Conclusion

In general any water soluble ethoxylated nonionic surfactant will inactivate the commonly used cosmetic preservatives when employed in their usual concentrations if tested on Sabouraud dextrose agar or Jaag medium against the organisms mentioned.

SOME USES OF PAPER CHROMATOGRAPHY FOR THE ANALYSIS OF COSMETICS*

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In Switzerland, cosmetics are subject to control based on the Federal decree of May 26, 1936, regulating trade in foodstuffs and various common substances, with particular reference to Article 467 of the decree. Medicinal cosmetics and those on the market with indications claiming curative properties are controlled by the Inter-cantonal Office for the Control of Medicaments (O.I.C.M.).

Article 467 states that preparations used for the care of the mouth, skin and hair, hair dyes and make-up must not contain metalloidal or toxic metallic (arsenic, antimony, lead, mercury, thallium) substances or harmful organic components.

Paragraph 2 of article 467 mentions the following organic components considered to be noxious: para-phenylene, diamine, formaldehyde, paraformaldehyde, pilocarpine and nitrobenzine, but this list is not exhaustive, for it ends with "etc." The Federal Public Health Service may at any time declare other organic components to be noxious and may forbid their use. Thus the use of thioglycerin in permanent wave liquids has been prohibited since 1956.

As regards coloring matters, cosmetics may in principle be colored only with foodstuff colorings listed in Article 441 of the above decree. Paragraph 6 of Article 467, however, allows the use of harmless coloring matters other than those used in foodstuffs, provided that their chemical composition is communicated to the Federal Public Health Service.

It will be agreed that Swiss legislation on cosmetics is very liberal.

The great variety of substances used for the composition of cosmetics makes their analysis exceedingly difficult. The "Swiss Foodstuffs Manual," 4th edition, 1939, containing official methods for analysis of foodstuffs

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and common substances, gives no method for the isolation and identification of coloring matters in cosmetics. We have therefore carried out research to perfect methods of isolation and identification of coloring matters by paper chromatography; this research has been extended to cover substances other than coloring matters, such as thioglycolic and thiolactic acids, G-11, etc.

We considered it useful to describe to cosmetic chemists some of the techniques used in our laboratory. We certainly do not wish to boast of having overcome all the analytical difficulties which arose, and this is particularly true of the tracing of coloring matters in the presence of quaternary compounds. A large number of problems remain unsolved, and from experience we can state that there is no general method of analysis for cosmetics: each cosmetic, because of its particular composition, constitutes a special case for analysis. For this reason the indications given must be considered merely as examples and not as techniques which can be applied in all cases.

Use of Paper Chromatography

We have used the simplest method, i.e., paper chromatography, by ascending development.

Generally two kinds of paper are used: Whatman No. 1 (thin paper) and Schleicher and Schuell No. 2093b (thick, very porous paper).

In certain cases, such as the separation of polyalcohols, it is an advantage to impregnate these papers uniformly with certain salts. With these salted papers we obtain better separation of substances and rounder spots with better delimitation and clear edges, since the diffusion of the substances under examination is diminished on the paper.

These salted papers are easily made. A solution of 0.1 M of the chosen salt (NaCl, KCl, K_2SO_4) is sprayed onto the suspended sheets of paper, which are then dried in an oven regulated at 80° C. At a distance of 3 cm. from the lower edge of the paper, we put a series of spots with intervals of 3 cm. containing the solution under examination or the reference material. Each spot is 5 cm. in diameter. The sheet of paper is then rolled into a cylinder and clipped so that the edges of the paper do not touch each other.

Vats

We use cylindrical glass vats (height 40 cm., diameter 14 cm.), closed by means of a glass disk, modeling clay being used to ensure watertight closure.

Solvents

We use the following solvents, which have been chosen after a large number of trials:

(*a*) *Coloring Matters*

FORMULA 1

Ethanol 96%, 80 ml.
Ammonia 25%, 8 ml.
Distilled water, 112 ml.

