ISSN 0037-9832

VOL 25 No 10 OCTOBER 1974

Journal of the Society of Cosmetic Chemists

Contents

CHECK THIS LIP GLOSS FORMULA

BASE FORMULA

Beeswax	2.5%
Lantrol[1]	3.0
Lanolin, anhydrous	27.0
Lecithin	1.2
Petrolatum, white	2.0
Ozokerite	16.0
Isopropyl palmitate	48.3
	100.0%

PRODUCT FORMULA

Base (above)	80.0%
Chroma-Lite®[2] pearlescent magenta	10.0
Pearl-Glo® pearlescent pigment	10.0
	100.0%

PROCEDURE FOR BASE

1. Weigh each part and combine.
2. Heat to 80° C. Continue heat and paddle gently until dispersed. May be stored in cool room for 90 days.

PROCEDURE FOR PRODUCT

Weigh base and heat to liquid. Weigh pigments. Slowly sift into base with rapid agitation. Pearl concentration may be increased or decreased by varying amount of base. Pour into containers and chill rapidly

Footnotes:

1. Malmstrom Chemical Corporation
2. Mallinckrodt Chemical Works. U.S. Pat. No. 3647492

RESULTS:

Here's an exceptional, versatile lip gloss. It gives luster to lips. But keeps the soft, natural look. It softens the skin. Can be put on with fingertips or applicator. For best filling appearance, should be hot-poured into pots, jars or other packaging.

By using two pearlescent pigments, the formula combines desireable shine with good economics and extraordinary simplicity of manufacture. The Pearl-Glo pearlescent pigment sets the degree of luster. Chroma-Lite pearlescent colors also add luster. You have 13 colors to choose. They're perfectly blendable to produce any shade you wish. Yet because the color and pearl are integrated, the messy job of milling in color is ended. Chroma-Lite pigments may be added by simple agitator mixing. Why not try them!

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England

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COSMETIC CHEMICALS

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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.

Development of a skin cream designed to reduce dry and flaky skin: J. D. MIDDLETON. *Journal of the Society of Cosmetic Chemists* **25** 519–534 (1974)

Synopsis—Dry and flaky skin can result from a low extensibility in the stratum corneum. Because the extensibility of corneum depends upon its water content, humectants are often added to skin products to increase the corneum water content. Measurements of extensibility and water holding capacity in isolated animal corneum showed that conventional humectants such as glycerol, sorbitol or sodium lactate can be effective but that the effect is lost on rinsing the corneum in water. It was found that isolated animal corneum adsorbed lactic acid and that the resulting increase in extensibility was retained after rinsing in water. In consumer tests, hand skin dryness and flaking was assessed by trained observers. Two weeks' use of hand lotions, containing sodium lactate or lactic acid adjusted to pH 4, resulted in less hand skin dryness and flaking than the use of control lotions. A lactic acid lotion also resulted in less dryness and flaking than a sodium lactate lotion.

The thin layer chromatographic detection and determination of an imidazolidinyl urea antimicrobial preservative: D. S. RYDER. *Journal of the Society of Cosmetic Chemists* **25** 535–544 (1974)

Synopsis—An antimicrobial preservative of the imidazolidinyl urea type is detectable in a variety of complex cosmetic and toiletry formulations by TLC. The method is specific for the above type of preservative, even in the presence of a number of other antimicrobials. A ten-fold increase in sensitivity of the ninhydrin reacted zones is achieved when using transmitted uv light as compared to visible daylight colours. The antimicrobial is quantitatively determined in a moisturizing lotion with a relative standard deviation of ±10% using densitometry.

Studies of the factors controlling the action of hair sprays—III: The influence of particle velocity and diameter on the capture of particles by arrays of hair fibres: R. W. RANCE. *Journal of the Society of Cosmetic Chemists* **25** 545–561 (1974)

Synposis—The velocity of an aerosol hair spray has been determined by measuring the gas velocity within the spray with a pitot-static tube. The velocity rises to a maximum at the centre of the spray cone and falls rapidly with increasing distance from the spray orifice. For a given distance from the orifice the velocity also falls with decreasing pressure of the aerosol pack.

Measurements of capture and penetration of hair spray droplets into a model array of hair fibres backed by a solid plate representing the scalp have shown that coarse sprays give better penetration than fine sprays. This is in contrast to the behaviour predicted by classical aerosol capture theory and reasons for the observed behaviour are discussed.

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

ORIGINAL SCIENTIFIC PAPERS

Factors which determine the skin irritation potential of soaps and detergents
Colin Prottey, B.Sc., Ph.D., and Terry Ferguson, L.R.I.C.

Evaluation of antiperspirant preparations under normal conditions of use
M. W. Steed, B.Sc., A.R.I.C.

The action and fate of sodium pyridinethione applied topically to the rabbit
H. C. S. Howlett, B.Sc. and N. J. van Abbé, F.P.S.

Percutaneous absorption of some anionic surfactants
D. Howes, B.Sc.

SUBJECT REVIEW PAPERS

Implications of the enlarged European Economic Community on the quality and safety of cosmetics and toiletries
D. M. Gabriel, B.Sc., F.R.I.C.

A survey of microbiological contamination in cosmetics and toiletries in the U.K. (1971): BASIL JARVIS, ALAN J. REYNOLDS, ANNETTE C. RHODES and MICHAEL ARMSTRONG. *Journal of the Society of Cosmetic Chemists* **25** 563–575 (1974)

Synopsis—One hundred and seventy-two toiletry and cosmetic items purchased in 1971 from retail outlets throughout England and Wales were examined microbiologically. Viable micro-organisms were not recovered from over 50% of the items tested and about 90% contained fewer than 1000 organisms g^{-1}. 75% of all powder preparations tested did not contain viable spores of anaerobic bacteria and none contained more than 300 spores of anaerobic bacteria g^{-1}. Of the anaerobes isolated, none was identified as *Clostridium tetani*. *Coliform bacteria* were not detected in any preparation of toothpaste or lipstick examined. Comparison of count from the top and bottom ends of metal foil tubed products showed almost identical counts in most cases, but in two instances significantly higher counts were observed in the top (nozzle end) sample. Further analyses were performed on six or twelve replicate items of a single brand of seven product types to check the inter-sample variation in count. The results obtained confirmed the overall level of colony count observed previously for these products; in some instances marked inter-sample variation in count was seen.

Journal of the Society of Cosmetic Chemists

This edition is published for

THE SOCIETY OF COSMETIC CHEMISTS
OF GREAT BRITAIN

by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL
Hon. Editor: J. M. Blakeway
Chesebrough Pond's Ltd, Victoria Road, London NW10 6NA

VOL. 25 OCTOBER 1974 No. 10

GENERAL NOTICES

Publication dates: The 'Journal of the Society of Cosmetic Chemists' is published on the 5th of each month.
Five issues for the Society of Cosmetic Chemists of Great Britain
56 Kingsway London WC2B 6DX.
Seven issues by the Society of Cosmetic Chemists
50 East 41 Street, New York, N.Y. 10017, U.S.A.

Issue No	*Publication Date*	*Country of Origin*
1	January	Great Britain
2	February	U.S.A.
3	March	U.S.A.
4	April	Great Britain
5	May	U.S.A.
6	June	Great Britain
7	July	Great Britain
8	August	U.S.A.
9	September	U.S.A.
10	October	Great Britain
11	November	U.S.A.
12	December	U.S.A.

Advertisements: All enquiries regarding advertisements in the British Editions of the Journal should be addressed to Blackwell Scientific Publications, Osney Mead, Oxford OX2 0EL.

Subscription: All members of the Society of Cosmetic Chemists of Great Britain receive one copy of each edition free. Further copies at non-member rates. Industrial and non-member subscribers: £21. Non-profit institutional subscribers: £14 per annum, post free Single issues: £2.10. If payments are made by bank transfer all charges shall be at the remitter's expense.

Missing numbers: Journals are despatched at Printed Paper rate. Claims for missing numbers can be entertained only from subscribers in the country of origin of the particular issue, and must be made within 30 days from date of issue. Members and subscribers are urged to give notice of change of address to the Publications Offices.

Responsibility for statements published: The Society of Cosmetic Chemists of Great Britain and its Hon. Editor assume no responsibility for statements or opinions advanced by contributors to this Journal.

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J. Soc. Cosmet. Chem. **25**, 519–534 (1974)

Development of a skin cream designed to reduce dry and flaky skin

J. D. MIDDLETON*†

Synopsis—Dry and flaky SKIN can result from a low extensibility in the STRATUM CORNEUM. Because the EXTENSIBILITY of corneum depends upon its water content, HUMECTANTS are often added to skin products to increase the corneum water content. Measurements of extensibility and water holding capacity in isolated animal corneum showed that conventional humectants such as glycerol, sorbitol or sodium lactate can be effective but that the effect is lost on rinsing the corneum in water. It was found that isolated animal corneum adsorbed LACTIC ACID and that the resulting increase in extensibility was retained after rinsing in water. In consumer tests, hand skin dryness and flaking was assessed by trained observers. Two weeks' use of HAND LOTIONS, containing SODIUM LACTATE or lactic acid adjusted to pH 4, resulted in less hand skin dryness and flaking than the use of control lotions. A lactic acid lotion also resulted in less dryness and flaking than a sodium lactate lotion.

INTRODUCTION

The function of the surface layer of skin, the stratum corneum, is to provide a protective layer over the surface of the body. This layer prevents excessive evaporative water loss and protects against physical and chemical

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insults in the environment. Because much of the body surface is continuously stretching and flexing the stratum corneum must be flexible and extensible in order to conform without cracking to changes in body posture.

The extensibility of corneum depends upon its water content (1, 2) and upon its temperature (3, 4). When the skin surface is exposed to cold or dry conditions, there must be a gradient of decreasing water content and temperature from the base of the corneum to the surface. This means that the corneum surface tends to be less extensible than the deeper layers so that surface cracks and flaking occur more frequently than deep cracks or chapping.

One objective of skin cream and lotions is, therefore, to maintain a high corneum water content and extensibility so that skin cracking and flaking is less likely to occur.

Several authors (2, 5–7) have shown that the water content of corneum depends upon the presence within the corneum of hygroscopic substances which can hold water in atmospheres of normal humidity. These hygroscopic substances are contained within the corneum cells by the cell walls which are permeable to water but not to electrolytes and they cannot be extracted unless the cell wall is damaged (2). The water held by the hygroscopic materials maintains the extensibility of the corneum (2). The cell wall can be damaged by physical disruption, by extracting its lipids with solvents or by prolonged treatment with detergents which also extract lipids (8). The loss of hygroscopic substances resulting from this damage reduces the water content and extensibility of corneum (2).

The knowledge of the way in which corneum normally holds water and maintains its extensibility enables one to postulate that extensibility of damaged corneum could be increased by adding hygroscopic substances and a number of skin preparations contain humectants such as glycerol and various mixtures simulating the natural hygroscopic substances. However, in intact corneum the natural hygroscopic substances are kept in by the cell walls. When hygroscopic materials are added to solvent- or detergent-damaged corneum they may penetrate into the corneum cells but are unlikely to be retained and will be washed out when the skin is exposed to water. Their effect on extensibility is therefore likely to be only temporary.

The objective of the work reported in this paper was to find a hygroscopic material which could increase the extensibility of solvent-damaged corneum and which would be retained by the corneum so that the increased extensibility would survive immersing the corneum in water. Solvent- rather than detergent-damaged corneum was selected because the damage is

greater and more reproducible. A further objective was to incorporate this material into hand lotion and compare the effect on dry and flaky skin with that of lotions containing no humectant or lotions containing humectants which were not retained by the corneum.

Experimental

Studies on isolated corneum—stratum corneum

Corneum was obtained from the rear footpads of guinea pigs. It was separated by incubating the whole footpad in 0.1 mol tris buffer, pH 7.2, containing 2 mol urea and 0.5% trypsin (BDH) for 18 h at 37°C (2). After this the soft underlying tissues could be scraped away and the resulting corneum was washed for 2 h in distilled water with only one change of water. It was solvent-damaged by immersion in diethyl ether at ambient temperature for 18 h, followed by immersion in distilled water at ambient temperature for 6 h. This procedure removed lipids and hygroscopic substances and reduced the water content and extensibility in humid atmospheres (2). This corneum was then used in experiments designed to increase water content and extensibility towards the levels for intact corneum.

Measurement of water content in humid atmospheres

The technique for measuring water content or water holding capacity has been described previously (2). Briefly, it consisted of equilibrating pieces of corneum at 81% r.h. and ambient temperature over saturated potassium bromide solution (9) to constant weight. The pieces of corneum were then transferred to a dry atmosphere over molecular sieve (type 4A) and re-equilibrated before weighing. Both equilibria take about 6 days to attain. The water holding capacity was expressed as mg water held 100 mg^{-1} dry weight of corneum at 81% r.h.

Measurement of extensibility in humid atmospheres

The technique for measuring extensibility has also been described previously (2). From each piece of footpad corneum a strip measuring 0.6×1.5 cm was cut with a stainless steel punch. Extensibility was measured on an *Instron Tensile Tester*. The strip of corneum was clamped between two pairs of jaws and was stretched at a constant rate of extension. The load

required to do this was measured with a tension cell and the load-extension curve was plotted automatically on a recorder. Extensibility was calculated from the initial part of the curve and expressed as percentage extension 100 g^{-1} load. Measurements were carried out at ambient temperature and in a controlled atmosphere at 81% r.h. This was achieved by enclosing the jaw assembly of the *Instron* in a polythene cabinet and recirculating air which was pre-equilibrated at 81% r.h. by passing it through columns of saturated potassium bromide solution.

After measuring extensibility, the water holding capacity was measured with the same pieces of corneum. The two pieces of corneum from the same animal were kept together. One was subjected to the treatment under investigation and the other served as a control. For statistical analysis the corneum from a number of animals was used. Each animal was taken as a statistical block and significant differences between treatment and control were assessed by analysis of variance.

Adsorption studies

The adsorption of materials by corneum was determined by measuring the decrease in concentration of an aqueous solution of the material in contact with corneum.

