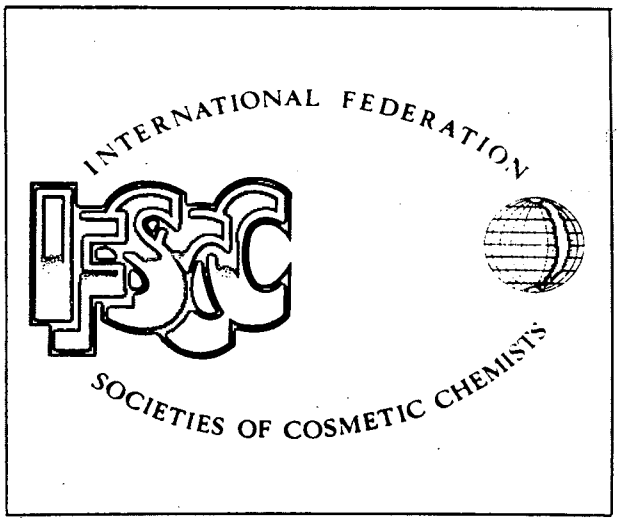
Cosmetic Raw Material Analysis and Quality

Volume I

Hydrocarbons, Glycerides, Waxes and Other Esters



Cosmetic Raw Material Analysis and Quality

Volume I: Hydrocarbons, Glycerides, Waxes and Other Esters

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The International Federation of Societies of Cosmetic Chemists

The International Federation of Societies of Cosmetic Chemists (IFSCC) is an international association which links national societies of cosmetic chemists and which is dedicated to international cooperation in cosmetic science and technology. Interest in the formation of the IFSCC arose in Paris in 1956 and culminated in the birth of the Federation in Brussels on September 8, 1959. At that time there were only eight countries entering the Federation: Belgium, Denmark, France, Great Britain, Spain, Switzerland and the United States. Maison G. deNavarre, of the United States, was elected as its first president.

Today, after thirty-five years, there are thirty-one societies and thirty-four countries belonging to the Federation (the Scandinavian Society, SCANCOS, represents four countries). Their Societies' members all meet the high standards of scientific and educational qualifications of the IFSCC, and they are supportive of its aims and programmes. The total number of individual members in all Societies of the Federation now exceeds 10,700. The IFSCC continues to grow as the cosmetics industry expands into new geographical areas, such as Eastern Europe, Asia, Africa and South America.

The IFSCC is committed to advancing the knowledge of cosmetic science and to encouraging the dissemination of this knowledge through its international meetings. It encourages strong cooperative relationships in the exchange of information between its member Societies and their members. It sponsors a number of educational programmes, besides its congresses and conferences. Monographs have been published and are being prepared by the Federation on a variety of key subjects in cosmetic science. Speakers are currently being sponsored by the IFSCC to lecture at meetings of the smaller and newer member Societies. The IFSCC Scientific Data Base, *KOSMET*, initiated by Mr Guy Aubin, a Past President, has grown rapidly and is accessed frequently by almost all member countries. It contains extensive information from scientific and trade journals. Awards are presented at all IFSCC congresses for the best technical presentations. The Federation is always open to suggestions from member Societies for expanding its educational and awards programmes.

The Federation has a bicameral governing body consisting of a Praesidium and a Council of Delegates from member Societies. The Council and the Praesidium meet annually in the Autumn in conjunction with the Congress or the Conference. The Council elects the officers of the Federation who in turn make up the Praesidium. The Praesidium also meets every Spring, in Europe.

The head office of the IFSCC constitutes the Secretariat of the Federation and is maintained in cooperation with the British Society of Cosmetic Scientists. The administrator of this office is Mrs Lorna K. Weston, the General Secretary of the IFSCC, and her assistant is Mrs Julia Ross. Member Societies are encouraged to be in close communication with the Secretariat to ensure full utilization of the benefits of membership in the Federation and generate a flow of information between Societies. The Secretariat publishes for the officers of each Society a quarterly IFSCC Newsletter incorporating information received from member Societies. In addition, a summary newsletter *Flash* is issued twice each year to all Societies for direct distribution to their membership.

The major meetings of the IFSCC are the Congress, held every other year in the even-numbered years, and the Conference, held in odd-numbered years. The Congress is a larger meeting usually lasting three or four days, while the Conference generally lasts two days or less. The first IFSCC Congress was held in 1960 in Munich, when 350 delegates attended; recent congresses have attracted over 1500. Attendance at conferences averages about 300 delegates.

The IFSCC actively pursues all means to further its goals of encouraging international communication and cooperation in the field of science and technology in the cosmetics industry. It welcomes contributions from its member Societies toward achieving these aims.

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The current list of benefactors, to whom we offer our profound thanks for their continuing support of the IFSCC, are shown below:

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The following companies also make a substantial contribution to the IFSCC in supporting members of the Praesidium, and our grateful thanks therefore also go to:

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General Introduction to the Monograph

Analytical chemistry has played an important role in the development of the cosmetics industry, and continues to do so. It is an essential aspect of quality control and product safety and has led to the discovery of new cosmetic ingredients. The results of analytical research have been summarized in the official compendiums of cosmetic ingredients and products in many countries. This monograph, entitled *Cosmetic Raw Material Analysis and Quality*, is published in seven volumes on behalf of the International Federation of Societies of Cosmetic Chemists (IFSCC). It summarizes the many technologies employed by both analytical experts and cosmetic scientists in general, for the analysis and quality assessment of cosmetic raw materials.

Each volume is devoted to a specific group of raw materials in order to provide a comprehensive description and illustration of analytical methodologies and to avoid duplication. Each volume is divided into six or seven main divisions which deal with different aspects of the various raw material classes within the particular group.

The series results from the collaboration of analytical experts in many member Societies of the IFSCC and thus provides a broad spectrum of approaches to the technologies employed in cosmetic raw material analysis. Whilst, as a consequence, the series sometimes lacks uniformity of coverage, this has been deemed a small price to pay for the greater benefit of presenting as many different technologies as possible and providing an understanding of quality criteria which are not dictated by specifications but which are nevertheless essential in ensuring the quality of the finished cosmetic product. Each volume also lists cosmetic raw materials with reference to the available compendiums currently published in the United States by the Cosmetic, Toiletry and Fragrance Association (CTFA), the *CTFA Compendium of Cosmetic Ingredient Composition*, and the *Japanese Standards of Cosmetic Ingredients* prevailing in Japan.

The IFSCC Scientific Advisory Committee would like to express its gratitude to the authors for their valuable contributions to each volume in the series.

Tasuku Takamatsu
Joint Monograph Coordinator

Other titles in preparation are:

Volume II: *Surface Active Agents*
Volume III: *Polysiloxanes*
Volume IV: *Polyols, Saccharides, Organic Acids, Amino Acids and Peptides*
Volume V: *Polymers*
Volume VI: *Inorganic Pigments and Powders*
Volume VII: *Additives*

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SCS (Society of Cosmetic Scientists of Great Britain)
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Contents

Reference Compendiums

Although the cosmetics industry, worldwide, is apt to regard many of the compendiums listed below as having legal status, it should be noted that only a few are official documents. The exceptions are the pharmacopoeias but they are limited in the number of cosmetic materials they contain. Although uniformity is desired, many countries have different laws governing the sale of cosmetic products, and enquiries should always be made in the country where the product is to be marketed.

In the United States, since the law requires ingredient labelling, the Cosmetic, Toiletry and Fragrance Association's (CTFA's) *International Cosmetic Ingredient Dictionary*, fifth edition, of established names is officially recognized as the primary source by the Food and Drug Administration (FDA), the authority responsible for regulating cosmetics labelling. Manufacturers of raw materials and cosmetics are required to substantiate claims for the safety of ingredients and products and they are guided by the published, scientifically obtained results of the CTFA-funded Cosmetic Ingredient Review (CIR) Expert Panel. Contrary to general opinion, the *CTFA Compendium of Cosmetic Ingredient Composition: Descriptions* volumes and the *Specifications* volume, though often used and copied in many countries, are not legal documents.

In Japan, cosmetic products are regulated and subject to premarket licensing using cosmetic ingredients approved by the Ministry of Health and Welfare (MWH). This body has established the Comprehensive Licensing Standard of Cosmetics by Category and parts 1-6 include specifications. The *Japanese Cosmetic Ingredient Dictionary* (Vols. 1-12) is an ongoing list of permitted substances.

In Europe, the European Commission issued a Cosmetics Directive (1976) which controlled many aspects of the sale of cosmetics and, under the 6th Amendment (1993), required an Inventory of Cosmetic Materials to be published by December 14, 1994 and all cosmetics to be labelled by January 1, 1997. Comité de Liaison des Associations Européennes de l'Industrie de la Parfumerie, des Produits Cosmétiques et de Toilettes (COLIPA), an organisation formed by representatives from the United Kingdom Cosmetic, Toiletry and Perfumery Association (CTPA) and similar associations in the other eleven European Community countries, has prepared this legal document. Most of the labelling names to be used, known as International Nomenclature Cosmetic Ingredient (INCI) names, are published in the *CTFA International Cosmetic Ingredient Dictionary*, fifth edition (1993).

Organisations

AOAC	Association of Official Analytical Chemists, 2200 Wilson Boulevard, Suite 400, Arlington, VA 22201-3301, USA
AOCS	American Oil Chemists' Society, PO Box 3489, Champaign, IL 61826-3489, USA
ASTM	American Society for Testing Materials, 1916 Race Street, Philadelphia, PA 19103-1187, USA
CAS	Chemical Abstracts Service, PO Box 02228, Columbus, OH 43202, USA
CIR	Cosmetic Ingredient Review, CTFA, 1101 17th Street, N.W., Suite 300, Washington, DC 20036-4702, USA
COLIPA	Comité de Liaison des Associations Européennes de l'Industrie de la Parfumerie, des Produits Cosmétiques et de Toilettes, European Commission, Brussels, Belgium

CTFA	Cosmetic, Toiletry and Fragrance Association, Inc., 1101 17th Street, N.W., Suite 300, Washington, DC 20036-4702, USA
CTPA	Cosmetic, Toiletry and Perfumery Association, 35 Dover Street, London WX1 3RA, UK
FDA	US Food and Drug Administration, Washington, DC, USA
JCIA	Japan Cosmetic Industry Association, Fourth Floor, Hatsumei Building, 9-14, Toranomon 2-chome, Minato-ku, Tokyo, Japan
NGD	Norme Grassi e Derivati, Stazione Sperimentale per le Industrie degli Olii e dei Grassi, Milano, Italy

Compendiums

The following compendiums give useful and varied information on some of the materials listed in this volume. It must be stressed that their relevant status should be checked in the country of origin. The compendiums referred to throughout this volume are shown with abbreviations in **bold**.

21CFR	*Title 21 of the Code of Federal Regulations - Food and Drugs* [revision as of April 1, 1993]
ARG	*Farmacopeia Nacional Argentina* (Pharmacopoeia of Argentina) [4th edition 1956]
AUS	*Osterreichisches Arzneibuch* (Pharmacopoeia of Austria) [9th edition 1960]
BEL	*Pharmacopeia Belgie* (Pharmacopoeia of Belgium) [5th edition 1962]
BP	*British Pharmacopoeia*, Pharmaceutical Press, 1 Lambeth High Street, London SE1 7JN, England [latest is 1993 edition]
BPC	*British Pharmacopoeia Codex* [1973 - now combined with the British Pharmacopoeia]
BRA	*Farmacopeia dos Estados Unidos do Brasil* (Pharmacopoeia of Brazil), [2nd edition 1959]
CIH	*CTFA International Cosmetic Ingredient Handbook* [latest edition is the 2nd, 1992 to which a supplement was issued in 1993]
CIR	*CIR Compendium*, CTFA Cosmetic Ingredient Review (see previous section) [1994, updated annually]
CLS	*The Comprehensive Licensing Standard of Cosmetics by Category*, Yakuji Nippo Ltd, 1 Kanda Izumicho, Chiyoda-ku, Tokyo, 101 Japan [Parts 1 to 6, 1986 to 1991]. An English language version is available.
CTFA CID	*CTFA International Cosmetic Ingredient Dictionary* [latest edition is the 5th, 1993]
CTFA	*CTFA Compendium of Cosmetic Ingredient Composition* , in four volumes. *Descriptions I, Descriptions II, Specifications,* and *Methods* [1990]
CZE	*Cesskoslovensky Iekopis, Pharmacopoea Bohemslovenica, Addendum* (Pharmacopoeia of Czechoslovakia) [3rd edition 1970]
DA	*Deutsches Arzneibuch* (Pharmacopoeia of Federal Republic of Germany) [7th edition 1972]
DDR	*Deutsches Arzneibuch, Deutsche Demokratische Republik* (Pharmacopoeia of Democratic Republic of Germany) [7th edition 1972]
EGY	*Egyptian Pharmacopoeia* [1953 and Addendum 1962]
EEC	European Economic Community Cosmetics Directive 76/768/EEC, as amended, Annex II to VII
EP	*European Pharmacopoeia*, The Council of Europe, Brussels [2nd edition 1980-1987]
FCC	*Food Chemicals Codex*, National Academy Press, Washington, DC, USA [2nd edition 1972 and 3rd edition 1981]
FI	*Farmakope Indonesia* (Pharmacopoeia of Indonesia) [2nd edition 1965]
FIN	*Soumen Farmakopea* (Pharmacopoeia of Finland) [7th edition 1956]
HUN	*Hungarian Pharmacopoeia* [6th edition 1966]
HP	*Homoepathic Pharmacopeia of the United States* [7th edition, revised 1964]
II	*Inventario Italiano degli Ingredienti Cosmetici*
IND	*Pharmacopoeia of India* [2nd edition 1966]
ITA	*Farmacopeia Ufficiale della Repubblica Italiana* (Pharmacopoeia of Italy) [8th edition 1972]

JCID	*Japanese Cosmetic Ingredient Dictionary* [Vols. I to XII, 1981 to 1991]
JP	*Japanese Pharmacopoeia* [12th edition 1991]
JSCI	*Japanese Standards of Cosmetic Ingredients* [2nd edition, 1984, to which supplements were added in 1986 and 1992]
JSCIS	Supplement to JSCI
KCID	*Korea Cosmetic Ingredients Dictionary*
KCLS	*The Comprehensive Licensing Standard of Cosmetics by Category of Korea* [1992]
KOR	*Korean Pharmacopoeia* [5th edition, 1992]
KSCI	*Korean Standards of Cosmetic Ingredients* [1986]
M3	*Mitteilung 3*, Third Report of the Dye-Stuff Commission of Colours for Cosmetics, German Research Association [1968, amended 1971]
MAR	*Martindale: The Extra Pharmacopoeia*, Pharmaceutical Press, 1 Lambeth High Street, London SE1 7JN, England [latest is 30th edition, 1993]
MEX	*Farmacopea Nacional de los Estados Unidos Mexicanos* (Pharmacopoeia of Mexico) [3rd edition, 1962]
MI	*Merck Index*, Merck Inc., Rahway, NJ, USA [latest edition is the 11th ,1990]
NED	*Codex Medicamentorum Nederlandicus* (Netherlands) [1950]
NF XVII	*The National Formulary*, United States Pharmacopeial Convention, Rockland, MD, USA [latest edition is the 17th, 1990]
NFJ	*Formulae Nationales Japoniacae* (National Formulary of Japan) [2nd edition, 1955]
PF	*Pharmacopoeia Gallica* (Pharmacopoeia of France) [7th edition, 1949]
PJ	*Pharmacopoeia of Japan* [7th edition, 1961-62]
PN	*Pharmacopeia Nordica* [1963]
POL	*Farmakopea Polska*, II (Pharmacopoeia of Poland) [4th edition, 1970]
POR	*Farmacopeia Portuguesa, Supplemento* (Pharmacopoeia of Portugal) [4th edition, 1961]
ROM	*Farmacopeea Romana* (Pharmacopoeia of Romania) (8th edition, 1965]
SNPF	*Dictionnaire de la Fédération de l'Industrie des Produits de Parfumerie, de Beauté et de Toilette* [Syndicat National Parfumerie Française, 1974]
TPF	*Specifications Standards*. Toilet Preparations Federation, London [now CTPA]
TSCA	*Toxic Substances Control Act Chemical Substances Inventory*, Environmental Protection Agency, Washington, DC, USA [May 1985]
USAN	*United States Adopted Names*, Cumulative List [1990]
USD	*The United States Dispensatory and Physician's Pharmacology* [27th edition, 1973]
USP	*United States Pharmacopeia* United States Pharmacopeial Convention, Rockland, MD, USA [latest is 22nd edition, 1990]
WHO	*Pharmacopeia Internationalis* (International Pharmacopoeia), World Health Organization [2nd edition, 1970]
YUG	*Pharmacopoea Jugoslavica* (Pharmacopoeia of Jugoslavia) [2nd edition, 1951]

Introduction

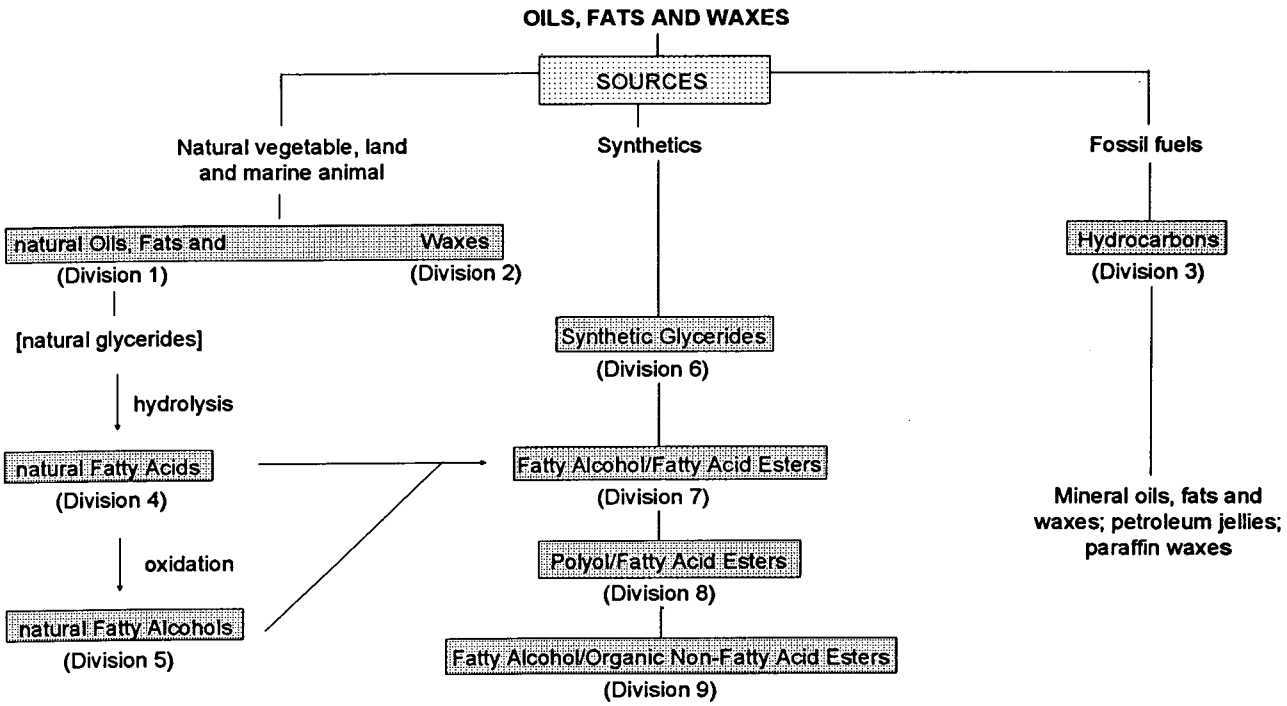
This volume outlines the analytical methods employed to identify and determine the quality of the fatty raw materials widely used in the cosmetics industry. These materials originate mainly from purified natural vegetable oils, land or marine animal oils, fats and waxes or petroleum oils and "waxes". Synthetic derivatives are developed from these natural sources.

The volume is divided into divisions, each of which deals with a specific group of raw materials. Many of the identification and quality tests for these materials are common to each division and are given in the appendix. However, a study of each relevant division should be made, including those relating to the specific molecules forming the fatty material. For example, additional information on waxes, and also on fatty alcohol/fatty acid esters, can be found in the divisions dealing with fatty acids and fatty alcohols.

Hilda Butler

Editor

Organic Fatty Materials Used in Cosmetic Products



Division 1: Natural Oils and Fats

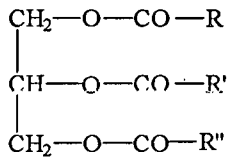
Angel Monclus

1 CHEMICAL DESCRIPTION, STRUCTURE AND USE IN COSMETICS

Natural oils and fats are fatty materials obtained from land or marine animal or vegetable sources and consist mainly of glycerides and esters of fatty acids.

1.1 Structure

Those substances in the group that are liquids at room temperature are named oils, and those whose texture is solid or semisolid are named fats, e.g. butter.



where R, R' and R" are fatty alkyl chains

1.2 Origins

1.2.1 Vegetable Sources

a) Annual plant seed oils

› Many common oils are obtained from whole seeds of different plants, e.g. sunflower, sesame, soya, grape. In the case of wheat only the germ part of the seed is used.

b) Oil-bearing trees.

› Olive, palm, and avocado pear are examples of oils obtained from the flesh of the fruits of trees, but the kernels of the fruits of almond, peach and apricot yield oils which are often used in cosmetics.

1.2.2 Animal Sources

Animal adipose tissues are rendered to give oils and fats; for example, mink oil and tallow from the ox. Other parts are used, such as liver to give cod liver oil. Lanolin oil is an external secretion and is extracted from sheep's wool.

1.2.3 Production

Three different methods are used for the production of oils and fats:

a) Rendering/boiling in water: This extraction method is mainly used for animal fats as they have a solid/semi-solid texture at room temperature; also, because they are insoluble in water, they form a layer on top which can be removed and purified.

b) Pressing: This process is mainly used for extraction from plant sources, but only those yielding 15-20% oil. It can be carried out with no added heat ('cold-pressed'). This method produces oils of the highest purity and integrity as neither high temperatures nor solvents are involved.

c) Solvent extraction: High yields are obtained by treating different sources with volatile solvents. The solvent must then be completely eliminated.

1.2.4 Purification

After extraction, oils and fats must be subjected to a refining process in order to improve their colour and odour and to improve physical and chemical stability. Sometimes they are finally hydrogenated for increased stability and to be more useful as a cosmetic raw material. Reduction of unsaturation lessens the chance of rancidity.

1.3 Uses

1.3.1 General

Natural fats and oils are the main source of the many fatty organic materials produced at present. Over 2000 are available for use in cosmetics.

The pure oils supply emolliency, possess moisturizing properties and also function as carriers of liposoluble vitamins, activators of skin metabolism and natural sun filters, etc. By physical separation of their constituent components, individual natural glycerides and free fatty acids are extracted. Hydrolysis of the glycerides yields saturated, unsaturated and some hydroxy fatty acids, together with glycerol.

1.4.2 Uses and Effects in Cosmetics

The properties of the different oils must be considered from the point of view of their action on human skin and the characteristics they impart to cosmetic products.

1.4 Raw Materials and References

Table 1.1 References to Natural Oils and Fats

Raw Material	Compendiums[1]
Almond Oil	CTFA; CLS/JSCI
Apricot Kernel Oil	CTFA
Avocado Oil	CTFA; CLS/JSCI
Burdock Seed Oil	CLS/JSCI
Cacao Butter	CTFA: CLS/JSCI
Camellia Oil	CLS/JSCI
Castor Oil	CTFA; CLS/JSCI
Coconut Oil	CTFA; CLS/JSCI
Corn Oil	CTFA; CLS/JSCI
Cottonseed Oil	CTFA; CLS/JSCI
Egg Oil/Egg Yolk Oil	CTFA; CLS/JSCI
Evening Primrose Oil	CLS/JSCI
Hydrogenated Castor Oil	CTFA; CLS/JSCI
Hydrogenated Coconut Oil	CTFA: CLS/JSCI
Hydrogenated Cottonseed Oil	CTFA
Hydrogenated Soy Oil	CLS/JSCI
Hydrogenated Vegetable Oil	CTFA; CLS/JSCI
Grape Seed Oil	CLS/JSCI
Mink Oil	CTFA; CLS/JSCI
Olive Oil	CTFA; CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

Table 1.1 *(contd.)* References to Natural Oils and Fats

Raw Material	Compendiums
Palm Kernel Oil	CLS/JSCI
Palm Oil	CLS/JSCI
Peanut Oil	CTFA; CLS/JSCI
Rice Bran Oil	CLS/JSCI
Rice Germ Oil	CLS/JSCI
Rosemary Oil	CLS/JSCI
Safflower Oil	CTFA; CLS/JSCI
Shea Butter	CLS/JSCI
Soybean Oil	CTFA; CLS/JSCI
Sesame Oil	CTFA; CLS/JSCI
Tallow	CTFA; CLS/JSCI
Wheat Germ Oil	CTFA; CLS/JSCI

2 THE PHYSICOCHEMICAL PROPERTIES AFFECTING USE IN COSMETICS

2.1 Physical Properties

a) Viscosity

In monophasic cosmetic forms the viscosity is somewhat complex. Each oil will have some influence on the final viscosity of the cosmetic affecting its spreading capacity, the after-feel and the thickness of the film after application. In biphasic cosmetic forms (emulsions), the oil will contribute to the viscosity of the fatty phase, affecting the rheology of the emulsion as well as the after-feeling on the skin.

b) Surface tension

Surface tension mainly defines the "wetting" properties of an oil or fat and the physicochemical properties of an emulsion, hydrophilic-lipophilic balance (HLB), etc. The "wetting "property is important for the dispersion of solids; for example, in colour cosmetics. Theoretically the effect of surface tension in an emulsion is much more difficult to define and the use of its measurement is more limited.

c) Spreading capacity

In principle spreading capacity refers to its capacity of being spread in the form of a thin film. As a standard measure, the number of times that the diameter of a drop placed on a specific surface grows has been determined. The spreading coefficient (SC) of an aqueous medium over an oily surface is calculated from the surface tension (ST) and the interfacial tension (IT) (with a given oil) by the following formula:

$$SC = ST_{oil} - (ST_{soln.} + IT_{oilsoln})$$

The greater the value of SC the greater the wetting and spreading power.

d) Absorption

The cosmetic meaning of this parameter refers especially to the after feeling obtained when applying an oil to the skin, (alone, or when in the finished product). Low absorptions give a fatty feel, while high ones feel "drier" and velvety to the touch.

e) Occlusive capacity
By this property the cosmetic oil acts as a protector, by preventing the loss of water through the skin. It constitutes one form of moisturising it. Many methods, both *in vivo* and *in vitro* have been proposed as an objective measure of this property.

f) Polarity
This property is related to the ones mentioned above, and will influence directly the emulsifying characteristics of each oil.

g) Melting point
Melting point is important in determining the skin-feel during application of the finished product. It can also affect the stability of solid finished products and also of emulsions.

2.2 Chemical Properties

Alkaline hydrolysis of the glyceride molecule yields soaps and glycerol. Mineral acid reaction with the soaps yields fatty acids.

The chemical characteristics of the fatty acids forming the glyceride molecule and any free acids affect the chemical behaviour of oils and fats. Unsaturation leading to oxidation can lead to rancidity. Unsaponifiable substances present can also affect the chemical reactions: for example, α- and γ-tocopherols act as antioxidants in corn oil.

3 TESTS FOR IDENTIFICATION AND QUALITY

The usual methods for identifying oils and fats may be divided into three groups.

3.1 Physical Tests

*3.1.1 **Organoleptic Characteristics*** (see Appendix, ***1, 2 and 3***)

a) Appearance
b) Colour
c) Odour

*3.1.2 **Physical Characteristics*** (see Appendix, ***6, 8, 11 and 16***)

a) Specific gravity
b) Refractive index
c) *Melting point*
d) Freezing point
e) Cloud point
f) Melting point of isolated fatty acids

3.2 Chemical Analyses

a) Saponification value (see Appendix, *22*)
b) Hydroxyl value (see Appendix, *23*)

This is the number of free hydroxyl groups occurring in the oil or fat, i.e. in the mono- and diglycerides, hydroxy acids, etc.

c) Iodine value (see Appendix, ***19***)

If the fat or oil contains unsaturated fatty acids, the iodine value (IV) can be used as an identification method. If not, it can be used as a test for unsaturated impurities.

d) Unsaponifiable fraction (see Appendix, **24***)*

Different natural oils and fats contain known specific unsaponifiables, e.g. sterols or vitamins. Isolation of these from the fatty material can be used to identify the oil or fat. When testing named materials, unexpected unsaponifiables indicate adulteration of the original sample.

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatographic Analysis

4.1.1 Thin-Layer Chromatography

Though this is not a specific technique for showing the separate elements in this group the differences between triglycerides and esters may be shown. Silica gel chromatoplates may be used, with *n*-hexane, diethyl ether and acetic acid as eluant and iodine vapour or sulfuric acid as developer.

The use of a flame-ionisation detector (FID) with thin-layer chromatography (TLC-FID) allows the separation of triglycerides from the unsaponifiables, e.g. fatty alcohols, hydrocarbons, etc.

4.1.2 Gas Chromatography

Gas chromatography is performed on the separated fatty acids or methyl esters (see Figure 1.1). The analysis may be made using a Carbowax-type column or with silicone using helium as a gas carrier and FID detector.

4.1.3 Column Chromatography

Column chromatography is generally used to separate the hydrocarbons.

4.1.4 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is used to separate mono-, di- and triglycerides.

4.2 Spectroscopy

4.2.1 Infrared Spectroscopy

The main functional groups in oils and fats are esters and alkyl chains (see Appendix, ***30***). The characteristic absorption bands are those of alkyl groups (2920 and 2850 cm^{-1}) methylene chains (1470cm^{-1}, 1390-1200 cm^{-1} and *ca.* 720 cm^{-1}) and esters (1750-1720 cm^{-1}, 1280-1100 cm^{-1}). (See Figure 1.2.)

Figure 1.1

Gas Chromatogram of Coconut Oil

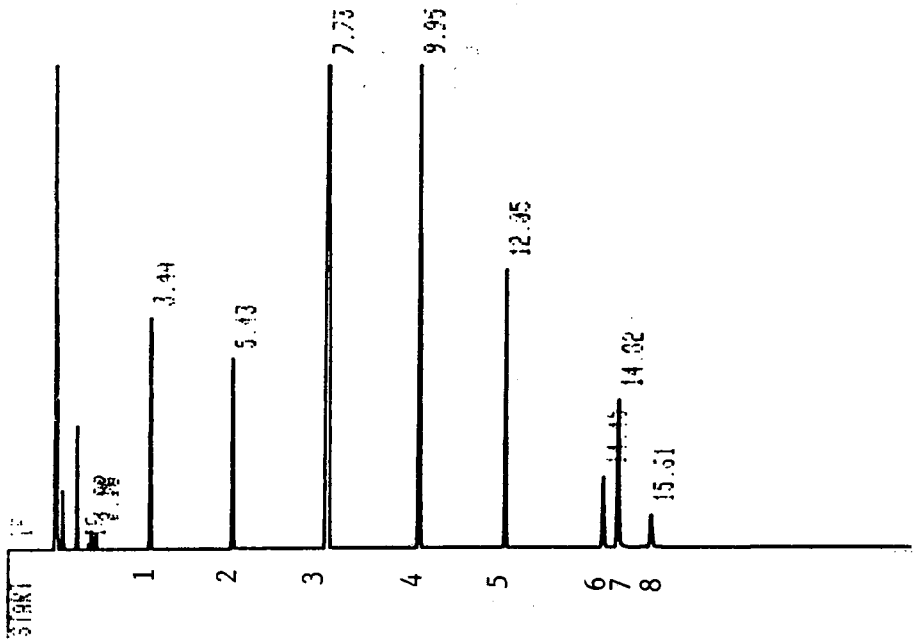
Column: F.F.A.P. 25 mts 0.3 mm ID;

Split: 1/100 Helium;

Detector: F.I.D.

Initial temperature: 100°C;
Rate: 10°C/min;

Final temperature: 220°C;
Final time: 20 min
Inject. temperature: 250°C;
Detect. temperature: 250°C



[1] C_8 Methyl Ester; [2] C_{10} Methyl Ester; [3] C_{12} Methyl Ester; [4] C_{14} Methyl Ester; [5] C_{16} Methyl Ester; [6] C_{18} Methyl Ester; [7] $C_{18(=)}$ Methyl Ester; [8] $C_{18(2=)}$ Methyl Ester

4.2.2 Ultraviolet–Visible (UV-VIS) Spectrophotometry

The UV spectrum of an oil or fat is generally measured over the range 220- 400 nm although the visible range up to 800 nm may also be covered by a single instrument. Conjugated double bonds absorb strongly in the region 230 nm. The samples are dissolved in 95 per cent ethanol for analysis. See Figure 1.3.

5 TESTS FOR IMPURITIES AFFECTING QUALITY

The quality of an oil or fat is generally determined by a range of tests as described in the international compendiums, and by the analytical tests for identification described above (¶3 and ¶4). The evaluation of the constants obtained should be compared with fresh standard samples and the raw material tested for its suitability in the finished formulation.

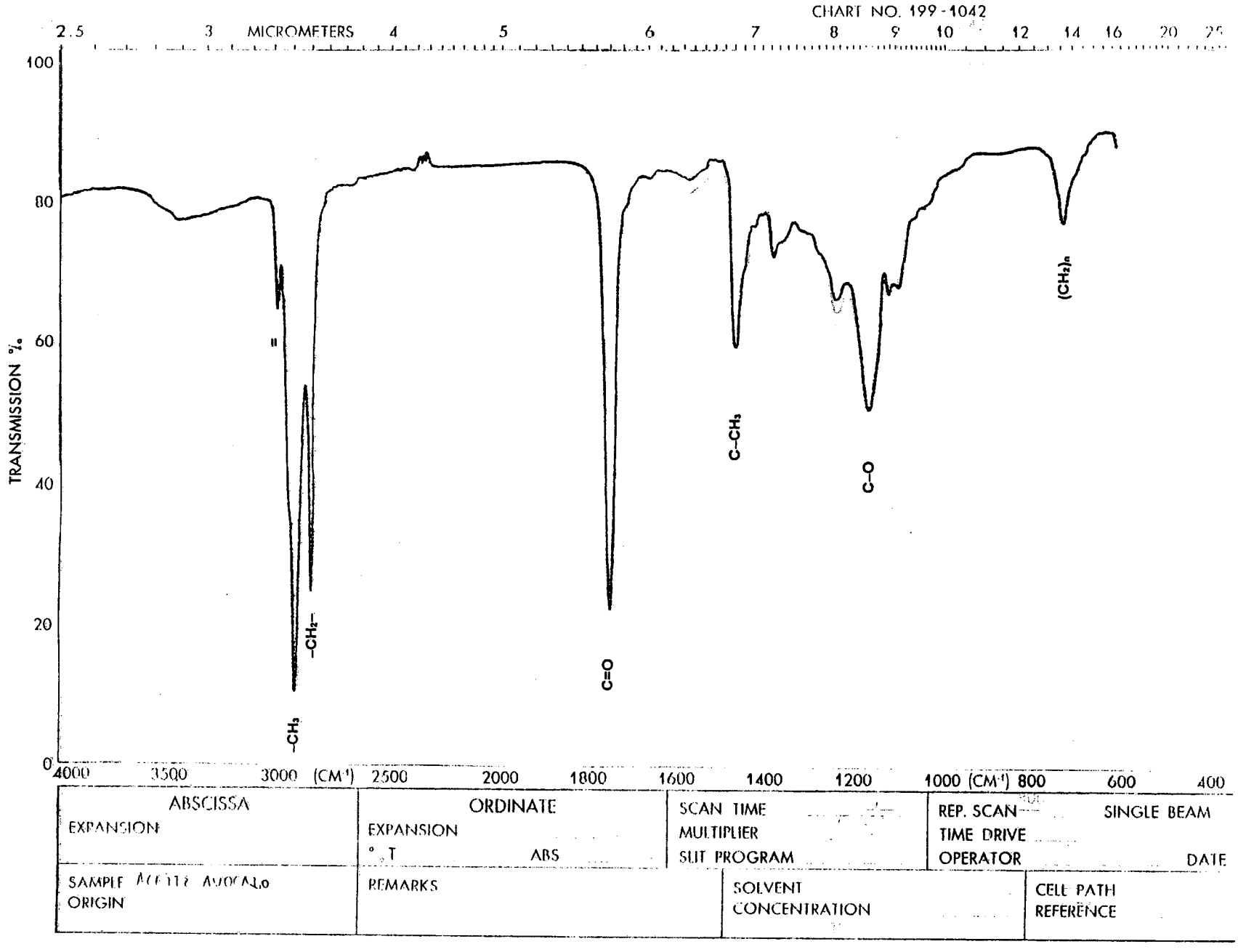


Figure 1.2 IR Spectrum of Avocado Oil

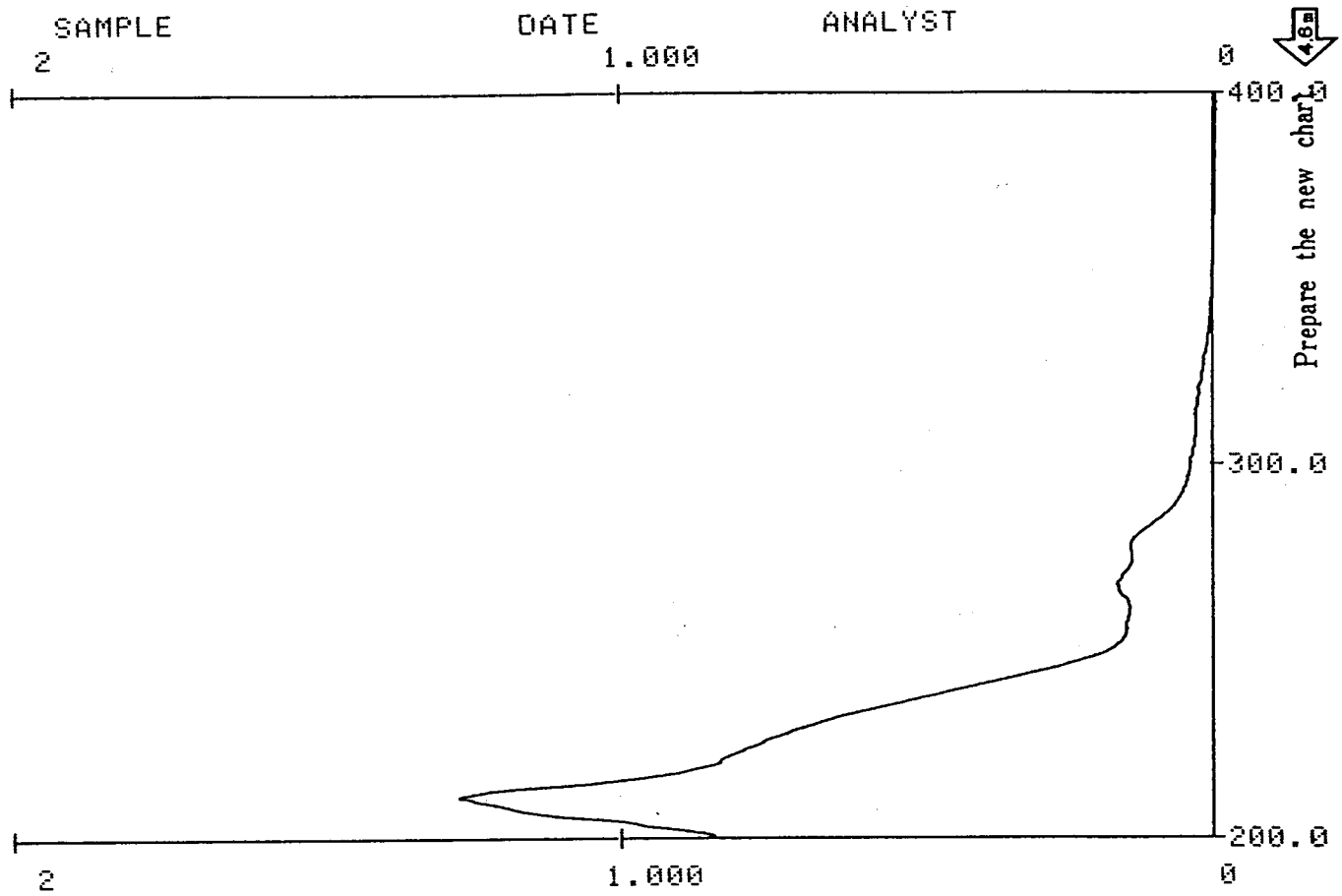


Figure 1.3 *UV-VIS Spectrograph of Avocado Oil. Sample dissolved in Ethyl Alcohol 0.1 g/50 ml*

5.1 Organoleptic and Physical Characteristics

The characteristics described under ¶3.1.1 and ¶3.1.2 above when compared with a standard sample, indicate quality. The variation of these properties may lead to a modification of the colour and texture or interfere with the perfume and modify the odour of the cosmetic.