This solvent is unsuitable for certain coloring matters such as foodstuff blues and colorings which are insoluble in water (red and fatty Sudans).

FORMULA 2

2 per cent ammonia solution saturated with methyl-iso- butyl ketone Shell.

This solvent is unsuitable for foodstuff blues and methyl violet B, and is not very suitable for orange I, tropeoline OO and Rhodamine B.

FORMULA 3

Acetone, 50 ml.
Conc. HCl, 5 ml.
Distilled water, 200 ml.

This solvent is unsuitable for the following coloring matters: navy blue, induline, chrysoidine, methyl violet B, geranium ultra fix (Gy).

The acidity of this medium causes the following coloring matters to disappear: eosine, phloxine and naphthol yellow. Once the development is finished it is therefore necessary to dry the sheet and then to plunge it into an atmosphere of ammonia in order to make the above coloring matters reappear.

FORMULA 4

Technical formic acid 85%, 80 ml.
Distilled water, 20 ml.

This medium ensures the separation of the following colorants: *p*-dimethylaminobenzine, Sudan G, Sudan I, fatty orange (Gy), Sudan II (Gy) and Sudan III (Gy).

(*b*) *Active Substances Contained in Sticks, Creams, Soaps and Deodorant Lotions (G-4, G-11, Anobial and Phenol Components)*

FORMULA 5

10 per cent ammonia solution saturated with ether.

(*c*) *Separation of Thioglycolic Acid from Thiolactic Acid in Permanent Wave solutions and Depilatory Creams*

FORMULA 7

Ethanol 96%, 160 ml.

Conc. ammonia, 10 ml.
Distilled water, 30 ml.

As examples we shall describe a few actual cases.

1. Identification of Coloring Matters in a Lipstick: Introduce into a test tube about 0.2 gr. of the lipstick, then 10 ml. of 50 per cent acetic acid, heat to boiling point only, shaking the test tube constantly, allow to cool in order to solidify fatty matter, then filter. The solution is agitated twice in a separatory funnel with 20 ml. petroleum ether (note that certain fatty reds are particularly soluble in the ethereal phase). The acid solution is poured into a porcelain dish, then evaporated to dryness over a water bath. When cold the residue is ground with 5 ml. alcohol at 50 per cent. The solution is centrifuged then decanted. The alcoholic solution is then transferred to the paper for chromatography according to the usual technique.

This method is unsuitable where the lipstick contains the coloring matter cyclamen Gy. We have observed that this coloring matter is destroyed by acetic acid when hot. This is apparent from the gradual fading in the color of the solution during heating. We therefore extract the coloring matter by melting 0.2 gr. of the lipstick in 10 ml. hot water. The aqueous solution is purified as before with petroleum ether, then evaporated to dryness in a porcelain dish. The residue is taken up by 50 per cent alcohol. The alcoholic solution is finally transferred to the chromatography paper. The fatty residue from the aqueous extraction is then treated with 50 per cent acetic acid, as above.

Liquid lipsticks. We have modified the method of extraction of coloring matters for liquid lipsticks, since these preparations contain no fatty matters. The procedure is therefore as follows: Pour one or two drops of the liquid onto 2–3 gr. of kieselguhr in a porcelain dish, stir with a glass rod, then dry over a water bath. Treat the warm colored kieselguhr with 50 per cent acetic acid, filter, evaporate the filtrate to dryness in a porcelain dish, and take up the residue with 50 per cent alcohol. Transfer the alcoholic solution onto the chromatography paper. After treatment with 50 per cent acetic acid, the kieselguhr remains highly colored. It is taken up with alcohol at 96° which dissolves the fatty coloring matter remaining on the kieselguhr; the alcoholic solution is then transferred to the chromatography paper.

In order to identify better the coloring matter extracted from a lipstick, it is necessary to put next to the spot made by the coloring matter the following reference colorings: eosine, phloxine (extra bluish geranium), rhodamine b, metanil yellow, orange II, etc. Our method has been tested by preparing with industrial products offered for this type of cosmetic fatty carriers of very different composition, to which we have added known coloring matters.

