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Journal of the Society of Cosmetic Chemists

Contents

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dermatology

HUMAN ORAL MUCOSA: Development, Structure and Function

C. A. SQUIER M.A. PH.D. M.I.BIOL., Senior Lecturer in Experimental Oral Pathology, The London Hospital Medical College; N. W. JOHNSON M.D.SC. F.D.S.R.C.S. PH.D., Reader in Experimental Oral Pathology, The London Hospital Medical College; and ROSAMUND M. HOPPS B.SC. PH.D., Lecturer in Experimental Oral Pathology, The London Hospital Medical College.

June 1976. 160 pages, 32 illustrations. Paper, about £4.00

This volume briefly describes the functions of the oral mucosa and then proceeds to explain how its structure fulfils those functions in the different regions of the oral cavity. Particular regard is paid to epithelial cell proliferation and the various processes of differentiation which lead to variations in the pattern of keratinisation. The book represents the only complete account of the mucosa produced for the undergraduate dental student but should also be of value to postgraduate students in both dentistry and dermatology.

Contents The functions of oral mucosa; The oral epithelium; The boundary between epithelium and connective tissue; Interactions between epithelium and connective tissue; The connective tissue; Embryological development and age changes in oral mucosa.

ORAL MUCOSA IN HEALTH AND DISEASE

Edited by A. E. DOLBY M.D. B.CH. F.D.S.R.C.S., Professor of Periodontology, Welsh National School of Medicine, Cardiff.

1975. 528 pages, 72 illustrations. £16.00

Considerable advances have been made in the past decade in the understanding of many of the diseases involving oral mucosa. A number of authors who have themselves made major scientific contributions to the subject have prepared this review, which should prove of value to clinicians, postgraduate students and research workers in several disciplines.

Contents Structure and function of normal oral mucosa; The physiological responsiveness of the oral mucosa: the role of saliva; Immunoglobulin systems of oral mucosa and saliva; Oral mucous membrane markers of internal disease; Oral cancer; Premalignant lesions of oral epithelium; Bacterial and viral diseases and the oral mucosa; Oral ulceration: immunological aspects; Disturbances of salivary gland secretion: Sjoren's syndrom; Candidal infection of the oral mucosa.

HISTOPATHOLOGY OF THE SKIN

W. F. LEVER M.D., Professor of Dermatology, Tufts University; and GUNDULA S. LEVER M.D., Assistant Professor of Dermatology, Tufts University.

Fifth Edition, 1975. 928 pages, 410 illustrations. Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria.

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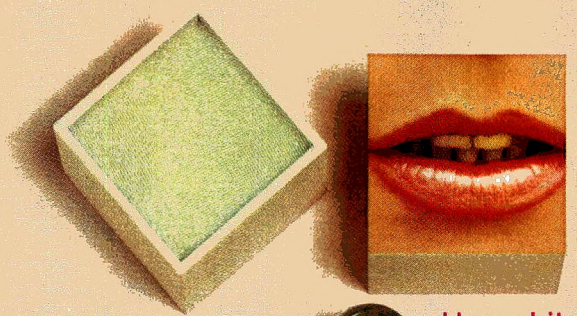

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal.

A study of damaged hair: V. N. E. ROBINSON. *Journal of the Society of Cosmetic Chemists* **27** 155–161 (1976)

Synopsis—A scanning electron microscope study of the morphological changes in human hair has revealed that these changes may be entirely attributable to the abrasion normally associated with hair grooming. In particular, shampooing and brushing can produce all of the observed changes detected in virgin hair. These changes are more deleterious in some cosmetically altered hairs. This appears to be attributable to a decrease in the disulphide bond content of these fibres, this decrease being brought about by the cosmetic treatment.

Analytical aspects of potentially risk-bearing substances in cosmetics: D. H. LIEM. *Journal of the Society of Cosmetic Chemists* **27** 163–208 (1976)

Synopsis—Analytical surveys have been made in the last three years to obtain a clearer picture of the use of potentially risk-bearing substances in cosmetics in furtherance of cosmetic legislation in the Netherlands.

Eye make-up colours have been identified by a set of characteristic standard reactions. Lipstick colours were identified by well-known chromatographic methods. Colour intermediates for the oxidative hair colourings were identified by two-dimensional thin-layer chromatography of the unpurified extract and the aromatic amines confirmed by glc analysis, which also permitted quantitative determination. A gas-liquid chromatography separation of phenolic intermediates is also described. Suntan preparations were analysed for the presence of uv-absorbers, browning agents and local anaesthesics.

A simple aerosol sampling method, prior to glc analysis, has been developed, thus permitting a total analysis of solvents and propellants in single liquid phase aerosols within an hour.

Analytical data of market samples is also presented.

The following papers have been accepted for publication in the *Journal*:

ORIGINAL SCIENTIFIC PAPERS

Evaluation of flavours in dental creams
Eric Baines, B.Sc.

The chemistry of human hair cuticle—III: The isolation and amino acid analysis of various subfractions of the cuticle obtained by pronase and trypsin digestion
J. A. Swift and B. Bews

Eye irritation tests. An assessment of the maximum delay time for remedial irrigation
R. E. Davies, B.Sc., M.I.Biol., S. R. Kynoch, B.Sc. and M. P. Liggett

SUBJECT REVIEW ARTICLES

Psycho-rheology—the relevance of rheology to consumer acceptance
J. V. Boyd, B.Sc., M.Sc., M.Inst.P.

Sex differences in odour perception
E. P. Koster, Ph.D.

Sex attractants in primates
Eric Barrington Keverne, B.Sc., Ph.D.

Journal of the Society of Cosmetic Chemists

VOL. 27 1976

The list of officers and council on the previous page replaces that published in *J. Soc. Cosmet. Chem.* **27** (1); when a re-run of the 1974–75 officers was inadvertently printed.

J. Soc. Cosmet. Chem. **27**, 155-161 (1976)

A study of damaged hair

V. N. E. ROBINSON*

Synopsis—A SCANNING ELECTRON MICROSCOPE study of the morphological changes in human HAIR has revealed that these changes may be entirely attributable to the ABRASION normally associated with hair grooming. In particular, SHAMPOOING and BRUSHING can produce all of the observed changes detected in virgin hair. These changes are more deleterious in some cosmetically altered hairs. This appears to be attributable to a decrease in the disulphide bond content of these fibres, this decrease being brought about by the cosmetic treatment.

INTRODUCTION

In a study of fine changes in the surface architecture of human hair due to cosmetic treatment, Swift and Brown (1) have illustrated the stages of breakdown of human hair during cosmetic treatments. The natural appearance of hair, when first formed, displays a scale structure with smooth edges. This is subsequently transformed into stages they have characterized by: jagged scale edges, partial scale erosion, complete scale erosion, fibre splitting into two roughly hemi-cylindrical components and gross longitudinal splitting into many fibrous elements. These changes are generally referred to as weathering and the predominant cause is thought to be exposure to sunlight (2), which may result in a variation of the chemical and physical properties from the root to the tip of the fibres (3, 4).

By means of before and after treatments and the examination of the same hair fibres in the SEM, Swift and Brown (1) have established that some of these

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variations are due to combing of the hair and concluded that some may be due to natural weathering processes. They have also briefly mentioned some of the changes introduced into hair by perming and bleaching treatments. In a series of dynamic *in situ* experiments in a scanning electron microscope, Brown and Swift (2) have further demonstrated the deleterious effects of combing out tangles in hair. Such combing can cause cuticle cell lifting in tightly-looped fibres, as well as snapping fibres transversely or through the initial formation of a longitudinal fracture, when excess tension is applied during the combing.

In a study of the structure and properties of normal adult hair, Wall and Hunter (5) have similarly illustrated the production of jagged edges in the cuticle, and have shown features on the cuticle that they have attributed to sun and atmosphere exposure. DiBianca (6) has demonstrated many different types of damaged hair ends, but has not studied how these different types of damage occurred. Robbins and Kelly (7) have analysed the amino acid content of cosmetically-altered hair. They found that bleached and permanent-waved hair contained less cystine than unaltered hair, and correspondingly more cysteic acid residues. Bleached hair also contained slightly less tyrosine and methionine than unbleached hair. Miyazawa, Nozaki and Tamura (8) have made a similar study of the amino acid composition of hair damaged by treatment with cold-waving and hair bleaching lotions. They have also observed a reduction in the cystine content of human hair, with one bleaching condition reducing the cystine content from the normal of 13.9% to 5.0%, corresponding to an approximately 64% reduction in the disulphide bond cross-linking of the protein molecules.

These studies have illustrated the extent of chemical changes and the magnitude of physical changes introduced to hair, but have not yet confirmed how these observed physical changes were introduced. There is a need to understand how and why these deleterious physical changes to hair fibres occur in order to be able to prevent the damage. Some of this information is presented in this study.

Experimental

Samples of Caucasian hair were used in this study. This hair included many fibres displaying various degrees of splitting and hair damage, and samples that displayed no damage visible to the unaided eye. No attempt was made to differentiate between hair of different texture or condition.

The fibres were mounted on stubs so that the tip, root and portions of the mid shaft of each fibre could be examined in a scanning electron microscope (JSM2). The mounted fibres were coated with approximately 500 Å of gold prior to examination. They were examined for variation of scale structure along and between fibres, extent of scale damage and removal and degree of splitting of ends. The findings were correlated with the known history of the hair, and

attempts were made to ascertain what caused the observed changes by reproducing the damage in controlled laboratory experiments.

The study was divided into two sections: the examination of virgin hair, that is, hair that had not been dyed, bleached, permed or otherwise treated, and cosmetically altered hair, hair that had received one or more of these types of treatments.

Results

Virgin hair

In common with the findings of Wall and Hunter (5) this study has shown that there was very little difference in fibre appearance between fibres from different people. The great variation detected was along fibres from root to tip and this variation was similar for all the fibres studied. This variation is reported below.

Fig. 1 shows a micrograph of a hair fibre, taken near the root, indicating the natural appearance of hair that is free from externally promoted defects (1). A few millimetres from the root the scale edges became jagged. For most of the fibres examined this jagged scale edge appearance, *Fig. 2*, represents the typical appearance of most of the fibre length.

Previous researchers (1, 2) have concluded that some of this change is due to the mechanical damage caused by brushing, combing and handling, and that some contribution to this deterioration may be due to weathering by exposure to rain, sunlight and dirt. Some of the fibres examined in this study had been shampooed and towel-dried three or four times per week, given minimal combing, approximately five comb strokes per day, were hardly ever exposed to sun or rain and had never been brushed. This abrasion of the scale edges still occurred in this hair, see *Fig. 2*. It seems probable that this deterioration of scale edge appearance was, in this case, due almost entirely to the wet abrasion associated with shampooing and towel drying.

To check the effect of wet abrasion on the cuticle, two experiments were performed. In one study, a child's hair was lightly shampooed, approximately once every week to minimize wet abrasion, and given minimal brushing and combing. The majority of the fibres examined from this child displayed a fibre appearance similar to that shown in *Fig. 1*. In the second experiment, a group of fibres from the above study were wet and rubbed vigorously between the hands to simulate shampooing and towel-drying. It was found that this action produced deterioration similar to that displayed in *Fig. 2*. From these observations it was concluded that the wet abrasion associated with shampooing and towel-drying was a dominant factor in the deterioration of scale edge appearance, at least amongst the persons studied.

With the exception of hair that had only been lightly shampooed, all other hair studied always displayed this deterioration of scale edges. However, some hair fibres displayed damage beyond that shown above. As reported by Swift and Brown (1), the next stages of fibre damage that were observed were partial, followed by complete scale removal, see *Fig. 3*. Again, by rubbing wet hair fibres in a manner that simulated shampooing, it was found that it was possible to remove completely the scale structure from the fibres. Similar rubbing of dry fibres did not produce the same degree of scale damage. Thus it seems probable that shampooing is a contributing factor to complete scale removal.

At this stage no assessment has been made of the role of hair brushing and combing in the damage to and removal of scales, although it seems probable (2) that these actions could also produce deterioration in the cuticle.

Amongst the hair fibres studied, it was found that complete scale removal only occurred in the last few centimetres. Once complete scale removal had occurred splitting of the fibre end into two, three or more longitudinal sections usually occurred, see *Fig. 4*.

Attempts were made to reproduce this type of damage. It was found that brushing a group of fibres that had been subjected to simulated shampooing caused the ends of some of the fibres to split, indicating that shampooing and brushing alone is sufficient to cause the ends of hair fibres to split. At this stage, no attempt has been made to ascertain which of these two actions is most responsible for the production of splitting, or what other procedures can cause splitting.

It was noted during the brushing trials that when a fibre had split longitudinally, it was common for the individual splits to fibrillate, see *Fig. 5*. Continued brushing caused these fibrillated regions to break, *Fig. 6*, thus producing a fibrillated end fibre.

The appearance of split ends could vary quite considerably from the simplified breakdown pattern shown in *Figs 4*, *5* and *6*. Some of these variations have been shown by Swift and Brown (1) and DiBianca (6). There is a shortening of fibres associated with the rupture of these fibrillated ends and this shortening appears to keep this type of damage restricted to the last few centimetres of a fibre shaft. These observations have illustrated that shampooing and brushing alone are sufficient to transform the hair from its natural state, *Fig. 1*, to split ends, *Figs. 5* and *6*. It seems probable that these grooming treatments of shampooing and brushing are the dominant factors in the physical deterioration of virgin hair. Weathering and exposure to sunlight would appear to be minor effects. If a sample of hair is just stored for hundreds of years, this type of damage does not occur (1), indicating that the deterioration is not an ageing process.

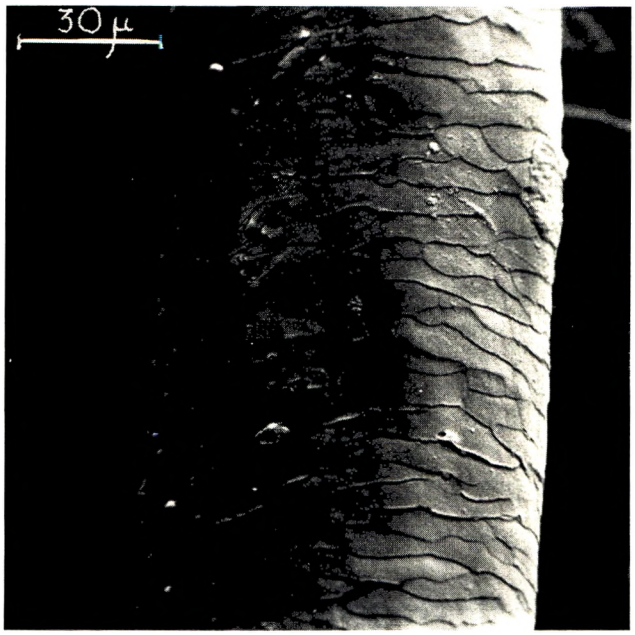

Figure 1. Typical appearance of human hair within a few millimetres of root. × 640.

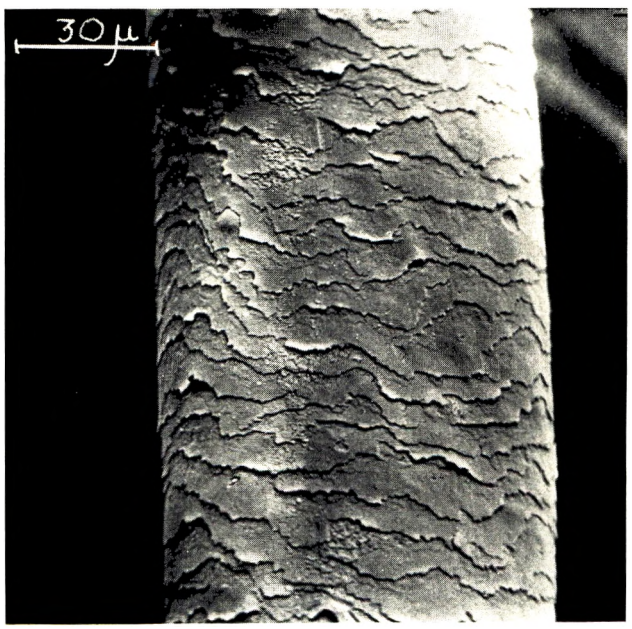

Figure 2. Typical appearance of human hair over most of the mid-shaft. The deterioration of scale edge appearance can be caused by shampooing alone. × 640.

(*Facing p.* 158)

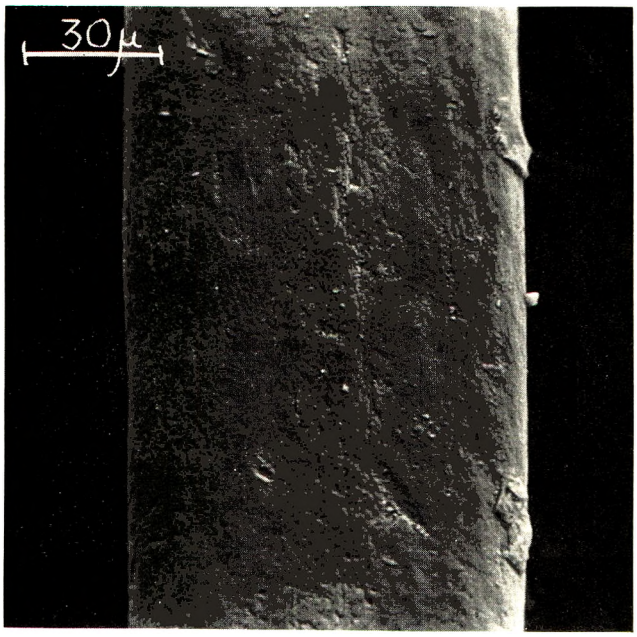

Figure 3. Complete cuticle removal near the ends of long hair. × 640.

Figure 4. A longitudinal split near the end of a fibre. × 480.

