J. Soc. Cosmet. Chem., **33**, 407-420 (December 1982)

Cosmetic analytical chemistry—coming of age

IRA E. ROSENBERG, *Clairol Incorporated, 2 Blachley Road, Stamford, CT 06922.*

Received October 28, 1982.

INTRODUCTION

The last 10 years have seen a rapid growth in both the activity and the importance of the analytical laboratories in the cosmetic industry. Two factors seem to have spurred this change. The first has its base in the increased types of raw materials available to cosmetic chemists. The fact that the 3rd Edition of the *C.T.F.A. Ingredient Dictionary* now contains 3,400 entries is a poignant reminder of progress in this area. The second has roots in the diversity of the analytical tools with which cosmetic laboratories are equipped nowadays. Advanced instrumentation coupled with microprocessor technology has allowed the researcher to probe more critically into the quality of supplied raw materials and formulated finished products. Techniques such as nuclear magnetic resonance spectroscopy (NMR), high pressure liquid chromatography (HPLC), mass spectroscopy (MS), and capillary gas chromatography (CGC) are standard equipment in many cosmetic laboratories. The influx of computers has given the analytical chemist greater flexibility in data acquisition and their evaluation and interpretation. Raw data can be stored and manipulated at will, yielding more extensive information per experimental run. Introduction of these instruments has also brought an increase in analytical sensitivity. Ten years ago, routine analysis or structure elucidation required milligram to gram quantities of sample. Today, nanogram to microgram quantities are needed to obtain the same information.

There has been a visible impact on the more traditional analytical tools, such as ultraviolet, visible, and infrared spectroscopy. Data stations now interface with these spectrometers, supplying computer assisted interpretation of data. Libraries of spectra are available which can be interfaced with the instruments and recalled at will for comparison with freshly generated spectra. A transformation of cosmetic analytical chemistry is emerging in all areas of the industry, from basic research to quality control.

The purpose of this review is to elaborate on the current trends in the use of advanced instrumentation and on the analysis of important cosmetic raw materials such as surfactants, cosmetic preservatives, fragrances, etc.

INSTRUMENTATION

For cosmetic chemists, packed column gas chromatography remains one of the most useful and quantitatively reproducible techniques for the separation and identification of volatile components in complex mixtures. Automatic injectors which are controlled by microprocessors can be interfaced at the front end of the gas chromatograph, and computing integrators with data storage capabilities can be interfaced at the back end. Dedicated systems of this configuration provide rapid and reliable results for routine quality control. Recently, capillary column gas chromatography has greatly improved the resolution over that obtained from packed columns. This technology has had the greatest impact in the area of fragrance analysis.

Unlike most analytical techniques which are developed in academia and utilized in industry, high pressure liquid chromatography found its origin and growth in industrial problem-solving. The majority of raw materials used in the cosmetic industry are non-volatile, and HPLC has proven to be of particular value in the analysis of these materials. The technique has the ability to separate mixtures of components, but instead of the moving phase being a gas, the moving phase is a liquid. Chromatographic separation occurs by interaction between sample molecules and the stationary phase residing in a metal column. These interactions are essentially absent in the moving phase of GC, but they are present in the liquid phase of HPLC, thus providing an additional variable for controlling and improving separation. Also, chromatographic separation is generally enhanced as the temperature is lowered because intermolecular interaction becomes more effective. A greater variety of fundamentally different stationary phases allows separation using a number of hydrophobic and hydrophilic solvents. Another advantage of high pressure liquid chromatography is the relative ease of sample recovery. Separated fractions are collected in open vessels. Recovery is quantitative, and the isolated fraction can then be analyzed by ancillary techniques such as infrared or mass spectroscopy. An array of direct detecting techniques such as visible and ultra violet absorption, refractive index, electrochemical, and fluorescent detection allows for greater specificity in sample analysis. Recent advances in column technology in which the column packing is radially compressed has increased both efficiency and resolution of HPLC separation. An excellent treatise on HPLC can be found in *Introduction to Modern Liquid Chromatography* by Snyder and Kirkland (1).

Nuclear magnetic resonance spectroscopy has been useful in the analysis of cosmetic raw materials, especially in the area of surfactants. The spectrum can show many absorption peaks whose relative positions can yield detailed information about the molecular structure. The number of signals gives information on the number of different kinds of protons in the molecule. The position of the signal gives information about the electronic environment of each kind of proton. The intensity of the signal tells how many protons of each kind there are; and the splitting of the signal into several peaks can tell us about the environment of a proton with respect to other, nearby protons. NMR has therefore found utility in structure elucidation and fingerprinting of organic compounds.

Considering its ultimate and absolute potential, it is not surprising that the mass spectrometer is rapidly becoming the most universal detector. The need for quality analysis in response to competitive and regulatory pressure has been a motivating force in bringing this technique into the cosmetic industry. The introduction of quadrapole mass spectrometers, which have a lower price tag than the more conventional magnetic

sector spectrometers, has enabled smaller companies to acquire this instrumentation. The interfacing of both packed and capillary gas chromatographs with the mass spectrometer followed by computer data acquisition gives us a powerful tool for simultaneous separation and absolute structure elucidation. Recent technology has also interfaced HPLC to the mass spectrometer and this combination should prove valuable in separation and identification of nonvolatile mixtures.

Both prism and grating infrared spectrophotometers have tradiationally been used for the characterization of cosmetic ingredients. Low cost Fourier transform infrared spectrometers are now available offering increased and constant resolution throughout the entire spectral range. Bench-top models can acquire spectra within seconds, and as with mass spectrometers, these systems are microprocessor and computer-assisted, allowing the chemist to search reference spectra for absolute identification.

A new breed of hyphenated techniques such as GC-MS, LC-MS, GC-FTIR, LC-FTIR, and GC-FTIR-MS are finding increased application in chemical analysis. These systems contain both non-destructive and destructive analysis in tandem allowing the chemist to obtain several forms of spectroscopic information by performing a single experiment. Just recently the development of automated analysis system incorporating ^{13}C-NMR, GC-MS and a computer has been reported on in detail (2).

