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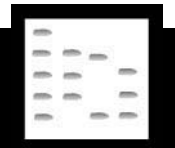
# COSMETICS AND TOILETRIES: CHROMATOGRAPHY



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Toiletries, used by millions of consumers for the daily care and hygiene of the body, include several products (mainly rinse products) with different formulative bases: soaps, shampoos, bath foams, toothpastes, deodorants.

Since surfactants are the basic materials used in toiletries (in soaps and shampoos they increase the washing properties of water; in shaving products they act as wetting and foaming agents; in bath oils they make the perfume water-soluble; in hair products they are used as conditioners), this article will deal with the techniques used for the analysis of these important constituents.

Leaving aside the rough, but still used, organoleptic testing (odour, colour, clarity, opalescence), analytical procedures for quality control may range from physical evaluations (specific gravity, refractive index, optical rotation, viscosity) to chemical analysis by standard volumetric and gravimetric methods, and instrumental analysis by chromatographic techniques (thin-layer, gas and liquid chromatography) and spectroscopic techniques (ultraviolet, infrared and nuclear magnetic resonance spectroscopy and mass spectrometry). Chromatographic and spectroscopic methods now find wide application, since toiletries and raw materials are complex mixtures, and there is a constant need to distinguish subtle structural differences in composition and determine impurities even if present in trace amounts.

The term surfactant (a contraction for surface-active agent) is used to describe organic chemicals that, when added to a liquid, change the interfacial properties of that liquid. Chemically surfactants can be divided into two major classes: nonionic (uncharged substances) which do not dissociate in aqueous solution, and ionic (charged substances) which dissociate and form ions, one of which becomes the actual surface-active agent. Ionic surfactants are classified by the nature of their charges in solution: anionic (negatively charged), cationic (positively charged), amphoteric (both positively and negatively charged).

## Anionic Surfactants

Anionic surfactants constitute about 65% of all surfactants manufactured and it is not surprising that the bulk of literature on surfactants deals with the analysis of these compounds. **Table 1** reports the main types of anionic surfactants used in toiletries.

The quality control of anionics in raw materials and in finished products is mainly quantitative and several methods that give a total estimate of active ingredients have been developed. The two-phase mixed indicator titration and other titrimetric analyses, such as direct colorimetric titration and precipitation titration are the simplest procedures, since they are sufficiently reliable, require little and inexpensive equipment, and can be used for both product development and quality control applications. The two-phase mixed indicator titration is based on the extraction of an anionic-indicator or cationic-indicator complex into a nonaqueous solvent (usually chloroform) in equilibrium with an aqueous solution of the unknown anionic surfactant or the titrating cationic surfactant. The method is not quantitative for compounds containing fewer than 12 carbon atoms when chloroform is the organic phase (in this case a mixed organic phase of 2 : 3 (v/v) chloroform–1-nitropropane must be used). Sodium, magnesium or sulfate ions at concentrations up to 0.4 mol $L^{-1}$ do not interfere, while chloride interferes above $5 \times 10^{-3}$ mol $L^{-1}$.

All the spectrophotometric procedures are based on the formation of a solvent-extractable compound

**Table 1** Anionic surfactants

| *Type* | *General structure* |
|---|---|
| Alkyl carboxylates (soaps) | $R–COO^-X^+$<br>$C_8–C_{18}$ fatty acids; salts with NaOH, KOH, $NH_4OH$, monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA) |
| Alkylethoxylated carboxylates | $R–(OCH_2CH_2)_nOCH_2COO^-X^+$<br>$R = C_8–C_{18}$ fatty alcohols<br>X = Na, K, $NH_4$, MEA, DEA, TEA |
| Alkyl sulfates | $R–O–SO_3^-X^+$<br>$R = C_8–C_{18}$ fatty alcohols<br>X = Na, K, Mg, $NH_4$, MEA, DEA, TEA |
| Alkylethoxylated sulfates (AES) | $R–(OCH_2CH_2)_n–O–SO_3^-X^+$<br>$R = C_8–C_{18}$ fatty alcohols<br>X = Na, K, Mg, $NH_4$, MEA, DEA, TEA |
| Alkylarylsulfonates | $R–C_6H_5–SO_3^-X^+$<br>$R = C_{10}–C_{12}$ |
| $\alpha$-Olefin sulfonates | $R–SO_3^-Na^+$<br>$R = C_{14}–C_{16}$ |
| Isethionates | $R–CO–O–CH_2CH_2–SO_3^-Na^+$<br>$R–CO = C_{12}–C_{18}$ fatty acids |

between the anionic surfactant and a coloured cationic species: Methylene blue, Methyl green, Toluidine blue, Rosaniline, Brilliant green and Methyl violet being the most widely used. These cationic compounds are not extractable as such by organic solvents, but in the presence of anionic species they form a stable, stoichiometric ion-association complex that is poorly soluble in water, because it has no nett charge. The complex is extracted into the organic solvent and the absorbance gives a direct measure of the surfactant present.

These techniques are not suited for establishing the qualitative composition of these surfactants, e.g. for differentiating homologues and oligomers, for characterization of single components of a surfactant mixture, or for detection and quantification of impurities (unreacted materials, by-products), and when such determinations are required for finished detergent formulations.

Hence, more specific and sensitive chromatographic techniques such as thin-layer chromatography (TLC), gas chromatography (GC) and liquid chromatography (LC) have been developed and are now widely accepted in the surfactant industry. Mass spectrometry (MS) and tandem mass spectrometry (MS-MS), although considered the techniques of choice for rapid characterization of surfactant mixtures, have not yet gained general acceptance as routine analytical techniques.

TLC, because of its rapidity and low cost, is particularly useful. Anionics (sulfates, sulfonates, soaps) can easily be separated under the following conditions (all ratios given are volume ratios).

*One-dimensional chromatography*

Alumina 60 F254 with isopropanol.

Silica gel 60 with propanol–chloroform–methanol–10 mol $L^{-1}$ ammonia (10 : 10 : 5 : 2); ethylacetate–acetic acid–methanol–10 mol $L^{-1}$ ammonia (45 : 5 : 2.5); ethanol–acetic acid (9 : 1).

Silica gel G containing 10% ammonium sulfate with chloroform–methanol–0.05 mol $L^{-1}$ sulfuric acid (80 : 19 : 1).

*Two-dimensional chromatography*

Silica gel with acetone–tetrahydrofuran (9 : 1) followed by propanol–chloroform–methanol–10 mol $L^{-1}$ ammonia (10 : 10 : 5 : 2).

Several spray reagents are used for detection and identification: Pinakryptol Yellow, Dragendorff, ninhydrin, leucomalachite green and iodine vapour.

This method can be applied to all classes of tensides as it distinguishes anionics from nonionics (fatty diethanolamides and ethoxylates) in shampoos, bath foams and soaps. The aqueous samples can be freeze-dried and extracted with a suitable solvent (ethanol–water) to minimize foaming.

Basically, anionics are analysed by reversed-phase LC using an ion-pairing agent and an organic solvent (acetonitrile, methanol, tetrahydrofuran)–water gradient. In reversed-phase ion pair chromatography, the addition of an appropriate ion–pairing reagent (tetrabutylammonium hydrogensulfate) to the mobile phase suppresses the ionic nature of the sample while introducing some charge to the nonpolar surface of the stationary phase. The retention of the resulting

ion pair is then controlled by pH, counterion concentration and mobile phase polarity. Linear and branched-chain ($C_4$–$C_{18}$) alkylbenzenesulfonates are separated by this method according to the length and structure of the alkyl chain, using as the mobile phase 0.1 mol $L^{-1}$ tetrabutylammonium hydrogensulfate (pH 5)–water–acetonitrile (gradient elution). At pH 5, the sulfonates and pairing reagent are completely ionized, as are the carboxylated surfactants, whose p$K_a$ values are somewhat higher (p$K_a \sim 4$). The structure and concentration of the pairing agent also influences the retention behaviour: increasing the lipophilicity increases the retention of the surfactant ion pair by enhancing its affinity for the nonpolar stationary phase. The increase in the concentration of the pairing agent will lead to greater coverage of the stationary phase surface and consequently to longer sample retention.

A limitation is that only the chromophoric components of such mixtures can be monitored by ultraviolet (UV) absorption detection. Nonchromophoric anionic surfactants such as alkyl sulfates or their corresponding alcohols and acids can be determined by LC after derivatization to 3,5-dinitrobenzoate esters or *p*-(methylthio)benzoate esters. The acidic forms of α-olefin sulfonates, alkyl sulfates, alkylethoxylated sulfates and alkyl phosphates react with 4-diazomethyl-*N*,*N*-dimethylbenzenesulfonamide to produce UV-absorbing derivatives that can be separated by reversed-phase LC. Direct detection (no derivatization) can be achieved by the use of a spectrofluorimetric detector operating at 225 nm (excitation) and 295 nm (emission) (reversed-phase $C_{18}$ (RP-18) column; mobile phase: 0.1 mol $L^{-1}$ sodium perchlorate in 80 : 20 methanol–water) or by a differential refractometer (refractive index) detector. Long–chain alkane sulfonates ($C_{12}$–$C_{20}$) are separated by this method using a phenyl column with a 75% methanol–25% 0.1 mol $L^{-1}$ sodium nitrate mobile phase.