5.2 Unsaponifiable Fraction

The unsaponifiable fraction, when different from standard, is a measure of adulteration.

5.3 Peroxide Value (see Appendix, *25*)

Assuming that peroxides or other similar compounds are products of the oxidation, the peroxide value can give a measure of the extent of the oxidation processes causing rancidity in oils and fats, particularly those containing unsaturated fatty components.

5.4 Acid Value

Depending on the type of oil or fat analysed, the fatty acid content is expressed as the amount of oleic, palmitic or lauric acid. If a more precise number is needed, the average molecular weight based on the accepted fatty acid composition of the oil or fat is used.

5.5 Moisture

Traces of water in fatty materials, even just moisture content, can cause hydrolysis producing

a) by-products of low molecular weight with a high olfactory level;

b) free fatty acids which may accelerate auto-oxidation.

5.6 Ash

Inorganic impurities remain in the ashes after burning fatty materials. If these are metallic compounds, they could act as catalysts for auto-oxidation processes.

6 DETERMINATION OF FATS AND OILS IN FINISHED PRODUCTS

Owing to the great variety of formulation types in the cosmetic field and to the similarity of components belonging to this group it is very difficult to identify oils and fats in a finished product. The presence of this type of ingredient may be confirmed by using all the related separation techniques (TLC, GC, HPLC, TLC-FID).

6.1 Separation of Oil-Soluble Materials

An organic solvent (*n*-hexane) is used to extract the oil-soluble materials. Problems may be encountered because of the emulsion formed between the water and fatty phases. The addition of electrolytes (e.g., sodium chloride) makes the separation easier. The extract is then evaporated to dryness in an oven at 105°C.

6.2 Analysis of the Dry Extract from ¶6.1

Analysis is carried out by the procedures in ¶4. If hydrocarbons or volatile esters are present they can be directly determined by GC and compared with internal standards for quantification. In most cases, if the formulation is complex interpretation may be difficult.

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Division 2: Natural Waxes

Angel Monclus

1 CHEMICAL DESCRIPTION

Natural waxes are mixtures of esters formed from long-chain fatty acids (higher than found in fats and oils) and monohydric fatty alcohols, with variable quantities of free fatty acids and free hydrocarbons. Some hydrocarbon waxes are also included in this division.

1.1 Structure

The structural formula of the main components, the mixed esters, is as follows:

$$R - O - CO - R'$$

where R is a fatty alcohol radical and R′ is a fatty acid radical.

1.2 Origins

Natural waxes are obtained from vegetable and animal tissues by means of fusion processes or extraction with solvents and subsequently refined and/or hydrogenated. For cosmetic use it is very important that they are decolorized and deodorized so that a standard material is produced which does not vary from batch to batch, though variations may occur according to the supplier.

1.3 Properties Useful in Cosmetic Product Formulation

The hardness of many of the natural waxes contributes not only to the mechanical properties of cosmetics in stick form (resistance to breakage, fusion point, etc.) but also modifies their consistency, pay-off and rheology determined by the characteristics of the melting, softening or flowing points of the waxes used.

When used to increase the viscosity of the external phase of a water-in-oil emulsion, waxes help to stabilize it; but although they also increase the viscosity of the oil phase in oil-in-water emulsions, their effect is not so marked as in the former case. Many of the waxes are employed to increase the viscosity of cosmetic oils to form gels resulting in water-free preparations. Hydrolysis of the esters with alkali gives rise to soaps, which makes them useful as emulsifiers.

$C_{30}H_{61}O\ CO\ C_{15}H_{31}$	+	KOH	=	$C_{15}H_{31}COOK$	+	$C_{30}H_{61}OH$
melissyl (or myricyl) palmitate				potassium palmitate		melissyl alcohol

The presence of alcohols, free fatty acids and sterols in some waxes, such as beeswax or lanolin wax, provide them with a certain hydrophilic character that makes them useful as emulsifiers in water-in-oil emulsions. Waxes are important cosmetically because of the good appearance their application gives to the skin.

Note: The use of borax with beeswax in cold creams gives rise to water-in-oil or oil-in-water creams, without the aid of secondary emulsifiers, according to the ratio of oil to water phase. Water-in-oil emulsions usually result when the aqueous phase is less than 45%; above this oil-in-water creams are obtained.

1.4 Raw Materials and References

Table 2.1 References to Natural Waxes

Raw Material	Compendiums[1]
Acetylated Lanolin	CTFA; CLS/JSCI
Bayberry Wax	CTFA
Beeswax	CTFA; CLS/JSCI
Candelilla Wax	CTFA; CLS/JSCI
Carnauba	CTFA; CLS/JSCI
Hydrogenated Jojoba Oil	CLS/JSCI
Hydrogenated Lanolin	CTFA: CLS/JSCI
Japan Wax	CTFA; CLS/JSCI
Jojoba Oil	CTFA; CLS/JSCI
Lanolin	CTFA; CLS/JSCI
Lanolin Oil (Liquid Lanolin)	CLS/JSCI
Mink Wax	CLS/JSCI
Montan Wax	CLS/JSCI
Rice Bran Wax	CLS/JSCI
Spermaceti	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

2 PHYSICAL AND CHEMICAL PROPERTIES

2.1 Physical Properties

The most important properties of natural waxes are those that modify consistency and rheology, which are determined by their melting, softening and flowing points, as well as by their hardness and resistance to breakage. In some specific fields, properties such as adherence or flexibility may be of interest. See also ¶1.3.

a) *In sticks:* The properties of waxes are such that a correctly proportioned mixture of them when blended in a stick gives it strength and maintains the stick form over a range of temperatures up to 50°C. A good blend will hold the oil phase so that it will not bleed or sweat yet be easy to apply.

Carnauba and candelilla are hard waxes which give strength and gloss and help the release of the stick from the mould. Beeswax is often used as a hardener but if it is not blended with other waxes the stick will not be glossy and will drag on the skin.

b) *In blocks:* Beeswax employed to stiffen but not harden increases flexibility and plasticity, which help in application. In moulded products a blend of waxes should be formulated that shrinks just enough to aid mould release, yet that is non-sticky.

c) *Creams and lotions:* The control of the ratio of waxes to oils and humectants in the oil phase of a cream or lotion can lessen tackiness on the skin.

2.2 Chemical Properties

Soaps are produced by the alkaline hydrolysis of esters and these form good emulsifiers; and if free alcohols, free acids or sterols are present, the added hydrophilicity makes them useful emulsifiers. Other chemical properties of natural waxes are discussed in ¶1.3.

3 PHYSICOCHEMICAL ANALYSIS FOR IDENTIFICATION AND QUALITY

The usual methods for identifying a wax may be divided into three groups (see Appendix for definitions and general methods).

3.1 PHYSICAL TESTS

3.1.1 Organoleptic Characteristics

a) Appearance

b) Colour

c) Odour

Variations in texture, colour and odour from the standard materials used in the formulation development of a cosmetic may lead to modifications in the texture, colour, or perfume of the finished product. As waxes are natural, their physicochemical properties tend to vary according to the region of origin and the time of year of production. It is advisable therefore, once a formula has been established, for a cosmetic manufacturer to standardise the quality and source and to obtain repeat deliveries from the same supplier.

3.1.2 Physical Characteristics

a) Melting Point

b) Hardness

c) Resistance to Breakage.

3.1.3 Chemical Analyses

a) Acid Value (see Appendix, ***21***)
Depending on the type of wax, the fatty acid content is expressed as the amount of oleic, palmitic or lauric acid. If a more precise number is needed, the average molecular weight based on the fatty acid composition of the fat is used.

b) Saponification and Ester Value (see Appendix, ***22***)
These are a measure of the ester linkages, and are expressed as the number of mg of potassium hydroxide needed to hydrolyze (saponify) 1g of substance.

c) Hydroxyl Value (see Appendix, ***23***)

d) Iodine Value (see Appendix, ***19***)
If a wax contains unsaturated fatty acids, the iodine value (IV) can be used as an identification method. If not, it can be used as a test for unsaturated impurities.

e) Unsaponifiable Fraction (see Appendix, ***24***)
This measures the hydrocarbons, higher aliphatic alcohols, sterols, pigments and vitamins because they cannot be saponified by caustic alkalis.

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

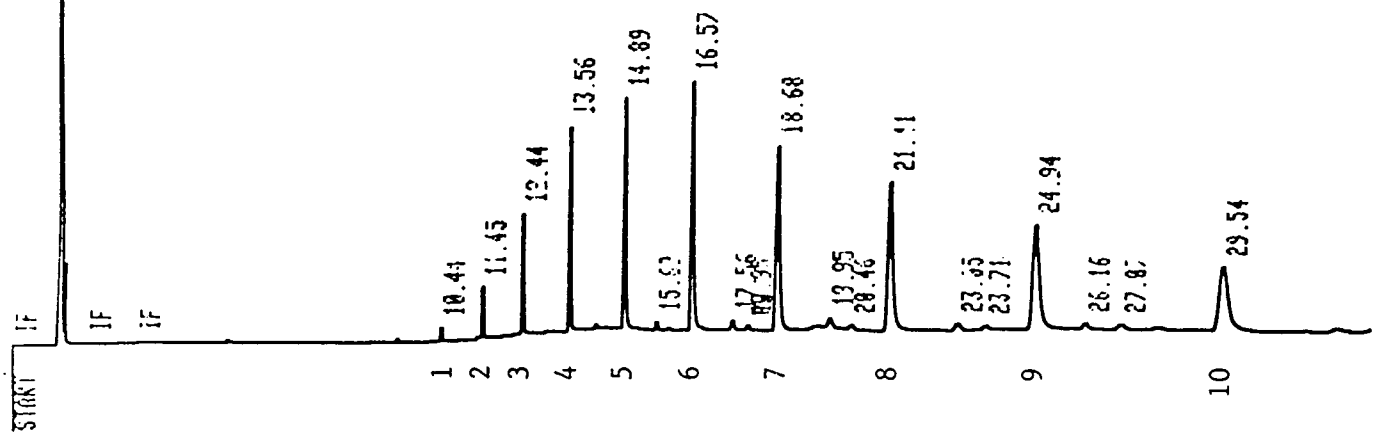
4.1.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a very suitable technique for separating different compounds in natural waxes.

The use of a flame ionization detector (TLC-FID) enables triglycerides, fatty alcohols, hydrocarbons and others to be separated.

4.1.2 Gas Chromatography

Gas chromatography of the hydrocarbons present as unsaponifiables and the separated fatty acids , or methyl esterification (Figure 2.1), identifies the wax. The analysis may be done using a Carbowax type column or silicone and using helium as a gas carrier and F.I.D. detector.



[1] C_{22} Hydrocarbon; [2] C_{23} Hydrocarbon; [3] C_{24} Hydrocarbon [4] C_{25} Hydrocarbon; [5] C_{26} Hydrocarbon; [6] C_{27} Hydrocarbon; [7] C_{28} Hydrocarbon; [8] C_{29} Hydrocarbon; [9] C_{30} Hydrocarbon; [10] C_{31} Hydrocarbon

Figure 2.1 *Gas Chromatogram of Candelilla Wax.* Conditions: *Column: F.F.A.P. 25 mts 0.3 mm ID; Split: 1/100 Helium; Detector: F.I.D. Initial temp.: 100°C; Rate: 10°C/min; Final temp.: 220°C;Final time: 20 min; Inject. temp: 250°C; Detect. temp: 250°C*

4.2 Spectroscopy

4.2.1 Infrared Spectroscopy

Each functional group in organic chemistry has characteristic absorption bands in infrared spectroscopy so that identification can be made of the main functional groups in waxes: i.e. fatty acids, fatty alcohols, esters and hydrocarbons. Figure 2.2 shows an infrared spectrum of beeswax. See also Appendix, ***30***.

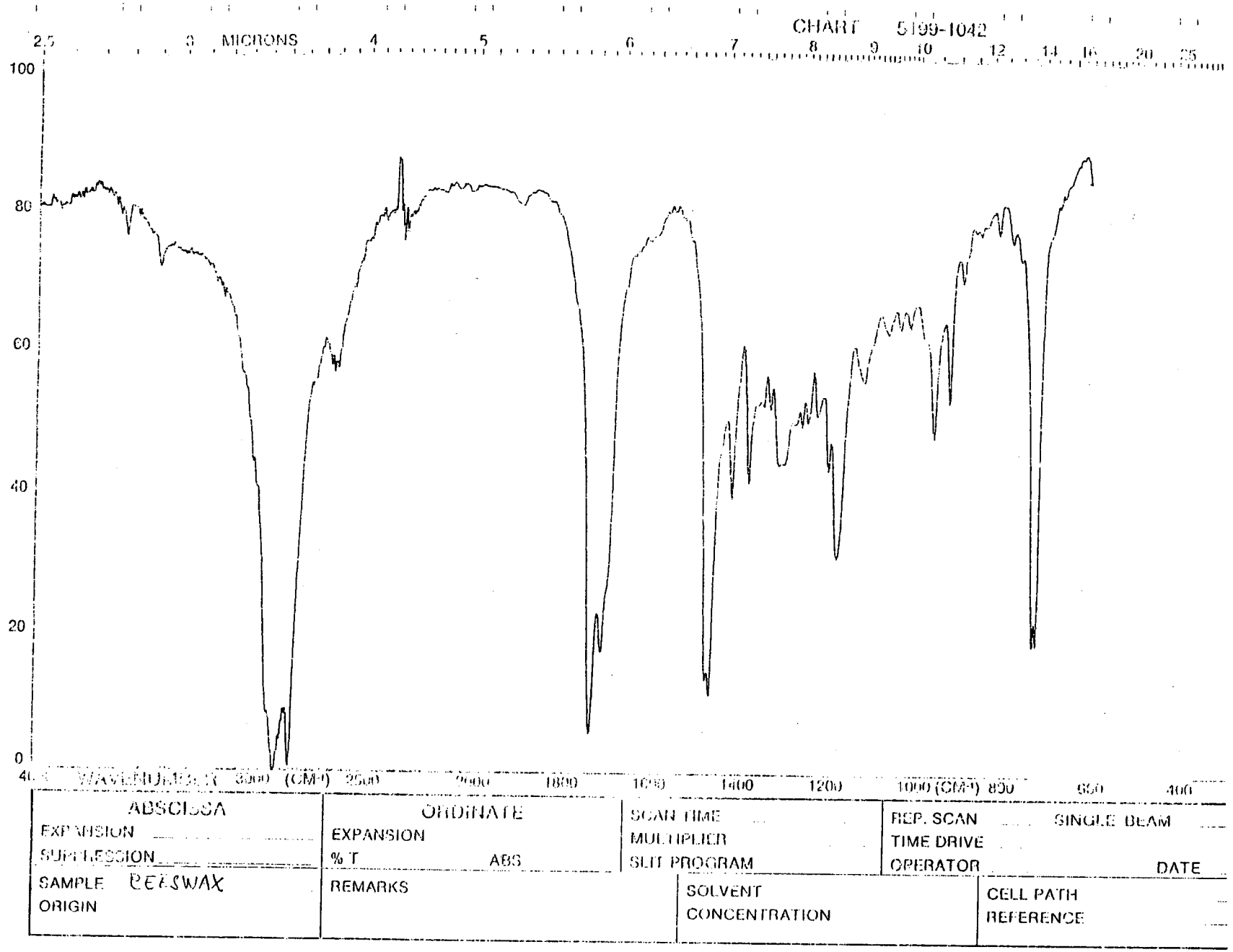


Figure 2.2 *IR Spectrum of Beeswax*

5 QUALITY ASSESSMENT AND FUNCTIONAL SUITABILITY

The quality of a wax is determined not by one analytical value but by the total results of all those described in the international compendiums. Not only must the quality be assessed but the following tests allow for an assessment of the successful function and suitability of the wax for the prospective cosmetic formulation.

5.1 Organoleptic and Physical Characteristics

The characteristics under ¶3.1 when compared with a standard sample indicate quality.

5.2 Unsaponifiable Fraction

The unsaponifiable fraction, when different from standard, is a measure of adulteration.

5.3 Peroxide Value (see Appendix, *25*)

Assuming that the material contains peroxides or other similar compounds are products of oxidation, the peroxide value can give a measure of the extent of the oxidation processes which cause rancidity in waxes containing unsaturated fatty acids.

5.4 Moisture

Traces of water in fatty materials, even just moisture content, can cause hydrolysis producing:

a) by-products of low molecular weight with a high olfactory level

b) free fatty acids which may accelerate auto-oxidation.

5.5 Ash

Inorganic impurities remain in the ashes after burning fatty materials. If these are metallic compounds, they could act as catalysts for auto-oxidation processes.

6 DETERMINATION OF WAXES IN FINISHED COSMETICS

6.1 Separation of Oil-Soluble Materials

An organic solvent (*n*-hexane) is used to extract the oil-soluble materials and the extract is evaporated to dryness in an oven at 105°C. The emulsion formed between the water and fatty phase can be broken down by the addition of electrolytes (sodium chloride) to make the separation easier.

6.2 Analysis of the Dry Extract from ¶6.1

The analysis is carried out by the procedures in ¶4.1 and ¶4.2. If hydrocarbons or volatile esters are present they can be directly determined by gas chromatography and compared with internal standards for quantification. In most cases, the interpretation may be difficult depending on the complexity of the formulation.

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Division 3: Hydrocarbons

Tasuku Takamatsu

1 CHEMICAL DESCRIPTION AND RAW MATERIALS CLASSIFICATION

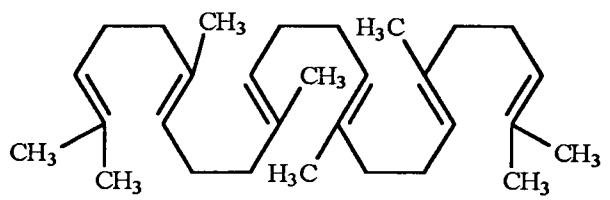
Hydrocarbons, as the name suggests, are organic compounds which contain only carbon and hydrogen atoms in the molecule.

1.1 Structure

The molecules can be saturated or unsaturated, open-chain (alkane, aliphatic or fatty) or closed-chain (alicyclic or aromatic):

open-chain	C_nH_{2n+2}	$C_nH_{2n+2-2x-4y}$
closed-chain	C_nH_{2n}	$CnH_{2n-2x-4y}$
	saturated	unsaturated

where n = no. of carbon atoms, x = no. of double bonds and y = no. of triple bonds



Squalene

1.2 Origins

Most of the hydrocarbons that are used in cosmetic formulations are saturated by-products of the petroleum industries, e.g. light and heavy paraffin oils, and paraffin wax. Other sources are derived from animals or plants; e.g. squalene from shark liver oil and beta-carotene found in crude palm oil. The petroleum industry by-products are sometimes known as mineral oils and waxes to distinguish them from those derived from animals or plants. They have been classed as oils, fats or waxes according to their physical state such as volatility, viscosity and appearance, which are described below. The lower members are gases and with increasing molecular weight are light to heavy oils, then semi-plastic e.g petrolatum (petroleum jelly) to waxy materials. Paraffin waxes are classed according to their melting points and these should be specified when used in a cosmetic formulation. (See Division 2 for the chemistry of true waxes which consist of fatty acid esters of fatty alcohols.)

1.3 Uses In Cosmetics

The saturated mineral hydrocarbons are very unreactive in nature, and can therefore be refined easily and obtained in colourless, odourless and tasteless form and used in cosmetic products in fairly large quantities as so-called base ingredients.

Squalane, $C_{30}H_{62}$, is a typical hydrocarbon obtained by the direct hydrogenation of squalene, $C_{30}H_{50}$ (see above), a main constituent of shark liver oil. Its main use in cosmetics is as a skin lubricant and similar, in this respect, to mineral oils. Lower hydrocarbons, such as *n*-butane, isobutane or their mixtures, have been used in aerosol cosmetics as propellants. Although the analytical methods are not included in this monograph, the ingredients are listed in the following section.

1.4 Raw Materials and References

Table 3.1 References to Hydrocarbons

Raw Material	Compendium[1]
Liquid Hydrocarbons	
Deodorized Kerosene	CTFA
Light Isoparaffin	CLS/JSCI
Light Liquid Isoparaffin	CLS/JSCI
Light Liquid Paraffin	CLS/JSCI
Mineral Oil	CTFA; CLS/JSCI
Pristane	CLS/JSCI
Squalene	CLS/JSCI
Squalane	CTFA; CLS/JSCI
Paste/Solid Hydrocarbons	
Petrolatum	CTFA; CLS/JSCI
Paraffin Wax	CTFA; CLS/JSCI
Microcrystalline Wax	CTFA; CLS/JSCI
Ozokerite	CLS/JSCI
Ceresin	CLS/JSCI
Polymer Hydrocarbons	
Polyethylene	CLS/JSCI
Polyisobutene	CLS/JSCI
Polyisoprene	CLS/JSCI
Propellant Hydrocarbons	
Isobutane	CLS/JSCI
Isopentane	CLS/JSCI
Liquefied Petroleum Gas	CLS/JSCI
Propane	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

2 PHYSICAL AND CHEMICAL ANALYSES FOR IDENTIFICATION AND QUALITY

The physical properties of hydrocarbons are very important for their identification and quality control. As hydrocarbons are chemically inactive, chemical tests do not usually provide information except with regard to impurities. The only useful chemical constants may be iodine values which indicate the presence and degree of unsaturation and the acid value which may indicate impurities. Many physical constants have been employed to ensure the quality and repeat effect of these raw materials when used in finished cosmetic products. For example, viscosity, specific gravity and refractive index are used for liquid hydrocarbons, and melting and setting points, softening point, solidification point, hardness, penetration and shrinkage tests, etc. for paste to solid hydrocarbons. See the Appendix for these tests methods and definitions.

3 MODERN TECHNOLOGICAL ANALYSIS FOR IDENTIFICATION AND QUALITY

3.1 Chromatography

3.1.1 Thin-Layer Chromatography

Thin layer chromatography (TLC) can be used for identification, although it cannot differentiate between the materials chemically categorized in this raw material class; and in addition, the hydrocarbons usually advance to the solvent interface even with development by the least polar solvent such as *n*-hexane and petroleum ether. The indicator to be used is an iodine vapour or phosphomolybdic acid. In order to separate unsaturated hydrocarbons from saturated ones, the silica gel TLC plate is impregnated with silver nitrate.

3.1.2 Gas Chromatography

Gas chromatography (GC) is the best tool for a pattern identification of the hydrocarbons, although it does not usually result in a complete separation even with a capillary column, particularly in the case of petroleum-derived hydrocarbons, which are the mixtures of normal paraffin, isoparaffin, olefin, cycloparaffin, etc. The mixture of normal paraffins gives a baseline separation. On the other hand, the presence of a large amount of isoparaffin, cycloparaffin, etc. in a gas chromatogram of microcrystalline wax is indicated by an appreciable, but slow, drift of the baseline. This comparison can be seen in Figure 3.1. The identified peaks by the normal paraffin standards provide a distribution of carbon numbers and the peak area composed by a baseline drift may be an index of the amount of isoparaffin, cycloparaffin, etc. present.

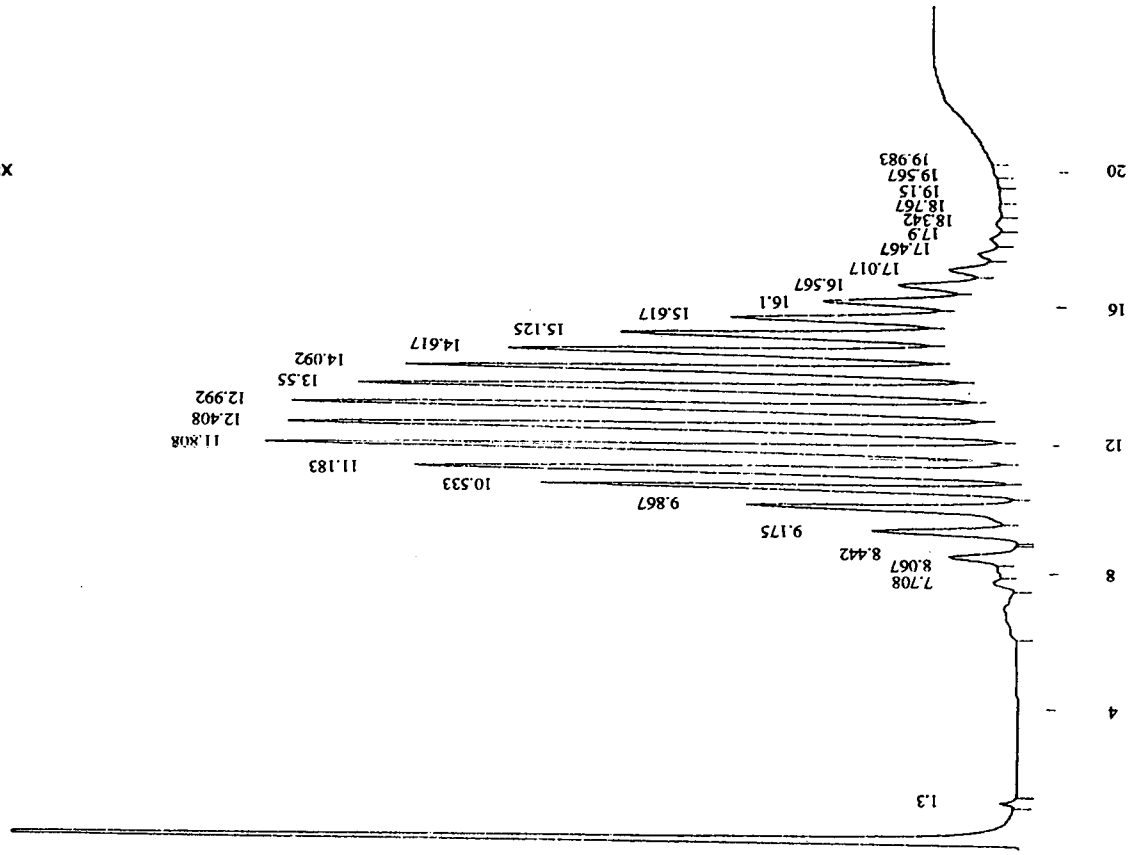
Pattern analysis is better performed by GC analysis with a packed column, because this method does not need too much resolution. In order to separate the normal paraffin from isoparaffin, although such separation is not a complete one, either adsorption by molecular sieve or urea adduction can be employed, which makes gravimetric determination possible and the subsequent GC analysis easier. Figure 3.2 is an example of molecular sieve separation of petroleum-derived wax.

GC can be used in quantitative analysis of certain hydrocarbons such as pristane, squalane etc., which are fairly pure compounds.

3.1.3 Gas Chromatography/Mass Spectrometry (GC-MS)

A structural elucidation of each constituent of petroleum-derived hydrocarbons could be achieved by GC combined with mass spectrometry (GC-MS). However, a fragmentation of an MS spectrum is difficult to interpret without well established standard samples. Separation by a capillary GC column is required to make interpretation of the MS spectrum easier. Figure 3.3 shows a typical capillary GC chromatogram of mineral oil, and, as a comparison, a separation by the packed column.

Microcrystalline Wax



Normal Paraffin Mixture

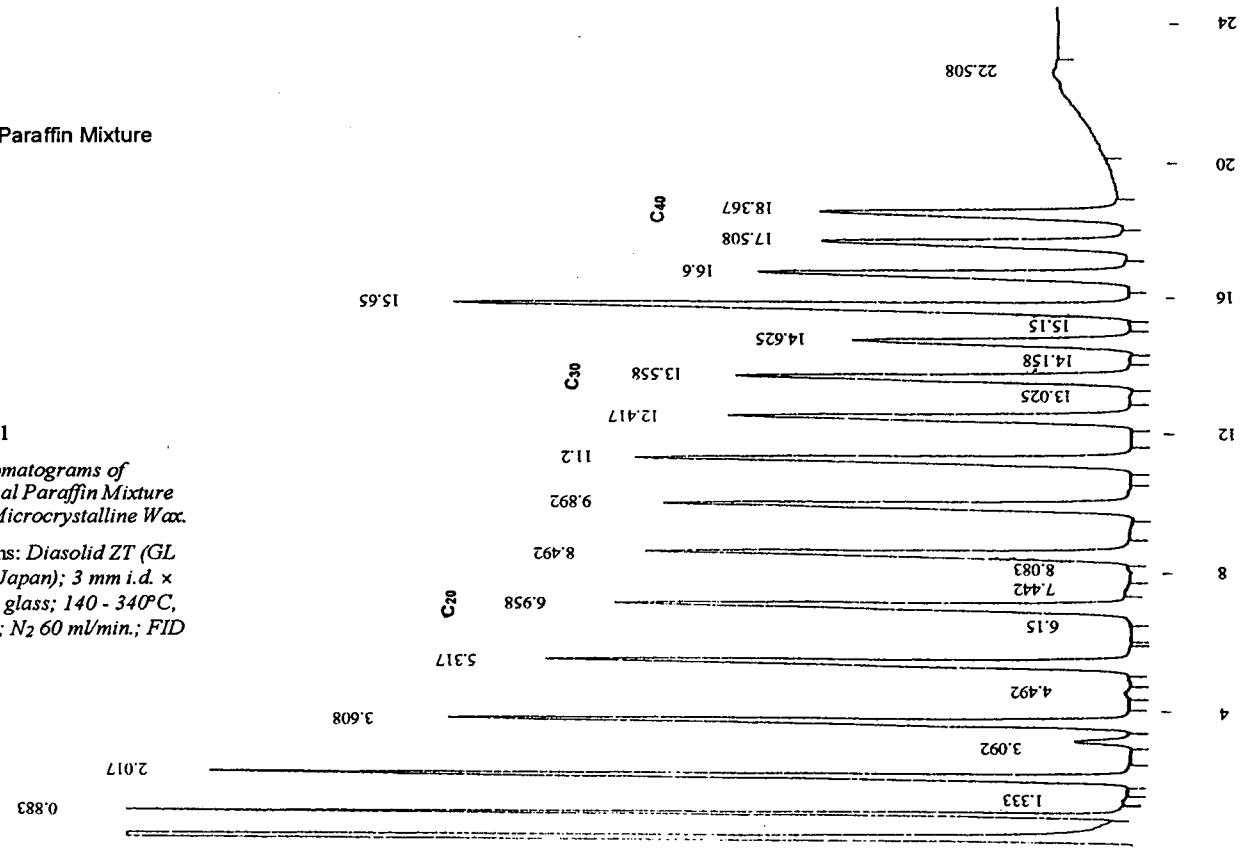


Figure 3.1

Gas chromatograms of (a) Normal Paraffin Mixture and (b) Microcrystalline Wax.

Conditions: *Diasolid ZT (GL Science, Japan); 3 mm i.d. × 0.5 m L.; glass; 140 - 340°C, 8°C/min.; N_2 60 ml/min.; FID*

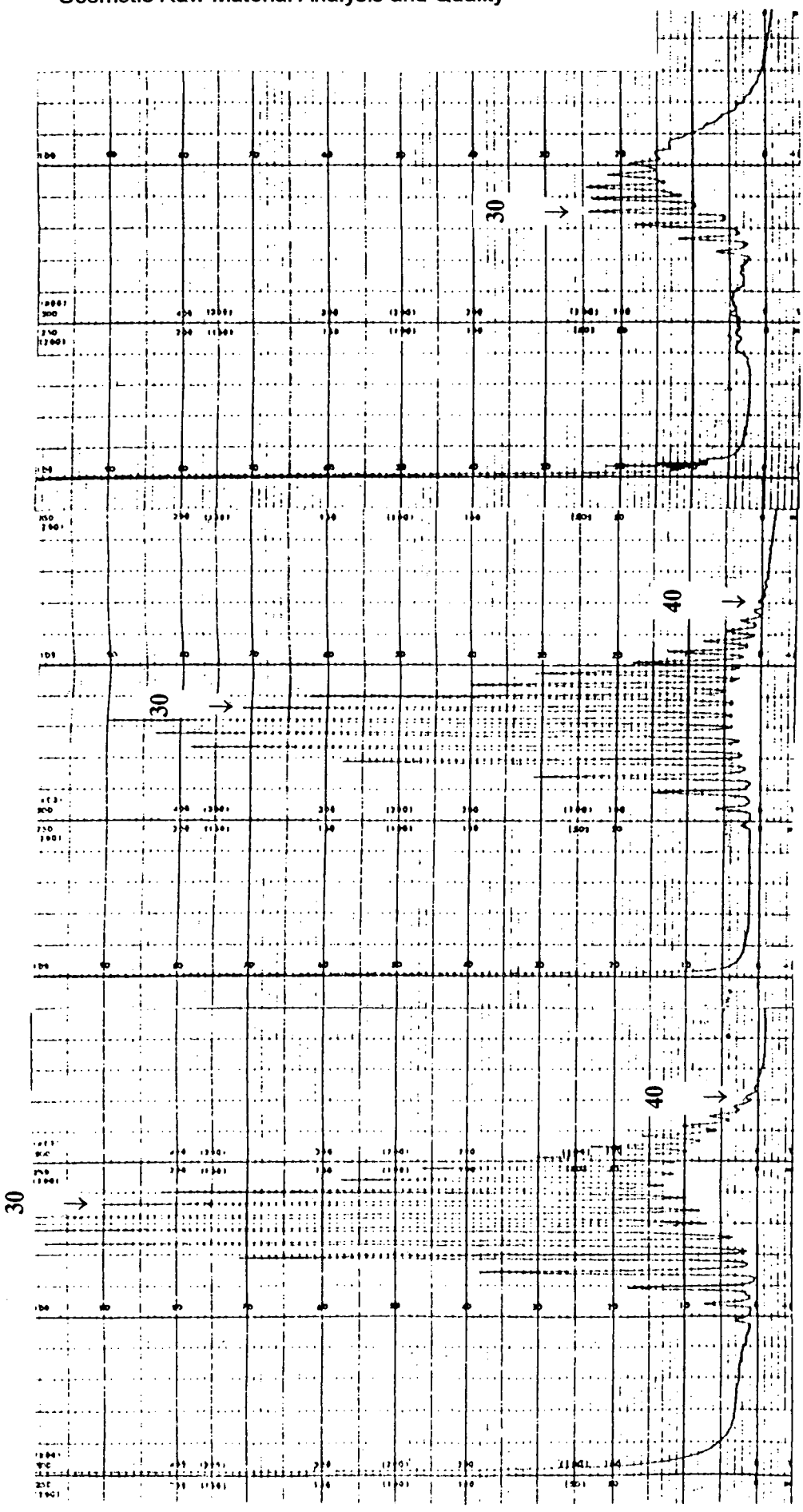
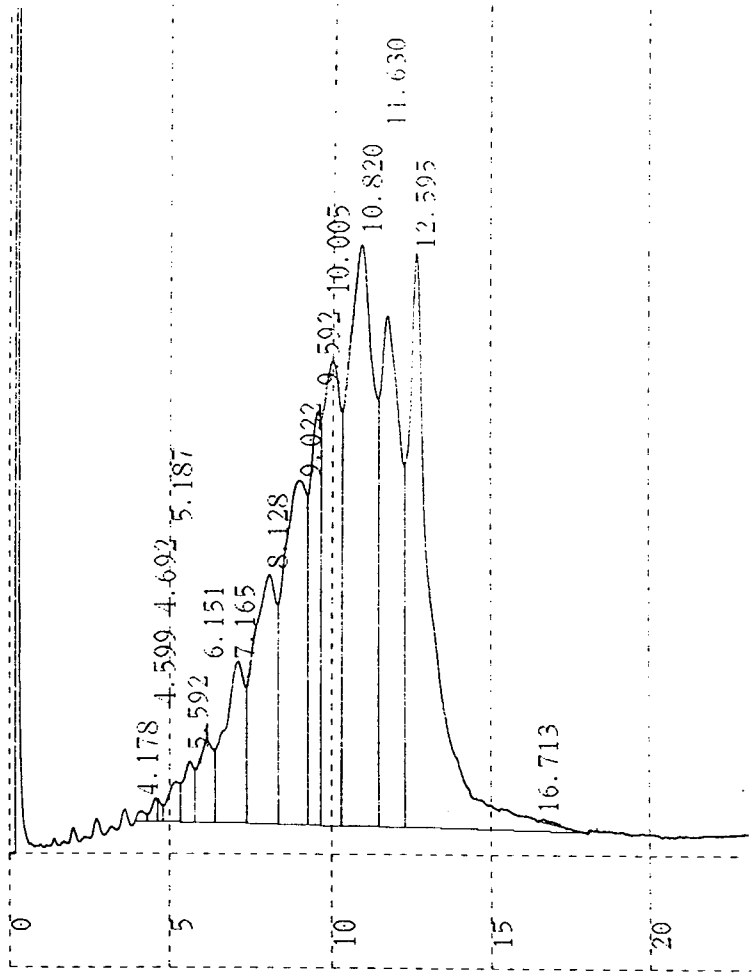


Figure 3.2

Gas chromatograms of petroleum-derived wax after molecular sieve separation.

