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J. Soc. Cosmet. Chem. **25** 297–306 (1974)

Studies of the factors controlling the action of hair sprays
II. The adhesion of hair spray resins to hair fibres

R. W. RANCE*

Synopsis—Measurements have been made of the ADHESION of HAIR SPRAY RESINS to HAIR FIBRES and the importance of adhesion in determining hair spray properties is discussed. The similarities between the technology in the bonded fabric field and in the hair spray field are considered and useful extrapolations concerning adhesion and other resin properties may be made. For good adhesion at least two requirements are apparently necessary. The viscosity of the resin solution must be low so that the resin remains in close interfacial contact with the fibre throughout the large dimensional changes occurring as the solvent evaporates. Also the resin itself must be sufficiently deformable to allow any stresses due to these dimensional changes to be dissipated and to enable the dried joint to withstand impact and bending. These factors, which emerge from earlier studies of adhesion, are found to be applicable in the present study.

INTRODUCTION

Polymer adhesion to wool fibres is known to be an important factor in the production of anti-felting properties on wool fabrics (1), and in the manufacture of non-woven textiles (2–4). Tikhomirov and Gusev have reported an investigation of the structural bonds and the properties

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of bonded fabrics, and consider that the strength of the bond between the binder and the fibre is one of the principal factors in the tensile strength of such fabrics (4). The strength of this bond depends mainly on the forces of adhesion which characterize the efficiency of the binder. These authors give results of measurements of the adhesion of polymeric binders to wool fibres and those results are among the first attempts at an experimental determination of the adhesion of polymers to natural fibres. Similar measurements on hair fibres do not seem to have been reported.

The requirements of a good adhesive have been defined (5, 6) as the ability to wet the adherend, to be solid at the temperature of usage, and to be sufficiently deformable to minimize stress concentrations during solidification. This latter requirement is also advantageous in the hairspray field since the resin bridges will be less liable to fracture during flexing of the hair fibres.

Good adhesion of a hairspray resin to hair combined with good tensile properties of the resin will promote strong and lasting hold, while poor adhesion will give poorer hold but easy elimination on brushing or combing.

The adhesive power of polymeric materials is normally assessed from tests on large specimens where the surface area of contact of the adhesive joint can be measured easily. Determination of adhesive strength becomes considerably more difficult when the adherend is a natural or synthetic fibre since the adhesive strength of a large specimen would be greater than the tensile strength of the fibre. The area of contact of the adhesive and the fibre surface must be kept small, therefore, but at the same time must be capable of accurate measurement.

Thus in measuring the adhesion of hairspray resins to hair fibres a technique must be used in which the fibres are introduced into a resin block so that the fibre is bonded along a given, measurable length. Such a technique has been developed by Shiryaeva, Gorbatkina and Andreevskaya (7) for the measurement of adhesive strength to glass fibres. This technique was adapted for use in the present study.

Experimental

A 10% w/w solution of hairspray resin was allowed to evaporate in a flat circular silicone rubber mould to yield a resin film about 300 μm thick. The dried film was cut into strips about 1 mm wide and 10 mm long while the film was still soft and flexible, and a small amount of the polymer

solution was applied to one side of two of the polymer strips. A single hair fibre was placed between and across the two strips, placed one on top of the other, so that it became cemented into a solid block of resin. Care was taken to ensure that the resin solution did not run along the fibre; the use of a fairly viscous solution helped to prevent this. In this way the fibre was coated with resin only over the width of the resin strip, i.e. about 1 mm. About 25 specimens were prepared for each resin studied. *Fig. 1* shows a completed specimen before testing.

The adhesion test specimens were left to dry for a few days and then the total surface area of adhesive bonding was measured for each specimen. The diameter of the fibre was measured by observation of the fibre cross-section under the microscope using an image splitting eyepiece (8). In order to do this the fibre was cut close to the point at which it emerged from the resin block on the side furthest from the root of the fibre and the end section at this point was examined. Owing to the well-known ellipticity of the hair fibres it was not considered sufficient to measure just one diameter. Instead the maximum and minimum diameters ($2a$ and $2b$) were measured and the circumference of the fibre calculated using the equation for the circumference of an ellipse:

$$\text{Circumference} = 2\pi\left(\frac{a^2+b^2}{2}\right)^{\frac{1}{2}}. \qquad (1)$$

The total surface area of the bond was then calculated by multiplying the circumference by the bonded length l. This bonded length was measured using a travelling microscope.

