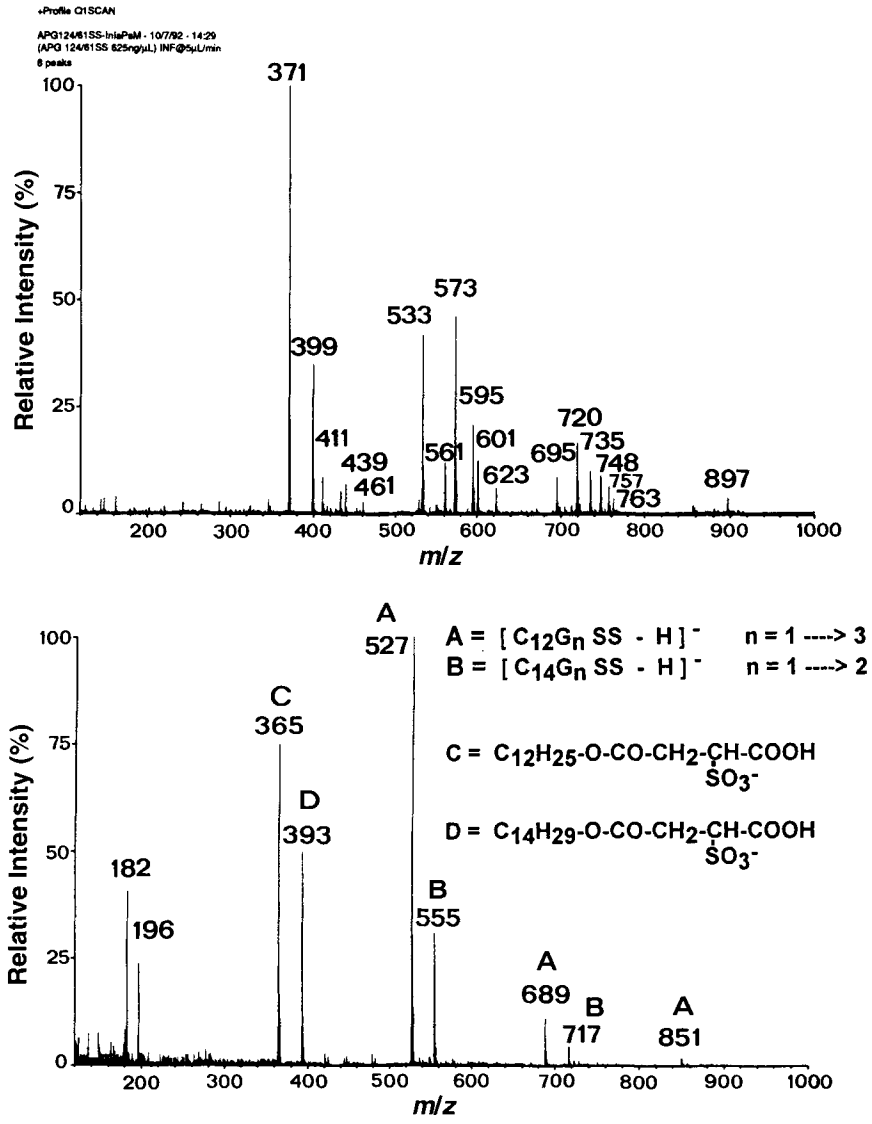


FIG. 3. Atmospheric-pressure ionization ion-spray mass spectra of alkylpolyglucoside esters of sulfosuccinic acid.

2) could no longer be detected. As observed with FAB–MS ionization with other sulfopolycarboxylate surfactants (1), APGSS were detected as disodium $[MNa_2]^+$ species, in which M is the APGSS ion, and as $[MNa_3]^+$, by further sodium attachment, with the ions:

m/z 573 $[C_{12}G_1SS + 2Na]^+$ and m/z 595 $[C_{12}G_1SS + 3Na]^+$; [13]

m/z 735 $[C_{12}G_2SS + 2Na]^+$ and m/z 757 $[C_{12}G_2SS + 3Na]^+$; [14]

m/z 897 $[C_{12}G_3SS + 2Na]^+$; [15]

m/z 601 $[C_{14}G_1SS + 2Na]^+$ and m/z 623 $[C_{14}G_1SS + 3Na]^+$; [16]

m/z 763 $[C_{14}G_2SS + 2Na]^+$ [17]

The ions at m/z 411 and 439, which belong to the same type of clusters $[MNa_2]^+$ (those at m/z 433 and 461 to the corresponding $[MNa_3]^+$ species), were the cationized molecular ions of lauryl and myristylsulfosuccinates. This indicates that the intermediate by-product alkylmaleates also underwent quantitative sulfonation.