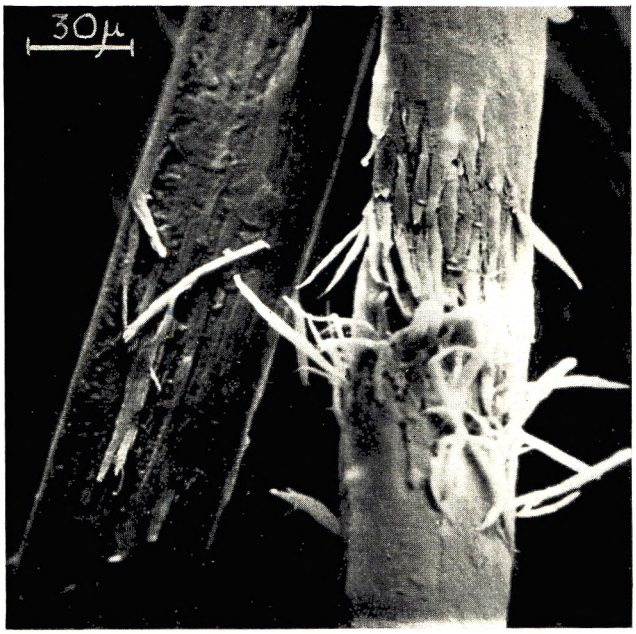

Figure 5. A hemicylindrically split fibre segment, fibrillating at cortical cell boundaries. × 480.

Figure 6. A fibrillated end fibre caused by the rupture of a fibrillating region. × 640.

Figure 7. Fibre splitting in permanently-waved hair. × 640.

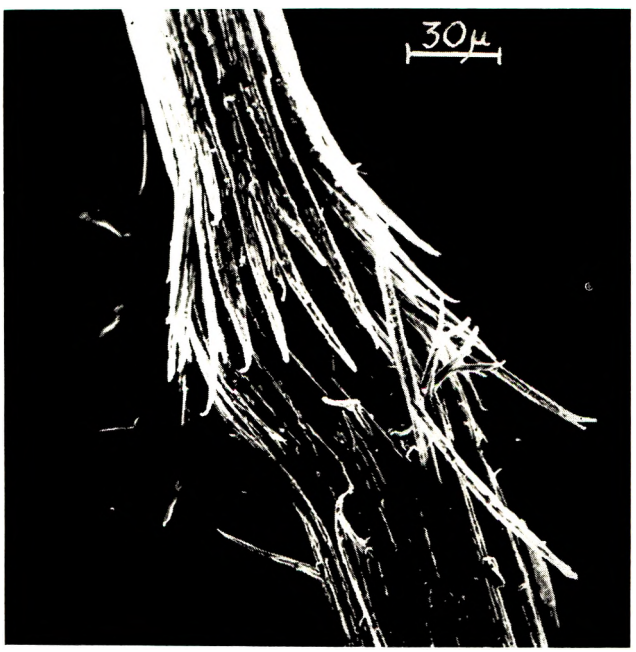

Figure 8. Shaft damage in permanently-waved hair. This damage was detected about 10 cm from the end of the fibre. × 400.

Cosmetically-altered hair

Cosmetically-altered hair had an appearance very similar to virgin hair, when no split ends were detected. The hair appearance shown in *Figs 1–6* for virgin hair, can also be seen in cosmetically-altered hair. However, there are also other types of breakdown associated only with cosmetically-altered hair.

Fig. 7 shows a split end from hair that has had one permanent-wave application. Unlike virgin hair, where splitting was only observed after almost all of the cuticle was removed, hair that had been permed or bleached was often observed to split whilst the cuticle was still clearly visible.

It was observed that splits and general shaft damage could also occur well away from the end. *Fig. 8* shows shaft damage detected about 10 cm from the end of a fibre that had been permed once and the perm did not 'take'.

The third important factor of cosmetically-altered hair was the extent of splitting. Virgin hair less than 15 cm long was hardly ever observed to split, whilst cosmetically-altered hair less than 15 cm long was often observed to split. This was detected as most likely to occur when a perm 'went wrong' or did not 'take', and on some bleached hair. Also, the splits and shaft damage associated with hair that had been permed or bleached, was generally observed to be far more severe than for virgin hair. That is, cosmetically-altered hair was observed to contain far more of the hair categorized as fly-away fibre (6) than virgin hair, for hair fibres of the same length.

The reason for this additional deterioration associated with cosmetically-altered hair is not immediately apparent. Swift and Brown (1) have shown that very minor changes in the surface structure of hair occur immediately after perming and bleaching, but these are not likely to be directly responsible for the observed additional damage. It is probable that these processes have chemically altered and weakened the hair and then during subsequent physical treatments, the altered hair is unable to withstand the abrasion normally associated with hair grooming to the same extent as can the virgin hair, and a greater degree of fibre splitting results.

Robbins and Kelly (7) and Miyazawa *et al.* (8) have found a reduction in the cystine content of bleached and permanent-waved hair. It is primarily the cystine content of keratin fibres, through the cross-linking associated with the disulphide bonds, that gives these protein fibres their high mechanical stability. It seems probable that the reduced fibre cross-link content associated with these treatments is responsible for loss of physical strength associated with these fibres.

Feughelman and Chapman (9) have shown that the relative cross-link density of keratin fibres can be ascertained by a determination of the diametral swelling of the fibres in 98% formic acid. This provides a convenient method for the determination of a relationship between fibre cross-link and splitting caused by the mechanical abrasion associated with hair grooming.

Virgin hair, when immersed in formic acid, swelled diametrally approximately 35% above the diameter of the dry fibre. Cosmetically-altered hair samples swelled to different amounts. Some fibres swelled the same as or only a little more than virgin hair, approximately 35–40% above the diameter of dry fibres. Other fibres were observed to swell to over 100% more than their dry diameter, indicating (9) an approximate 50% loss of disulphide cross-link content. Miyazawa *et al.* (8) have also observed similar reductions in the cross-link content of cosmetically-altered hair.

As a general observation, it was noticed that the fibres displaying greatest diametral swelling in formic acid also displayed the greatest tendency to split. No direct correlation was obtained between diametral swelling in formic acid and degree of splitting because it appears that the degree of splitting depends upon grooming conditions as well as disulphide bond content, and therefore no direct correlation could be expected to exist. It was also noticed that persons with hair that swelled more in formic acid were less satisfied with the appearance and condition of their hair. It seems likely that the cross-link content of the hair is at least partially responsible for the condition and manageability of the hair. That is, a reduction of the cross-link content of the hair, which may be introduced by some cosmetic treatments, results in a reduction of the ability of fibres to withstand the abrasive forces normally associated with hair grooming, causing an increase in hair damage during grooming, and appears to result in a loss of manageability of the hair.

Discussion

The results presented above indicate that the structural changes observed in hair fibres can be due entirely to the mechanical abrasion associated with normal hair grooming. This study has demonstrated that shampooing of virgin hair is sufficient in itself to damage and completely remove the cuticle. It is, of course, probable that some other treatments may also produce the same effect, but these have not yet been exhaustively investigated. It also appears that brushing of the hair contributes to damage to the cortex, and is at least partially responsible for the production of split ends in hair. No attempt has yet been made to determine the relative roles played by all of the hair grooming processes in breakdown of hair fibres.

Some cosmetically-altered hair is more prone to mechanical breakdown during grooming than virgin hair. This hair exhibits a greater tendency to swell in formic acid, indicating a loss of cross-link content resulting from the cosmetic treatment. It appears that the cosmetic treatment has reduced the cross-link content of the hair and that this has rendered the hair less able to withstand the mechanical abrasion associated with hair grooming, resulting in earlier splitting of the fibres.

Hydrogen bonds represent another useful form of cross-linking. Hydrogen bonds are reversibly broken every time hair is wet and are reformed again when it dries. Because of the decrease in hydrogen bonding, wet hair is completely unmanageable. Observations made during this study have indicated that wet hair is far more susceptible to damage during grooming than dry hair, and it would thus appear that minimal handling and brushing of wet hair could reduce the extent of physical damage to the hair.

Conclusion

This study has shown that the mechanical deterioration of hair which results in the production of split ends can be entirely attributable to the abrasion associated with hair grooming. It has also indicated that the increased tendency of hair to split after cosmetic treatments, particularly perming and bleaching, appears to be due to a reduction in the cross-link content of the fibres.

To prevent this mechanical damage minimum brushing and shampooing would appear to be desirable. Prevention of damage to the disulphide bond content of the hair fibres would also appear to be desirable to reduce splitting and improve the manageability of hair. There would also appear to be some advantages in increasing the cross-link content of hair by the introduction of cross-linking molecules. The additional cross-linking would retard fibre swelling when wet and possibly increase the wet abrasion resistance of the hair. It is also possible that such a treatment would improve the manageability and condition of the hair as well as being able to impart a permanent change of shape to the fibres. Studies in these directions are continuing.

(*Received: 24th February 1975*)

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J. Soc. Cosmet. Chem. **27** 163–208 (1976)

Analytical aspects of potentially risk-bearing substances in cosmetics

D. H. LIEM*

Presented at the IFSCC VIIth International Congress on 'Cosmetics—Quality and safety' organized by the Society of Cosmetic Chemists of Great Britain at London on 26–30th August 1974

Synopsis—Analytical surveys have been made in the last three years to obtain a clearer picture of the use of POTENTIALLY RISK-BEARING SUBSTANCES in cosmetics in furtherance of COSMETIC LEGISLATION in the Netherlands.

EYE MAKE-UP COLOURS have been identified by a set of characteristic standard reactions. LIPSTICK COLOURS were identified by well-known CHROMATOGRAPHIC methods. Colour intermediates for the OXIDATIVE HAIR COLOURINGS were identified by two-dimensional THIN-LAYER CHROMATOGRAPHY of the unpurified extract and the aromatic amines confirmed by glc analysis, which also permitted quantitative determination. A GAS-LIQUID CHROMATOGRAPHY separation of phenolic intermediates is also described. SUNTAN PREPARATIONS were analysed for the presence of uv-absorbers, BROWNING AGENTS and local ANAESTHESICS.

A simple AEROSOL sampling method, prior to glc analysis, has been developed, thus permitting a total analysis of solvents and propellants in single liquid phase aerosols within an hour.

Analytical data of market samples is also presented.

INTRODUCTION

Analytical data gives a picture of the use of potentially risk-bearing substances in cosmetics, which might be different from literature and textbook sources. At the time the Dutch Cosmetic Bill became valid in September 1968 not

* Government Food Control Station, H. B. Blijdensteinlaan 2, Enschede, Holland.

much was known about the actual use of these materials. It was decided to start analytical market surveys of several classes of cosmetics in order to contribute to the establishment of a well-balanced legislation in Holland.

The work will continue for several years, but it was thought valuable to make the analytical experiences available to those who are interested in the field of analytical cosmetic chemistry.

Eye Make-up Colours

Identification

Application of colours in the area of the eye should be carried out with the utmost care and with the best non-toxic colours available, in order to minimize the hazard of damage to this vital organ.

In contrast to lipstick colours no practical data are available on the kinds of pigments that are in actual use in today's eye cosmetics. At the beginning of 1972 an analytical survey of these colours was started. As no analytical methods were available such a system was developed. To get an idea what kind of colours might be used an inventory was compiled which is tabulated in *Table I* (1–5). Many are inorganic insoluble pigments. From this point of view instrumental analysis of the metal elements would give much information. Emission spectroscopy, X-ray fluorescence and X-ray diffraction methods are the methods of choice. On the other hand it should also be possible to make an analysis along classical chemical lines. A 'non-instrumental' methodology has been used but excluding the identification of the whites (except for TiO_2). This can only be done satisfactorily by instrumental analysis.

Apparatus and reagents

Apparatus for thin-layer chromatography
Microburner
Platinum needle
Platinum disk
Homogenizer (for instance Ultra Turrax: TP 18/2, Janke & Kunkel. W. Germany)
Kjeldahl flasks (25 ml)
Microscope
Vitreosol or quartz crucibles (*ca* 20 ml)
Centrifuge

Polyamide powder MN–SC6 or similar
Lead acetate/cotton plugs
Sodium Peroxide *granules* (Merck 6563 for instance)

Table I. Inventory of eye make-up colours
(numbers refer to colour index numbers)

GREEN
77288 Chromic oxide
77289 Chromic oxide hydrate
75810 Copper chlorophyllene
77013 Ultramarine green
— Copper versenate
— Titanated mica (green)
Synthetic organic colours and their lakes.
e.g.
42170
44090

VIOLET
77007 Ultramarine violet
77745 $Mn_3(PO_4)_2.7H_2O$
77742 $Mn(NH_4)P_2O_7$
Synthetic organic colours and their lakes.
e.g.
73385

YELLOW-ORANGE
77489 Iron oxide yellow
75300 Turmeric
75120 Anatto
75130 Carotene
— Titanated mica (gold)
Synthetic organic colours and their lakes.
e.g.
11920
19140

BROWN
77492 Iron oxide brown
— Burnt sienna
— Burnt umber
— Caramel

PEARL
— Guanine
— Titanated micas/talc
— BiOCl, or precipitated on mica
77480 Gold powder
77820 Silver powder
77400 Copper/bronze powder
77000 Aluminium powder

BLUE
77007 Ultramarine blue
77346 Cobalt aluminate
75510 Ferricferrocyanide
— Titanated mica (blue)
Synthetic organic colours and their lakes.
e.g.
42735
42090
Sudan blue II

RED
75470 Carmine
77491 Iron oxide red
77007 Ultramarine red
— Titanated mica (red)
Synthetic organic colours and their lakes.
e.g.

12120	15850	45430
12150	15865	45170
12085	15880	45425
	15630	

BLACK
77499 Iron oxide black
77267 Bone black
77266 Carbon black
— Vegetable black
— Graphite

WHITE
— Talc
77891 TiO
77947 ZnO_2
— Zn carbonate
77005 Kaolin
77002 Aluminum oxide
77120 $BaSO_4$
77713 $MgCO_3$
77220 $CaCO_3$
77231 Gypsum
— SiO_2
— Tin oxide
— Stearates of Li, Mg, Ca, Al, Zn

Sulphuric acid conc.
Sulphuric acid 4 M
Hydrochloric acid 6 M
NaOH 8 M
Nitric acid 50%

Diphenylcarbazide 1% in ethanol
Hydrogen peroxide 3%
Nessler reagent (K_2HgI_4) for ammonia
Ammonium molybdate 0.5% in conc. sulphuric acid

Methanol
Petroleum ether (40/60)
Benzene
Carbon tetrachloride
Dimethylformamide
Glycerol
Borax
Potassium bisulphate
Potassium ferrocyanide

Methods

Additives should first be removed from the sample and the pigments isolated. There are three procedures to do this, which depend on the physicochemical properties of the sample. In these operations centrifuging is an important step, since the striated sediment will in general give visual information of the separated components.

Removal of additives and isolation of the pigments. Procedure of removal of additives depends on the physicochemical properties of the sample:

Hydrophilic (miscible with water):	procedure A
Lipophilic (immiscible with water):	procedure B
'Wax cake' for instance mascaras:	procedure C

Procedure A. Mix 1 g sample with 40 ml water. Homogenize with blender or Ultra Turrax homogenizer. Centrifuge in 45 ml tube for 15 min at 3–4000 rev/min.

N.B. The striated sediment will give valuable information, in particular, of which components the pigment-mixture consists. The analysis will proceed according to these assessed colour components of the dried sediment.

The supernatant liquid might be coloured. If the colour can be adsorbed with Polyamide powder (procedure under *Table III*), the colour is in its water soluble form. If not the colour might be a suspension of small particles of dye-lakes, which is mainly present in the sediment.

Procedure B. Mix 1 g sample with 20 ml petroleum ether. Heat gently on a water bath to defat the powder thoroughly. Decant the petroleum ether. Repeat the defatting procedure twice more. Dry the powder and suspend in 40 ml water. Proceed as under A.

Procedure C. 'Wax cake' mascaras might contain chromic and iron oxides, and the different kinds of carbon blacks. For the identification of the carbon type

method A or B must be used. For the identification of chromic or iron oxide the sample should be ashed on a platinum dish to remove additives and the carbon.

The next step is to assess the colour components visually by observation of the original sample and of the striated centrifugal sediment. Assessment should be within the following defined colour groups:

GREEN	RED	BLACK
BLUE	YELLOW-ORANGE	PEARL
VIOLET	BROWN	WHITE

For example bluegreen should be assessed Blue and Green. The assessment of colour components of the sample is necessary to guide the analysis. *Table II* gives for each colour component the possible groups of colour compounds.

Table II. Possible groups of colour-compounds for the assessed colour components

GREEN	YELLOW-ORANGE
Chromium-oxides	Iron-compounds
Ultramarines	Organic colours
Organic colours	
	BROWN
BLUE	Iron-compounds
Ultramarine	Manganese-compounds
Iron-compounds	
Cobalt-compounds	BLACK
Organic colours	Carbon
	Iron-compounds
VIOLET	
Ultramarines	PEARL
Manganese-compounds	Pearlescent compounds
Organic colours	
	WHITE
RED	White compounds
Iron-compounds	
Organic colours	

All the possible groups of compounds should now be investigated systematically by characteristic reactions and methods that belong to each group of compounds.

Characterization reactions and methods

Ash. Ash a small sample on a platinum dish, heated by a microburner. Observe the colour changes during the heating and after cooling.

Sulphuric acid 4M. Add a small amount of the isolated pigment to $\frac{1}{2}$ ml of the acid. Observe before and after gentle heating. Gas evolution and colour changes

might occur. H_2S gas can be smelt and chemically confirmed by the browning of lead acetate/cotton plugs.

Hydrochloric acid 6M. Heat a little of the ash residue (1) with ½ ml of the acid. Coloration might occur.

NaOH 8M. Add a little of the isolated pigment to ½ ml of NaOH 8M. Gas evolution or coloration might occur.

Aqua regia. (50% Nitric acid + HCl 6M, 3 : 1 v/v.). A little of the isolated pigment is added to ½ ml of aqua regia. The pigment might dissolve and the liquid colour.

Dimethylformamide (DMF). Add a little of the isolated pigment to 1 ml of DMF. Coloration of the solvent might occur.

Methanol. Add a little of the isolated pigment to 1 ml of methanol. Coloration of the solvent might occur.

Sodium peroxide melt. Melt a small amount of the ash residue (1) with several granules of sodium peroxide in a vitreosol or quartz crucible. Observe colour after cooling. If characteristic reactions should be obtained with this melt, all of the excess sodium peroxide should first be decomposed by excessive heating.

Borax bead. Melt borax powder on a platinum wire loop with a little of the ash residue (1). Observe the colour of the hot bead and after cooling.