An alternative approach to separating and detecting aliphatic anionic surfactants is ion interaction chromatography (reversed-phase column) with an aromatic ion-pairing agent also acting as chromophore for UV detection at 254 nm (cetylpyridinium chloride, phenethylammonium ion).

Anion exchange chromatography with indirect detection is not a common approach for aliphatic sulfonates, although it is simpler than ion pair chromatography. Using hydrogenphthalate, sulfosalicylic acid or *m*-sulfobenzoic acid in 60 : 40 acetonitrile–water as mobile phase, $C_2$–$C_8$ sulfonates can be separated on a strong cation exchange column with indirect UV absorbance detection at 297, 320 and 298 nm, respectively. $C_6$–$C_{12}$ aliphatic sulfonates and sulfates can also be analysed using sodium naphthalenedisulfonate–acetonitrile as the mobile phase on a polymeric fluorocarbon–amine cross-linked weak anion exchange silica column with either indirect conductivity or photometric detection modes.

The solid-phase reagent (SPR) procedure has been introduced recently as a new method of postcolumn conductivity detection of alkylsulfates and alkylsulfonates. SPR, an aqueous suspension of submicrometre particles of a polymeric cation exchange material in the hydrogen form, is pumped into the eluent stream coming from the column (silica-based reversed-phase). The postcolumn reaction transforms the tetrabutylammonium alkyl sulfate or sulfonate into the corresponding free acid. This changes the analytes into more conductive species and tetrabutylammonium borate eluent to the low conductivity boric acid. The conductivity detection method with SPR makes it possible to employ gradient elution for separation of complex mixtures.

GC and GC-MS cannot be applied directly to the analysis of anionic surfactants since these compounds are too polar and nonvolatile to be amenable either to GC or to conventional electron-impact MS (desulfonation with acids, alkali fusion sulfochlorination, methylation, pyrolysis-GC are well known methods of prederivatization for GC analysis). The use of soft ionization techniques such as field desorption (FD) and fast atom bombardment (FAB) is highly suited for characterization of these polar compounds by MS.

FAB-MS (in positive or negative ion mode) can be used to analyse complex anionic mixtures without prior separation of the components, since it gives abundant deprotonated (negative) or cationized (positive) molecular ions, and no fragmentation. As is shown in the case of a mixture of ethoxylated alcohol ($C_{12}/C_{14}$) sulfates (**Figure 1**), the technique not only gives the complete pattern of oligomer distribution (length of the alkyl and/or of the ethoxylate chain), but also information about the purity of the raw material (the c and d series in Figure 1B are the cationized molecular ions of unreacted materials, the unsulfated ethoxylated fatty alcohols). Ethoxylated alcohol sulfates are easily detected by this technique in finished detergent formulations, even in the presence of other surfactant types (i.e. amphoteric tensides), as has been demonstrated for shampoos.

## Nonionic Surfactants

Nonionic surfactants constitute the second most important class of tensides: although their foaming properties are low in respect to those of anionics, they

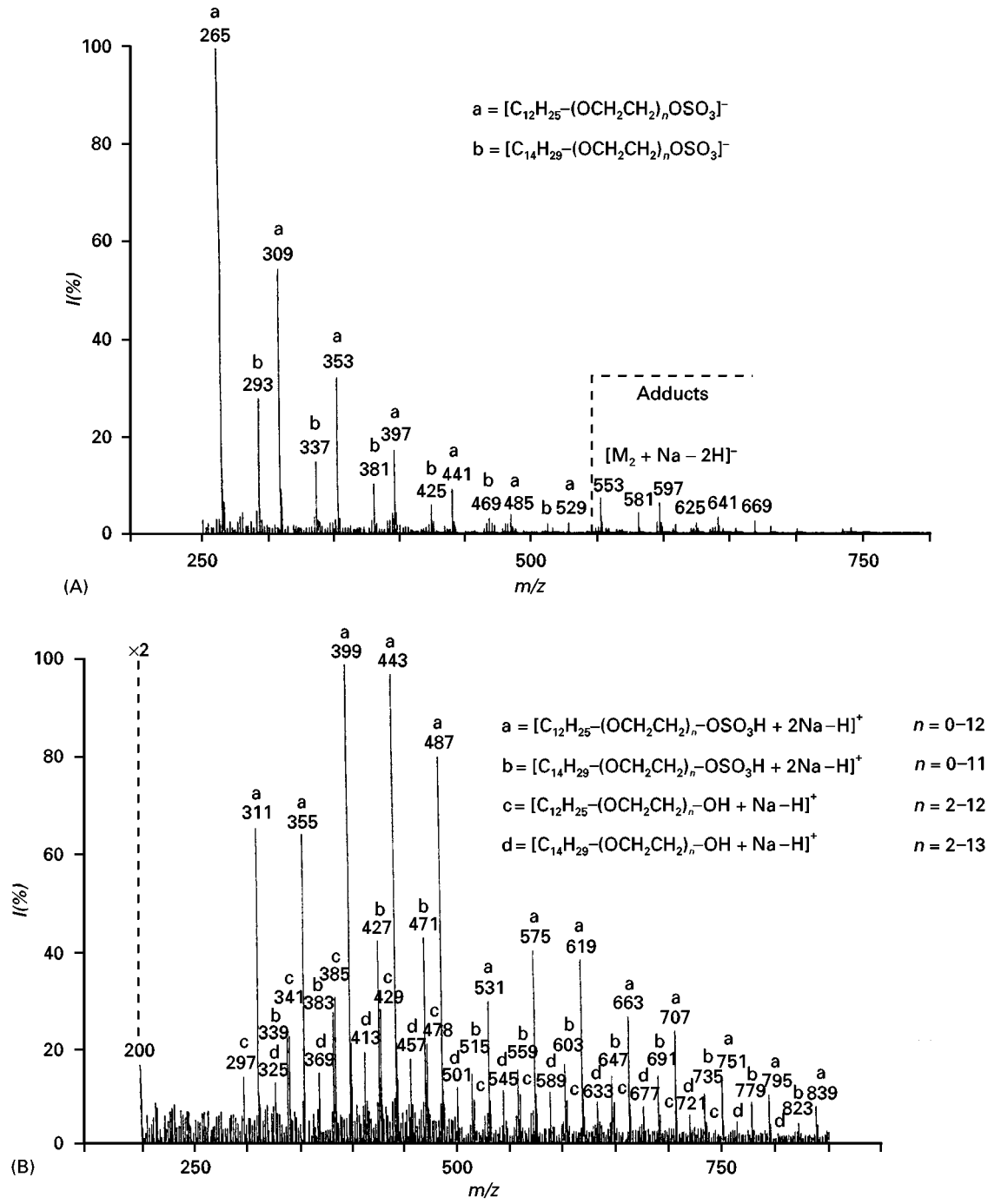



**Figure 1** (A) Negative-ion and (B) positive-ion FAB mass spectra of ethoxylated alcohol sulfates (anionic surfactants). (From Maffei Facino *et al.*, 1989.)

are widely used in detergent formulations (especially in bath foams) to increase viscosity and as foam boosters. **Table 2** reports the chemical classification of the most important nonionic surfactants.

Spectrophotometric methods for the quantitative analysis of nonionic surfactants are popular: they are based on complex formation with tungstophosphoric acid, molybdophosphoric acid, picric acid, Malachite green, potassium tetracyanatozincate and ammonium tetrathiocyanatocobaltate (III). This last reagent gives better results than tungstophosphoric acid and than the potentiometric method with Dragendorff's reagent. It can be applied for determination of ethoxylate compounds in detergent solutions: the sur-

**Table 2** Nonionic surfactants

| *Type* | *General structure* |
|---|---|
| Ethoxylated alcohols | $R–O(CH_2CH_2O)_nH$<br>Reaction product between ethylene oxide and fatty alcohols |
| Ethoxylated alkylphenols | $R–C_6H_4–O(CH_2CH_2O)_nH$<br>Reaction product between ethylene oxide and alkylphenol ($R = C_8/C_9$) |
| Alkanolamides | $R–CO–N–(CH_2CH_2OH)_n \quad n = 1,2$<br>Reaction product of fatty acids ($C_{10}–C_{18}$) with mono- or diethanolamine |
| Alkylglycosides (glucose ethers) | $R–O–(Gluc)_n$<br>Reaction product between glucose and fatty alcohols ($R = C_8–C_{14}$) |

factant is extracted from the aqueous solution with chloroform and the extract is treated with the reagent to form a blue complex that is then analysed spectrophotometrically. The absorbance is not affected by temperature, electrolytes or dilution.