In the negative-ion mass spectrum (Fig. 3, bottom panel), the base peak at m/z 527 (A series) was relative to the C_{12}-monoglucosylsulfosuccinate anion $[C_{12}G_1SS]$ and was accompanied by peaks at m/z 689 and m/z 851, the homologous di- and triglucosyl derivatives. The ions of the B series at m/z

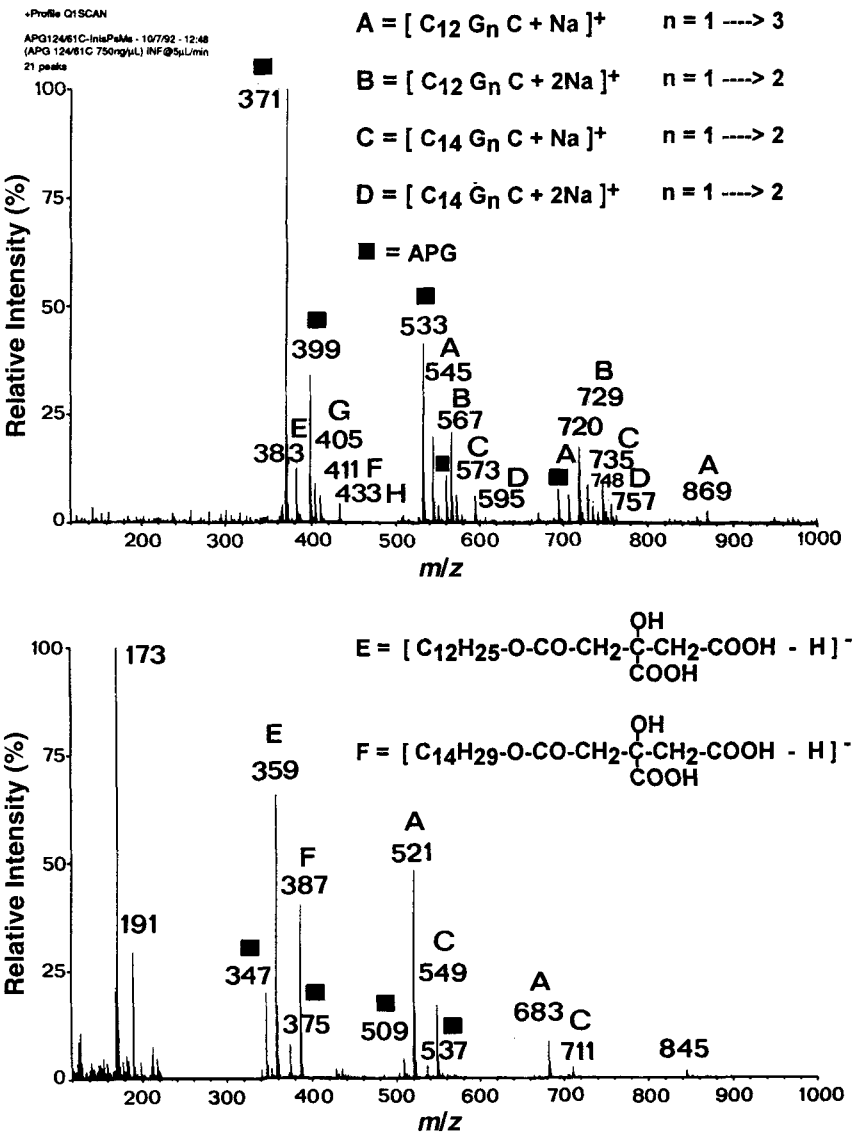


FIG. 4. Atmospheric-pressure ionization ion-spray mass spectra of alkylpolyglucoside esters of citric acid.

555 [$C_{14}G_1SS$] and *m/z* 717 [$C_{14}G_2SS$] are due to the corresponding myristyl derivatives. The abundant ions C and D at *m/z* 365 and *m/z* 393 are the deprotonated molecular ions of lauryl and myristylsulfosuccinates.

Because all these compounds possess two ionizable functions, they can give rise to multiply-charged ions, i.e., the $[M - 2H]^{2-}$ ions of the corresponding free acids. These doubly-charged species were detected for the C and D ions only, at *m/z* 182 and 196.

As evidenced by the API–MS approach, the final product is a multicomponent mixture of nonionic (APG) and anionic (APGSS and alkylsulfosuccinates) surfactants.

No residual free fatty alcohols were detectable by GC analysis in the final product.