Dissolution. Heat a small amount of the ash residue (1) with a mixture of *ca* 0.25 g $KHSO_4$ and *ca* 0.25 conc. sulphuric acid, in a small kjeldahl flask over a small flame of a microburner. Cool and dilute with water.

Thin-layer chromatography. Details of tlc identification will be given under chapter 3: lipstick colours.

Metal analysis. This will not be discussed in this paper.

Microscope. Suspend the isolated pigment in glycerol. Observe plate structures and colour interference under the microscope. Compare with reference samples.

Observations

Chromium-oxides
77288 Cr_2O_3
77289 $2CrO_3.3H_2O$

Ash. Both pigments turn grey during the ashing, which becomes dull-green after cooling.

Sulphuric acid 4M. Chromic oxides are insoluble and remain unchanged by this reagent.

NaOH 8M. Chromic oxides are insoluble and colour persists in this reagent.

Sodium peroxide melt. The melt is yellow coloured, due to the formation of a chromate. Cool the melt and dissolve in sulphuric acid 4M, until reaction is acid. Boil the solution until the excess peroxide is decomposed. Cool and add diphenylcarbazide reagent. A violet colour indicates chrome.

Iron compounds
77510 Ferric ferrocyanide
77489 Yellow-iron oxide. Ochre $Fe_2O_3.nH_2O$
77491 Red iron oxide. Fe_2O_3
77492 Brown iron oxide. Fe_2O_3
77499 Black iron oxide. Fe_3O_4

Ash. All iron oxides turn brownblack to black during the ashing which turn deepbrown after cooling.

Sulphuric acid 4M. This reagent is only of importance to distinguish Prussian blue (Ferric ferrocyanide) and Ultramarine blue. Prussian blue retains its colour, Ultramarine fades.

HCl 6M. Heat the pigments in this reagent until dissolved. The hot solution is yellow. Dilute with 5× its volume with water. The yellow colour fades. Add several crystals of K-ferro cyanide. A blue precipitate indicates iron.

NaOH 8M. As reaction no 2, this reaction will distinguish Prussian blue and Ultramarine blue. Prussian blue turns redbrown, but Ultramarine retains its blue colour.

Ultramarines
77007 Ultramarine blue, violet, pink

Ash. All Ultramarines turn grey during the ashing, which turns blue after cooling.

Sulphuric acid 4M. All Ultramarines decompose with dilute acid even without heating. The residue is white. H_2S gas evolution occurs, which can be smelled and characterized by the browning of lead acetate/cotton plugs.

NaOH. Ultramarines retain their colour in this reagent, in contrast to Prussian blue (see under Fe-compounds).

Mn-compounds
77745 Mn-phosphate $Mn_3(PO_4)_2.7H_2O$
77742 Mn-violet $Mn\ NH_4\ P_2O_7$ —Burnt Umber Mn containing iron oxide

Ash. Both violet Mn phosphates turn white after the ashing. Burnt umber however retains its colour.

Sulphuric acid 4M. This reaction confirms the phosphate. Mix the ash residue with this reagent. Add Amm. molybdate reagent. Heat. A yellow colour indicates phosphate.

NaOH 8M. This reaction confirms the NH_4 radical. Mix with the reagent. Heat gently. NH_3 gas will escape, which can be shown by the yellow–brown coloration of a hanging drop Nessler reagent.

Sodium peroxide melt. The reaction is only of importance of the violet Mn-compounds. The melt is green

coloured, due to the formed manganate. Destroy excess peroxide by heating. Cool. Dissolve melt in 2 ml water. The colour is still green. Acidify. The colour turns into violet, caused by permanganate.

Metal analysis. If burnt umber is confirmed, metal analysis should be made. The ratio Fe/Mn might give an indication of the umber origin.

Co-compounds
77346 Cobalt aluminate. $CoO.Al_2O_3$

Ash. Cobalt aluminate is the only eye make-up colour that retains its beautiful blue colour during and after the ashing.

Borax bead. The colour of the borax bead is deep blue.

Carbon
77266 Carbon black
77267 Bone black —Vegetable black

Ash. The residue after ashing is of importance. Carbon black has no residue; Bone black leaves 80% of white residue. Vegetable black leaves a much smaller amount of residue.

Sulphuric acid 4M. This reagent is only of importance to confirm the ash residue of bone black, which contain phosphates. A small amount of the ash residue is heated with $\frac{1}{2}$ ml sulphuric acid 4M and NH_4 molybdate reagent. The phosphates give a yellow coloration.

Organic colours
75470 Carmine. Al/Ca lake of carminic acid
75810 Cu-chlorophyllene
There are many synthetic organic colours possible.

Sulphuric acid 4M. Colouring of the dilute acid after heating might show the presence of an organic colour.

Dimethylformamide. Colouring of this solvent by a small amount of the isolated pigment might show the presence of an organic colour. Filter. Concentrate by evaporation (in the hood!) on a waterbath and use the solution for tlc. N.B. Many lakes are appreciably soluble in dimethylformamide.

Methanol. This solvent will dissolve the unsulphonated organic colours, leaving the lakes undissolved. Concentrate the solution and proceed with tlc for identification.

Thin-layer chromatography. Use the extract of 6 and 7 for tlc using the systems of chapter 3: identification of lipstick colours.

Pearl-pigments
77000 Aluminum powder
77400 Copper/bronze powder

Ash. Aluminum powder burns with flashes, leaving a white oxide. If there is a strong orange colour, BiOCl might be present. This compound turns white again after heating for a short time, but if heating is excessive the colour remains yellow after ashing. TiO_2 and ZnO turns

77820 Silver powder	also yellow during the ashing, but not so intense as with
77480 Gold powder	BiOCl. The yellow colour turns white again after cooling.
BiOCl, as such or precipitated on mica	*Sulphuric acid 4M* will dissolve BiOCl. Then add Na_2S. A brown precipitation of Bi_2S_3 occurs. Copper and bronze powder will dissolve with a blue colour, which deepens after addition of ammonia.
TiO_2 precipitated on mica or talc (Titanated mica) White–Blue–Red Green–Gold	*HCl 6M*. Titanated gold micas contain a little iron oxide. Heat it with a little HCl 6M, dilute with equal volume of water and add several crystals of K. ferrocyanide. After some time a green–blue coloration occurs.
Guanin, pearl essence 2.Amino, 6.Oxy purine	*NaOH 8M*. This is an excellent reaction for Aluminum powder, which generates hydrogen. This reaction of Al with a base is much better than with an acid. Guanin dissolves in NaOH 8M. Titanated mica retains its colour in this reagent.
	Aqua regia. This is only important to dissolve gold or silver powder.
	Dissolution. This is the best method to confirm Ti. After cooling dilute with 5 × water and add several drops of 3% H_2O_2. A specific yellow–orange coloration occurs.
	Metal analysis. This will give information for talc or mica.
	Microscope. Mica platelets will show beautiful interference colours under the microscope. Compare with reference substances.

Market survey of eye make-up. Analytical results

Sampling from the market was done in January 1972. There were nine brands with 111 samples. All colour tints of three brands were taken. Of the other brands the brown, grey and black colours were omitted, because they were chemically less interesting. All kinds of products were sampled: liquid liner, pencil liner, shadow liquid, shadow compact powder, shadow stick, shadow ointment, mascara wax cake, mascara liquid.

Two unidentifiable samples were found in the 111 samples—one blue, one violet.

There were 33 GREEN eye make-up preparations
- 28 contain chromic oxides
- 7 contain iron oxides
- 2 contain ultramarines
- 3 contain aluminum powder

1 contains BiOCl on mica
15 contain titanated micas
3 contain lakes of organic colours CI 19140
CI 42090

There were 31 BLUE eye make-up preparations
14 contain chromic oxides
2 contain ferric ferrocyanide
27 contain ultramarines
2 contain aluminum powder
6 contain BiOCl on mica
5 contain titanated micas
2 contain lakes of organic colours CI 14290
CI 42090

There were 20 VIOLET eye make-up preparations
3 contain iron oxides
15 contain ultramarines
3 contain Mn-violet
1 contains aluminum powder
3 contain BiOCl on mica
7 contain titanated micas
2 contain lakes of organic colours CI 42090
CI 75470 (Carmine)
CI 45170
1 contains unidentified pigments

There were 3 RED/BROWN eye make-up preparations
2 contain iron oxides
3 contain ultramarines
2 contain titanated micas
1 contains a lake of an organic colour CI 45425

There were 3 BLACK eye make-up preparations
1 contains iron oxides
3 contain carbon black

There were 5 GREY eye make-up preparations
3 contain iron oxides
2 contain ultramarines
1 contains aluminum powder
1 contains BiOCl on mica
2 contain carbon black

There were 16 BROWN eye make-up preparations
14 contain iron oxides
2 contain bronze powder
6 contain titanated micas

Lipstick Colours

Identification

Many excellent papers exist on the paper and thin layer chromatographic identification of lipstick colours. For this reason the choice of methods made for their identification in the 36 samples (18 brands) of lipsticks purchased in February 1972 is only briefly described.

A selection of 40 of the most important lipstick colours was made, based on several recent publications in this field. Reference substances were obtained by the kind cooperation of several manufacturers and laboratories. The list of these reference colours is in *Table III*.

There are two important groups of lipstick colours worth consideration from an analytical aspect. Xanthene colours are the principal compounds for indelible types of lipstick. It appears that the industrial reference colours in several instances contain minor substances, which are clearly visible on the tlc plates (see *Fig. 1*). In the actual separation of the sample extracts, this phenomenon might interfere in the identification of a minor spot.

A second important group of colours in lipsticks are the lakes of the water soluble dyes. Most of these dyes occur as water-insoluble lakes in lipsticks, because of the undesirability of 'bleeding' on the place of application. Most of the lakes are also practically insoluble in other organic solvents, such as ethanol, chloroform or benzene. Fortunately hot dimethylformamide (DMF) will dissolve many lakes to an appreciable extent thus permitting extraction for tlc analysis.

Extraction of lipsticks

The Lehmann method (7) was used. It gives five fractions; the fat fraction is discarded; the main (DMF) fraction and the 'lake' fraction are analysed by tlc and the pigment fraction is analysed by the same chemical methods as used for eye make-up.

Mix and heat 100 mg of sample with 10 ml dimethylformamide (dmf). Filter. Filtrate = *a* and residue = *b*. Defat *a* by shaking with 2 × 15 ml nHexane. Separate. Hexane-fraction is *c* and dmf-fraction is *d*.

Table III. The most important lipstick colours

1	2	3	4	5	6	7	8	9	10	11
12075	—	DC–Or 17	III–b3	Permanent orange	7	–	+	+	–	+
12085	C–Rot 1	DC–Red 36	III–a1	Flaming red	–	+	+	+	–	+
12120	Cex–Rot 1	(DC–Red 35)	IV–a1	Helioechtrot RN	4	–	+	–	–	+
12150	(C–Rot 2)	—	IV–a2	Solvent red 1	–	–	–	–	–	–
13065	Cex-Gelb 10	Ext. DC–Y 1	—	Metanil yellow	–	–	–	–	+	+
14720	C–Rot 54	Ext. DC–R 10	III–a3	Azorubin	–	+	–	–	+	–
15510	Cex–Or 8	Ext. DC–Or 4	III–b6	Orange II	3	+	+	+	–	+
15525	C–Rot 8	—	III–a5	Pigment red 68	–	+	–	–	–	+
15585	Cex–Rot 18	DC–Red 8/9	III–a7	Lake Red C	11	–	+	+	–	+
15630	Cex–Rot 33	DC–R 10/13	III–a8	Litholred lakes	6	–	+	+	–	+
15850	C–Rot 12	DC–Red 6/7	III–a9	Litholrubin B	3	+	+	+	–	+
15880	C–Rot 14	DC–Red 34	III–a11	Deep maroon	6	+	–	+	–	+
15980	C–Or 9	—	III–b7	Orange GGN	8	–	–	–	+	+
15985	C–Or 10	—	III–b8	Sunset yellow	–	–	–	+	–	+
16185	L–Rot 3	FDC–Red 2	III–a12	Amaranth	–	–	–	–	–	+
16255	L–Rot 4	—	III–a13	Cochenille red	–	–	–	–	–	+
19140	C–Gelb 10	FDC–Y 5	III–b9	Tartrazine	8	–	–	+	+	+
26100	—	DC–Red 17	—	Toney red	–	–	+	–	–	+
28440	C–Schw. 6	—	III–d1	Brill. schwarz BN	–	–	–	–	–	–
42051	C–Blau 20	—	III–c1	Patentblau V	–	–	–	–	–	+
42090	—	(FDC–Blue 1)	III–c2	Brill. blue FCF	1	–	+	+	–	+
42735	Cex–Blau 14	—	IV–c5	Acid blue 104	–	–	–	–	–	+
45160	Cex–Rot 26	—	—	Rhodamin 4GD	–	–	–	–	–	–
45170	Cex–Rot 27	—	III–a15	Rhodamin B	1	–	+	+	+	+
45220	Cex–Rot 30	—	—	Acid red 50	–	–	–	–	–	+

Table III continued

1	2	3	4	5	6	7	8	9	10	11
45350	Cex–Glb 16	DC–Y 7/8	III–b10	Uranin	–	–	–	–	–	+
45365	(C–Rot 26)	—	—	Dichlorofluoresc	–	–	–	–	–	+
45370	C–Rot 27	DC–Or 5	III–a16	Eosin S 10	16	+	+	+	+	+
45380	C–Rot 30	DC–Red 21/22	III–a17	Eosin Yellowish	17	+	+	+	+	+
45386	C–Rot 31	—	—	Eosin S	–	–	–	–	–	+
45396	C–Or 7	—	III–b22	Eosin 3G	1	+	–	–	–	+
45400	C–Rot 32	—	—	Eosin BN	–	–	–	–	–	–
45405	(C–Rot 33)	—	III–a18	Phloxine P	–	–	–	–	–	+
45410	C–Rot 34	DC–Red 27/28	III–a19	Phloxine	2	+	+	+	+	+
45425	C–Rot 35	DC–Or 10	III–a26	Eosin S 15	–	+	–	–	+	+
45430	C–Rot 38	FDC–Red 3	III–a20	Erythrosine	6	+	+	+	+	+
45440	C–Rot 37	—	—	Rose Bengale	–	+	–	–	+	+
73015	—	FDC–Blue 2	III–c6	Indigotine	–	–	–	–	–	–
73360	C–Rot 28	DC–Red 30	III–a21	Helindone Pink	–	–	–	+	–	+
75470	C–Rot 50	—	III–a22	Carmine	–	–	–	–	–	–

1. Colour index number.
2. W. Germany. Mitteilungen III der D. Forschung Gem.
3. USA. Food and Drug Administration Reference.
4. Proposed EEC list for cosmetics (28.12.1971). III = definitely accepted; IV = provisionally accepted.
5. Trivial names.
6. Found in our 36 samples (18 brands). This report.
7. Found by Lehmann 1970 (lit. ref. 7).
8. Found by Silk 1965 (lit. ref. 9).
9. Found by Cotsis and Garey (lit. ref. 10).
10. Found by Deshusser and Desbaumes 1966 (lit. ref. 8).
11. Found by Perdih 1972 (lit. ref. 12).

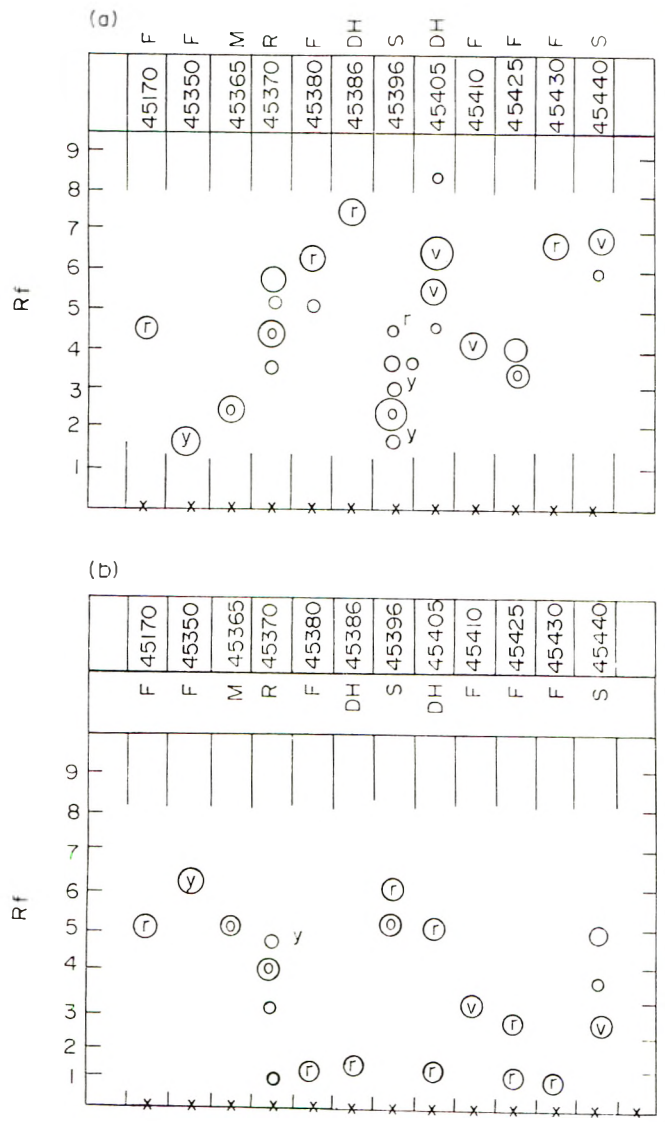


Figure 1. Tlc of reference xanthene colours. All spots are fluorescent under uv light (254 and 360 nm). (a) Tlc system: silica 10 cm precoated plates. Solvent: (vol.) 15 ethylacetate, 6 methanol, 3 conc. ammonia. (b) Tlc system: cellulose 10 cm precoated plates. Solvent: (vol.) 20 Na-citrate (2.5%), 3 methanol, 5 conc. ammonia. Daylight colours: r = red, o = orange, y = yellow, v = violet. Ciphers are Colour Index numbers. Letters: origin of reference substances (lab. or manuf.): F = U.S. Food and Drug Adm. S, DH and M are manufacturers.

FAT FRACTION — Fraction *c* is the FAT FRACTION.