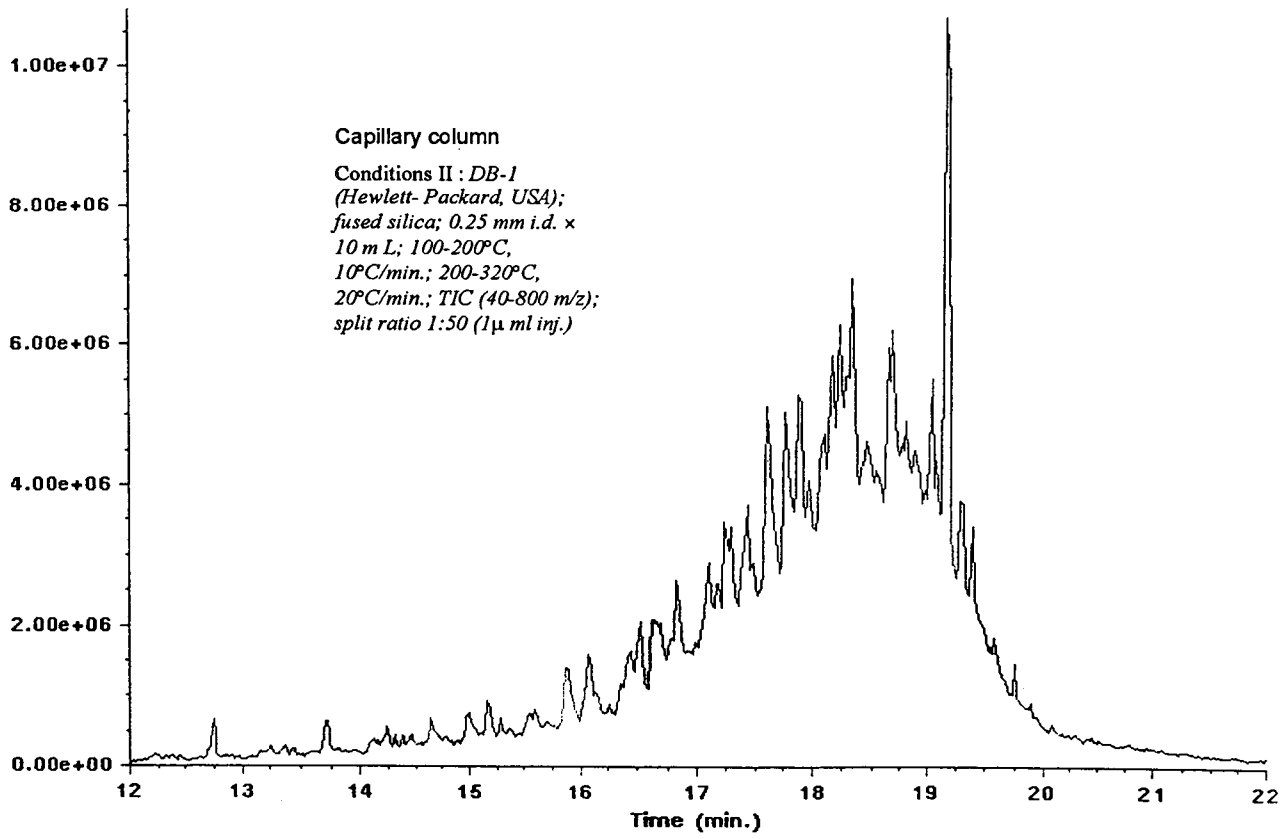
Figure 3.3

Gas chromatograms of mineral oil by capillary and packed columns



Packed column

Conditions I : *Diasolid ZT (GL Science, Japan); 3 mm i.d. x 0.5 m L.; glass; 140-340°C; 8°C/min., N_2 60 ml/min.; FID*



Capillary column

Conditions II : *DB-1 (Hewlett- Packard, USA); fused silica; 0.25 mm i.d. x 10 m L; 100-200°C, 10°C/min.; 200-320°C, 20°C/min.; TIC (40-800 m/z); split ratio 1:50 (1µ ml inj.)*

3.2 Spectroscopy

3.2.1 Infrared Spectroscopy

Infrared spectroscopy (IR) can also be used as an identification test for hydrocarbons by confirming several characteristic absorptions for alkyl chains (2920 and 2850 cm^{-1}) and methylene chains (1470, 1390-1200 and around 720 cm^{-1}). Unsaturated hydrocarbons can be distinguished by absorption at 2980 cm^{-1} shown on the shoulder of 2920 cm^{-1} absorption and also at 880 cm^{-1}. When the hydrocarbons are crystalline, many absorption peaks are observed, depending upon their carbon numbers, which appear between 1390-1200cm^{-1} and their crystalline structure is also judged from absorption around 720cm^{-1}, which appears in doublet for crystalline and in singlet for either paste or liquid hydrocarbons.

3.2.2 ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) is no more advantageous for hydrocarbon analysis than other analytical methods. However, ^{1}H- and/or ^{13}C-NMR signals relating to (CH)=(CH), CH_2-(CH)-CH_2 provide information regarding the presence of unsaturated or branched-chain structures. Nevertheless, especially for a pure compound such as squalane, the ^{13}C-NMR spectrum gives a characteristic pattern for identification.

4 FURTHER TESTS FOR QUALITY AND IMPURITIES

4.1 Chemical Constants

Tests may be useful to check the neutrality of the material for any acids or alkalis remaining which were used during the refinement process. In such cases, the tests are usually conducted as limit tests, but not as tests for chemical constants.

The hydrocarbon samples are dissolved in hot ethanol and the neutrality of the solution or the separated ethanol layer is assessed. For the unsaturated hydrocarbons such as squalene or its unsaturated impurities, the iodine value can be used.

The brown ring test
Petroleum-derived hydrocarbons are usually tested by heating small samples with concentrated sulfuric acid in a test-tube. In the presence of unsaturated, aromatic fatty chemicals and nitronaphthalene as impurities from a petroleum source, and also oils and waxes from other sources, a dark-brown ring appears at the interface of the sample and the sulfuric acid. The lighter the colour, the fewer the impurities.

4.2 Loss on Ignition

Inorganic impurities can be examined by gravimetrically in the residue from the sample after ignition or heating with sulfuric acid: this method is only applicable for such impurities at a higher level than 0.1%. If hazardous inorganic impurities are to be examined at very low level such as 1-10 ppm, specific test methods have to be established, for example, arsenic (As) and lead (Pb) by atomic absorption spectroscopy.

4.3 Other Impurities

Other impurities that are generally tested for in the petroleum-derived hydrocarbons are sulfur compounds and polynuclear aromatic hydrocarbons, both of which are contained in

crude petroleum oil and are removed through refinement processes. Sulfur compounds are examined by colouring reactions with lead monoxide in sodium hydroxide solution. The test method employed for polynuclear aromatic hydrocarbons is UV spectrophotometry.

4.4 Physical Constants

No other quality criteria for impurities can be considered at this moment, other than those described in the previous paragraphs, but hydrocarbons have been used in large amounts as the base ingredients in many different cosmetic products and their physical properties greatly affect the physical properties of the finished products. Since chemical analysis does not usually give much information, other than that of carbon chain distribution, measurements of physical constants have been used effectively as quality criteria, as described in ¶2.

5 ANALYTICAL METHOD FOR HYDROCARBONS IN COSMETIC PRODUCTS

5.1 Solvent Extraction

The first step to be taken in the analysis of hydrocarbons in finished cosmetic products is to separate the oil-soluble materials from the cosmetic products, which can be done as follows.

The product is first evaporated to dryness in an oven (105°C) or on a water bath and oil-soluble materials are then extracted with organic solvent such as *n*-hexane, benzene or chloroform. The separation of the extract from the residue is done by filtration, centrifugation or other physical means and then the extract is evaporated to dryness by appropriate measures for further analysis (see ¶5.2).

5.2 Silica Gel Column Chromatography

The oil-soluble portion of the cosmetic product prepared according to the method described in ¶5.1 can be subject to a silica gel column chromatography to isolate the hydrocarbons from other oil-soluble materials. For this separation, *n*-hexane or petroleum ether elution is usually employed and it can be done quantitatively, since the recovery of the hydrocarbons from silica gel column is almost 100%. For column chromatography, besides silica gel, many other adsorbents, such as alumina or Florisil, can be used. The isolated hydrocarbons can be identified by GC and MS as in ¶3.1.3.

If a pure hydrocarbon such as squalane is to be tested, it can be directly determined by GC analysis by dissolving the sample in an appropriate solvent and comparing it with an internal standard.

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Division 4 : Fatty Acids

Seung Jung Kim

1 CHEMICAL DESCRIPTION, STRUCTURE AND USE IN COSMETICS

Fatty acids are carboxylic acids with the general structure:

R-COOH

They are mostly obtained from natural sources, i.e. land and marine animal or vegetable fats and oils, by hydrolysis and subsequent purification. Those having alkyl chains shorter than seven carbon atoms are not considered 'fatty' acids. Natural fatty acids may be *saturated* or *unsaturated,* terms which divide them into two broad classes on the basis of the absence in the former or presence in the latter of double and triple bonds in their hydrocarbon chains. They generally contain an even number of carbon atoms though recent research has shown that a few with odd numbers are found in nature in small quantities in vegetable oils but are more common in fish oils.

The fatty acids most commonly encountered as solids or liquids in natural fats have common names related to their natural occurrence. They are obtained by processing selected fats and oils such as coconut, soybean, cottonseed, corn, and other vegetable oils, and beef tallow, where they are originally present as triesters of glycerol (triglycerides) which form the main constituents of the oils; up to 98% in refined oils and 95% in those unrefined.

Fatty acids are a common source of raw materials both as the free acids and for the synthesis of many cosmetic ingredients. They are used in cosmetics in the form of their salts, i.e., as soaps, which are frequently formed *in situ* from the acid and a suitable alkaline-metal or alkaline-earth. These salts are emulsifiers and suspending agents and some possess foaming characteristics which are desirable in shaving preparations and can be used in skin and hair cleaning products.

Free fatty acids are used to produce superfatted soaps and as opacifiers and thickening agents. Other physical properties such as oiliness, texture and melting point are useful not only in affecting the feel of a cosmetic on the skin but also the stability of an emulsion.

1.1 Saturated Fatty Acids

1.1.1 Normal Alkanoic Acids

The saturated fatty acids, if they contain no branching side chains, are referred to as normal alkanoic acids. The empirical formula for all of the members of the normal saturated fatty acid series is $C_nH_{2n}O_2$ where n may be any even or odd integer. Since all the acids of the series, except formic, consist of an alkyl chain and terminal carboxyl group, they may be conveniently represented by the formula RCOOH and all members above acetic by the formula $CH_3(CH_2)_nCOOH$. The lower members of the series are liquids at ordinary temperatures, but as the series is ascended the individual members become increasingly more viscous and ultimately crystalline solids. The lower members are soluble in water and exhibit weakly acidic properties compared to the strongly dissociated inorganic acids. All of the even-numbered acids from acetic to octatriacontanoic occur either in the free or combined state in nature, and all of them from butyric to octatriacontanoic are present either as glycerides or as monoesters in fats or waxes.

Until very recently only two fatty acids with an odd number of carbon atoms had been reported to be constituents of natural fats. These were valeric and margaric. The former was subsequently proved to be an iso-acid and the latter a mixture of palmitic and stearic acids. However, in the past decade all of the odd-numbered acids from pelargonic to nonadecanoic have been reported to be minor components of a number of fats including human hair fat,

ox fat, and fish liver oils. It is probable that an increasing number of such acids will continue to be reported from a greater diversity of sources. Listed below in Table 4.1 are some cosmetic raw materials which fall into the category of normal alkanoic acids with their common names together with the available official compendiums in which they occur

1.1.2 Alkyl Alkanoic Acids

In recent years increasing numbers of branched-chain acids have been identified from natural sources, including wool fat,[1] butter fat,[2] mutton fat[3] and ox perinephric fat.[4] A large number of such acids has also been prepared synthetically. Low molecular weight branched-chain acids (less than 7 carbon atoms) have been isolated from petroleum[5] and higher ones are believed to exist.

Most of the naturally occurring branched-chain acids have only a single branching alkyl group, generally methyl, but several polyalkyl branched acids have been isolated and others have been prepared synthetically. Wherever branched-chain acids have been found to occur in natural fats, it is in very small amounts, except in the case of isovaleric acid (dolphin and porpoise oils, hop oil and tobacco). The origin and natural function of these acids are obscure.

1.1.3 Monohydroxy Alkanoic Acids

Investigations during the past decade have indicated that hydroxy acids, both saturated and unsaturated, occur more frequently as constituents of natural lipids than was formerly thought to be the case. The lower molecular weight hydroxy acids are found in nature but not as constituents of fats and waxes. The most widely occurring of the lower acids is lactic, an acid of considerable commercial importance. Higher molecular weight hydroxy acids have been shown to be present in various natural waxes, for example, beeswax, caranday, candelilla, carnauba, licuri or ouricury, conifer waxes, jalop, tree bark waxes and wool wax, as constituents of brain lipids (cerebrosides), and in some seed fats. Small amounts are also found unexpectedly in fats and oils, probably as a result of atmospheric oxidation.

Occasionally they may form precipitates in fatty oils after long standing especially when exposed to light and elevated temperatures.

1.1.4 Polyhydroxy Alkanoic Acids

Compared with the number of known natural and synthetic monohydroxyalkanoic acids, only a few polyhydroxy saturated acids are known. Of these even fewer have been found as constituents of natural fats.

Certain polyhydroxy acids, such as: dihydroxyacetic, $(HO)_2CHCOOH$, in reality the hydrated form of the aldehyde acid, glyoxylic, OCHCOOH; glyceric acid, $HOCH_2CHOHCOOH$; α-,γ-dihydroxybutyric acid, $HOCH_2CH_2CHOHCOOH$; and the various sugar acids (pentonic, hexonic, etc.) are of interest to the organic and biological chemist. With few exceptions our knowledge of the higher molecular weight, polyhydroxy fatty acids has been obtained from studies of the oxidation of unsaturated fatty acids. The vicinal dihydroxy fatty acids and tetra- and hexahydroxy fatty acids are formed by oxidation at the double bonds of unsaturated acids with potassium hydroxide or osmium tetraoxide under mild conditions.

1.1.5 Ketoalkanoic Acids

Few ketoalkanoic acids have been isolated from natural sources and none is of commercial importance.

1.1.6 Alkanedioic Acids

The alkanedioic or dicarboxylic acids, although not common constituents of fats and waxes, are nevertheless of considerable importance in the chemistry of the fatty acids. The lower members of the alkanedioic acid series are found in some plants, especially in foliage and roots, both free and in the form of potassium and calcium salts. Intermediate and higher members of the series have been reported as constituents of certain waxes (Japan wax, sumach berry wax) and natural resins. The alkanedioic acids form a series corresponding to the general formula $(CH_2)_n(COOH)_2$. In the lowest member of the series, oxalic or ethanedioic, the value of n is zero. The esters of succinic and adipic acids are useful in cosmetics.

1.2 Alkenoic Unsaturated Fatty Acids

A considerable number of unsaturated acids exists in nature, especially in fats and waxes, that are differentiated from those in the previous division by the presence of one or more double or triple bonds in their carbon skeletons. In addition, several series of polyunsaturated acids containing one to six centres of unsaturation occur in nature.

The presence of carbon-carbon unsaturated bonds in these acids markedly alters their chemical and physical properties compared with those of their saturated counterparts. In some, these linkages impart special biological activity and to others they impart properties which are of utilitarian value from the industrial point of view.

Because of the large number and diversity of the unsaturated fatty acids it is necessary to group them into classes for ease of description and comparison of their chemical and physical properties. The alkenoic (ethylenic, olefinic) acids have been classified on the basis of the number of carbon to carbon double bonds in the normal carbon chain, and are designated as monoalkenoic (monoethenoic); alkadienoic (dienoic, diethenoic); alkatrienoic (trienoic, triethenoic); etc.

1.2.1 Monoalkenoic Acids

This group encompasses the largest number of known naturally occurring and synthetic unsaturated acids. The majority of the unsaturated acids that have been isolated from plant and animal sources have a double bond between the ninth and tenth carbon atoms. Of these, oleic, $C_{18}H_{34}O_2$, and palmitoleic, $C_{16}H_{30}O_2$, are by far the most commonly occurring and best known of this series. Their wide distribution in nature and frequency of occurrence make them comparable with stearic and palmitic acids, the predominant acids of the saturated alkanoic series.

1.2.2 Alkadienoic Acids

One series comprises the di-unsaturated or alkadienoic acids, also referred to as dienoic, diethenoic, and diolefinic acids. Though the number occurring in natural fats is small compared to the number of monoalkenoic acids found in 1.2 (a), the structures of the former are less diverse than those of the latter.

Except for sorbic acid, which occurs in nature but not as a constituent of natural fats, most of the naturally occurring acids of this group contain 18 or more carbon atoms. The best

known and most widespread of these acids is linoleic, the presence of which in sufficient proportions confers siccative properties to an oil.

1.2.3 Alkatrienoic Acids

The normal alkatrienoic (triethenoic, trienoic) acids are characterized by the presence of three double bonds in the hydrocarbon chain. With few exceptions these acids are found only in vegetable oils. Because of their relatively high degree of unsaturation they readily undergo oxidation and polymerization when exposed to air and elevated temperatures.

1.2.4 Alkatetraenoic Acids

With one exception, the known alkatetraenoic (tetraethenoic, tetraenoic) acids are found only in animal fats and principally in marine animal oils. The ready oxidation of fish oils is due to the presence of these and more highly unsaturated acids containing 16 to 26 carbon atoms, but principally to those with 20 and 22 carbon atoms.

1.2.5 Other Alkenoic Acids

Several other kinds of ethylenic unsaturated fatty acids exist. These are alkapentaenoic acids, monoalkyl alkenoic acids, polyalkyl alkenoic acids, hydroxy alkenoic acids, keto alkenoic acids and epoxy alkenoic acids, etc,.

1.3 Other Fatty Acids

1.3.1 Alkynoic (Acetylenic) Unsaturated Fatty Acids

The known naturally occurring alkynoic (acetylenic, ethynoic) acids comprise a group of acids having hydrocarbon chains containing one or more triple bonds, double and triple bonds, or double and triple bonds and a hydroxyl group. In addition to several natural acids, an extensive number of related acids have been prepared synthetically, especially for use as intermediates in the synthesis of keto acids.

1.3.2 Miscellaneous Commercial Fatty Acid Blends

A number of other acids are available which are prepared from natural oils by chemical processing - Hydrogenated Coconut Oil, for example, by hydrogenation.

1.3.3 Alicyclic-Substituted Fatty Acids

A number of acids having a five-carbon ring attached at the terminal or ω-carbon atom of a saturated or unsaturated aliphatic chain have been found in natural fats. These acids are confined principally to the group of oils known collectively as chaulmoogra oils which have been used in various forms for the treatment of human leprosy. At least seven such acids have been isolated from natural sources and a large number of related acids have been prepared synthetically.

1.4 Raw Material and References

Table 4.1 References to Fatty Acids (technically pure)

Raw Material	Formula	Appearance	Compendiums[1]
Alkanoic acids			
Capric Acid	$CH_3(CH_2)_8COOH$	crystalline solid	CLS/JSCI
Lauric Acid	$CH_3(CH_2)_{10}COOH$	solid	CTFA; CLS/JSCI
Myristic Acid	$CH_3(CH_2)_{12}COOH$	waxy solid	CTFA; CLS/JSCI
Palmitic Acid	$CH_3(CH_2)_{14}COOH$	waxy solid	CTFA; CLS/ JSCI
Isostearic Acid	$(CH_3)_2CH(CH_2)_4COOH$	liquid	CTFA; CLS/JSCI
Hydroxystearic Acid	$CH_3(CH_2)_5CHOH(CH_2)_{10}COOH$	solid	CLS/JSCI
Stearic Acid	$CH_3(CH_2)_{16}COOH$	waxy solid	CTFA; CLS/JSCI
Behenic Acid	$CH_3(CH_2)_{20}COOH$	waxy solid	CLS/JSCI
Lignoceric Acid	$CH_3(CH_2)_{22}COOH$	solid	CLS/JSCI
Alkenoic acids			
Oleic Acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	liquid	CTFA; CLS/JSCI
Linoleic Acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	liquid	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

Table 4.2 References to Fatty Acids (natural blends)

Raw Material	Compendium
Coconut Acid	CTFA; CLS/JSCI
Corn Acid	CTFA
Cottonseed Acid	CTFA
Lanolin Acid	CTFA; CLS/JSCI
Safflower Acid	CLS/JSCI
Tall Oil Acid	CTFA
Tallow Acid	CLS/JSCI

2 PHYSICAL AND CHEMICAL PROPERTIES

2.1 Physical Properties

The physical properties of fatty acids are of practical importance for a number of reasons. Many technical applications of fatty materials, including their uses in edible products, depend on the oiliness, surface activity, solubility, melting behaviour, or other physical properties peculiar to long alkyl chain compounds. In the manufacture of commercial fat products, heat treatment or other purely physical treatment, with or without accompanying phase transformations, is a common adjunct to chemical processing.

Recent years have seen physical methods of testing and analysis replace less accurate and more time-consuming chemical methods, and in some cases such methods serve as powerful tools for the extraction of information unobtainable through a strictly chemical approach.

Perhaps the most important group of physical properties are those associated with solid-liquid and liquid-solid phase changes, or the process of melting and solidification. [6,7]

2.2 Chemical Properties

Fatty acids containing eight or more carbon atoms are capable of undergoing most of the reactions of the simpler members of the series such as acetic or propionic acids. Owing to the decreasing water solubility of the higher members of the series, conditions must be changed (i.e., high pressure or solvent solution) for conventional reactions to take place.

The main functional group of fatty acids is the carboxyl group which when reduced gives fatty alcohols; reaction with halides gives alkyl acid chlorides; and with ammonia alkyl amides are formed. Esterification occurs with alcohols, glycols and glycerol giving in the latter case tailor-made glycerides useful as emulsifiers.

Gaseous hydrogen in the presence of a catalyst (nickel, platinum or palladium) adds to the double bond transforming unsaturated acids into partially or completely saturated acids – so called "hardened" acids. In general other acids with extra functional groups exhibit, in addition, the properties of those groups.

Soaps are made from fatty acids in a continuous process integrating the initial step of high pressure fat splitting and fatty acid distillation with subsequent saponification and processing of neat soap.

3 PHYSICAL AND CHEMICAL ANALYSES FOR IDENTIFICATION AND QUALITY

3.1 Physical Tests

3.1.1 Appearance (see Appendix, *1*).

To match standard .

3.1.2 Colour (see Appendix, *2*)

With the use of efficient fractional distillation technology, the colour of fatty acids is frequently improved over that of the parent fats and oils; therefore, more lighter-shade colour-test methods are usually required for fatty acids than for fats and oils. Older methods, such as the Gardner and Lovibond systems still find use, although in recent years there appears to have been an emphasis for the use of the platinum-cobalt scale and the photometric index systems of colour definition.

The ASTM recommends the platinum-cobalt colour test method in its Method D 1209 for fatty acids in protective coatings. The most recent test method for fatty acid colour is the most scientific method since it eliminates human judgment; it is also precise and rapid. AOCS Method Td 2a-64 (Reapp. 73) is the photometric index method. The colour of fatty acids is designated as a photometric index expressed as 100 × the absorbance at each of two wavelengths, 440 and 550 nm. It is being used increasingly in the specification literature of United States fatty acid producers.

3.1.3 Odour (see Appendix, *3*)

Although the need for organoleptic evaluations of fatty acid odour is, in general, less critical than for fats and oils, the demand for product stability, accompanied by a requirement for bland-smelling products, is increasing for fatty acids also. This is probably a result of their use in cosmetics and toiletries, and also in food, where their use in food additives continues to grow.

3.1.4. Viscosity and Oiliness (see Appendix, *4*)

Viscosity is a measure of internal friction in molecules. Dunstan, Hilditch, and Thole[8] noted that a plot of the logarithm of the viscosities of saturated fatty acids against their molecular weights was approximately linear.

Viscosities of binary, ternary, and quaternary mixtures of C_{12}-C_{18} fatty acids obey the "principle of congruence" in that the viscosity of each mixture depends only on the average chain length.[9]

3.1.5 Surface and Interfacial Tension (see Appendix, *5*)

Surface tension increases with increase in chain length and decreases with increase in temperature and is essentially linear. The interfacial tension also decreases with increase in temperature.

3.1.6 Density and Expansibility (see Appendix, *6*)

The density of both fatty acids and glycerides is greater the lower their molecular weight and the higher their unsaturation.

3.1.7 Melting Points (see Appendix, *8*)

The melting points of fatty acids increase with increasing chain length and decrease as the acids become more unsaturated. The melting points of the component fatty acids are reflected in a general way in the melting points of their methyl and ethyl esters, mono-, di-, or triglycerides, and fatty alcohols. Fatty acids are *polymorphic.*

3.1.8 Titre (see Appendix, *10*)

Titre, defined as a solidification point under somewhat empirical test conditions, is probably the oldest and most generally used characteristic for both fatty acids and fats and oils, although it is now apparent that its use is declining somewhat, at least for fatty acids. The general test methods are AOCS Tr la-64 (Rev.78) and ASTM D1982. As applied to fatty acids it is not necessary to saponify, acidulate and dry the fatty acids, as is the case with titre determination of fats and oils. However, if it is suspected that the sample contains fat or monoglyceride, the saponification step must be included. Rarely, however, is this the case, and it is usually necessary only to filter and dry the sample before measurement, with care taken not to reheat more than once.

3.1.9 Softening Point (see Appendix, *9*)

A ring-ball method for the determination of softening point (often referred to as melting point), as in ASTM D 36, is intended for bituminous materials, but is also suited for fatty acid pitch.

3.1.10 Thermal Properties

3.1.10.1 Heat Stability (see Appendix, *17*)

The measurement of colour development as a function of heating the fat.

3.1.10.2 Heat of Combustion (see Appendix, *12*)

The heats of combustion of the saturated fatty acids increase with increase in the chain length of the acids, and vary from about 24700 kJ/kg (5900 cal/g) for butyric acid to about 37300 kJ/kg (8900 cal/g) for lauric, 40200 kJ/kg (9600 cal/g) for stearic and 41000 kJ/kg (9800 cal/g) for behenic acid. Data for unsaturated acids are sparse, and purity of the fatty acids is not always high; thus the results given for them should be accepted with reservation. Values for the unsaturated acids are slightly lower than for saturated acids of the same chain length, about 39600 kJ/kg (9450 cal/g) being recorded for oleic acid and 39100 kJ/kg (9350 cal/g) for linoleic acid.

3.1.10.3 Specific Heat (see Appendix, *12*)

Fats or simple derivatives such as fatty acids or methyl esters at temperatures just above their melting points have specific heats of about 0.5 or somewhat higher.

3.1.10.4 Vapour Pressure and Boiling Points (see Appendix, *13*); *Heat of Vaporization* (Appendix, *12*)

Vapour pressure, boiling point, and heat of vaporization are among the most important properties of fats and their derivatives, both theoretically and practically. It is only since the 1930s that systematic and reliable determinations have been made of these most basic and important properties.

Fatty acids are even more volatile than the corresponding mono- and triglycerides, and they can be distilled readily under reduced pressure. Large quantities of fatty acids are purified on a commercial scale by vacuum distillation with or without the assistance of steam. Fractional distillation is a convenient tool for separating fatty acids that differ in chain length by two or more carbon atoms. Fatty acids do not, however, behave as ideal mixtures and show deviations from Raoult's law.[10,11]

3.1.11 Smoke, Fire, and Flash Points (see Appendix, *14*)

These are useful temperatures to record when identifying fatty materials or assessing their behaviour for storage or use.

3.1.12 Solubility and Miscibility (see Appendix, *15*)

At temperatures above their melting points, fatty acids are miscible in all proportions with many organic solvents, such as hydrocarbons, esters, ethers, ketones and chlorinated solvents.

3.1.13 Refractive Index (see Appendix, *16*)

It is not only useful for identification purposes and for establishing purity, but also for observing the progress of reactions of fatty acids, such as catalytic hydrogenation and isomerization. The relationship between the refractive index and the structure and composition of fatty acids and glycerides may be generalized as follows:

i. The refractive indices of fats and fatty acids increase with increase in the length of the hydrocarbon chains, but the difference between adjacent members becomes smaller with increase in molecular weight.

ii. The refractive indices of fats and fatty acids increase with number of double bonds and with increase in conjugation.

3.2 Chemical Tests for Identification and Quality

3.2.1 Moisture (see Appendix, *18*)

The Karl Fischer method of water analysis eliminates the need for heat and is rapid and convenient.

3.2.2 Iodine Value (see Appendix, *19*)

AOCS Method Tg la-64 (Rev. 73) is the Wijs method. As with the method used with normal fats and oils, so for fatty acids the excess of reagent used in the test is critical. Between 100 and 150% excess of reagent is used for normal fatty acids and 115 to 135% excess reagent for conjugated acids.

It cannot be assumed that the iodine value of fatty acids containing conjugated unsaturation is that due to the total unsaturation present: α-β unsaturated acids are known to show incomplete addition of iodine.

3.2.3 Unsaturation by Hydrogenation

With fatty acids the use of catalytic hydrogenation is sometimes preferred where interfering substitution reactions are suspected in the determination of unsaturation by halogen addition.

3.2.4 Thiocyanogen Value (see Appendix, *20*)

Prior to 1939 this value was much used in conjunction with iodine value determination to estimate oleic, linoleic, and linolenic acid contents in many vegetable-derived fatty acids, but the absorption of thiocyanogen is now known to be less than that originally thought, and it was largely displaced in the 1960s by the ultraviolet spectrophotometric method (AOCS Method Tj 1a-64 (Reapp. 73), and the latter, in turn, has been largely supplanted by the GLC method (AOCS Method Cc 2-66 or ASTM D 1983).

3.2.5 Acid Number (Acid Value) and Free Fatty Acid (see Appendix, *21*)

Acid number determination is perhaps the most frequently used test method applied to fatty acids. When the acid number is determined on samples free of inert or neutral matter, the information obtained is of value in indicating the mean molecular (equivalent) weight of the fatty acids present.

When only fatty acids are present in the sample (fractionated fatty acids frequently meet this requirement), the average chain length is revealed from the acid number, and the technologist has a potential clue to the origin of the sample. The acid number has its greatest value, however, when it is used in conjunction with other supporting analytical data such as saponification number, unsaponifiables, and homologue distribution (GLC analysis) for a more complete indication of the nature and composition of the sample.

AOCS Method Te la-64 (Reapp.73) and ASTM D 1980 are the two standard test methods for the determination of acid number. They are applicable to all fatty acids and polymerized fatty acids.

3.2.6 Saponification Number (Value) and Ester Number (Value) (see Appendix, *22*)

Principally, saponification numbers are useful in providing information relating to the proportion of glycerides and acids that are present in a given sample. Used in conjunction with acid number, this indication is of optimum usefulness; the combined data are indicative of the mean molecular weight of the acids in the sample. Short-chain acids such as lauric acid have high saponification numbers, whereas longer-chain acids such as stearic acid give correspondingly lower numbers.

When the saponification number is expressed as percent oleic acid, it is occasionally known as *total fatty acid*. The term *saponification equivalent* is sometimes used and represents the number of grams of sample that will react under these saponification conditions with 56.1 grams of potassium hydroxide. Expressed this way, the numerical results are a measure of the average equivalent weight of the fatty acids. The saponification number is identical to the acid number when the fatty acid is entirely free from ester-like contaminants. The difference between the saponification number and the acid number is called the ester number (or ester value) and for fatty acids commercially available today this number is rarely more than a point or two, with the saponification number higher than the acid number.

When applied to certain fatty acid derivatives, such as dimer acid esters, the saponification number determinations are carried out in higher boiling solvents such as ethylene glycol.

The AOCS recommends that Method Tl la-64 (Corr.79), originally intended for industrial oils and derivatives, be used for fatty acids. The ASTM equivalent method is D 1962.

3.2.7 Hydroxyl and Acetyl Numbers (see Appendix, *23*)

The hydroxyl number is of use in the analysis of crude or partially esterified fatty acid-alcohol mixtures, the analysis of hydroxy-substituted fatty acids, (e.g., hydroxystearic acid), and the analysis of partially esterified polyhydroxy compounds, among others. AOCS Method Cd 13-60 (Reapp.73) and ASTM D 1957 are applicable to hydroxystearic acids and many other hydroxy-containing substances.

The older acetyl number (value), as outlined in AOCS Method 4-40 (Reapp. 73) and intended for acetylated fats and oils, is rarely applied to fatty acids, as the utility and the need are completely satisfied by the hydroxyl number determination.

3.2.8 Unsaponifiables (see Appendix, *24*)

Unsaponifiables are materials found in fatty acids and oils, eg, aliphatic alcohols (C_{12} and higher), sterols, methyl sterols, natural hydrocarbons such as squalene, long-chain alcohols, triterpene alcohols, pigments, and other trace materials that cannot react with caustic alkalies but are soluble in ordinary solvents. They are to be considered impurities in certain cases.

Their analysis and assay depend on the fact that they are soluble in the common low-boiling "fat solvents" and they are determined by methods based on extraction of them from the unsaponifiable matter left as residue in the saponification of the fat products.

There are six AOCS standard methods for the determination of unsaponifiable matter that use various solvents depending on the content and the nature of the unsaponifiable matter. American Oil Chemists' Society Method TK la-64 (Reapp. 73) or ASTM Method D 1965, intended originally for tallow and grease fatty acids, is recommended for all acids, drying oils and polymerized fatty acids but, in practice, has not been applied generally to all members of the fatty acid class. In this method seven successive extractions of the alcoholic aqueous saponification medium are carried out with petroleum ether (AOCS Specification H 2-41) or until such time as extractions afford less than 0.005 g residue. A correction for any free fatty acid content of the combined residues is made.

3.2.9 Fatty Acid Composition

The fatty acid composition of fatty acid mixtures with 8-24 carbon atoms can be determined for both saturated and unsaturated fatty acids by GLC (AOCS tentative method Cel-62).

Fatty acid composition, although not normally a specification of fatty acids, is now routinely employed to describe the properties of fatty acids. Knowledge of acid composition has been available since the early forties through the use of high-vacuum fractional distillation for the separation of saturated and monoenoic fatty acids and ultraviolet spectrophotometry for the more unsaturated acids. Methods for determinations of physical and chemical properties of fatty acids are also described by ASTM in the Standard Methods (for Analysis of Fats and Oils) and by the Association of Official Analytical Chemists.

The melting points of fatty acid mixtures vary according to the chain lengths, crystalline structures, and proportions of saturated fatty acids in the mixture. Crystalline structures are termed polymorphic since they are easily altered by heat treatment. Fatty acid mixtures with the same titre may have varying iodine values. For instance, pure oleic acid has the same iodine value (*ca.* 90) as a 1:1 mixture of stearic (iodine value = 0) and linoleic acids (iodine value = 180).

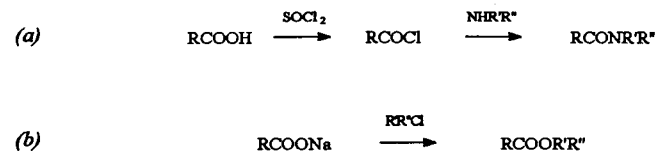
Both acid and saponification values are measures of average molecular weight. However, mixtures of fatty acids can differ widely in composition yet yield very similar values. These values are also influenced by the unsaponifiable content and cannot be relied upon for quantitative information concerning composition.

3.2.10 Further Chemical Tests for Identification

A great number of carboxylic acids can be identified by conversion to derivatives which have a characteristic colour or melting point. Cheronis, Entrikin, and Hodnett[12] describe in detail the reactions for preparing the derivatives of the carboxyl group.

Generally, these derivatives fall into four classes: (a) amides and substituted amides; e.g., 1- and 2-naphthylamides, *p*-bromoanilides, *p*-toluidides, which are formed by the reaction of the carboxyl group with ammonia or amines; (b) esters e.g., *p*-bromophenacyl esters, formed by the reaction of the arylalkyl halides and the sodium salts of the acids; (c) salts of the carboxylic acids with bases; e.g., 2,4-dinitrophenyl hydrazides; and (d) a variety of derivatives, e.g., S-benzylthiouronium, formed by reaction of the carboxyl group with various functional groups.

The most frequently used derivatives belong to the first two classes (a) and (b); examples are given below. In (a), in order to form amides and substituted amides, the carboxylic acids are first converted to the acid chloride by treatment with thionyl chloride and then reacted with ammonia or an amine:



4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

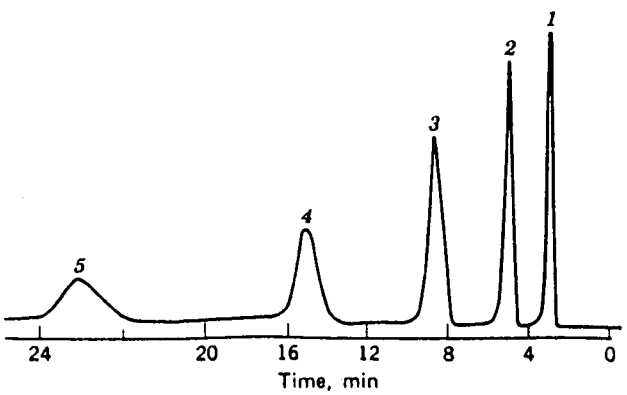
4.1.1 Thin-Layer Chromatography

When qualitative information is required about a complex mixture of carboxylic acids, thin layer chromatography may be the best method to obtain it. For example, a mixture of fifteen carboxylic acids was separated by a method involving a two-dimensional system.[13] Electrophoresis in a formic acid buffer in the first dimension was followed by thin layer chromatography in isoamyl alcohol saturated with formic acid in the second dimension. Upon completion of the process, the carboxylic acids were detected by observation under ultraviolet light.

4.1.2 Ion-Exchange Chromatography

Aliphatic carboxylic acids are generally weakly adsorbed by ion exchange resins. Nevertheless strong cation exchange resins are suitable for their separation from one another. Samuelson[14] found that the adsorption of aliphatic monobasic acids on sulfonated phenol-formaldehyde type resins increases with increasing chain length.

Weakly basic anionic exchange resins are useful for the separation of strong mineral acids from organic acids. Strongly basic anion exchange resins retain all acids; the weaker organic acids can then be eluted by displacement with stronger acids.



1. Capric Acid; 2. Lauric Acid; 3. Myristic Acid; 4. Palmitic Acid; 5. Stearic Acid.

Figure 4.1

Chromatogram obtained in the analysis of C_{10} - C_{18} aliphatic mono-basic acids on a packed 4 ft. x 1/4 in. OD column containing 20 wt% diethylene glycol adipate and 3 wt% phosphoric acid on 60/80 mesh Chromosorb W; helium carrier gas, 55 ml/min at column outlet; thermal conductivity detector; temperatures: injection block 265°C, column 222°C, detector 225°C.