APGC. Top panel of Figure 4 shows the positive-ion API fingerprint of APGC, with four diagnostic series of ions:

A = *m/z* 545 [$C_{12}G_1C + Na]^+$, *m/z* 707 [$C_{12}G_2C + Na]^+$, *m/z* 869 [$C_{12}G_3C + Na]^+$; [18]

B = *m/z* 567 [$C_{12}G_1C + 2Na]^+$, *m/z* 729 [$C_{12}G_2C + 2Na]^+$; [19]

C = *m/z* 573 [$C_{14}G_1C + Na]^+$, *m/z* 735 [$C_{14}G_2C + Na]^+$; [20]

D = *m/z* 595 [$C_{14}G_1C + 2Na]^+$, *m/z* 757 [$C_{14}G_2C + 2Na]^+$ [21]

In this case too, the esterification of the intermediate APG with citric acid is not quantitative, because (i) the starting APG is still easily detected: cationized molecular ions at *m/z* 371 (base peak: [$C_{12}G_1 + Na]^+$) and at *m/z* 399, 533, 561 and 695, accompanied by the typical solute–solute clusters at *m/z* 720 and 748; (ii) citric acid also reacts with the free fatty alcohols present in the APG intermediate to give the expected by-products lauryl and myristyl citrates, present in the API

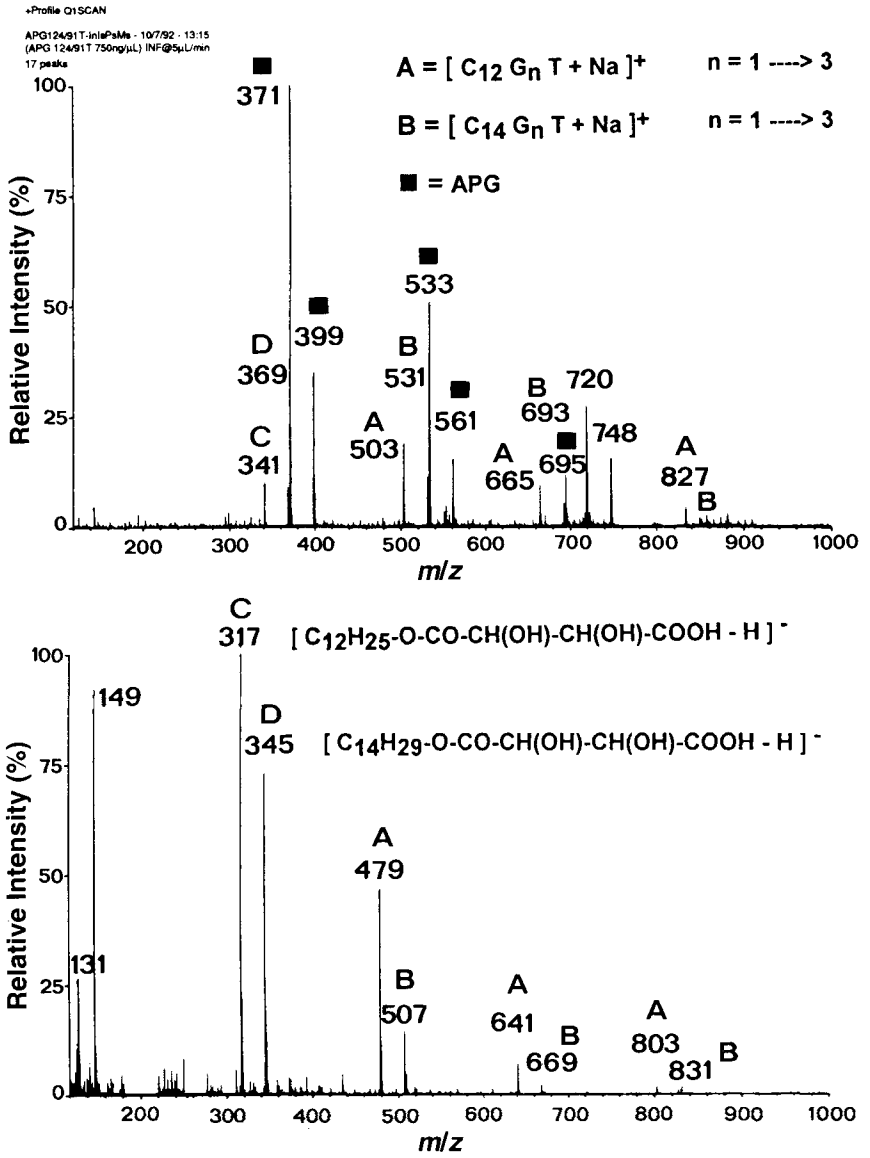


FIG. 5. Atmospheric-pressure ionization ion-spray mass spectra of alkylpolyglucoside esters of tartaric acid.

mass spectrum in both monocationized $[M + Na]^+$ (ions E and F at *m/z* 383 and 411) and dicationized $[M + 2Na]^+$ form (ions G and H at *m/z* 405 and 433).