Several electrochemical and potentiometric techniques are also available for quantitative analysis of nonionic tensides: for example, using a barium ion selective electrode it is possible directly to quantitate the surfactant in the range of $2 \times 10^{-5}$ to $1 \times 10^{-3}$ mol $L^{-1}$. A typical potentiometric titration is based on formation of insoluble complexes with molybdophosphoric acid: the sample dissolved in aqueous ethanol containing barium chloride is treated with an excess of the complexant and the unreacted molybdophosphoric acid is titrated potentiometrically with diantipyrylmethane, using a platinum indicator cathode.

In the quality control of ethoxylated compounds, it is important to determine their composition, since they are manufactured as mixtures of homologous compounds that differ in the length of the ethoxylate and/or the alkyl chain. Several spectroscopic nuclear magnetic resonance (NMR) and infrared (IR) and chromatographic techniques (TLC, GC, LC) and supercritical fluid chromatography (SFC) are available to determine the degree of ethoxylation and the distribution of homologues in nonionic surfactant mixtures.

Both IR spectroscopy and NMR spectroscopy may be used for the determination of the average molecular mass ($M_r$), the average degree of condensation ($x$), and the hydrophilic/lipophilic balance (HLB) of nonylphenol ethylene oxide condensates. The IR method is based on the regression between the logarithm of the surfactant properties and the logarithm of the ratio of the heights of the bands at 840 and 960 $cm^{-1}$, corresponding to aromatic C–H vibrations: the first band is greater than the second for compounds of smaller degree of condensation, but this relationship becomes inverted as the degree of polymerization increases.

The NMR approach involves the calculation of absolute integrals of three types of hydrogen atoms: from these integration values it is possible to calculate the degree of condensation and the alkyl residue composition, as each surfactant molecule contains four aromatic hydrogen atoms that can be used as an internal reference to determine the number of hydrogen atoms corresponding to the remainder of the peaks.

TLC with a flame ionization detector (FID) has been applied for the separation and quantitative determination of nonionic surfactants containing an average number of oxyethylene units not higher than 8.0. The oligomers are separated on Chromarod S-II (silica gel-coated rods) with double development; (a) benzene–ethyl acetate (6 : 4) for 10 cm from the start; (b) ethyl acetate–acetic acid–water (8 : 1 : 1) up to a distance of 8 cm. After development, the rods are passed through a FID operating with hydrogen (160 mL $min^{-1}$) and air (2 L $min^{-1}$). The major advantage of LC in the analysis of nonionic surfactants lies in its ability to separate and quantitate alcohol or alkylphenol ethoxylate oligomers that differ in the length of the ethoxylate chain. While alkylphenol ethoxylates can readily be identified by UV detection, aliphatic compounds, since they do not posses significant UV absorption, must be derivatized prior to LC (for example by esterfication with 3,5-dinitrobenzoyl chloride). Reversed-phase LC with refractive index detection has been proposed to establish the retention behaviour of a wide range of ethoxylated and/or propoxylated adducts: there is a linear relationship between the logarithm of the capacity factor and the degree of polymerization of the ethoxylated and/or propoxylated $C_{12}$, $C_{16}$, $C_{18}$ alcohols, ethylene oxide–propylene oxide copolymers, poly(ethylene glycol)s and poly(propylene glycol)s. This can be used not only for the prediction of chromatographic separation, but also for the estimation of the degree of polymerization and of the length of the alkyl chain.

Recently, the evaporative light-scattering (ELS) detector, also known as the mass detector, was

introduced as a universal detector for separation and quantification of all surfactant species. The detector measures light refracted by the nonvolatile particles after the effluent from the LC is nebulized and the carrier solvent is evaporated. The detector gives an equal and linear response factor for each class of surfactant that is independent of molecular mass (the amount of refracted light is proportional to the concentration of the analyte species).

Alcohol ethoxylates have been characterized by GC as acetate derivatives on a packed column or as silylated derivatives using a fused silica capillary column. However GC gives only a partial fingerprint, since only low molecular mass components can be detected (the free alcohols and short-chain ethoxylated homologues, up to approximately 12 ethylene oxide oligomers).

High temperature capillary gas chromatography and SFC are new alternative procedures for the analysis of these compounds that are thermally unstable or have low volatility. The alcohol and ethoxylate distributions, mean molecular mass and average number of moles of ethylene oxide can be calculated rapidly with both the methods (polyglycols with average molecular masses of 2000–2500 Da have been successfully analysed by SFC). Advantages and limitations of the SFC and high temperature capillary GC procedures can be summarized as follows: (1) for routine quality control analyses of known alcohol ethoxylates, both techniques appear to be equally suited; (2) SFC is time-saving because derivatization is not required, although for complex mixtures derivatization improves resolution (acetylation by means of acetic anhydride and pyridine or silylation with bis(trimethylsilyl)trifluoroacetamide and pyridine); (3) the GC technique is able to resolve $C_{12}$–$C_{18}$ alcohol ethoxylate oligomers, thus avoiding ambiguous identification of components. **Figure 2** shows the chromatographic profiles of a $C_{12}/C_{13}$ alcohol ethoxylate with an average of 6.6 moles of ethylene oxide obtained by SFC and by high temperature capillary GC after silylation.

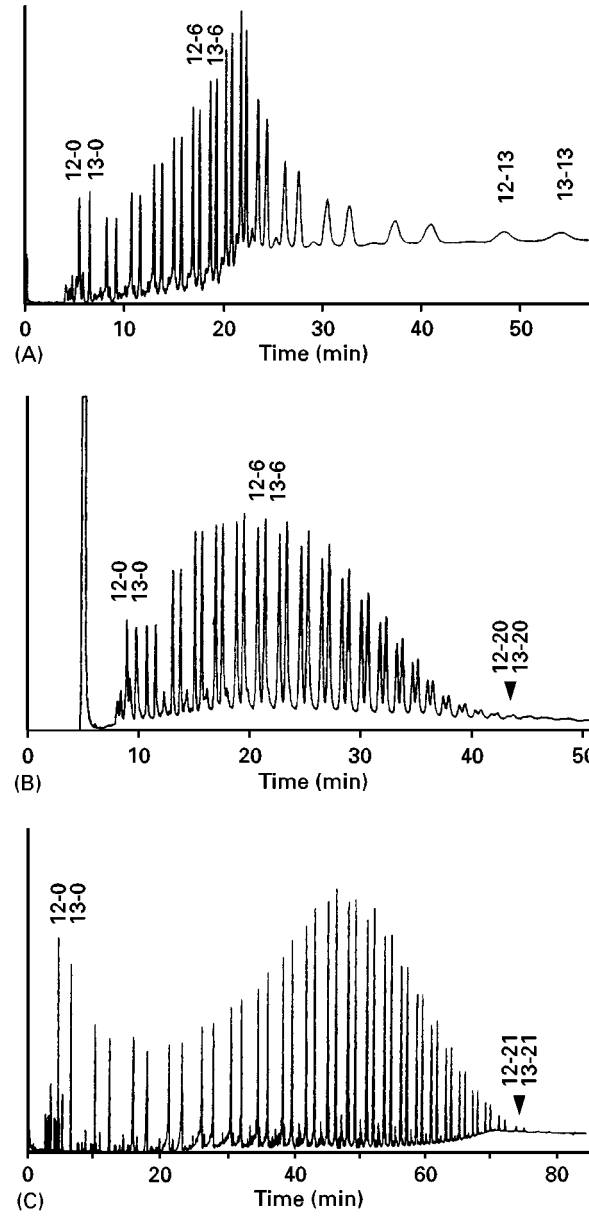



**Figure 2** Analysis of ethoxylated alcohols (nonionic surfactants) by GC, high temperature GC and SFC. (A) Capillary GC of a silylated $C_{12}/C_{13}$ alcohol ethoxylate with an average of 6.6 moles of ethylene oxide. (B) Capillary SFC of the same mixture (underivatized). (C) Capillary high temperature GC of the same mixture (after silylation). (From Silver AH and Kalinoski HT (1992) *Journal of the American Oil Chemist's Society* 67: 599–608.)

Among the mass spectrometric methods, the use of conventional GC-MS electron impact (EI) ionization is limited to nonionic surfactants with a low degree of ethoxylation. Compounds with a high degree of ethoxylation (20–25 units) cab be identified directly in raw materials and in finished detergent formulations by soft ionization techniques such as direct chemical ionization (DCI), FD and FAB. This last, in positive ion mode, furnishes the complete pattern of oligomer distribution of ethoxylated compounds, since it gives a series of ions at 44-Da intervals (protonated and/or cationized molecular ions only, with no fragmentation). In addition FAB-MS (in positive or negative ion mode) gives direct characterization of alkylpolyglycosides, a new generation of highly polar nonionic tensides not amenable to analysis by conventional chromatographic methods. The method, which is based on unambiguous molecular mass determination of the single components (protonated or deprotonated molecular ions), allows the definition of length of both the alkyl and the glucosidic chains (up to 10 glucose units).