Source: L.D.Metcalfe, *J. Gas Chromatography*, 1(1), 7 (1963)

Courtesy Preston Technical Abstracts Co.

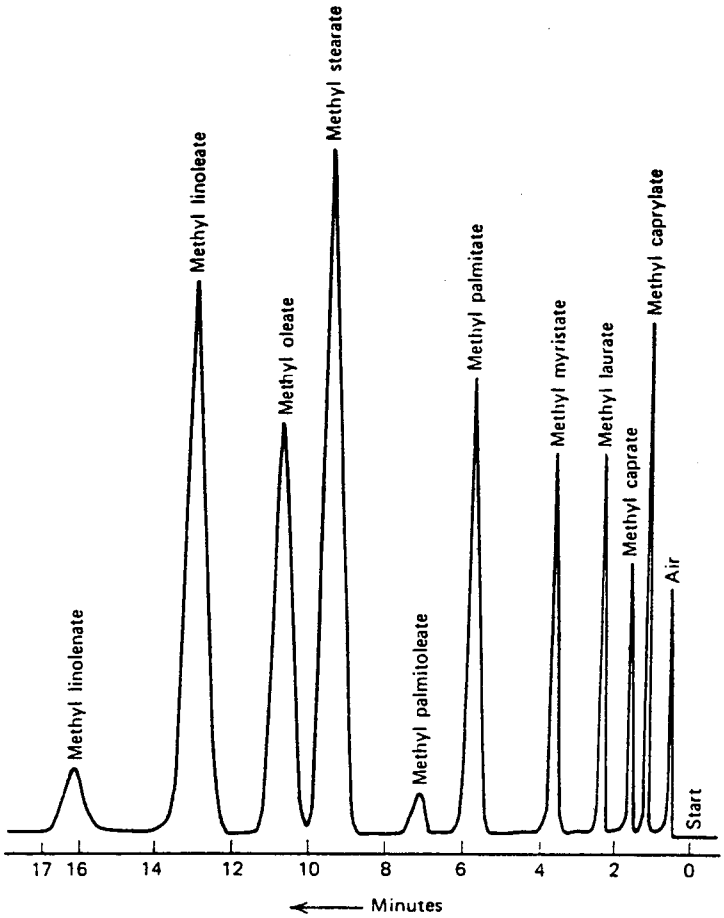


Figure 4.2

Analysis of a mixture of fatty acid methyl esters.

Conditions: *2 m × 1/4 in. OD packed column containing 20% Reoplex 400 on Celite 60/80 mesh; column temperature 210°C; thermal conductivity detector.*

4.1.3 Gas Chromatography

Although the first publication of gas chromatography by James and Martin[15] dealt with the analysis of free fatty acids through C_{12}, carboxylic acids are now seldom analysed in free form by this technique; they are usually converted to their methyl esters prior to analysis. There are three reasons why the analysis of carboxylic acids is more difficult than the analysis of the methyl esters.

First, the volatility of the carboxylic acids is lower than that of the methyl esters; secondly, there is a significant tendency for carboxylic acids to react or interact with the surfaces of the chromatographic support or even with the interior surfaces of the structural material of the column; finally, lower carboxylic acids have a tendency for dimerization. A good compilation of the problems of carboxylic acid analysis by gas chromatography is given by Supina.[16]

4.2 Spectroscopy

4.2.1 Infrared Spectroscopy

Carboxylic acids are easily identifiable by infrared spectroscopy. Table 4.2 lists the type of characteristic groups analysed and the corresponding frequencies. As this table indicates, these frequencies are associated either with the hydroxyl or with the carbonyl group. Because

of abnormally strong hydrogen bonding, the O-H stretching vibrations are distorted and thereby typical for carboxylic acids. Since free carboxylic acids are generally present as dimers, the carboxyl frequencies are different from those normally encountered.

Structural changes in the α and β positions to the carboxyl group can be detected but are not so clearly identifiable as with the ketones.

4.2.1 Ultraviolet Spectrophotometry

Although ultraviolet absorption of unconjugated unsaturated compounds has limited utility, examples of which will be given later, the greatest utility of this type of absorption, as well as visible absorption spectroscopy, is limited to the study of compounds containing conjugated bonds.

Table 4.3 Characteristic Groups and the Corresponding Infrared Frequencies

Group	Frequency range, cm^{-1}
carboxylic acid	
OH stretching vibrations (free)	3560-3500
OH stretching vibrations (bonded)	2700-2500
C=O vibrations	
saturated aliphatic acids	1725-1700
α-halogen substituted aliphatic acids	1740-1720
α,β-unsaturated acids	1715-1690
aryl acids	1700-1680
acids showing internal hydrogen bonding	1670-1650
C-O stretching vibrations or OH deformation	1440-1395
vibrations	1320-1211
OH deformation (out of plane)	950-900
	1610-1550
	1420-1300

Observation of the ultraviolet spectrum of any fatty acid, ester, glyceride, or natural product from which these compounds are derived can answer such questions as: (a) Does the sample contain any unsaturated conjugated material? (b) If conjugated substances are present, what is the order of conjugation? (c) If there is evidence of diene conjugation, what does Woodward's rule imply about the nature of the substituents? According to this rule, the position of maximal absorption of a normal diene can be calculated simply by adding 5 nm to the position of the maximum for butadiene, CH_2=CH-CH=CH_2, (*ca.* 217 nm) for each substituent, and 5 nm for each exocyclic bond present in the diene system. Extension of the conjugated system to a trienoic conjugation, -C-C=C-C=C-C=C-, results in a shift in the absorption to about 268 nm, while a tetraenoic system absorbs at about 315 nm, pentaenoic at about 346 nm, and hexaneoic at about 374 nm (d) If triene conjugation is detected, is this

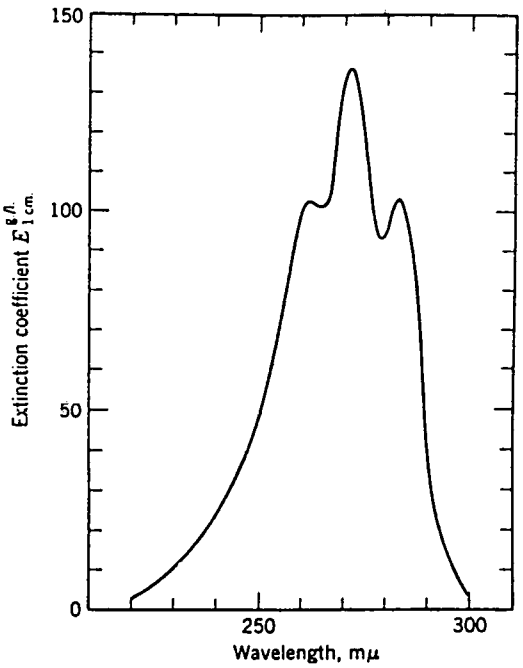


Figure 4.3

Spectral absorption curve (ultraviolet), American tung oil in cyclohexane

conjugation in a single ring, i.e., is the material aromatic or aliphatic ? (e) If diene conjugation involves a nonaromatic ring, what do the extensions of Woodward's rule imply concerning the possibility of the unsaturation being entirely within the ring, esocyclic on the ring, or exocyclic to it? It is apparent that any property of matter that can be utilized to answer such questions would find several applications in the field of fatty acid chemistry.

Unsaturated fatty materials absorb light in the ultraviolet region of the spectrum. This region is between about 200 and 400 nm, the lower value being set by the limitation of ordinary available instruments. In the case of nonconjugated and saturated materials, the absorption is weak and general and cannot be used for analytical purposes; ultraviolet spectral data on numerous nonconjugated compounds have been reported.[17-19] Conjugated double bonds, however, give rise to intense, well defined absorption maxima which permit quantitative determination of conjugated compounds in mixtures. 9,11-Octadecadienoic acid has a strong absorption band at about 233 nm. A typical ultraviolet absorption curve for tung oil, which is rich in eleostearic (9,11,13-octadecatrienoic) acid, is shown in Figure 4.3.[20] The major

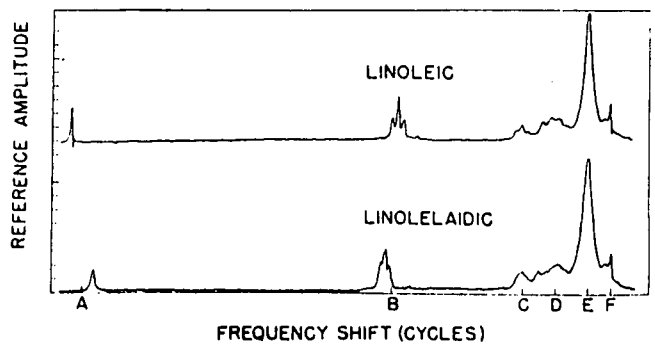


Figure 4.4

Nuclear magnetic resonance spectra of linoleic and linolelaidic acids

(Courtesy J.N.Shoolery and J.E.Callan)

absorption peak is at about 270 nm with two others at about 260 and 280 nm. Parinaric acid with four conjugated double bonds shows similar intense absorption but at somewhat longer wavelengths.

4.2.3 Fluorescence Spectroscopy

The fluorescence excited by ultraviolet radiation has been suggested as an aid in the identification of fats and oils. For this purpose the fluorescent colours of several fatty acids have been compiled. For some of the more common acids these are: formic: pale lilac; acetic: colourless; propionic: colourless; butyric: weak yellow; caproic: red violet; lauric: vivid violet; palmitic: pale yellow-grey; stearic: white with a violet tinge; oleic: strong violet; erucic: vivid violet; and lactic: pale lilac. No particularly useful application seems to have been made of this property. The colours of the fluorescence of natural oils depend upon that of the acids which they contain and these have been used for the detection of adulterants or contaminants.

4.7. Nuclear Magnetic Resonance Spectroscopy

In Figure 4.4 the high resolution nuclear-magnetic resonance spectra of linoleic and linolelaidic (*cis, cis*-9,12- and *trans, trans*-9,12-octadecadienoic) acids are reproduced. The peaks represent the protons with different types of chemical bondings and the area below each peak reflects the number of protons in each type of chemical bonding.

5 TESTS FOR IMPURITIES

5.1 Trace Metals (See Appendix, *35*)

Recent developments in the technique of atomic absorption analysis have been extraordinary. This technique offers accurate, rapid analysis of trace metals without the need for time-consuming ashing steps. The AOCS offers two atomic absorption spectrophotometric methods for trace metals.

5.2 Analytical Methods to Detect Unwanted Constituents (not Necessarily Toxic)

The natural constituents in fat and oil products that are objectionable for reasons other than for toxicity include those that occur as fatty acid components of the glycerides and those that are non-oil in chemical nature. Among the former are cyclopentenoid fatty acids such as those found in chaulmoogra and gorliseed oils; erucic acid in certain rapeseed oils; and *trans*-unsaturated, polymerized, and oxidized oils. The analytical methods that find largest use in the separation and identification of these components are the GLC methyl ester methods (ASTM D 1983 and AOCS Method Ce 2-66) and other separation techniques. Varying degrees of objection to non-oil minor constituents are manifest. The contaminants include moisture, nonsaponifiable matter, trace metals, ash, rosin and stilbenes in tall oil or its fatty acids, waxes, phosphorus and sulfur compounds, chlorophyll, β-carotene, and a few others.

5.3 Unsaponifiable Matter

Impurities in fatty acids outlined in ¶3.2.8 which are not normally found in a standard sample and could be considered as adulteration.

6 ANALYTICAL METHOD FOR FATTY ACIDS IN COSMETIC PRODUCTS

Chloroform Extractable Material: The investigation of the substances that can be extracted with chloroform permits the identification and determination of the most important components present in creams and lotions. The procedure consists of successive extractions; it is outlined below. It is applicable only to soap emulsions because other types of emulsifiers, such as alkyl sulfates, quaternary ammonium compounds, complex silicates, and polyoxyethylene-type surfactants, may emulsify the chloroform.[21]

Procedures

Weigh a 3 g sample into a 250 ml separatory funnel, add 50 ml of water, acidify with concentrated hydrochloric acid, and extract four times with 35 ml portions of chloroform. Combine the chloroform extracts in a second separatory funnel and wash once with 10 ml of water. Add the washing to the extracted aqueous solution and save. Filter the chloroform extracts through a cotton plug into a tared 250 ml beaker, evaporate to dryness on a steam bath with the aid of a stream of air, dry for 10 min at 105°C, cool, weigh and express as chloroform extractable material. The chloroform extracts all fatty materials but not such substances as alkanolamines, inorganic salts, and polyhydroxy compounds.

Chloroform Extract. To the chloroform extractable material, add 25 ml of 95% ethanol, 1 g of potassium hydroxide, and 50 ml of benzene, and saponify by standing for 2 hr on a steam bath. Transfer the entire mixture to a separatory funnel having a teflon stopcock. Add 50 ml of hot water, shake well, and draw off the aqueous layer into a second separatory funnel. Extract the aqueous solution twice with 50 ml portions of hot benzene. Reserve the extracted aqueous solution.

Combine the benzene extracts, and wash three times with 30 ml portions of 3:7 ethanol-water. Shake very gently during the first washing to avoid emulsification. Add the washings to the reserved extracted aqueous solution. Filter the washed benzene extract through a cotton plug into a tared 250 ml beaker, evaporate to dryness on a steam bath with the aid of a stream of air, and dry for 10 min at 105°C. Cool, weigh, and express as unsaponified material. Run an infrared spectrum and look for hydrocarbons and alcohols. Dissolve a weighed portion of the unsaponified material in warm heptane and allow to cool to room temperature. If there is any precipitate, filter through a filter paper, washing the paper with heptane. Dissolve the residue on the filter paper by passing hot chloroform through the paper into a tared beaker.

Evaporate to dryness, weigh, and express as heptane-insoluble alcohols. Separate the substances present in the heptane solution by column chromatography, on an alumina column. Acidify the reserved extracted aqueous solution with concentrated hydrochloric acid and extract with three 30 ml portions of chloroform. Test an aliquot of the extracted aqueous solution for glycerol. Carry out the test for polyoxyethylene compounds as outlined below from another aliquot of the extracted aqueous solution. Wash the combined chloroform extracts with water. Filter the washed chloroform extract through a cotton plug into a tared 250 ml beaker, evaporate to dryness on a steam bath with the aid of a stream of air, and dry for 10 min at 105°C. Cool, weigh, and express the residue as fatty acids.

Identification. When the fatty acids have been separated, it often becomes necessary to identify them. In the case of the saturated acids, this is done preferably by determining the melting points, mixed melting points, and possible other physical constants of the acids or certain of their derivatives. The latter include amides, anilides, and hydrazides. Other reagents that have been used to give reaction products suitable for identification are phenacyl bromides, S-benzyldithiouronium chloride, and *o*-phenylenediamine. Monoethenoid acids

are often characterized as dihydroxy acids following oxidation with dilute alkaline permanganate or performic acid. Polyunsaturated acids are mainly identified as their bromides.

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Division 5: Fatty Alcohols

Roberto Leonardi

1 CHEMICAL DESCRIPTION, STRUCTURE AND USE IN COSMETICS

Fatty alcohols are represented by hydrocarbons in which a hydroxyl group replaces a hydrogen attached to a carbon atom:

ROH

1.1 Origins of the Types Used in Cosmetics

1.1.1 "Natural" Alcohols

"Natural" alcohols are so-called because they are mainly obtained by the reduction of fatty acids obtained from vegetable and animal organisms and, as a consequence of these natural sources, they usually consist of an even number of carbon atoms[1] with the OH group in position one of the alkyl chain. The alkyl chain can be linear or branched, saturated or unsaturated. An example of methyl branching is found in sebum.[2] When double bonds exist in nature the configuration is generally *cis*.

Hydrolysis of extracts of natural oils, fats and waxes (mainly triglycerides and esters) produces mainly fatty alcohols, fatty acids and glycerol. The fatty acids, or their methyl esters, obtained in this way are very often reduced to give alcohols.

Other "natural" fatty alcohols, – aliphatic, cyclic or aromatic – are derived from the transformation of petroleum (mineral oil) products. Though non-fatty, the alcohols with 1 to 4 linear chain carbon atoms, i.e. methyl, ethyl, propyl and butyl alcohols respectively, are, nevertheless, natural; they result from fermentation processes of materials such as sugar beet, grapes, cereals and sugar cane.

1.1.2 Synthetic Alcohols

Other alkyl linear or branched-chain alcohols are produced which consist of an odd number of carbon atoms. In addition the OH groups can be either primary, secondary or tertiary. Synthetic alcohols containing an even number of carbon atoms are also available.

1.1.3 Polycylic Alcohols

The other groups of alcohols used in cosmetics that are included in this division are sterols, other polycyclic alcohols and alcohols containing aromatic rings (see Tables 5.1 and 5.2). For example, the sterols are polycyclic alcohols of which the basic structure is cyclopentaneperhydrophenanthrene, and the most representative compound is cholesterol which is derived from lanolin.

The unsaponifiable fraction of natural oils and of wool fat yields sterols. Their biological structure derives from isoprenic units. Other alcohols can have different origins: for example, abietic alcohol originates from rosin.

1.1.4 Selected Alcohols

Other alcohols considered as preservatives, such as terpene alcohols of vegetable origin and alcohols with vitamin action, are not described in this division. (See, however, Volume VII).

1.1.5 Phenolic Alcohols

The last class of compounds are phenols which have the OH group linked directly to an aromatic ring.

1.2 Use in Cosmetic Products

The use of fatty alcohols in cosmetic products depends essentially on their molecular structure characteristics; for example, the following categories have distinctive properties:

(i) aliphatic short-chain alcohols

(ii) aliphatic long-chain alcohols

(iii) polycyclic alcohols (sterols and terpene alcohols)

(iv) other alcohols

1.2.1 Aliphatic Short-chain Alcohols

They are especially used as carriers of particular compositions (e.g. perfumes or aftershave lotions) and as solvents or antibacterials. *Ethanol* is an important commodity both as a solvent and an antiseptic agent. (See note under 1.2.c for its biological activity.)

1.2.2 Aliphatic Long-chain Alcohols

a) Formulation uses

They are used as stabilizers, emulsifiers and emollients and as substances to increase viscosity. In oil-in-water emulsions they are especially added as viscosity enhancers, but act mainly as co-emulsifiers being unsatisfactory alone. In water-in-oil emulsions they can be used as primary emulsifiers; very often they are useful also as solubilizers and dispersants: all these properties depend on their surfactant action.

b) Use in skin-care products

They are used as emollients or in lip products because of their particular velvety sensation on the skin.

1.2.3 Polycyclic Alcohols (Sterols and Terpene Alcohols)

Many of these alcohols are used both for their emollient and hydrating properties such as lanolin and particularly lanolin derivatives, such as lanolin alcohols which contain a minimum of 30% cholesterol, cholesterol itself and alcohols and the unsaponifiable fractions of many oils and fats.[3,4]

Note: Many research studies have been conducted on the biological activity of some compounds, particularly shorter-chained compounds and the sterols, e.g. ethyl alcohol and cholesterol; these properties can be partly used to substantiate their beneficial use in cosmetics.

Also, given the biological and toxicological potential of these substances, there are many enzymatic techniques reported that exploit the possibility of using these alcohols as substrate in biological reactions.[61,79,88,119-121,104,110]

1.2.4 Other Alcohols

The use of other alcohols varies according to their properties. Some are solvents, others substances with germicidal power (phenol) and yet others are useful additives, e.g. vitamins.

1.2.5 Cosmetic Applications

Table 5.1 Applications of Fatty Alcohols

Name (CTFA CID)	CAS number	Formula	Application
Abietyl Alcohol	666-84-2	$C_{20}H_{32}O$	
Alcohol (Ethanol)	64-17-5	C_2H_6O	Solvent, preservative
Behenyl Alcohol	661-19-8	$C_{22}H_{46}O$	Emollient, thickener
Benzyl Alcohol	100-51-6	C_7H_8O	
n-Butyl Alcohol	71-36-3	$C_4H_{10}O$	Solvent
t-Butyl Alcohol	75-65-0	$C_4H_{10}O$	Solvent
C9-11 Alcohols	68551-08-6		
C12-13 Alcohols			Emollient
C12-15 Alcohols			Thickener
C12-16 Alcohols	68855-56-1		Emollient, opacifier, thickener
C14-15 Alcohols			Emollient
C20-40 Alcohols			Binder, dispersant
C30-50 Alcohols			Binder, dispersant
C40-60 Alcohols			Binder, dispersant
Caprylic Alcohol	111-87-5	$C_8H_{18}O$	Solvent, thickener
Cetearyl Alcohol	8005-44-5		Emollient, emulsifier,opacifier,thickener
Cetearyl Alcohol	67762-27-0		
Cetyl Alcohol	36653-82-4	$C_{16}H_{34}O$	Emulsifier, emollient, opacifier, thickener
Cetylarachidol		$C_{36}H_{74}O$	
Cholesterol	57-88-5	$C_{27}H_{46}O$	Emollient
Coconut Alcohol	68425-37-6		Conditioner
Decyl Alcohol	112-30-1	$C_{10}H_{22}O$	Solvent, thickener
Decyltetradecanol	58670-89-6	$C_{24}H_{50}O$	Emulsifier
7-Dehydrocholesterol	434-16-2	$C_{27}H_{44}O$	
Dihydroabietyl Alcohol	26266-77-3	$C_{20}H_{34}O$	
Dihydrocholesterol	80-97-7	$C_{27}H_{48}O$	
Dihydrolanosterol		$C_{30}H_{52}O$	
Dodecyltetradecanol		$C_{26}H_{54}O$	
Hexyl Alcohol	111-27-3	$C_6H_{14}O$	Solvent, Thickener
Hexyldecanol		$C_{16}H_{34}O$	
Hydrogenated Tallow Alcohol			Thickener
Isocetyl Alcohol	36311-34-9	$C_{16}H_{34}O$	Emulsifier, dispersant, binder
Isopropyl Alcohol	67-63-0	C_3H_8O	Solvent
Isostearyl Alcohol	27458-93-1	$C_{18}H_{38}O$	Emollient
Jojoba Alcohol			Emollient
Lanolin Alcohol	8027-33-6		Emollient, emulsifier, moisturizer
Lanosterol	79-63-0	$C_{30}H_{50}O$	
Lauryl Alcohol	112-53-8	$C_{12}H_{26}O$	Thickener
Methyl Alcohol	67-56-1	CH_4O	
Myricyl Alcohol	593-50-0	$C_{30}H_{62}O$	
Myristyl Alcohol	112-72-1	$C_{14}H_{30}O$	Emollient, thickener
Octyldecanol		$C_{18}H_{38}O$	Emulsifier, emollient
Octyldodecanol	5333-42-6	$C_{20}H_{42}O$	Emulsifier, emollient
Oleyl Alcohol	143-28-2	$C_{18}H_{36}O$	Emollient, lubricant, solubilizer
Pentadecyl Alcohol		$C_{15}H_{32}O$	
Phenethyl Alcohol	60-12-8	$C_8H_{10}O$	
Phenol	108-95-2	C_6H_6O	
Phenylisohexanol	55066-48-3	$C_{12}H_{18}O$	
Propyl Alcohol	71-23-8	C_3H_8O	Solvent
Stearyl Alcohol	112-92-5	$C_{18}H_{38}O$	Emulsifier, thickener
Tallow Alcohol			Thickener
Tetradecyleicosanol		$C_{34}H_{70}O$	
Tetradecyloctadecanol		$C_{32}H_{66}O$	
Tridecyl Alcohol	112-70-9	$C_{13}H_{28}O$	
Undecyl Alcohol	112-42-5	$C_{11}H_{24}O$	
Undecylenyl Alcohol	112-43-6	$C_{11}H_{22}O$	
Undecylpentadecanol	68444-33-7	$C_{26}H_{54}O$	

1.3 Raw Materials and References

Table 5.2 References to Fatty Alcohols

Raw Material	Formula	Appearance	Compendium[1]
Normal Alcohols			
n-Butyl Alcohol (1-Butanol)	$CH_3(CH_2)_2CH_2OH$	liquid	CTFA; CLS/JSCI
n-Hexyl Alcohol (1-Hexanol)	$CH_3(CH_2)_4CH_2OH$	liquid	CTFA
Caprylic Alcohol (1-Octanol)	$CH_3(CH_2)_6CH_2OH$	liquid	CLS/JSCI
n-Decyl Alcohol (1-Decanol)	$CH_3(CH_2)_8CH_2OH$	liquid	CLS/JSCI
Lauryl Alcohol (1-Dodecanol)	$CH_3(CH_2)_{10}CH_2OH$	crystalline solid	CTFA; CLS/JSCI
Myristyl Alcohol (1-Tetradecanol)	$CH_3(CH_2)_{12}CH_2OH$	crystalline solid	CTFA; CLS/JSCI
Cetyl Alcohol (1-Hexadecanol)	$CH_3(CH_2)_{14}CH_2OH$	waxy solid	CTFA; CLS/JSCI
Stearyl Alcohol (1-Octadecanol)	$CH_3(CH_2)_{16}CH_2OH$	solid	CTFA; CLS/JSCI
Cetearyl Alcohol (Cetyl/Stearyl Alc.))	$CH_3(CH_2)_{14\text{-}16}CH_2OH$	solid	CTFA; CLS/JSCI
Oleyl Alcohol (9-Octadecen-1-ol)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CH_2OH$	liquid	CLS/JSCI
Arachidyl Alcohol (1-Eicosanol)	$CH_3(CH_2)_{18}CH_2OH$	solid	CLS/JSCI
Behenyl Alcohol (1-Docosanol)	$CH_3(CH_2)_{20}CH_2OH$	solid	CLS/JSCI
Branched-chain Alcohols			
Isopropyl Alcohol (2-Propanol)	$CH_3CHOHCH_3$	liquid	CTFA; CLS/JSCI
Isocetyl Alcohol (Isohexadecanol)	$C_{16}H_{33}OH$	liquid	CTFA; CLS/JSCI
Isostearyl Alcohol (Isooctadecanol)	$C_{18}H_{37}OH$	liquid	CTFA; CLS/JSCI
Isooctyldodecyl Alcohol (Isoeicosanol)	$C_{20}H_{41}OH$		CLS/JSCI
Other Alcohols			
Abietyl Alcohol	$C_{20}H_{31}OH$		CTFA
Benzyl Alcohol	$C_6H_5CH_2OH$	liquid	CTFA; CLS/JSCI
Cholesterol	$C_{27}H_{45}OH$	solid	CTFA; CLS/JSCI
Cinnamyl Alcohol (Cinnamic Alcohol)	$C_6H_5CH{=}CHCH_2OH$	crystalline solid	CLS/JSCI
Dihydroabietyl Alcohol	$C_{20}H_{33}OH$		CTFA; CLS/JSCI
Dihydrocholesterol	$C_{27}H_{47}OH$		CLS/JSCI
Eucalyptol	$C_{10}H_{18}O$	liquid	CTFA
Eugenol	$C_{10}H_{12}O_2$	liquid	CLS/JSCI
Geraniol	$C_{10}H_{17}OH$	liquid	CLS/JSCI
Jojoba Alcohol	$C_{20}H_{39}OH/C_{22}H_{43}OH$	liquid	CLS/JSCI
Lanolin Alcohol		semisolid	CTFA; CLS/JSCI
Lanosterol	$C_{30}H_{49}OH$	crystalline solid	CLS/JSCI
Oryzanol	$C_{40}H_{58}O_3$	powder	CLS/JSCI
Phytosterol		solid	CLS/JSCI
Phenethyl Alcohol (2-Phenylethanol)	$C_6H_5CH_2CH_2OH$	liquid	CLS/JSCI
Sitosterol		solid	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

2 PHYSICAL AND CHEMICAL PROPERTIES

The chemical and physical properties of fatty alcohols depend essentially on the OH group, which gives polarity to the molecule and the capability of forming hydrogen bonds. for these reasons they are more aggregated than related homologous hydrocarbons, but always present a certain volatility allowing analysis by gas chromatography.

Those with short chains are liquid with low boiling points which rise as the aliphatic chain becomes longer until the higher fatty alcohols are solid at the temperature of the environment.[1,5-7,9-12] Nevertheless liquid high molecular weight fatty alcohols with branched chains and double bonds can be found.

The OH group make these molecules easily oxidizable, changing them first to aldehydes and, subsequently, to acids: reactions that are much used for qualitative and quantitative determinations. Another typical reaction of fatty alcohols is the reaction with acids which leads to the formation of esters: it is used to determine hydroxyl value.[8,9,19,26]

3 CHEMICAL AND PHYSICAL ANALYSES FOR IDENTIFICATION AND QUALITY

3.1 Physical Tests

3.1.1 Appearance, Colour and Odour

To match standard (see Appendix, *1,2*,and *3*)

3.1.2 Viscosity of Liquid Compounds[3,33] (see Appendix, *4)*

In addition to characterization this property can affect the properties of finished cosmetic products.

3.1.3 Melting Point[8,9,30] *and Calorimetric Characteristics of Higher Alcohols* (see Appendix, *8*)

The melting point is useful in the recognition of fatty alcohols.[30] However, frequently they are mixtures of substances, even if simple ones, so we speak about 'melting interval' rather than melting point. Much more useful are the determinations that can be arrived at by differential scanning calorimetry (DSC), such as observing the fraction which is liquid at various temperatures.

3.1.4 Refractive Index (see Appendix, *16*)

This datum can be very useful for the identification of these substances.[9,23,32] It has the great advantage of being quick, although not very selective. The procedure is carried out at 20°C for liquid samples, and at 40° and 60°C for solid samples.

3.2 Chemical Tests

3.2.1 Water/Moisture Content (see Appendix, *18*)

The moisture content should be at a minimum and limits may be given in specifications for the materials. The Karl Fischer method is quick and suitable for low limits.

All the various fatty alcohols may contain some water as impurity and various methods can be used to evaluate it. The loss in weight at a temperature of 100°C is clearly not applicable to lower alcohols because of their volatility. More specific methods must therefore be applied, such as Karl Fischer's method[9,83] or spectrophotometric methods.[91]

3.2.2 Iodine Value (see Appendix, *19*)

The iodine value gives a measure of the unsaturation, i.e. the quantity of double bonds, in the sample. When compared with a standard, the iodine value[20,27] gives an indication of the presence of extraneous substances.

3.2.3 Acid Value (see Appendix, *21*)

The acid value[22,29] can be a limit value for quality or a measure of impurity when compared with a fresh standard sample.

3.2.4 Saponification Value (see Appendix, *22*)

The saponification value[21,28] can indicate the quality of the raw material: as with acid value, this must be within a certain limit.

3.2.5 Hydroxyl Value (see Appendix, *23*)

The hydroxyl value depends on the relation between OH groups and other structures present in the molecule and on the possible presence of extraneous substances.[5-12,19,26]

3.2.6 Other Specific Identification Tests

Before the use of mass spectrometry and spectroscopic and chromatographic methods, various reactions were used to recognize the alcohols and their molecular structure.

a) Dichromic acid colour test

Chromic anhydride (chromium trioxide) or potassium dichromate in diluted sulfuric acid, is added to the sample and within a few minutes the mixture changes from a clear orange solution to an opaque greenish one:[9,10] this reaction can be applied only to primary and secondary alcohols and not to tertiary ones.[1]

b) Iodoform colour test

An unknown fatty alcohol treated with NaOH and iodine will react, producing a yellow precipitate of iodoform, if a methyl group and a hydrogen atom are present at an α-OH group in the molecule

c) Acetic anhydride colour test

For sterols and the lanolin derivatives these type of tests are also useful for their characterization : for the latter a rapid chemical test is represented by a colour development when such derivatives are treated with acetic anhydride in chloroform and sulfuric acid, or in chloroform solution with sulfuric acid alone.[9]

d) Ferric chloride colour test

For phenols, a typical reaction is represented by treatment with ferric chloride and subsequent generation of colours.[13]

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

4.1.1 Thin-Layer Chromatography

The recognition of fatty alcohols by means of TLC is achieved through systems in general use such as the silica gel stationary phase with the mobile phase, represented by a mixture of ether/ethyl ether/acetic acid (90:10:1). This system is very useful for separating fatty alcohols from other fatty components, such as hydrocarbons, triglycerides, esters and acids.

Alcohols have an R_f (see Appendix, *26*) about 0.2 while other less polar substances, such as esters and triglycerides have a higher R_f. As developer, iodine is useful for unsaturated substances, or bromthymol blue with ammonia vapours, rhodamine B or 2-7 dichlorofluorescein with ultraviolet light, sulfuric acid heated at 130°C.[38]

TLC methods can be applied to unsaponifiable fractions of natural oils and fats, to separate alcohols and sterols from other substances: silica gel with a mobile phase petroleum ether/ethylic ether/ formic acid (60:40:1) is used, as is benzene/acetone (95:5) or *n*-hexane/ethyl ether (65:35).[34]

These methods can also be used on a preparatory scale to obtain samples for further gas chromatographic analysis. in particular, also a mixture of *n*-hexane/ethyl ether (60:40) can be used on plates developed in a refrigerator or by means of 80% potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid and heated at 130°C.[39] Different chromatographic detector systems have recently been reported in the literature.[83,122,135,149,157]

4.1.2 Gas Chromatography

One of the preferred analytical methods for characterizing fatty alcohols, is gas chromatography:[8,9] because of the relative volatility of all these substances, it can be used for both low-boiling and high-boiling fatty alcohols, as well as for sterols.

a) Low-boiling alcohols

A substantial bibliography exists for analysis by both capillary and packed-column GLC, with various stationary phases for alcohols including methyl, ethyl, propyl, butyl, isobutyl and *tert.*-butyl types. This is mainly because of current interest in their biological and environmental[15,16,53-56,66,70-72,77,84,89,90,92-94,96,101,102,106] properties. Some of the analyses referred to are directly applicable to finished products.

Methods of treating the samples vary,[76] according to their origin: in cosmetic products,[69] there is a risk with pressurized products of contamination of materials and problems with residual solvents. For those alcohols which develop trimethylsililethers, a derivative reaction is sometimes employed.[103] The *headspace* technique is often used when searching for residual solvents or vapours in the atmosphere.[60,65,73,78,85]

b) High-boiling alcohols

Identification and assays of higher fatty alcohols are reported in collections of official methods[2,34,35] and other methods are suggested in the relevant literature. These methods can be applied to raw materials for cosmetic use. Generally a derivative reaction, that leads to the formation of trimethylsililethers is preferred; these are then injected into the gas chromatographic apparatus, at a working temperature of about 180-260°C.

When alcohols are part of complex lipidic chains (matrixes, complex lipidic matrixes), it is necessary to separate them by methods used for the determination of unsaponifiables and further by TLC.[25,36]

Important reports in the literature on gas chromatographic analysis for lipids describe many variations in the treatment of samples, possible derivatives and types of column, stationary phases and detectors to use;[40,50-52,57,68,74,82,107,109,144-146] these variations enable the separation of fatty alcohols on the basis of

- their molecular weight
- any unsaturation
- the kinds of alkyl chains
- stereoisomerism
- enantiomorphism

The coupling of gas chromatography and mass spectrometry (MS – see ¶4.2.5) allows the precise identification of the substances being analysed.[62,111,113]

c) Sterols

GLC analysis is one of the most selective and sensitive methods for sterols particularly if it is coupled to a mass spectrometer.[108] There are official methods for determining the content of sterols in fatty substances, but after separation of the matrix from other components of the unsaponifiables.[37] This is another case where the methods can be applied to raw materials for cosmetic use, for quality as well as identification. Further references on this subject are available.[114-117,150,152-154]

4.1.3 Supercritical Fluid Chromatography

SFC is a relatively new technique that has been developed to offer the advantages of both gas chromatography and liquid chromatography, with the aim of eliminating the disadvantages of both. Usage of this technique is reported.[64,137]

4.1.4 High-Performance Liquid Chromatography

HPLC is a well known and very sensitive and selective analytical technique, even though it is less generally used than gas chromatography. The very low or null adsorption of aliphatic alcohols in the ultraviolet spectrum makes HPLC difficult to use because the UV spectrum is often the detector in HPLC apparatuses.[41,124,126,139,140,151,155,156] Therefore, besides the usual methods which make use of the measurement of the refractive index,[42,43,142] other methods have been developed in which pre- or post-column derivatives are formed with substances carrying chromaphore groups,[44] such as 3,5-dinitrobenzochloride to obtain 3,5 dinitrobenzoates,[45] or with phenylisocyanate to obtain alkylphenyl-urethanes.[46] Also, fluorescent reagents have been used; in this way selectivity and sensitiveness have been improved by using a fluorimetric detector.[58,95,112]

A further way of using HPLC and the ultraviolet detector in alcohol analysis is by indirect detection, a technique that records the substances by measuring the negative absorption due to their passage in the detector when a chromaphoric substance is present in the mobile phase.[105]

The type of apparatus that has become popular in recent years is the light- scattering detector, in which an electrical signal originates from the agitation of a light beam that crosses the vaporized mobile phase issuing from the column.[125] Other studies have considered the possibility of producing enzymatic detectors[127] or amperometric detectors.[159] Enzymatic detectors exploit the capacity of the alcohol to act as a biological reaction substrate.

HPLC can be used both with a direct-phase column (to give lipidic class separation)[47,48] or with a reverse-phase column that is more selective. Unlike aliphatic alcohols, alcohols with aromatic substituents (e.g. benzyl alcohol) or phenols, present high-level absorption in UV and are thus easy to analyse by HPLC-UV.[59,67,118,131,132,136,158]

4.2 Spectroscopy

4.2.1 Infrared Spectroscopy

By means of infrared spectroscopy it is possible to obtain interesting data concerning the characterization of fatty alcohols. They absorb at precise frequencies: the OH bond associated with hydrogen bonds presents a vibration absorption in the zone between 3200 and 3600 cm^{-1}, generally with wide and intense bands; when it is not associated, the absorption band is generally clear, about 3600 cm^{-1}.[1] The absorption band for the vibration of the simple bond CO is in the zone between 1000 and 1200 cm^{-1} and, generally, it is wide: its position depends on the molecular structure of the alcohol.

A considerable improvement of this technique has been obtained by using a Fourier transform infrared spectrometer (FTIR) which by analysing electronically the signal of the infrared light absorbed or reflected by the sample (Attenuated Total Reflectance Fourier Transform Infrared Spectrometry – AFTIR) is a method of much increased sensitivity and selectivity;[63,98] Raman spectroscopy is also reported in applied studies.[97]

4.2.2 Near-Infrared Reflectance Analysis

Analysis by means of near-infrared reflectance (NIRA) spectroscopy is used for the recognition and determination of single components of complex matrixes: these analyses exploit the interaction between the material and electromagnetic energy with the wave length intermediate between visible and infrared. There are examples reported in literature of this technique being applied to cosmetic products.[100]

4.2.3 Ultraviolet Spectrophotometry

Ultraviolet (UV) spectrophotometry cannot be applied directly to the alcohols because of low absorption: it is therefore necessary to utilize derivatives of compounds that contain chromophore groups: for example, by using UV spectrophotometry to measure the colour change obtained from the reaction of methyl alcohol with chromotropic acid[20] the methyl alcohol can be determined quantitatively.