2. Identification of G-11 (2,2′-Dioxy-3,5,6,3′,5′,6′-hexachlorodiphenylmethane) in a Stick Deodorant: *Extraction of G-11.* Since the composition of deodorant cosmetics is extremely variable, we have not been able to perfect a general method. The one given here is suitable for sticks composed of a solid solution of a soda soap in ethanol. Modifications will be necessary in the case of creams, lotions and handsoaps.

Extraction technique. Cut up 0.5 gr. of the stick in thin shavings into a small porcelain dish. Dry the shavings in a vacuum drying oven or over a silica gel at 50° C. Grind the dried product with 15 ml. dry acetic ether, using a glass rod. Filter, and evaporate the solvent over a water bath, then redissolve the residue in 5 ml. of 0.1 *N* caustic soda. Shake this alkaline solution with 15 ml. petroleum ether. Separate the aqueous solution by centrifuging. Acidify with a few drops of dilute HCl and extract the G-11 with 10 ml. acetic ether. Dry the solution with a pinch of sodium sulfate, then filter and distill. Redissolve the crystalline residue in $^1/_{10}$ ml. acetic ether. The solution is then transferred to chromatography paper by means of a capillary tube. Utilize solvent 5, taking care to put in the center of the vat a small beaker containing ordinary ether saturated with 10 per cent ammonia.

Duration of chromatography: $3^1/_2$ hours
Height of liquid column: 25–26 cm.

Dry the sheet at 60° C. in an oven, then bring out the spots by spraying onto the sheet an 0.1 per cent acetone solution of 2.6-dibrom-N-chloro-*p*-quinoneimine followed immediately by the spraying of buffer alkaline solution (1 part 1 per cent sol. caustic soda and 2 parts buffer sol.—13 gr. boric acid, 15 gr. potassium chloride, 1.7 gr. caustic soda in 1 liter distilled water).

The spots appear blue on a white ground, except for Anobial (chlorinated salicyl anilide) which is identifiable from its brilliant blue fluorescence in ultraviolet light.

This solvent permits the separation of G-11 from the following substances:

	Rf
G-11	0.59
G-4	0.73
Sustane	0.67
Parachlorometacresol	0.80
Anobial	0.27
p-hydroxybenzoic acid	0.94
Salicylic acid	0.91
Sensitivity 1–5 micrograms G-11	

3. Search for and Separation of Thioglycolic and Thiolactic Acids in Permanent Wave Water: Dilute 1 ml. of the permanent wave solution

with 5 ml. distilled water, verifying that the solution is alkaline, then extract the essences and fatty emulsionants by shaking with 20 ml. ether oxide without peroxide, wash the ethereal layer with 5 ml. distilled water; add to the ether 0.4 ml. ammonia. The ammonium salt of thioglycolic acid is precipitated. It is extracted with 1 ml. distilled water. The aqueous layer is used for the chromatography. Use solvent No. 7.

Duration of chromatography. The duration is about 14 hours, the experiment being stopped when the column of liquid attains a height of 26 cm.

Indicator		Ml.
I	0.1-*N* silver nitrate	50
	10% ammonia	50
II	10% solution of sodium chloride	

Remove the sheet from the apparatus and dry it in a current of warm air from a hair-dryer. Spray indicator I on to the dry sheet. Dry, then spray the sheet with the sodium chloride solution, leave to dry and expose the sheet to daylight in front of a window.

The spots appear dark violet on a mauve ground. Above 50 gamma the center of the spot is yellowish.

Results. The indices Rf obtained for thioglycolic and thiolactic acid are the following:

	Rf	
Thioglycolic acid	0.28	0.02
Thiolactic acid	0.42	0.02

Sensitivity. Five-gamma thioglycolic or thiolactic acid still give a visible spot.