This method of measuring the bonded surface area took no account of the surface roughness of the fibres. It has not been possible to measure the true area of contact and all of the measurements are based on the assumption of a smooth-surfaced fibre.

After drying for 14 days the specimens were mounted in the jaws of an *Instron* tensile tester and extended at a constant rate of 15 mm min^{-1}. The load required to break the adhesive bond (F) was recorded and the adhesive strength of the specimen calculated from the formula:

$$\sigma_{ad} = \frac{F}{S} \qquad (2)$$

where σ_{ad} is the specific adhesion in kg cm^{-2}, F the load required to break the bond and S the surface area of the bond.

When testing the specimens the fibre was always pulled in the same direction; that is, the root end of the hair fibre was held in the lower pair of jaws and the resin in the other pair. In this way the motion of the fibre was with, and not against, the cuticle cells. This corresponds to the action of combing or brushing the resin out of the hair, when the resin is pulled away from the hair fibre by the comb moving towards the tip of the fibre.

On examining the specimens under the microscope after testing, a hole was usually clearly visible in the resin block where the fibre had been cemented (see *Fig. 2*) and no resin was left adhering to the fibre. This showed that the breaks were adhesive in nature.

Through all the tests a consistent behaviour was observed. In each case the specimens either broke down by adhesive failure at the resin-fibre interface or the adhesion was so great that the bond remained stable up to loads at which fibre breakage occurred. This latter behaviour was observed in a few cases where thin fibres and large bond lengths were encountered, but no examples of resin material fracture itself were observed. This is consistent with the observations of Hearle and Newton (3) on model systems of bonded fibres in their investigations on nonwoven fabrics.

Results

The value of adhesive strength for a particular resin was determined by taking the arithmetic mean of about 25 specimens. *Fig. 3* shows a typical set of results for hair spray resin C plotted as surface area of bonding against specimen break load. Although there is scatter in the results, as with all adhesion measurements, the correlation between surface area and break load can be clearly seen. The larger the bonded surface area the greater the break load of the specimen.

Fig. 4 shows the same data plotted as a distribution curve of adhesive bond strength against frequency. The frequency, Δn, is the number of specimens with strengths within a given interval. The specific adhesion was divided into intervals of 2.5 kg cm^{-2} and the resulting frequencies referred to the midpoints of the intervals.

Several commercially available hair spray resins were studied by the above method. *Table I* lists the adhesive strengths of these resins together with the number of specimens used in each case, and the average total surface area of the bonds, S. The final column of *Table I* lists the glass transition temperatures (T_g) for each of the resins.