Weighed pieces of dry corneum were immersed in solutions of the material under investigation using a solid-solution ratio of approximately 10 mg in 1 cm^3 of solution. At the end of the experimental period the mixtures were centrifuged and the supernatants analysed for the material under investigation. Sorbitol and glycerol were analysed by a periodate titration method (10) and aromatic carboxylic acids by measuring their ultra-violet absorption at the wavelength of their absorption maxima using an *Optica CF 4R spectrophotometer*. Lactate was determined by the method of Barker and Summerson (11) or by using sodium DL lactate-$2C^{14}$ (Radiochemical Centre, Amersham) and determining the concentration by comparing with standards in a *Packard Liquid Scintillation Spectrophotometer model 3224* with 1 cm^3 of lactate solution added to 10 cm^3 of Bruno-Christain scintillator (12).

Control aliquots which had not been in contact with corneum were analysed using the same solution in each case. The results were expressed as mg of material adsorbed mg^{-1} dry weight of corneum under the conditions of the experiment.

Consumer tests with lotions

Three consumer tests were carried out, each comparing the effects of three lotions on housewives' hands. In each test, all three had the same oil phase and two of the lotions contained humectant in the aqueous phase and the third did not and served as a control.

At least 100 women took part in each test. Each woman used each of the three lotions for a period of 2 weeks. There were equal numbers of women using the lotions in each of the six possible sequences. The amount of hand dryness and flaking was assessed at the start of the test and after two weeks' use of each lotion and the effects of the lotion were compared.

In order to assess the effect of a lotion on skin dryness and flaking there must be some initial dryness and flaking. The hands of about double the number of women required were examined and those with the most hand skin dryness and flaking were selected. The tests were all carried out in winter to obtain the maximum amount of skin dryness.

The method of assessing hand skin dryness and flaking has been reported previously by Gibson (13). It consists of a trained assessor scoring six areas of each hand according to the following scheme:—0 = no relevant visible damage; 1 = slight dryness; 2 = marked dryness; 3 = slight flaking; 4 = marked flaking/slight cracking; 6 = severe cracking. The areas of the hand assessed are: back of hand, thumb web, other webs, back of fingers, palm, and front of fingers. There is no clear distinction between any of the grades of dryness and flaking, but with practice, assessors can become consistent in their scoring. The 12 areas on each panellist are summed to give total hand score.

To obtain a proper statistical balance for the experiment, the women who were to take part in the test were arranged in decreasing order of hand score. They were then allocated to one of the six possible sequences of hand lotion usage by taking the first six on the list and allocating them at random to one of the six sequences. This process was repeated down the list. In this way the mean and range of hand scores in each sequence was approximately the same.

In each test the same person assessed the hands and did not know which lotion the panellists had been using. Also the panellists themselves received coded products and did not know which lotions contained the humectants.

Using the six possible sequences of hand lotion usage allows the statistical analysis of the results to take account of any variations in the overall mean hand score with time. Such variations may occur as the result of

changes in the weather. In order to ascertain whether there are significant differences between hand lotions, the statistical analysis must take account of differences between subjects in their mean hand scores and also the differences between the two weekly hand scoring sessions. In an analysis of variance, each of the six sequences of hand lotion usage was analysed as a randomized block experiment with some missing observations, subjects corresponding to blocks and scoring sessions to treatments. The treatment means which are given by this analysis are the scoring session means corrected for differences between subjects. They contain information on differences between lotions as well as between sessions. These results were then analysed as a generalized latin square experiment, sequences corresponding to rows, sessions to columns and lotions to treatments. The treatment means which are given by this analysis are the overall mean hand scores for each lotion corrected for differences between subjects and for differences between scoring sessions.

Results

Effect of normal humectants on isolated corneum

In the first series of experiments, the effect of a number of humectants on the water holding capacity and extensibility of solvent-damaged guinea pig footpad corneum was investigated. *Table I* shows the effect of immersing the corneum for 30 min in 5% aqueous solutions of glycerol, sorbitol, sodium lactate and the sodium salt of 5-pyrollidone-2-carboxylic acid (NaPCA). Glycerol and sorbitol are commonly used humectants in skin creams. NaPCA (14) and lactate (5) are important constituents of the natural hygroscopic material in intact corneum. In each experiment, the effects of the humectant solution were compared with those of water on a number of replicate pairs of corneum pieces. The table indicates where there were statistically significant differences between the effects of humectant and water, as assessed by analysis of variance. All the humectants increased the extensibility of corneum under these conditions. With the exception of sorbitol, they also significantly increased the water holding capacity.

Table II shows the effect, in a different series of experiments, of immersing the treated corneum in water. Solvent-damaged corneum was immersed in 5% solutions of the humectants for 30 min, followed by immersion in water for 30 min. Control pieces of corneum were immersed in water for two successive periods of 30 min.

Table I. Effect of 5% humectant solutions on water holding capacity and extensibility at 81% r.h. of solvent-damaged guinea pig footpad corneum

Humectant	Water held (mg 100 mg^{-1} dry corneum)	Extensibility (% per 100 g load)
Glycerol	19.9 (9)	1.24 (10)
Water	17.4* (9)	0.60* (10)
Sorbitol	18.6 (8)	0.82 (8)
Water	17.5 (8)	0.53* (8)
Sodium lactate	21.6 (9)	1.71 (20)
Water	17.8* (9)	0.43* (20)
NaPCA	23.3 (10)	2.07 (10)
Water	17.5* (10)	0.54* (10)

Figures in brackets represent number of replicates.
* Significant difference ($P < 0.05$) between humectant and water.
Treatment times 30 min.

Table II. Effect of rinsing on water holding capacity and extensibility at 81% r.h. of solvent-damaged guinea pig footpad corneum with 5% humectant solutions

Treatment	Water held (mg 100 mg^{-1} dry corneum)	Extensibility (% per 100 g load)
Glycerol : Water	Not done	0.76 (20)
Water : Water		0.76 (20)
Sorbitol : Water	16.5 (10)	0.44 (10)
Water : Water	17.1 (10)	0.42 (10)
Sodium lactate : Water	17.2 (18)	0.48 (30)
Water : Water	17.5 (18)	0.41 (30)
NaPCA : Water	15.6 (10)	0.33 (10)
Water : Water	15.5 (10)	0.41 (10)

Figures in brackets are numbers of replicates.
All treatment times were 30 min.

In no case was there a significant difference between the water holding capacity or extensibility of corneum treated with humectant followed by water and corneum treated with water alone. The supposition is that water removes the added humectant and this results in a loss of the increased water holding capacity and extensibility.

Adsorption studies

The results given above indicate that any beneficial effect of most humectants will only be temporary and that the effect will be lost when the skin is immersed in water. A more effective humectant would be one which is adsorbed by the corneum so that it is not easily rinsed out.

The adsorption of glycerol, sorbitol and sodium lactate on to solvent-damaged corneum was studied by determining the reduction in concentration of the humectant in an aqueous solution in contact with corneum. No reduction in the concentration of these three humectants could be detected, indicating that there was no adsorption to the corneum. This observation was consistent with the loss of effect on water holding and extensibility after rinsing corneum treated with the three humectants. In the next series of experiments the adsorption of aromatic carboxylic acids was investigated in the same manner. Carboxylic acids have been shown to adsorb to hair keratin (15) and aromatic compounds were selected as examples of carboxylic acids because of the ease of estimating their concentration in aqueous solution by ultra-violet absorption.

Table III shows the adsorption of three aromatic carboxylic acids after immersing the corneum in an aqueous solution of the acid for 4 h at a concentration which gave a convenient reading on the spectrophotometer.

Table III. Adsorption of aromatic carboxylic acids by solvent-damaged corneum

Acid	Concentration (mmol)	Wavelength (nm)	Adsorption (mg acid mg^{-1} dry corneum)
Phthalic	0.5	278.5	0.0041
Salicylic	0.2	295	0.0089
Mandelic	3.0	256	0.0021

The results showed that there was some adsorption of carboxylic acids. The acids investigated were not hygroscopic. Further experiments were therefore carried out with a hygroscopic acid, lactic acid.

In preliminary experiments using Barker and Summerson's method (11) for determining lactic acid, an average adsorption of 0.056 mg lactic acid mg^{-1} corneum was obtained after immersing corneum in 0.5% lactic acid solution for 2 h. The adsorption was investigated in more detail using C^{14} labelled lactate which allowed a simpler and more accurate analytical determination of lactate and lactic acid.

Table IV shows a comparison in duplicate experiments of the uptake of lactic acid by solvent-damaged and intact corneum after immersing the corneum in 0.01 mol acid (0.09%) for 2 h and for 24 h.

Table IV. Adsorption of lactic acid by solvent-damaged and intact corneum

Time (h)	Adsorption (mg lactic acid mg^{-1} corneum)	
	Solvent-damaged	Intact
2	0.0089, 0.0089	0.0007, 0.0012
24	0.0094, 0.0093	0.0016, 0.0021

The results of the method using C^{14} lactic acid agree with those using the Barker and Summerson method in that there is approximately one fifth of the adsorption at one fifth of the concentration of lactic acid. The results show a much greater adsorption of lactic acid by solvent-damaged corneum than by intact corneum. This is consistent with the cell walls preventing the passage of lactic acid in intact corneum. After 2 h there is little further increase in adsorption by damaged corneum, but this is not true for intact corneum.

The effect of pH on adsorption of lactic acid is shown in *Table V*. The pH of lactic acid solutions was adjusted with sodium hydroxide and final concentration of lactic acid plus sodium lactate was 0.01 M. Solvent-damaged corneum was immersed in the solutions for 2h.

Table V. Effect of pH on adsorption of lactic acid by solvent-damaged corneum

pH	Adsorption (mg lactic acid mg^{-1} corneum)
2.9	0.009
3.0	0.009
3.4	0.0085
3.8	0.006
3.95	0.0045
4.2	0.0034
4.55	0.0020
4.85	0.0036
5.1	Zero
6.2	Zero

Each figure is the mean of three experiments.
Corneum immersed in 0.01M lactic acid/lactate solution for 2 h.

The adsorption of lactic acid decreases as the pH increases. Above pH 5 there is no detectable adsorption. These observations are consistent with the adsorption of undissociated lactic acid. The pH of lactic acid is 3.88 and above pH 5 there will be very little undissociated lactic acid in solution.

Effect of lactic acid solution on water holding capacity and extensibility

The adsorption studies showed that lactic acid was adsorbed by solvent-damaged corneum. The effect of lactic acid on corneum water holding and extensibility was then determined. Pieces of guinea pig footpad corneum were immersed in a 10% W/V solution of lactic acid for 30 min and water holding and extensibility were measured (*Table VI*). In a separate experiment, the effect of a subsequent 30 min immersion in water was investigated. In both experiments the effect of lactic acid was compared with that of water. The results of separate experiments showing the effect of 10% sodium lactate, which is not adsorbed, are included for comparison. *Table VI* shows the results.

Table VI. Effect of 10% lactic acid and sodium lactate solution on water holding capacity and extensibility of solvent-damaged corneum at 81% r.h.

Treatment	Water held (mg 100 mg^{-1} dry corneum)	Extensibility (% per 100 g load)
Lactic acid	34.0 (6)	30.6 (6)
Water	18.9* (6)	0.5* (6)
Lactic acid then water	16.9 (10)	1.1 (10)
Water	15.6 (10)	0.6* (10)
Sodium lactate	47.3 (10)	29.8 (10)
Water	19.1* (10)	0.9* (10)
Sodium lactate then water	18.0 (10)	0.85 (10)
Water	18.0 (10)	0.61 (10)

Figures in parentheses are numbers of replicates.
All treatment times were 30 min.
* Denotes significant difference ($P < 0.05$).

Both lactic acid and sodium lactate caused large increases in water holding and extensibility, provided that the corneum was not rinsed after treatment. After a 30 min rinse the effect of the sodium lactate had been lost, but the corneum treated with lactic acid still had a significantly greater extensibility than corneum treated with water, although much of the effect

had been lost during the rinsing period. No detectable increase in water holding capacity remained after rinsing corneum treated with lactic acid.

These experiments showed that adsorption of lactic acid resulted in the corneum maintaining an increased extensibility after rinsing in water under conditions where the effect of other humectants was lost.

Effect of hand lotions containing lactic acid on corneum water holding and extensibility

The pH of lactic acid itself is too low for incorporation into hand lotions. Hand lotions containing lactic acid were prepared by partially neutralizing lactic acid with sodium hydroxide to give a pH of 4, and incorporating this into the aqueous phase of a lotion to give a product containing 10% by weight of the lactic acid-sodium lactate mixture, calculated as lactic acid. Similar lotions containing 10% sodium lactate, and a control lotion containing water in place of the lactic acid or lactate, were also prepared.

The lotions were rubbed into both sides of pieces of solvent-damaged guinea pig footpad corneum for a total period of 90 s with the fingertips while wearing rubber gloves. Excess lotion was removed by wiping with tissues and the treated corneum was either equilibrated at 81% r.h. or rinsed by immersion in water for 30 min before equilibration. The control pieces of corneum from the same animals were either left untreated or were immersed in water for 30 min as appropriate. *Table VII* shows the effect of the lotions on corneum water holding capacity and extensibility.

Table VII. Effect of hand lotions on water holding and extensibility of solvent-damaged guinea pig footpad corneum at 81% r.h.

Treatment	Water held (mg 100 mg^{-1} corneum)		Extensibility (% per 100 g load)	
	Unrinsed	Rinsed	Unrinsed	Rinsed
Control lotion	17.6 (9)	18.6 (12)	0.68 (10)	0.93 (12)
Untreated	17.7 (9)	18.1 (12)	0.59 (10)	0.87 (12)
Lactic acid lotion	17.4 (16)	18.3 (19)	1.19 (10)	0.98 (11)
Untreated	16.4* (16)	18.3 (19)	0.58* (10)	0.59* (11)
Sodium lactate lotion	19.9 (10)	18.1 (11)	0.81 (10)	0.74 (11)
Untreated	17.8* (10)	17.4 (11)	0.59* (10)	0.75 (11)

Figures in parentheses are numbers of replicates.
Treatment time with lotions was 90 s, rinsing time was 30 min.
* Denotes significant difference ($P < 0.05$).