SURFACTANTS

Surfactants are one of the most widely used classes of cosmetic raw materials. Comprehensive reviews are available to the reader (3,4); defined as compounds containing both a "hydrophilic" and "hydrophobic" group, the molecules can locate between the interface of an organic and aqueous phase. The materials are used as components in shampoos, conditioners, lotions, and creams. The anionic surfactants which are a large portion of this class of compounds are usually sulfate esters of long chain fatty alcohols having the general formula:

$$CH_3(CH_2)_n—OSO_3^-Na^+ \quad \text{where n = 11, 13, 15, or 17.}$$

Since most are derived from natural sources, they are usually mixtures of homologs, and their effectiveness and physical properties depend markedly on the alkyl chain length distribution. Initial analytical approaches have focused on the anionic portion of the molecule, and the greatest number of methods reported in the literature involve the use of dyes as a complexing agent.

Thus, early literature reported that colored, water-insoluble salts were formed between methylene blue and anionic surfactants and that the reaction products were soluble in organic solvents such as chloroform (5). This observation was developed into a colorimetric determination with the sensitivity of the method being 20 ppm of surfactant (6,7) and is the basis for the anionic titration method currently and frequently used in the cosmetic industry. The determination of anionic surfactants using polarography has also been explored, but the reduction in height of the half-wave potential of methylene blue, buffered at pH 4.5, by the addition of anionic surfactants, was shown to be non-specific (8).

The use of infrared spectroscopy for the determination and identification of surfactants has grown over the years. The major drawback is in the case of mixtures of several

components; the spectrums become complex and diffuse, and it becomes increasingly difficult to recognize anything other than particular functional groups. Consequently, the infrared spectrum of a commercial surfactant may bear only a slight resemblance to its isolated major component. Useful information can be obtained if water is carefully removed from the sample, since the presence of even traces of moisture can mask surfactant absorptions in the 3400-3000 cm^{-1}, 1700-1500 cm^{-1}, and 600 cm^{-1} region (all associated with H_2O). The more common assignments of functional groups associated with anionic surfactants are the hydroxyl-OH stretching frequency occurring between 3400-2400 cm^{-1}. This band is usually complex and broad. The CH- stretching frequency is usually superimposed on the low side of the broad OH- band. Absorption arising from the CH- stretching of the methylene (CH_2) and methyl (CH_3) groups on the carbon chain occurs between 2950-2750 cm^{-1}. Unsaturation can be observed at 3020 cm^{-1}, and its intensity relative to the methylene antisymmetric stretch at 2920 cm^{-1} can give an indication as to the degree of unsaturation (9-14).

More recently, gas chromatography has played an increasingly important role in the characterization of anionic surfactants, the best known being sodium dodecyl sulfate. This material can be easily hydrolyzed in acid media to yield the original fatty alcohol. It has been shown that about 98% of sodium lauryl sulfate is hydrolyzed in 1N HCl at 100° in seven hours (15). The hydrophobic oil is extracted from the reaction solution with suitable solvents, then gas chromatographed with or without pretreatment. Mixtures of fatty alcohols have also been separated by forming the acetates (16).

Further work has been reported in the literature on the analysis of alkyl sulfates using on-line pyrolysis gas chromatography with and without reagents (17). At 650°C pyrolysis gas chromatography of commercial sodium lauryl sulfate gave peaks for the C_{12} and C_{14} fatty alcohols and the C_{12} and C_{14} α-olefins. On-line P_2O_5 pyrolysis gas chromatography gave peaks due only to a mixture of olefins. The formation of the alkyl alcohols was not observed.

A second class of anionic surfactants used widely in cosmetics are the ethoxylated ether sulfates of fatty alcohols, obtained by reacting the alcohol with ethylene oxide followed by sulfation. The general formula for these compounds is:

$$CH_3(CH_2)_n—O(CH_2—CH_2-O)_mSO_3^-Na^+ \quad \text{where } n = 11, 13, 15, 17 \quad \text{and } m = 1-20.$$

Naturally occurring sources usually contain the C_{10}, C_{14}, and C_{16} alcohols in varying amounts. If one ethoxylates lauryl alcohol to an average of 2 ethylene oxide (EO) units, the number of reaction products obtained is considerable. Not only is the 2EO derivative present, but the homologs of the 1, 3, 4, 5, and 6EO derivatives are also formed. Impurities of the C_{10}, C_{14}, and C_{16} alcohols along with their homologous EO derivatives are also present. Since cosmetic products are delicately balanced formulas, differences in the homologous ratio can affect both stability and performance of the product. This has generated research into the analysis and distribution of the homologous series contained in this class of surfactants.

As with the alkyl sulfates, the alkyl ether sulfates can be hydrolyzed back to the ethoxylated alcohols. Gas chromatography of the hydrolyzed mixture on the porous polymer tenex or the liquid phase OV-17 has been used to separate the underivitized C_{12}EO oligomers with up to 8 ethylene oxide units, while the trimethylsilyl ether of the C_{12} alcohol with up to 16EO units have been separated on Dow Corning high vacuum grease as the liquid phase (18).

Researchers have found better resolution with capillary GC by converting the hydroxyl group to the methyl ether. The methylation was carried out with methyliodide and dimethylsulfonyl anion (19,29). An OV-101 glass capillary column was used for the separation, and a commercial C_{12}-C_{14} alcohol ethoxylated with an average of 3 moles of ethylene oxide gave eleven peaks under these conditions. This method is quantitative and therefore suitable for fast routine quality control (21).

Mass spectroscopy has been used for absolute identification of the separated peaks obtained from the hydrolysis of ethoxylated sodium alkyl ether sulfates. Traditional electron impact mass spectrometry on these compounds does not show a molecular ion. However, we have demonstrated in our laboratory that isobutane chemical ionization mass spectroscopy can be used to obtain the molecular weights of the separated components. Under these conditions, the alcohols show a strong (M − 1) ion whereas the ethoxylated alchols show a strong (M + 1) ion (22). We have also demonstrated (23) that silylation of the hydrolyzed surfactant will yield electron impact spectra that are suitable for structure elucidation. There is no doubt that mass spectroscopy yields the most complete picture of the chemical distribution of this class of surfactant.