Many methods for colorimetric determination of alcohols of low molecular weight appear in the literature.[123,128] Derivatives with chromophoric substances are used to detect alcohols after separation in HPLC (see ¶4.1.4).

Phenols: since phenols absorb ultraviolet light, it is possible to make a direct determination.[129,130,138,148]

4.2.4 Proton Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy works in the molecular field of protons widely used to investigate the structure of substances. Examples are reported in the literature in which quantitative and qualitative results can be obtained.[143] The technique is based on the measure of the interaction between magnetic fields, radio frequency fields and the material.

According to the respective energy of these fields it is possible to investigate the variations in energy of different atoms. Protonic NMR works in the molecular field of protons and therefore is very often used in association with IR spectroscopy[1] for their detection.

4.2.5 Mass Spectrometry

By means of this technique it is possible to determine the molecular structure of compounds as well as their molecular weight.[86,133] Probably, the most widely used analytical application is the coupling of mass spectrometry with gas chromatography (MS-GC – see ¶4.1.2)

4.3 Electrochemical Analysis

Electrochemical analysis is another method that is used for low-boiling alcohols and sterols. Information is available in the literature.[80,87,160]

5 TESTS FOR IMPURITIES

5.1 Heavy Metals (see Appendix, *35*)

Fatty alcohols may be contaminated by heavy metals from either the technological processes they have undergone or the environment. Various bibliographical references consider different methods of treating the sample. Atomic Absorption Spectroscopy and Plasma Spectroscopy are the most recent.[81,147] Spectrophotometric determinations[141] are useful; also, studies are being conducted using luminescence.[99]

Conventional techniques,[8,9] using the reaction with sulfuric acid, give the result as the sum of the heavy metals but do not give the separate quantities of the single metals present.

5.2 Other Extraneous Substances

5.2.1 Ash (see Appendix, *36*)

This is a classic test used to detect the presence of extraneous substances in organic matrixes: the residual is determined after high temperature treatment.

5.2.2 Oxidation (see Appendix, *25*)

Fatty alcohols with double bonds can undergo oxidation processes which can adversely affect the quality of the raw material – especially if auto-oxidation processes have already started. This could lead to unpleasant consequences in storage and even to instability of the finished products[3] if used in manufacture.

Depending on the development stage of the auto-oxidation, various tests are suitable for detecting the substances produced; normally the peroxide number, Kreis' assay and the *p*-anisidine number are used.[24,31]

a) Peroxide number – this measures the beginning of the auto-oxidation reaction

b) Kreis' assay – a colorimetric reaction with phloroglucine, or

c) *p*-Anisidine number – a colorimetric reaction with *p*-anisidine

Test (c) detects aldehydes or ketones, resulting from further auto-oxidation.

Recent studies have shown that HPLC can be used.[49,134]

6 ANALYTICAL METHODS FOR FATTY ALCOHOLS IN FINISHED PRODUCTS

6.1 Extraction of Alcohols from the Matrix

With the exception of the analytical techniques (FTIR, NIRA) applied to fatty matrices such as finished products, analyses of fatty alcohols in cosmetic products always start by separating them from the matrix. A simple method that is often employed involves extraction by means of a solvent:[17] the cosmetic sample dissolved in water/ethanol is then extracted by means of petroleum ether, in which fatty substances are collected – obviously, in the case of alcohols with polar groups, the extraction may be incomplete.

A very usual method for cosmetic products is solid phase extraction: small columns are packed with mobile phases for HPLC, which, depending on the kind and quantity of the solvent used, selectively retain the various types of substances. Once they have been isolated and purified from most of the undesired substances, the alcohols can be analysed by means of the techniques described below (see ¶7.2)

6.2 Analysis of Extracted Alcohols

A method for separating the free fatty alcohols in products based on anionic or cationic surfactant is ion-exchange column chromatography: the ion-exchange resin column (e.g. "Amberlite") will retain the ionic substances allowing the non-electrically charged substances to pass; the maximum separation will occur at the correct p*H* and even in a common separatory funnel, in which the sample is dissolved in water/ethanol and treated with "Amberlite". It is then extracted by means of carbon tetrachloride.[18]

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Division 6: Synthetic Glycerides

Luigi Rigano

1 CHEMICAL DESCRIPTION, STRUCTURE AND USE IN COSMETICS

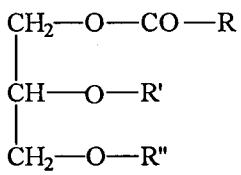
The mono, di- and triglycerides treated in this division are normally synthetic or semi-synthetic raw materials obtained either by partial hydrolysis of naturally occurring glycerides or by synthesis.

The classical definition of glycerides includes compounds having in their chemical structure glycerol with the hydroxyl groups esterified by one, two or three acid radicals. Their chemical behaviour is determined by the presence of one or more ester bonds, and also, in the case of mono- and diglycerides, of two or one hydroxyl groups.

Triglycerides that occur naturally in animals and vegetable fats and oils are treated separately in this monograph, under Division 4. Glyceryl esters of inorganic acids, including phosphoric, are not described in this division; because of their special properties they are treated according to their use in cosmetic products. Polyglycerides and ethoxylated glycerides will also be treated in a separate volume (see Volume II).

1.1 Structural Formula

The general formula of these compounds is



Where R = an alkyl radical
R' and R" = any acyl radicals, different or equal to -CO-R, or alkyl radicals or hydrogen atoms.

They can be fluid, semi-solid or solid products, according to the length, number and type of alkyl chains, and either saturated or unsaturated, linear or branched, and with or without additional functional groups. When the acyl group R" is different from group -CO-R, the central carbon atom is optically active and the molecule may exist as optically active enantiomers.

From the chemical point of view, the class can be divided into

a) triglycerides, with the triester structure;

b) mono- and diglycerides, with alcoholic and ester functions; and

c) mono- and diglycerides, with ether and ester functions.

1.2 Sources

The primary sources of these compounds are natural lipidic materials, from vegetals like coconut oil, or animal fats, like tallow, treated with a hydrolytic process to separate one or two acid moieties, or hydrogenated to give saturated structures.

Other formation processes consist of direct esterification of glycerol with a fatty acid, transesterification of fatty acid methyl esters with glycerol, or chemical synthesis starting

from propylene and the chosen fatty acid. In many cases mixtures of mono- and diesters are obtained, and the corresponding free hydroxyl groups can be at the 1 or 2 position in the case of monoesters, and at the 1 and 2 or 1 and 3 position in the case of diesters. Ether glycerides are obtained by esterification of the corresponding glyceryl ethers.

1.3 Uses In Cosmetics

The physical properties and use of synthetic glycerides in cosmetics depends on their high polarity (particularly the mono- and diglycerides). They are employed:

- as emulsifiers or coemulsifiers;
- as modifiers of the polarity of oily phases;
- to impart hydrophilicity to fatty or waxy mixtures, improving their application to human skin;
- in pigmented products they give good wettability and compatibility with powders;
- some monoesters have antimicrobial properties;

Triglycerides are often used as lubricants, emollients and spreading aids.

1.4 Raw Materials and References

1.4.1 Mono- and/or Diglycerides

Table 6.1 References to Mono- and/or Diglycerides

Raw Material	Compendiums[1]
Cottonseed Glyceride	CLS/JSCI
Glyceryl Cocoate	CLS/JSCI
Glyceryl Erucate	CLS/JSCI
Glyceryl Hydroxystearate	CLS/JSCI
Glyceryl Isostearate	CLS/JSCI
Glyceryl Laurate	CLS/JSCI
Glyceryl Myristate	CLS/JSCI
Glyceryl Oleate	CTFA; CLS/JSCI
Glyceryl Ricinoleate	CTFA; CLS/JSCI
Glyceryl Stearate	CTFA; CLS/JSCI
Safflower Glyceride (Safflower Oil Fatty Acid Glyceride)	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

1.4.2 Triglycerides

Table 6.2 lists only the most common raw materials. Note that CTFA Adopted Names/INCI Names (see page viii) are frequently defined as *tri-* + the common name of the glyceride: *Tristearin* instead of Glyceryl Tristearate.

Table 6.2 References to Synthetic Triglycerides

Raw Material	Compendiums[1]
Glyceryl Triacetylricinoleate	CLS/JSCI
Glyceryl Tricocoate	CLS/JSCI
Glyceryl Tritallowate	CLS/JSCI
Hydroxystearin (Glyceryl Tri[12-Hydroxystearate])	CLS/JSCI
Triacetin (Glyceryl Triacetate)	CLS/JSCI
Tribehenin (Glyceryl Tribehenate)	CLS/JSCI
Tricaprylin (Glyceryl Tricaprylate)	CLS/JSCI
Triisopalmitin (Glyceryl Triisopalmitate)	CLS/JSCI
Triisostearin (Glyceryl Triisostearate)	CLS/JSCI
Trilaurin (Glyceryl Trilaurate)	CLS/JSCI
Trimyristin (Glyceryl Trimyristate)	CLS/JSCI
Trioctanoin (Glyceryl Trioctanoate)	CLS/JSCI
Triolein (Glyceryl Trioleate)	CTFA
Tripalmitin (Glyceryl Tripalmitate)	CLS/JSCI
Tristearin (Glyceryl Tristearate)	CLS/JSCI
Triundecanoin (Glyceryl Triundecanoate)	CTFA; CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

2 PHYSICAL AND CHEMICAL PROPERTIES

2.1 Physical Properties

See ¶3.1. For behaviour and uses in cosmetics, see ¶1.3.

2.2 Chemical Properties

Synthetic glycerides give the classical reaction of esters: saponification to the corresponding carboxylic acids and, in the case of mono- and diglycerides, of alcohols (they can be esterified with acetic anhydride). Other properties are due to the acyl chain characteristics, e.g. its unsaturation.

3 ANALYTICAL METHODS FOR IDENTIFICATION AND QUALITY

The methods for identification of a glyceride consist of

a) physicochemical measurements to characterize some parameters which are typical of the product; and of

b) comprehensive chemical determinations on the glyceride molecule as a whole molecule, and also on the fatty acid moiety linked with glycerol;

c) general methods for identification such as nuclear-magnetic resonance, mass spectrometry, infrared spectroscopy;

d) specific chemical tests for glyceride identification (see ¶3.2.4).

3.1 Physical

3.1.1 Colour (see Appendix, *2*)

Colour varies between white to slightly yellow.

3.1.2 Odour (see Appendix, *3*)

Their odour is faint or slightly fatty.

3.1.3 Viscosity (see Appendix, *4*)

It is more a quick method for comparison between very similar materials than a tool for identification or characterization. Nevertheless it is often given in manufacturers' specifications.

3.1.4 Relative Density (or Specific Gravity) (see Appendix, *6*)

Their specific gravity is less than unity. Thus, C_8-C_{10} triglyceride has the value 0.947-0.952.

3.1.5 Flash Point (see Appendix, *14*)

This measure is meaningful for safety reasons, especially storage.

3.1.6 Melting Point (see Appendix, *8*)

The melting point of glycerides depends mainly on the acyl chain length and type and less on the number of acyl residues attached to glycerol: C_8-C_{10} triglyceride is a liquid at room conditions, while glyceryl monostearate is a white, wax-like solid which melts at 56-58°C or between 55 and 70°C for commercial samples. It is related to the constancy of purity of the raw material and/or to the constant composition of the acyl residues.

3.1.7 Solubility (see Appendix, *15*)

Glycerides are generally soluble in hot organic solvents, such as alcohol, benzene, ether or acetone, or mineral or fixed oils. They are insoluble in water, but become dispersible in the presence of small amounts of soap or suitable surface active agents. Solubility at room temperatures depends on the structure and the amount of free hydroxyl groups.

3.1.8 Refractive Index (see Appendix, *16*)

In general the refractive index indicates the constancy of composition and purity of the raw material, rather than the actual composition of the molecule. Measurements are made at temperatures at least 10°C higher than the melting point of the substance.[1]

The refractive index is considered only for liquid products at room conditions; it can vary between 1.446 and 1.448 for C_8-C_{10} triglyceride.

3.2 Chemical Analysis

3.2.1 Iodine Number (or Value)[2] (see Appendix, *19*)

The iodine number is related to the degree of unsaturation of the product.

3.2.2 Saponification Number (or Value)[3] (see Appendix, *22*)

The saponification number is usually very high (more than 100): C_8-C_{10} has the value 320-345, while glyceryl monostearate has a saponification index between 150 and 190.

3.2.3 Hydroxyl Number (or Value) (see Appendix, *23*)

In the case of pure monoglyceride substances the hydroxyl value indicates the esterification ratio; in the case of blends it is indicative of constancy of composition, when considered together with the iodine and saponification values.

3.2.4 Specific Chemical Tests for Identification of Glycerides

a) One example[4] that is generally used for all glycerides (and glycerol) is heating with potassium bisulfate: acrolein, which has a characteristic odour, develops.

b) Another simple test involves heating in 5% ethanolic solution with 2.5 times its volume of dilute sulphuric acid for 30 minutes, then cooling: the esterifying acid separates on the top, and can be solubilized and extracted with 1.5 volumes of ether and eventually identified with general methods for acids, or by odour, when characteristic.

c) Also alkaline hydrolysis is possible: in this case by reflux heating, followed by acidification and extraction with *n*-hexane. Volatile acids can be recognized by gas chromatography.

3.2.5 Total Fatty Acids

The total fatty acid content is obtained by saponification of the glyceride with potassium hydroxide in hydroalcoholic solution, followed by acidification with hydrochloric acid in the presence of a methyl orange indicator and subsequent extraction with petroleum ether.[4] After evaporation of the solvent, the residue is weighed. This value is useful for batch to batch control, while more precise indications of the nature of the fatty-chain components are given by the methods below (see ¶4).

3.2.6 Fatty Acid Composition

See ¶4.1.2.

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

4.1.1. Thin Layer Chromatography (see Appendix, *26*)

For mono-, di- and triglyceride mixtures, thin layer chromatography allows the separation of mono-, di- and triglycerides by using silica gel-made slabs (eluent *n*-hexane:ether 7:3 to which 1.5 ml of formic acid is added) and the separation of the triglycerides is related to their degree of esterification by using argentation thin-layer chromatography (Ag-TLC).[5]

4.1.2. Gas Chromatography (see Appendix, *27*)

The most up-to-date analytical techniques for studying the quantitative compositions of mono-, di- and triglycerides employ gas chromatography[6,7] and high-performance liquid chromatography (HPLC). In particular, inverse-phase gas chromatography over an RP18 column allows for the separation of the different triglycerides in relation to both their alkyl chain length and the degree of unsaturation of the fatty acids.[8,9]

The identification of the separate fatty acids in the total from ¶3.2.5 can be obtained by gas chromatography[10] using their carefully prepared methyl esters[6]. In the case of a fatty acid with a different chemical nature from that prescribed by the reference method, it is necessary to compare it with the standard substance in order to determine the corresponding retention volumes and make the appropriate variations in the operating conditions. When using a precisely weighed standard as reference (usually heptadecanoic acid is used), this method allows a quantitative determination of every individual fatty acid.

4.2. Spectroscopy

4.2.1 Infrared Spectroscopy

The infrared (IR) spectra of triglycerides show, besides the bands related to the hydrocarbon structure of the fatty-acid radicals, those of the functional groups. In the first group there are

(a) a strong band at 3030 cm^{-1} caused by stretching vibrations of the C-H link;

(b) a single or double band at 1449 and at 1428 cm^{-1} related to the C-H bonding;

(c) a band which appears between 1388 and 1351 cm^{-1} caused by the symmetrical deformation vibrations of the methyl group; and

(d) another band at 719 cm^{-1} typical of the hydrocarbons which are in chains of 4 or more consecutive carbons and caused by the CH_2 oscillating mode.

Bands related to the functional groups are the C=O stretching band, which appears between 1748 and 1739 cm^{-1}, and a group of other bands between 1250 and 1111 cm^{-1} related to the C-O links, including a more pronounced band (strong) at 1162 cm^{-1}.[29] In the case of mono- and diglycerides a band is present caused by the stretching of the O-H which appears between 3400 and 3200 cm^{-1}.[30] Some examples[31] are shown in Figures 6.1, 6.2 and 6.3.

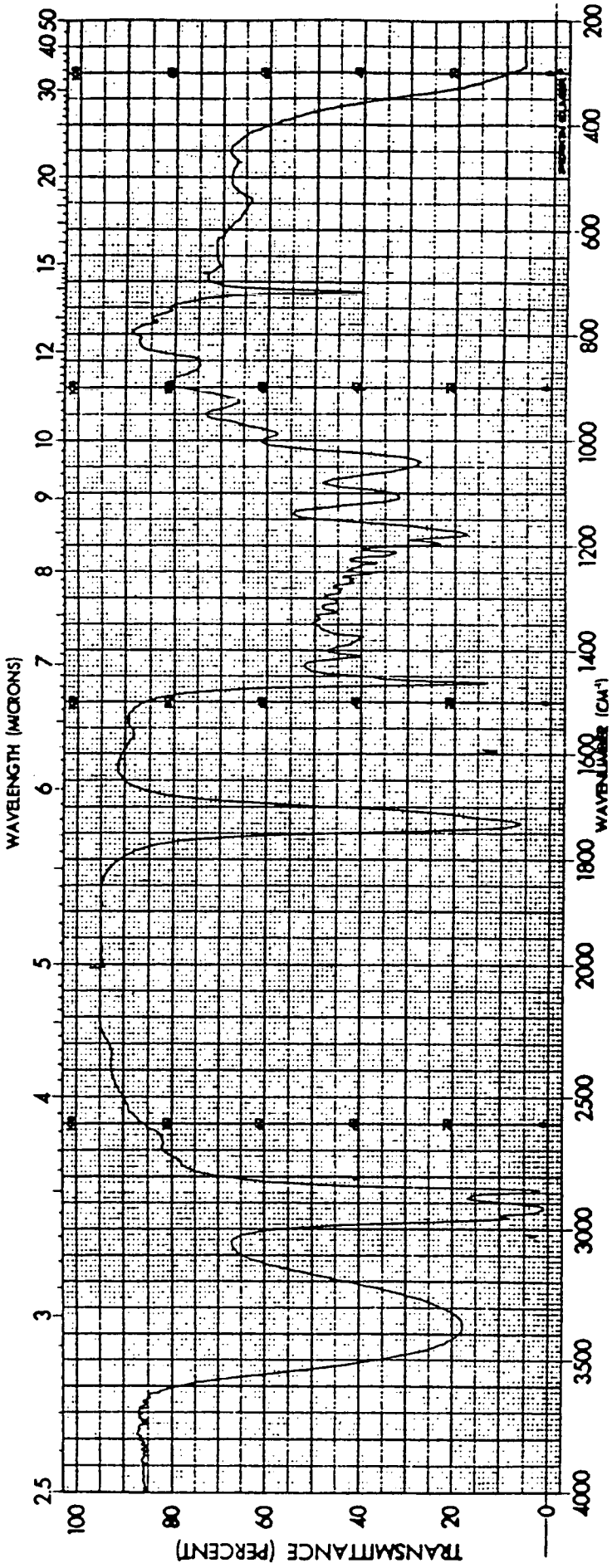


Figure 6.1

Infrared spectrum of Glyceryl Stearate

Product: Glyceryl Stearate
Phase: Solid
Method: Capillary Film on KBr Disks
Reference: Air
Instrument: Perkin-Elmer Model 621
Optics: Grating

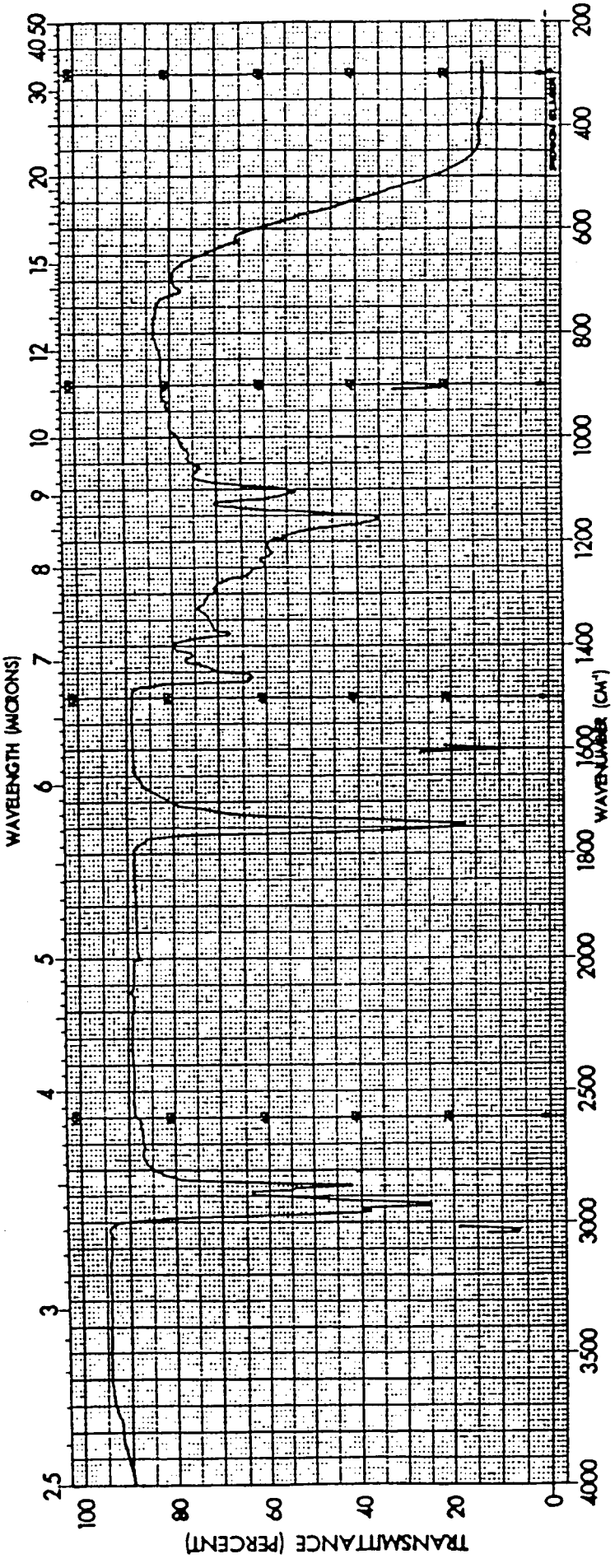


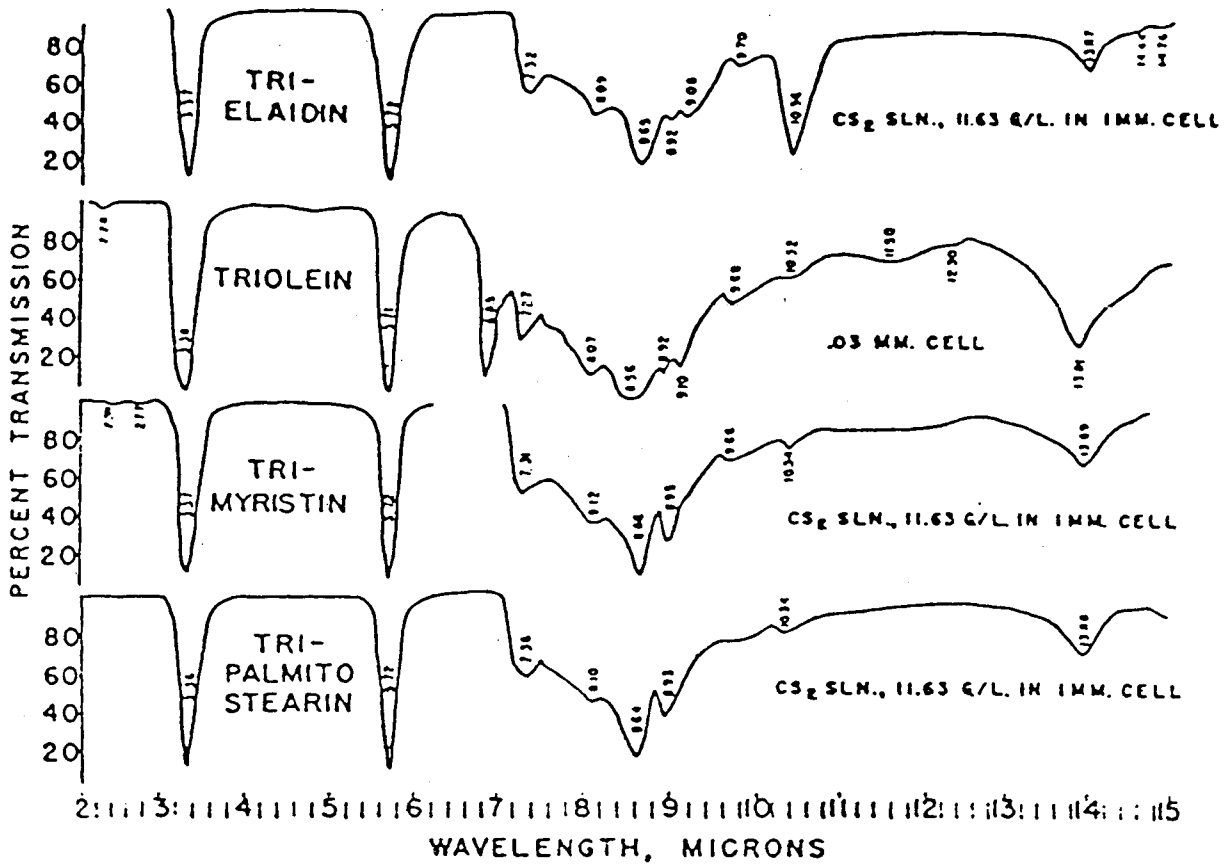
Figure 6.2

Infrared spectrum of Caprylic/Capric Triglyceride

Product: Caprylic/Capric Triglyceride
Phase: Liquid
Method: Capillary Film Between NaCl Plates
Instrument: Perkin-Elmer Model 621
Optics: Grating

Figure 6.3

Infrared absorption spectrums of pure long-chain triglycerides.



4.2.2 Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) can give information on the structural characteristics of glycerides. ^{1}H-NMR analysis enables the degree of esterification of glycerides to be determined,[11] and analysis by ^{13}C-NMR allows the location of the double bonds and the nature of the configurational isomers of the unsaturated fatty acid to be established.[12-21] To determine and assign the resonances of the partial glycerides, particular sequences of impulses such as the DEPT (Distortionless Enhancement by Polarization Transfer) must be used to distinguish the primary carbon atoms from the secondary and tertiary ones.

4.2.3 Mass Spectrometry

Another technique of glyceride identification and analysis is mass spectrometry which, with the use of electronic impact, allows the determination, on the base of characteristic fragments, of the esterification location; and to identify the acyclic groups located at the 1 and 2 positions;[22,23] and in the case of chemical ionization, caused by the presence of molecular ions with elevated intensity, gives precise information about the molecular weight of the glycerides, even in the case of mixtures.[24] Because of the high identification specificity typical of mass spectrometry, the most effective way to analyse complex mixtures appears to be to link this technique with gas chromatography[25,26] and to high-resolution liquid chromatography,[27,28] thus exploiting the great separational powers typical of these last two techniques.

5 QUALITY DETERMINATIONS

The quality of a glyceride can be determined by several tests that are particularly concerned with the presence of foreign substances, of unreacted starting raw materials used for industrial production, the oxidation level, and alkyl chain distribution. Purity criteria can also be established by tests for the presence of heavy metals. The performance of the raw material in the finished cosmetic product and its influence on the final product characteristics can also be determined by tests.

5.1 Moisture/Water

The moisture content of glycerides can be determined by three different methods:

(a) loss of weight by heating at 105°C[32] (in this case the value found also includes the content of volatile substances);

(b) water by azeotropic distillation with xylene[33] (this cannot be used for substances containing less than 0.5% of water or with samples that distil at the test temperature or that are soluble in water); and

(c) Karl Fisher's method[34] for products with a low content of water.

5.2 Ash

The ash content, which shows the presence of mineral substances not related to the organic matrix, e.g. those deriving from the production apparatus, is obtained by calcination of the product after combustion and carbonization.[35] Heavy metals are of special importance for the allergenic potential of some of them (e.g. Ni, Co).

5.3 Inorganic Acidity

Inorganic acidity due to the presence of mineral acids and/or to water-soluble fatty acids is measured by titration with an alkali hydroxide the aqueous phase obtained on extraction with water of the petroleum ether solution of the product.

In the case of some self-emulsifying glycerides, alkaline soaps are present which can be similarly titrated by standard acid solution.

5.4 Organic Acidity

Organic acidity, which follows the partial synthesis processes, is determined by titration of the glyceride, dissolved in an alcohol-ether mixture, with alkali hydroxide solution.[36] The value obtained can be expressed in terms of the main fatty acid present in the glyceride structure; or, for a more precise determination, the alkali salt can be extracted several times with water, then the solution made acid by an excess of hydrochloric acid. The floating acid can be extracted with ethyl ether and, after drying and evaporation of the solvent, the type of acid can be analysed for molecular weight and structure by the methods described under GC (¶4.1.2).

5.5 Free Glycerol

As in the case of organic acidity free glycerol is derived from the partial synthesis process and can be determined by two methods: (a) the aqueous solution of free glycerol obtained by extracting the glycerides with water can be titrated with periodic acid;[37] or, (b) by the use of gas chromatography.

5.6 Oxidation

The state of oxidation of a glyceride is a very important factor for quality evaluation, since it could be the cause of instability in the finished product. Many tests have been developed which measure the auto-oxidation at different stages of development. Of the chemical ones the most commonly used are the peroxide number,[38] the Kreis assay,[39] and the *p*-anisidine number, which detects the presence of aldehydes and ketones.[40]

Instruments of great relevance for the determination of the oxidation capability of glycerides have became available recently. These can determine, through measurements of conductivity, the volatile acids that are formed when insufflating air into the product kept at constant temperature. The time needed for the acids to be formed measures the amount of oxidation.

For the future, high-performance liquid chromatography HPLC[41,42] and gas chromatography are being studied for the measurement of epoxides.[43]

5.7 Heavy Metals

Heavy metals, such as cadmium, lead, nickel, arsenic, antimony and mercury can be determined by atomic adsorption techniques (AAS). After prior incineration cadmium, chromium, lead and nickel can be determined by the flame-AAS technique, and arsenic and antimony by the hydride-AAS technique. For mercury the determination is by flameless-AAS technique, and follows digestion in nitric acid solution.[32]

6 GUIDELINES FOR THE ANALYSIS OF GLYCERIDES IN FINISHED PRODUCTS

The separation of glycerides from cosmetic formulations can be carried out by extracting the dried product with ethyl ether and then separating the soluble part by thin-layer chromatography using eluents such as *n*-hexane-ethyl ether 95:5, benzene-acetone 95:5, or *n*-hexane-ethyl ether 70:30. Identification of the single bands may be achieved by using more suitable reference substances and also by extracting the spots with chloroform-methanol 3:1 and subsequently applying infrared spectroscopy.

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(42) A. Gasparoli, and E. Fedeli. *Riv. Ital. delle Sostanze Grasse* 63 643 1986
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Division 7: Fatty Alcohol/Fatty Acid Esters

Tasuku Takamatsu

1 CHEMICAL DESCRIPTION, STRUCTURE AND USES IN COSMETICS

This raw material class is chemically described as an ester of a fatty alcohol and a fatty acid.

1.1 Structure

In general, fatty acid/fatty alcohol esters conform to the chemical formula:

$$RCOOR'$$

where R and R' stand for alkyl chains of fatty acid and fatty alcohol respectively.

1.2 Origins

The *fatty acids* used as starting material for ester synthesis usually have carbon chains of eight or above and may include either unsaturated bonds or branched structures. They are mainly derived from natural sources and therefore mainly contain even carbon numbers.

When synthetic fatty acids are used, they are usually branched-chain fatty acids. Typical examples are 2-ethylhexanoic acid and 2-heptylundecanoic acid (isostearic acid). Lower carbon acids such as acetic acid, propionic acid, etc. are seldom used for synthesis of esters for cosmetic uses except for (a) modification of an unreacted hydroxyl group in a glyceride or polyol ester (see Divisions 6 and 8); (b) esters of dibasic acids (see Volumes II and V); and (c) esters of lower hydroxy acids (Volume VII).

Similarly the *fatty alcohol* portions have the same characteristic origins. However, lower alcohols than those with eight carbon atoms are frequently used for esters in cosmetic products. Typical examples are isopropyl alcohol esters of fatty acids. Isopropyl alcohol is derived from propylene which is obtained from the "cracking" of petroleum.

1.3 Uses in Cosmetics

These esters have been widely used in many types of cosmetic products as basic ingredients. Their consistency ranges from low to high viscosity liquids through soft pastes to solids. They are used to stiffen creams, as opacifiers in shampoos, as plasticizers in setting lotions, and, most importantly, as conditioners and emollients in many types of cosmetics.

1.4 Raw Materials and References

The following list of typical esters (Table 7.1) is limited to those that have been described in the official compendiums for cosmetic ingredients. Many other cosmetic esters not listed in this division are available commercially.

2 PHYSICAL AND CHEMICAL PROPERTIES

The physical properties of esters are determined by both the fatty alcohol and acid portions.They are very important since the resulting esters affect the physical properties of the finished products.

Not only are the chemical constants very important for the quality control of esters but some, for example the saponification value or ester value, can be used for identification since they represent the molecular weight of the ester under test.

Table 7.1 References to Fatty Alcohol/Fatty Acid Esters

Raw Material	Compendiums[1]
Arachidyl Propionate	CTFA; CLS/JSCI
Butyl Myristate	CTFA; CLS/JSCI
Butyl Stearate	CTFA; CLS/JSCI
Cetearyl Octanoate	CLS/JSCI
Cetyl Acetate	CLS/JSCI
Cetyl Caprylate	CLS/JSCI
Cetyl Myristate	CLS/JSCI
Cetyl Octanoate	CLS/JSCI
Cetyl Palmitate	CTFA; CLS/JSCI
Cetyl Ricinoleate	CLS/JSCI
Decyl Myristate	CLS/JSCI
Decyl Oleate	CTFA; CLS/JSCI
Ethyl Isostearate	CLS/JSCI
Ethyl Linoleate	CLS/JSCI
Ethyl Oleate	CTFA; CLS/JSCI
Ethyl Stearate	CLS/JSCI
Hexyl Isostearate	CLS/JSCI
Isocetyl Myristate	CLS/JSCI
Isocetyl Palmitate	CLS/JSCI
Isocetyl Stearate	CTFA; CLS/JSCI
Isodecyl Isononanoate	CTFA; CLS/JSCI
Isodecyl Neopentanoate	CLS/JSCI
Isodecyl Oleate	CTFA; CLS/JSCI
Isohexyl Laurate	CTFA
Isohexyl Palmitate	CTFA
Isopropyl Isostearate	CTFA; CLS/JSCI
Isopropyl Lanolate	CTFA; CLS/JSCI
Isopropyl Linoleate	CTFA; CLS/JSCI
Isopropyl Myristate	CTFA; CLS/JSCI
Isopropyl Palmitate	CTFA; CLS/JSCI
Isostearyl Isononanoate	CTFA
Isostearyl Isostearate	CLS/JSCI
Isostearyl Laurate	CLS/JSCI
Isostearyl Myristate	CLS/JSCI
Isostearyl Neopentanoate	CTFA; CLS/JSCI
Isostearyl Palmitate	CLS/JSCI
Isotridecyl Isononanoate	CTFA; CLS/JSCI
Isotridecyl Myristate	CLS/JSCI
Methyl Hydroxystearate	CTFA
Methyl Ricinoleate	CLS/JSCI
Myristyl Myristate	CTFA; CLS/JSCI
Octyl (2-Ethylhexyl) Hydroxystearate	CTFA; CLS/JSCI
Octyl (2-Ethylhexyl) Isopalmitate	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

Table 7.1 *(contd.)* References to Fatty Alcohol/Fatty Acid Esters

Raw Material	Compendiums
Octyl (2-Ethylhexyl) Palmitate	CTFA; CLS/JSCI
Octyl (2-Ethylhexyl) Pelargonate	CLS/JSCI
Octyl (2-Ethylhexyl) Stearate	CTFA; CLS/JSCI
Octyldodecyl Erucate	CLS/JSCI
Octyldodecyl Myristate	CLS/JSCI
Octyldodecyl Neodecanoate	CLS/JSCI
Octyldodecyl Oleate	CLS/JSCI
Octyldodecyl Ricinoleate	CLS/JSCI
Oleyl Oleate	CLS/JSCI
Stearyl Heptanoate	CTFA; CLS/JSCI
Stearyl Octanoate	CLS/JSCI
Stearyl Stearate	CLS/JSCI

3 PHYSICAL AND CHEMICAL TESTS FOR IDENTIFICATION AND QUALITY

3.1 Physical Tests

Tests for physical properties such as viscosity (see Appendix, *4*), hardness (penetration) and melting point (Appendix, *8*) are necessary to ensure the quality of cosmetic products and, by comparison with standard fresh materials, can be used to identify the raw material.

3.2 Chemical Analysis

No useful chemical analytical methods for the identification of esters exist except for the chemical constants described below. The constants obtained by testing the component parts of the ester can be used to identify it.

For example, after *saponification* by alcoholic potassium hydroxide (Appendix, *22*) the fatty alcohol portion of the ester can be extracted by organic solvents such as *n*-hexane or petroleum ether, and the fatty acid portion extracted after neutralization of the remaining solution with hydrochloric acid. These can then be subjected to further tests: *acid value* (Appendix, *21*) for the acid portion and *hydroxyl value* (Appendix, *23*) for the alcohol portion. (See also Divisions 4 and 5).

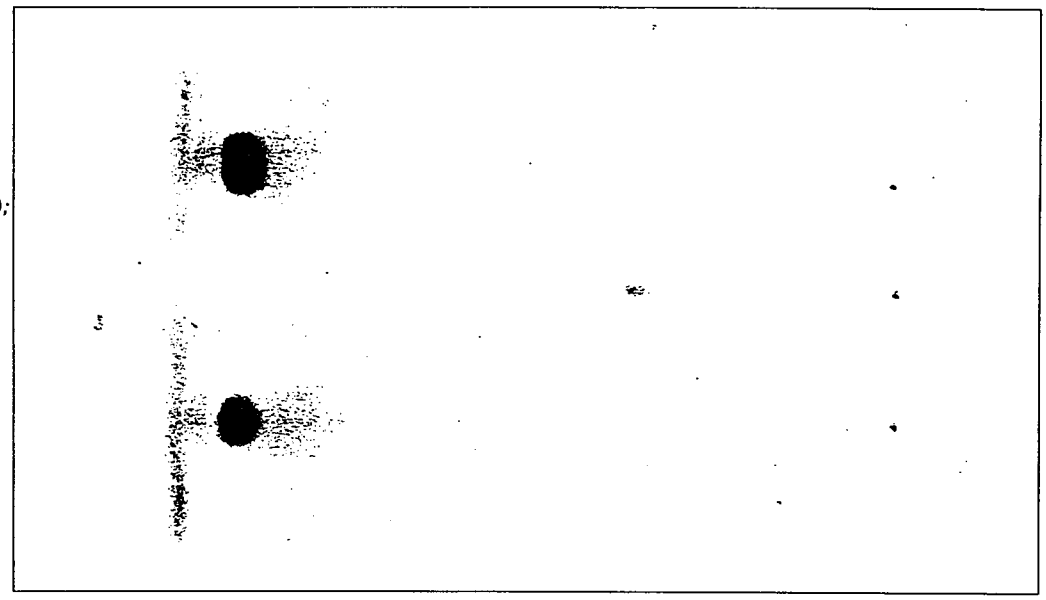
The acid value not only identifies the component acid but before saponification measures any free fatty acid. Similarly, the hydroxyl value indicates free fatty alcohol before saponification and the component alcohol after saponification when the ester is not composed of any hydroxy acid and/or diols. The *iodine value* (Appendix, *19*) can be used for identification if the fatty alcohols and/or fatty acids composing the ester are unsaturated. If not, it can show unsaturated impurities.