The results showed that the control lotion had no effect on water holding or extensibility. Both the lactic acid lotion at pH 4 and the sodium lactate lotion increased water holding and extensibility. After rinsing for 30 min, the corneum treated with lactic acid lotion had retained an increased extensibility, and that treated with the sodium lactate lotion had not. As with the lactic acid solution, the increased water holding capacity caused by the lactic acid lotion was lost after rinsing.

These results indicated that a relatively short treatment (90 s) with a lotion at pH 4 containing lactic acid resulted in an increased extensibility and that after a relatively prolonged rinsing in water (30 min) the extensibility was still higher than that of control pieces of corneum.

Effectiveness of lactic acid hand lotions in consumer tests

In the first consumer test, 143 women used each of three hand lotions for 2 weeks. The lotions were a control, a sodium lactate lotion and a lactic acid lotion. The effect of these lotions on corneum water holding and extensibility was shown in *Table VII*. *Table VIII* shows the mean hand scores after using the three lotions.

Table VIII. Consumer test 1. Mean hand scores after using hand lotions

Hand lotion	Hand score
Control	12.3
Sodium lactate	9.6
Lactic acid	9.0
Difference required for significance ($P = 0.05$)	1.2

143 women completed the test.
Lotions contained 10% lactic acid/sodium lactate.

The mean hand score after using the control lotion was significantly higher, i.e. there was more hand skin dryness and flaking than after using the sodium lactate or lactic acid lotion. The lactic acid lotion resulted in a lower mean hand score than the sodium lactate lotion, but the difference between the two was not statistically significant. This test, therefore, showed that the presence in a hand lotion of a humectant, such as sodium lactate, which is not adsorbed by corneum, can result in less skin dryness and flaking. It did not demonstrate that a humectant which is adsorbed results in a better hand condition than one which is not.

Although the first consumer test was carried out in winter, the weather was exceptionally warm and there was relatively little skin dryness and flaking amongst the panellists. It was thought that this may have reduced the sensitivity of the test.

A second test was carried out during the following winter using the same humectants incorporated into a different lotion. *Table IX* shows the mean hand scores after 2 weeks' use of each lotion.

Table IX. Consumer test 2. Mean hand scores after using hand lotions

Hand lotion	Hand score
Control	13.2
Sodium lactate	11.9
Lactic acid	10.7
Difference required for significance ($P = 0.05$)	0.9

156 women completed the test.
Lotion contained 10% lactic acid/sodium lactate.

The weather was colder during the second test and the hand scores were slightly higher. The second test confirmed the result of the first test in showing that a hand lotion containing humectant can result in less hand skin dryness and flaking than a control lotion. In the second test, the lactic acid hand lotion resulted in a lower hand score than the sodium lactate lotion. This indicates that a humectant which is adsorbed by the corneum can prevent skin dryness and flaking to a greater extent than one which is not adsorbed.

In a third test, the effect of lowering the lactic acid content from 10% to 5% was investigated. Lotions containing 10% or 5% of the lactic acid-sodium lactate mixture at pH 4 were compared with a control lotion. In the expectation that colder weather would result in higher hand scores, this test was carried out in Scandinavia in winter. *Table X* shows the mean hand scores after using the lotions for 2 weeks.

Despite the cold weather in Scandinavia, the mean hand scores were much lower than in the previous tests carried out in the U.K. The probable explanation for this is that in Scandinavia, women protect their hands by wearing gloves much more than they do in the U.K. Although there was relatively little skin dryness and flaking, the effectiveness of the lactic acid lotions could still be demonstrated. This indicates that lactic acid lotions are

Table X. Consumer test 3. Mean hand scores after using hand lotions

Hand lotion	Hand score
Control	6.42
5% lactic acid	5.36
10% lactic acid	5.32
Difference required for significance ($P = 0.05$)	0.90

107 women completed the test.

not only effective for women with severe skin dryness, but can also be expected to be of benefit in cases where there is relatively little dryness.

Discussion

The results of the experiments on isolated animal corneum showed that increasing the water holding capacity by the addition of humectants resulted in an increased corneum extensibility. The experiments also indicated that the use of a humectant, lactic acid, which was adsorbed by the corneum allowed the treated corneum to be subjected to a prolonged washing without all the effect being lost. The results of the animal experiments predicted that lotions containing humectants, such as sodium lactate, which are not adsorbed might be less effective than lotions containing humectants such as lactic acid which are adsorbed. The results of the consumer tests showed that these predictions were valid and that animal corneum is a useful model system for studying effects in the human.

The precise mechanism of action of lactic acid in reducing hand skin dryness and flaking is not clear. The results on animal corneum (*Tables VI* and *VII*) showed that corneum treated with lactic acid and then rinsed still retained an increased extensibility but there was no residual increase in water holding capacity. The explanation may be that the quantity of lactic acid adsorbed to the corneum after rinsing is too small to hold sufficient water for the gravimetric method to detect. Alternatively, the adsorbed lactic acid may itself have a direct effect on extensibility. Some separate, unpublished experiments on non-hygroscopic carboxylic acids, such as mandelic acid, indicated that these acids can increase extensibility of animal corneum without increasing water holding capacity, and that the increased extensibility survives rinsing the corneum. This suggests that lactic acid may have a direct effect on a corneum extensibility without influencing the water content.

It is extensibility and not water content which is important for the corneum in its resistance to flaking and cracking. The water content is only important in so far as it affects extensibility. Lactic acid, therefore, appears to have a dual action on the corneum. It increases extensibility by increasing the water holding capacity in the same way as conventional humectants. This effect is lost on rinsing the corneum in water, but is sufficient to result in some effect on skin dryness and flaking as shown in the consumer tests with sodium lactate lotions. The second effect, which is not shown by conventional humectants, is that the adsorbed lactic acid increases extensibility. In treated and unrinsed corneum, there is no evidence of an additive effect between the extra water and the adsorbed lactic acid, and the effect of the adsorbed lactic acid only becomes apparent on rinsing. This means that the effect of lactic acid is likely to be more persistent than that of sodium lactate, and should therefore result in less skin dryness and flaking. This was confirmed in the results of the second consumer test where the comparison of the sodium lactate and lactic acid lotions showed that the lactic acid lotion results in a significantly better hand skin condition.

(Received: 8th March 1974)

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The thin layer chromatographic detection and determination of an imidazolidinyl urea antimicrobial preservative

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Synopsis—An ANTIMICROBIAL PRESERVATIVE of the IMIDAZOLIDINYL UREA type is detectable in a variety of complex cosmetic and toiletry formulations by TLC. The method is specific for the above type of preservative, even in the presence of a number of other antimicrobials. A ten-fold increase in sensitivity of the ninhydrin reacted zones is achieved when using TRANSMITTED UV LIGHT as compared to visible daylight colours. The antimicrobial is quantitatively determined in a moisturizing lotion with a relative standard deviation of $\pm 10\%$ using DENSITOMETRY.

INTRODUCTION

Cosmetics provide an ideal medium for the growth of microorganisms. The manufacturer must guard against the microbial contamination of his product during manufacture and storage and also against contamination by the consumer. Thus antimicrobial preservatives are added which are designed to be effective against a wide spectrum of microorganisms over a long period of time.

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Many non-polar preservatives are in common use and several authors have reported tlc methods for their detection. König (1) separated and identified 16 halogenated aromatics. Karlskind, Valmalle and Wolff (2) quantitatively estimated six halogenated aromatics in soap by means of tlc and spectroscopic methods. Graber, Domsky and Ginn (3) identified zinc omadine and five halogenated aromatics in personal care products. Hexachlorophane, tribromosalicylanilide and trichlorocarbanilide have been detected and quantitatively determined by Schwarze (4) using tlc and ion exchange procedures. In addition, Porcaro and Shubiak (5) developed a uv/liquid chromatographic method for the estimation of hexachlorophane in nanogram quantities using a dianisate ester derivative. Wolf and Senionow (6) detected six halogenated aromatics in soap using High Pressure Liquid Chromatography, although Irgasan CF_3 and trichlorocarbanilide were not separated. Little has been reported of the more difficultly-detected and quantified non-aromatic, polar, hydrophilic antimicrobials, particularly of the imidazolidinyl urea type.

The first commercial member of this family is Germall 115.* This preservative is claimed to be a broad spectrum antimicrobial which is non-toxic, non-irritating and is particularly effective in emulsions and protein-containing formulations. It concentrates in the microbial supporting aqueous phase and its efficiency is not impaired by the presence of non-ionic emulsifiers and proteins.

For quality control purposes and for the identification of antimicrobials in unknown formulations, it was necessary to develop a rapid and specific identification and quantification procedure for imidazolidinyl urea in a wide variety of personal care products. Because of the complexity of the formulations to which this preservative may be added, i.e. lotions, creams, hair conditioners, shampoos, deodorants etc., a comprehensive colorimetric method was impracticable. Thus tlc was used, since no sample preparation was necessary and the method was applicable to new products without change in the procedure.

Experimental

Apparatus

Thin layer plates: 20×20 cm 0.25 mm Silica gel F_{254} (Merck) used as received without further activation.

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Separating chambers: For 20 × 20 cm plates (Desaga).
UV viewer for transmission: Blak-Ray Transilluminator (Shandon Southern Instruments Ltd).
UV viewer for reflectance: Blak-Ray Chromato-Vue (Shandon Southern Instruments Ltd).
Chromoscan densitometer with thin layer attachment: Joyce Loebl and Co. Ltd.

Reagents

Germall 115 (Imidazolidinyl urea).
Flow solvent: chloroform : methanol : acetic acid : water; 50 : 30 : 10 : 10.
Spray reagent: Ninhydrin—0.3 g ninhydrin in 95 ml n-butanol and 5 ml acetic acid.

Procedure

Sample application

5 µl of a 10% solution or suspension of the sample in methanol-water (70 : 30) is applied using a Drummond Microcap pipette. When the samples examined are of a viscous nature, it is necessary to use the rubber bulb in order to fill and dispel the solutions. The size of the applied spot is normally 0.5 cm diameter. Sample and reference solutions are spotted alternately along the plate at a height of 2 cm from the edge of the plate. The spots are well dried after application using a warm-air dryer.

Reference solutions

Solutions are prepared of the product under test without imidazolidinyl urea and the product containing varying known quantities of imidazolidinyl urea from 0.1% to 0.6%.

Chamber

A Desaga chamber for 20 × 20 cm plates is lined with filter paper, saturated in the flow solvent and allowed to equilibrate for 30 min before use.

Development

The chromatogram is allowed to develop for 50 min, in which time the solvent front travels approximately 9 cm.

Visualization

The plate is briefly dried using a warm-air dryer and sprayed with the ninhydrin spray until well wetted. The plate is heated at a temperature of 150°C for 20 min, allowed to cool and then viewed in uv light of 366 nm by transmission. The imidazolidinyl urea preservative is seen as two pale yellow fluorescent zones at Rfs of 0.27 and 0.35.

Densitometric measurements

These are performed on a Chromoscan densitometer with a thin layer attachment. The following operating conditions are used. Chromoscan: light source 12 V, 100 W, tungsten halide lamp, filter 3.0 O.D., optical wedge 0–0.5 O.D., gain 5, cam A.

Thin layer attachment

Light source uv mercury lamp type ST 75, aperture 1 mm × 17 mm, uv filter 300–400 nm between the light source and specimen, Kodak Wratten uv filter No. 2E between the specimen and detector, specimen expansion ratio 1 : 1. The measurements are carried out using the reflectance mode.

Results and discussion

During the optimization of the method, various alternative systems were attempted. Laboratory-prepared plates were compared with precoated plates. The laboratory coated plates were prepared by mixing 52 g of Merck, Silica gel 'G' F_{254} with 110 ml of distilled water in a Waring blender and ten glass plates were then coated using a Camag automatic spreader set at a wet layer thickness of 300 μm. The plates were left at room temperature for 20 min and finally dried for 1 h at 105°C in an air-blown oven. It was found that although satisfactory for samples containing 0.5% imidazolidinyl urea, the laboratory-prepared plates did not display the necessary sensitivity at the lower concentrations of preservative. This may in part be due to the increased initial size of the applied spot on laboratory-prepared plates, which more readily absorb the viscous sample solutions than do the polyvinyl alcohol bound precoated layers. Alumina laboratory-prepared plates were also examined but these failed to produce a satisfactory separation.

On investigating various sample solutions, water alone usually yielded an excessive quantity of bubbles and a solution which was too viscous to pipette conveniently. The urea compound was found to be insufficiently soluble in methanol alone to be certain of recovery from emulsified products,

but as the preservative is up to 50% aqueous soluble, the relatively small proportion of water in the mixture, methanol-water (70 : 30) was found to be satisfactory and was used for all the products examined.

It was shown to be essential to dry the spots well, after application, otherwise distortion and lack of sensitivity resulted. No decomposition of the imidazolidinyl urea zones was detected on heating the applied spots with a warm-air dryer, even when heated to a considerably greater extent than that required for a good chromatogram.

As imidazolidinyl urea was almost insoluble in any solvent other than water, in which it was very soluble, the correct flow solvent required a certain water content but this needed to be modified by a non-polar solvent, chloroform was chosen as a convenient solvent after considering its position in the eluotropic series. These two solvents were rendered homogeneous by the mutually-miscible solvent methanol.

A mixture of chloroform-methanol-water (45 : 45 : 10) was found to give satisfactory Rf values but rather diffuse spots. As a change in pH will often produce a 'sharpening up' of diffuse spots, the following solvents were investigated.

Chloroform-methanol-acetic acid-water (20 : 30 : 40 : 10), although compacting the spots, produced an increase in Rf's and failed to separate the preservative from other components in the formulations. Chloroform-methanol-ammonia (50 : 40 : 10) gave very low Rf's and even after the carefully-attempted removal of the ammonia, a poor background colour reduced the limits of detection considerably. Chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10) having a pH of 2.5 was found to yield compact zones separated from all other visible components. This solvent also gave a good separation of the imidazolidinyl urea components in Germall itself.