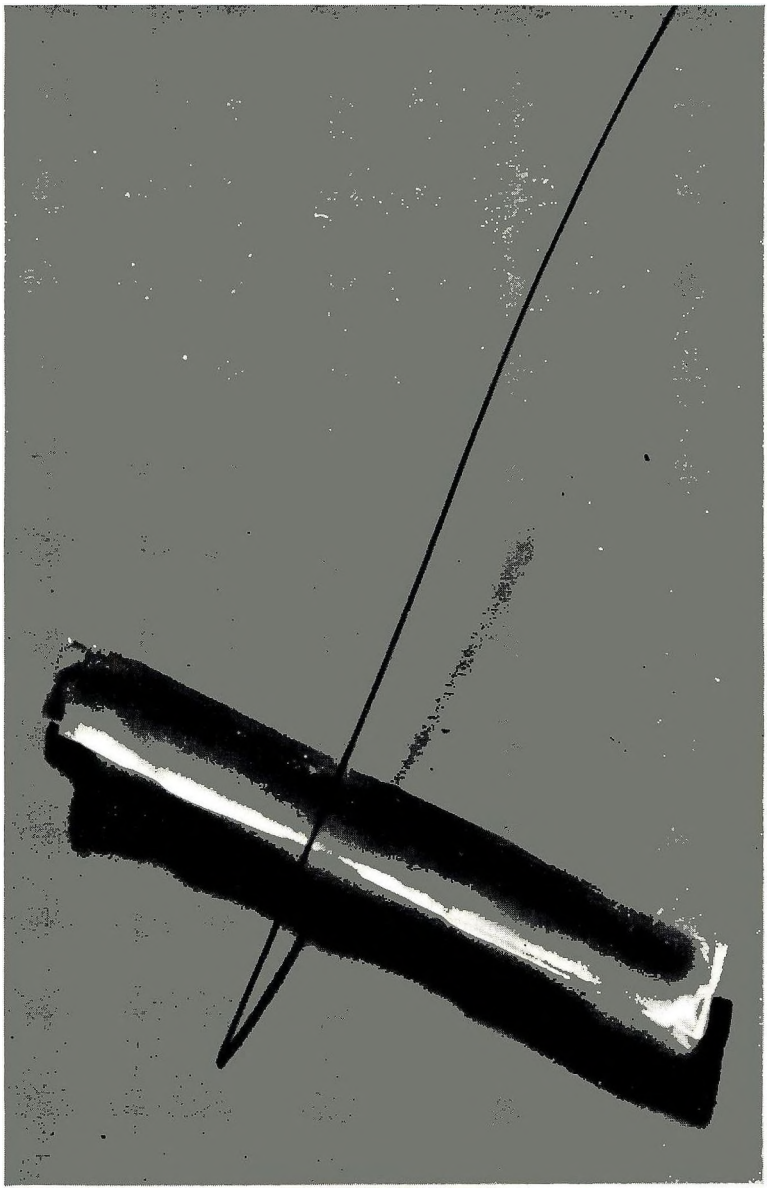

Figure 1. Completed adhesion test specimen before testing. ×7.

(*Facing p.* 300)

Figure 2. Resin block after withdrawing the hair fibre in the adhesion test. Adhesive break. (View from above.) × 200.

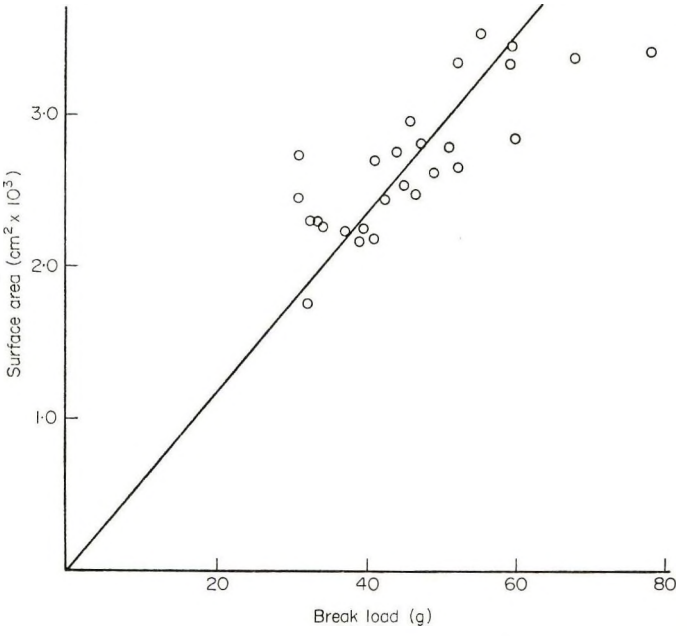

Figure 3. Results of adhesion test on 27 specimens of resin C. Plotted as surface area of bond against break load.