Free poly(ethylene glycol)s (PEGs) are the main contaminants of ethoxylated derivatives and are frequently found in the products obtained from them, because they can be formed as side-products in the reaction of ethylene oxide with the hydrophobic component (in which case they are present as a mixture of homologous polymeric derivatives with a molecular mass distribution that depends on the reaction conditions); they can be added intentionally to obtain specific performances of the final product; and they can arise from the decomposition of adducts in the synthetic reaction or during the processing. Hence determination of free PEGs is important not only from the viewpoint of routine quality control of the manufacturing process, but also for the determination of the suitability of surfactants for specific purposes. Among the procedures used for the separation of PEGs from adducts and the unreacted starting material, a simple method involves extraction of an ethyl acetate solution of surfactants with 5 mol $L^{-1}$ sodium chloride, followed by extraction of the aqueous phase with chloroform, evaporation of the solvent and gravimetric determination of PEGs (accurate temperature control is required).

Column LC is faster and more reproducible for the separation of free PEGs from the other components of the mixture. Silica, hydrophobized with dichlorodimethylsilane, with chlorobenzene as the stationary phase, separates PEGs from their adducts using acetone–water–acetic acid as the mobile phase. Utilizing reversed-phase chromatography on silanized silica gel, and 30% aqueous isopropanol as mobile phase, PEGs are eluted, while adducts are desorbed with 96% ethanol. Partition chromatography, with ethyl acetate as the mobile phase and cellulose as support for the stationary phase (30% sodium chloride solution), is used for the determination of PEGs in adducts of fatty alcohols, alkylphenols, fatty acids and alkanolamides.

By applying hexane–isopropanol–water mobile phases of controlled composition (different ratios of hexane to isopropanol), either ethylene oxide adduct (EOA) or PEG oligomers can be separated on a bonded diol phase, and their distributions evaluated (refractive index detection). The PEG or EOA oligomers can easily be separated up to the 30-mer even without gradient elution, and ethoxylated surfactants (fatty alcohols, fatty acids, fatty acid monoethanolamides and alkylphenols) up to an ethoxylation degree of 20.

Another important contaminant of ethoxylated derivatives (both anionic and nonionic) is 1,4-dioxane: according to the European Economic Community Directive on Cosmetics, commercial products must be free from this compound, since it is carcinogenic in rats and mice. 1,4-Dioxane is formed by dimerization of ethylene oxide during the process of alcohol/phenol ethoxylation and might be found in the final detergent formulations via the use of ethoxylated fatty alcohol sulfates as cleansing agents.

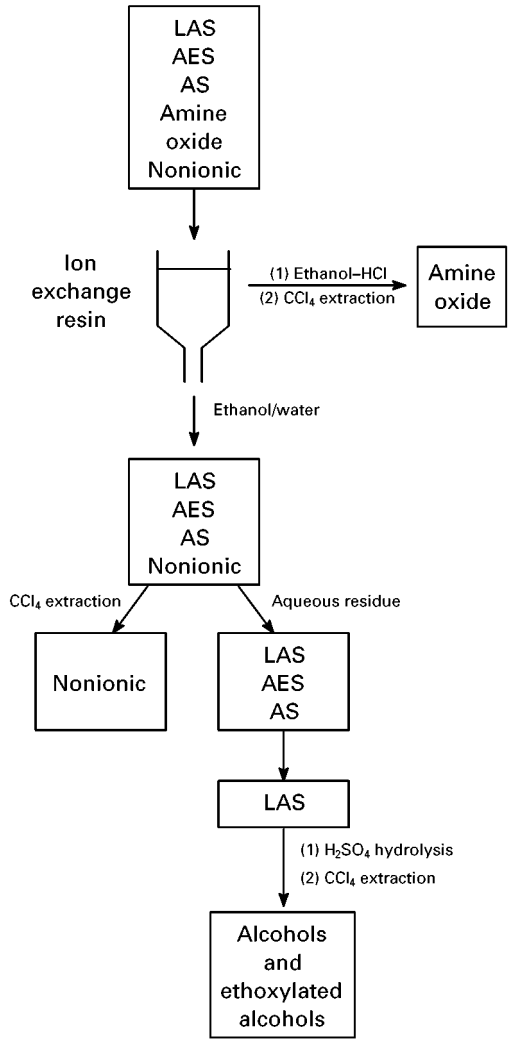
1,4-Dioxane is commonly determined by GC. A simple method applied to shampoos, requiring minimal sample preparation (dilution with water containing the internal standard isobutanol and direct injection), is carried out with packed column (15% OV-1 on 100/120 Chromosorb WHP) and FID (injection temperature 185°C; detector temperature 325°C; temperature programme 85°C (2 min), 5°C $min^{-1}$ to 95°C, followed by clean-up step). Linearity is in the concentration range 1–250 mg $kg^{-1}$; limit of detection 1 mg $kg^{-1}$.

An alternative technique than can be applied to different cosmetic matrices is GC-MS with selected ion monitoring (SIM); prior to analysis, rapid and efficient purification from the interfering materials of the cosmetic products is achieved by use of combined silica/octadecylsilica cartridges (limit of detection 3 mg $kg^{-1}$).

**Scheme 1** shows a procedure useful for separation and quantitation of a hypothetical detergent product (liquid or powdered) formulated with different types of active ingredients: amine oxide, ethoxylated alcohols (nonionic), alcohol sulfate (AS), ethoxylated alcohol sulfates (AES) and linear alkyl sulfonates (LAS). Under basic and neutral conditions, amine oxide behaves as a nonionic material, while under acidic conditions it acts as a cationic agent.

A sample ($\sim$5 g) of liquid detergent or of the alcohol–soluble material (1–2 g) from a powdered detergent is dissolved in a minimum volume of ethanol–water (1 : 1) and passed through a strong cationic ion exchange column (Dowex 50WX4, 200–400 mesh, sulfonic acid form). Elution with ethanol–water (1 : 1) separates anionic and nonionic surfactants from amine oxide selectively absorbed on the resin. The amine oxide is eluted from the column with 1 mol $L^{-1}$ ethanolic hydrochloric acid and the eluate, after neutralization, is extracted with carbon tetrachloride. The isolated amine oxide fraction can be further characterized by NMR, IR or GC and quantified by a titration method: under acidic conditions amine oxides are determined as quaternaries with a standard alkylbenzene sulfonate and methylene blue indicator (see Cationic Surfactants). This method does not distinguish amine oxides from their precursor alkyldimethylamines: the latter can be analysed by gas liquid chromatography (GLC).

If the amine oxide distribution and average molecular mass are unknown, they can be determined by packed column (Apiezon L on 60/80 Chromosorb W.




**Scheme 1** Separation of different types of surfactants. AS = alcohol sulfates; AES = ethoxylated alcohol sulfates; LAS = linear alkyl sulfonates.

HMDS) GC: these compounds pyrolyse to 1-olefins (column temperature 280°C; injection temperature 240°C; detector temperature 330°C) and pyrolysis is essentially complete over the range of $C_{12}$ to $C_{18}$ alkyl chains. Alkyldimethylamines do not decompose in these conditions and their peaks are well separated from olefins: therefore determination of the precursors should be possible by the use of a suitable internal standard.

The aqueous alcohol phase containing free nonionic ethoxylated alcohols, sulfated anionic and sulfonated anionic material is extracted with carbon tetrachloride to separate nonionic surfactants, which can then be analysed according to one of the methods mentioned previously. The aqueous alcohol residue containing only sulfated and sulfonated anionic materials is concentrated *in vacuo* to remove the alcohol and then hydrolysed with 1 mol $L^{-1}$ sulfuric acid. Hydrolysis converts all the sulfated anionic material to ethoxylated alcohols or fatty alcohols (the sulfonated anionic fraction is not affected by acid hydrolysis), which, after neutralization, can be recovered by carbon tetrachloride extraction.

The remaining ethanol–water phase containing sulfonated species is evaporated and the sulfonate is recovered and weighed by a salting out procedure; alternatively, it can be qualitatively and quantitatively analysed by the methods previously described.

## Cationic Surfactants

Cationic surfactants are devoid of detergent or foaming properties, but are excellent hair conditioners: for these reasons their use in toiletries is limited to formulations of specific shampoos. **Table 3** shows the main types of cationic surfactants used in cosmetics.