Non-ionic surfactants consist of ethylene oxide (or propylene oxide) capped with a hydroxyl group on one end and a long chain alkoxy group or alkyl phenol group at the other end. Examples of this class are alkyl ether alcohols, or the ethoxylated octyl and nonylphenols. These surfactants are very amenable to analysis by gas chromatography and other advanced analytical techniques (24). An excellent paper has been published on the applications of mass spectroscopy to the analysis of non-ionic surfactants, using electron impact solid probe mass spectroscopy. The authors characterized the most common ions obtained from ethoxylated alcohols, octyl and nonylphenols, fatty amines and ethoxylated fatty alcohols (25).

The degree of ethoxylation in ethoxylated non-ionic surfactants is an important parameter which influences product performance and stability. Nuclear magnetic resonance spectroscopy has been used for the determination of this parameter (26–29). The technique is based on the observation that a proton placed in an external magnetic field can be aligned with the external field. Energy can be absorbed by the proton and flipped so that the alignment is against the external field. This is the less stable situation. Upon realignment with the external field, energy is released. These spectra are usually obtained as a plot of signal intensity vs. change in strength of applied magnetic field. The CH_3 protons of trimethylsilane (TMS) are used as an internal standard and are arbitrarily set at 0 ppm or 10γ. Since TMS contains 9 equivalent protons, a strong singlet is obtained. The methylene protons associated with the ethylene oxide chain are shifted downfield to 6.0–6.5γ, while the methylene protons from the alkyl chain occur at 8.0–8.5γ.

By silylating the terminal alcohol with a strong silylating agent (i.e., Bis-(trimethylsilyl) trifluoroacetamide), one can ratio the integrated areas in the NMR for the 9 methyl protons of the derivitizing agent, with either the integrated areas for the protons from the ethylene oxide chain or the protons from the alkyl chain. Average alkyl chain length or average degree of ethoxylation can then be calculated. If the surfactant is octyl or nonylphenol, the integrated area corresponding to the four aromatic protons, which are shifted downfield to 2.0–3.0γ, can also be used for the ratioing. This technique has also been applied to propylene oxide adducts of alkyl phenols, fatty alcohols, and ethoxylated mercaptans.

A substantial improvement in the resolution of the proton magnetic resonance spectra can be obtained by the addition of so-called "shift reagents" (30-32). Typical shift reagents are complexes of the rare earth metals, europium and praseodymium with ligands such as 2,2,6,6-tetramethyl-3,5-heptanedione or 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione. These are available commercially as standard reagents for NMR. They induce large changes in the chemical shifts of the NMR spectra of compounds which possess functional groups with free electron pairs capable of forming a coordinate bond with europium or praseodynium ions: for example, alcohols. The improved resolution results in the largest shift occurring for the protons closest to the coordination site. The first application of shift reagents to this field was the structural study of the ortho and para-alkyl isomers of phenol polyglycol ethers. The spectrum of the ortho isomers showed greater shift when complexed than the para isomers (33).

Several researchers have reported on the use of HPLC for the characterization of surfactants (34,35). Studies have been done on esters of polyoxyethylene monododecyl ether prepared by reaction with 3,5-dinitrobenzoylchloride followed by HPLC of the ethoxylated species (36). Compounds containing more than 12EO units were difficult to chromatograph. Ethoxylated alkylphenols have also been separated using this esterification technique. The 3,5-dinitrobenzoylchloride was allowed to react for 30 minutes at 65°C in 20 ml of pyridine, followed by extraction in THF and separation by HPLC. A Lichrosorb RP-5, 5 um size column (Merck, Darmstadt, G.F.R.) with a mobile phase of acetonitrile/water (6:4) was used. The column was thermostatted at 50°C. Work has been reported on the separation of surfactant homologs by HPLC using an ODS/silica column (37). The authors were successful in obtaining separation of nine typical surfactants. Water/methanol adjusted to pH 2.2 with phosphoric acid and water/methanol containing 0.4M NaCl were used as the mobile phase. Further work on the separation of EO oligomers by HPLC has been done with isocratic and gradient elution (38,39).

α-Olefinsulfonates (AOS) are one of the newest class of surfactant raw materials being used in cosmetic products. The surfactant is manufactured by sulfation of α-olefins obtained from either cracking of or polymerization of ethylene. The surfactants are thus mixtures of isomers and homologs having a wide distribution of chain lengths (C_{10} to C_{20}). Desulfonation of AOS has not been successful. However, successful GC analyses have been obtained after hydrogenation followed by the formation of the volatile sulfonyl chloride. The hydrogenation was followed by IR using the disappearance of the 965 cm^{-1} band, and the GC analysis of the final mixture of the sulfonyl chlorides was performed using a 3% SE-30 column (40,41).

The term "cationic surfactant" refers to compounds containing at least one hydrophobic long chain alkyl group and a positively charged nitrogen. Generally referred to as "Quats," these materials are incorporated into cosmetic hair formulations imparting manageability and anti-static properties. Because of their inherent bacteriostatic properties, these compounds are also used as sanitizing agents, antiseptic agents, germicides, and fungicides.

Temperature-programmed gas chromatography has been the general technique of choice for qualitative identification. The technique is hard to apply to quaternary ammonium salts without first fragmenting the compound into more volatile species. Attempts have been made to degrade certain quaternary ammonium compounds

directly on a hot alkaline column (42). Near quantitative (about 90%) degradation of alkyldimethylbenzylammonium halides into alkyldimethylamines and benzyl halides has been accomplished (43).

The majority of quantitative methods available for this class rely upon the extraction of a relatively non-polar salt or complex formed by the surface active cation with an anion having a characteristic absorption in the visible or ultraviolet region (3). Many of these anions are the basic forms of acid-base indicators, and this principle is behind most of the methods used in the cosmetic industry.

Some reports appear in the literature on the use of mass spectroscopy for the determination of long chain quaternary amines, offering greater information on the chemical nature of this class of surfactants (44,45).