To fraction *d* an equal volume of water is added. It contains colours. Purify by adsorbing the colours on Polyamid powder 1 g (MN-SC6, Machery and Nagel). Wash with 2×10 ml water and 2×10 ml methanol in a microcolumn. Elute colours with 25 ml of (methanol–conc. ammonia, 95/5 vol./vol.)

DMF-FRACTION — This is the DMF-FRACTION, which is the main fraction of the colours in the lipstick. Concentrate and use the extract for tlc. In some instances the Polyamid powder might still be coloured. Elute with 25 ml dichloromethane. Concentrate. This is the

DCM-FRACTION — DCM-FRACTION, which contains unsulphonated colours.

The residue *b* contains residual lakes and pigments. Heat with 25 ml of (methanolammonia conc., 95/5 vol). Filter.

LAKE-FRACTION — Concentrate the filtrate. This is the LAKE-FRACTION.

The residue still contains a part of the lakes and also the inorganic pigments. The residue is ashed to

PIGMENT FRACTION — remove organic matter. The PIGMENT FRACTION is then obtained.

Analytical scheme for the extracted fractions

FAT FRACTION: Discard.

DMF-FRACTION: Apply tlc with the systems of *Figs 1* and *2(a)*. The xanthene colours 45370 and 45380 are well identified. The yellow colour 19140 and the blue 42090 should be confirmed by the system of *Fig. 2(c)*. The difficult separation of 15980 and 15985 (Orange GGN and Sunset yellow) can only be done by the system in *Fig. 2(d)*. The other orange colours are well identified by the systems of *Fig. 2(a)* and *(b)*.

DCM FRACTION: These unsulphonated colours are identified with the tlc system of *Fig. 2(e)*.

LAKE FRACTION: As under DMF FRACTION.

PIGMENT FRACTION: In most instances TiO_2, titanated micas and iron oxides will be present. Identify as described under the eye make-up colours earlier.

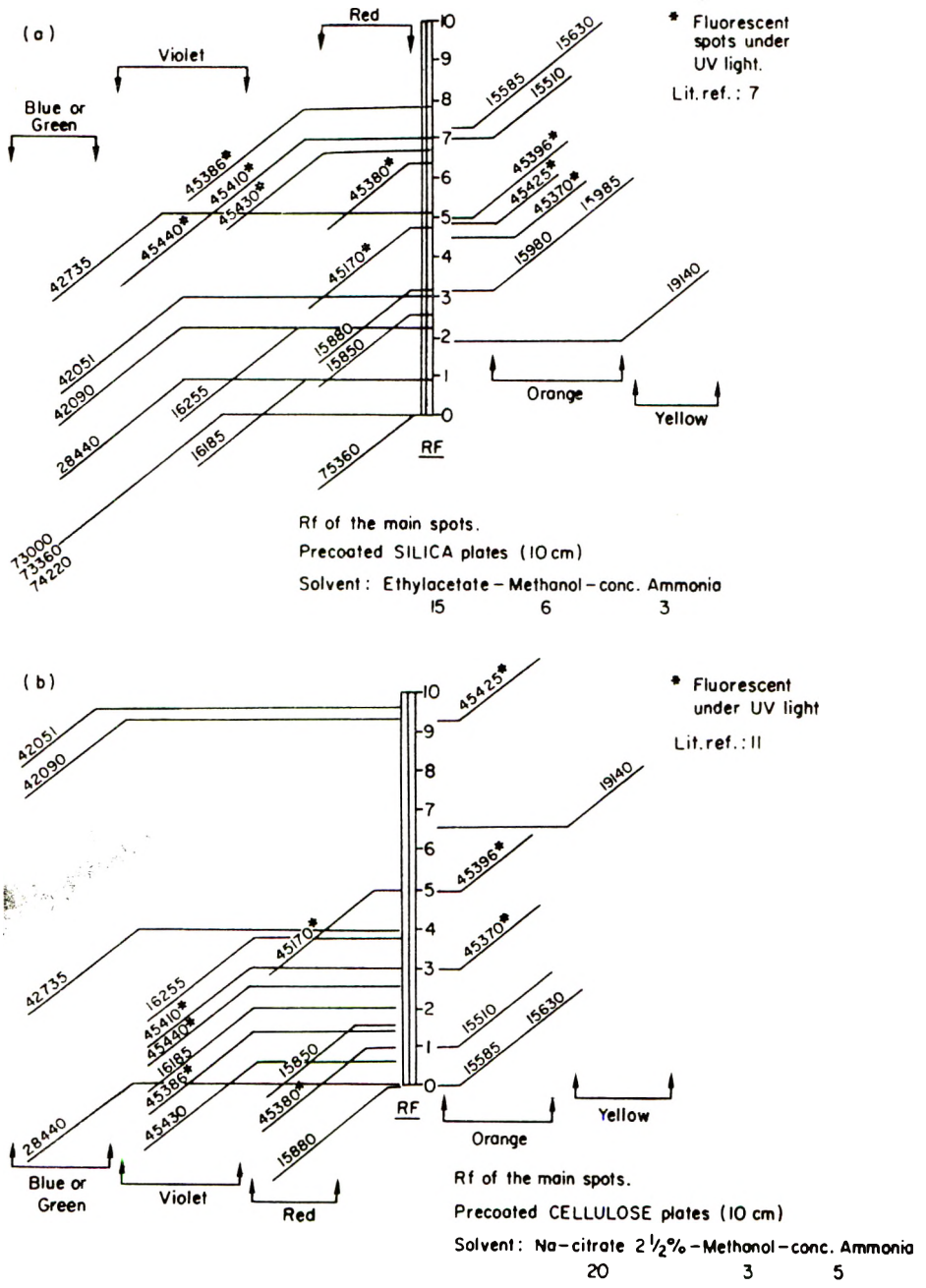
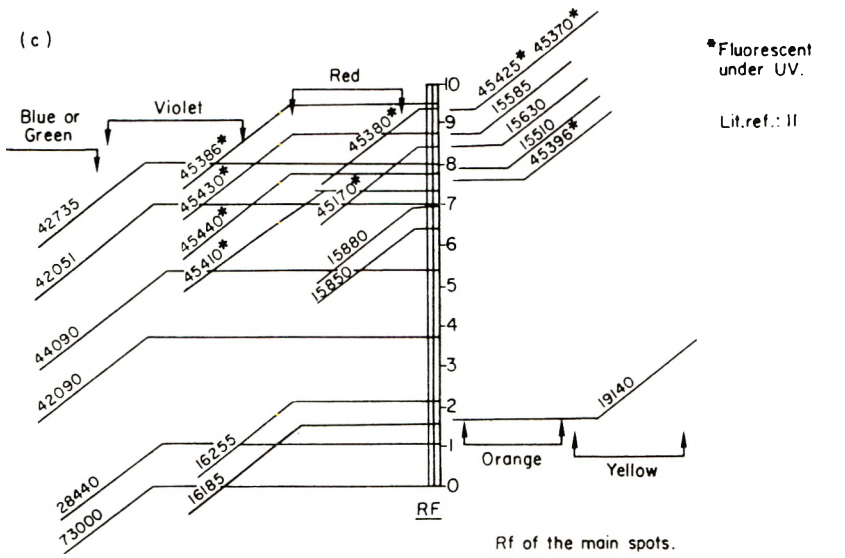

(a)
Red
Violet
Blue or Green
* Fluorescent spots under UV light.
Lit.ref.: 7
42735
45440*
45386*
45410*
45430*
45380*
45170*
42051
42090
28440
16255
16185
15880
15850
73000
73360
74220
75360
RF
15630
15585
15510
45396*
45425*
45370*
15985
15980
19140
Orange
Yellow
Rf of the main spots.
Precoated SILICA plates (10 cm)
Solvent: Ethylacetate – Methanol – conc. Ammonia
15 6 3
(b)
* Fluorescent under UV light
Lit.ref.: 11
42051
42090
45425*
19140
45396*
45370*
42735
45170*
16255
45410*
45440*
16185
45386*
45430
15850
45380*
15880
28440
RF
15510
15630
15585
Orange
Yellow
Blue or Green
Violet
Red
Rf of the main spots.
Precoated CELLULOSE plates (10 cm)
Solvent: Na-citrate 2½% – Methanol – conc. Ammonia
20 3 5

Figure 2a, b.


(c)
*Fluorescent under UV.
Lit.ref.: 11
Red
Violet
Blue or Green
45386*
45430*
45440*
45410*
45380*
45170*
42735
42051
44090
42090
15880
15850
45425*
45370*
15585
15630
15510
45396*
19140
Orange
Yellow
28440
73000
16255
16185
RF
Rf of the main spots.
Precoated CELLULOSE plates 10 cm
Solvent: n.Butanol – Pyridin – Water
6 4 3

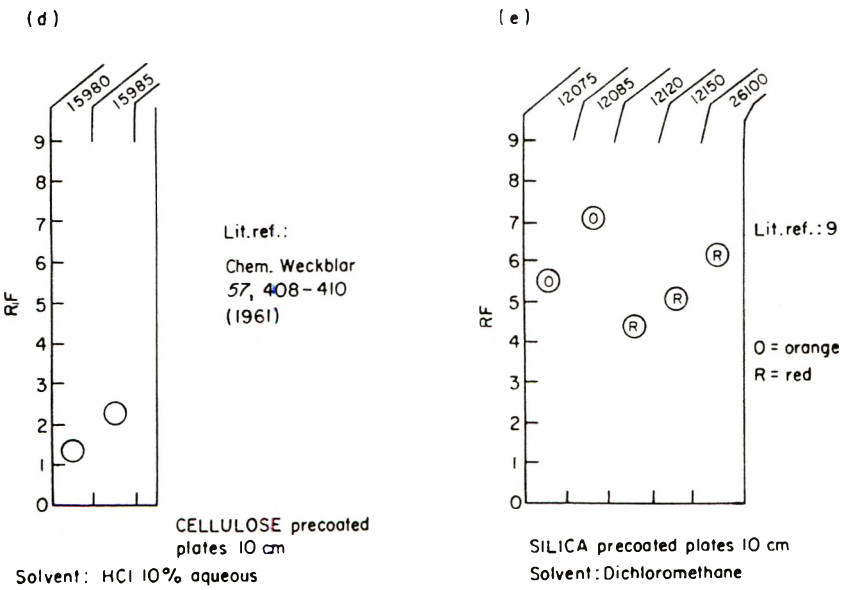

(d)
15980
15985
Rf
Lit.ref.:
Chem. Weckblar
57, 408-410
(1961)
CELLULOSE precoated plates 10 cm
Solvent: HCl 10% aqueous
(e)
12075
12085
12120
12150
26100
RF
O
R
Lit.ref.: 9
O = orange
R = red
SILICA precoated plates 10 cm
Solvent: Dichloromethane

Figure 2c–e.

The choice of the tlc systems is not difficult, because many excellent systems exist. After some preliminary work a final choice was made which is given in detail in *Fig. 2(a)–(e)*. Tlc matching with reference colours will be much aided by these tables, and also by *Fig. 1*. Confirmation should always be made by tlc of mixed spots, namely the sample extract + reference standard.

Analytical results: lipstick colours

THE ANALYSIS OF OXIDATIVE HAIR COLOUR INTERMEDIATES

It is well known that the oxidative hair colour intermediates have a significant potential toxicity. More than 100 compounds are mentioned in literature (13, 15, 16, 18). Most of them are substituted derivatives of benzene, naphthalene or pyridine. *Table IV* lists the most important possibilities. It is not likely however that all of them are used in modern hair colour preparations. To get an idea of the compounds most frequently in use, 80 samples of 10 brands of the hair colourants for home use as well as for professional use were examined. Most were cremes in tubes and some of them were viscous liquids.

Twenty-five reference compounds were selected based on the most recent papers on this subject (see *Table V*). The second step was to build up an analytical scheme. There exists a number of excellent tlc identification systems (14, 16, 17, 19) in the literature. The final choice was made after careful preliminary experimentation.

Analytical scheme for the identification of hair colour intermediates

Tlc	Preliminary tlc: *estimation of the concentration levels*. 20 min precoated plates, 10 cm, one-dimensional. Two-dimensional tlc: *main identification*. Self-made 20 × 20 cm plates, Silica H, visualization iodine vapour. $2\frac{1}{2}$ h.
Glc-aromatic amines	*For confirmation.* No clean-up of extract necessary. Selective column. Separation of OFD . MFD . PFD . 26DAP . 34TDA . 25TDA . 24TDA . 2CPFD . 24DAA. Polyphenols and aminophenols do not respond. Quantitative estimation possible.
Glc-polyphenols	*For confirmation.* Special clean-up necessary. Removal of amines, aminophenols, etc. Silylation of the phenols. OV–210 column. Separation of PCT . RES . HCH . PGL . PHL . AN . BN. (130°C) (HCH and CRES cannot be separated at 130°C, but at 100°C resolution is good). A quantitative estimation is possible.

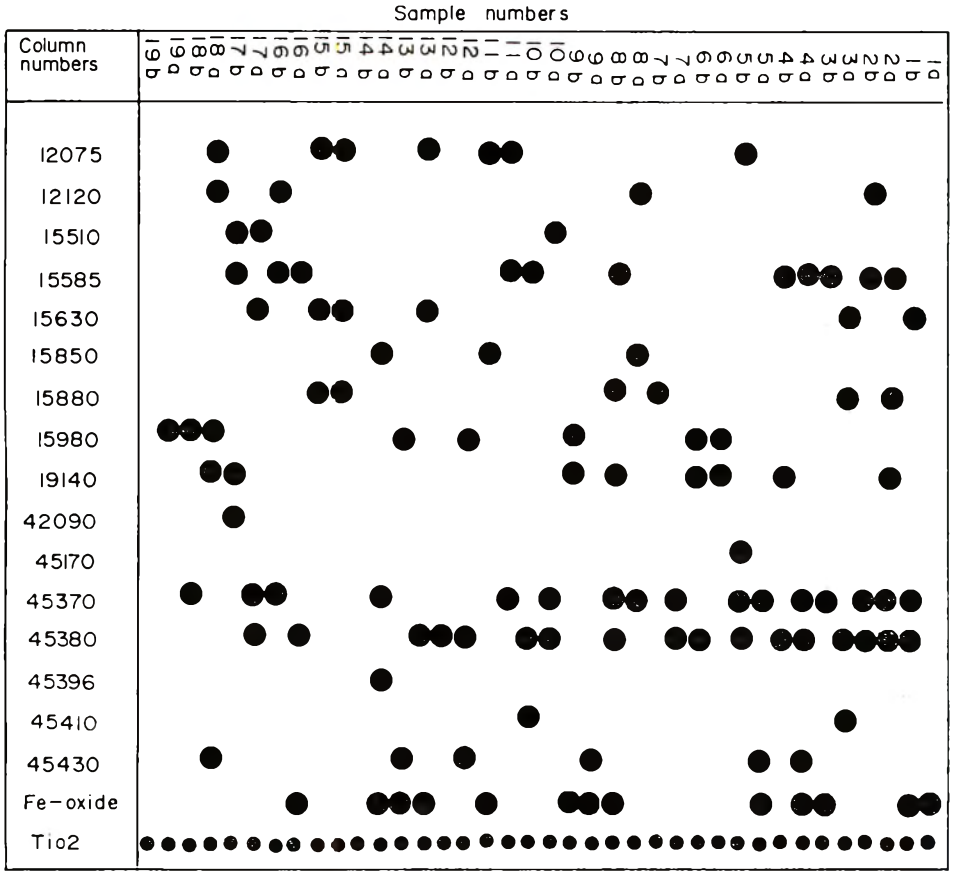


Figure 3. Analytical results: lipstick colours. Analysis of the colour composition of 38 commercial lipstick samples.

Experimental

The determination starts with a quick (*ca* 20 min) estimation of the concentration levels by tlc on 10 cm precoated plates and visualization by iodine vapour. This makes it possible to determine optimum amounts for the spots of the main identification procedure.

Thin-layer chromatography. Two-dimensional tlc on 20 × 20 cm silica plates was chosen for this main identification. The 'crude' extracts were 'cleaned-up' in the first direction, thus permitting excellent resolution in the second. The spots are easily visualized by iodine vapour, which colours all the classes of intermediates, the aromatic diamines, the aminophenols as well as the polyphenols.

Table IV. The most important types of oxidative hair colour intermediates

Substituents	No. of isomers	Position	Base	Abbreviation commonly used
Diamino	3	ortho	phenylenediamine	OFD
		meta	phenylenediamine	MFD
		para	phenylenediamine	PFD
Dihydroxy	3	ortho	dihydroxybenzene = pyrocatechol	PCT
		meta	dihydroxybenzene = resorcinol	RES
		para	dihydroxybenzene = hydroquinone	HCH
Trihydroxy	3	1.2.3	trihydroxybenzene = pyrogallol	PGL
		1.2.4	trihydroxybenzene = hydroxyhydroquinone	
		1.3.5	trihydroxybenzene = phloroglucine	PHL
Hydroxy amino	3	2.	aminophenol	2AF
		3.	aminophenol	3AF
		4.	aminophenol	4AF
Methyl diamino	6	2.3	toluylenediamine	
		2.4	toluylenediamine	24TDA
		2.5	toluylenediamine	25TDA
		2.6	toluylenediamine	
		3.4	toluylenediamine	
		3.5	toluylenediamine	
Hydroxy diamino	6	2.3	diaminophenol	
		2.4	diaminophenol = amidol base	24DAF
		2.5	diaminophenol	
		2.6	diaminophenol	
		3.4	diaminophenol	
		3.5	diaminophenol	
Diamino methyl ether	6	2.3	diaminoanisole	
		2.4	diaminoanisole	24DAA
		2.5	diaminoanisole	
		2.6	diaminoanisole	
		3.4	diaminoanisole	
		3.5	diaminoanisole	
Nitro diamine	6	3 nitro	1.2.phenylenediamine	
		4 nitro	1.2.phenylenediamine	4NOFD
		2 nitro	1.3.phenylenediamine	
		4 nitro	1.3.phenylenediamine	
		5 nitro	1.3.phenylenediamine	
		2 nitro	1.4.phenylenediamine	2NPFD
Hydroxy	2	α	naphthol	AN
		β	naphthol	BN
Diamino	6	2.3	diaminopyridine	
		2.4	diaminopyridine	
		2.5	diaminopyridine	
		2.6	diaminopyridine	26DAP
		3.4–3.5	diaminopyridine	

Table V. List of reference compounds: oxidative hair colour intermediates

OFD	o.Phenylenediamine	2AF	2.aminophenol	PCT	pyrocatechol
MFD	m.Phenylenediamine	3AF	3.aminophenol	RES	resorcinol
PFD	p.Phenylenediamine	4AF	4.aminophenol	HCH	hydroquinone
24TDA	2.4.tolylenediamine	M4AF	4.methylaminophenol	PHL	phloroglucine
25TDA	2.5.tolylenediamine		(metol base)	PGL	pyrogallol
24DAF	2.4.diaminophenol	2NPFD	2.nitro.p.phenylene-	AN	naphthol α
	(amidol base)		diamine	BN	naphthol β
24DAA	2.4.diaminoanisole	4NOFD	4.nitro.o.phenylene-	CRES	chlororesorcinol
26DAP	2.6.diaminopyridine		diamine		
2CPFD	2.chloro.p.phenylene-				
	diamine				

There is one important factor that is worth mentioning. The silica support must be carefully considered to obtain good resolution and good shapes of the spots. We did not have satisfaction with several kinds of precoated supports, including self-coated silica with gypsum binder. The purest kind of silica (without binder) (for instance Silica HR, Merck) gave the best results. Colouring of the iodine vapour of the spots on these self-made plates will also give much more beautiful colours (in particular the starting colours), which aids the identification procedure. Precoated plates will only give brownish colours with iodine vapour for all the components.