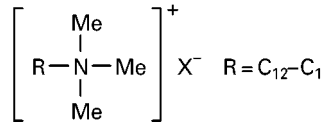
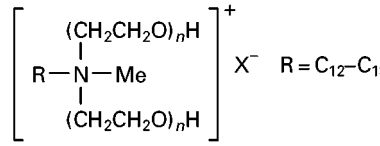
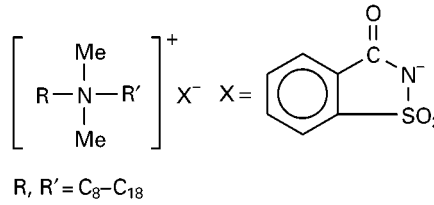
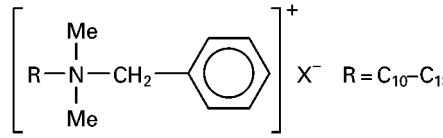
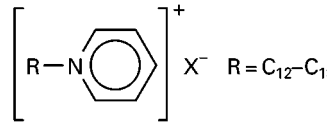
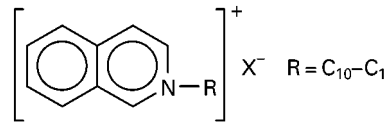
The ion pair extraction technique has proved suited for the determination of cationic surfactants by two-phase titrations and/or by spectrophotometry (the method is based on extraction of an ion pair between surfactant and dye, which is the basis of the well known Epton Methylene blue and The Cosmetic, Toiletry and Fragrance Association (CTFA) mixed indicator method). In the two-phase titration of cationics by lauryl sulfate in the presence of a suitable indicator dye (Methylene blue, Thymol blue, Bromophenol green, disulfine blue–dimidium bromide), the dye–surfactant ion pair is extracted almost completely by the organic solvent chloroform or methylene chloride.

When the titrant (an oppositely charged surfactant) is added, surfactant–surfactant ion pair formation takes place. The end point is indicated when enough titrant is added so that the small amount of the dye present is displaced from the dye–surfactant ion pair and returns to the aqueous phase. Alternatively, the dye–surfactant ion pair can be spectrophotometrically determined after chloroform extraction from the aqueous solution.

Using Bromophenol blue as dye indicator, it is possible to quantitate cationic surfactants and the corresponding amines when both are present in a detergent mixture. It has been shown that with long-chain quaternary ammonium compounds (cetyltrimethylammonium bromide), Bromophenol blue forms two different compounds: in alkaline solutions a blue di(cetyltrimethylammonium) salt, but in acid solution a yellow mono(cetyltrimethylammonium) salt.

Hence cationics can be estimated spectrophotometrically in two different ways, as the blue di-salt in

**Table 3** Cationic surfactants

| *Type* | *General structure* |
|---|---|
| Alkyltrimethylammonium halides |  |
| Alkylethoxylated ammonium halides |  |
| Dialkyldimethylammonium halides/saccharinates |  |
| Alkylbenzyldimethylammonium halides |  |
| Alkylpyridinium halides |  |
| Alkylisoquinolinium halides/saccharinates |  |

alkaline solutions (absorbance maximum at 606 nm) and as the yellow mono-salt in acid solutions (absorbance maximum at 416 nm).

Separate estimations of the quaternaries (which do not hydrolyse) and the amine salts (which can hydrolyse easily in alkaline solutions) can be carried out working at different pH values: a higher pH will decrease the contribution of the amine even more because the higher the pH, the greater the hydrolysis of the amine salts into the amine and removal by the organic solvent. Determination of the amine salts in the presence of cationics can be carried out by estimating the total cationics in acid solution and the quaternaries only in alkaline solution: the amine content is obtained by difference. The spectrophotometric determination of cationic surfactants with Orange II as dye indicator has the same kind of applications: dye salts are determined at 490 nm after chloroform extraction from aqueous solutions of surfactants and excess of Orange II dye: Orange II reacts with a 1 : 1 stoichiometry with cationic tensides and the molar absorptivity and the wavelength of maximum absorbance for the dye salts in chloroform are independent of the reacting surfactant. Isolation of dye salt in chloroform can be also used as a means of estimating average equivalent weights of commercial cationic surfactants.

By selective changing of the pH, the method might be applied for quantification of cationic precursors such as amines and amine oxides of amphoteric sur-

factants and of mixtures of amine and quaternary ammonium compounds.

The prerequisite for the extraction method is the formation of a lipophilic surfactant–dye ion pair which is then extracted into chloroform or methylene chloride. However, there are many cationic polymers used as hair conditioners that do not form lipophilic ion pairs, such as cationic polypeptides, and in addition many surfactants form an emulsion during extraction with lipophilic solvents, causing problems in determining the end point. In all these cases, quantitative analysis of cationic surfactants can be performed by a potentiometric method using a 'surfactant' electrode and sodium dodecyl sulfate as titrant.

Quaternary ammonium salts are not amenable as such to GC because of their low volatility and limited thermal stability. Long chain quaternary ammonium compounds undergo extensive but reproducible decomposition in a classical gas chromatographic system, to tertiary amines and alkyl halides. This chromatographic behaviour has led to the development of an analytical approach carried out with dedicated instruments such as a Curie point pyrolyser or a filament pyrolyser coupled to a GC-MS system, which has been applied both for structure elucidation (distribution of homologous compounds) and for quantitative determination of cationic surfactants in various matrices.

Long chain *N*-alkylpyridinium (alkyl = $C_{10}$–$C_{18}$) salts can be determined by GLC of the reduction products obtained by treatment with sodium tetrahydroborate and nickel(II) chloride. The procedure is useful for routine analysis of *N*-alkylpyridinium salts, as the reduction to perhydrogenated derivatives takes place quantitatively and cleanly in aqueous media at room temperature with easily handled reagents.

The most promising and convenient approach is LC, although its application is limited to UV-absorbing quaternaries (quantification of both UV- and non-UV-absorbing quaternaries can be achieved with a LC system coupled to a conductivity detector). Both normal-phase ion pair LC and reversed-phase LC have been used for analysis of cationics: reversed-phase chromatography is common but problematic, since these compounds mostly elute from octadecylsilica columns as badly tailing peaks.

The addition of ion-pairing agents and/or quaternary amines to the mobile phase generally does not eliminate this unwanted phenomenon. The substitution of an octadecylsilica by a polymeric polystyrene–divinylbenzene column was found to afford a considerable improvement in the peak shapes. For example, a homologous series of alkylbenzyldimethylammonium and alkylpyridinium halides with $C_{10}$–$C_{18}$ alkyl groups can be separated by employing porous microspherical poly(styrene–divinylbenzene) gel as the stationary phase and 0.5 mol $L^{-1}$ perchloric acid in methanol as the mobile phase (the logarithm of the capacity factor for each homologous series is directly proportional to the alkyl chain length). **Figure 3** shows the reversed-phase liquid chromatograms of a homologous series ($C_{12}$–$C_{18}$) of *n*-alkylbenzyldimethylammonium chlorides obtained under different experimental conditions.

The mass spectrometric soft ionization techniques (FD, FAB) allow a rapid and unequivocal structure elucidation of the components of a mixture of cationic surfactants. FAB in the positive ion mode gives unambiguous spectra, with abundant molecular ions and no fragmentation, furnishing detailed information on the length of both the alkyl and the ethoxylate chains in polyethoxylated derivatives (these last compounds are frequently used as hair conditioners).

## Amphoteric Surfactants

By definition, amphoterics are surfactants that have anionic or cationic properties depending on the pH and that have an isoelectric point. Because of the highly nucleophilic character of oxygen, amine oxides also have salt formation potential, and for this reason their analysis is similar to that of amphoterics.

**Table 4** shows the main amphoteric types produced today: alkylamido and alkyl betaines (and their respective amine oxides), alkylamido- and alkylsulfobetaines, amphoglycinates (formerly imidazolines).

Among the surfactants, amphoterics are those more prone to contamination from intermediates, since their synthesis involves several reaction steps.

Alkylamidobetaines are synthesized from the intermediate amidoamines, which in turn are obtained by reaction of fatty acids with amines (mainly dimethylaminopropylamine); the amidoamines react with sodium monochloroacetate in aqueous solution (alkaline medium) to give betaine derivatives (eqn [I]):

$$\begin{aligned} &R\text{–}COOH + H_2N\text{–}C_3H_6\text{–}N(CH_3)_2 \rightarrow \\ &\quad R\text{–}CONH\text{–}C_3H_6\text{–}N(CH_3)_2 \\ &+ Cl\text{–}CH_2\text{–}COO^-Na^+ \rightarrow \text{betaines} \end{aligned} \qquad [I]$$

The corresponding amine oxides are prepared by oxidation of the intermediates amidoamines with hydrogen peroxide in aqueous solution.