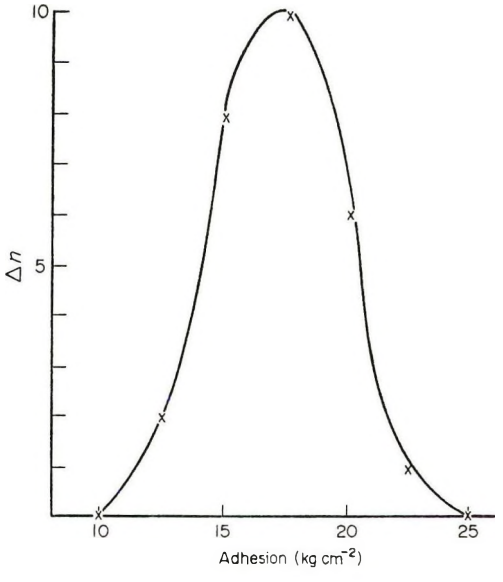

Figure 4. Adhesion of resin C to hair fibres. Adhesive strength distribution curve. (The frequency, $\triangle n$, is the number of specimens with strengths within a given interval. The adhesion was divided into intervals of 2.5 kg cm^{-2} and the resulting frequencies referred to the midpoints of the intervals.)

Table I. Experimental results for adhesion of hair sprays to hair fibres

Resin	S_{av} ($cm^2 \times 10^3$)	No. of specimens	σ_{ad} ($kg\ cm^{-2}$)	T_g (°C)
E	2.88	29	21.8±3·3	24
H	2.88	22	18.9±2.9	32
D	2.88	26	18.5±2.5	40
C	2.68	27	17.1±2.5	47
F	2.85	24	16.8±3.3	—
A	3.01	21	16.6±3.6	42
I	2.57	28	15.4±2.2	59
B	3.05	25	15.0±1.9	51
G	2.80	27	14.5±2.7	34

It is interesting to consider the relationship between the adhesive strengths and the glass transition temperatures of the resins. This relationship is shown in *Fig. 5*. It will be seen that generally the softer resins (lower T_g) have the higher adhesive strengths.

Another relationship worthy of consideration is that between the adhesive strength of the resin and the viscosity of its solution. *Table II* shows the viscosities of certain of the resin solutions in ethanol at 40% w/w concentration. These measurements were carried out using a Ferranti cone and plate viscometer.

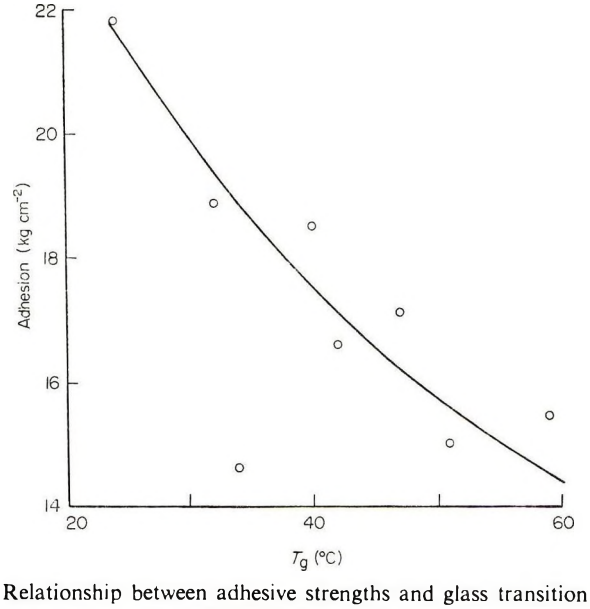

Figure 5. Relationship between adhesive strengths and glass transition temperatures (T_g) of hair spray resins.

Table II. Comparison between adhesive stengths of resins and the viscosities of 40% w/w solutions of the resins in ethanol

Resin	Adhesive strength (kg cm^{-2})	Viscosity at 40% w/w (cP)
E	21.8	360
D	18.5	450
B	15.0	450
C	17.1	630
F	16.8	925
G	14.5	1900

Discussion

The importance of fibre wettability and adhesion of resin to the fibres is now well accepted in the fabrics field. In view of the similarity between hair spray action and the formation of bonded fabrics it seems useful to consider such factors in the hair spray situation. Wettability and spreading have been considered in a previous communication (19) and adhesion of hair spray resins to hair fibres has been the subject of the present study.