Procedure. The solvent for extract or reference solutions is always methanol : ethylacetate = 1 : 4 vol. Extracts and reference solutions should always be preserved with several crystals of sodium-metabisulphite.

Reference solutions: Make 0.1% standard solutions of the substances of *Table V* in the above solvent mixture.

Standard mixture: A mixture of 0.1% each of PFD, 2AF, 3AF, 4AF, RES, AN.

Extraction: Mix 1 g of the product and 4 ml of the solvent. Heat a little on a waterbath. Add *ca* 250 mg sodium-metabisulphite and *ca* 500 mg sodium-sulphate exsicc. Shake the mixture vigorously. Use the clear liquid for tlc. *This extract can only be kept for ONE day.*

Optimal spotting is very important. For 20 × 20 cm plates 5 µg is the optimal amount for these tlc plates (except for 2NPFD ½ µg; for 3AF.4NOFD.HCH. AN.BN. 10 µg, and PGL 15 µg). For small plates (10 cm) half of these amounts.

Mixed spots of extracts and reference solutions can be used for confirmation.

Preliminary tlc. The purpose of preliminary tlc on precoated tlc plates (10 cm) is the estimation of the concentration level in the product, in order to permit optimal spotting in the main identification procedure.

It is made with the following spots of the extracts: 1–2–5–20 µl of the extract and a reference mixture spot of 2 µg of 25TDA–3AF–RES. Solvent same as in

the first direction of the 2-dimensional procedure. Visualization is by iodine vapour. Determine the optimal amount of the extract and use twice this amount for the 20 × 20 cm tlc plates.

Two-dimensional tlc. The spots on the two-dimensional tlc plate are identified by relative positions to the standard-mixture pattern. This is shown on *Fig. 4.* Colouring observation in the iodine tank will give some aid. It is important to remove all residual acetic acid of the second solvent from the plate (by heating for 10 min approx. at 50°C) in order to achieve optimal colouring.

The conditions used were the following. Silica H plates (self-made) 20 × 20 cm, 0·25 mm thick, activated 1 h at 105°C. First direction: (unsaturated tank) Acetone-chloroform-toluene 35–25–40 vol. 1 h. Dry 15 min. Second direction: (saturated tank) 1 h 45 min. Chloroform Acetic acid–H_2O 50–45–5. Dry 10 min at 50°C, until smell of acetic acid has disappeared.

Visualization: Put in empty chromatographic tank with several crystals of iodine. Observe starting and definite colours. Leave $\frac{1}{2}$ h in tank. Take it out. Excess iodine will disappear, leaving the definite colours of the spots.

PFD	starts green, change to violet/brown
MFD	yellow/brown
25TDA	starts green, change to violet/brown
24TDA	yellow/brown
24DAF	violet/brown
24DAA	yellow/brown
M4AF	violet/brown
AN	violet
BN	yellow
RES	grey/brown
HCH	yellow
PGL	brown
2NPFD	red
4NOFD	orange
2AF	yellow/brown
3AF	brown
4AF	violet/red.

Optimal results depend on the quality of the tlc plates. We have had poor resolution with several kinds of precoated plates, as well as with self-made plates with gypsum binder, as well as for colour display by iodine vapour, and the shape of spots.

Gas-liquid chromatography—amines. Doubts might still remain, especially with difficult pairs, such as resorcinol/hydroquinone, 2.5.toluylene diamine/2.4.di-aminoanisol/m. phenylenediamine, 2.4.toluylene diamine/4.aminophenol.

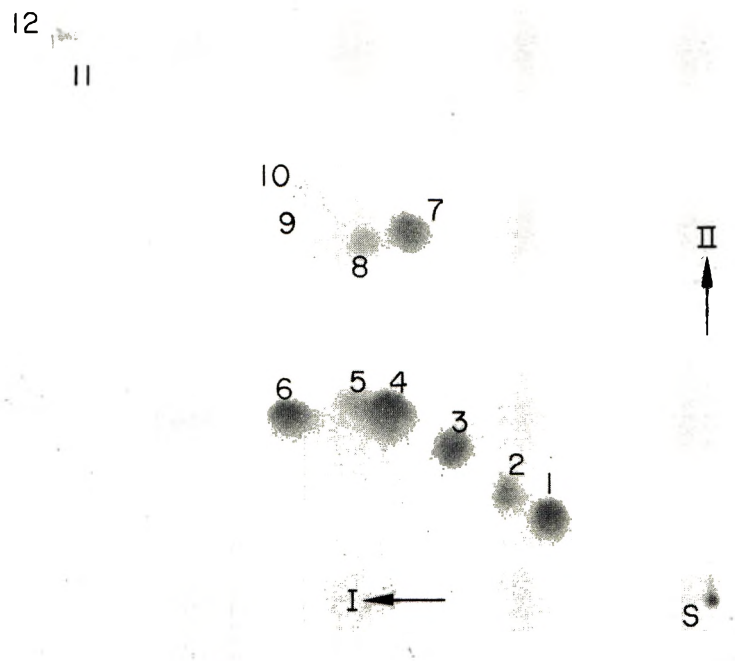


Figure 4. Two-dimensional resolution of a reference mixture. 1, p.phenylenediamine; 2, 2.5.toluylenediamine; 3, 4.aminofenol; 4, 3.aminofenol; 5, 2.nitro.p.phenylenediamine; 6, 2.aminofenol; 7, pyrogallol; 8, 4.nitro.o.phenylenediamine; 9, hydroquinone; 10, resorcinol; 11, b-naphthol; 12, a-naphthol.

(*Facing p.* 184)

Confirmation of the identity of the components by glc of the important aromatic diamines and separately of the polyphenol groups was then obtained.

The aromatic diamines are separated on an alkaline column (Apiezon L–KOH)[1]. The aminophenols and the diphenols do not give a response on this column, so that a good confirmation can be made of the identified aromatic diamines by injecting a freshly made crude extract. After several injections the column should be cleaned at a higher temperature, in order to avoid the appearance of 'ghost' peaks which generally belong to former injections. The following injection-sequence scheme during the day is highly recommended. Quantitative work is also possible with this column, which however requires precleaning of the extract. The internal standard used is 2 chloro.p.phenylene diamine. The precision of the method, however, is not very high (19, 20, 21).

The glc column is selective for the aromatic diamines. Aminophenols and the polyphenols do not give any response.

The same extraction as for tlc was used, except that ethyl-acetate only was used as solvent. *Freshly made extracts should be used.*

Experimental

Glc conditions: Column 150 cm, 0.25 inch cuts. diam., stainless steel. Filling: Apiezon L 6% and KOH 10% on Chromosorb WHP 80/100, which is prepared as follows: Dissolve 420 mg Apiezon L in some toluene. Dissolve 700 mg KOH in a small amount of water and much ethanol. Mix both solutions and add to 5880 mg Chromosorb WHP 80/100. Dry the mixture in a rotating vacuum evaporator, keeping the mixture as granular as possible. Column temperature: 150°C isotherm. Carrier gas: Nitrogen. Detector FID.

Glc reference mixture: a solution in ethylacetate, containing per 100 ml: 45 mg PFD—50 mg MFD—80 mg 25TDA—70 mg 24TDA—190 mg 24DAA. A chromatogram of this mixture (0.7 µl) is shown in *Fig. 5*.

As fouling of the column occurs, it must be cleaned periodically. Otherwise 'ghost peaks' appear after several injections. It is therefore necessary to work exactly according to the following scheme:

Injection of the glc reference mixture: 10 min (and not longer).
Injection of extract of the first sample: 10 min (and not longer).
Injection of the second sample extract: 10 min (and not longer).
Injection of the third sample extract: 10 min (and not longer).
Cleaning the column at 250°C from 30 min.

The quantitative determination of 25TDA is described, but the method can also be modified for the other aromatic diamines. An internal standard (2.Chloro. p.phenylenediamine) is used.

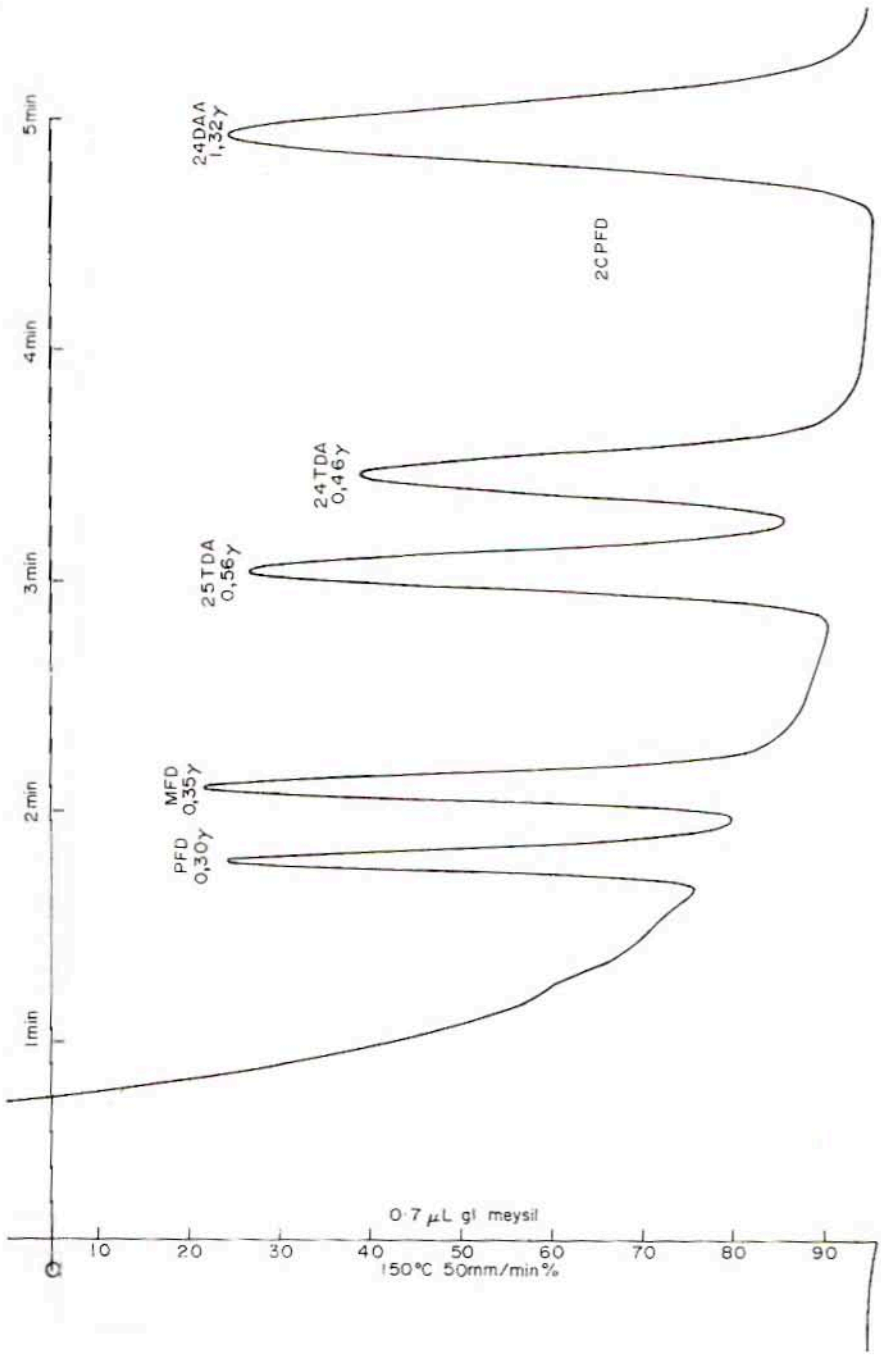


Figure 5. Glc separation of aromatic amines (standard mixture 0.7 μl).

Internal standard solution: 0.1% in ethylacetate of 2CPFD.
Ref. 25TDA solution: 0.15% in ethylacetate.

Standard curve: Prepare standard mixture by successive mixing of (internal standard solution) + (Ref. 25TDA solution) + (ethylacetate): 5–1–4, 5–2–3, 5–3–2, 5–4–1.

These standard mixtures contain respectively per µl:

0.150 0.300 0.450 0.600 µg 25TDA.

Extraction: Weigh *m* g of the sample, which must contain 1–6 mg 25TDA. (For quantitative work this extract must be used within 1 h.) Mix this in a 10 ml volumetric flask with 5 ml internal standard solution and sufficient ethylacetate to a volume of 10 ml. Homogenize if necessary by heating on a waterbath. Add 1 g of anhydrous sodium sulphate, mix vigorously. Carry out the glc according to the projected injection sequence. Calculate the peak height ratio 25TDA/2CPFD. Find the equivalent µg 25TDA from the standard curve (*a* µg 25TDA). The % 25TDA is (a/m) %.

$\frac{\text{height 25TDA}}{\text{height 2CPFD}}$ (A)	µg 25TDA per µl (B)	A/B
0.61	0.156	3.88
1.18	0.312	3.78
1.81	0.468	3.87
2.41	0.624	3.87
	Mean A/B	3.85
	Variation coeff.	1.3%

The reliability of this method for our uncleaned extracts, however, is not better than 5%, due to the retardance of the solvent peak to the baseline, which is caused by the impurities in the extract.

Gas-liquid chromatography—phenols. The polyphenols can be separated, after silylation, on an OV 210 column (50% Trifluoropropyl methyl silicone). In contrast to the aromatic diamines, crude extracts cannot be used in the glc procedure, because many components of the basic support are silylated and interfere with the glc procedure. It is therefore necessary to clean the extracts as described in the following procedure.

The polyphenols are isolated from the aromatic diamines, the aminophenols and also from impurities of the creme—or liquid-base of the hair colourant. The purified phenol fraction is then silylated and separated on silicone column purified phenol fraction is then silylated and separated on silicone column (OV–210). Satisfactory resolution is achieved between resorcinol and hydroquinone.

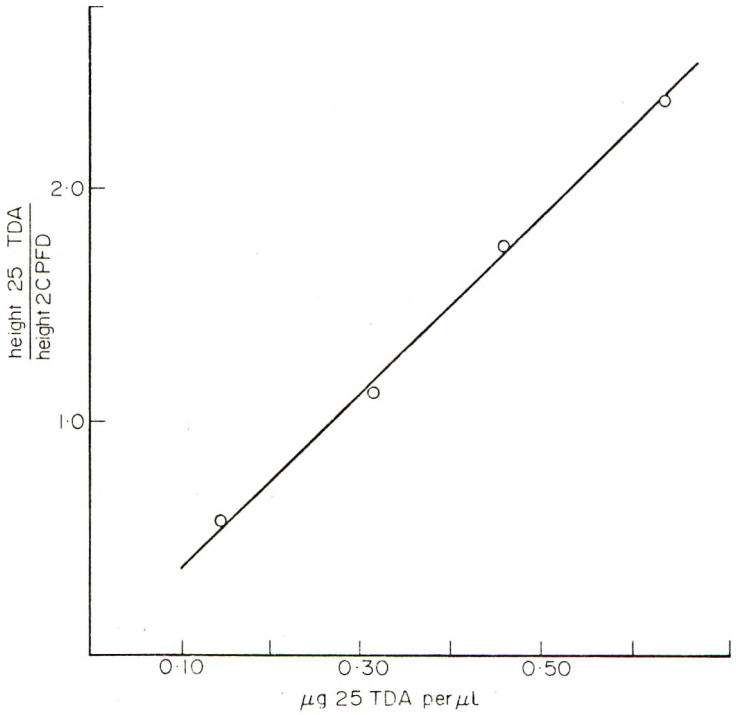


Figure 6. Estimation of 2.5. toluene diamine by glc.

Experimental

Shake in a 250 ml separatory funnel: (4 g sample + 6 ml NaOH 1M + *ca* 500 mg Na-ascorbate to prevent oxidation + 100 ml dichloromethane). Shake vigorously. Wait at least 30 min for the separation of the phases. Discard the dichloromethane fraction. Acidify the aqueous fraction with 0.6 ml 35% HCl. Extract with 50 ml, 25 ml, 25 ml dichloromethane successively. Dry the dichloromethane fractions with Na-sulphate exsicc. Evaporate the clear extract on a waterbath until dry.

Proceed with the silylation procedure in a small (approx. 5–10 ml) vial which can be closed tightly. Dissolve the dried residue of the extract in 4 ml ethylacetate and add successively: 0.2 ml Hexamethyldisilazane (HMDS) and 0.1 ml Trimethylchlorosilane (TMCS). Close the vessel and heat on a waterbath of 60°C for 5 min. Cool. The mixture is ready for glc.

Glc-conditions: Column length 150 cm, $\frac{1}{4}$ inch, filled with 10% OV–210 on Chromosorb WHP 80/100, glass. Column temperature 130°C isotherm. FID detector, temp. 230°C. Injectionport: 210°C. Carrier gas: Nitrogen 0.75 atm. Hydrogen: 30 ml/min. Air 300 ml/min.