In a similar way, alkylbetaines are prepared by carboxylation (with sodium chloroacetate) of the

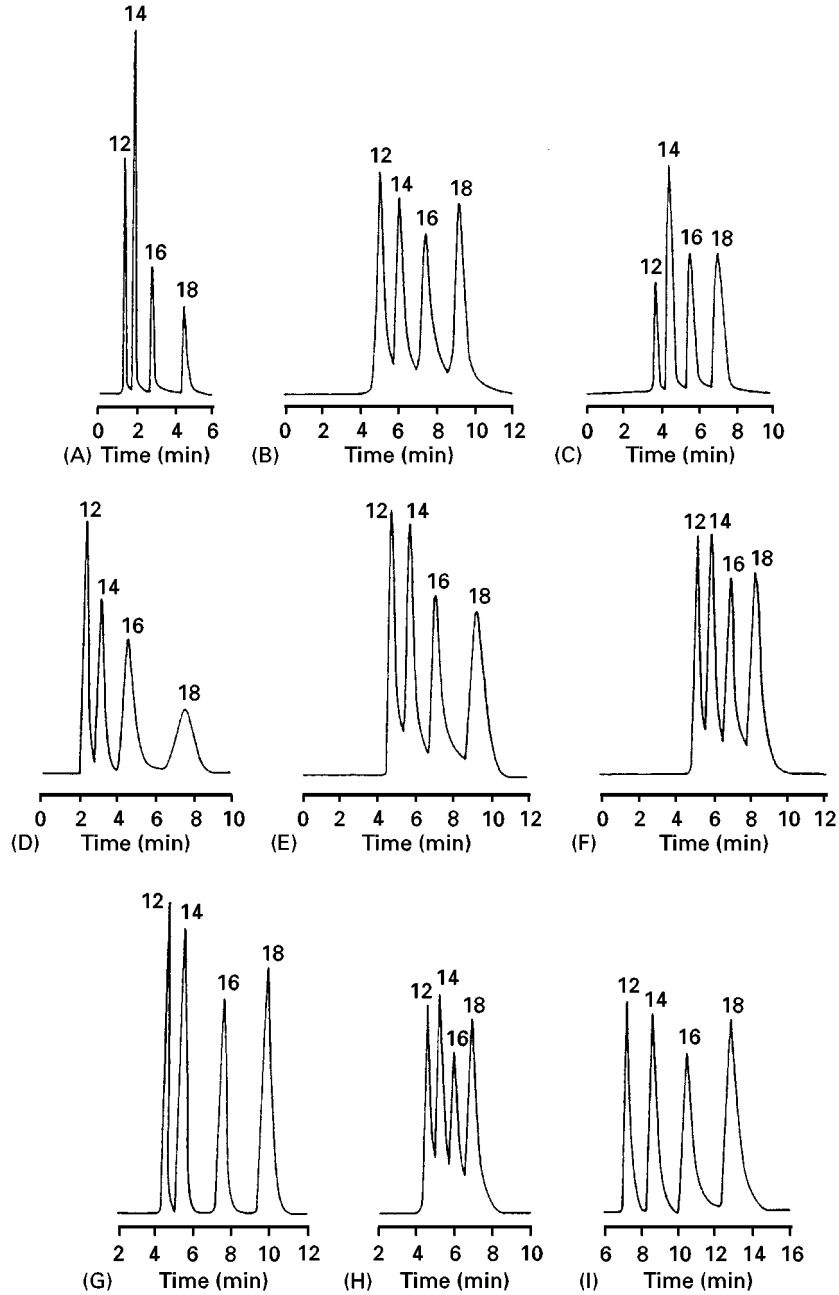



**Figure 3** Reversed-phase LC peaks of a homologous series of *n*-alkylbenzyldimethylammonium chlorides (cationic surfactants). LC conditions: all mobile phases contain 0.1 mol $L^{-1}$ sodium perchlorate (pH 3); (A) acetonitrile–water (9 : 1); (B) acetonitrile–water (1 : 1); (C) acetonitrile–water (7 : 3); (D) methanol–water (9 : 1); (E) methanol–water (3 : 2); (F) methanol–water (17 : 3); (G) THF–water (3 : 2); (H) THF–water (1 : 1); (I) THF–water (3 : 2) (THF = tetrahydrofuran). Stationary phases: octadecylsilica (A, D, G); cyanopropylsilica (B, E, H); phenylpropylsilica (C, F, I). (From Abidi SL (1985) *Journal of Chromatography* 324: 209–230.)

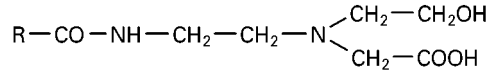
alkyldimethylamines according to eqn [II]:

$$\mathrm{R{-}COOH + NH_3 \xrightarrow[-2H_2O]{} R{-}C{\equiv}N \rightarrow R{-}CH_2{-}NH_2 \xrightarrow[+2H_2]{} R{-}CH_2{-}N(CH_3)_2 \xrightarrow[+CH_3X]{} \quad [II]}$$

Sulfobetaines are synthesized according to eqn [III]:

$$\mathrm{R{-}COOH + H_2N{-}C_3H_6{-}N(CH_3)_2 \rightarrow R{-}CO{-}NH{-}C_3H_6{-}N(CH_3)_2 + Cl{-}CH_2{-}CH{=}CH_2 \rightarrow [R{-}CO{-}NH{-}C_3H_6{-}N(CH_3)_2{-}CH_2{-}CH{=}CH_2]^+Cl^- + NaHSO_3 \rightarrow \text{sulfobetaine} \quad [III]}$$

**Table 4** Amphoteric surfactants

| *Type* | *General structure* | |
|---|---|---|
| Alkylbetaines | $R–N^+(CH_3)_2–CH_2COO^-$ | $R = C_{12}–C_{18}$ |
| Alkylamine oxides | $R–N(CH_3)_2 \rightarrow O$ | |
| Alkylamidobetaines | $R–CO–NH–(CH_2)_n–N^+(CH_3)_2–CH_2–COO^-$ | R–CO = fatty acids |
| Alkylamidoamine oxides | $R–CO–NH–(CH_2)_n–N(CH_3)_2 \rightarrow O$ | |
| Amphoglycinates |  | R–CO = fatty acids |
| Sulfobetaines | $R–CO–NH–(CH_2)_n–N^+(CH_3)_2–C_3H_6–SO_3^-$ | R–CO = fatty acids |

The position of the sulfo group is not certain, and the resulting amphoteric surfactants are thought to be a mixture of the 2- and 3-sulfopropylated quaternary ammonium compounds.

Amphoglycinates are prepared by reaction of fatty acids with aminoethylethanolamine to give intermediate cyclic compounds, imidazolines (it is common knowledge that this first step does not produce the linear amides). By carboxylation with sodium chloroacetate in aqueous solution, ring opening occurs with formation of amphoglycinates that are not of uniform composition.

Isoelectric point determination is the first measure to identify amphoteric surfactants: this can be carried out by conductivity titration, potentiometric titration or electrophoresis (isoelectric focusing). By potentiometric titration, the following isoelectric points have been determined: alkylamidobetaines $\sim 7.0$; alkylbetaines $\sim 6.0$; alkylamidoamine oxides $\sim 8.5$; alkylamine oxides $\sim 9.0$.

TLC on silica gel plates with chloroform–methanol–ammonia (30 : 50 : 2) or ethanol–chloroform–ammonia (45 : 40 : 15) as mobile phases rapidly distinguishes and identifies different amphoterics, even when present in detergent formulations; detection is with 0.1% Bromophenol blue followed by treatment with 0.1% sodium periodate in aqueous solution.

IR spectroscopy is an alternative method for identification of amphoterics: in the case of amino oxides, special precautions must be taken during preparation of the samples (freeze-drying and not drying at 105°C must be used to remove the water, otherwise the N–O bond will be broken). The typical N–O bands are at approximately 960 and 930 $cm^{-1}$ for both alkylamido and alkylamine oxides; for the alkylamido derivatives, the secondary amide bands at *c.* 3300, 1640 and 1550 $cm^{-1}$ are diagnostic; the characteristic bands of betaines are at 1605, 1402, 1340 $cm^{-1}$ (carboxylate bands), 890 $cm^{-1}$ (quaternary N band), and 3275, 1633 and 1549 $cm^{-1}$ (secondary amide bands, only present in alkylamidobetaines).

The alkyl distribution in alkylamidobetaines, amidoamine oxides and amphoglycinates can be evaluated by GC, while alkylbetaines, sulfobetaines or amine oxides are preferably analysed by LC. The determination of alkyl distribution in amide derivatives is carried out after hydrolysis (with concentrated hydrochloric acid) of the amide bonds: the free fatty acids are then converted into the corresponding methyl esters by derivatization with conventional methods (such as methanol–sulfuric acid).