Previous work by several authors on the adhesion of high polymers to such substrates as cellulose give useful indications as to the explanation of the present observations. Thus, McLaren (9) found that other things being equal (e.g. dipolarity and chemical composition of the polymers) the lower the viscosity of a material the more likely will it remain adapted to the interface during evaporation of the solvent. The lower the viscosity the more will the adhesive forces predominate over the cohesive forces within the adhesive which would tend to disrupt dipole-dipole attraction at the interface (10, 11). The transition from a solution of resin to a condition of solvent-free resin involves large dimensional changes and the material must possess sufficiently low viscosity at relatively high resin concentrations in order to remain in intimate contact with the surface during evaporation (12).

Inspection of *Table II* shows that in the main these trends are borne out by the experimental data. The lower the viscosity of the solution the stronger the adhesive joint.

'Deformability' proposed by McBain and Lee (13) expresses a highly

desirable property of an adhesive. Deformability of the dried resin film enables the joint to withstand impact or bending. Deformability during drying is important to the formation of strong joints. During drying internal stresses can be set up in the adhesive material if it is not deformable. The stresses are formed by the volume changes and when the joint is subsequently stressed it can, in extreme cases, fly to pieces.

If we can assume that deformability is related to the softness of the resin as measured by the glass transition temperature, then the above trend is also borne out by the present experimental data.

The surface of an adherend is rarely perfectly smooth but has numerous small interstices. For complete wetting of the surface by the adhesive these interstices must be filled by the liquid. Polymer-solvent mixtures often become viscoelastic solids even with as much as 15% of solvent remaining and with further loss of solvent the mixture passes through its glass transition temperature. Stresses then begin to arise at the adherend/adhesive interface and these stresses diminish the external force required to break the adhesive joint.

In the case of incomplete wetting of the adherend surface some of the interstices are not filled with adhesive before the mixture passes through its glass transition temperature. The stresses are then localized at the edges of these interstices. The lower the viscosity of the adhesive mixture the faster the spreading and the easier it is for the interstices to be filled.

When the adhesive completely wets the adherend before it passes through its glass transition temperature, the stresses are not localized at the edges of the interstices and a stronger joint results. It follows that low viscosity and low glass transition temperature both promote good adhesion.

A commercial application of the above principles has been demonstrated by Alexander (14). It was shown that polymethyl methacrylate and polystyrene can render wool unshrinkable if the correct quantity of plasticizer is present. The concentration of plasticizer was found to be critical. Starting with pure resin the shrinkage of wool decreased from 31.8% to 5.4% at 30% diethyl phthalate content and then rose again as more plasticizer was included. Similarly copolymers of butadiene and methyl methacrylate, within a narrow range of compositions, were capable of producing excellent non-shrinkability, while the two homopolymers, and copolymers of the wrong composition, did not produce the effect. The physical properties of the polymer obviously play a vital part in obtaining the desired effects and the polymer must be neither too hard and brittle, nor too soft and rubbery.

Conclusions

The technology of non-woven bonded fabrics and of the production of fabrics with anti-felting properties may be usefully compared to the processes occurring when hair sprays are applied to hair. Thus, Möschler (15) and Tikhomirov (16) have both demonstrated that the production of bonded fabrics involves the formation of resin bridges between fibres and at fibre intersections.

The resin bonds in non-woven fabrics can be formed in the same manner as those formed when using a hair spray, i.e. from a solution of the resin, or by alternative techniques such as the use of polymer melts or aqueous dispersions.

Various workers have attempted to characterize the types of bonding found in bonded fabrics (17, 18). There appear to be three main types of bond; simple, parallel and complex (17). The simple bonds involve only two fibres, approximately at right angles; parallel bonds involve two fibres bonded side by side, while complex bonds involve more than two fibres which are sufficiently close to be included in the same resin bond.

Measurements of adhesion of hair spray resins to hair fibres have shown that for several commercially available resins the adhesive strengths are in the range 14–22 kg cm^{-2}. Tikhomirov and Gusev (4) have reported the value 40.1 kg cm^{-2} for the adhesion of epoxy resin to wool fibres.