Example of retention times:

Pyrocatechol: 2.3 min
Nitrobenzene: 2.9 min
Resorcinol: 3.7 min
Hydroquinone: 4.1 min
Pyrogallol: 5.1 min
Phloroglucinol: 10.7 min
a-Naphtol: 12 min
b-Naphtol: 13 min.
See *Fig. 7.*

Quantitative Estimations

Quantitative determinations of resorcinol and hydroquinone are possible with nitrobenzene as an internal standard.

Standard curve: Silylate 4 mixtures: each containing 2 ml of 0.25% v/v nitrobenzene and 2 ml of a solution in ethylacetate of successively: 0.6—0.9—1.2—1.5 mg Resorcinol and the same amounts of hydroquinone. Inject 2 µl for glc.

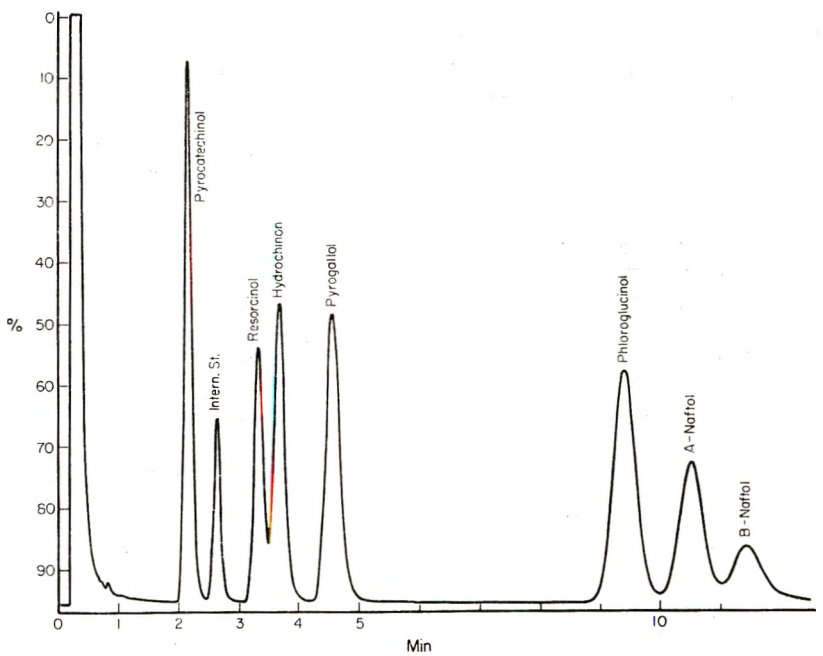


Figure 7. Glc separation of a silylated reference mixture of several polyphenols used in oxydative hair colourants.

Determination: The amount of Resorcinol (Res) or Hydroquinone (HCH) in the sample should be 0.4–1.6 mg. Prepare the purified extract as described under 'Experimental'. Dissolve the residue in 2 ml ethylacetate. Add 2 ml of the solution of the internal standard and silylate. Use 1–2 µl for glc injection.

Calculation: Find the peak height ratio RES/Nitrobenzene or HCH/Nitrobenzene. Find the corresponding mg RES or HCH in the standard curve. As the weight of the sample is known, the % can be calculated.

Recoveries of 94% of the RES in experimental samples was achieved.

Difficulties with chlororesorcinol and hydroquinone. In one sample a peak appeared on the hydroquinone place, after the resorcinol peak. The separation, however, was better than usual. The recorder reached the baseline, which was not the case in the standard chromatograms. The column temperature was lowered from 130°C to 100°C, a mixture of silylated hydroquinone and the extract was injected. Three peaks were clearly separated: resorcinol-hydroquinone and the quasi hydroquinone peak. Further trials with reference compounds, and tlc information led to the identification of 2.Chlororesorcinol. See *Fig. 8.*

Analytical results—oxidative hair colour intermediates

The results of the identification of our samples are tabulated below. Surprisingly p.phenylenediamine was absent. The most important components are still 2.5.toluylenediamine, the three isomeric aminophenols, resorcinol, α-naphthol and 2 nitro.p.phenylenediamine.

Brand A (12 samples):
- 11 with 25TDA
- 12 with 3AF
- 12 with RES
- 2 with 2NPFD
- 1 with 4NOFD

Brand B (9 samples):
- 7 with 25TDA
- 4 with 3AF
- 5 with RES
- 4 with 2NPFD

Brand C (5 samples):
- 4 with 25TDA
- 3 with 3AF
- 3 with RES
- 1 with 2NPFD

Brand D (7 samples):
- 5 with 25TDA
- 2 with 3AF
- 3 with RES
- 3 with 2NPFD

Brand E (8 samples):
- 5 with 25TDA
- 2 with 2AF
- 3 with 4AF
- 3 with 2NPFD
- 8 with RES

Brand F (11 samples):
- 10 with 25TDA
- 1 with 2AF
- 3 with 3AF
- 4 with 4AF
- 5 with RES
- 1 with AN

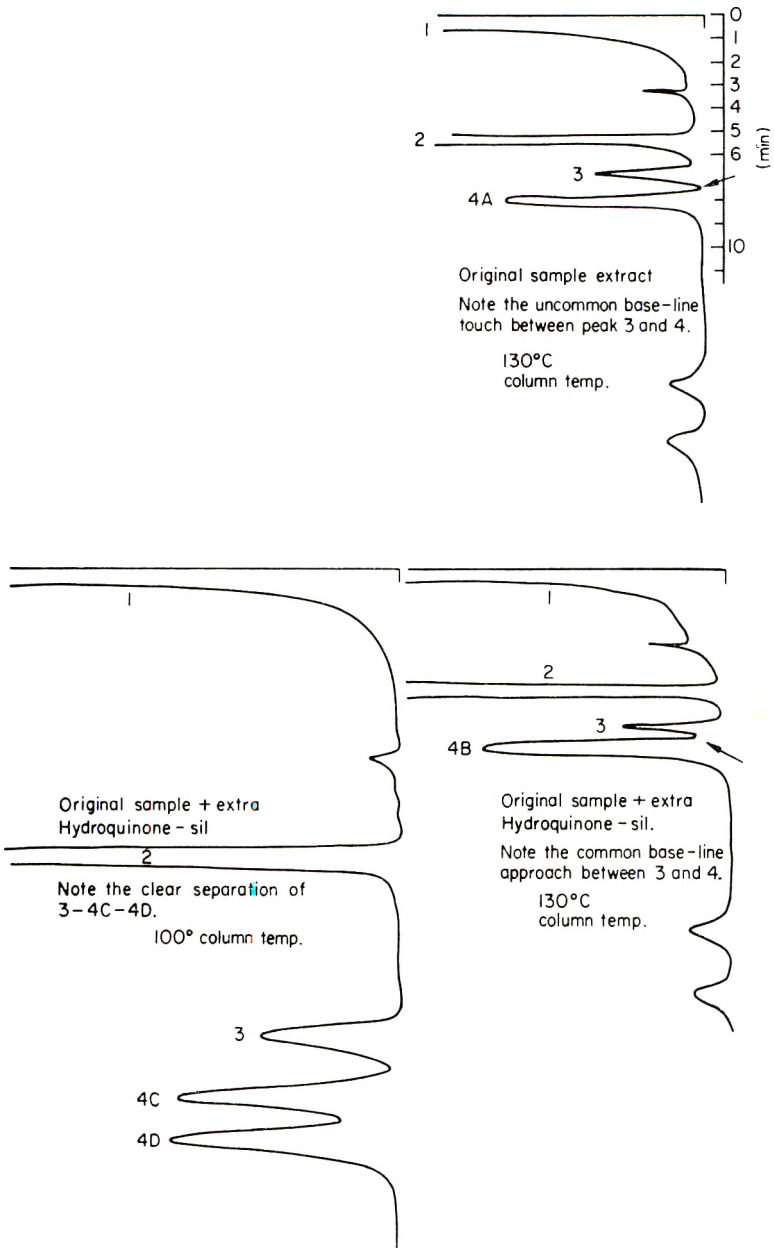


Figure 8. The problem of the quasi-hydroquinone peak of sample no. 25. 1. Solvent peak (ethyl acetate). 2. Nitrobenzene (internal standard). 3. Resorcinol-sil. 4A. Quasi-hydroquinone-sil. 4B. As 4A + superposed hydroquinone-sil. 4C. Hydroquinone-sil. 4D. The quasi-hydroquinone-sil peak, identified as chlororesorcinol-sil.

Brand G (6 samples):
- 6 with 25TDA
- 1 with 2AF
- 5 with 3AF
- 4 with 4AF
- 1 with 2NPFD
- 1 with AN
- 2 with 24DAA

Brand H (12 samples):
- 6 with 25TDA
- 6 with 2NPFD
- 6 with RES
- 1 with HCH
- 2 with AN
- 1 with CRES

Brand I (9 samples):
- 7 with 25TDA
- 7 with 3AF
- 1 with 2NPFD
- 7 with RES

Brand J (2 samples):
- 2 with 25TDA
- 2 with 3AF
- 2 with HCH
- 1 with AN

Unidentified spots: 8

Conclusions: No p.phenylenediamine was found.
2.5.Toluylenediamine and Resorcinol were the most important compounds.
The 3 isomeric aminophenols are frequently used.
2.nitro.p.phenylenediamine is much used, 4.nitro.o.phenylenediamine less.
Of minor importance are: α-naphthol, hydroquinone, chlororesorcinol, 2.4.diaminoanisol.

Abbreviations:
- 25TDA = 2.5.Toluylenediamine
- 2AF = 2.Aminophenol
- 3AF = 3.Aminophenol
- 4AF = 4.Aminophenol
- 2NPFD = 2.Nitro.p.Phenylenediamine
- 4NOFD = 4.Nitro.o.Phenylenediamine
- RES = Resorcinol
- CRES = 2.Chlororesorcinol
- HCH = Hydroquinone
- AN = α-Naphthol
- 24DAA = 2.4.Diaminoanisole.

The analysis of suntan preparations

Suntan preparations may contain several classes of potentially hazardous substances to man, such as sunscreens (uv absorbers), browning agents (mainly dihydroxyacetone), and local anaesthesics (for instance ethyl p.aminobenzoate or benzocaine and lidocaine). These cosmetics are applied on large surfaces of the

body, by old and by young people, and intensively during several weeks annually. At that time the skin is exposed to the sun, the air and the water. It is possible that under such rigorous conditions of use the toxicological hazards of the active components will increase.

To get an idea of the diversity in the use of the sunscreens in particular, analytical data of some 66 suntan preparations (24 brands) was collected. The identification was made mainly by tlc-glc matching with industrial sunscreens as references, and combined with chemical group reactions of two important classes of sunscreens. By the kind cooperation of the chemical industry, some 27 sunscreens were collected for reference analysis (*Table VI*). This number, however, is far below the possibilities mentioned in literature (22, 23) (*Table VII*). The list considered is given in *Tables VI* and *VII*.

Table VI. List of reference industrial sunscreens

Firmenich	Solprotex 1, 2, 3	Salicylates.
Givaudan	Parsol mcx	2.ethylhexyl p.methoxycinnamate.
	Parsol ultra	Mixture, not known.
	Givtan F	2.ethoxyethyl p.methoxycinnamate.
Laserson/Sabety	Ecranosol	Salicylates.
	Solecran	Unknown.
Felton	Sunarome	2.ethylhexylsalicylate.
GAF	Uvinul ms40	2.hydroxy.4.methoxy.5.sulphonic acid. benzophenone.
	Uvinul n539	2.ethylhexyl.2¹.cyano.3.3¹.diphenylacrylate.
	Uvinul 400	2,4¹.dihydroxybenzophenone.
Norda/Schimmel	Angstrol	Salicylates.
	Filtrosol A, B, Triple	Salicylates.
Naarden	Solisoline A, B	Unknown.
Merck	Eusolex 3573	4.phenyl.2.carbonicacid isoctyl ester.benzophenone.
	Eusolex 4360	Unknown.
	Eusolex 161	3.4.dimethoxyphenyl-glyoxylate Na.
	Eusolex 232	2.phenylbenzimidazoline.
Rhone-Poulenc	Rhoditan L	Salicylates.
Dragoco	Prosolal S9	Mixture of phenylacrylic and oxybenzoic esters.
Merck	Eusolex 6653	Dibenzalazin.

Thin-layer chromatography of sunscreens

In the tlc system used, several dark spots from a single extract usually appeared under uv light. To detect a sunscreen spot the following procedure was used. The spot is scraped off, extracted with 3 ml of methanol, filtered, and the uv

Table VII. Inventory of sunscreens mentioned in the literature (22, 23)

A. Para amino benzoic acid and esters: ethyl, butyl, propyl, glyceryl.
B. N.N.Dimethyl para amino benzoic acid ester: isoamyl.
C. Ortho aminobenzoic esters (anthranilic acid esters): methyl, menthyl, phenyl, benzyl, linalyl, cyclohexenyl, bornyl, isobornyl.
D. Salicylic acid esters: menthyl, homomenthyl, phenyl, benzyl, glyceryl, dipropylene glycol, 2.ethylhexyl.
E. Cinnamic acid esters and derivatives: 2.ethylhexyl p.methoxy cinnamate, benzyl cinnamate, 2.ethoxy ethyl p.methoxy cinnamate, menthyl cinnamate, benzyl p.methoxycinnamate, i.butyl salicyl cinnamate, propyl p.methoxycinnamate, butyl cinnamoyl pyruvate, α-phenyl cinnamonitrile, 2.ethylhexyl 2^1.cyano 3.3^1.diphenylacrylate.
F. Dihydroxycinnamic derivatives: umbelliferone, methylumbelliferone, methyl aceto-umbelliferone.
G. Trihydroxycinnamic acid derivatives: esculetin, β.methyl esculetin, dafnetin, glycosides of esculin and dafnin.
H. Benzophenone derivatives: 2.4^1.dihydroxybenzophenone, 4.phenylbenzophenone, carbonic acid isooctylester.
I. Quinine oleate, tannate, stearate, bisulphate.
J. Coumarin derivatives: 7.hydroxy-, 7.methyl-, 3.phenyl.
K. Digalloyltrioleate.
L. Dibenzalazine.
M. Dibenzalacetophenone, benzalacetone.
N. Benzimidazolen, phenylbenzimidazolon sulphonic acid Na.
O. 2.Phenyl benzoxazole, methylnaphthoxazole.
P. 2.Phenyl benzothiazole.
Q. Stilbene.
R. 2.Acetyl bromo indazole.
S. 8.Hydroxy quinoline, 2.phenyl quinoline.
T. Butyl carbityl 6^1.propyl piperonyl ether.

absorption spectrum determined between 250 and 320 nm. At such a low concentration level (approx. 30 μg per 3 ml = 10 ppm) a sunscreen still has an absorption maximum at near 300 nm. Other dark spots of the chromatogram, such as from preservatives, will not have an appreciable absorption at 300 nm (see *Fig. 9*).

Experimental

Extraction: Dissolve 1 g of the sample in 4 ml methanol. Heat gently. Add 1 g Na_2SO_4 exsicc. Mix thoroughly. Use upper methanol layer for tlc or glc.

Reference solution: Use 1% w/v solutions of reference sunscreens in methanol. If the sunscreen is not soluble in methanol, dissolve by adding small amounts of water to the mixture until a clear solution is obtained.

Tlc-plates: Glass plates covered with a 0.25 mm layer of silica GF (with fluorescent indicator), and activated at 105°C for ½ h.

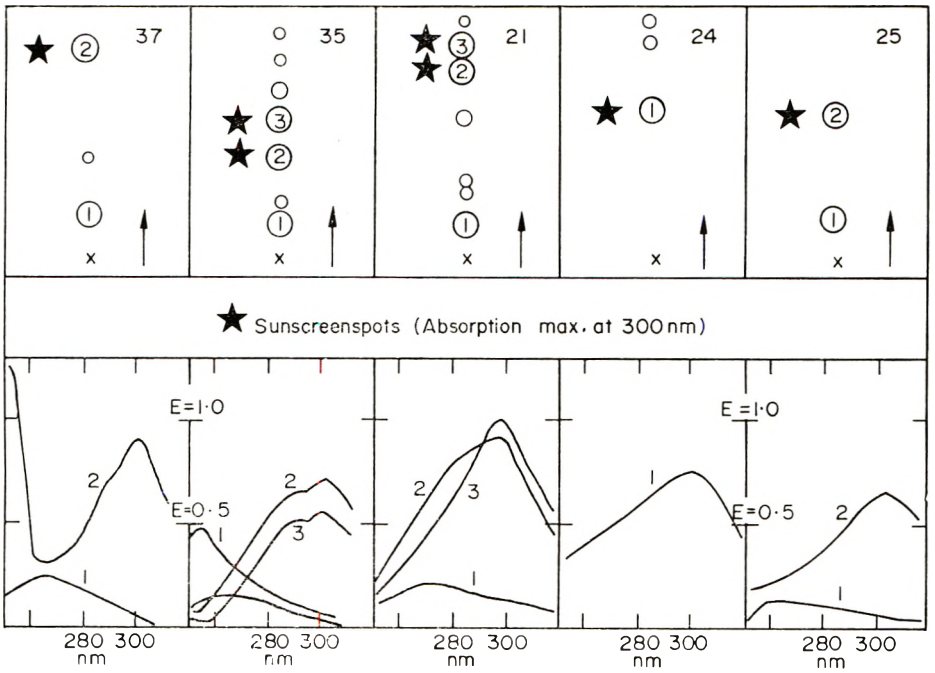


Figure 9. Detection of a sunscreen spot on a tlc plate. Scrape off the spot from the glass plate. Extract with 3 ml of methanol. Filter. Determine the uv spectrum of the solution in a 1 ml quartz cell, between 250 and 320 nm. If at this low concentration level (approx. 10 ppm) an appreciable absorption occurs at 300 nm, the tlc spot is a sunscreen. Examples: tlc of the samples no. 37, 35, 21, 24, 25; uv spectra of the spots.

Solvent system: Diisopropylether-n.Hexane-Acetic acid (20+80+1 vol). Unsaturated tank.

Spots: For the extracts or reference solutions 5–10 µl.

Visualization: Uv 254 nm.

Gas-liquid chromatography of sunscreens

Gaschromatographic conditions. Column: 6% Apiezon L and 10% KOH on Chromosorb W 60–80. 150 cm diam. 0.25 inch, copper.