Reversed-phase LC (RP-18 column) is successfully used for characterization of all amphoterics, both in raw materials and in cosmetic formulations, using methanol–water (80 : 20) (**Figure 4**) or methanol–aqueous sodium hypochlorite as mobile phase. Alkylamido products (including alkylamidoamine oxides) can be detected by absorbance at 215 nm, while for alkyl distribution in alkylamine compounds a refractive index detector is recommended.

Direct analysis of amphoterics (sulfobetaines) in combination with coconut and tallow soaps can be carried out by reversed-phase LC (detection by differential refractometry) using methanol–water (85 : 15) as the mobile phase containing 0.2% (v/v) acetic acid (pH $\sim$ 4). At this pH value, tallow and coconut soap mixtures are analysed as fatty acids and are easily separated from the sulfobetaine components.

Ionic and amphoteric surfactants can also be separated on reversed-phase columns with 2-naphthalenesulfonic acid as counterion in the mobile phase (aqueous methanol) and detected by UV absorbance and differential refractometry. The simultaneous use of both UV and refractive index detectors allows ion pairing (ionic) and nonpairing (amphoteric) components in a mixture to be distinguished.

In the case of sulfobetaines, it is possible to separate the final product from reagents and intermediates: neither amphoterics nor long-chain fatty acids form ion pairs with the counterion in the mobile phase and they are detected by differential refractometry only. The intermediates amidoamine and long chain allyl

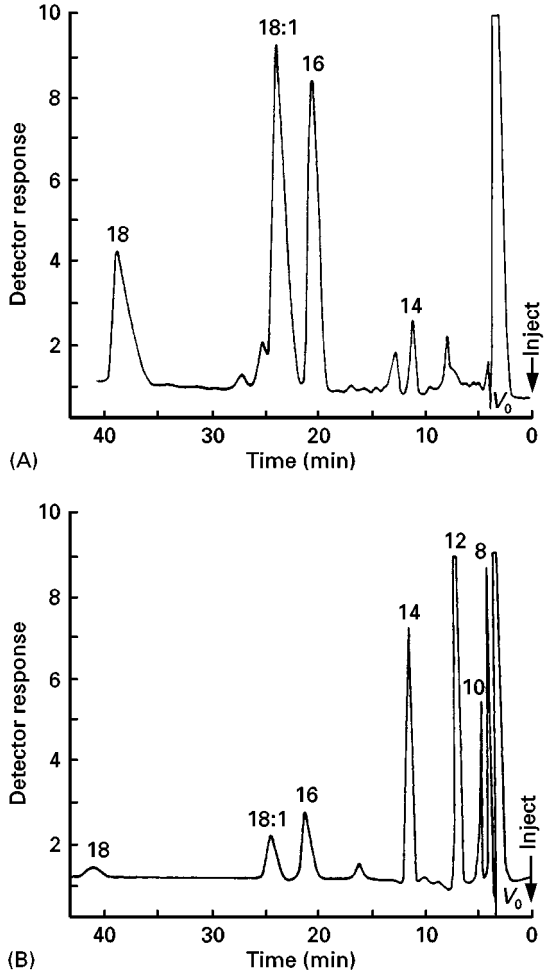



**Figure 4** Reversed-phase LC peaks of (A) tallow-derived sulfobetaines and (B) coconut oil-derived sulfobetaines (moblie phase methanol–water, 80:20; refractive index detection). (From: Parris N *et al.* (1977) *Analytical Chemistry* 49: 2228–2231.)

quaternary ammonium chloride are detected as ion pairs by both UV absorbance and refractive index detection. Detection of ionizable surfactants as UV-absorbing ion pairs improves detection limits 100-fold over those obtained by differential refractometry.

Amphoterics are frequently contaminated with various by-products: free fatty acids; free amines (long chain amines and long chain amidoamines); and free chloroacetic acid. The determination of free fatty acids is limited to amide products, and especially to alkylamidobetaines (in this case the presence of residual amounts of free fatty acids is not a drawback, since these compounds positively affect the viscosity characteristics of the alkylamidobetaine in combination with anionics). Fatty acids can be determined as methyl esters by GC after extraction with diethyl ether of the dried product.

Unlike alkyl dimethylamines, whose presence in alkylbetaines and alkylamino oxides is undesirable, residual levels of long chain amidoamines in alkylamidobetaines are 'cosmetically' acceptable (they increase viscosity and have foam booster properties).

Titration methods for the determination of amine residues are primarily used for alkylbetaines or alkylamine oxides. In the method of Metcalfe, the total amine content is determined by titration (in isopropanol as solvent) with hydrochloric acid in isopropanol. After quaternarization of the residual tertiary amines with methyl iodide at 50°C, and repeated titration with acid, the total amine oxide content is determined; the tertiary amine content can be determined by subtraction (limit of detection approximately 0.5%).

LC furnishes a more selective and sensitive determination of these amine residues in all classes of amphoterics. The separation is carried out on a reversed-phase column ($C_{18}$) with hexane–isopropanol (60 : 40) as mobile phase containing 2 mmol $L^{-1}$ octanosulfonic acid (for the ionic pairing of the amines); detection is by UV absorbance at 215 nm for amidoamines and refractive index for alkylamines.

Alternatively, postcolumn detection can be employed for primary, secondary and tertiary amines, but not for quaternaries: the compounds separated by the LC column are first converted into the corresponding *N*-chloramines with hypochlorite; the *N*-chloramines are then treated with iodide to form triiodide, which can be monitored by its absorbance at 355 nm.

Chloroacetic acid residues can be evaluated by titration or by chromatographic methods. In the titration method, the first step involves estimation of total chlorine content by silver nitrate, after sodium hydroxide hydrolysis of the sample (2 h under reflux: under these conditions chloroacetic acid is hydrolysed to glycolic acid and chloride). The titration of an unsaponified sample gives the chloride content. The amount of 'organic chloride' corresponding to chloroacetic acid is obtained by subtracting the chloride content from the total chlorine. The detection limit of the method lies at 0.03% organic chloride, equivalent to 0.08% (800 mg $kg^{-1}$) chloroacetic acid. Using ion chromatography with a conductivity detector (amino exchange column with a hydroxide gradient elution), the limit of detection reduces to approximately 20 mg $kg^{-1}$.

The low volatility of alkylbetaines hampers the use of conventional EI and chemical ionization (CI) MS for structure determination. The pyrolytic behaviour of this class of compounds has been studied under EI conditions: the most important pyrolytic process

is the intermolecular isomerization to tertiary aminoesters $(CH_3)_2N–CH_2–COOCH_3$. Although pyrolysis EI spectra are useful for structure confirmation of pure compounds, they have limited or no utility for the analysis of mixtures of constituents of unknown chain length, since the spectra are dominated by the ions generated by C–N cleavage and the intensities of the molecular ions of the esters are low.

An alternative approach to structure determination is FD-MS, which gives as prominent ions the protonated species $[M + H]^+$; intermolecular alkyl transfer also occurs during field desorption, resulting in mass spectra containing structurally diagnostic adduct ions (methyl, ethyl, propyl groups linked to nitrogen readily undergo intermolecular transfer to give $[M + CH_3]^+$, $[M + C_2H_5]^+$ and $[M + C_3H_7]^+$). The presence in the mass spectra of several other