Certain general trends are noticeable from the results. For example, the greatest adhesion is found for resins which give solutions of lower viscosity. This is in agreement with previous observations on the adhesion of high polymers to such substrates as cellulose and it is considered essential that a good adhesive should have a low viscosity in solution in order that the resin remains in intimate contact throughout the dimensional changes accompanying the loss of solvent. Intimate interfacial contact is essential for good adhesion since the adhesive bonds act only over very small distances.

Similarly the adhesive strength shows a general increase with the softness or deformability of the resin. Again reference to earlier adhesion studies shows that deformability allows stresses, set up on drying of the adhesive, to be dissipated. If such stresses cannot be dissipated they may cause shattering of the joint when any subsequent stress is applied. Deformability is also needed to allow flexing or bending of the joint. This latter condition will promote lasting hold in a hair spray.

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REFERENCES

(1) Feldtman, H. D. and McPhee, J. R. The spreading and adhesion of polymers on wool. *Text. Res. J.* **34** 634 (1964).
(2) Tikhomirov, V. B. The influence of adhesion on the strength of cemented fibrous materials. *Dokl. Akad. Nauk. SSSR* **167** 207 (1966).
(3) Hearle, J. W. S. and Newton, A. Non-woven fabric studies. XVI: The behaviour of model systems of bonded fibres. *Text. Res. J.* **38** 488 (1968).
(4) Tikhomirov, V. B. and Gusev, V. E. Analysis of the structural and mechanical properties of bonded non-woven fabrics. *Tech. Text. Ind. USSR* **1** 103 (1966).
(5) McBain, J. W. and Hopkins, D. G. *J. Phys. Chem.* **29** 188 (1925).
(6) McBain, J. W. and Lee, W. B. Adhesives and adhesion. Mechanical properties of films of adhesives. *Ind. Eng. Chem.* **19** 1005 (1927).
(7) Shiryaeva, G. V., Gorbatkina, Yu. A. and Andreevskaya, G. D. Methods for determining the adhesion of polymers to the surface of glass fibres. *Russ. J. Phys. Chem.* (English trans.) **37** 123 (1963).
(8) Rance, R. W. Particle size distribution measurement of hair sparys using an image-splitting particle size analyser. *J. Soc. Cosmet. Chem.* **23** 197 (1972).
(9) McLaren, A. D. Adhesion of high polymers to cellulose. *J. Polymer Sci.* **3** 652 (1948).
(10) Josefowitz, D. and Mark, H. Stickiness, tackiness and spinnability. *India Rubber World* **106** 33 (1942).
(11) McLaren, A. D. and Hofrichter, C. H. Temperature dependence of the adhesion of high polymers to cellulose. *Ind. Eng. Chem.* **40** 329 (1948).
(12) McLaren, A. D. and Hofrichter, C. H. Theory of the adhesion of high polymers to cellulose. *Pap. Trade J.* **125** 96 (1947).
(13) McBain, J. W. and Lee, W. B. Adhesives and adhesion: relation of joint strength to tensile strength of films. *J. Soc. Chem. Ind. London* **46** 321T (1927).
(14) Alexander, P. Non-shrink processes for wool. *J. Soc. Dyers Colour.* **66** 351 (1950).
(15) Möschler, W. The structure and properties of bonded fabrics. *Deut. Textiltech.* **18** 74 (1968).
(16) Tikhomirov, V. B. Manufacture and properties of non-woven textile materials made with thermoplastic binders. *Deut. Textiltech.* **17** 300 (1967).
(17) Hill, D. D. and Michie, R. I. C. Non-woven fabric studies. IX: Characterization of binder distribution. *Text. Res. J.* **36** 787 (1966).
(18) Zeronian, S. H. and Wilkinson, J. Non-woven fabric studies XI: Binder distribution effects. *Text. Res. J.* **36** 866 (1966).
(19) Rance, R. W. Studies of the factors controlling the action of hair sprays. *J. Soc. Cosmet. Chem.* **24** 501 (1973).