Temperature column: isotherm 230°C. Injection port: 240°C. Detector: 250°C (flame ionization).

Carrier gas: Nitrogen.

Extraction: as under tlc. Reference solution: as under tlc. Injection: ½–1 µl.

The same column is used as for the aromatic diamines. (See hair colourants.) Benzocaine gives a good response. Not all of the reference sunscreens gave a response, but that was unimportant, as glc matching was the main purpose.

Chemical analysis of sunscreens

Group reactions for p.aminobenzoic acid and esters, and salicylate esters were carried out.

P.aminobenzoic acid/esters. Ehrlich reagent (1% dimethylaminobenzaldehyde in 10% HCl) gives a strong Yellow/Orange colour. Application directly on the sample or as a spray on a tlc plate.

N.B. Many other compounds which are non-uv absorbers with aromatic amine groups show a positive Ehrlich reaction, e.g. sulphonamides.

The possibilities for sunscreens are: p.Aminobenzoic acid, ethyl p.Aminobenzoate (= benzocaine), glyceryl p.aminobenzoate. N.N.dimethyl p.aminobenzoic acid esters do not give a reaction.

Salicylic acid esters. After saponification, the free salicylic acid will give a violet colour with 1% $FeCl_3$.

Experimental. Boil 0.5 g sample with 5 ml 8% ethanolic KOH under reflux from 30 min. Cool, dilute with 40 ml water. Neutralize to pH 5–7. Add 1 drop 1% $FeCl_3$. A violet colour appears, which will persist after the addition of an equal volume of ethanol (phenol gives a violet colour that fades with ethanol).

General problems

By means of a simple tlc/glc matching many of the sunscreens could be identified. An example is given in Fig. 10. Not all the sunscreens could be identified, mainly because the range of reference compounds was not sufficient.

Benzocaine in sunscreens

In several samples of two brands, the analytical data indicate the presence of benzocaine. This substance has sunscreen properties, but acts as a local anaesthetic as well. The presence of benzocaine was confirmed by its ir spectrum and its glc response, after isolation by column chromatographic means.

Identification of benzocaine (ethyl p.aminobenzoate). Two samples (1 and 48) gave a strong yellow/orange coloration with Ehrlich reagent. Such a positive reaction, however, might also be given, not only by benzocaine, but also by the free p.aminobenzoic acid or its glyceryl ester, both of which are known sunscreens.

Tlc separation of these three substances was achieved with the following solvent system:

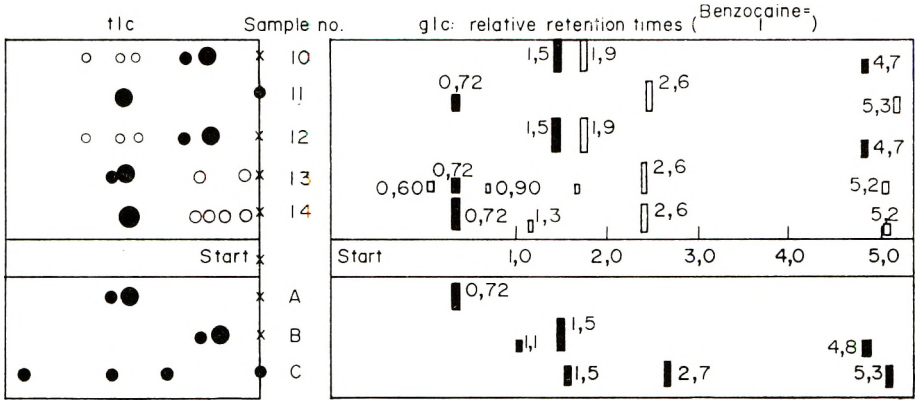


Figure 10. Example of tlc/glc matching for the identification of sunscreens. Sample no. 10 contains B (Givtan F). Sample no. 11 contains A (Eusolex 3573) and possibly C (Solprotex 1). Sample no. 12 contains B (Givtan F). Sample no. 13 and 14 contain A (Eusolex 3573) and possibly C (Solprotex 1).

Diisopropylether-n.Hexane-Acetic acid 75 : 35 : 1, by volume. Visualization was with Ehrlich reagent.

		Sample no.	
	Rf	1	48
Benzocaine	0.55	+	+
p.Aminobenzoic acid	0.45	–	+
Glycerol ester of pAB	0	–	+

Saponification of both samples, followed by extraction and tlc, left only one spot of the free acid.

Procedure: Boil ½ g of the sample with 5 ml 8% ethanolic KOH during 30 min. Cool and dilute with 20 ml water. Acidify with 4M HCl to a pH of 1–3. Extract the free acid with 2 × 10 ml chloroform. Evaporate the chloroform fraction to 1 ml, and use this for tlc.

Benzocaine was isolated from both samples by means of column chromatography. The isolated fractions of Benzocaine (Fraction I, 4 of sample no. 1 and Fraction I, 3 of sample no. 48) were confirmed by its ir spectra and its response on glc.

Procedure: Use 2 × 20 cm glass columns. Fill with 15 g Silicagel Merck (diam. 0.05–0.20 mm) suspended in n.Hexane. Add a mixture of (1 g sample + 1 g Na_2SO_4 exsicc. + 5 ml n.Hexane) on the column. Elution proceed as described below.

The column fractions are concentrated and controlled by tlc (as above).

Dihydroxyacetone in suntan preparations

The browning agent dihydroxyacetone can be detected on tlc plates of cellulose by the same method as for the separation of sugars. It is interesting to note that with one of the samples this tlc system shows the presence of a mixture of synthetic organic colours, namely a grey, a red and a yellow spot.

Method. Extraction: Dissolve 1 g of the sample in a mixture of 0.5 ml water and 3.5 ml methanol. Mix thoroughly. Use aqueous methanolic layer for tlc.

Reference solution: Dissolve 400 mg dihydroxyacetone in a mixture of 2 ml water and 23 ml methanol. (1 µl = 20 µg dihydroxyacetone.)

Tlc plates: Glass plates with 0.3 mm coating of cellulose MN 300. For the coating of 5 plates 20 × 20 cm: mix 15 g cellulose MN 300 with 90 ml water. Dry at 90°C until thoroughly dry.

Solvent system: Used by Raadsveld and Klomp (27) for tlc of sugars: Water-Ethylacetate-Pyridin (15 : 60 : 25, vol). Unsaturated tank. Time about 45 min for 15 cm path.

Spotting: Extract 10 µl; reference solution 5 µl.

Visualization: Spray with a mixture of: 1.3 ml H_3PO_4 85% + 0.93 ml aniline + 100 ml 70% ethanol. Then heat the plates at 100°C for 15 min.

Dihydroxyacetone	Rf 0.85: Tomato red
Glucose:	0.30: Brown
Rhamnose	0.60: Green.

Results

The analytical results on a range of commercial products are finally tabulated in *Table VIII.* In 20% of the samples more than one sunscreen is used in a product, and which might be present in both phases of the emulsion. From the tlc data the conclusion can be drawn that many of the industrial sunscreens as offered by the chemical industry are not always single compounds, but mixtures of 2, 3 or sometimes even 5 components. The browning agent dihydroxyacetone was found in 8 samples of 4 brands. Benzocain was found in 6 samples of 2 brands, but lidocain has not been detected. Due to an incomplete library of reference compounds several sunscreens remain unidentified.

The analysis of propellants and solvents in hairspray aerosols

Aerosol cosmetics which take an important place in our society contain major proportions of propellants and solvents. These compounds have toxicity hazards if inhaled and could also be fire hazards because of the flammability of several of

Table VIII. Analytical results: suntan preparations. Identification of sunscreens and dihydroxyacetone (Market samples, spring 1971: 66 samples of 24 brands)

	Samples no. (per brand)																							
	1–7	8–9	10–14	15–18	19	20	21–26	27–28	29	30–31	32	33–35	36–38	39–40	41–47	48–49	50	51–52	53–56	57	58	59–60	61	62–66
Dihydroxyacetone	×												×	×				×						
Unidentified	?				?		?	?									?	?						
Solprotex 3																								
Solprotex 2																								
Solprotex 1			×																					
Benzocaine	×															×								
Glyc.pAB																×								
p.AminoBenz. acid																×								
Parsol ultra	×			×			×			×							×					×		
Parsol mcx				×																				
Giv-tan F	×	×	×												×			×						
Ecranosol																								
Solecran																								
Sunarome											×		×	×										
Uvinul ms40																								
Uvinul n539																								
Uvinul 400																								
Angstrol																								
Filtrosol B																								
Filtrosol A											×		×	×										
Filtrosol triple A																								
Solisoline B																								×
Solisoline A																								
Eusolex 3573	×		×									×			×					×				×
Eusolex 161		×													×									
Eusolex 4360						×						×												
Eusolex 6653	×						×								×									
Eusolex 232																					×			
Rhoditan L																								
Prosolal S9																								
Salicylates	×		×						×		×		×	×					×				×	

the compounds. It was of interest to study the aerosol cosmetics on the market in order to estimate the real danger of these products. The composition of some 60 samples of hair spray aerosols was examined in Spring 1973.

Gas chromatographic analysis is the method of choice and excellent papers have been published in this field (28, 29). Several columns have been described which give a satisfactory resolution of the propellants and solvents used. The difficulty of the analysis, however, is in the sample handling prior to the gas chromatographic analysis.

To transfer the pressurized liquid of an aerosol from the container to the injection port of the gas chromatograph, one can use a closed system, such as has been described by Cannizzaro and Lewis (28). The aerosol can is pierced below the liquid level by a special 'can piercing unit'. The pressurized liquid is then led via a metering liquid sampling valve to the injection port of the gas chromatograph.

An alternative way of bringing the pressurized liquid to the gas chromatograph is the use of an intermediate transfer vessel made of glass, without damaging the can, and injecting the pressurized liquid by means of a special syringe. Schubert and Keitel (29) used a small glass serum bottle for this intermediate transfer. The work described used a small all-glass aerosol bottle (10 ml), such as is used for perfume aerosols, but without a dip tube. This system has been described in another paper and only the most important points are noted here, (*Fig.* 11). The outfit is simple and inexpensive. The special gas syringe (0–25 µl) has a small Teflon sliding valve at the end.

The glc system used is the same as that of Cannizzaro and Lewis, with small modifications. The column was only 4 m in length. A precolumn was used to

Table IX. Glc-conditions

Gas chromatograph Carlo Erba, Fractovap 2200. Catharometer-detector: bridgecurrent 150 mA. Temp. 200°C.

Inj. port: 125°C.

Precolumn attached before the main column to prevent fouling: 30 cm in length, 4 × 6 mm diameter, stainless steel, Chromosorb WHP 60/80. To be replaced after 100 injections.

Main column: 400 cm in length, 4 × 6 mm in diameter, filled with 20% Hallcomid M18 on Chromosorb WHP 60/80. Column temp. 65°C isotherm.

Carrier gas: Helium 50 ml/min.

Time for one injection: 30 min.

Example of retention times (min):

Propane	1.9	Acetone	8.1
Propellant 12	2.0	Methanol	12.5
isoButane	2.2	Dichloromethane	14.6
Dimethylether	2.2	Cyclohexane (i. stand.)	18.4
Propellant 11	5.1	Ethanol	20.4
norm. Butane	2.8	Methylchloroform	23.0
		isoPropanol	25.5

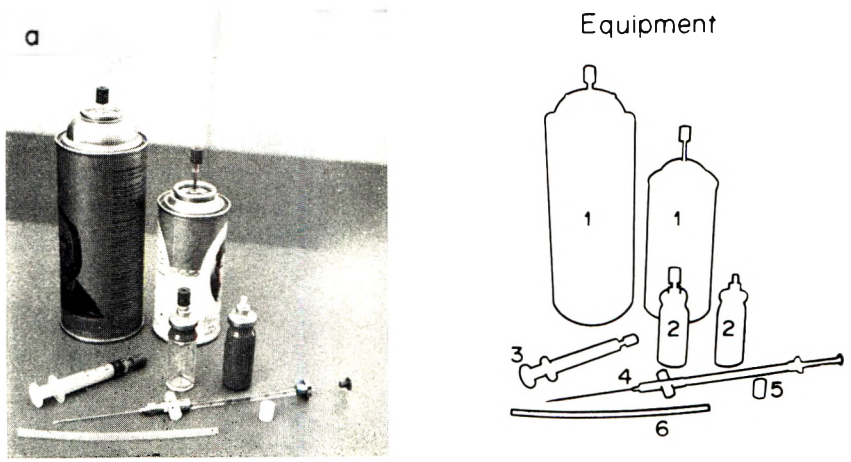
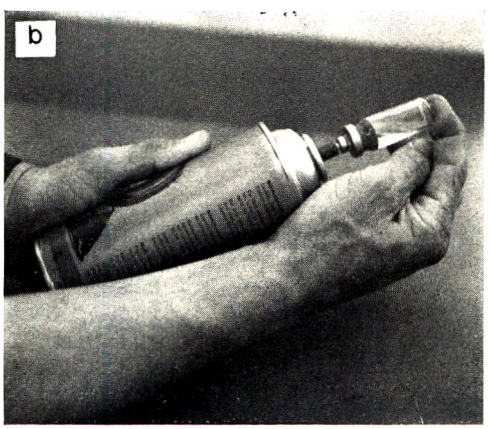


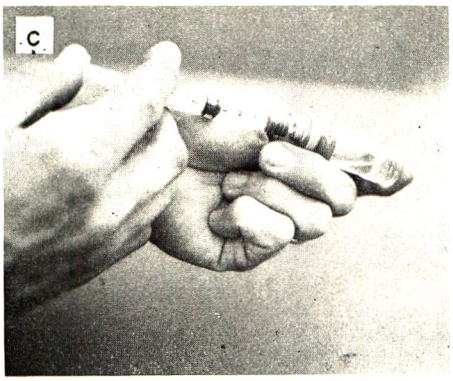
Figure 11. Sampling equipment and procedure prior to glc analysis.

(*a*) 1. Aerosol samples. Sprayhead removed. Transfer pieces (polyethylene) on.
2. All-glass aerosol bottle 10 ml. Vessel for intermediate transfer. With ordinary male valve, without dip tube.
3. Plastic syringe (2 ml). Disposable hospital type.
4. Pressure-Lok A2 Gas Syringe. 25 µl. ['Precision Sampling', Baton Rouge, Louis., USA.]
5. Polyethylene transfer piece.
6. Polyethylene tubing.

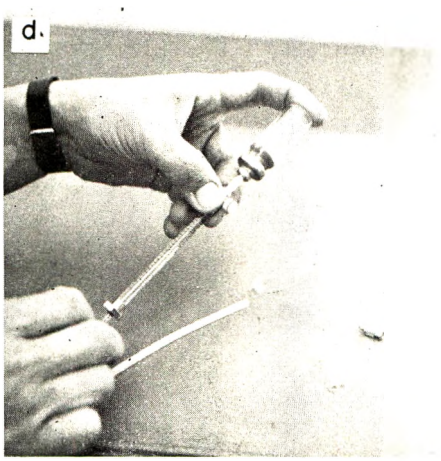


(*b*) Weigh, cool and evacuate the small glass aerosol bottle (2). Remove the spray nozzle of the aerosol sample (1). Introduce by means of plastic transfer pieces, about 8 ml of the pressurized liquid into the small aerosol bottle. Weigh accurately and find the weight of the transferred sample (= m gram).

(*Facing p.* 200)



(*c*) Introduce about 1 ml cyclohexane (internal standard) into the small aerosol bottle by means of a plastic disposable syringe (without needle), and plastic transfer pieces (3 and 5). Weigh accurately and find the weight of the internal standard. No phase separation should occur in the liquid mixture. If so, too much cyclohexane has been added. Sampling should be repeated with less cyclohexane.



(*d*) The Pressure-Lok A2 syringe can now be filled with the pressurized liquid mixture in the small glass aerosol bottle. Disconnect the needle of the syringe. Push with the thumb the syringe valve to position 'open'. Slowly move up and downwards the piston several times, taking care that it reaches the syringe bottom completely each time. This is an extremely important operation, which should be carefully done, in order *to take a representative* sample of the mixture. No gas space should be present in the syringe. Close the syringe valve with the thumb at approx. 20 µl. Control for leaks by waiting a minute and holding the syringe piston in a fixed position. No 'gas' space should appear in the syringe. Injection into the gas chromatograph can now be done. Put the needle on the syringe, inject into the septum. Push the valve on 'open' and immediately push down the piston.