A number of spray reagents were investigated, several being general reagents, whilst the remainder were intended to be more specific for the imidazolidinyl urea grouping. Of the twelve reagents tried, few successfully detected less than 25 μg of imidazolidinyl urea (*Table I*). Only Erlich's reagent and ninhydrin are sufficiently sensitive to detect 0.5% imidazolidinyl urea in a product, i.e. 2.5 μg for the loading used. The sensitivity of the ninhydrin spray is increased by a factor of ten when viewed by transmitted uv light of 366 nm. There seems to be little record of compounds which react with ninhydrin being more sensitively detected using uv light rather than colours visible in daylight. It seems likely that the difference in sensitivity of detection found when viewed by reflected and transmitted uv light may be mainly due to the difference in intensity of light on the plate,

Table I. The limits of detection of imidazolidinyl urea type (*Germall* 115) using various reagents

Reagent	Colour of reaction	Limit of detection
Ninhydrin (daylight)	Red	2.5 μg
Ninhydrin (reflected UV)	Yellow	1 μg
Ninhydrin (transmitted UV)	Yellow	0.25 μg
Dragendorff's reagent	No reaction	—
Blue Salt Irga B	No reaction	—
Cobalt thiocyanate	Blue	25 μg
Erlich's reagent	Yellow	2.5 μg
Silver nitrate	No reaction	—
Iodine	No reaction	—
Fluorescein	No reaction	—
Rhodamine 6G	Yellow in UV	10 μg
Prochazka reagent	No reaction	—
Chromic acid	No reaction	—
Pinacryptol yellow	Blue in UV	10 μg

i.e. 450 μW cm^2 at 18 in for reflectance and 1900 μW cm^2 at the surface for transmission.

The extent to which plates were sprayed was found to be critical, thus in order to establish optimum conditions, plates were sprayed until varying states of wetness were obtained.

Plates which were observed to be not wetted, i.e. not darkened, plates which were just wetted and even plates which were fairly well wetted, gave poor results. Well-wetted plates and those which were soaked, i.e. of a shiny appearance were satisfactory. The correct spray was therefore judged to be well wetted until the first shiny appearance was observed, which normally required 20 ml of reagent for a 20 × 20 cm plate, although due to variance in spraying techniques the appearance of the plate rather than the volume used is the better criterion.

Chromatograms heated at 105°C, the normally recommended temperature for ninhydrin failed to give good sensitivity even when heated for up to 90 min. At 150°C little reaction was noted for up to 10 mins heating but the maximum sensitivity was achieved after 15 min and no change was detected after further heating for 30 min. Thus heating at 150°C for 20 min is preferred. The spots once developed are stable for several days if protected from daylight.

As when examined at a higher loading, the preservative revealed six components, i.e. two major and four minor, the possibility of decomposition

due to chromatography was examined. A two-dimensional chromatogram was carried out using the solvent chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10) in both directions. All the components were found to lie in a diagonally straight line, showing that no new components were formed during the chromatographic run. In addition, solutions kept for several days showed no detectable decomposition compared to freshly-prepared solutions. The detection of imidazolidinyl urea when added to several cosmetic and toiletry products was attempted. These products included a moisturizing lotion containing fifteen ingredients, some of which were themselves complex mixtures and known to include an aliphatic amine, a mixture of parabens, two dyes, a lanolin product, an emulsifier, long chain alcohols and esters, poly hydroxy compounds and aloe, polysaccharides, proteins, amino acids and vitamins. An egg shampoo was also tested, as unlike some antimicrobials, the efficiency of imidazolidinyl urea is not impaired by the presence of proteins. The other products tested were, a deodorant foam bath, a hand cream, a squeeze-on deodorant, a roll-on deodorant and an aerosol deodorant (Fig. 1).

Imidazolidinyl urea was successfully detected down to at least 0·1% in all the products tested and only the roll-on deodorant and the aerosol deodorant exhibited any distortion of the preservative zones.

The preservative was easily detected in the presence of other antimicrobials (*Table II*), including parabens with which it is recommended for joint use, as a synergistic effect in efficiency has been observed.

The detection system was specific for the imidazolidinyl urea type of antimicrobial when compared with other antimicrobials and additives, as none of those tested revealed a similar distinctive coloration in uv light, even though a number reacted with ninhydrin to form colours visible in daylight.

Quantitative

Although for routine quality control, a simple, visual comparison of spots with the appropriate standard spots is both rapid and sufficiently accurate, a more precise densitometric determination was also investigated. For all quantitative work, the sample was run with replicate spots alternately spaced with standard solutions, which consisted of the 'blank' product doped with known quantities of imidazolidinyl urea. The chromatographic conditions were as for the previous qualitative work.

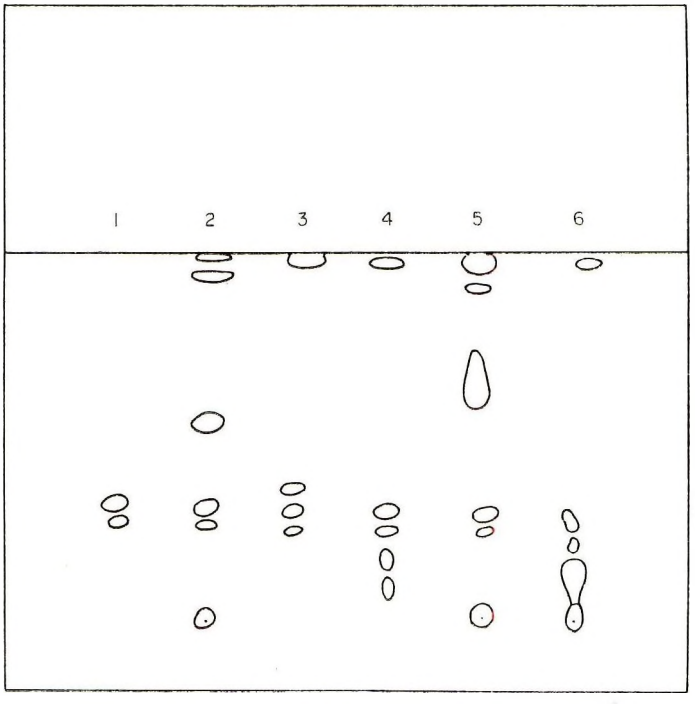

Figure 1. Diagram of the detection of imidazolidinyl urea type (Germall 115) added to personal care products. System: pre-coated silica gel; flow solvent = chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10); detection = ninhydrin, viewed by transmitted uv light 366 nm. 1 = Germall 115; 2 = hand cream; 3 = foam bath; 4 = moisturizing lotion; 5 = egg shampoo; 6 = deodorant.

The main difficulty encountered in achieving reproducible results is in obtaining a regular and easily definable baseline. This in turn is due to the background colour on the plate which varies in the direction of the solvent flow.

It was thought that adding ninhydrin to the flow solvent might improve the uniformity of the background colour and also remove errors due to uneven spraying. Unfortunately the reverse was found to be the case, the background intensity and variance being increased using this procedure.

A partial answer to the problem was achieved by drawing a pencil line approximately 2 cm above the line of the imidazolidinyl urea spots and a line parallel to this through the origins of the spots. This enabled a base line for each applied spot to be drawn on the graph, joining the responses of the two pencil lines, i.e. before and after the Germall peaks. The maximum peak

Table II. Antimicrobial preservatives chromatographed using the conditions required for the imidazolidinyl urea type (*Germall* 115)

Anti-microbial preservative	UV 254 nm	Ninhydrin	Rf × 100
Germall 115 (Imidazolidinyl urea type) (traces)	−	+	27 + 35 (0 + 15 + 60 + 72)
Sorbic acid	−	+	86
Dichlorophene	+	−	87
Hexachlorophene	+	−	88
Chlorcresol	+	−	90
Chlorxylenol	+	−	91
Trichlorocarbanilide	+	−	88
Irgasan DP 300 (2,4,4¹-trichloro-2¹-hydroxy diphenyl ether)	+	−	92
Methyl paraben	+	−	85
Propyl paraben	+	−	88
Propylene glycol	−	−	69
Tribromosalicylanilide	+	−	92
Irgasan CF3 (4,4¹-dichloro-3-trifluoromethyl-carbanilide)	+	−	89
Cetrimide	−	+	65
Bronopol, (2-bromo-2-nitropropane-1,3-diol)	+	−	80
Phenoxyethanol	+	−	91

Flow solvent, chloroform-methanol-acetic acid-water (50 : 30 : 10 : 10).
+, Detected; −, not detected.

height above this base line was recorded and the results of the standard spots were used to construct a calibration graph. A typical calibration graph of the preservative added to a moisturizing lotion is shown in *Fig. 2.*

Although most plates gave a straight line graph for values from 0.5 μg to 2.5 μg, in some cases the curve 'flattened out' at the higher values giving rise to poor reproducibility. The relative standard deviation for ten determinations of the urea product in a moisturing lotion at the level of 0.5% was found to be ±10%. One determination was considered to be the mean of four replicate sample spots on one plate calculated from a calibration graph of standards run on the same plate. Although the relative deviation was high, it may be considered acceptable, as the percentage of preservative in any formulation will always be at a low level.

Conclusion

Using the tlc method described, it is possible to detect the imidazolidinyl urea type of antimicrobial preservative in a wide variety of cosmetic and

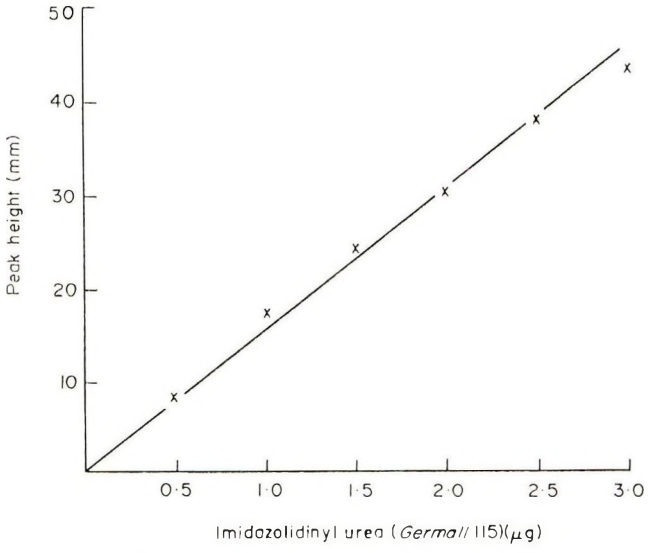

Figure 2. A calibration graph of imidazolidinyl urea type (Germall 115) added to a moisturizing lotion.

toiletry formulations. The uv colour reaction is specific for imidazolidinyl urea, even in the presence of a number of other antimicrobials. The preservative may be quantitatively determined with sufficient accuracy for routine quality control by either visual comparison or densitometric measurements.

(*Received: 12th March 1974*)

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J. Soc. Cosmet. Chem. **25** 545–561 (1974)

Studies of the factors controlling the action of hair sprays—III: The influence of particle velocity and diameter on the capture of particles by arrays of hair fibres

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Synopsis—The VELOCITY of an AEROSOL HAIR SPRAY has been determined by measuring the gas velocity within the spray with a PITOT-STATIC TUBE. The velocity rises to a maximum at the centre of the spray cone and falls rapidly with increasing distance from the spray orifice. For a given distance from the orifice the velocity also falls with decreasing pressure of the aerosol pack.

Measurements of CAPTURE and PENETRATION of hair spray droplets into a model ARRAY OF HAIR FIBRES backed by a solid plate representing the scalp have shown that coarse sprays give better penetration than fine sprays. This is in contrast to the behaviour predicted by classical aerosol capture theory and reasons for the observed behaviour are discussed.

INTRODUCTION

This paper is one of a series which describes studies of the factors influencing the action of hair sprays. Previous papers in this series (1, 2) have been concerned with the events occurring after the hairspray droplets

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have been deposited on the hair, and studies of the spreading of hairspray resin solutions on hair (1), and of the adhesion of hairspray resins to hair fibres have been reported (2). In another publication (3) the author has described measurements of the particle size distribution of hair sprays.

The present study is concerned with measurements of the velocity of particles within the sprays, and with the capture of the particles by arrays of hair fibres. It is shown that the capture and penetration of the particles into an array of fibres depends on both the size of the particles and their velocity.

Particle capture by fibre assemblies is generally treated by considering a model system in which a particle-laden gas stream is drawn through a filter composed of the fibres. In experimental studies it is convenient to control as many parameters as possible and monodisperse aerosols, travelling at well-defined air-stream velocities, are captured in a model filter in which fibres of uniform diameter are arranged with a regular interfibre spacing.

In the present study we have not restricted ourselves to such a model system and have studied the capture of actual hair spray particles produced by pressurized aerosol packs. The particles were captured by grids composed of hair fibres arranged in a roughly parallel configuration but without any regular interfibre spacing. In order to characterize the sprays we have measured the mass median diameter of the particles, and assessed the velocity of the particles by measuring the velocity of the particle-laden gas stream with a Pitot-static tube.

THE THEORY OF PARTICLE CAPTURE BY FIBRES

Recently Light (4) has reviewed the forces which determine the movement of an aerosol particle subsequent to the initial velocity imparted by its source. The combined effect of forces such as gravity, drag, inertia, diffusion and electrostatic charges determines the path followed by a particle. For a comprehensive discussion of these forces the reader is referred to this article and we shall only consider here those forces which influence the deposition of hair spray droplets on hair fibres.

As pointed out by Light, the particle size is perhaps the most important variable which is within the control of the formulator and for deposition on small surfaces, such as human hair, the particle diameter should be of the same order of magnitude as the surface dimension.

The particles in hair sprays are generally quite large. The mass median diameters are commonly in the range 60 to about 300 μm, and there is rarely greater than about 10% by weight of the spray with diameters less

than 10 μm. For such large particles the forces aiding capture are mainly gravity, inertia and direct interception.