PRESERVATIVES

An important aspect of cosmetic formulation is the incorporation and analysis of antimicrobial agents in raw materials and finished products. Early analytical work in this area used primarily thin layer chromatography for the separation and identification. Separation of twenty-five preservatives was accomplished by TLC on silica gel with a limit of detection of approximately 0.1-0.5 ug, using benzene-acetone as the solvent system (46). One report has looked at fifty antimicrobials divided into nine different groupings (47). It was found that silver nitrate could be used as a spray reagent for the identification of organic-halogen preservatives, and that gas chromatography could be successful in separating the silyl derivatives of phenolic compounds. Formaldehyde, an important preservative in cosmetic systems, is not detected by chromatographic methods but can be visualized easily by color reactions with a 1% solution of 4-amino-3-hydroquino-5-mercapto-1,2,4-triazole. This reaction can also be used for formaldehyde donors such as Bronopol®, Dowicil 200®, Germall 115®, hexamine, and MDH Hydantoin®. A method using fluorometric determination of formaldehyde-releasing cosmetic preservatives also appears in the literature (48).

More recently, advanced instrumentation is proving useful for the separation and quantitation of these materials. A method has been published on a fast and rapid analysis for the methyl and propylparabens (49). It involves sample solubilization in the THF/EtOH mobile phase, and separation by HPLC. The detection levels for methylparaben are approximately 200 pg using UV detection at 254 nm. The same detection system using 45/55 acetonitrile/water mobile phase on a Sepralyte® C-18 column has been used to separate the methyl, ethyl, propyl, and butyl parabens simultaneously. Since these preservatives are often used in various combinations, HPLC affords a fast and rapid simultaneous analysis for these compounds. There is no doubt that instrumental techniques will continue to be explored for the analysis of preservative systems.

FRAGRANCES

Fragrance companies have traditionally led the industry in the development of methodology for the analysis and identification of compounds in essential oils. The greatest impact has come from the development of capillary gas chromatography. This

technique has enabled the analytical chemist to separate most of the components in a volatile mixture. Identification is accomplished by comparing the retention time of the unknown on several columns with that of a standard. Detection systems that have been used with capillary GC are based on thermal conductivity, hydrogen flame ionization, argon ionization, electron affinity, and coulometric principles. Several review articles and books have been published on this subject (50-52).

The increase in use of mass spectrometers throughout the industry is primarily due to the development of quadrupole mass filters capable of separating ions on the basis of their mass to charge ratio (m/e) solely by means of an electric field (53). The development of this type of mass filter into commercially available instruments has lowered the cost of mass spectrometers. Coupled with computers capable of processing information rapidly, the mass spectrometer has become a universal detector for absolute identification. The coupling of capillary GC to the mass spectrometer has allowed this technique to emerge as the primary approach to fragrance analysis. Standard libraries of compounds are available for computer comparison of data, or libraries containing only fragrance compounds can be built. With these libraries residing within the computer, reverse searching capabilities are available, along with the ability to quantitate volatile mixtures.

A second emerging technique for the analysis of fragrance is GC-FTIR. The separation is again accomplished by capillary GC, followed by introduction of the sample into a light pipe where an infrared spectrum is obtained. As in mass spectroscopy, libraries of IR data are becoming available, along with computer search programs. Several researchers are combining both these techniques so that the IR and MS of a separated component can be obtained from a single run. The computer can then cross-check both the IR and MS of a given separated compound and assign the most probable structure based on both of the spectroscopic techniques employed.

More recently, HPLC used alone or interfaced with a mass spectrometer has shown promise in the analysis of fragrance compounds (54).

AMINO ACIDS, PROTEINS, AND POLYMERS

A number of cosmetic product categories over the last several years have incorporated protein, amino acids, and polymers as raw materials in their formulations. The analysis and quality control of these ingredients is of increased importance to the cosmetic analytical chemist. The traditional method for the analysis of amino acids is based on elution chromatography from buffered columns of ion-exchange resin. The separated components are reacted with ninhydrin and quantitated by visible spectrophotometric detection. The analysis of proteins by this method is accomplished by hydrolyzing the material in hydrochloric acid followed by separation and quantitation of the amino acids.

The latest approach to amino acid analysis has used HPLC combined with either pre-column or post-column derivitization. Researchers have separated the phenylthiohydantoin derivatives of all 20 common amino acids using a reverse phase C_{18} column eluted with a concave ethanol gradient in aqueous ammonium acetate at pH = 5.1 (55). Researchers have separated the amino acids normally found in protein hydrolysates within 45 minutes using normal-phase chromatography on NH_2-silica (56). Detection was accomplished with either ninhydrin or o-phthalaldehyde. Reproducibility of the

retention times allowed peak-height or peak-area measurements to be used for quantitation in the range of 10 pmol to 25 nmols. Pre-column conversion of the amino acids to their damsyl derivative followed by HPLC also has given good separation of all 20 common amino acids (57).

Gas chromatography coupled with flame photometric detection is used for the analysis of sulfur-containing amino acids (58).

High pressure (performance) liquid chromatography of proteins is becoming more prevalent in the literature, with the experimentation focusing on various support phases and buffer systems. For example, peptides varying in size from di- to decapeptide have been separated on phenyl-corasil, Poragel PN, and Poragel PS using reverse phase conditions with acetonitrile-water (59) as the mobile phase. Researchers have modified this method with the addition of phosphoric acid to the mobile phase (60,61).

The analysis of proteins has also been reported using gel-permeation chromatography (62) and nuclear magnetic resonance spectroscopy (63).

In the case of gel permeation chromatography, the chemist is using the separation technique of HPLC to obtain molecular weight distribution (64) rather than identification of the fraction separated. Cationic polymers used in hair fixatives can be analysed using gel-permeation chromatography to obtain an average molecular weight. Polymer chain length is no doubt related to product performance; therefore, improved methods of analysis using gel permeation chromatography for charged polymers should be forthcoming.

TRACE CONTAMINANTS

Trace analysis of cosmetic raw materials is one of emerging concern for the cosmetic analytical chemist. During the past several years, the regulatory climate has involved the cosmetic industry in three major areas: the analysis of nitrosamines, the analysis of dioxane, and the analysis of specific trace contaminants in raw material dyes used in cosmetic finished products. The analytical methodology used to determine the level of contaminants of concern will be discussed.