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

4.1.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a proper tool for identification of the ester, although it cannot differentiate the materials chemically categorized in this class and needs well established standard materials for comparison. The conditions usually employed for this analysis are silica gel TLC in combination with a mixture of petroleum ether and ethylether as a developing solvent and either iodine vapour or phosphomolybdic acid as an indicator. A spot of the ester can be identified at an R_f value (see Appendix, *26*) somewhere in between those of hydrocarbons (at solvent front) and triglycerides (at Rf of 0.5), as indicated in Figure 7.1. Should the unsaturated esters be separated from the saturated ester, a silica gel impregnated with silver nitrate may be used.



Mixture of Isopropyl Myristate and Oleyl Oleate at both sides and Olive Oil (Triglyceride) in the centre

Figure 7.1

Thin-Layer Chromatogram of Fatty Acid and Alcohol Ester

Conditions: Kieselgel 60 (Merck); Petroleum Ether:Ethylether (90:10); I_2 vapour

4.1.2 Gas Chromatography

Gas chromatography (GC) may be the best tool for identification of the esters. If the total numbers of carbon atoms in the ester are less than 35, the ester can be analysed by GC directly using almost any silicone stationary phase without any pretreatment of the ester. However, the availability of a well established standard is a prerequisite for this analysis. If the standard is not available, a methylene unit value can be employed as an alternative index to retention time. In Table 7.2, methylene unit values are summarized for several typical esters, which were obtained from the analysis using OV-17, a type of capillary column, as the stationary phase.

In addition to a direct GC analysis of the ester, fatty acid and fatty alcohol portions of the ester can also be analysed by GC after conversion into either the methyl ester for acids or acetate for alcohols and trimethylsilyl derivatives for both. A preparation of such derivatives can be performed after saponification of the ester, according to paragraph 3.1, and *trans*

(methyl) esterification using sodium or potassium methylate. Analytical conditions are shown elsewhere in the divisions of fatty acids or fatty alcohols.

Table 7.2 Methylene unit values of typical esters

Esters	MU value	
Isopropyl Myristate	19050	
Cetyl 2-Ethylhexanoate	25500	
2-Ethylhexyl Palmitate	25790	For the homologues, the methylene unit (MU) value can be estimated by adding 1000 to the known MU value for one carbon increase, and *vice versa*
Stearyl 2-Ethylhexanoate	27490	
2-Hexyldecyl Palmitate	30760	
2-Octyldodecyl Laurate	32580	
2-Heptylundecyl Palmitate(1)	33600	
2-Octyldodecyl Myristate	34500	
2-Heptylundecyl Palmitate(2)	34560	
2-Octyldodecyl Palmitate	36430	
2-Decyltetradecyl Myristate	38340	

In Figure 7.2, GC chromatograms of a mixture of several esters are shown for both packed and capillary column. When either the fatty acid or the alcohol portion of an ester have a branched carbon chain structure or unsaturated bonds, GC can separate them either from a straight carbon chain structure or saturated esters. However, it again depends on the availability of the standard sample to identify the ester. An effective tool to resolve the above problem is Mass Spectrometry (MS) especially when it is hooked with GC (see ¶4.1.3).

4.1.3 Gas Chromatography/Mass Spectrometry

Examples of Gas Chromatography-Mass Spectrometry (GC-MS) spectra are shown in Figure 7.3. It can be seen in the sample spectrum that a difference in fragmentation of a carbon chain can elucidate a chemical structure when such is needed for the identification of the ester.

As shown in the previous chromatograms, separation of the mixture of the esters can be achieved by a packed column, although it is far better when analysed by capillary column as shown in Figure 7.3, which can also separate 2-ethylhexanoic palmitate and cetyl 2-ethylhexanoate that have the same carbon numbers. However, isocetyl palmitate cannot be well separated even by capillary column because of the many isomers present.

When the ester is described as RCOOR', ionic fragments such as RC(OH)O-, $RC(OH)_2$-, RCO-, R'OCO-, R'(-H) etc. are keys for identification of the ester, branched chain structure and position of unsaturation. A comparison should be made of fragmentations of alkyl chains having the same carbon numbers, but with different branched chain and degree of unsaturation. A ratio of the molecular ions of the esters having long alkyl chains is usually very low in GC/Electron Impact (EI) MS and fragment ions are therefore also keys for determination of molecular weight.

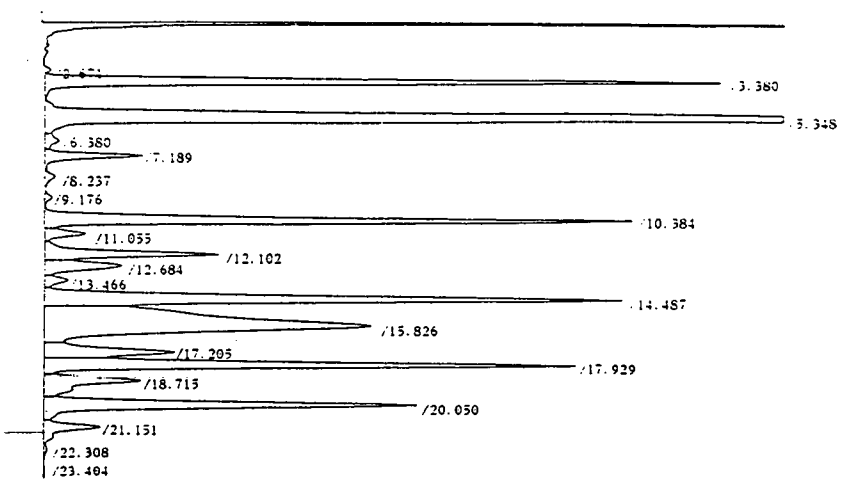
Figure 7.2

GC Chromatograms of mixtures of fatty alcohol/fatty acid esters

Condition I:

3. 4 min: *Isopropyl Myristate*
5.3 min: *Hexyl Laurate*
10.4 min: *Cetyl 2-Ethylhexanoate*
14.5 min: *Decyl Oleate*
15.8 min: *Isocetyl Palmitate*
17.9 min: *2-Heptylundecyl Palmitate*
20.1 min: *Oleyl Oleate*

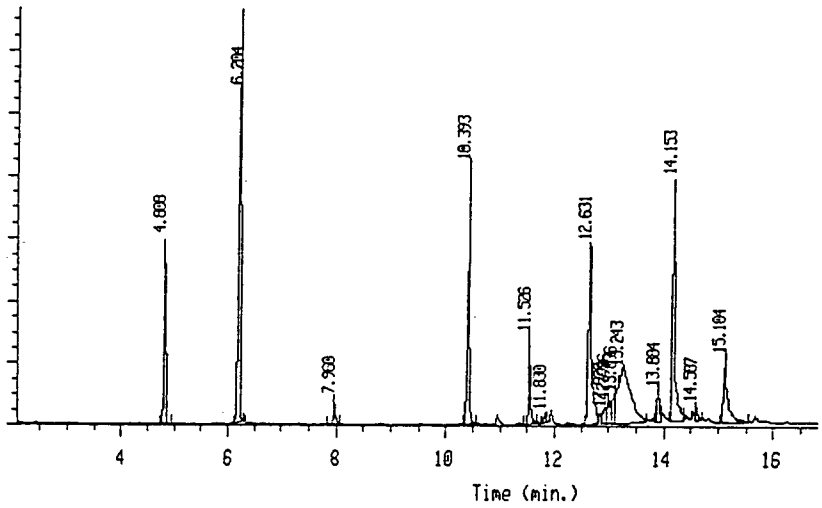
Diasolid ZT (GL Science, Japan); 3 mm i.d. × 0.5 m L.; Glass; 140-340°C; 8°C/min.; N_2 60 ml/min.; FID



Condition II

4.8 min: *Isopropyl Myristate*
6.2 min: *Hexyl laurate*
10.4 min: *Cetyl 2-Ethylhexanoate*
12.6 min: *Decyl Oleate*
13.2 min: *Isocetyl Palmitate*
14.1 min: *2-Heptylundecyl Palmitate*
15.1 min: *Oleyl Oleate*

DB-1 (Hewlett-Packard, USA); Fused silica 0.25 mm i.d. × 10 m; 100-200°C, 10°C/min.; 200-320°C, 20°C/min.; TIC (40-800 m/z); Split ratio – 1:50 (1 µl inj.)



4.2 Spectroscopy

4.2.1 Infrared Spectroscopy

Infrared spectroscopy (IR) can also be used as an identification test for esters by confirming several characteristic absorptions for alkyl chains (2920 and 2850 cm^{-1}), methylene chains (1470, 1390-1200 and around 720 cm^{-1}), and ester carbonyl (1740, 1180 and around 1100 cm^{-1}) etc. A characteristic absorption pattern in the ester region may give further information to distinguish the esters from other esters such as triglycerides. Isopropyl esters give a strong characteristic absorption at 1110 cm^{-1} and unsaturated esters can be distinguished by absorption at 2980 cm^{-1} which appears on the shoulder of 2920 cm^{-1} absorption. When the esters are crystalline, carbon numbers of the esters can be estimated from numbers of absorption appearing between 1390-1200 cm^{-1} and their crystalline structure is also judged

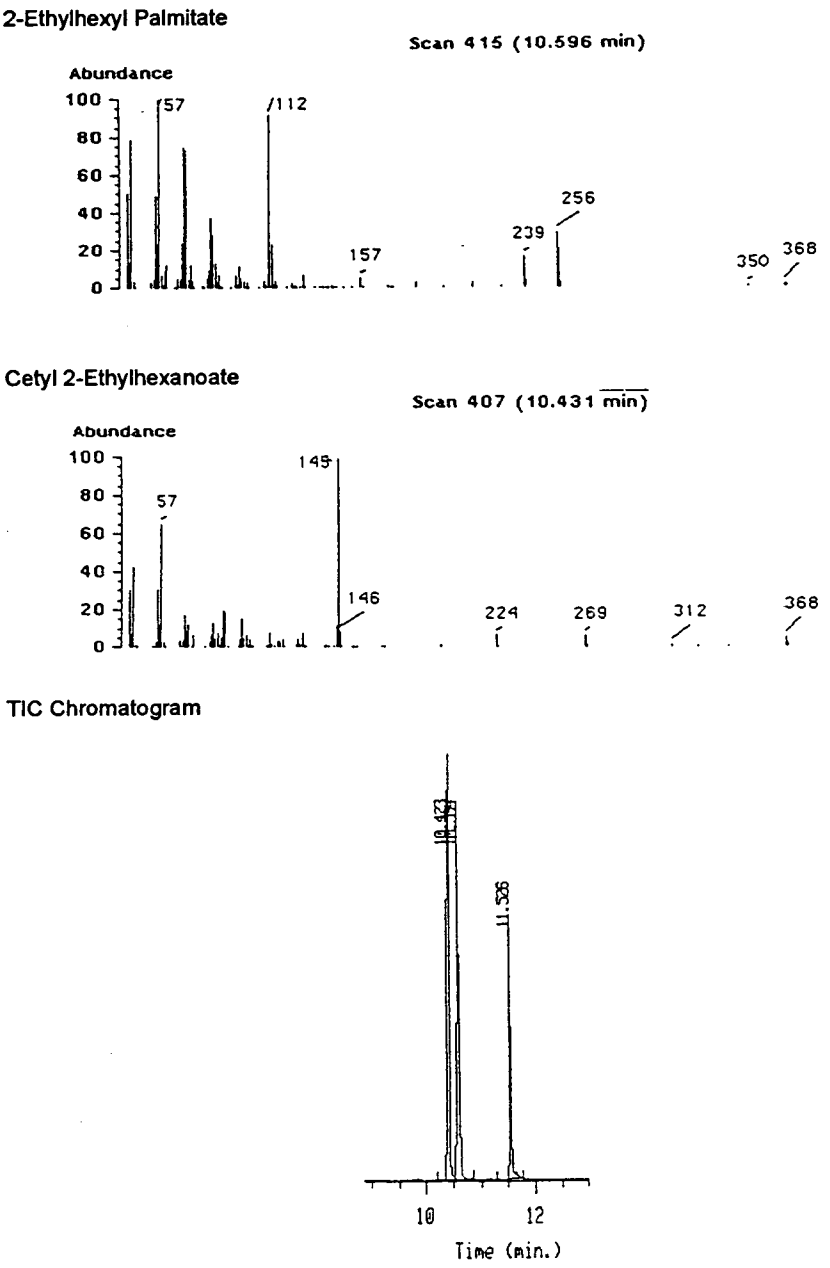


Figure 7.3

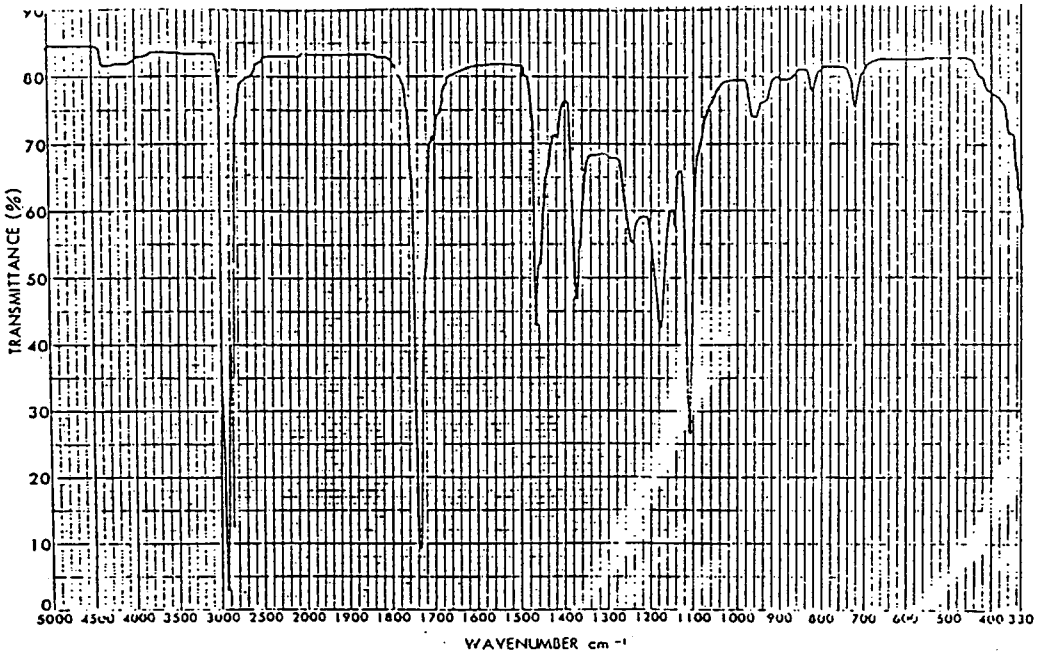
EI Mass Spectrum of 2-Ethylhexyl Palmitate and Cetyl 2-Ethylhexanoate

from absorption around 720 cm^{-1}, which appears in doublet for crystalline and in singlet for either paste or liquid.

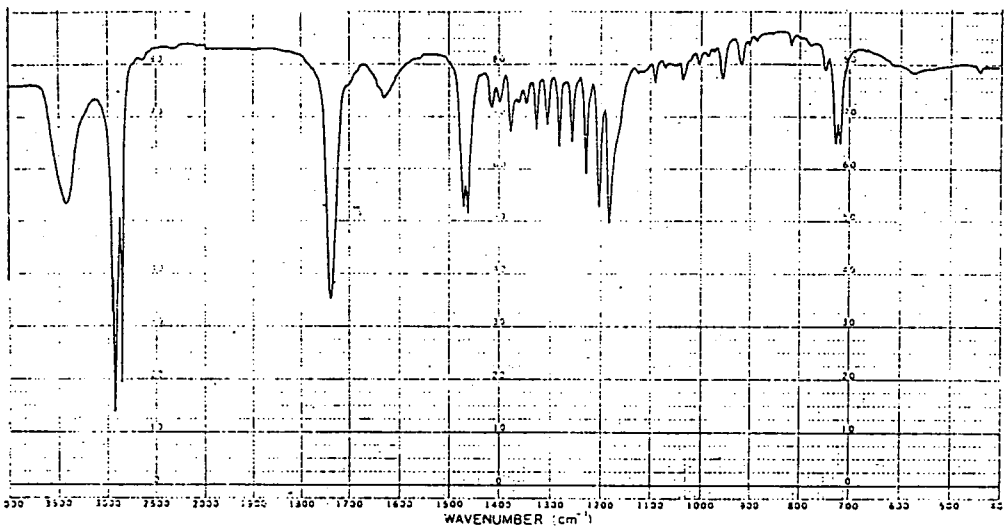
In Figure 7.4 IR spectra of typical esters are shown, where comparison of isopropyl esters, crystalline myristyl myristate and liquid oleyl oleate is made.

Figure 7.4
IR Spectra of typical esters

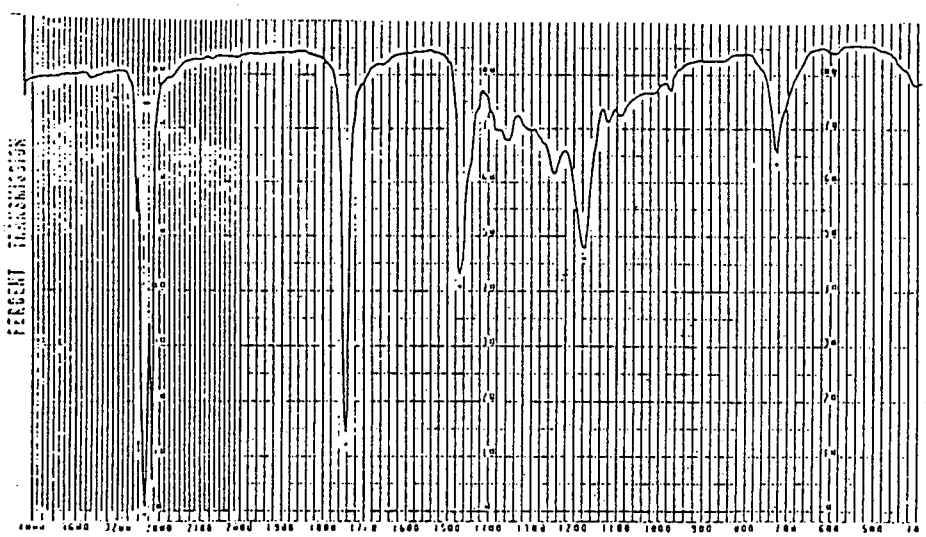
Isopropyl Myristate



Myristyl Myristate



Oleyl Oleate



4.4 ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance spectroscopy (NMR) does not give more information than IR, GC and GC/MS for the esters with straight alkyl chains. However, in the case of those esters with branched or unsaturated alkyl chains, ^{1}H- and/or ^{13}C-NMR provides useful information suggesting the presence of unsaturation, e.g. -(CH=CH)-, or branched chains, e.g. (CH_3)- or $-CH_2-(CH)-CH_2-$. They are also very useful for distinguishing these esters from other frequently used polyol esters.

5 TESTS FOR IMPURITIES AND QUALITY

5.1 Chemical Constants

Impurities to be examined are very few in this raw material class since they can be checked with several chemical constants described in the paragraph 3.2. These are, for example, free fatty acids by acid value, esters with different carbon chain other than those specified by either ester value or saponification value, degree of unsaturation by iodine value, etc. If odour or smell of the ester are important criteria for quality, the peroxide number (see Appendix, *25*) can be incorporated into the specification.

5.2 Loss on Ignition

Inorganic impurities can be examined by gravimetric method after it is ignited or heated with sulfuric acid. This method is applicable to those impurities at levels higher than 0.1%. If hazardous inorganic impurities are to be examined at very low level such as 1-10 ppm, specific test methods have to be established, for example, arsenic (As) and lead (Pb) by Atomic Absorption Spectroscopy.

5.3 Loss on Drying

If volatile compounds like moisture are to be controlled, a simple method such as gravimetric method for weight loss on drying for a certain period of time can be employed.

5.4 Others

If the raw materials pass the above constants and tests for purity and do not affect the performance, physical properties and safety in use of the finished cosmetic product, no other tests need be considered, since generally both the ester and its separate components will have undergone clinical tests before marketing and will have been widely used in cosmetics.

6 ANALYTICAL METHOD FOR ESTERS IN COSMETIC PRODUCTS

6.1 Solvent Extraction

In order to separate the ester from the finished cosmetic products, the first step to be taken is to separate any oil-soluble materials from cosmetics, which can be done by firstly evaporating the products to dryness in an oven or on a water-bath and then extracting oil-soluble materials with organic solvent such as *n*-hexane, benzene or chloroform. A separation of the extract from the residue can be done by filtration, centrifugation, etc. and then the extract is evaporated to dryness by appropriate measures for further analysis.

6.2 Silica Gel Column Chromatography

An oil-soluble portion of the cosmetic product which is prepared according to the method described in ¶6.1 can be subjected to a silica gel column chromatography to isolate the ester from other oil-soluble materials. For this separation, a stepwise elution is usually applied starting from *n*-hexane, benzene, chloroform and acetone and then to methanol, where the ester is normally isolated into the benzene fraction. Such stepwise elution can be achieved by mixing *n*-hexane and ethylether in different ratio, and for column chromatography. Many other adsorbents other than silica gel, such as alumina, Florisil, etc. have been utilized. After the ester is isolated from the cosmetic product it can be subjected to the methods described especially in paragraphs ¶4.1 to ¶4.3 for identification. Quantification can also be done gravimetrically and it can also be supplemented by GC analysis if well established standards are available.

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Division 8: Polyol/Fatty Acid Esters

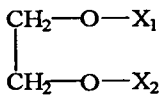
Toshiaki Iso

1 CHEMICAL DESCRIPTION, STRUCTURE AND USE IN COSMETICS

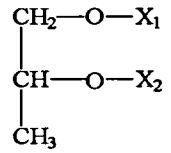
Many different types of polyol fatty acid esters are used as ingredients in cosmetic products mainly for their oily or waxy properties. This division describes polyol fatty acid esters other than natural glycerides and mainly those that are fully esterified. Some partial esters with remaining hydroxyl groups are discussed in Volume II, Surface Active Agents.

1.1 Structural Formulas

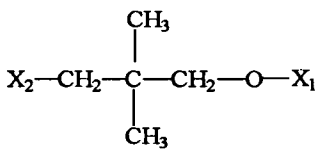
Polyol/fatty acid esters consist of different types of polyols esterified with a variety of fatty acids to form the required compound. The polyol molecular portions of the raw materials in this division are ethylene glycol, propylene glycol, neopentyl glycol, trimethylolpropane, and pentaerythritol. The remaining portions can be straight and/or branched-chain, or saturated and/or unsaturated fatty acids that normally have between 8 and 22 carbon atoms and are similar to the ones in the division on fatty alcohol and fatty acid esters (Division 7). The structures of these esters are shown below.



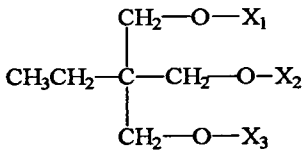
Ethylene glycol esters



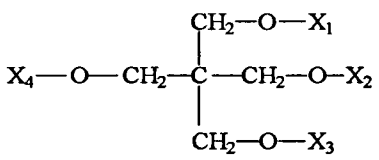
Propylene glycol esters



Neopentyl glycol esters



Trimethylolpropane esters



Pentaerythritol esters

$X_1 =$ —COR_1

$X_{2\text{-}4} =$ —$COR_{2\text{-}4}$ or —H

1.2 Sources and Production

The polyol fatty acid esters can be classified as semi-synthetic chemicals, since the polyol portion is totally synthetic, as is the final esterification process, but the fatty acids are mainly derived from natural sources. When synthetic fatty acids are used, they are usually branched-chain fatty acids such as 2-ethylhexanoic (octanoic) acid and isostearic acid.

Esterification is normally obtained by the dehydration of polyol and fatty acid. The reaction is produced with an acid catalyst (in some cases, benzene or toluene is used for azeotropic dehydration), followed by distillation, deodorization and refining.

1.3 Uses in Cosmetics

These materials possess properties and characteristics useful in cosmetic products and have therefore been produced commercially and thoroughly studied. Different compounds have been adjusted to improve their use with other materials already available for finished products, i.e. feel on the skin, stability, compatibility, etc.

These esters are less oily than other oils and fats with similar effects in cosmetic formulation and are suitable for products that aim to produce a smooth feeling on the skin. Normally, they have advantages over natural fats and oils such as favourable colour, odour, and stability in auto-oxidation. Furthermore, they may be used as co-emulsifying agents, dispersion agents for inorganic pigments and as binding agents for makeup foundations.

1.4. Raw Materials and References

Listed below are the names of raw materials for cosmetic products that fall into this category and their official literary titles.

Table 8.1. References to Polyol/Fatty Acid Esters

Raw Material	Compendiums[1]
Ethylene Glycol esters	
Dioctanoate (2-Ethylhexanoate)	CLS/JSCI
Dioleate	CLS/JSCI
Distearate	CLS/JSCI
Ditallowate	CLS/JSCI
Propylene Glycol esters	
Dicaprylate	CTFA; CLS/JSCI
Dicaprylate/Dicaprate	CTFA; CLS/JSCI
Dicocoate	CLS/JSCI
Diisostearate	CLS/JSCI
Dioleate	CLS/JSCI
Dipelargonate	CTFA; CLS/JSCI
Neopentyl Glycol esters	
Dicaprylate	CLS/JSCI
Diooctanoate	CLS/JSCI
Trimethylolpropane esters	
Triisostearate	CLS/JSCI
Trioctanoate	CLS/JSCI
Pentaerythritol esters	
Tetraisostearate	CLS/JSCI
Tetramyristate	CLS/JSCI
Tetraoctanoate	CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

2 CHEMICAL AND PHYSICAL PROPERTIES

The chemical and physical properties of esters are determined by both the polyol part of the molecule and the composition of the fatty acids which form the esters. See also ¶3.1 and ¶3.2.

3 IDENTIFICATION AND QUALITY CONTROL OF RAW MATERIALS

3.1 Physical Tests

The physical constants specific gravity (see Appendix, *6*), refractive index (Appendix, *16*), optical rotation, and melting point (Appendix, *8*) are used as identification and quality tests and are very important for the quality assurance of cosmetic products. Physical properties such as viscosity (Appendix, *4*) and surface tension (Appendix, *5*), as well as polarity-related properties, are important factors that influence the effect of finished products in use.

3.2 Chemical Tests

Chemical properties, especially chemical constants, are very important for the quality control of raw materials. Saponification value and ester value (see Appendix, *22*) are used as identification tests since these measure the amount of fatty acids and give the molecular weight data. If the raw materials are composed of unsaturated fatty acids, the iodine number (Appendix, *19*), which is an index of the unsaturation level, can also be used as the identification test. If not, it can be used as the test for unsaturated fatty acids regarded as impurities. The hydroxyl value (Appendix, *23*) is especially important as the esterification level index when the ester is not composed of any hydroxy acids, and the acid value (Appendix, *21*) is used as the weight index of existing free fatty acid.

There have been no useful chemical analytical methods for identifying the ester types except for the measurement of physical and chemical constants mentioned in the previous division. Therefore, the fatty acid portion and polyol portion are normally identified separately.

3.2.1 Separation of Fatty Acid Portion of Ester from Polyol Portion

After saponification by alcoholic potassium hydroxide, and neutralization by hydrochloric acid, the fatty acid portion is extracted using solvents such as *n*-hexane or ethyl ether, and identified by the acid value and by spectrometry and chromatography, as explained in ¶3.2.2.

3.2.2 Identification of Polyol Portion

It is difficult to separate the polyol after extraction of the fatty acids from the aqueous solution containing it and the neutralized salt. Therefore, the polyol portion is usually presented for chromatography without further separation. Chromatography is useful when the fatty acid and polyol are composed of multiple substances.

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

4.1.1 Thin-Layer Chromatography

Thin-layer chromatography (TLC) requires standard samples and their data. It is, however, a useful method for identification of esters. The conditions usually employed for this analysis are silica gel TLC in combination with a mixture of *n*-hexane and ethyl ether (for example, 90:10, 85:15, etc.) as a developing solvent. For detection, either the heating method (120 to 150°C) after spraying 50% sulfuric acid or the iodine evaporation method is used. A spot of the polyol fatty acid ester can be identified by the R_f value. This R_f value is closer to that of triglyceride and lower than that of higher alcohol esters.

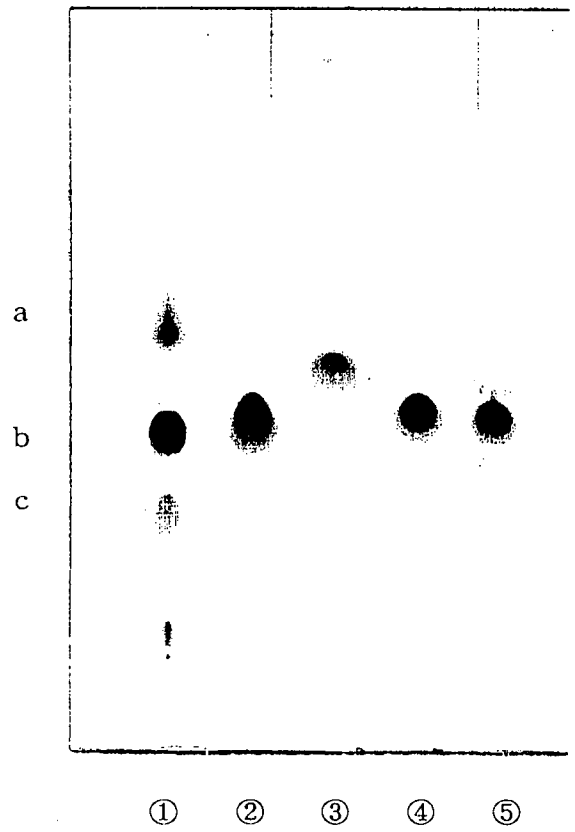
Figure 8.1 shows an example of TLC analysis. As a comparison of R_f values, spots of mixtures of isopropyl myristate, olive oil (triglyceride), and 2- octyldodecanol were identified at the same time.

Figure 8.1

Thin-Layer Chromatogram of Polyols/Fatty Acid Esters

Conditions:

Silica Gel G (Analtech);
n-Hexane:Ethylether (85:15);
I_2 vapour



[1] Standard mixture
(a) Isopropyl Myristate
(b) Olive Oil
(c) 2-Octyldodecanol
[2] Propylene Glycol Dicaprate
[3] Neopentyl Glycol Dioctanoate
[4] Trimethylolpropane Trioctanoate
[5] Pentaerythrityl Tetraoctanoate

4.1.2 Gas Chromatography

Gas chromatography (GC) is an excellent means of identifying esters. If the total number of carbons in the ester is less than 60, almost all esters can be analysed by GC directly using methyl silicone as stationary phase, without any pre-treatment of the ester. Since the esters are identified by comparing retention times of the standard samples, the availability of standard samples and their data is a prerequisite for this analysis. The retention index and methylene unit value are sometimes employed as the data of standard samples. The packed column was once mainly used as the GC column; however, the capillary column has a superior separating ability and has become the leading column of late.

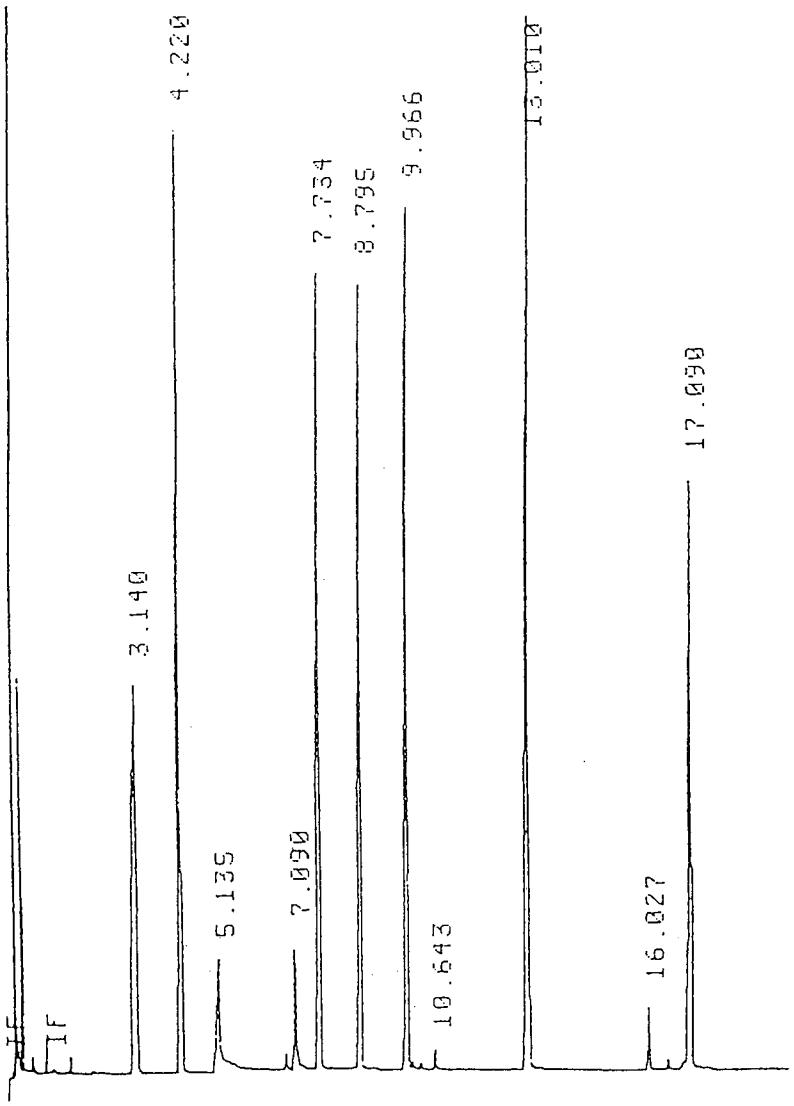
The GC chromatogram of an esters mixture with its measurement conditions is shown in Figure 8.2. The precision factor of the GC analysis is high. However, the sample's retention index and methylene unit value to be obtained is not always the same as those of the standard samples. Although the difference is 1% or less, it has to be taken into account. Therefore, the GC analysis alone cannot take ester identification beyond the inference stage. Also, the

Figure 8.2

GC Chromatogram of Polyol/ Fatty Acid Esters Mixture

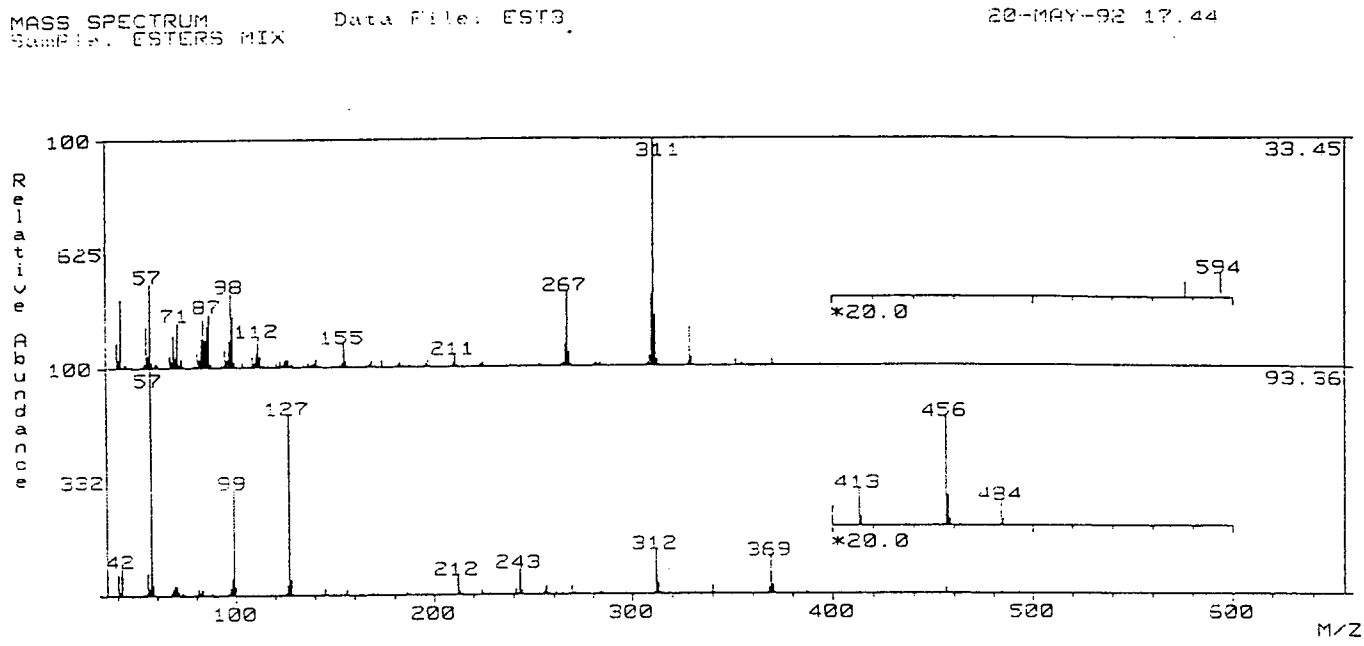
Conditions:

DB-1 (Methyl Silicone); Fused Silica; 0.25 mm i.d. x 5 m; 150-340°C; 10°C/min.