Gravitational settling contributes significantly to the removal of particles in excess of 10 μm from a flowing stream. However, settling is only of importance when the stream is flowing over a horizontal surface and will contribute little to the capture of particles by fibres. Inertial impaction and direct interception are thus the major mechanisms controlling the capture of hair spray particles by hair fibres, and it is useful to consider these more fully in order to define the dependence on the particle diameter and velocity.

First we shall consider inertial impaction. When a particle-laden gas stream approaches an obstacle placed in its path, the gas stream alters its path to flow around the obstacle. Because of its inertia, a particle will not be able to follow completely the flow lines of the gas and may leave these flow lines sufficiently to impact on the obstacle. The probability of collision depends on two parameters (4), the Reynold's number which defines the pattern of the gas streamlines, and its dependence on the stream velocity, and the inertial impaction parameter. The inertial impaction parameter is defined as:

$$P = \frac{d^2 \rho V_0}{18 \eta D} \tag{1}$$

where d is the particle diameter, V_0 the gas stream velocity, ρ the density of the particle, η the gas viscosity and D the obstacle dimension, which for a fibre is the fibre diameter.

Light (4) has shown how the efficiency of capture may be calculated from the Reynold's number and the inertial impaction parameter. For a hair fibre of 100 μm diameter Light gives the capture efficiencies for particles of various sizes approaching at speeds of 10,100 and 1000 cm s^{-1} (Table 5 of ref. 4). The efficiency increases with increasing particle diameter and velocity and reaches 100% for a 10 μm particle at 1000 cm s^{-1} or a 50 μm particle at 100 cm s^{-1}.

Next we shall consider the contribution of direct interception to the total capture. When the size of the aerosol particles approaches the diameter of the fibres, capture by direct interception becomes significant and increases the capture occurring by inertial impaction. Because of the way in which capture efficiency is defined:

$$\text{efficiency} = \frac{\text{cross-sectional area of stream from which particles are removed}}{\text{cross-sectional area of fibres projected upstream}}$$

it is possible for the total efficiency to be greater than 100%. For example, let us assume that the inertia of all the particles is so great that they continue to travel in straight lines when the streamlines diverge around the obstacle. Then all particles whose centres are within the projected area of the fibre will be captured. In addition those particles whose centres are within a distance $d/2$ of the surface of the fibre will also be captured. The total capture efficiency is then $\frac{D + d}{D}$.

Inertial interception becomes significant for $d/D > 0.1$ and increases with increasing diameter of the particles.

From the foregoing analysis we can see that the capture of hair spray particles by hair fibres can be predicted to increase with increase in both the diameter and velocity of the particles. Conversely, maximum penetration into an array of fibres requires the use of small, low-velocity particles.

Experimental

Measurement of particle velocities in aerosol sprays produced by pressurized packs

The determination of the velocities of particles in an aerosol spray is difficult since there is a distribution of velocity across the spray cone. Particles at the centre of the spray have the highest velocity while those at the outside have the lowest velocity since here the particle-laden gas stream is in contact with the stagnant air of the surrounding atmosphere. We can thus expect a parabolic velocity distribution similar to that shown by a fluid moving through a pipe under laminar flow conditions. The situation is further complicated by local turbulence which is apparent in the spray, particularly on the outside of the cone.

Attempts to measure the particle velocities using a high-speed photographic technique were of limited use, owing to the restricted depth of field, together with the problems outlined above. Instead we eventually chose to measure the velocity of the gas stream carrying the particles rather than the velocity of the particles themselves. The work thus contains the assumption that the particles travel isokinetically with the gas stream. This restriction is probably of little significance when compared with the overall accuracy of the velocity measurements.

The Pitot tube was first described by Henri de Pitot in 1732. Pitot measured velocities by immersing two open tubes to the same depth in flowing water, as shown in *Fig. 1*. The lower opening in one of the tubes was perpendicular to the flow and the rise in water in this tube was taken as an indication of the static pressure p_s of the fluid. The other tube was bent through 90° so that its lower opening faced into the flow direction. The rise in water level in this tube was taken to be an indication of the total pressure p_t, i.e. the sum of the static and dynamic pressures, where the dynamic pressure $\frac{1}{2}\rho_0 V^2$ is the pressure equivalent of the kinetic energy of the flowing stream. The difference in water levels is thus a measure of the velocity of the fluid.

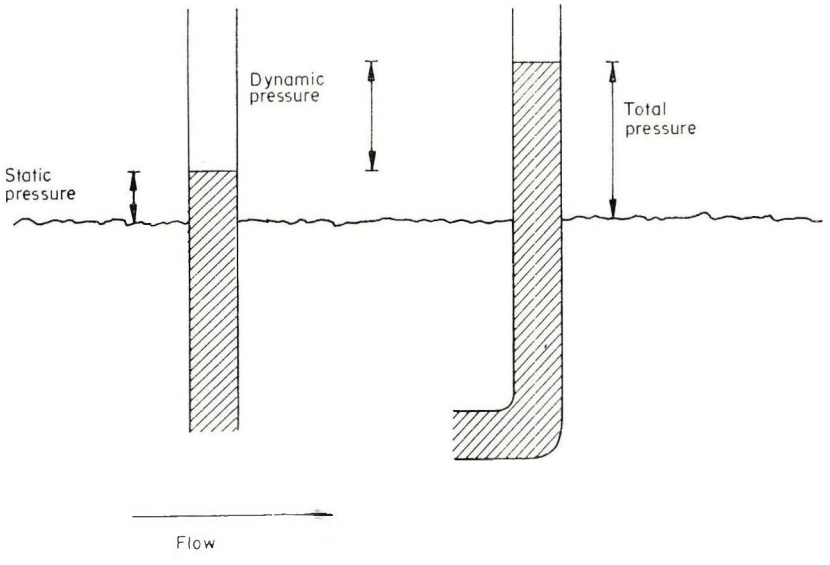

Figure 1. The Pitot-static tube method for measuring the velocity of a fluid stream.

Thus
$$p_t = p_s + \tfrac{1}{2}\rho_0 V^2 \qquad (2)$$
where ρ_0 is the density of fluid and V its velocity.

The instrument used in this investigation combines both tubes in one unit as shown in *Fig. 2*. The pressure differences $(p_t - p_s)$ produced are very small, especially at the lower velocities, and have to be measured on a specially designed inclined manometer. The particular instrument used was

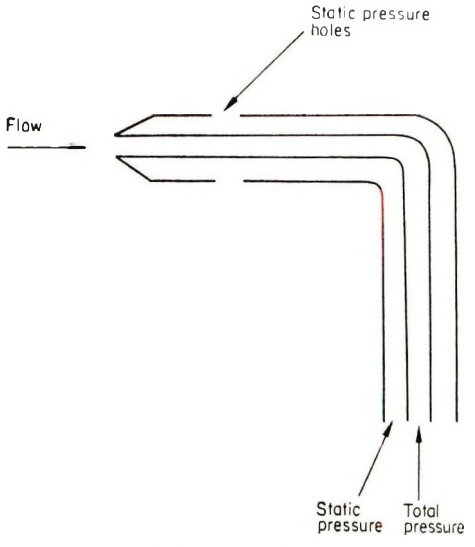

Figure 2. A combined Pitot-static tube.

the 'Portable airflow testing set Mark 4',* incorporating two adjustable limb manometers, each with several different inclinations. The manometer tubes were filled with a red dyed blend of paraffin having a specific gravity of 0.787 at 60°F. The static pressure tube was connected to the top of the manometer and the total pressure tube to the bottom so that only the differential pressure was measured. The scales of the manometer were calibrated in inches of water and an alternative scale was also supplied so that air velocities in ft min^{-1} could be read directly from the instrument.

Pressurized packs were filled according to the specifications shown in *Table I.*

The Pitot-static tube was placed within the aerosol spray cone at a given distance from the atomizing nozzle, and the aerosol button actuated. The

Table I. Details of filling of aerosol cans for velocity measurements

Pressure ($kN\ m^{-2}$)	Resin-alcohol concentrate (%)	Propellant (%)	*Freon* 11/ *Freon* 12
152	40	60	45/15
221	40	60	35/25
290	40	60	24/36
359	40	60	14/46

* Airflow Developments Ltd.

Pitot tube was moved within the cone until the maximum pressure was recorded on the manometer. The reading was then noted and the button released. The procedure was repeated at several distances from the nozzle so that the velocities of the aerosol gas stream were obtained as a function of distance from the nozzle. The maximum velocity within the cone was always measured since there is a velocity profile across the cone and a velocity less than the maximum could be obtained, depending upon the position of the Pitot tube.

It was found necessary to wash out the Pitot tube with alcohol after each measurement before the resin solution had time to dry and partially obstruct the gas flow into the tube.

Measurement of the penetration of the hair spray particles into an array of hair fibres

A model filter system was constructed to simulate a mass of hair fibres backed by the scalp. The filter consisted of six separate arrays of fibres which were then placed together. Each array consisted of about 200 hair fibres stretched across a circular brass ring of 47.5 mm internal diameter, and 1.5 mm wall thickness. The fibres were placed roughly parallel, and secured between two rings with Araldite epoxy resin. No attempt was made to obtain a uniform spacing between adjacent fibres. When completed, each array was marked and numbered so that the complete filter could be reproducibly assembled. A sheet of thin aluminium foil, attached across a seventh ring, acted as a back plate representing the scalp. This plate was placed behind the sixth filter stage to leave a 1.5 mm gap. This gap allowed some gas to pass through the filter whilst still maintaining a back pressure amongst the fibres.

Penetration measurements were made in the following way. The filter assembly was dismantled and washed thoroughly with alcohol. After drying to constant weight the individual filters, and back plate, were weighed separately and the whole unit reassembled. A further brass ring was placed in front of the first filter to prevent spray depositing directly on its former, and the spray from an aerosol can, placed at a given distance, was directed at the filter. The unit was dismantled after being allowed to dry. Each part was then reweighed to constant weight, to obtain the weight of resin deposited at each stage.

Several different types of spray were used. These were produced by varying both the actuator button and the internal pressure of the aerosol pack. Details of the various combinations used are listed in *Table II.*

Table II. Summary of actuators and products examined in penetration experiments

Actuator	Type	Orifice diameter (cm)	Pressures (kN m^{-2})
Precision 2-piece	Swirl chamber	0.045	145–372
PKN-38	Swirl chamber	0.040	152–372
	Mechanical break-up	0.075	165–359

The results of the penetration experiments were analysed in the following way.

Consider that in time t a total of (N_0)g of spray approaches the first filter. A fraction Δx of the particles will be removed by the fibres in the first filter, so that the weight collected will be $(N_0)\Delta x$, and the total weight passing to the second stage will be $(N_0)\,(1 - \Delta x)$. Assuming that a further fraction Δx is removed at each subsequent stage, the weight penetrating the second filter is:

$$N_0(1 - \Delta x) - N_0\Delta x(1 - \Delta x) = N_0(1 - \Delta x)^2 \tag{3}$$

and the weight penetrating filter number y is:

$$N = N_0(1 - \Delta x)^y. \tag{4}$$

The initial weight, N_0, is obtained by summing all the weights captured on the individual filters together with that on the back plate, assuming that no material escapes through the gap between the final filter and the back plate.

From equation (4) we obtain the penetration at any stage y:

$$\text{penetration} = \frac{N}{N_0} = (1 - \Delta x)^y \tag{5}$$

$$\therefore \log \frac{N}{N_0} = y \log (1 - \Delta x). \tag{6}$$

Thus a plot of $\log \frac{N}{N_0}$ against filter number y should be linear and the slope will be a measure of the overall penetration of the spray into the filter.

Results and discussion

Velocity measurements

Figure 3 shows that the velocity of an aerosol spray varies with the distance from the actuator and with the pressure of the aerosol pack. The results shown are for pack pressures of 152, 221, 290 and 359 kN m^{-2} and for a *Precision Standard* RTBU type actuator. The hair spray formulation consisted of 5.6% crotonic acid/vinyl acetate copolymer in IMS with a product/propellant ratio of 40/60, the propellant being the particular mixture of *Freon 11* and *Freon 12* required to give the desired pressure.

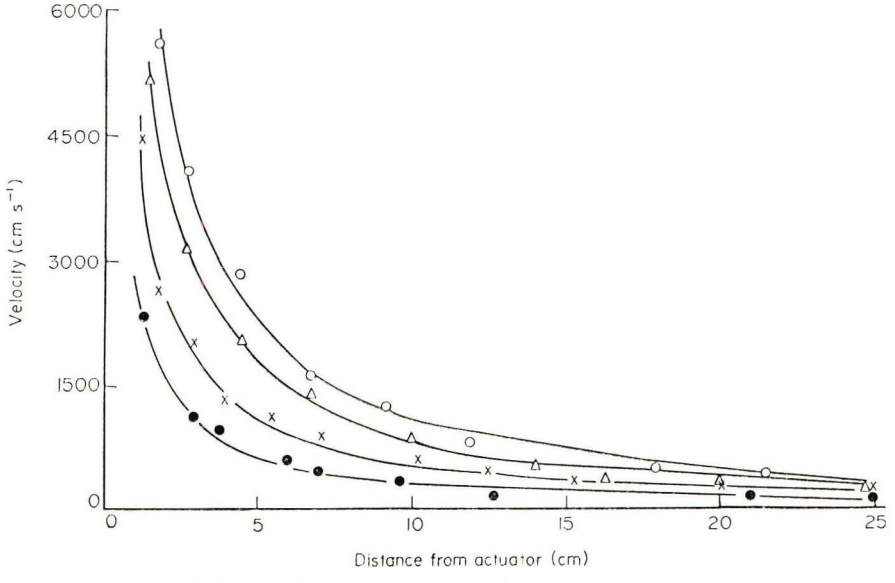

Figure 3. Variation of velocity of aerosol sprays with distance from the actuator. Experimental points: O, 359 kN m^{-2}; △, 290 kN m^{-2}; X, 221 kN m^{-2}; ●, 152 kN m^{-2}.

Figure 4 shows the velocity profile across the spray cone for the same aerosol packs. These measurements were taken at a distance of 50 mm from the actuator by placing the can on a turntable calibrated in degrees. In each case the aerosol button was initially lined up with the Pitot tube by eye, and the position was taken to be zero degrees. Velocity measurements were then taken on each side of this zero.