For the past five years, the Cosmetic, Toiletry and Fragrance Association (CTFA) Nitrosamine Task Force has been studying trace level contamination of cosmetic raw materials, with their major focus the determination of N-nitrosodiethanolamine (NDElA). Since NDElA is a polar-nitrosamine, its determination stands apart from the general methodology for the analysis of nitrosamines. Volatile nitrosamines have been detected by gas chromatography employing either a nitrogen specific detector or a thermal energy analyzer (TEA) (65,66). Initial research which found trace levels of NDElA in cosmetic products used a TEA (67). The instrument contains a pyrolytic oven which cleaves the weak N═N═O bond to produce NO. An inert gas is used to sweep the NO into an ozonator which then forms activated NO_2. When the NO_2 decays to the ground state, the emitted energy is detected. A gas chromatograph is used at the front end of the pyrolysis oven for the initial separation. This instrument for the most part is nitrosamine specific and is currently the leading analytical technique used to meet government standards for volatile nitrosamines.

The cosmetic chemists' problem is more difficult, since NDElA is polar and appears sometimes in trace quantity in complex mixtures. The CTFA has developed methodol-

ogy which uses a thermal energy analyzer for the analysis of seven classes of cosmetic raw materials (68): ethanolamines, ethanolamides, monoethanolamine salts, triethanolamine salts, amphoteric compounds, quaternary ammonium compounds, and morpholine.

NDElA and other polar nitrosamines are very amenable to separation by reverse phase HPLC using either water or water/alcohol mobile phases (69). The water/alcohol mobile phase provides good solubility for the raw material, eliminating the need to perform multiple isolation steps prior to analysis. Quantitation of the nitrosamine is by UV detection. Methods are in the literature for the analysis of NDElA in ethanolamines, alkanoladines, and cosmetic products (70–74). Likewise, pology raphy and conductivity detectors have been used for the analysis of NDElA (75,76).

Since the initial findings of NDElA in cosmetics, other nitrosamines have also been shown to be present in trace quantities, and methodology has been developed for each nitrosamine (77). These have all been polar compounds, and both water soluble and oil soluble nitrosamines have been studied. Some methods are available for total nitrosamine in cosmetic raw materials and finished products; however, these are generally not rapid and certainly not specific (78).

By far the most absolute method for nitrosamine detection is mass spectrometry and, when possible, should be used to confirm the presence of the nitrosamine of interest. Researchers have reported the determination of NDElA by first forming its disilyl derivative followed by GC/MS analysis (79).

The second half of the nitrosamine problem, that of nitrite analysis, has also been explored. The standard colorimetric test developed by Greiss (80) and its subsequent modifications (70,81) can determine low ppb levels of nitrite, while derivative formation followed by fluorescent spectroscopy can measure in the picogram region (82). The CTFA has published nitrite methodology which is specific for cosmetic raw materials (83).

More recently, trace analysis for 1,4-dioxane has become significant for the cosmetic analytical chemist. 1,4-dioxane can be present, at trace levels, in some types of ethylene oxide condensates, and this broad class of compounds is widely used in both the food and cosmetic industry. Presently, the "Birkel procedure" (84), which consists of vacuum distillation of a sample followed by gas chromatographic analysis, is the accepted validated procedure. The total analysis time per sample is 2–3 hours with a 0.5 ppm limit of detection. Several other methods have been generated through the CTFA (85). Samples of widely used cosmetic ingredients (i.e., sodium laureth sulfate, Polysorbate 60, and PEG-8) were chosen for study. A total of seven generally different analytical techniques were employed, including the Birkel and modified Birkel procedures. Other methods generated used GC/MS with perdeuterotoluene as an internal standard (86), purge and trap procedures followed by GC, direct GC injection, headspace GC, and atmospheric azeotropic distillation followed by GC (87,88). The CTFA study showed that these alternate procedures yielded results comparable to the Birkel method, except for the purge and trap technique. It was felt that with some additional methods development work, the purge and trap technique could also be improved to the point where it too would be satisfactory.

The last couple of years has seen an increased concern within both industry and government in establishing the safety of the dyes and colors used in cosmetic products.

This concern has recently been highlighted with respect to D & C Green #6 (1,4-bis[(4-methylphenyl)amino]-9,10-anthracenedione), which is listed in the Code of Federal Regulations (CFR) for use in drugs and cosmetics. Because it is a certified dye, each commercially-prepared batch of this color is subject to FDA certification. This material is formed by reacting 1 mole of quinizarin with 2 moles of p-toluidine. D & C Green #6 has been shown to be safe for external use; however, literature reports have demonstrated that p-toluidine is a carcinogen in mice (89). Residual amounts of reactants such as p-toluidine are commonly found in this color, and trace levels are unavoidably present even in highly purified reagent grade material. Until recently, there were no validated methods available for detecting trace levels of p-toluidine in D & C Green #6, thus the reluctance of the FDA to "permanently" list this color for use in consumer products. Several methods have since been reported (90,91) which can detect p-toluidine at the 500 ppb level and probably lower. The methods involve separation by HPLC followed by UV or fluorescence detection. This approach by the regulatory agencies of requiring analytical methodology for hazardous trace components in compounds shown to be safe is becoming standard, and the cosmetic analytical chemist will become more involved in developing trace analytical techniques for quality control of these raw materials.

REFERENCES

(1) L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography,* 2nd Edition (John Wiley & Sons, Inc., New York, 1979).

(2) R. Namba, H. Nishiya, A. Shibamoto, Y. Morikawa, S. Tahara, and T. Mitsui, "Development of new automated analysis system of cosmetics by means of computer," IFSSC 12th International Congress, Paris, September 13-17, 1982.

(3) *Anionic Surfactants—Chemical Analysis,* Surfactant Science Series, Vol. 8, J. Cross, Ed. (Marcel Dekker, New York, 1977).

(4) J. Cross, *Cationic Surfactants,* E. Jungermann, Ed. (Marcel Dekker, New York, 1970), pp 419-482.