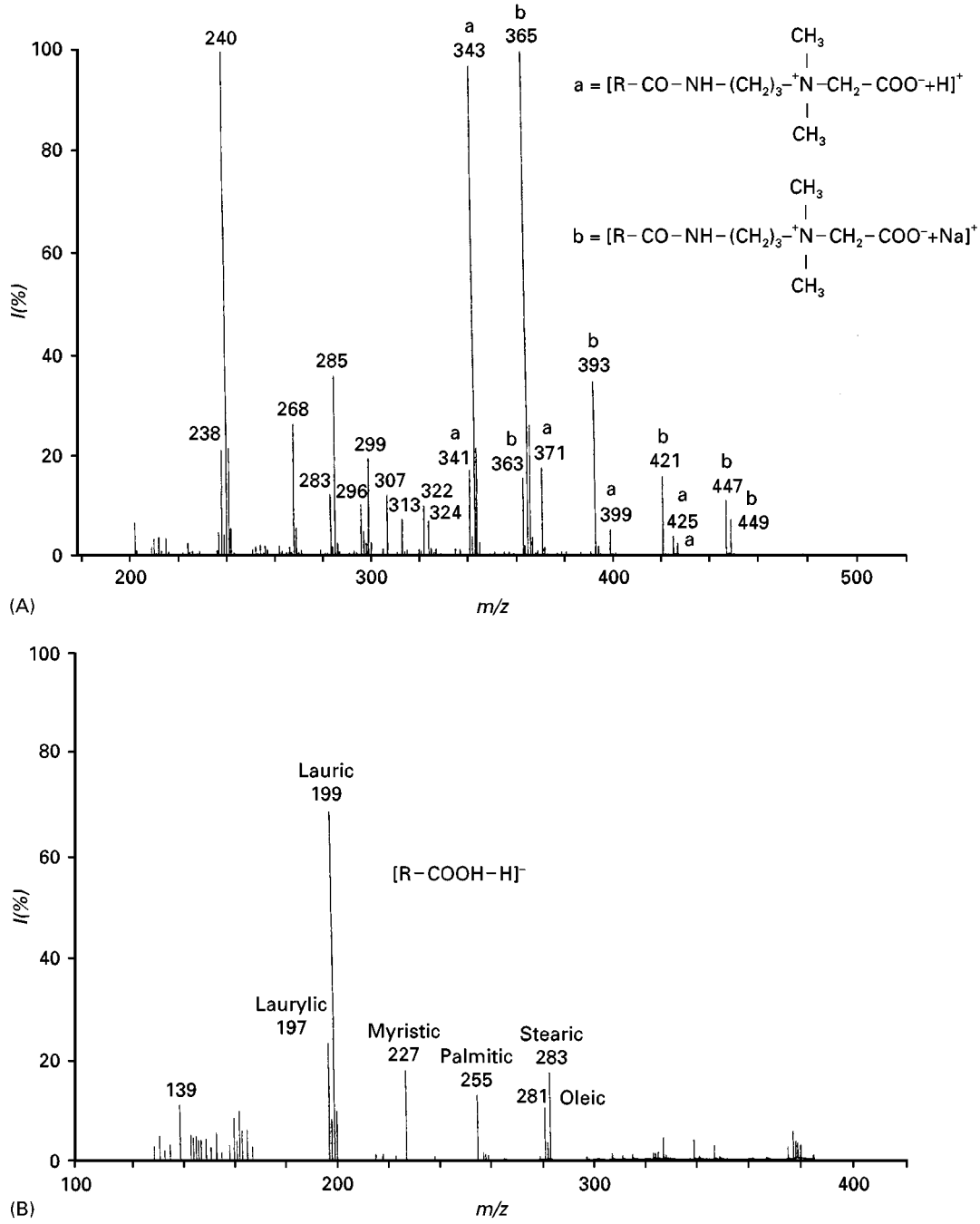



**Figure 5** (A) Positive ion and (B) negative ion FAB mass spectra of cocamidopropylbetaine (amphoteric surfactants). (From Maffei Facino *et al.*, 1989.)

adduct and fragment ions (whose relative intensities strictly depend on emitter current) complicates the analysis of complex mixtures by this technique.

FAB-MS in the positive or negative ion mode is more promising, since gives not only an immediate fingerprint of the alkyl distribution in a mixture of amphoteric surfactants, but also direct information on the presence of contaminants.

As is shown with a commercial sample of cocamidopropylbetaine, in the positive ion mode (**Figure 5A**) the protonated (a series) and cationized (b series) molecular ions of the propylamidobetaine derivatives of coconut fatty acids ($C_{12}$–$C_{18}$) can easily be detected; the mass spectra also contain a few ions (at *m/z* 238, 240, 268, 296, 322, 324) that are due to fragmentation reactions (loss of a dimethylaminoacetic acid residue and loss of the carboxylic group) and some ions that, as determined by MS-MS (parent scan mode) correspond to the protonated molecular species of the dimethylaminopropylamide derivatives $[R–CO–NH–(CH_2)_3–N(CH_3)_2 + H]^+$ of fatty acids present in the mixture as unreacted materials (ions at *m/z* 283, 285, 313).

Negative FAB ionization cannot be used for identification of amphoteric surfactants because these compounds do not give $[M–H]^-$ ions, but it is an excellent tool for a rapid detection of unreacted fatty acids, which under these conditions give abundant deprotonated molecular ions $[M–H]^-$ (*m/z* 143 caprylic; *m/z* 171 capric; *m/z* 197 laurylic; *m/z* 199 lauric; *m/z* 227 myristic; *m/z* 255 palmitic; *m/z* 281 oleic; *m/z* 283 stearic acid) (**Figure 5**B). Where alkyl ($C_{12}$–$C_{14}$) betaines and cocamidopropylbetaine have been identified, this approach can also be successfully applied for the rapid detection of amphoteric surfactants in finished detergent formulations.

## Recent Developments

The more recent developments in the field of surfactants analysis, in raw materials, in detergent formulations, or in environmental samples, are all based on the application of new mass spectrometric soft ionization techniques (thermospray and atmospheric pressure ionization (API)). These techniques are more rapid and versatile than conventional FAB-MS, which is dependent upon the surface activity of the sample in a given viscous liquid matrix and requires time-consuming screening of the matrix compounds to achieve maximal ionization response.

Thermospray mass spectrometry coupled to reversed-phase LC has been applied for the quantitative determination of linear primary alcohol ethoxylate (AE) surfactants in environmental samples at levels from 25 to 102 ppb (total AE), corresponding to a range of individual AE concentration from 60 ppt to 2.17 ppb. The method is able to distinguish highly branched propylene-based alcohol ethoxylates from isomeric linear ethylene-based alcohol ethoxylates.

Positive ion atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has been successfully applied to the determination of the oligomer distribution of alkylphenol polyethoxylates and fatty alcohol polyethoxylates. Positive ion and negative ion API-MS techniques have been used for quality control of the individual steps of the manufacturing process (intermediates and final products) of new classes of anionic surfactants, the alkylpolyglucoside esters of sulfosuccinic, citric and tartaric acid. With both techniques, the complex mixtures can be injected directly into the ion source without prior chromatographic separation, and the constituents are 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.

*See also:* **II/Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry. **Chromatography: Liquid:** Derivatization; Detectors: Refractive Index Detectors; Ion Pair Liquid Chromatography. **Chromatography: Thin-Layer (Planar):** Spray Reagents. **Extraction:** Solid-Phase Extraction. **III/Fatty Acids: Gas Chromatography. Flame Ionization Detection: Thin Layer (Planar) Chromatography. Surfactants:** Chromatography; Liquid Chromatography.

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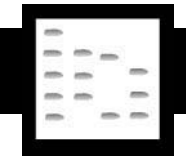
# CRUDE OIL: LIQUID CHROMATOGRAPHY



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## Introduction

Chromatographic methods that utilize liquid mobile phases include open-column liquid chromatography, high performance liquid chromatography (HPLC), size exclusion chromatography (SEC) and thin-layer chromatography (TLC). These techniques have been widely applied for the evaluation of crude oils (as well as their subfractions) for their quality, processability or hazards. This overview covers various approaches to the characterization of crude oils by these techniques. Specific applications, operational advantages and limitations of these methods are also highlighted.

The major applications of open-column liquid chromatography and HPLC to the characterization of crude oils and related materials including residua, topped crude oils, coal liquids or shale oils involve preparative fractionation for the determination of hydrocarbon types or class separation to be followed by the determination of important subgroups and individual components. There are also numerous reports where analytical HPLC with various detection schemes has been applied to the quantitative characterization of crude oils as well as other fossil fuels.

Crude oils are usually fractionated into several compound classes according to their molecular structures. A majority of class separations have dealt with the determination of saturates, aromatics, resins (or polars) and asphaltenes (SARA). Saturates consist of paraffinic and naphthenic compounds. If olefins are present in the sample, they are usually grouped with saturates. Aromatics range from alkylbenzenes (and other monoaromatics) to polycyclic aromatic hydrocarbons (PAHs). The polars are usually aromatic in nature and consist of compounds that may contain nitrogen, sulfur and oxygen as heteroatoms. Asphaltenes are highly condensed aromatic structures.

Conventional TLC with silica and alumina adsorbents provides separation of components from crude oils based on their polarity. TLC with flame ionization detection (TLC-FID) has been applied for the determination of hydrocarbon types. SEC has been particularly useful for the characterization of heavy crude oil fractions.

## Open-Column Liquid Chromatography

Crude oils have been fractionated into saturates, aromatics, resins and asphaltenes using open-column liquid chromatography. Asphaltenes are *n*-pentane, *n*-hexane- or *n*-heptane-insolubles depending on the *n*-alkane used. The *n*-alkane-soluble materials, termed maltenes, are usually fractionated on a silica or alumina column using appropriate solvents. In general, saturates are extracted with an *n*-alkane (such as *n*-hexane) followed by elution of aromatic and polar fractions with solvents or solvent mixtures of higher eluotropic strengths. Quantitative data are obtained by the gravimetric determination of each fraction after evaporation of solvent or solvent mixture. Rotary evaporation under mild vacuum is a common practice for the concentration of the collected fractions.

A crude oil separation scheme is shown in **Figure 1**. Maltenes are obtained by precipitation of asphaltenes from the crude oil using *n*-heptane. Using column liquid chromatography on alumina, and solvents or solvent mixtures as indicated in Figure 1, fractions enriched with saturates, aromatics I and II and polars can be obtained. The aromatics I fraction contains