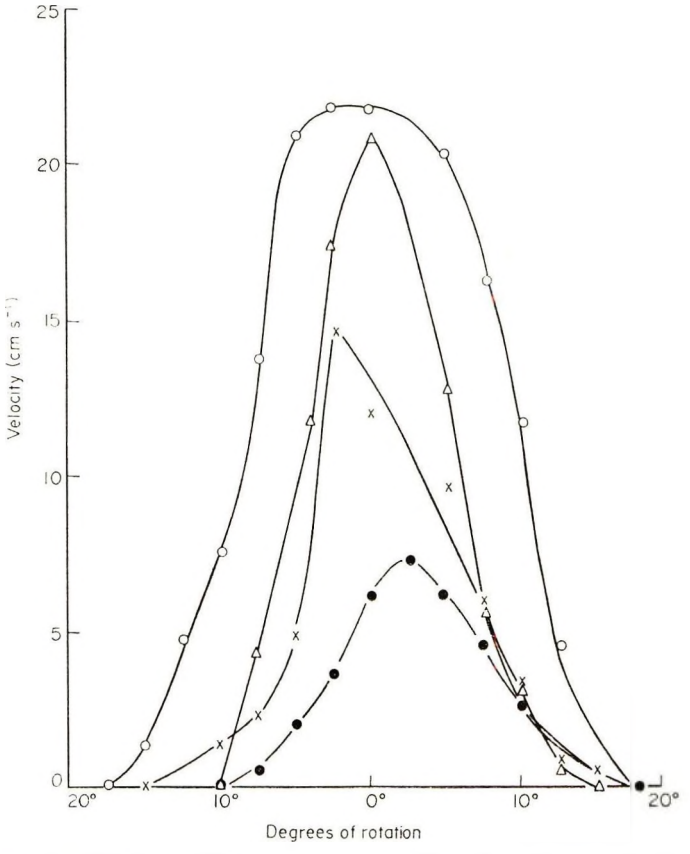

Figure 4. Velocity profile across spray cone 5 cm from the actuator for a *Precision* Standard RTBU actuator. Experimental points: ○, 359 kN m^{-2}; △, 290 kN m^{-2}; X, 221 kN m^{-2}; ●, 152 kN m^{-2}.

Figure 3 shows that the velocity is very high close to the actuator and falls rapidly with increasing distance from the actuator. For the high pressure packs (290 and 359 kN m^{-2}) the velocity 10 mm from the orifice is of the order of 6000 cm s^{-1}. At 250 mm the velocity falls to betweeen 100 and 500 cm s^{-1} depending on the pack pressure. The velocity profiles across the spray cone (*Fig. 4*) show that the velocity rises to a maximum at the centre of the spray cone. Both figures show that the maximum velocity in the cone increases with increasing pressure of the aerosol pack.

Figure 5 shows the velocity profile across the spray cone for a 221 kN m^{-2} pack fitted with a *Precision* two-piece actuator.

From a knowledge of the diameter of the actuator orifice and the discharge

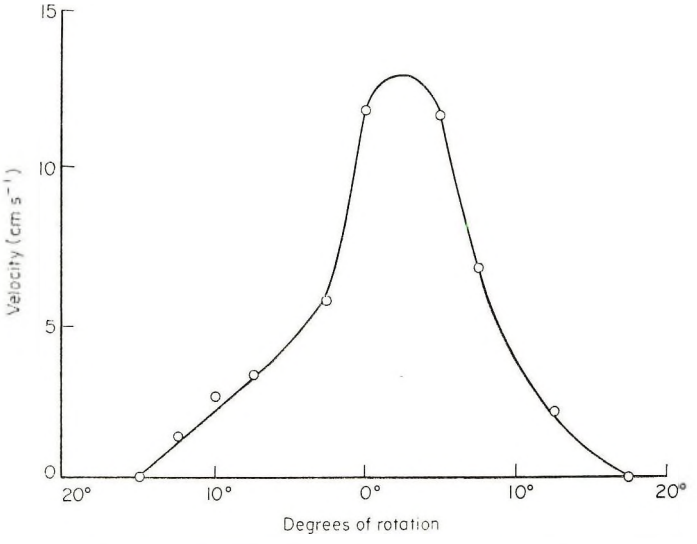

Figure 5. Velocity profile across spray cone 5 cm from the actuator for a *Precision* 2-piece actuator. The pressure was 221 kN m^{-2}.

rate of the aerosol it is possible to calculate the discharge velocity of the sprays. *Table III* shows data for the *Precision Standard* actuator which had an orifice diameter of 0.041 cm. The formulation density was taken as 1.1 g cm^{-3}. The final column of *Table III* shows the values of the maximum velocity of the sprays at a distance of 20 mm. The measured velocities are several times greater than the calculated values. This evidence suggests that the product does not emerge from the can as a continuous jet but as a mixture of liquid and propellant gas. The expansion chamber which precedes the atomizer probably allows partial evaporation of propellant before the

Table III. Comparison of calculated and measured discharge velocities for the standard RTBU actuator

Pressure (kN m^{-2})	Discharge rate (g s^{-1})	Calculated velocity (cm s^{-1})	Measured velocity 2 cm from orifice (cm s^{-1})
152	0.92	645	1700
221	1.03	722	2600
290	1.11	778	3900
359	1.15	806	5200

product enters the orifice. The product then leaves the actuator in a partially atomized condition as a mixture of gas and liquid. The mixture has a lower overall density and consequently higher discharge velocity.

Penetration measurements

Penetration measurements were made with sprays produced by aerosol packs fitted with swirl chamber actuators of the *Precision* two-piece and *Aerosol Research* PKN 38 design and with a conventional mechanical break-up actuator with an exceptionally large orifice diameter of 0·75 mm. Each actuator was used with aerosol cans packed with 5.6% crotonic acid/vinyl acetate copolymer in IMS with a product/propellant ratio of 40/60, and with mixtures of *Freon 11* and *Freon 12* to give pressures ranging from about 138 to 359 kN m^{-2}.

A series of experiments was performed with the model fibre array at a distance of 150 mm from the actuator.

The results for the series of experiments are shown in *Fig. 6* where the data is presented as plots of log N/N_0 versus filter number.

In general linear plots, as predicted by the theoretical analysis, are obtained. Deviations occur for the poorly atomized sprays produced by the mechanical break-up button with 0.75 mm orifice diameter at the lower pressures. These sprays are jet-like and the capture theory breaks down since capture of individual droplets is no longer the controlling mechanism.

Figure 6 shows certain trends which can be seen by simple inspection. The sprays produced by the 0.75 mm orifice diameter mechanical break-up actuator were more penetrating than those produced by either of the swirl chamber actuators at equivalent pressures. Furthermore, for a given type of actuator the penetration generally increases with decreasing pressure of the aerosol pack. Both of these observations indicate that coarse sprays, containing large droplets, are more effective in producing penetration into the array of fibres. This is directly opposite to the effects expected from theoretical consideration.

Direct comparison of the penetration plots allows a ranking order of penetration to be obtained. This order is shown in *Table IV*.

Particle capture theory predicts that the efficiency of capture increases with increasing inertia of the particles, that is with increasing diameter and velocity of the particles. Our data, on the other hand, indicate that this condition does not apply for particles encountered in aerosol hair sprays. Thus the coarser the spray the more penetrating it proves to be.

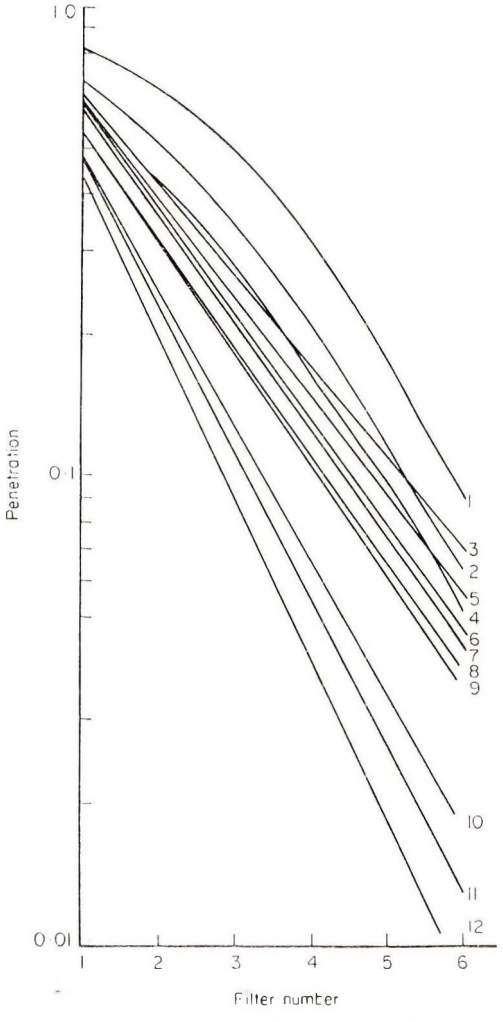

Figure 6. Penetration of hair spray droplets into model fibre array, placed 15 cm from the actuator.

Actuator	Pressure (kN m^{-2})	Actuator	Pressure (kN m^{-2})
1. Mechanical break-up	165	7. *Precision* 2-piece	214
2. Mechanical break-up	262	8. *Precision* 2-piece	283
3. *Precision* 2-piece	165	9. *Aerosol Research* PKN 38	234
4. Mechanical break-up	303	10. *Precision* 2-piece	372
5. *Aerosol Research* PKN 38	165	11. *Aerosol Research* PKN 38	303
6. Mechanical break-up	359	12. *Aerosol Research* PKN 38	372

Table IV. Penetration of sprays into model filter placed 150 mm from actuator

Ranking order	Actuator/ pressure (kN m^{-2})	d (μm)	V (cm s^{-1})	$d^2 V$ ($cm^3 s^{-1}$)
1	MBU/165	1000	200	2.00
2	MBU/262	500	400	1.00
3	P2P/165	270	200	0.146
4	MBU/303	121	450	0.066
5	PKN-38/165	75	400	0.023
6	MBU/359	81	600	0.039
7	P2P/214	225	400	0.203
8	PKN-38/283	160	200	0.051
9	P2P/234	100	450	0.045
10	P2P/372	60	600	0.022
11	PKN-38/303	80	450	0.029
12	PKN-38/372	63	600	0.024

To investigate the dependence of penetration on the inertia of the particles more fully, it is necessary to consider both the velocity and the dimensions of the particles. If a particle is projected into still air with an initial velocity V cm s^{-1}, and if the particle motion subsequently obeys Stokes law, then the distance travelled by the particle before coming to rest is known as the 'stop distance' and is given by:

$$\text{'stop distance'} = 307\, d^2\, \rho\, V \qquad (7)$$

where d is the particle diameter in cm and ρ is the particle density in g cm^{-3}. The quantity d^2V is a measure of the inertia of the particle.

Since the velocity and size of the particles within each spray both follow a distribution it is not a simple matter to calculate an inertia value which can be rigidly applied to each spray. Perhaps the best that can be hoped for is to use some average value of particle diameter and velocity for each spray. This may be done conveniently with respect to the particle size by calculating the mass median diameters of the sprays from particle size distribution measurements as shown previously (3). It is much more difficult to obtain an average velocity for each spray and the best that could be obtained in the present study was to measure the maximum velocity of the spray at a distance of 150 mm from the actuator. The values of the mass median diameter and maximum velocity for each spray are listed in *Table IV* together with the calculated values of d^2V. It can be seen that the general

trend is for the penetration to decrease with decreasing values of d^2V. A more quantitative picture can be obtained by considering actual values of the penetration. For example *Fig. 7* shows the penetration N/N_0 after the fourth filter, plotted against d^2V. The values of d^2V are plotted on a logarithmic scale because of the large range of values encountered (200–20 000).

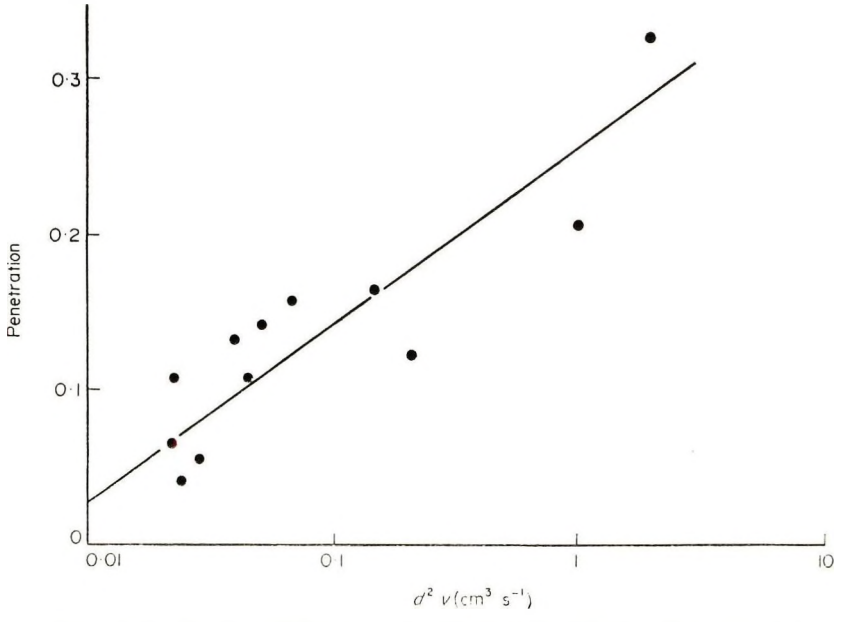

Figure 7. Dependence of the penetration through the first four filters on the product d^2V.

From our experiments we thus find that the theory of capture of aerosol particles does not apply for the capture and penetration of hair spray droplets in arrays of hair fibres. With these systems the greater penetration of coarse sprays is apparently due to the much larger inertia of their particles, which is in turn mainly due to their larger diameters.