(5) J. H. Jones, General colorimetric method for the determination of small quantities of sulfonated or sulfated surface active compounds, *J. Assoc. Offic. Ag. Chemists,* **28,** 389-409 (1945).

(6) G. P. Edwards, W. E. Ewers, and W. W. Mansfield, Determination of sodium cetyl sulfate and its solution in water, *Analyst,* 77, 205-207 (1952).

(7) K. Burger, Methods for quantitative micro-determination and trace detection of surface active compounds. I. Detection and determination of very small amounts of anionics and cationics in aqueous solution, *Z. Anal. Chem.,* **196,** 15-21 (1963).

(8) G. S. Buchanan and J. C. Griffith, Polarographic estimation of anionic detergents, *J. Electroanal. Chem.,* **5,** 204-207 (1963).

(9) *Laboratory Methods in Infrared Spectroscopy,* 2nd Edition, R. G. J. Miller and B. C. Stace, Eds. (Heyden and Sons, Ltd., London, 1972).

(10) D. Hummel, *Identification and Analysis of Surface Active Agents by Infrared and Chemical Methods* (Interscience Publishers, New York, 1962).

(11) L. J. Bellamy, *The Infrared Spectra of Complex Molecules,* 2nd Edition (Methuen and Co., Ltd., London, 1958).

(12) W. Brugel, *An Introduction to Infrared Spectroscopy* (Methuen and Co., Ltd., London, 1962).

(13) N. B. Colthup, L. H. Daly, and S. E. Wiberley, *Introduction to Infrared and Raman Spectroscopy* (Academic Press, New York, 1964).

(14) R. G. Sinclair, A. F. McKay, G. S. Myers, and R. N. Jones, The infrared absorption spectra of unsaturated fatty acids and esters, *J. Am. Chem. Soc.,* 74, 2578-2585 (1952).

(15) M. Aoki and Y. Iwayama, Determination of ionic surface active agents with dyes. IV. Applicability of fluorescein dyes and indicator, and stability of anionic detergents in solution, *Yakugaku Zasshi* (Tokyo), **80,** 1749 (1960) (in Japanese).

(16) W. E. Link, H. M. Hickman, and R. A. Morrissette, Gas-liquid chromatography of fatty derivatives. II.

Analysis of fatty alcohol mixtures by gas-liquid chromatography, *J. Am. Oil. Chem. Soc.*, **36**, 300 (1969).
(17) T. H. Liddicoet and L. H. Smithson, Analysis of surfactants using pyrolysis-gas chromatography, *J. Am. Oil. Chem. Soc.*, **42**, 1097 (1975).
(18) J. Torrnquist, Quantitative analysis of polyethylene glycol monododecyl ethers by gas chromatography after silylation, *Acta Chemica Scandinavica*, **23**, 1935-1942 (1969).
(19) S. I. Hakomori, A rapid permethylation of glycolipid polysaccharide catalyzed methylsulfonyl carbanion in dimethylsulfoxide, *J. Biochem.*, **55**, 205-208 (1964).
(20) K. Sjõberg, A stable solution of methylsulfinyl carbanion, *Tetrahedron Letters*, **51**, 6383-6384 (1966).
(21) G. Schomburg *et al.*, Gas chromatographic analysis with glass capillary columns, *J. Chrom.*, **122**, 55-72 (1976).
(22) I. E. Rosenberg and E. Fu, Unpublished research.
(23) I. E. Rosenberg and J-S. Wang, Unpublished research.
(24) H. J. Vonk *et al.*, Modern analytical methods for ethoxylated surfactants, *T. Mezhdunar. Kongr. Poverkhn.-Akt. Veshehestrum*, **1**, 435-449 (1977).
(25) E. Julia-Danés and A. M. Casanovas, Application of mass spectroscopy to the analysis of nonionic surfactants, *Tenside Deterg.*, **16**, 317-323 (1979).
(26) C. K. Cross and A. C. Mackay, Analysis of alkyl ethoxylates by NMR, *J. Am. Oil Chem. Soc.*, **50**, 249-250 (1973).
(27) H. Walz and H. Kirschnek, Nuclear resonance spectroscopy as a valuable complement to infrared and ultraviolet analysis of surface active compounds, *Proc. 3rd Intern. Congr. Surface Active Substances, Koln*, **3**, 92-98 (1960).
(28) A. R. Greff, Jr. and P. W. Flanagan, The characterization of non-ionic surfactants by NMR, *J. Am. Oil Chem. Soc.*, **40**, 118-120 (1963).
(29) M. M. Crutchfield, R. R. Irani, and J. T. Yoder, Quantitative application of high resolution proton magnetic resonance measurements in the characterization of detergent chemicals, *J. Am. Oil Chem. Soc.*, **41**, 129-132 (1964).
(30) C. C. Hinckley, Paramagnetic shifts in solution of cholesterol and the dipyridine adduct of trisdipivalomethanatoeuropium (III). A shift reagent, *J. Am. Chem. Soc.*, **91**, 5160-5162 (1969).
(31) J. K. M. Sanders and D. H. Williams, A shift reagent for use in nuclear magnetic resonance spectroscopy. A first order spectrum of n-hexanol, *Chem. Commun.*, 422-423 (1970).
(32) J. K. M. Sanders and D. H. Williams, Tris(dipivalomethanato)europium. A paramagnetic shift reagent for use in nuclear magnetic resonance spectroscopy, *J. Am. Chem. Soc.*, **93**, 641-645 (1971).
(33) G. E. Stolzenberg, R. G. Zaylskie, and P. A. Olson, Nuclear magnetic resonance identification of O,P-isomers in an ethoxylated alkylphenol nonionic surfactant as tris(2,2,6,6-tetramethylheptane-3,5-dione)europium III complex, *Anal. Chem.*, **43**, 908-912 (1971).
(34) C. P. Terweij-Groen, S. Heemstra, and J. C. Kraak, Distribution mechanism of ionizable substances in dynamic anion exchange systems using cationic surfactants in high performance liquid chromatography, *J. Chromatogr.*, **161**, 69-82 (1978).
(35) K. Nakamura, Y. Morikawa, and I. Matsumoto, Rapid analysis of ionic and non-ionic surfactant homologs by high performance liquid chromatography, *J. Am. Oil Chem. Soc.*, **58**, 72-77 (1981).
(36) A. Nozawa and T. Ohnuma, Improved high performance liquid chromatographic analysis of ethylene oxide condensation by their esterification with 3,5-dinitrobenzoyl chloride, *J. Chromatogr.*, **187**, 261-263 (1980).
(37) K. Nakamura and Y. Morikawa, Separation of surfactant mixtures and their homologs by high pressure liquid chromatography, *J. Am. Oil Chem. Soc.*, **59**, 64068 (1982).
(38) J. F. K. Huber *et al.*, Rapid separation and determination of nonionic surfactants of the polyethylene glycol-monoalkyl phenyl ether- type by column liquid chromatography, *Anal. Chem.*, **44**, 105-110 (1972).
(39) K. Aitzetmuller, Application of moving-wire detectors for the liquid chromatography of fats and fatty acid derived oleochemicals, *J. Chrom. Sci.*, **13**, 454-461 (1975).
(40) J. J. Kirkland, Analysis of sulfonic acids and salts by gas chromatography of volatile derivatives, *Anal. Chem.*, **32**, 1388 (1960).
(41) T. Nagai, S. Hashimoto, I. Yamane, and A. Mori, Gas chromatographic analysis for alpha-olefin sulfonates, *J. Am. Oil Chem. Soc.*, **47**, 505 (1970).
(42) L. D. Metcalf, The direct gas chromatographic analysis of long chain quaternary ammonium compounds, *J. Am. Oil Chem. Soc.*, **40**, 25 (1963).