3.14 min: Propylene Glycol Dioctanoate
4.22 min: Neopentyl Glycol Dioctanoate
7.73 min: Propylene Glycol Dicaprate
8.80 min: Neopentyl Glycol Dicaprate
9.97 min: Trimethylolpropane Trioctanoate
13.01 min: Pentaerythrityl Tetraoctanoate
17.09 min: Ethylene Glycol Distearate

Mass Spectrum



TIC

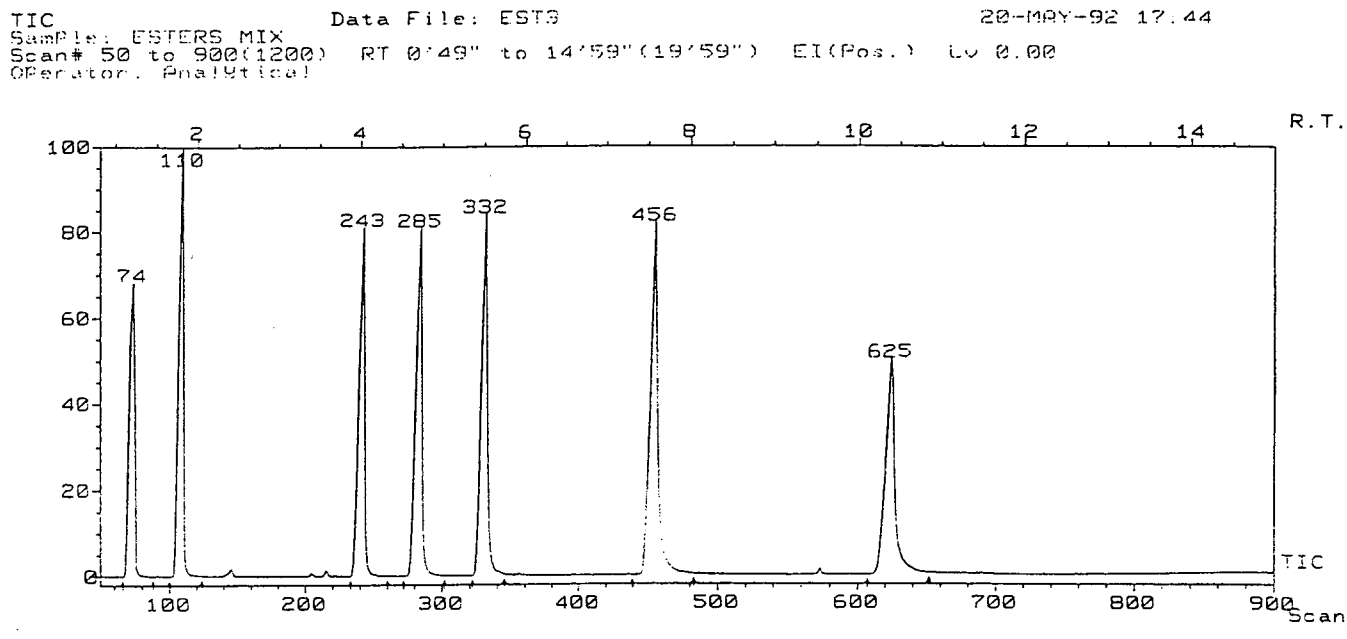


Figure 8.3 *Mass Spectra of Trimethylolpropane Trioctanoate and Ethylene Glycol Distearate*

retention indexes and methylene unit value of some substances are not much different. For further accurate confirmation, it is better to use other analyses such as infrared (IR) and nuclear magnetic resonance (NMR) together with the GC analysis.

4.1.3 Gas Chromatography/Mass Spectrometry

An effective and different tool to resolve the problem referred to in ¶4.1.1 is mass spectrometry (MS). Especially, analysis which uses a device directly coupling the GC and MS (GC-MS) is most effective. Since the MS can provide data including molecular weight and the mass number of the partial structure of a molecule, it is an effective analysis method even when the standard sample is not available.

In the case of esters normally the molecule ion peak is either small or does not appear except in esters of low molecular weight. Since the molecular ion peak does not easily appear for polyol fatty acid esters, the fragment ion peak is the key to identification of the ester. Two peaks, namely RCO and M-RCOO, among many fragment ion peaks are comparatively large and important. Therefore, the structure is determined by the data provided by MS and the estimate of molecular weight based on data such as retention time by GC.

Figure 8.3 shows an example of the mass spectrum. In the spectrum of trimethylolpropane trioctanoate, m/e = 127 is RCO and m/e = 369 is M-RCOO. In the spectrum of ethylene glycol distearate, m/e = 267 is RCO and m/e = 311 is M-RCOO.

4.2 Spectroscopy

4.2.1 Infrared Spectroscopy

Infrared (IR) spectroscopy is a very useful method for the identification of esters as they have common characteristic absorption bands according to their structure (1740, 1170 and around 1100 cm^{-1}).

A useful method for distinguishing the ester type can be obtained by the absorption pattern around 1300 to 1100 cm^{-1}. The absorption in this range shows characteristic patterns depending on the types of fatty acid and polyol forming the esters. For example, the absorptions around 1170 cm^{-1} of esters composed of 2-ethylhexanoic acid appears in a sort of doublet form.

Furthermore, a characteristic absorption pattern in the ester region may provide further information for distinguishing the esters from other polyol esters such as triglycerides. Comparison of the spectrum of the sample with that of a standard ester is the most assured way for accurate identification. Since there are many esters, computers can be used for quick and accurate spectrum searches.

Figures 8.4, 8.5, and 8.6 show typical IR spectra. Neopentyl glycol dicaprate (Figure 8.4) and neopentyl glycol dioctanoate (Figure 8.6), differ in their fatty acid portions. Neopentyl glycol dioctanoate (Figure 8.6) and pentaerythrityl tetraoctanoate (Figure 8.5) differ in their polyol. Both differences clearly appear at absorptions around 1300-1100 cm^{-1}

4.2.2 1H- and 13C-Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) can provide important data for determining chemical structures. Therefore, NMR is a better identification test method, demonstrating its superior abilities especially for identifying the esters in this division.

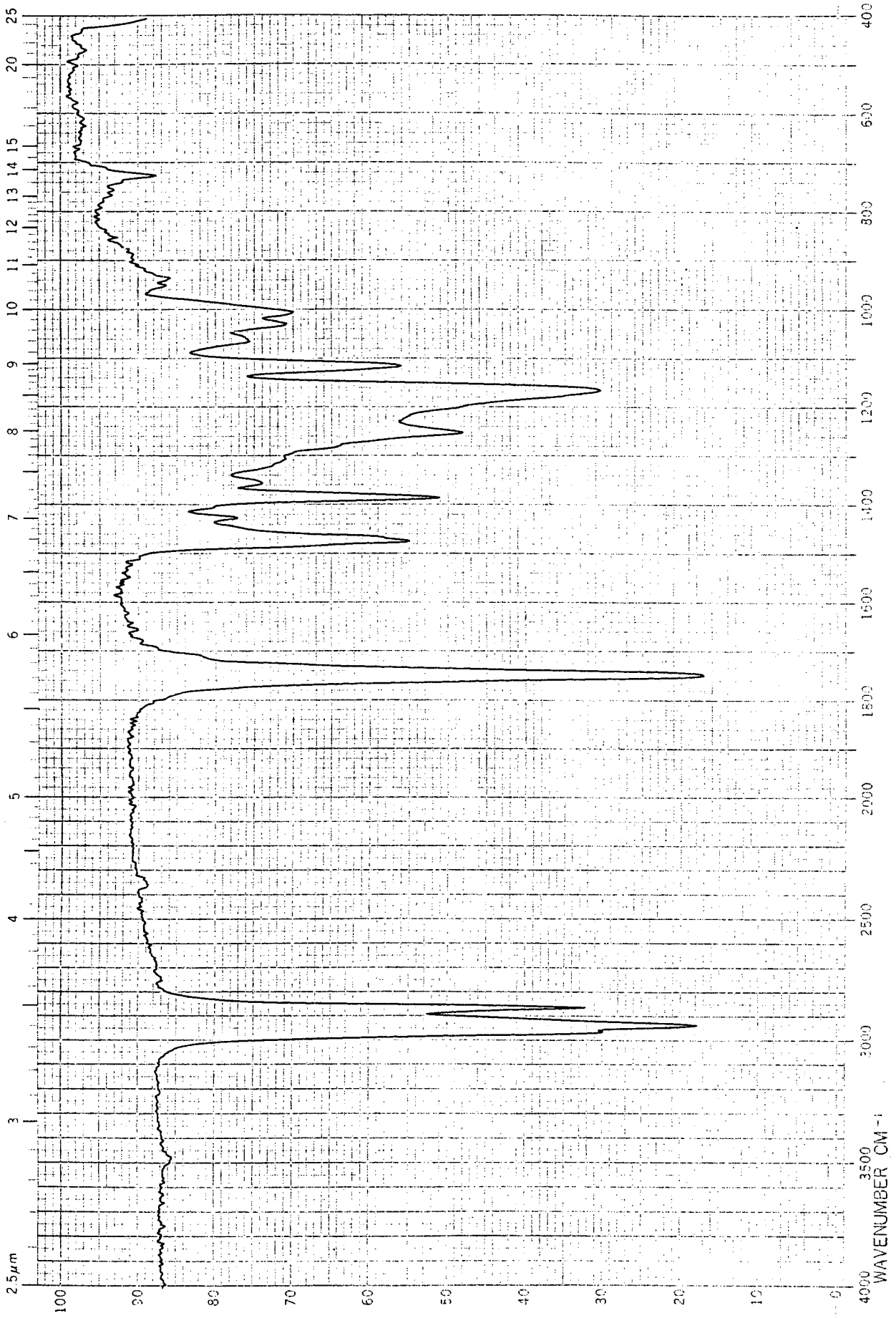


Figure 8.4

IR Spectrum of Neopentyl Glycol Dicaprate

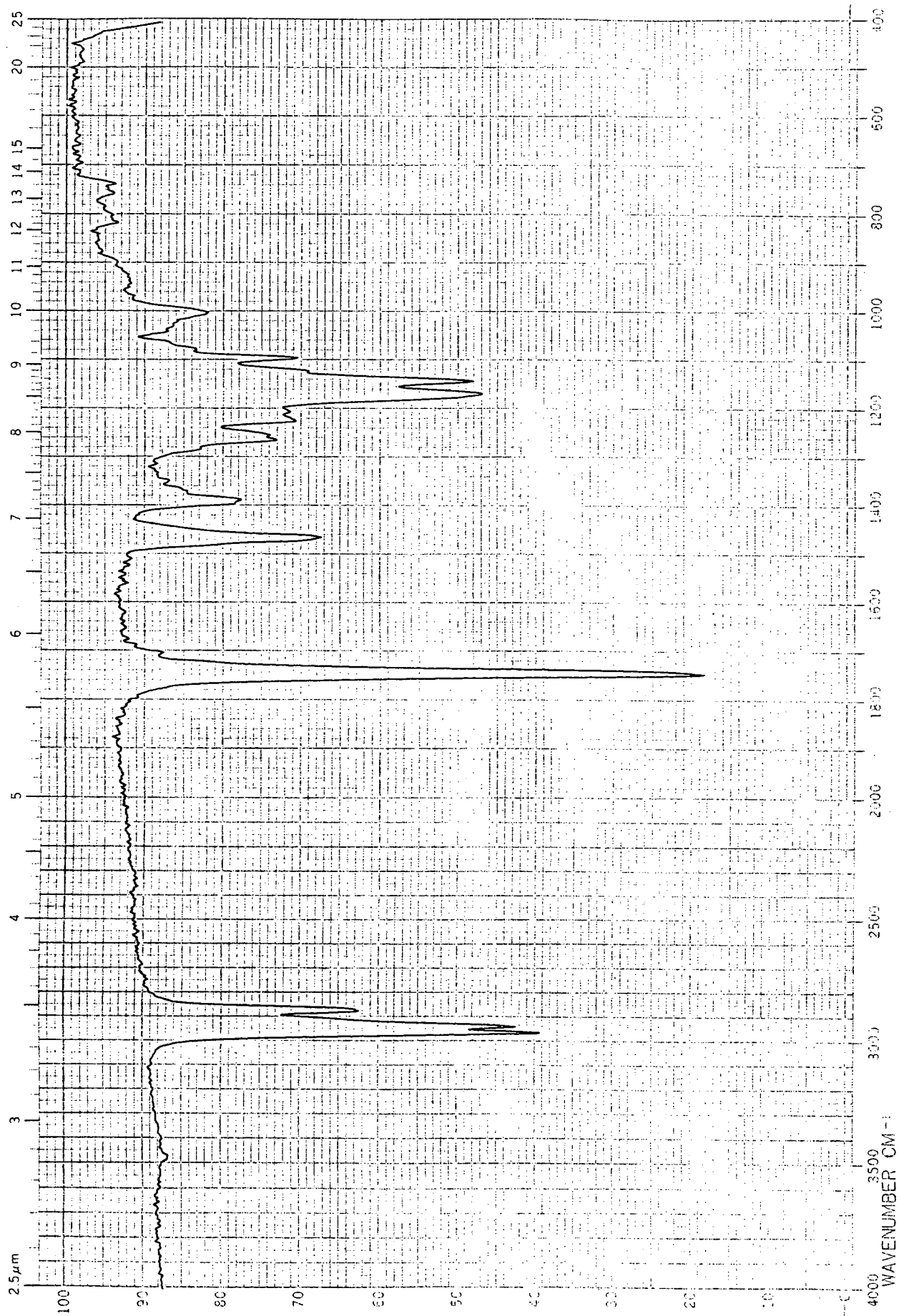


Figure 8.5

IR Spectrum of Pentaerythrityl Tetraoctanoate

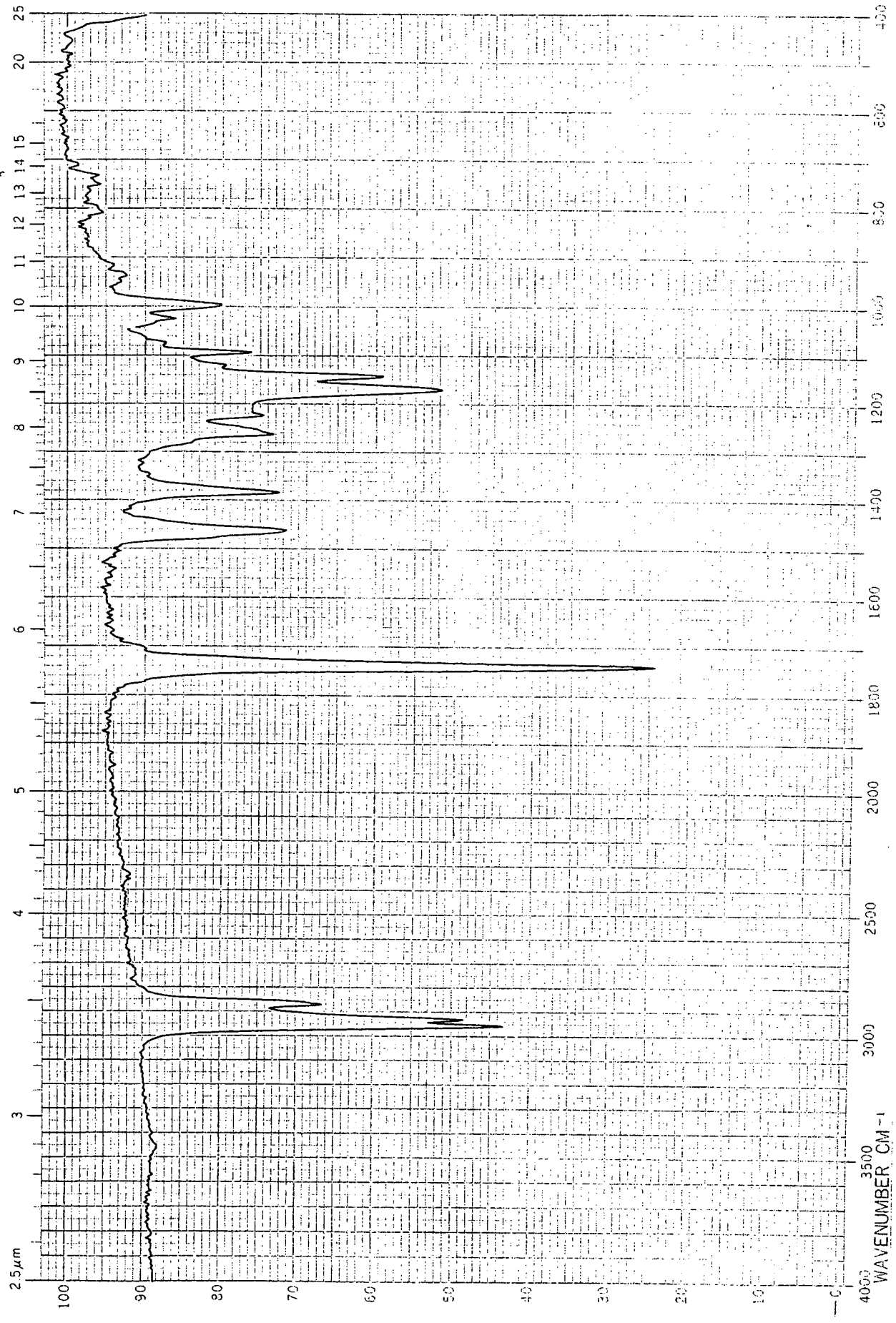


Figure 8.6

IR Spetrum of Neopentyl Glycol Dioctanoate

^{1}H-NMR provides data relating to the existence and environment of each proton in a molecule. For example, signals appear near 0.9 ppm for the methyl proton, which is the end group of alkyl; near 2.3 ppm for the methylene proton, which is adjacent to carbonyl; near 4 ppm for the methylene proton, which is adjacent to ester oxygen; and near 5 ppm for the methine proton. Moreover, characteristics (singlet, doublet, triplet, or multiplet) appearing in the signal patterns follow fixed rules in accordance with the surrounding environment. The signal intensity is in proportion to the number of protons. Therefore, the chemical structure of esters can sometimes be determined by ^{1}H- NMR alone.

Figure 8.7 shows the ^{1}H-NMR spectrum of the propylene glycol dioctanoate and neopentyl glycol dioctanoate and their signal reversion.

^{13}C-NMR provides data relating to the existing environment of each carbon atom in a molecule. For example, signals appear around 10 to 20 ppm for the methyl carbon, when it is an end group of an alkyl group; around 20 to 35 ppm for the methylene carbons of alkyl chains; and around 160 to 220 ppm for carbonyl carbon. When the measurement is performed by the INEPT (Insensitive Nucleus Enhancement Through Polarisation Transfer) method, signals of the quaternary carbon disappear and the methylene carbon can be expressed by a negative signal. However, most important are the signals which represent the carbon atoms in the polyol structure and they are very useful for distinguishing the types of polyol.

Figure 8.8 shows the ^{13}C-NMR spectrum of pentaerythrityl tetraoctanoate. Each of ten carbon types is clearly seen to appear separately. The signals around 12 and 14 ppm are of the methyl carbon; the four signals at 22 to 32 ppm and the signals around 61.5 ppm are of the methylene carbon; the signals around 42 ppm are of the quaternary carbon; the signals around 47 ppm are of the methine carbon; and the signals around 176 ppm are of the carbonyl carbon.

5 TEST METHODS FOR IMPURITIES

5.1 Chemical Constants

Esters that can be defined as impurities in the raw materials described in this division are, for example, partial esters which contain unesterified hydroxyl groups in their molecules (caused by insufficient esterification reaction during the manufacturing process) and free fatty acid. These impurities can be checked by the hydroxyl value and acid value (see Appendix, *23* and *21*, respectively).

The presence of fatty acids of differing numbers of carbons can be detected by the ester value and saponification value (see Appendix, *22*). The unsaturated fatty acids as impurities can be detected by the iodine value (Appendix, *19*). With substances such as unsaturated fatty acid esters, which are easily affected by oxidation, the peroxide number will be an important quality control item (see Appendix, *25*). The peroxide value indicates the content of peroxide produced during the initial stage of auto-oxidation. Raw materials with a high peroxide value may be detrimental to the odour and colour of products.

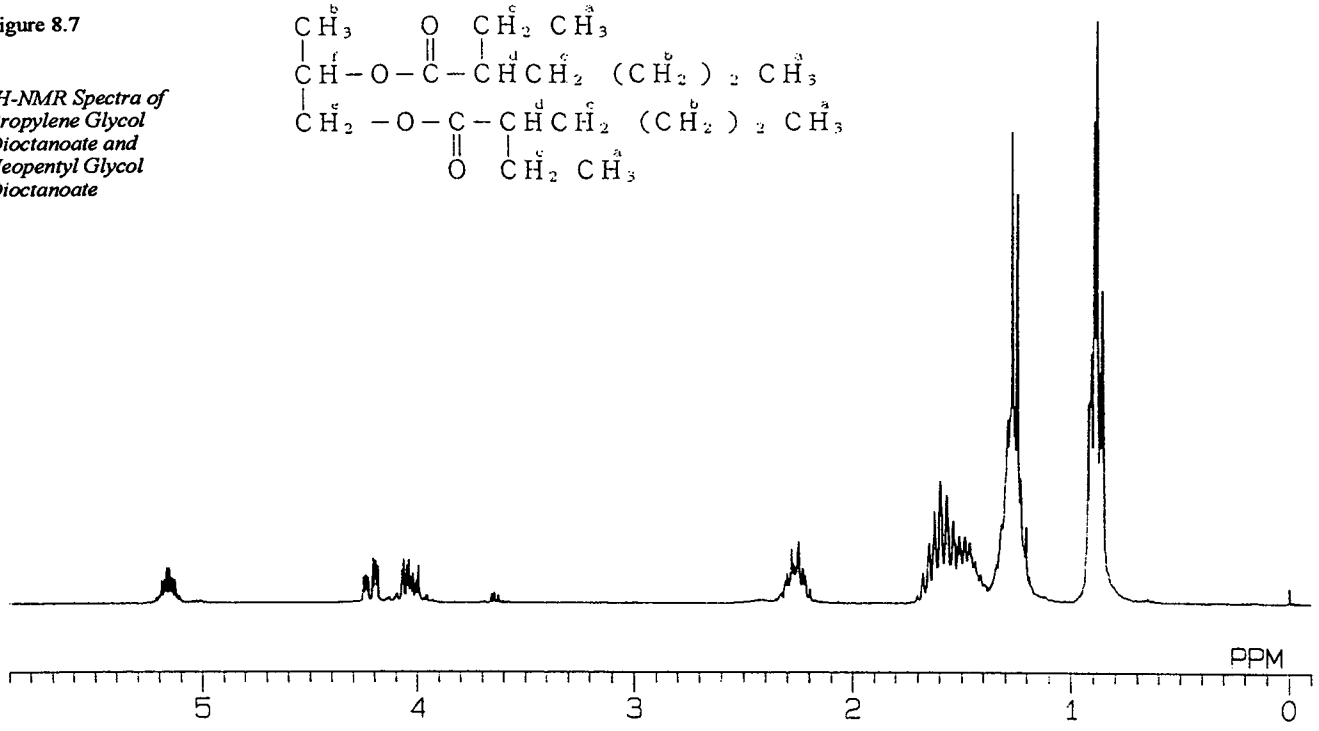
5.2 Inorganic Impurities

The measurement of remaining impurities after a sample is ignited or heated is useful for finding out the content of inorganic impurities (see Appendix, *35*).

Figure 8.7

^{1}H-NMR Spectra of Propylene Glycol Dioctanoate and Neopentyl Glycol Dioctanoate

Propylene Glycol Dioctanoate



Neopentyl Glycol Dioctanoate

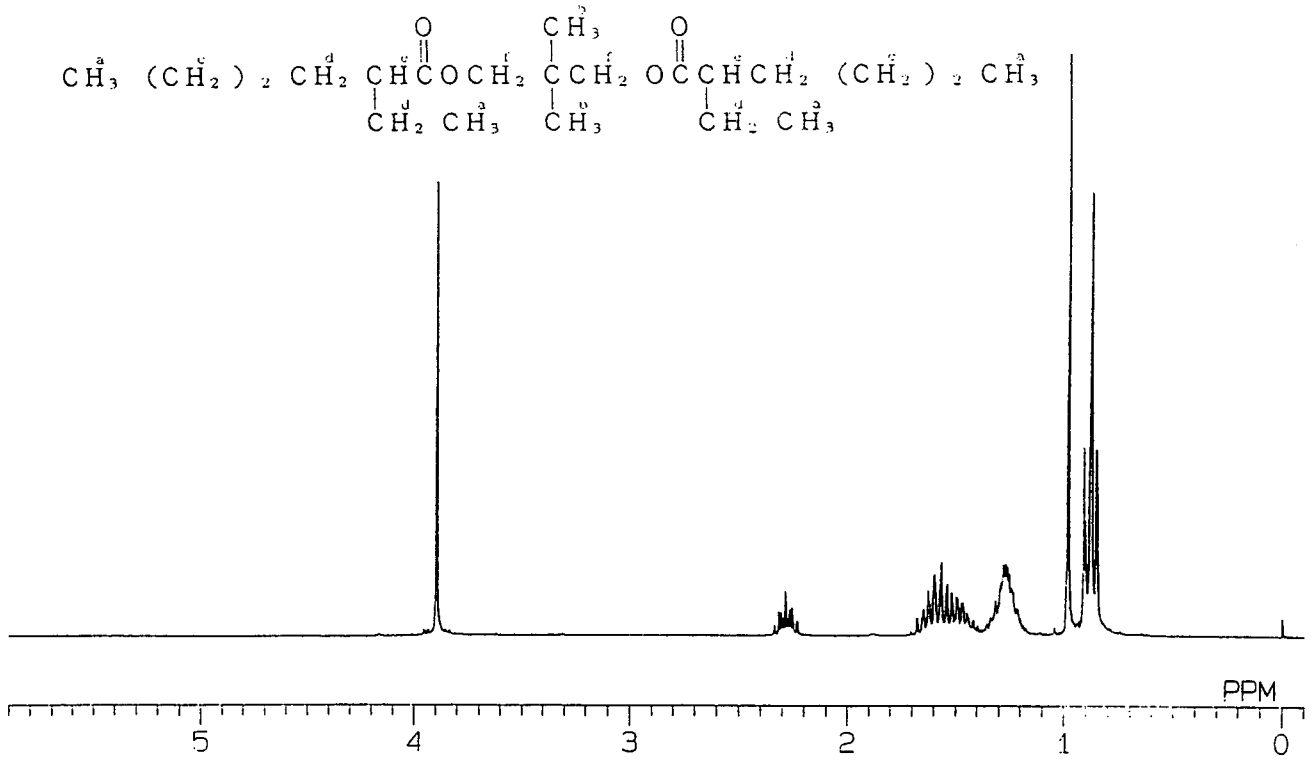
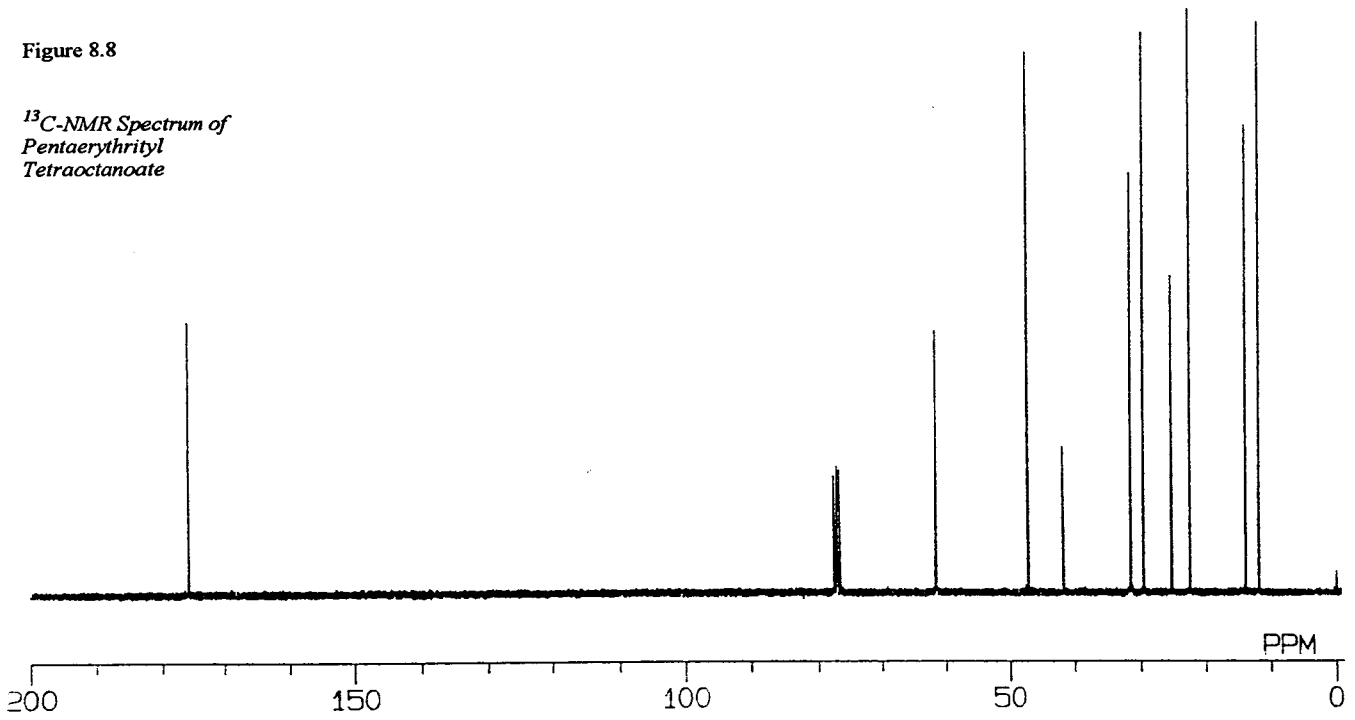
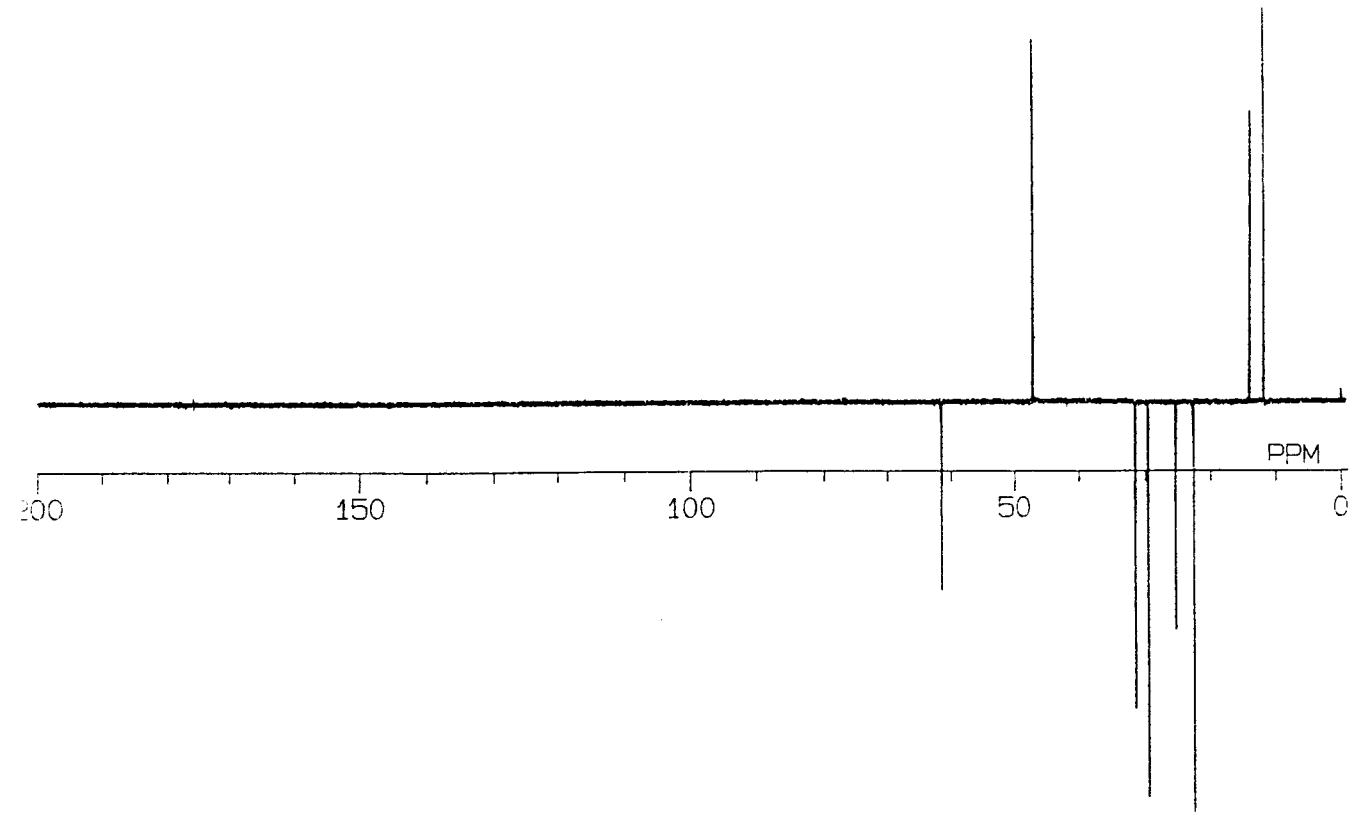


Figure 8.8

^{13}C-NMR Spectrum of Pentaerythrityl Tetraoctanoate





This method is applicable to identifying impurities at the level of 0.1% or more. If hazardous metals such as lead (Pb) or arsenic (As) are to be examined, an analysis method at ppm level is necessary. Atomic absorption spectroscopy, which is superior in sensitivity and selectivity, has been established for analysing lead. Although arsenic can be determined by atomic absorption spectroscopy, accessory equipment is required. Arsenic is rather too special for this analysis method. A colorimetric analysis, which is an improved Gutzeit method, is more generally used.

5.3 Loss On Drying

If substances like moisture or esters of low molecule weight with low boiling points are present as impurities, the weight loss can be determined after drying the materials for a certain period of time.

6 ESTER DETERMINATION IN COSMETIC PRODUCTS

6.1 Solvent Extraction

In order to separate esters from finished cosmetic products, the first step is to separate oil-soluble materials from cosmetics by extraction with solvents (see Appendix, *37*).

6.2 Silica-Gel Column Chromatography

An oil-soluble portion (of cosmetic products) prepared in accordance with the method described in the previous division (¶6.1 and Appendix, *37*) is a mixture of several substances (hydrocarbon, ester, triglyceride, etc.). Silica gel column chromatography can be applied to isolate the ester from other oil- soluble materials.

Elution is performed by changing the mixing ratio of *n*-hexane and ethyl ether (95:5, 9:1, 7:3, 1:1, and so on). The higher alcohol fatty acid esters are obtained when the ratio of *n*-hexane to ethyl ether is 95:5. The polyol fatty ester can be obtained when the ratio is 9:1. Triglyceride also starts dissolving when the ratio is 9:1. Therefore, to obtain the polyol fatty acid ester only, silica gel column chromatography should be performed again while gradually changing the ratio of *n*-hexane and ethyl ether. Normally, however, the fraction obtained at 9:1 is used to achieve qualitative and quantitative analyses in connection with TLC, GC, GC-MS, and NMR analyses. The quantitative results are obtained through consideration of the GC and NMR analyses by measuring the weight of the fraction from silica-gel chromatography.

7 BIBLIOGRAPHY

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Division 9: Fatty Alcohol/Organic Non-Fatty Acid Esters

Roberto Leonardi

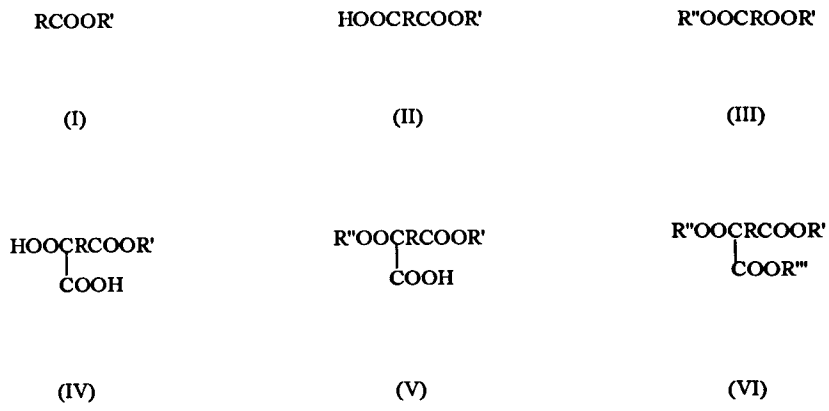
1 CHEMICAL DESCRIPTION, STRUCTURE AND USE IN COSMETICS

Fatty alcohol/organic non-fatty acid esters are mainly synthetic materials used industrially and in the laboratory.[1-8] Some vegetable oils and fats can contain, besides alcohol and fatty acid esters, esters with non-fatty organic acids.

The esters of polyhydric alcohols are covered in Vol. I, Division 8 and Vol. II, Division 6. The esters of fatty acids are covered in Vol. I Division 7. The esters of polyether alcohols are treated in Vol. II, Divisions 1, 3 and 6.

1.1 Structure

Fatty alcohol/organic non-fatty acid esters can be represented by the following structural formulae:



In (I) RCO- represents the organic non-fatty acid part of the molecule, e.g.benzoic acid, and OR' the fatty alcohol part. Besides the esters formed by only one fatty alcohol and a monofunctional acid, there are diesters and triesters; in other words molecules formed by a di- or tri-functional acid, e.g. phthalic acid, (II) and (III), and citric acid (IV), (V) and (VI) respectively, and one or more alcohols. -OR', OR'' and OR'''.

1.2 Origins[9]

By synthesis:

a) By direct esterification of an acid and an alcohol.

$$RCOOH + R'OH = RCOOR' + H_2O$$

b) By transesterification of an ester in the presence of an alcohol.

$$RCOOR'' + R'OH = RCOOR' + R''OH$$

c) By the transformation of an acid derivative.

$$RCOCl + R'OH = RCOOR' + HCl$$

d) The fatty alcohols are derivatives of natural oils and fats and petrochemicals, and are used industrially.

1.3 Uses in Cosmetics

1.3.1 Regreasing Substances - Emollients

Similar to fatty acid/fatty alcohol esters (Division 7), they can be used as regreasing and emollient agents in emulsions or anhydrous compounds. They help the spreading of a cosmetic product onto the skin surface and improve its feel.

1.3.2 Emollient - Moisturizers

Depending on the characteristics of the acid part of the molecule, these compounds can have skin-moisturizing properties, particularly if they possess additional hydroxy groups or unesterified acid groups which have high hydrophilicity.

1.3.3 Plasticizers

Some compounds of this series are used as plasticizers both in cosmetic products and in packaging materials. They improve the softness of lipogels and act as crystallization modifiers in sticks and plastic materials.