In the negative-ion mass spectrum (Fig. 4, bottom panel), the corresponding carboxylate anions E and F at *m/z* 359 and 387 and the deprotonated molecular ions $[M - H]^-$ of APG at *m/z* 347, 375, 509 and 537 are evident.

No ions other than the carboxylate anions of the laurylmono-, di- and triglucosyl esters of citric acid (A ions at *m/z* 521, 683 and 845) and those relative to the myristyl species (C ions at *m/z* 549 and 711) could be detected in the mass spectrum, thus excluding other minor by-products due to diesterification of citric acid with APG and/or fatty alcohols. In the low mass range, the structure of the deprotonated molecular ion of citric acid could be assigned to the abundant ion at *m/z* 191 and that of the corresponding $[M - H_2O]^-$ fragment to the base peak at *m/z* 173.

APGT. The qualitative composition of the APGT mixture is similar to that of APGC, because, as shown in the positive-ion API mass spectrum (Fig. 5, top panel), it contains: (i) the cationized molecular ions $[M + Na]^+$ of the tartaric esters of laurylmono-, di- and triglucosides that differ by 162 u (A ions at *m/z* 503, 665, 827); (ii) the $[M + Na]^+$ ions relative to the homologous C_{14} derivatives (B ions at *m/z* 531, 693, 855); (iii) unreacted APG: ions at *m/z* 371, 533, 695 $[C_{12}G_n + Na]^+$, at *m/z* 399, 561 $[C_{14}G_n + Na]^+$and at *m/z* 720, 748 (adducts); and (iv) the $[M + Na]^+$ ions of lauryl (C = *m/z* 341) and myristyl (D = *m/z* 369) esters of tartaric acid, the main expected by-products in the synthesis of APGT (Scheme 1).

Again, in the negative-ion mode (Fig. 5, bottom panel), the most abundant ions were those relative to carboxylate anions C and D at *m/z* 317 (the base peak) and 345 (C12/C14 alcohol tartrates), A at *m/z* 479, 641, 803 $[C_{12}G_nT - H]^-$, B at *m/z* 507, 669, 831 $[C_{12}G_nT - H]^-$. The intense ion at *m/z* 149 might correspond to the $[M - H]^-$ of tartaric acid and that at *m/z* 131 to its $[M - H_2O]^-$ fragment.

Hence, both the final products APGC and APGT are mixtures of nonionic and two different types of anionic surfactants.

The results of this study evidence that API–MS with ionspray is highly suited for the analysis of nonvolatile, highly polar nonionic (APG) and anionic tensides such as the alkylpolyglucoside esters of sulfosuccinic, citric and tartaric acids, because it provides direct characterization of all components of the final surfactant mixture without its prior chromatographic separation into different classes. Although the positive-ion mass spectra can be somewhat complicated by the presence of more than one signal for each chemical entity, due to the formation of clusters with different metals, or solute–solute clusters, this is not a drawback, because more information can be obtained for each constituent; definitive structure attribution is easily achieved by the complementary information obtained in negative-ion detection, which gives simpler and unequivocal mass spectra dominated by deprotonated molecular ions.

The chief advantages of this innovative mass-spectrometric approach for the definition of the qualitative composition of raw materials (i.e., for differentiating homologues from oligomers, and for detection of impurities, such as unreacted materials, by-products) are sensitivity (full scan spectra are obtained by infusion of only a few ng/μL of the compounds) and, above all, rapidity (total analysis time within 10 min), because it overcomes the aforementioned limitation of FAB–MS: the choice of the matrix compound. This last point determines whether sample ions are observed at all (frequently more than one matrix must be used to detect structurally unrelated compounds concomitantly present in a complex mixture) and, given that sample ions are observed, it greatly affects the level of background noise.

In addition, the API–MS technique, because of its ability to evaluate a broad spectrum of known and/or theoretical product impurities and of potential degradation products, can be applied to the validation of the manufacturing process, especially when the final product is constituted by several entities belonging to chemically different homologous series. Because of its versatility, it is a valid alternative to the well-established hyphenated techniques (LC–MS, SFC–FTIR, SFC–MS) for the analysis of multicomponent mixtures.

The "real time" MS acquisition of an unequivocal fingerprint of the intermediate products is of standing importance for (i) development of alternative synthetic strategies; (ii) optimization of the reaction conditions; and (iii) removing of those impurities of significant concern.

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