(43) D. Grossi and R. Vece, The gas chromatographic analysis of primary, secondary, and tertiary fatty amines and of compounding quaternary ammonium compounds, *J. Gas Chromatog.*, **3,** 170 (1965).
(44) R. J. Cotter, G. Hansen and T. R. Jones, Mass spectral determination of long chain quaternary amines in mixtures, *Anal. Chim. Acta,* **136,** 135-42 (1982).
(45) N. N. Daoud, P. A. Crooks and P. Gilbert, Identification of component alkyl chains within commercial samples of benzalkonium chloride mixtures by chemical ionization mass spectrometry, *J. Pharm. Pharmacol.,* **33,** 8P (1981).
(46) C. H. Wilson, Identification of preservatives in cosmetic products by thin layer chromatography, *J. Soc. Cosmet. Chem.,* **26,** 75-81 (1975).
(47) D. H. Liem, Analysis of antimicrobial compounds in cosmetics, *Cosmet. and Toilet.,* **59,** 59-62, 67-68, 70-72 (1977).
(48) E. P. Sheppard and C. H. Wilson, Fluorometric determination of formaldehyde-releasing cosmetic preservatives, *J. Soc. Cosmet. Chem.,* **25,** 655-666 (1974).
(49) M. W. Dong and J. L. DiCesare, Very high speed liquid chromatography. III. Quantitative analysis of parabens in cosmetic products, *J. Chromatogr. Sci.,* **20,** 49-54 (1982).
(50) Y. Masada, *Analysis of Essential Oils by Gas Chromatography and Mass Spectroscopy* (John Wiley & Sons, Inc., New York, 1976).
(51) B. J. Gudzinowicz, M. J. Gudzinowicz, and H. F. Marten, *Fundamentals of Integrated GC-MS, Vol. 7* (Marcel Dekker, Inc., New York, 1977).
(52) W. Jennings and T. Shieamoto, *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography* (Academic Press, New York, 1980).
(53) W. Paul and H. Steinwedel, Ein Neues Massenspektrometer Ohne magnetfeld, *Z. Naturforschung,* **(8a),** 448-450 (1953).
(54) B. B. Jones, B. C. Clark, and G. A. Iacobucci, Semipreparative high-performance liquid chromatographic separation of a characterized flavor mixture of monoterpenes, *J. Chromatog.,* **178,** 575-578 (1979).
(55) J. Fohlman, L. Rask, and P. A. Peterson, High pressure liquid chromatographic identification of phenylthiohydantoin derivatives of all twenty common amino acids, *Anal. Biochem.,* **106,** 22-26 (1980).
(56) G. J. Hughs, K. H. Winterhalter, E. Boller, and K. J. Wilson, Amino acid analysis using standard high performance liquid chromatography, *J. Chromatogr.,* **235,** 417-426 (1982).
(57) D. W. Hill, F. H. Walters, T. D. Wilson, and J. D. Stuart, High performance liquid chromatographic determination of amino acids in the Picomole range, *Anal. Chem.,* **51,** 1338-1341 (1979).
(58) S. I. Bonvell and R. H. Monheimer, A gas-liquid chromatographic analysis of sulfur-containing amino acids employing flame photometric detection, *J. Chromatogr. Sci.,* **18,** 18-22 (1980).
(59) J. J. Hansen, T. Greibrokk, B. L. Currie, K. Nils-Gunnar Johansson, and K. Folkers, High pressure liquid chromatography of peptides, *J. Chromatogr.,* **135,** 155-164 (1977).
(60) W. S. Hancock, C. A. Bishop, R. L. Prestidge, and D. R. K. Harding, High pressure liquid chromatography of peptides and proteins. II. The use of phosphoric acid in the analysis of underivatized peptides by reverse phase high pressure liquid chromatography, *J. Chromatogr.,* **153,** 391-398 (1978).
(61) F. E. Regnier and K. M. Gooding, High performance liquid chromatography of proteins, *Anal. Biochem.,* **103,** 1-25 (1980).
(62) T. Hashimoto, H. Sasaki, M. Aiura, and Y. Kato, High speed aqueous gel-permeation chromatography of proteins, *J. Chromatogr.,* **160,** 301-305 (1978).
(63) B. A. Coles, Protein determination by nuclear magnetic resonance, *J. Am. Oil Chem. Soc.,* **57,** 202-204 (1980).
(64) I. J. Levy and P. L. Dubin, Molecular weight distribution of cationic polymers by aqueous gel permeation chromatography, *Ind. Eng. Chem. Prod. Res. Deve.,* **21,** 59-63 (1982).
(65) "N-nitroso compounds—analysis and formation," IARC Scientific Publication No. 3, International Agency for Research on Cancer, Lyon, France, 1972.
(66) "Environmental N-nitroso compounds—Analysis and formation," IARC Scientific Publication No. 14, International Agency for Research on Cancer, Lyon, France, 1976.
(67) T. U. Fan, U. Goff, L. Song, D. H. Fine, G. P. Arsenault, and K. Biemann, N-nitrosodiethanolamine in cosmetics, lotions and shampoos, *Food Cosmet. Tox.,* **15,** 423-430 (1977).
(68) MRI Reports NFT-1-NFT-7. Available through the Cosmetic, Toiletry and Fragrance Association, 1110 Vermont Avenue, N.W., Washington, D.C. 20005.