There are at least two effects which may be responsible for the observed behaviour. Firstly, since the fibre array is backed by a solid plate representing the scalp the particle-laden gas stream will not be able to pass right through the array. The gas flow lines will be deflected around the array, carrying with them the smaller particles. The larger particles will be able to leave these flow lines more easily and enter the array of fibres. Particles which enter will then travel through the array mainly due to their own inertia

since the gas within the array will probably be stagnant. The higher the inertia of the particles the further will they be able to penetrate. This effect will also be shown on the head. Many of the smaller particles will be deflected away by the gas flow around the head and only the larger particles, or the smaller particles at the very centre of the spray, will be deposited on the hair.

The second effect which could produce greater penetration of the larger particles is incomplete capture of the particles by the hair fibres. The aerosol capture theory assumes that those particles which contact a fibre are completely captured, but it is very likely that large high velocity particles might shatter on impact with the fibres, producing several smaller droplets which penetrate further into the array. Only a fraction of the initial droplet is retained at the first impact. This effect would become of greater significance when the particles approach or become larger than the diameter of the fibres, a condition which exists for many of the sprays studied.

Conclusions

Measurement of the velocity of aerosol sprays using a Pitot-static tube to measure the velocity of the gas stream rather than the actual particle velocity have shown that there is a velocity distribution across the spray cone. The velocity rises to a maximum at the centre of the cone and this maximum falls off with increasing distance from the actuator, and with decreasing pressure of the aerosol pack for a given distance from the actuator.

The capture of hair spray droplets by arrays of fibres backed by a solid plate representing the scalp does not agree with the behaviour predicted from classical aerosol capture theory, that is that the fine sprays containing small droplets would be more penetrating than coarse sprays. In practice it has been found that coarse sprays are more capable of achieving penetration into the fibre array.

It has been found that the penetration increases with increasing value of the product d^2V where d is the mass median diameter of the aerosol spray, and V is the maximum velocity of the spray at a distance of 150 mm from the actuator. This was the experimental distance used between the actuator and the fibre array and corresponds approximately to the spraying distance used by the consumer.

The observed capture behaviour can be explained in terms of the greater inertia of the larger particles which is necessary to carry the particles into the array of fibres. Normal aerosol capture experiments use a filter

which is open at both ends so that the gas stream and the particles can flow right through. Larger particles are then more efficiently captured by a combination of the inertial and direct interception mechanisms. When the array of fibres is backed by a solid plate most of the gas stream will be deflected around the front of the array. Small, low inertia particles will tend to follow the gas flow lines and any particles which do enter the array will travel only a small distance before losing their remaining inertia since the air within the array will be largely stagnant. Large particles will leave the gas flow lines much more easily to enter the fibre array and will then travel further because of their greater inertia.

A second factor which could help to achieve greater penetration with larger particles is splitting of droplets on impact with hair fibres. This splitting is liable to be greater the greater the particle inertia. The droplet fragments so produced are then capable of further penetration into the array.

(*Received: 2nd May 1974*)

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A survey of microbiological contamination in cosmetics and toiletries in the U.K. (1971)

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Synopsis—One hundred and seventy-two toiletry and cosmetic items purchased in 1971 from retail outlets throughout England and Wales were examined microbiologically. Viable MICRO-ORGANISMS were not recovered from over 50% of the items tested and about 90% contained fewer than 1000 organisms g^{-1}. 75% of all POWDER PREPARATIONS tested did not contain viable SPORES of ANAEROBIC BACTERIA and none contained more than 300 spores of anaerobic bacteria g^{-1}. Of the anaerobes isolated, none was identified as *Clostridium tetani*. *Coliform bacteria* were not detected in any preparation of toothpaste or lipstick examined. Comparison of counts from the top and bottom ends of metal foil tubed products showed almost identical counts in most cases, but in two instances significantly higher counts were observed in the top (nozzle end) sample. Further analyses were performed on six or twelve replicate items of a single brand of seven product types to check the inter-sample variation in count. The results obtained confirmed the overall level of colony count observed previously for these products; in some instances marked inter-sample variation in count was seen.

Introduction

Although many cosmetic, toiletry and pharmaceutical preparations contain preservatives (1–4) microbiological spoilage can still occur (5, 6).

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In some instances microorganisms in cosmetic preparations, whether present initially or transferred to the product by the user, have been implicated as the aetiological agents of disease. Wilson and co-workers (7, 8) have demonstrated that eye cosmetics may serve as a possible vector in transmission and persistence of microorganisms in clinical infections of the eye. An outbreak of tetanus in babies has been attributed to the use of talcum powder contaminated with *Clostridium tetani* (9). Other examples of the contamination of non-sterile drugs and cosmetics are given by Bruch (10, 11).

In 1967 the Toilet Preparation Federation and the Society of Cosmetic Chemists of Great Britain established a Select Committee to report on matters relating *inter alia* to the quality and safety of cosmetic preparations. One aspect of the work of this committee was to advise on the desirability for the establishment of microbiological standards for such products (12). Although many manufacturers have data on the microbiological quality of products immediately post manufacture, such information is not generally available. Furthermore, it may bear only a superficial relationship to the microbial quality of the products as purchased by the user. The present investigation was undertaken in 1971 at the instigation of the Select Committee to assess the incidence of contamination in a restricted range of cosmetic and toiletry preparations on sale to the general public.

Materials and methods

Provision of cosmetics

Products were purchased by representatives of the Toilet Preparations Federation in six areas of England and Wales. Two units (one of large size and one of small size whenever possible) were taken for each product from a large and a small retail outlet respectively. In some cases, additional items were provided for analysis. In total 172 cosmetic items were examined. Further items of selected products were purchased locally to investigate the inter-sample variation in counts.

Sampling of products

The outside surfaces of all containers were swabbed with 70% v/v ethanol before opening.

In general, 1 g samples were taken for each product, but in some instances 10 g or 0.1 g samples were examined. The sampling procedures used were as follows.

Talcum powder

An adequate quantity of the powder was shaken into a sterile petri dish, mixed and a representative sample was weighed into a tared bottle.

Loose and compressed face powder

After aseptically removing the seal, a sample of powder was scraped from the entire surface of the product into a sterile petri dish. For mixed samples the complete contents of the container were ground using a sterile pestle and mortar.

Complete make-up

Bottled products were mixed by inversion 20 times through an arc of 1 ft and a representative sample was removed using a wide-bore sterile pipette. For tubed products a large sample was extruded into a sterile bottle, mixed thoroughly and a sample for analysis was taken with a sterile spatula.

Face and hand creams, cake mascara and eye shadow

Products were sampled by taking a surface scrape as detailed above for face powder.

Liquid products (shampoo, bath oils, eye shadow)

The samples were mixed by inversion and an aliquot was removed by pipette.

Toothpaste and other tubed products

Samples were removed aseptically by extrusion through the nozzle. A second sample of each product was obtained by aseptically removing the crimped end of the tube and extruding a suitable sample.

Soap cakes

These were scraped with a sterile scalpel to remove wafer thin shavings from the entire surface. The shavings were mixed and a representative sample was taken for analysis.

Aerosol shaving soap products

These were voided into sterile wide neck jars. The sample was mixed and a 1 g aliquot weighed into a sterile wide neck bottle. After addition of 9 ml diluent, a few drops of sterile Antifoam A was added and the contents were mixed by swirling.

Lipstick

The whole or half surface of a lipstick was sampled by swabbing with a calcium alginate wool swab which was then transferred to sterile Calgon–Lubrol broth (see *Table I*) to effect solution of the alginate and dispersal of the lipstick 'fat'.

Table I. Diluents used in the analyses of cosmetics and toiletries

Sample type	Examination	Diluent	Footnote
Talcum and face powders	Aerobic counts	Tween-Peptone	1
	Anaerobic counts	Tween-RCM or RCM	2
Water-based creams and emulsions	Aerobic counts	Tween-Peptone	1
Eye cosmetics			
Toothpastes			
Bath oil, detergents	Aerobic counts	Peptone	3
Shampoo, soap			
Lipstick	Aerobic counts	Calgon-Ringer-Lubrol	4
Oil-based creams and emulsions	Aerobic counts	Lubrol broth	5

Composition of diluents

(1) Tween-Peptone: 0.1% w/v Peptone solution (pH 7.0) containing 0.1% v/v Tween 80.
(2) Tween-RCM: Reinforced Clostridial Medium (Oxoid), containing 0.1% v/v Tween 80. RCM: Reinforced Clostridial Medium (Oxoid).
(3) Peptone: 0.1% w/v Peptone solution, pH 7.0.
(4) Calgon-Ringer-Lubrol: 1 tablet of Calgon-Ringer (Oxoid) dissolved in 6 ml distilled water plus 4 ml 4% w/v Lubrol W solution.
(5) Lubrol Broth: 4 ml 4% w/v Lubrol W solution plus 5 ml Nutrient Broth (Oxoid)—see Ref. (2).

Microbiological procedures

In general the methods used were those recommended by Van Abbé *et al.* (2). Ten-fold serial dilutions were prepared in an appropriate diluent (*Table I*). Aerobic bacterial colony counts were made by pour plate technique on plate count agar (PCA; Oxoid). Plates were incubated in duplicate at 30°C for 3 days and at 37°C for 2 days. In later experiments counts of

bacteria were made at 25°C for 5 days instead of at 30°C for 3 days. Counts of yeasts and moulds were made on Sabouraud Dextrose Agar (SDA; Oxoid) incubated for 5 days at 25°C.

Counts of anaerobic sporeforming bacteria were made on Reinforced Clostrial Agar (RCA; Oxoid) after pasteurization (30 min at 75°C) of dilutions made up in freshly steamed Reinforced Clostridial Medium (Oxoid). The plates were incubated for 3 days at 37°C in an atmosphere of 95% hydrogen plus 5% CO_2. Clostridia were identified by the methods described by Willis (13). Dilutions of lipstick and toothpaste samples were tested for the presence of presumptive coliforms by inoculation into MacConkey Bile Salt Broth (Oxoid) which was incubated at 37°C and examined after 1 and 2 days.

After incubation the number of colonies was recorded for each plate. Arithmetic mean counts were derived for each item from those plates having from 30 to 300 colonies. In the case of samples with low counts the number of colonies recorded on the first dilution tested was used to derive the count. In some instances, the presence of an antimicrobial agent in the product was shown by a carryover effect where the count at high dilution was sometimes greater than the count at low dilution. Repeat analyses were always undertaken in such cases.

Mould growth on compressed eye make-up

The remainder of each sample after surface scraping was aseptically dissected into three portions, each of which was placed on a moistened sterile filter paper in a petri dish. The surface of the make-up was moistened with sterile water and the samples were incubated in a moist atmosphere for several weeks at 25°C; sterile water was added as necessary to the samples.

Statistical analysis

When replicate samples were tested, the significance of the difference in mean count for samples from different sources (e.g. surface scrape or mixed sample) or for replicate plates incubated at different temperatures was tested using two methods. For those results where large numbers of 'sterile' items (i.e. < 10 cfu g^{-1}) were obtained, Wilcoxson's Signed Ranks test was used (14). Where mainly definitive results were obtained, the mean difference of the $\log_{10}$ counts from zero was tested using Student's t. Counts of <10 g^{-1} were in all cases assigned the numerical value of 10 in calculations of the t value.

Results

A total of 190 analyses were carried out on the 172 items purchased during the survey. The additional 18 tests were conducted to compare the counts on top and bottom samples from all tubed products, e.g. toothpaste, hair dressing, etc. A further 60 analyses were made on items of selected products purchased locally.

Aerobic bacteria

The distributions of aerobic bacterial colony counts at 30° and 37°C are summarized in *Tables II and III*. Over 80% of the items tested contained fewer than 300 cfu g^{-1}. Viable bacteria were not recovered from over 50% of the items examined. No difference was seen in the bacterial colony counts on samples from the top and bottom ends of 16 tubed products. Most samples yielded no viable bacteria from either end of the tube but in one case colony counts of 2.6×10^4 and 3.0×10^4 cfu g^{-1} were recorded for the top (nozzle end) sample at 30° and 37°C respectively whereas counts from the crimped end sample were 1.32×10^5 and 1.34×10^5 cfu g^{-1} respectively. In this instance general contamination of the product prior to filling may have occurred. In contrast, significantly different counts were noted in two items. The counts at 37°C on these items were 1.65×10^4 and 2.5×10^3 cfu g^{-1} for the top samples and 10 and 35 cfu g^{-1} respectively for the bottom samples. In these two instances it is probable that the nozzle end of the tubes was contaminated prior to filling with the product. Coliform bacteria were not detected in 0.1 g of any toothpaste sample nor on any lipstick sample examined. The predominant microorganisms isolated from high count products were Gram negative non-sporing rods, but no attempt was made to identify the organisms.

Anaerobic bacteria

Samples of each item of talcum powder, face powder and 'complete make-up' were examined for spores of mesophilic anaerobic bacteria. The distribution of counts is summarized in *Table IV*. Statistically significant counts (>300 cfu g^{-1}) were not obtained from any product examined in the survey, but such counts were later observed in the repeat analyses (see below). Selected colonies of anaerobes were subcultured and examined both microscopically and culturally. The organisms were typically mesophilic

Table II. Distribution of aerobic colony counts on PCA incubated for 3 days at 30° C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range			
		< 300	300–1000	1001–10 000	>10 000
Powders					
Talcum powder	12	12 (100)	0 (0)	0 (0)	0 (0)
Face powder and rouge	14	12 (86)	0 (0)	2 (14)	0 (0)
Complete make-up	11	7 (64)	0 (0)	3 (27)	1 (9)
Creams and lotions					
Hand and body lotion	21	19 (90)	1 (5)	1 (5)	0 (0)
Face cream	17	12 (70)	2 (12)	1 (6)	2 (12)
Skin perfume	6	4 (67)	2 (33)	0 (0)	0 (0)
Hair cream and dressing	16	15 (94)	1 (6)	0 (0)	0 (0)
Shaving cream and foam	6	5 (83)	0 (0)	0 (0)	1 (17)
Eye make-up					
Mascara, eyeliner and eye shadow	20	13 (65)	3 (15)	1 (5)	3 (15)
Soaps and detergents					
Bath oil and detergent	18	16 (89)	0 (0)	1 (6)	1 (6)
Shampoo and hair colourant	13	12 (92)	0 (0)	1 (8)	0 (0)
Soap	6	6 (100)	0 (0)	0 (0)	0 (0)
Miscellaneous					
Toothpaste	6	6 (100)	0 (0)	0 (0)	0 (0)
Lipstick*	6	6 (100)	0 (0)	0 (0)	0 (0)
Total	172	145 (84)	9 (5)	10 (6)	8 (5)

* cfu/lipstick surface, not per g.

clostridia. *Cl. tetani* was not detected but 16 of the 50 isolates examined were identified as *Cl. perfringens.*

Yeasts and moulds

The distribution of counts of yeasts and moulds is presented in *Table V.* Of those items containing viable organisms, most were contaminated more heavily with yeasts than with moulds, the level of mould contamination rarely exceeding 50 cfu g^{-1}. Growth of moulds on moistened samples of cake mascara, rouge, and similar products did not occur during a three-month period at 25°C. On three occasions, heavy growth of bacteria occurred on plates of SDA. In each instance the bacteria were Gram negative rods, which also comprised the predominant flora of the items tested (liquid eye make-up). Such growth could have been avoided by the use of an antibiotic-containing medium (15).