(*Facing p.* 201)

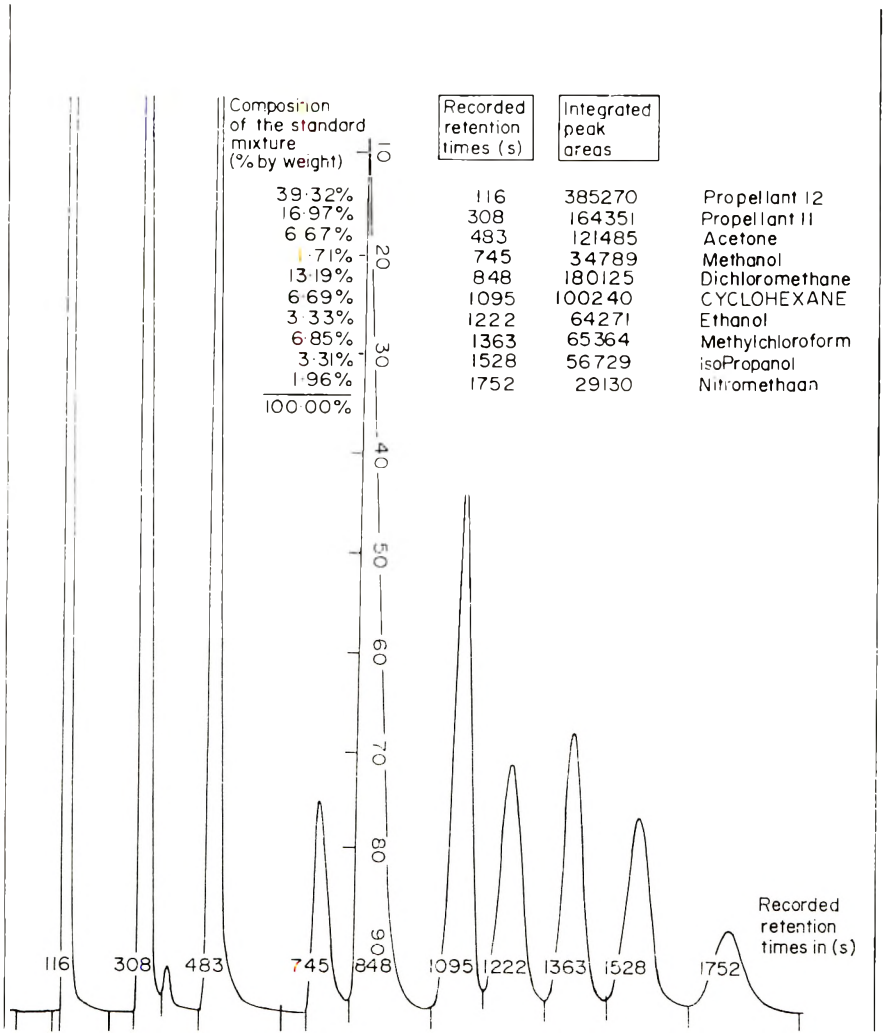


Figure 12. Example of the determination of correction factors F. 20 µl of a standard mixture of known composition are injected. The correction factors F are calculated by the formula: $F_1 = \frac{x_1}{s} \cdot \frac{A_s}{A_1}$, in which F_1 is the correction factor of compound 1; x_1 is the weight per cent of compound 1 in the mixture; s is the weight per cent of internal standard in the mixture (Cyclohexane); A_1 is the integrated peak area of compound 1; A_s is the integrated peak area of the internal standard cyclohexane. The calculated correction factors are:

Propellant 12 = 1.53	Methanol = 0.74	Methylchloroform = 1.57
Propellant 11 = 1.55	Dichloromethane = 1.10	isoPropanol = 0.87
Acetone = 0.82	Ethanol = 0.78	Nitromethane = 1.01

prevent fouling of the main column. The resolution for the solvents and propellants of hairspray aerosols was satisfactory, except for Propellants 12/114, and for isobutane/dimethylether (*Table IX*).

Before proceeding with the analysis, some important questions—specific to this type of analysis—had to be considered.

How reliable is a glc-injection with the system? To answer this question *one* transfer vessel was filled with a hairspray aerosol sample (+ internal standard) and 10 successive injections of 20 µl were made. From the integrated glc-data the % of the solvents and propellants were calculated. Statistical evaluation gave the following coefficients of variation: P12 (1.4%), P11 (1.3%), DCM (1.5%), EtOH (2.5%). It can be concluded that the sampling and injecting system is quite reliable (*Table X*).

Table X. Reliability of 10 successive injections of the same liquid mixture

One small (10 ml) all-glass aerosol bottle was filled with a hairspray aerosol sample and an internal standard added. The liquid mixture was analysed by 10 successive injections according to the method and the integrated peak areas were used to calculate the % of the volatile components. The calculated % are:

1	27.4% P12	54.8% P11	5.25% Dichloromethane	6.76% Ethanol
2	27.1	54.5	5.16	6.79
3	27.3	55.0	5.22	6.91
4	27.5	55.2	5.45	7·21
5	27.2	55.5	5.25	7.14
6	26.8	54.1	6.34+	6.74
7	26.5	53.5	5.76+	6.69
8	27.3	55.1	5.23	7.10
9	27.8	55.9	5.27	7.02
10	27.3	55.0	5.24	6.92
Mean	27.2	54.9	5.26	2.69
Standard deviation	0.4	0.7	0.08	0.20
Variation coefficient	1.4%	1.3%	1.5%	2.5%

+ Abnormal results.

Experimental

Can an aerosol sample be analysed at any emptying stage, or in other words will the composition of an aerosol change during use? To find the practical answer one container was sampled several times according to the method, while releasing it between the sampling. The results of the analysis of the five small aerosol bottles for the intermediate transfer are given in *Table XI*. It proves that sampling can be done at any emptying stage, except when the can is nearly empty.

Table XI. Composition of a hairspray aerosol during its use

A single hairspray aerosol is sampled five times for analysis, at different stages of emptying. The following data were obtained:

	'Full' = 530 g net weight	Removed 200 g	Removed 300 g	Removed 400 g	'Empty' 30 g left
Propellant 12	29.8%	29.4%	29.3%	28.5%	23.8%
Propellant 11	45.0	45.1	45.1	45.8	46.6
Acetone	0.30	0.29	0.30	0.23	0.28
Dichloromethane	22.7	22.6	22.7	22.5	26.6
Ethanol	4.4	4.4	4.0	4.1	4.7

Is the injected volume of importance? Generally speaking, the introduction of an internal standard makes the volume of the injected liquid of no importance. But in this kind of analysis another important factor must be considered, namely that the 'dead' volume of the needle must be negligible in comparison with the injected volume. Experimentally the minimum volume was 15 µl. In all our experiments the volume is standardized to 20 µl to obtain optimal reliability.

Is one general standard (cyclohexane) acceptable for such a mixture of different kinds of organic compounds (alkanols, alkanones, hydrocarbons, fluorinated and chlorinated hydrocarbons)? The linearity of the response for certain practical concentration levels was checked by analysing three different reference

Table XII. Reliability of the use of cyclohexane as a general internal standard

Three different standard mixtures, containing nine compounds and cyclohexane were analysed by glc. From the integrated peak areas the correction factors F were calculated by the formula $F = x_1/S)\ (A_s/A_1)$.

	% by weight			Correction factors			Concentration levels
	A	B	C	A	B	C	
Propellant 12	39.3	21.4	28.4	1.52	1.53	1.47	20–40%
Propellant 11	17.0	8.9	26.6	1.58	1.55	1.53	10–30%
Acetone	6.7	18.0	13.3	0.86	0·84	0·80	5–20%
Methanol	1.7	4.6	3.2	0.77	0.74	0.72	1–5%
Dichloromethane	13.2	18.0	6.6	1.19	1.12	1.10	5–20%
Cyclohexane	6.7	6.1	6.6	1	1	1	
Ethanol	3.3	4.5	1.9	0.78	0.81	0.79	2–5%
Methylchloroform	6.8	12.4	9.9	1.55	1.57	1.57	5–15%
isoPropanol	3.3	4.6	1.9	0.84	0.87	0.84	1–5%
Nitromethane	2.0	1.5	1.6	1.03	1.00	1.18	1–2%

Conclusion: For these levels of concentration cyclohexane as a general internal standard is acceptable, except for nitromethane.

mixtures. The calculated results are tables in *Table XII* in which it can be concluded that for these levels of concentration cyclohexane is acceptable as a general internal standard in our procedure.

Method of determination of linearity of glc response. The determination of the correction factors F, by simple injection of standard mixtures of which the composition is known exactly was first obtained. Several standard mixtures were made before and stored in 100 ml glass aerosol bottles without dip tubes, so that they can be used over and over again. The analysis of such a standard mixture and the calculation of the correction factors F is given in *Fig. 12.* The correction factors should be determined daily for optimal reliability. They might vary a little, depending on the slightly different glc conditions day by day.

Since every injection on the gas chromatograph takes 30 min for total response, sampling in the intermediate transfer vessels can be done in the meantime, including the addition of the internal standard. Of each sample 20 µl amounts should be injected and the % calculated from the integrated data as shown in *Fig. 13.* By this single injection a total quantitative analysis of the propellants solvents is accomplished within half an hour.

A simple analytical control has been devised for each determination, namely by summation of the found %, including the % of the non-volatile residue which should be determined separately by simple gravimetric analysis. If the sum of the percentages differs more than 5% from the theoretical 100%, the analysis should be repeated, including the sampling procedure. This simple control has been proved valuable during our analytical survey, as fault analysis can be avoided.

Results

The results of the analysis of the 60 hairspray aerosols are pictured in *Fig. 14*, which shows histograms of the levels found in the samples.

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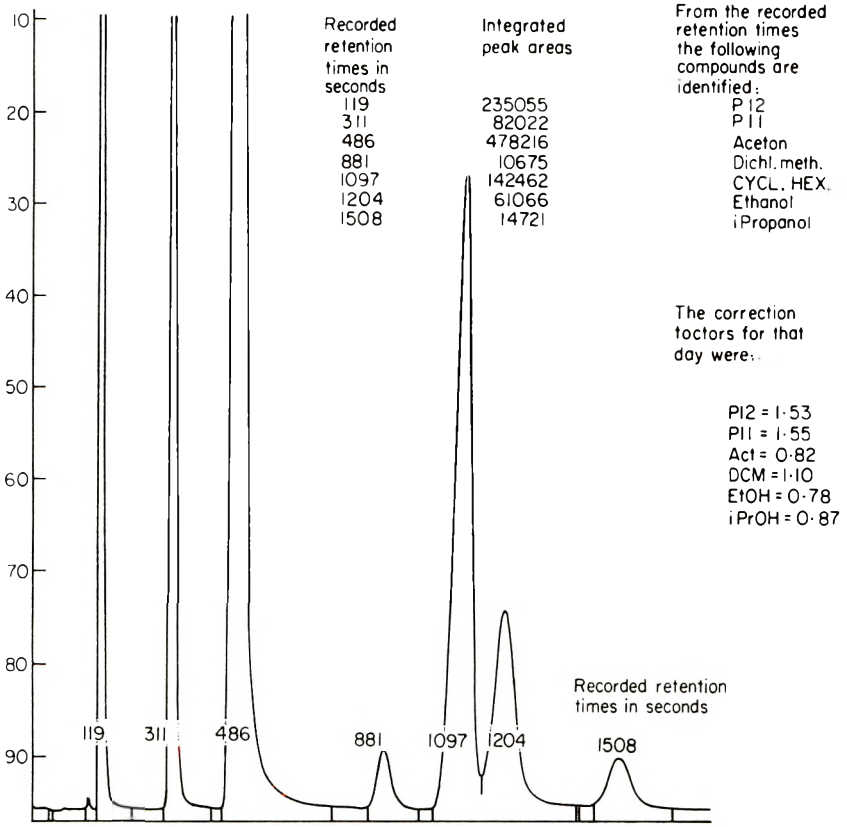
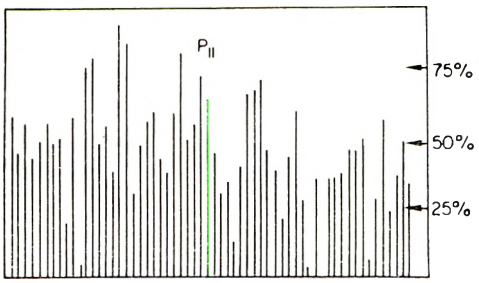


Figure 13. Example of the glc analysis of a hairspray aerosol sample no. 34. The weight per cent were calculated with the formula $\% = (A_1 . F_1/A_s) . (s/m) . 100\%$ in which A_1 = integrated peak area of compound 1; F_1 = correction factor of compound 1; A_s = integrated peak area of Cyclohexane; s = weight in grams of Cyclohexane; m = weight in grams of the sampled mixture.

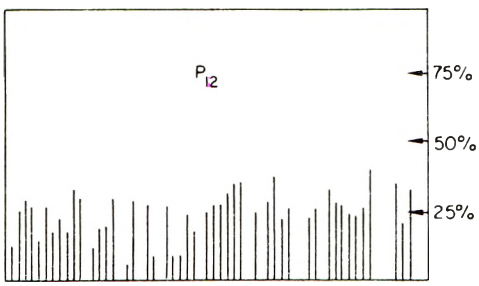
The following data were obtained:

Propellant 12	35.5%
Propellant 11	12.7%
Acetone	39.3%
Dichloromethane	1.2%
Ethanol	4.9%
isoPropanol	1.3%
For the purpose of analytical control, we determined the non-volatile residue by gravimetric analysis and found—Residue	1.9%
Total found	96.8%

This sum of per cent differs less than 5% from the 100%, so the analysis is acceptable.

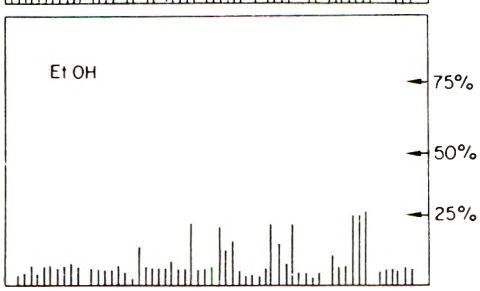


Propellant 11 (Trichlorofluoromethane) has a boiling point of 23·8°C. The histogram profile shows clearly that this compound is the most important propellant/solvent in hairspray aerosols. Average % between 30 and 60%; in 4 samples more than 80%!



Propellant 12 (Dichlorodifluoromethane) is an important propellant in hairspray aerosols. Found in 80% of the samples; average 20–25%.

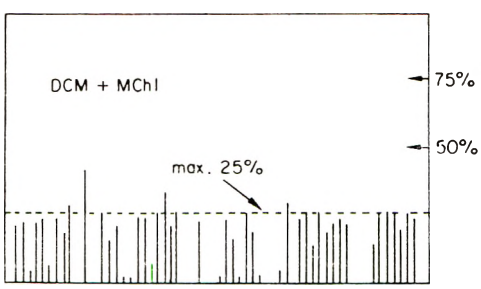
Dimethylether: Two samples contain dimethylether which is only recently introduced as a propellant.



Ethanol is an important solvent. Almost all samples contain ethanol at a level of 10%. Some samples contain 25%, but higher levels do not occur.

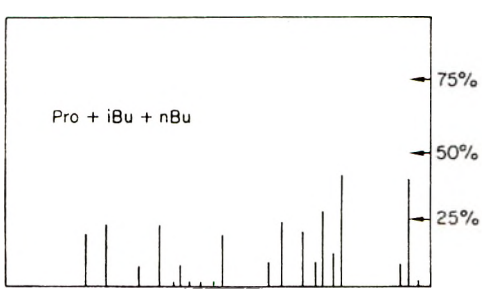
Methanol. Small amounts (less than 0·5%) are found in 45% of the samples, probably as a denaturant of ethanol or isopropanol.

Dichloromethane + methylchloroform (DCM+MChl): These chlorinated solvents are used instead of ethanol to lower the price of the hairspray aerosol. The Dutch Cosmetic Bill states a maximum of 25%. Two samples contain more, namely 42·8% and 34·2%. A quarter of our samples do not contain chlorinated solvents.



Hydrocarbons (Propane+nButane+iButane) are used as propellants in 25% of the samples. In one sample even more than 40%, but the average % is 20%.

Inflammable compounds (Sum of hydrocarbons-dimethylether-aceton-methanol-ethanol-isopropanol). Only four samples exceed the 45% inflammable compound which is accepted by aerosol technologists as an upper limit for the fire safety of aerosols.



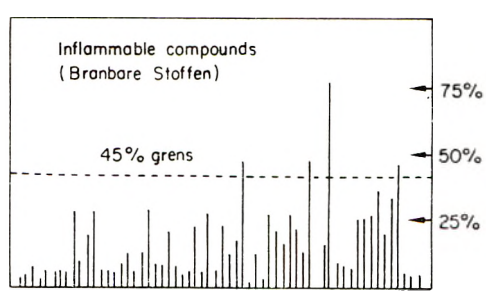


Figure 14. Histograms of the results: total analysis of propellants and solvents of hairspray aerosols (60 samples).

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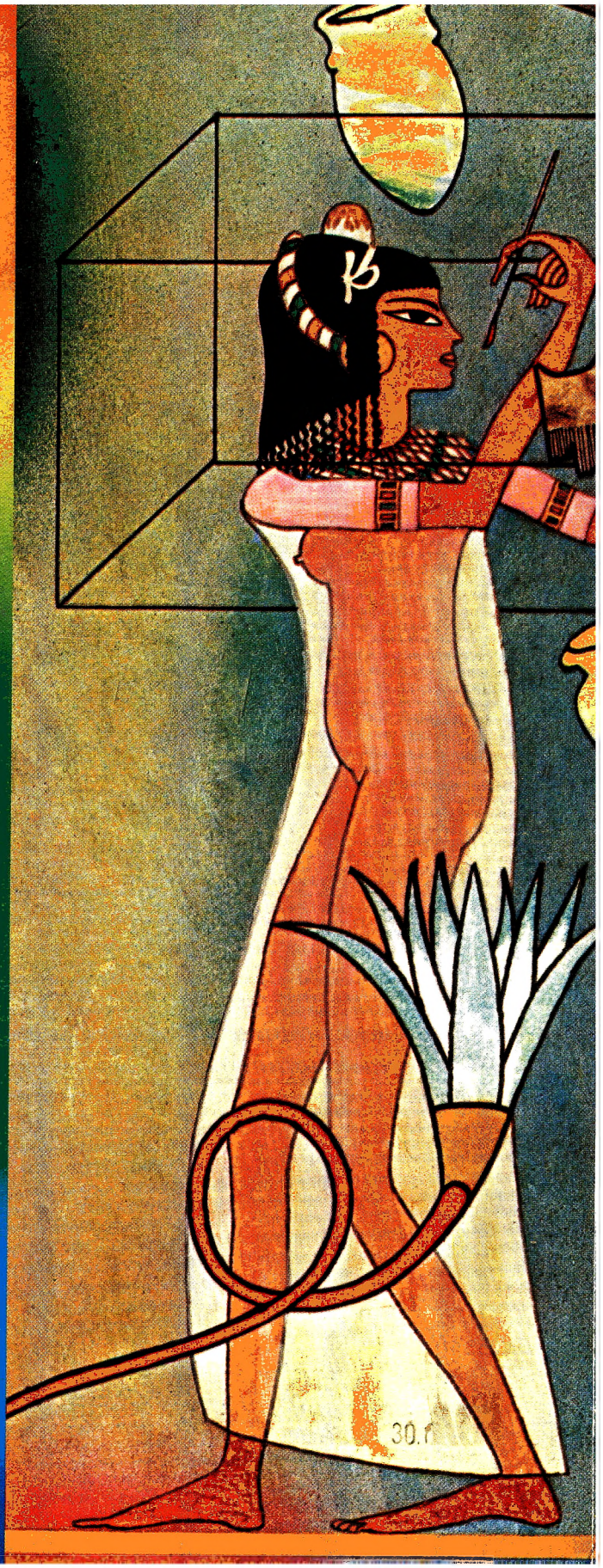

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