(69) P. Rahn and W. Mitchell, High performance liquid chromatography in cosmetic analysis, *Drug Cosmet. Indus.*, **123**, 56-66, 126, 130 (1978).
(70) I. E. Rosenberg, J. Gross, T. Spears, and U. Caterbone, Methodology development for the determination of nitrite and nitrosamines in cosmetic raw materials and finished products, *J. Soc. Cosmet. Chem.*, **30**, 127-135 (1979).
(71) I. E. Rosenberg, J. Gross, T. Spears, and P. Rahn, Analysis of nitrosamines in cosmetic raw materials and finished product by high pressure liquid chromatography, *J. Soc. Cosmet. Chem.*, **31**, 237-252 (1980).
(72) I. E. Rosenberg, J. Gross, and T. Spears, Analysis of N-nitrosodiethanolamine in linoleamide DEA by high pressure liquid chromatography and U.V. detection, *J. Soc. Cosmet. Chem.*, **31**, 323-327 (1980).
(73) Y. Fellion, J. DeSmedt, and N. Brudney, "An HPLC-UV method for the direct evaluation of N-nitrosodiethanolamine in some cosmetic products and raw materials, N-nitroso compounds analysis, formation and occurrence," IARC Scientific Publication No. 31, International Agency for Research on Cancer, Lyon, France, 1980, pp 435-444.
(74) M. Tunick, H. S. Veale, and G. W. Harrington, Qualitative detection of N-Nitrosodiethanolamine in cosmetic products, *Fd. Chem. Toxic.*, **20**, 473-474 (1982).
(75) H. J. Chou, R. L. Yates, R. J. Gajan, and H. M. Davis, Differential pulse polarographic determination of N-nitrosodiethanolamine in cosmetic products, *J. Assoc. Off. Anal. Chem.*, **65**(4), 850-854 (1982).
(76) R. H. Bennett and E. S. Peterson, "Determination of nitrosamines by liquid chromatography using a photoconductivity detector," Paper presented at the 21st Rocky Mountain Conference on Analytical Chemistry, August 2, 1979, Denver, CO.
(77) S. S. Hecht and J. B. Morrison, N-nitroso-N-methyldodecylamine and N-nitroso-N-methyltetradecylamine in hair care products, *Fd. Chem. Tox.*, **20**, 165-169 (1982).
(78) V. H. Baptist and R. Brown, Nitrosamine determination by the use of conventional equipment, *J. Soc. Cosmet. Chem.*, **31**, 219 (1980).
(79) I. Schmaltz, S. Abidi, and D. Hoffman, Tumorigenic agents in unburned processed tobacco: N-nitrosodiethanolamine and 1,1-dimethylhydrazine, *Cancer Letters*, **2**, 125-132 (1977).
(80) P. Griess, Bernerkungen zu der Abhandlung der H. H. Weselsky und Benedikt: 'Ueber einige azovervindungen,' *Ber. Dtsch. Chem. Ges.*, **12**, 426-428 (1879).
(81) R. R. Gadde and B. Patel, A simple nitrite assay method for the screening of raw materials commonly used in creams and lotions, *J. Soc. Cosmet. Chem.*, **30**, 385-391 (1979).
(82) J. H. Wiersma, 2,3-Diaminonaphthalene as a spectrophotometric and fluorometric reagent for the determination of nitrite ion, *Anal. Letters*, **3**(3), 123-132 (1970).
(83) MRI Reports NFT-10; NFT-11. Available through the Cosmetic, Toiletry and Fragrance Association, 1110 Vermont Avenue, N.W., Washington, D.C. 20005.
(84) T. Birkel, C. Warner, and T. Fazio, Gas chromatographic determination of 1,4-Dioxane in Polysorbate 60 and Polysorbate 80, *J.H.O.Ac.*, **62**, 931-936 (1979).
(85) Report of the Dioxane Round-robin Program. Available through the Cosmetic, Toiletry and Fragrance Association, 1110 Vermont Avenue, N.W., Washington, D.C. 20005.
(86) B. A. Waldman, Analysis of 1,4-dioxane in ethoxylated compounds by gas chromatography/mass spectrometry using selected ion monitoring, *J. Soc. Cosmet. Chem.*, **33**, 19-25 (1982).
(87) J. J. Robinson and E. W. Ciurczak, Direct gas chromatographic determination of 1,4-dioxane in ethoxylated surfactants, *J. Soc. Cosmet. Chem.*, **31**, 329-337 (1980).
(88) M. L. Stafford, K. F. Guin, G. A. Johnson, L. A. Sanders, and S. L. Rockey, Analysis of 1,4-dioxane in ethoxylated surfactants, *J. Soc. Cosmet. Chem.*, **31**, 281-287 (1980).
(89) E. K. Weisburger *et al.*, Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity, *J. Environ. Path. and Toxicol.*, **2**, 325-356 (1978).
(90) E. Cox, High performance liquid chromatographic determination of quinizarin, p-toluidine and D & C Violet No. 2 in D & C Green No. 6, *J.A.O.Ac.*, **62**, 1338-1341 (1979).
(91) *Federal Register*, **47**, No. 64, 14140 (1982).