1.4 Raw Materials and References

Table 8.1 References to Fatty Alcohol/Organic Non-Fatty Acid Esters

Raw Material	Compendium[1]
Acetylcitric Acid *ester of*	
Butyl Alcohol (Acetyl Tributyl Citrate)	CLS/JSCI
Ethyl Alcohol (Acetyl Triethyl Citrate)	CTFA; CLS/JSCI
Adipic Acid *ester of*	
Isobutyl Alcohol (Diisobutyl Adipate)	CLS/JSCI
Isocetyl Alcohol (Diisocetyl Adipate)	CLS/JSCI
Isooctyl Alcohol (Diisooctyl Adipate)	CTFA; CLS/JSCI
Isopropyl Alcohol (Diisopropyl Adipate)	CTFA; CLS/JSCI
Isostearyl Alcohol (Diisostearyl Adipate)	CLS/ JSCI
Citric Acid *ester of*	
Ethyl Alcohol (Triethyl Citrate)	CLS/JSCI
Lactic Acid *ester of*	
C12-15 Alcohols (C12-15 Alkyl Lactate)	CTFA
Cetyl Alcohol (Cetyl Lactate)	CTFA
Myristyl Alcohol (Myristyl Lactate)	CTFA
Maleic Acid *ester of*	
Diisostearyl Alcohol (Diisostearyl Maleate)	CLS/JSCI
Propionic Acid *ester of*	
Arachidyl Alcohol (Arachidyl Propionate)	CTFA
Sebacic Acid *ester of*	
Isooctyl Alcohol (Diisooctyl Sebacate)	CTFA; JSCI
Isopropyl Alcohol (Diisopropyl Sebacate)	CTFA; JSCI
Succinic Acid ester of	
Ethoxyethyl Alcohol (Diethoxyethyl Succinate)	CLS/JSCI
Isooctyl Alcohol (Diisooctyl Succinate)	CTFA; CLS/JSCI

1. See p. viii for an explanation of the abbreviations used for compendiums.

2 PHYSICAL AND CHEMICAL PROPERTIES

These organic fatty alcohol/non-fatty acid esters have physicochemical properties that can be used to identify them, for their application and for their quantitative determination and by comparing the test results with those of standard materials they can be used for quality assessment.

2.1 Physical Properties

The physical properties of fatty alcohol/organic non-fatty acids esters depend on various factors:

a) the kind of fatty alcohol and the length of its chain;

b) the kind of acid.

a) The lipophilicity of the compounds is greater the longer the chain; also in this case the presence of lateral free OH groups increases the hydrophilicity.

b) Very differing characteristics can be given to esters by various acids. They can be:

- more or less lipophilic;
- more or less hydrophilic (presence in the molecule of COOH or free OH groups);
- more or less hydrolysis resistant.

These compounds are frequently liquid at ambient temperatures and volatile at high temperature[9-16] (see ¶4.1.2 and Appendix, *22*).

2.2 Chemical Properties

Hydrolysis is the main reaction of the esters which converts it into the two components: acid and alcohol or alcohol mixture. This reaction, which can occur on the skin surface, releases -OH and -COOH groups and increases the hydrophilicity of the mixture. It is also used in the quantitative estimation of esters (see saponification value, ¶3.2.4).

3 PHYSICAL AND CHEMICAL TESTS FOR IDENTIFICATION AND QUALITY

3.1 Physical Tests

Physical tests (see Appendix, *1, 2, 3, and 4*) cover :

3.1.1 Appearance
3.1.2 Odour
3.1.3 Colour
3.1.4 Viscosity
3.1.5 Melting Point

The melting point can be used to identify solid esters at ambient temperature. Tests can be carried out by differential scanning calorimetry, (DSC), e.g., the ratio of liquid to solid at different temperatures can be very useful when identifying liquid esters at ambient temperature.

3.1.6 Refractive Index

The refractive index can be very useful for identification,[13,17,18] It is very quick, but not a very selective test. See Appendix, *16*.

3.2 Chemical Tests

3.2.1 Hydrolysis

Hydrolysis is the main reaction for identifying the esters. It is used to determine the saponification value,[9,19,20] (see ¶3.2.4 and Appendix, *22*).

3.2.2 Iodine Value[21,22] (see Appendix, *19*)

This determination measures the number of double bonds present in the sample. It is used for identification and also quality when the value does not match the standard specification.

3.2.3 Acid Value (see Appendix, *21*)

3.2.4 Saponification Value (see Appendix, *22*)

The saponification value depends on the ratio of the ester groups and the other structures present in the molecule (aliphatic chain length, etc., and the possible presence of foreign substances, i.e. quality.

3.2.5 Hydroxyl Value (see Appendix, *23*)

4 MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

4.1 Chromatography

4.1.1 Thin-Layer Chromatography

The identification of esters by Thin-Layer Chromatography (TLC) is generally made through systems used for lipophilic compounds; corrective procedures must be found for more hydrophilic esters, such as hydroxy esters. Esters usually have an R_f (see Appendix, *26*) of approximately 0.8 which can vary according to the molecular structure.

As detector, iodine is useful for unsaturated substances, or bromothymol blue with ammonia vapours, rhodamine B or 2-7 dichlorofluorescein and ultraviolet light, or sulfuric acid heated 130°C.[23]

These systems can also be used to prepare samples for further gas chromatography (see fatty alcohols, Division 5). Many recent studies on the use of different chromatographic and detection systems are reported in the literature.[6,24,25]

4.1.2 Gas Chromatography (see Appendix, *27*)

Gas Liquid Chromatography (GLC) is one of the preferred analytical methods for characterization because of the relatively easy vaporisation of the esters. Analytical methods vary depending on the type of molecules concerned. There are many literature reports, both official methods and research papers.[4,26-47]

4.1.2 High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a well-known sensitive and selective analytical technique, even though it is less used than GLC. It is particularly used for esters which absorb in the ultraviolet spectrum because of the extensive use of this detector.

4.1.3 Other Techniques: Micellar Electrokinetic Chromatography

Other techniques, such as Micellar Electrokinetic Chromatography are also employed. They are described in the literature.[48-50]

4.2. Spectroscopy

4.2.1 Ultraviolet Spectrophotometry

Saturated esters have a low absorbance in this part of the spectrum, but ultraviolet (UV) spectrophotometry can be used when the acid part of the molecule shows absorption.

Many methods of ester spectrophotometric determinations are reported in the literature.[24,53-55] UV spectrometers are used to detect the esters after separation by HPLC. Some studies even report applications to saturated esters.[56]

4.2.2 Infrared Spectroscopy

Fatty alcohol/organic non-fatty acid esters present the most characteristic absorption in the infrared field:

- Vibration of the double bond, C=O, produces a clear and intense peak in the zone around 1700-1750 cm^{-1}; its position depends on the molecular structure.
- Two intense absorption bands for the vibration of the single bond, C-O, are in the zone between 1050 and 1300 cm^{-1}.
- If OH groups are present, the oxygen-hydrogen bond in association with hydrogen bonds presents a vibration absorption in the zone between 3200 and 3600 cm^{-1}, with bands generally wide and intense; if it is not associated, the absorption band is generally around 3600 cm^{-1}.
- The presence of free fatty acid groups can be seen by absorption in the zone around 1650-1700 cm^{-1}. This technique can be considerably improved by using Fourier transform infrared radiation (FTIR) instruments.
- An electronic analysis of the infrared signal absorbed or reflected by the sample, using attenuated total reflectance Fourier transform infrared spectrophotometry (ATRFTIR), gives increased sensitivity and selectivity which can also be used for GLC detection.
- Raman spectroscopy is reported in some application studies,[57] frequently combined with other techniques.

4.2.3 Proton Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) is widely used to investigate the structure of compounds and is therefore used for fatty alcohol/organic non-fatty acid esters.

Proton NMR is very useful, especially when associated with the infrared spectroscopy which on its own is not very selective for some constituents of the molecule.

4.2.4 Mass Spectrometry

Mass Spectrometry (MS) enables the determination of the structure as well as the molecular weight of a compound to be made.[62,63] The use with GLC is probably its most widely used analytical application.

4.3 Electrochemical Analysis

Electrochemical Analysis (i.e., Differential Pulse Voltametry and Differential Pulse Polarographic Analysis) is another technique used and mentioned in the literature.[51,52]

5 FURTHER TESTS FOR QUALITY

5.1 Moisture/Water Content

The test for moisture content is really only significant for hydrophilic esters. For esters that are not very volatile the loss of weight at 100°C gives an indication of water content.

A quick and useful method, specific for small amounts of moisture is the Karl Fischer method[13] (see Appendix, *18*)

5.2 Heavy Metals

Owing to both technological processing and the environment, contamination by heavy metals occurs in raw materials designed for cosmetic use (see Appendix, *35*).

- Atomic Absorption Spectroscopy (AAS) is the usual procedure to detect heavy metals after preparation of the sample either by combustion, acid attack in solvents, or extraction via metallic complexes.
- Spectrophotometric determinations of metallic complexes which absorb in the UV range are also useful.
- Conventional methods[13,58] give, from the weight of dried residue after heating with sulphuric acid, the total amount of heavy metals present, whereas spectroscopical methods give the quantity of a single ion.

5.3 Ash

High temperature combustion to constant weight allows the determination of noncombustible materials (see Appendix, *36*).

5.4 Oxidation

Fatty alcohols with double bonds can undergo oxidation processes which can adversely affect the quality of the raw material – especially if auto-oxidation processes have already started. This could lead to a deterioration in colour and odour, a change in properties in storage and even to instability of the finished products[6] if used in manufacture.

Depending on the stage of auto-oxidation reached, various tests can be applied to detect the substances produced. The peroxide number, Kreis' assay and the *p*-anisidine number[59,60] are the tests most often used (see Appendix, *25*).

6 METHODS OF ANALYSING ESTERS IN COSMETIC PRODUCTS

6.1 Extraction of Esters from the Matrix

a) A simple method of analysing esters is by extraction with a solvent;[61] the cosmetic sample dissolved in water/ethanol is extracted by petroleum ether, in which the fatty substances collect. Note that in the case of esters with polar groups, the extraction can be incomplete.

b) Another method is by solid base extraction: small columns are packed with mobile phases for HPLC, which, depending on the kind and quantity of solvent used, selectively retains the various types of substances.

6.2 Analysis of Extracted Esters

Once they have been isolated, or in any way purified from the most unwanted substances, the desired components are analysed by the above identifying techniques (see ¶3 and ¶4).

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Appendix

Analytical Methods and Definitions

PHYSICAL AND CHEMICAL TESTS FOR IDENTIFICATION AND QUALITY

A Physical Tests

1 Appearance
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3 Odour
4 Viscosity
5 Surface and Interfacial Tension
6 Density and Expansibility
7 Dilatometry
8 Melting Point or Freezing Point
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10 Titre
11 Cloud Point
12 Specific Heat Capacity, Latent Heat and Heat of Combustion
13 Vapour Pressure and Boiling Point
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16 Refractive Index
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19 Iodine Value
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21 Acid Number (Acid Value) and Free Fatty Acid
22 Saponification Number (Value) and Ester Number (Value)
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MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

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27 Gas Chromatography
28 Supercritical Fluid Chromatography
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D Spectroscopy

30 Infrared Spectroscopy
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DETERMINATION IN COSMETIC PRODUCTS

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PHYSICAL AND CHEMICAL TESTS FOR IDENTIFICATION AND QUALITY

*Standardized methods are available in the Official and Tentative Methods of the American Oil Chemists' Society (*AOCS Methods*) to determine the following chemical and physical properties of fats, oils, and fatty acids: titre, acid and iodine values, colour, stability, saponification value, unsaponifiables, and fatty acid composition. The published methods of the American Society for Testing Materials are indicated by* ASTM Methods. *In Italy, Norme Grassi e Derivati, Stazione Sperimentale per le Industrie degli Olii e dei Grassi (Milan), publishes methods which are referred to in this volume as* NGD Methods.

Some of the tests give constants applicable to single raw materials so that deviations from standard can not only identify the substance but can also be indicative of quality, e.g. refractive index.

The physicochemical properties of oils and fats, because of their natural origin, tend to vary according to the region of source and the time of year. It is best therefore, once a formula has been established, for a cosmetic manufacturer to standardize the quality and source and to obtain repeat deliveries from the same supplier.

A PHYSICAL TESTS

Appearance, Colour, Odour

Variations in texture, colour and odour from the standard materials used in the formulation development of a cosmetic may lead to modifications of the texture, colour, or perfume in large-scale manufacture of the finished product.

1 Appearance

Texture to match standard.

2 Colour

To match standard. With the use of efficient fractional distillation technology, the colour of fatty acids is frequently improved over that of the parent fats and oils; therefore, more lighter-shade colour-test methods are usually required for fatty acids than for fats and oils. Older methods, such as the Gardner and Lovibond systems, still find use, although there appears to be an emphasis for the use in recent years of the platinum-cobalt scale and the photometric index systems of colour definition.

The Gardner colour standards, developed originally for the measurement of colour of drying oils, and ,later, fatty acids and derivatives, can be expected to be used indefinitely because of the reluctance of the coatings industry to modify raw material standards.

The AOCS Method Td la-64 (Corr. 76) describes the test method said to be applicable to natural and synthetic drying oils, fatty acids and oil derivatives "which do not differ appreciably from the standards." It is based on the use of 18 colour standards supplied by either of two reliable supply houses.

The American Society for Testing Materials (ASTM) recommends the use of the Gardner method in its Method D 1544. It defines the Gardner series in terms of Commission Internationale de l'Eclairage (CIE) chromaticity coordinates, then gives preference to 1963

permanent glass standards for applicability to fatty acids used in protective coatings (ASTM Method D 1467).

For drying oils, ASTM Method D 555 is used; for rosin oils, D 1131; and for tall oil, D 803, with the older iron-cobalt standards replaced by the 1963 standards. In 1953, the ASTM had adapted colour standards using chloroplatinate instead of ferric chloride-cobalt chloride in tubes 1-8 because of the better colour stability of chloroplatinate over the iron-cobalt standards. However, the chloroplatinate standards were not sufficiently soluble to permit concentrations for tubes above 8 and they were replaced by the permanent 1963 glass standards.

Two test methods for the determination of colour in fats and oils – AOCS Method Cc 13a-43 (Reapp.73), the FAC standard colour method primarily for animal fats, and AOCS Method CC 13c-50 (Corr. 77), a photometric method used only for cottonseed, soybean, and peanut oils are also used for the measurement of fatty acid colour.

3 Odour

Characteristic – or match with a fresh standard sample. The demand for product stability, accompanied by a requirement for bland-smelling fatty materials, is increasing. This is probably as a result of their use in cosmetics, toiletries, and also in food, where their use in food additive derivatives continues to grow. Recently, sensory analysis has been applied to make statistically significant odour evaluations.

4 Viscosity

The coefficient of viscosity, etc., is defined as the force per unit area required to maintain a unit difference of velocity between two parallel layers that are a unit distance apart. In centimetre-gram-second (cgs) units when force per unit area is in dynes per square centimetre, velocity is in centimetres per second and distance is in centimetres, the coefficient of viscosity is expressed in poises.

In modern terms of Système Internationale d'Unités (SI units), the coefficient of viscosity is measured in newton seconds per metre squared:

$$\eta = N\ s\ m^{-2} = 10 \text{ poises}$$

where the pressure is one newton per square metre, the velocity is metres per second and the distance is one metre.

Viscosity of a certain fluid substance can also be defined as the inside friction which takes place among its particles when they flow one against the other. It can be put as absolute viscosity (or dynamic viscosity) or as kinematic viscosity equal to the ratio absolute viscosity/density.

5 Surface and Interfacial Tension and Spreading Coefficient

Surface tension increases with increase in chain length and decreases with increase in temperature as does the interfacial tension. The relationship between surface tension and temperature is essentially linear.

The spreading coefficient (SC) of an aqueous medium over an oily surface is calculated from the surface tension (ST) and the interfacial tension (IT) (with a given oil) by the following formula:-

$$SC = ST_{oil} - (ST_{soln.} + IT_{oilsoln})$$

The greater the value of SC the greater the wetting and spreading power.

6 Density and Expansibility

In absolute terms density is expressed in grams per cubic centimetre; relative density denoted as *d* at a specific temperature is commonly used for fats and oils and is the ratio of the mass of the substance to the mass of an equal volume of distilled water at 4°C. In European literature the specific gravity *d* at 15°C is commonly used. In American practice it is usually expressed as *d* at 25°C unless the fat is not completely melted at 25°C, in which case the density at 60°C is usually determined. This method, which is applicable to products that are completely fluid at the test temperatures, is carried out with a pycnometer (NGD C19, 1979).

Solid products that are completely insoluble in water can also be measured. The volume occupied by a certain known weight of solid is determined by the difference between the initial weight of the water contained in the pycnometer plus the weight of the solid and the weight of the solid plus the remaining water left in the pycnometer after the addition of the solid. Entrapped air bubbles must be avoided.

(Weight of water + product weight) - (Weight of residual water + product) = Product volume

Density, *d* = Product weight/Product volume

7 Dilatometry

The technique for observing changes in density or volume with varying temperature and time is known as dilatometry and the instrument used is known as a dilatometer[1,2] Dilatometry is useful not only in obtaining fundamental physicochemical information such as melting points and phase changes, but also has become one of the few direct practical ways for characterizing and controlling the consistency of commercial fat mixtures.

8 Melting Point or Freezing Point

Melting Point (M.P.) – the temperature at which a solid begins to liquefy

Freezing Point (F.P.) – the temperature at which a liquid begins to solidify

The melting point, when measured by a gradual heating of the product in a capillary glass test tube, is determined by two temperatures: that at which the product begins to flow (flow point) and that at which it becomes perfectly clear.

When complex mixtures are concerned, the drop-point is more frequently used. The Ubbelohde thermometer, which allows the first drop of molten product to flow through a small hole when its viscosity is low enough, or a similar automatic device (Mettler), in which the first drop interrupts an electric circuit by operating a photo-cell, give the so-called drop-temperature.

The melting point is an important physical characteristic of fatty compounds which is useful for identification purposes and important in many technological applications of fatty

materials. Pure fatty acids, esters, and glycerides (and mixed fats as well) are polymorphic; that is, they solidify in different crystalline forms, which are often sufficiently stable to exhibit distinctive melting points, densities, heats of fusion, X-ray diffraction patterns, etc.

9 Softening Point

A ring-ball method is used for the determination of softening point (often referred to as melting point), as in ASTM D 36.

10 Titre

Titre, defined as a solidification point under somewhat empirical test conditions, is probably the oldest and most generally used characteristic for both fatty acids and fats and oils, although it is now apparent that its use is declining somewhat, at least for fatty acids. The general test methods are AOCS Tr la-64 (Rev. 78) and ASTM D1982.

11 Cloud Point

The temperature at which substances, soluble at higher temperatures, begin to separate from the solution.

12 Specific Heat Capacity, Latent Heat and Heat of Combustion

Specific heat is the ratio of the thermal capacity of a substance to the thermal capacity of water at 15°C and, since it is a ratio, is dimensionless.

It is also the old term for what is now called *specific heat capacity* – the heat energy required to raise unit mass of a substance through one kelvin. The SI units are J/(kg.K), although in practice the use of kilojoules is recommended. In cgs units it is calories per gram per degree Centigrade. The calorie is defined as the quantity of heat needed to raise the temperature of 1 g of water by 1°C.

Specific latent heat is the amount of heat required to change unit mass of substance from solid to liquid, or liquid to vapour without change of temperature. The SI units are joules per kilogram (J/kg). The *specific latent heat of fusion* is the amount of heat necessary to convert unit mass of solid into its liquid state without rise of temperature and, similarly, *specific latent heat of vaporization* is the amount of heat necessary to convert liquid into vapour.

The *heat of combustion* is the amount of heat evolved by 1 mole of a substance when it is completely burned in oxygen at constant volume. In SI units it is usually measured in kilojoules per gram.

13 Vapour Pressure and Boiling Point

Vapour pressure and boiling point are among the most important properties of fats and their derivatives, both theoretically and practically. It is only since the 1930s that systematic and reliable determinations have been made of these most basic and important properties.

The *vapour pressure* of a substance is the pressure exerted by a vapour in equilibrium with its solid or liquid at a particular temperature.

The *boiling point* of a liquid is the temperature at which the vapour pressure is equal to the atmospheric pressure.

14 Smoke, Fire, and Flash Points

The smoke, fire, and flash points of a fatty material are measures of its thermal stability when heated in contact with air.

The *smoke point* is the temperature at which smoking is first detected in laboratory apparatus protected from drafts and provided with special illumination. The temperature at which the material smokes freely is usually higher.

The *flash point* is the temperature at which the volatile products are evolved at such a rate that they are capable of being set on fire when heated over a naked flame but not of supporting combustion. This can be determined in an open vase[3] (Cleveland method, NGD C 18) or in a closed one[4] (Pensky-Martens method, NGD C 30).

The *fire point* is the temperature at which the volatile products will support continued combustion.

15 Solubility and Miscibility

At temperatures above their melting points, fats and fatty acids are miscible in all proportions with many organic solvents, such as hydrocarbons, esters, ethers, ketones, and chlorinated solvents.

The solubility of a substance in a nonaqueous system[5] depends primarily on the melting point and heat of fusion of the solute, and secondarily on those characteristics of the solvent that determine the degree of mutual affinity of solvent and solute molecules.

16 Refractive Index

The refractive index of a substance is determined as the ratio between the sine of the angle of incidence and that of the angle of refraction when a beam of light of fixed wavelength passes from the air into the substance. Measurements are made at temperatures at least 10°C higher than the actual melting point of the substance (NGD C27, 1976).

The refractive index of fats and fatty acids is an important characteristic because of the ease and speed with which it can be determined precisely, the small quantity of sample needed, and its relationship to structure. It is useful for identification purposes and for establishing purity, and also for observing the progress of reactions, such as catalytic hydrogenation and isomerization.

The relationship between the refractive index and the structure and composition of fatty acids and glycerides may be generalized as follows:

- 1. The refractive index of fats and fatty acids increases with increase in the length of the hydrocarbon chains, but the difference between adjacent members becomes smaller with increase in molecular weight.
- 2. The refractive index of fats and fatty acids increases with the number of double bonds and increase in conjugation.

17 Heat Stability

The measurement of colour development as a function of heating the fat. This test is used for fatty acids, which can be examined by heating at 205°C under certain controlled conditions followed by estimation of the Gardner colour.

B Chemical Tests

18 Moisture

The moisture and "moisture and volatile matter" methods have been developed to provide accurate moisture assays in minimum test time. The older methods involve the evaporation of water by heating with or without use of vacuum for the quickest drying without damage to the solids by decomposition, charring, or loss of weight as the result of pyrolysis of portions of the oil. The choice of drying temperature is critical in these determinations. The Karl Fischer method of water analysis eliminates the need for heat and is rapid and convenient.

19 Iodine Value

AOCS Method Tg la-64 (Rev. 73) is the Wijs method employing iodine monochloride in glacial acetic acid (Wijs solution) and expresses the iodine values as centigrams of iodine absorbed per gram of sample, or grams of iodine per 100 grams of sample. Like the method used with normal fats and oils, the excess of reagent used in the test is critical for fatty acids. This is titrated with sodium thiosulphate with a starch indicator.

This number indicates the quantity of double bonds present, except when conjugation occurs (α/β unsaturated acids are known to show incomplete addition of iodine.)

The Hanus method uses iodine monobromide in acetic acid and is an official method of the AOAC. For both the Wijs and Hanus methods the addition of mercuric acetate as catalyst cuts the analysis time from 30 min to 1-2 min.[6]

20 Thiocyanogen Value

The thiocyanogen value determination is based on the addition of thiocyanogen to olefins. Unlike halogen addition (as in the iodine value test) it is not stoichiometric but provides useful information and was once widely adopted, in conjunction with iodine value, as a measurement of unsaturation in fats.[7]

It was largely replaced in the 1960s by ultraviolet spectrophotometry (AOCS Method Tj 1a-64 Reapp. 73) and the latter has, in turn, been largely supplanted by the GC method (AOCS Method CC 2-66 or ASTM D 19983).

21 Acid Number (Acid Value) and Free Fatty Acid

The term is defined as the milligrams of potassium hydroxide required for the neutralization of the free carboxyl groups in 1 gram of the sample.

The acid number has its greatest value, however, when it is used in conjunction with other supporting analytical data such as saponification number, unsaponifiables, and homologue distribution (GC analysis) for a more complete indication of the nature and composition of the sample.

There is a continuing trend to use the term *acid number* in place of the term "acid value" and, although the two terms are used interchangeably, the former is perhaps preferred because it illustrates the additive nature of this characteristic when used in conjunction with *saponification number* (for "saponification value"), *hydroxyl number* (for "hydroxyl value"), and so on.

22 Saponification Number (Value) and Ester Number (Value)

Saponification number, interchangeable with saponification value, is the number of milligrams of potassium hydroxide required to react completely "with all the reactive groups" in 1 gram of a sample.

The test is carried out in alcoholic alkali at the reflux temperature for a period of 30-60 min, or "until the sample is completely saponified as judged by the clarity and homogeneity of the saponification medium". Alkali is consumed for carboxylic acid groups in the fatty acids, and also for other ester-like groups in oils and partial glycerides as well as lactones and "estolides" and the like.

The term *saponification equivalent* is sometimes used and represents the number of grams of sample that will react under the saponification conditions with 56.1 grams of potassium hydroxide.

Natural waxes, which are the esters of long-chain alcohols and fatty acids, saponify slowly and incompletely under the saponification conditions to yield nonhomogeneous saponification media containing long-chain alcohols; therefore, mixtures of fatty acids, and waxes cannot be submitted to the usual saponification number analysis.

23 Hydroxyl and Acetyl Numbers

The hydroxyl number is defined as the number of milligrams of potassium hydroxide equivalent to the hydroxyl groups in 1 gram of sample.

The determination is carried out by reacting the substance with a known amount of acetic anhydride in pyridine solution. The excess of acetic anhydride, after hydrolysis to acetic acid, is titrated with a solution of potassium hydroxide, phenolphthalein indicator.

The number of mg of potassium hydroxide is therefore equivalent to the acetylatable hydroxyl groups found in 1 g of substance (NGD C 79, 1986).

AOCS Method Cd 13-60 (Reapp. 73) and ASTM D 1957 applicable to oils, mono- and di-glycerides, and hydroxy-substituted fatty acids, e.g. hydroxystearic acids, are used for hydroxyl number determination for many hydroxy containing substances.

The number is also of use in the analysis of crude or partially esterified fatty acid-alcohol mixtures, and the analysis of other partially esterified polyhydroxy compounds, among others.

24 Unsaponifiables

Unsaponifiables (AOCS Tk la-64T) are materials found in fatty materials, e.g, aliphatic alcohols (C12 and higher), sterols, pigments, and hydrocarbons that cannot react with caustic alkalies but are soluble in ordinary solvents.

25 Oxidation[7]

Fatty compounds can undergo oxidation processes which may adversely affect the quality of the raw material and lead eventually to rancidity - especially if auto-oxidation processes have already started. This could lead to deterioration in storage and even to instability of the finished products when used in manufacture.[8]

The *peroxide number* corresponds to the quantity of active oxygen in a fat (expressed in milli-equivalents) per one thousand grams of sample. It is determined by measuring the quantity of hydriodic acid that becomes oxidized in an acid environment: the resulting iodine is titrated with thiosulfate.

a) Accelerated Oxidation Tests and Peroxide Number

Accelerated oxidation tests are used by material suppliers to determine the stability of a fat or for evaluating their preservation by antioxidants. Known as the "aeration test", the "active-oxygen method" or the "Swift stability test", the sample is aerated continuously at 97.8°C and the time required for a specific peroxide number to develop is determined by titration at certain time intervals. The peroxide number chosen as the end-point should represent an average value for the beginning of rancidity for the kind of fat under test.

b) Auto-oxidation

Depending on the development of auto-oxidation many different tests are available for its detection at various stages (AOCS Cd 8-53; NGD C 36, 1979). The following are most commonly used:

Peroxide number
This is the test which measures the beginning of the auto-oxidation reaction.

Kreis' Assay
1 ml of the oil, or melted fat, is shaken with 1 ml of concentrated hydrochloric acid for 1 min.; then 1 ml of a 0.1% solution of phloroglucinol in ether is added and shaking continued for a further minute. A pink or red coloration in the lower acid layer indicates the presence of oxidation – the amount of which is roughly proportional to the intensity of the colour. AOCS recommend that the colour be measured by a tintometer.

The results of this test do not necessarily mean that the sample is rancid. Jones described a modification of the method suitable for cosmetic preparations.[9]

p-*Anisidine Colorimetric Reaction*
This detects substances, such as aldehydes or ketones, resulting from a further development of auto-oxidation. The colour is given by the optical density at 350 nm, multiplied by 100, of the following solution in 1 cm cells: 1 g of sample dissolved in 100 ml of the solvent/reagent mixture (isooctane and *p*-anisidine in acetic acid solution).

c) Oxirane Oxygen

The AOCS does not recommend the test method involving ring opening of epoxide groups with hydrogen bromide in glacial acetic acid, which finds limited use in the analysis of epoxidized oils (AOCS Method Cd 9-57, Rev. 79). This method is sensitive and subject to too many interferences: for example, peroxides, hydroperoxides, cyclopropenoids, conjugated unsaturated compounds, and soaps interfere. On a sample known to be free of these substances, the analysis can be carried out if sufficient care is taken.

In view of the need to standardize the reagent before each determination, the instability of the reagent, and the possible loss of hydrogen bromide during the titration, this analysis has not been considered sufficiently satisfactory. It appears that a standard instrumental method of analysis for the oxirane functional group could be developed to fill this void.[10]

d) Other methods

Future trends indicate that high pressure liquid chromatography and gas chromatography can be used to detect oxidation.

MODERN TECHNOLOGICAL METHODS FOR IDENTIFICATION AND QUALITY

C Chromatography

A method of chemical analysis in which a mobile phase, carrying the mixture/ substance to be analysed, is caused to move in contact with a selectively absorbent stationary phase. During the progress of the mobile phase the components being carried become absorbed on the stationary phase and separated, and can be identified; in some cases they can be quantified.

26 Thin-Layer Chromatography

Thin-layer chromatography (TLC) requires comparison to be made with the data of standard samples for identification of the components of a mixture. It is very useful for the separation of fatty compounds, such as hydrocarbons, triglycerides, other esters and acids, and for separating fatty alcohols from them. In addition, TLC methods can be applied to unsaponifiable fractions of natural oils and fats to separate alcohols and sterols from other substances. Specific methods are described in Divisions 1 to 9 of this volume.

The stationary phase

The stationary phase consists of a thin layer of silica gel (or Florisil – a form of magnesium silicate, alumina, or other absorbent materials) on a glass plate.[11] The coated plates must be air-dried and then activated in an oven at 102°C for 30 min to remove traces of moisture. This must be repeated just before use since silica gel readily absorbs moisture (even the breath of the operator can affect the results).

The mobile phase

The mobile phase consists of mixtures of solvents most suitable for the substances to be analysed.[11] If the absorbent is kept constant the ability to separate lipids according to their functional groups depends on the polarity of the solvents:

- *n*-hexane/diethyl ether is a mixture often used in the ratio, for example, of 90:10 or 85:15;
- also used is petroleum ether/diethyl ether/acetic acid in the ratios of 90:10:1;
- for unsaponifiable fractions in natural fats, petroleum ether/diethyl ether/formic acid (60:40:1) or hexane/diethyl ether (65:35) can be used (NGD C 75, 1989).

These methods can also be used on a preparatory scale to obtain samples for further gas chromatographic analysis.

Developers
After selective absorption from the mobile phase the plate is developed in various ways:[11]

- the most favoured is by iodine vapour – useful for unsaturated substances;
- also used is bromothymol blue with ammonia vapour;
- or by rhodamine B or 2-7 dichlorofluorescein and ultraviolet light;
- free fatty acids have been visualised by spraying with 0.5% bromothymol blue in 2% aqueous citric acid;
- or by spraying with aqueous sulfuric acid and charring in a heater at 130°C;
- or by means of 80% sulfuric acid saturated with potassium dichromate in a heater at 130°C.

The *Rf (value)* is the ratio of the distance moved by a particular solute to that moved by the solvent front. Alcohols have an Rf of about 0.2 while other less polar substances, such as esters and triglycerides, have a higher Rf value.

A spot of a polyol fatty acid ester can be identified by the Rf value. Its Rf value is closer to that of triglyceride and lower than that of higher alcohol esters.

27 Gas Chromatography

Gas chromatography (GC) is an analytical procedure which depends on the principal that different materials pass through a packed tube of an inert material at different rates.

The stationary phase consists of a narrow tube containing support material of uniform size (e.g diatomaceous earth) that has been coated with a non-volatile liquid, the whole apparatus being maintained in a thermostatically-controlled oven. The sample to be analysed is carried through the tube in an inert gas (e.g. argon or oxygen-free nitrogen). Solid and liquid samples are vaporised before introduction on to the column and components in a mixture selectively absorbed: some pass through more quickly than others.

The use of very sensitive detectors enables gas chromatography (GC) to be applied to sub-microgram quantities of material. GC requires standard samples with their data to compare for identification.

Because of hydrogen bonding, fatty acids in an unionized state are associated as dimers. For this reason they are usually analysed in the form of their methyl esters, which eliminates the possibility of such dimers being present.

28 Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a relatively new technique that has been developed to offer the advantages of both gas chromatography and liquid chromatography, aiming to eliminate the disadvantages of both. Usage of this technique has been reported. [72][145]

29 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC), once called high-pressure liquid chromatography, is a scaled-down and automated version of liquid chromatography. The method is rapid and the large number of theoretical plates results in high resolution. As with other

chromatographic techniques, the equipment has to be calibrated and does not provide an absolute identification of the chemical identity of the material under test.

Quantification is easier than in thin-layer chromatography. Lipids can be separated by HPLC that in GC would be decomposed at the higher temperature. It has low running costs, high efficiency and sensitivity and the low flow rates allow the column to be linked directly to a mass spectrometer. The separated components can be collected and examined spectroscopically.

D Spectroscopy

Spectroscopy is the study of spectra, including the excitation of the spectrum, its visual or photographic observation, and the precise determination of the wavelengths.

30 Infrared Spectroscopy

In infrared (IR) spectroscopy a sample, in a solvent or as a solid in a pellet of potassium bromide, is scanned and the absorbance recorded as a function of wavelength. The resulting spectrum provides information on the interatomic bonds, which have characteristic frequencies that fall within the infrared range. For example, methylene chains absorb at 1470cm^{-1}, 1390-1200 cm^{-1} and around 720 cm^{-1}. Hydroxyl resonance, as in fatty alcohols, occurs at 3300 cm^{-1}; carbonyl resonance occurs at around 1700 cm^{-1}. However for fatty acids, containing both of these groups, resonance is displaced and broadened so that it overlies the C-H absorbance at 3000 cm^{-1}. Other values can be found in the appropriate sections of each division of this volume.

The most accurate method of identifying an ingredient by IR spectroscopy is by comparison with a standard sample. The use of a computer enables quick and accurate searches to be made.

31 Nuclear Magnetic Resonance Spectroscopy

A sample subjected to a magnetic field causes the nuclei of various atoms to orientate in one of two energy levels. If energy is applied to the nuclei they will change their orientation. This is the basis of nuclear magnetic resonance (NMR) spectroscopy. The energy wavelengths causing the transition correspond to radiofrequencies and the precise frequency depends on the relationship of the nucleus to other nuclei or atoms in the molecule. Under controlled conditions, a spectrum can be obtained from which the structure of the molecule can be deduced.

The technique is usually used to determine the position of hydrogen (proton or ^{1}H-NMR) or carbon (^{13}C-NMR), for which the compound is labelled with the heavy isotope of carbon. Proton NMR is useful in association with IR spectroscopy which, when used alone, is not very selective for some constituents of the molecule.

NMR is used for hydrocarbons, fatty acids, fatty alcohols, glycerides and other esters (see the relevant division of this volume, where some have figures illustrating NMR spectra.)

32 Near Infrared Reflectance Spectroscopy

Near infrared spectroscopy indicates work carried out between 0.78 and 3 μm. The absorption bands in this region are mainly harmonics of bands in the infrared region. They are quite

sharp and are of value in the quantitative analysis of various functional groups, particularly those containing hydrogen atoms.

33 Ultraviolet Spectrophotometry

The technique of ultraviolet (UV) spectrophotometry relies on the fact that many molecules absorb ultraviolet light and by scanning samples with UV radiation spectra can be obtained which are characteristic of the particular molecules being scanned.

Information provided by UV spectroscopy is usually limited to showing the presence or absence of an aryl group or an aliphatic chain containing conjugated double bonds. Some discussion of this, including Woodward's rule, is given in the division on Fatty Acids (see p. 48).

34 Mass Spectrometry

A mass spectrometer is an instrument used to determine the isotopic ratio in a given material, or to monitor the amount of a heavy isotope in an isotopically-labelled substance.

The material is bombarded with a stream of high energy electrons which leads to its fragmentation. The fragments, of various weight and charge, are characteristic for a given material and can be separated by a magnetic field according to their mass/charge (m/z) ratios.

MS allows determination of isomer distribution, the location of side chains and the degree of branching. However, it is a very expensive and specialized analytical technique requiring highly-trained operators and is not for the smaller laboratory. The linking of the separated products obtained by gas chromatography (GC-MS) has proved of immense value to industry and there are several mentions of this technique in the various divisions.

IMPURITIES AFFECTING QUALITY AND PERFORMANCE

35 Metallic Impurities

The natural content of fat and oil products arises largely from the soils in which the vegetable plants were grown. If this were the only source of metal contamination, product quality problems would be minuscule. However, traces of iron and copper appear in many fat and oil products, and, irrespective of their source, their presence is objectionable because both play an adverse role in metal catalyzed auto-oxidation; cause fat and oil deterioration, odour and off-taste development in food materials; and occasionally cause color development. As little as 0.1 ppm of copper in margarine is said to be deleterious to flavour stability on storage, and maximum levels should be below 0.02 ppm for good product stability. Consequently, fat and oil products must be monitored to keep the level of these metals low. Tin, copper, and nickel are components of common catalysts widely used in fat and oil technology and must be removed from the finished products.

The detection of environmental contamination and pollution of important food materials by heavy metals is becoming increasingly important. Even cod liver oil contains traces of naturally derived arsenic. Heavy metals such as cadmium, lead, nickel, arsenic, antimony and mercury can be determined by atomic absorption (AAS) techniques. After prior incineration cadmium, chromium, lead and nickel can be determined by the flame-AAS technique and arsenic and antimony by the hydride-AAS technique. For mercury the

determination is by the flameless-AAS technique, and follows digestion in nitric acid solution (NGD C 36, 1976).

36 Loss on Ignition

One to 5 g of the sample is gradually heated or ignited with sulfuric acid as required, and turned completely into ashes. When cool these are placed in the desiccator and weighed repeatedly until constant.

DETERMINATION IN COSMETIC PRODUCTS

37 Solvent Extraction

In order to separate fatty materials from finished cosmetic products, the first step is to separate oil-soluble materials from cosmetics. This can be done by first removing volatile materials in the products in a water bath and then extracting oil-soluble materials by dissolution with organic solvents such as ethyl ether or chloroform followed by filtration. After removing the solvent, the extracted oil-soluble materials are subjected to the next analysis step

38 Qualitative and Quantitative Analysis

The fraction obtained at 40.a is used to achieve qualitative and quantitative analyses in connection with the TLC, GC, GC/MS, and NMR analyses. The quantitative results are obtained through consideration of the GC and NMR analyses by measuring the weight of the fraction from silica gel chromatography.

See the practical method in Division 4, Fatty Acids (p. 50).

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