Table III. Distribution of aerobic colony counts on PCA incubated for 2 days at 37° C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range			
		< 300	300–1000	1001–10 000	>10 000
Powders					
Talcum powder	12	12 (100)	0 (0)	0 (0)	0 (0)
Face powder and rouge	14	12 (86)	0 (0)	2 (14)	0 (0)
Complete make-up	11	8 (73)	1 (9)	1 (9)	1 (9)
Creams and lotions					
Hand and body lotion	21	20 (95)	0 (0)	1 (5)	0 (0)
Face cream	17	16 (94)	0 (0)	0 (0)	1 (6)
Skin perfume	6	5 (83)	1 (17)	0 (0)	0 (0)
Hair cream and dressing	16	14 (87)	1 (6)	1 (6)	0 (0)
Shaving cream and foam	6	5 (83)	0 (0)	0 (0)	1 (17)
Eye make-up					
Mascara, eyeliner and eye shadow	20	13 (65)	4 (20)	2 (10)	1 (5)
Soaps and detergents					
Bath oil and detergent	18	17 (94)	0 (0)	1 (6)	0 (0)
Shampoo and hair colourant	13	12 (92)	0 (0)	1 (8)	0 (0)
Soap	6	6 (100)	0 (0)	0 (0)	0 (0)
Miscellaneous					
Toothpaste	6	5 (83)	0 (0)	1 (17)	0 (0)
Lipstick*	6	6 (100)	0 (0)	0 (0)	0 (0)
Total	172	151 (88)	7 (4)	10 (6)	4 (2)

* cfu/lipstick surface, not per g.

Table IV. Distribution of colony counts of clostridia from powders on RCA incubated for 3 days at 37°C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range		
		< 10	10–300	>300
Talcum powder	12	8 (67)	4 (33)	0 (0)
Face powder and rouge	14	9 (65)	5 (35)	0 (0)
Complete make-up	11	11 (100)	0 (0)	0 (0)
Total	37	28 (75.6)	9 (24.4)	0 (0)

Table V. Distribution of colony counts for yeasts and moulds on SDA at 25°C

Product	No. of items	No. and (%) of items with colony counts g^{-1} within the range		
		< 300	300–1000	>1000
Powders				
Talcum powder	12	12 (100)	0 (0)	0 (0)
Face powder and rouge	14	12 (86)	1 (7)	1 (7)
Complete make-up	11	10 (91)	1 (9)	0 (0)
Creams and lotions				
Hand and body lotion	21	21 (100)	0 (0)	0 (0)
Face cream	17	17 (100)	0 (0)	0 (0)
Skin perfume	6	6 (100)	0 (0)	0 (0)
Hair cream and dressing	16	15 (94)	1 (6)	0 (0)
Shaving cream and foam	6	5 (83)	0 (0)	1 (17)
Eye make-up				
Mascara, eye liner and eye shadow	20	19 (95)	0 (0)	1 (5)
Soaps and detergents				
Bath oil and detergent	18	15 (83)	1 (6)	2 (11)
Shampoo and hair colourant	13	13 (100)	0 (0)	0 (0)
Soap	6	6 (100)	0 (0)	0 (0)
Miscellaneous				
Toothpaste	6	5 (83)	1 (17)	0 (0)
Lipstick*	6	6 (100)	0 (0)	0 (0)
Total	172	162 (94)	5 (3)	5 (3)

* cfu/lipstick surface, not per g.

Further analyses of selected items

Of the products examined during the survey, seven were selected for further investigation on the basis of the levels of contamination observed. Several units of each product were purchased locally from a number of different retail outlets. They were examined essentially as described above except that aerobic bacterial counts were made at 25°C rather than at 30°C and in some instances comparison was made of surface and mixed samples of the items. The distribution of counts is presented in *Table VI.*

The brand of liquid eye liner and bath detergent were selected because of the extremely high count observed previously in both items of each of these products. Further tests showed that the mean counts of the replicate items examined were also high (*c.* 10^5 cfu g^{-1}), but that the counts from different units ranged from no viable bacteria recovered to a count in one instance of 1.2×10^6 at 25°C. Significantly lower counts were observed at 37°C than at 25°C for these products.

Table VI. Replicate analyses of selected products

Product type	No. of items	Mean and (range) of colony forming units g^{-1} at		
		25°, PCA	37°, PCA	25°, SDA
				24Y
Bath detergent	6	100 345 (< 10–205 000)	< 10	< 10–80Y
Face cream				
Surface sample	6	10 (< 10–40)	31 (< 10–100)	NT*
Mixed sample	6	21 (< 10–45)	13 (< 10–35)	NT
Foundation cream	6	65 (< 10–210)	298 (< 10–700)	110Y, 65M† (< 10–320Y) (< 10–180M)
Toothpaste	6	< 10	10 (< 10–60)	< 10
Skin lotion	6	< 10 (< 10–10)	33 (< 10–95)	< 10
Eyeliner	12	121 758 (< 10–1 460 000)	41 (< 10–270)	27Y (< 10–160Y) 37° Anaerobic, RCA
Compressed face powder				
Surface sample	6	498 (280–660)	352 (215–470)	NT
Mixed sample	6	311 (90–550)	225 (85–390)	1180 (360–2700)

* NT, Not tested.
† Y = Yeasts; M = moulds.

Examination of replicate units of single brands of toothpaste, skin lotion/face cream and foundation cream for which counts below 300 cfu g^{-1} had previously been recorded, provided confirmatory evidence for the low levels of contamination of these items. The counts obtained from surface samples and mixed samples of the face cream did not differ markedly and were in any case below the level of statistical significance for the plate count method.

The replicate units of one brand of compressed face powder were also examined using both surface sample and mixed sample procedures. The counts obtained at 25° and 37°C were higher in most instances from surface samples than from mixed samples. However, although the difference was significant at the 5% level for counts at 37°C, no statistically significant

difference was observed for the counts made at 25°C. The counts of anaerobic bacteria on these units were much higher (mean count 1180 cfu g^{-1}) than had been observed previously.

Observations on packaging of the products studied

The majority of products were packaged such that post-packaging contamination would not be likely to occur before the product was used. Attempts to correlate colony counts with type of packaging were unsuccessful. Although a few products were clearly coded, this was not evident in the majority of items examined and the absence of coding would make difficult any retrospective attempt by the manufacturer to check against the production batch if consumer complaints subsequently occurred.

There were no obvious differences between colony counts from units of different size nor from small or large retail outlets. Although differences in brand gave rise to differences in the levels of contamination of particular product types it was not possible to determine the incidence of contamination for any particular brand because of the small number of items examined for individual brands.

Discussion

This survey demonstrates that in 1971 about 90% of a diverse range of cosmetic products and toiletry preparations contained fewer than 1000 viable microorganisms per g of product and that over 50% of the items examined were essentially 'sterile'. Of those items which were contaminated, few contained more than 10^5 organisms g^{-1}. The most heavily contaminated products were specific brands of eye make-up (especially liquid eyeliner), bath detergent and complete make-up. Unfortunately, tests for the presence of specific organisms such as *Pseudomonas aeroginosa* were not made on these products during the present investigation. From random selection of items it is not possible to determine whether the observed contamination reflects poor manufacturing conditions, post-process contamination or overlong storage by the retailer. For those products where high colony counts were observed also in the repeat examinations it is probable that the high colony counts reflect poor manufacturing conditions. Tests to assess whether these products might have become spoiled by growth of the contaminating organisms was not undertaken, but other workers (4, 5)

have demonstrated that deterioration can occur in products which are inadequately preserved.

The incidence of significantly contaminated samples (i.e. containing >300 cfu g^{-1}) in the present investigation is similar to the levels previously reported from the U.S.A. Wolven and Levenstein (16) reported an incidence of contamination of 24.4% (61 out of 250 items examined) whilst Dunnigan and Evans (17) observed contamination in 33 (19.5%) out of 165 items of cosmetic examined. Unfortunately, although the latter workers identified the predominant microflora they gave no information of the nature of the contaminated products. More recently, Wolven and Levenstein (18) have shown a much lower incidence of contamination of cosmetic products in the U.S.A., only eight (3.5%) of 223 items examined being contaminated. It is not unreasonable to suppose that similar improvements in the quality of cosmetics may have occurred during the past two years in the U.K. In particular, awareness of the need to ensure good microbiological quality in raw materials, especially natural pigments and fillers, will have had an effect on the levels of microorganisms present in many products (D. Spooner, personal communication).

In devising any 'Code of Good Manufacturing Practice' the absence of specific pathogens must be considered in addition to control of the overall level of microbial contamination of the product. When complete product sterility is not feasible, cosmetic and toiletry preparations should be free from viable pathogens such as *Pseudomonas aeroginosa*, salmonellae, *Escherichia coli*, *Staphylococcus aureus* and certain clostridia. Raw materials of mineral origin, such as talc, may be contaminated with spores of soil clostridia including *Cl. tetani* (19) and *Cl. perfringens*. Whilst *Cl. tetani* contamination of talc is known to have caused at least one outbreak of tetanus in babies (9, 19) the significance of *Cl. perfringens* spores is less clear. Strains of *Cl. perfringens* are known to cause gas gangrene in man and animals, the route of entry to the body tissues being via wounds and abrasions in the skin (20). However, the minimum infective dose of *Cl. perfringens* strains is probably considerably above the level at which any area of skin would become contaminated by a cosmetic preparation containing a relatively low number of spores per gram.

Acknowledgments

The authors are indebted to the Select Committee of the Toilet Preparation Federation and the Society of Cosmetic Chemists of Great Britain for

sponsoring this investigation and for permission to publish the results. We are grateful to Mr A. Rangnikar for technical assistance during the investigation.

(Received: 5th April 1974)

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1974 Publication Prize

The Publication Prize has been awarded to T. J. Elliott, B.Sc., Ph.D., of Beecham Products Ltd for his paper 'The use of a Laboratory Model to evaluate the factors influencing the performance of Depilatories'. *J. Soc. Cosmet. Chem.* **25** (7) 1974.

The 1973/1974 President of the Society of Cosmetic Chemists of Great Britain, Mr G. A. C. Pitt, M.Sc., F.R.I.C., M.B.I.M., presenting the 1974 Publications Prize to Dr T. J. Elliott of Beecham Products Limited.

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Book review

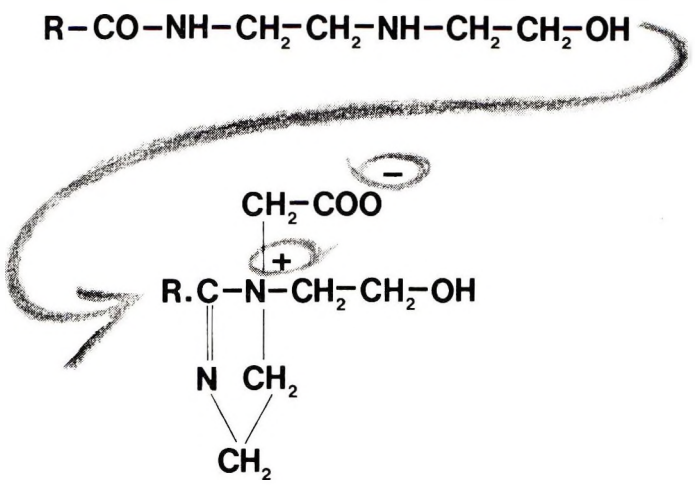
CHEMIST AND DRUGGIST DIRECTORY 1974

The Directory is a well-established reference source in general practice pharmacy; such sources could easily become outdated nowadays but the Chemist and Druggist Directory tries hard to avoid this. Some features are of specialized interest to pharmacists and medical practitioners, such as the comprehensive guide to tablet and capsule identification. Other sections are of more general utility, covering perhaps a wider range of information than that provided by the equivalent compendia specially prepared for use in the laboratory. For example, the Manufacturers & Suppliers Index includes chemical and machinery suppliers as well as manufacturers and distributors of packaging components and photographic goods; past experience suggests that the index, though not exhaustive, is helpful in providing details of the most likely sources for many laboratory needs, even rather off-beat ones; the 'buyer's guide' section is effectively classified in this respect, making it quite easy to locate suppliers for miscellaneous items like cycloserine, lobelia herb, metering pumps, laboratory mixers, rubber stamps and surgical dressings.

Other information of possible value to cosmetic chemists, includes details of a wide variety of professional and trade organizations and of the larger retail and wholesale outlets. The section giving forensic and general information might also prove useful at times, including as it does a good deal of information on the Poisons Rules and the Medicines Act. Sadly, the last page of the Directory, giving Postal Information, has already been outdated by the process of inflation.

Obviously there are some features of little interest to cosmetic chemists but a good deal, on the other hand, is certainly relevant and not always easy to locate elsewhere.

N. J. VAN ABBÉ



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