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Relative merits of 'in use' and laboratory methods for the evaluation of antimicrobial products

R. A. COWEN*

Presented on 13th November 1973 in Nottingham at the Symposium on 'Evaluation of Product Performance', organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The relationship between the resistance of cultures grown in the laboratory and on the skin is examined in the light of factors known to influence RESISTANCE OF BACTERIA under laboratory culture. Examples are taken from tests designed to measure the efficacy of HAIR SHAMPOOS, DEODORANTS, BATH ADDITIVES and ANTISEPTICS to show that little correlation exists between activity measured in the laboratory and in practice. Both LABORATORY TESTS and 'IN-USE' TESTS should be carried out side by side as a means of identifying factors which influence the results. With this information design of more realistic tests should be possible.

Introduction

The methods used for the evaluation of the antimicrobial activity of a product can be divided into three groups.

Simple laboratory tests.

Simulated 'in-use' tests.

In-use tests.

The latter, by definition, refer solely to the evaluation of the product under conditions of usage and not for the description of other types of

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test (e.g. simulated 'in-use' tests). The distinction between the simple laboratory and simulated 'in-use' test is more difficult. It may be argued that any modification of a simple laboratory test, for instance by adjustment of pH, or by addition of body fluids, which bring it closer to the conditions of usage convert the test from a simple laboratory test to a simulated 'in-use' test. Equally, whilst a particular test may be classified as an 'in-use' test for one group of products the same test can only be regarded as a simulated 'in-use' test when applied to a totally different group of products designed for a different purpose. Thus the 'Glove Juice Test' as used by Lowbury, Lilly and Bull (1) is an 'in-use' test when applied to the evaluation of a surgical scrub. However, if the same test is applied to the evaluation of some other type of antimicrobial product, designed for instance as a deodorant, the test must be regarded as a simulated 'in-use' test since factors are present in the test which will not be present in the 'in-use' situation. For the purposes of the present paper simulated 'in-use' tests will be considered along with laboratory tests, and these will be compared with true 'in-use' tests.

The trend, in evaluating finished products making antibacterial claims, is to move away from the use of laboratory and simulated tests and to employ whenever possible 'in-use' tests. This follows the belief that only an 'in-use' test can positively evaluate the true antimicrobial activity of a product. Unfortunately, 'in-use' tests are more difficult to perform than laboratory tests or simulated 'in-use' tests. Moreover, they involve considerably more time and usually a panel of volunteers is required. Consequently, laboratory tests are still widely used during the development stages of a product. It may be argued, however, that if these tests fail to evaluate the final product satisfactorily, they may equally well fail to select the most suitable product during the development period.

The reason for the failure of laboratory tests in the evaluation of an antimicrobial product rests largely with a lack of knowledge of the factors governing performance in actual practice. Once these factors are established there is little doubt they can be applied to design of more realistic *in vitro* methods. At the same time more information is required of the factors influencing performance in *in vitro* methods. By this interplay it should be possible to design much more realistic laboratory tests, but in order to achieve this there is a need for both laboratory tests and 'in-use' methods to be carried out side by side. Illustrations from laboratory tests and 'in-use' tests being carried out by the author's laboratory are used to indicate the kind of factors which influence performance.

Resistance and sensitivity of bacteria

Simple laboratory tests are usually performed according to the rules of well-established protocols and strict attention is usually paid to details like the temperature at which bacteria are grown, the temperature at which the test is carried out, the age of the culture etc., but attention to this kind of detail is not sufficient.

Our knowledge of the relationship between the resistance of a culture of a particular organism grown in the laboratory and the same organism present on the skin is very scant. *Table I* illustrates the concentration of parachlorometaxylenol (PCMX) required to kill the same strain of *Pseudomonas aeruginosa* when grown in two different culture media. A factor of 10 is involved in the concentration effectively killing the culture yet the only difference between the two culture media resides in the concentration of magnesium ions. The media growing (*Table I*) the more resistant culture

Table I. Concentration of PCMX required to kill culture of *Ps aeruginosa* in medium containing either 4 ppm Mg^{2+} or 40 ppm Mg^{2+}

Organism	Culture medium contains	
	40 ppm Mg^{2+}	4 ppm Mg^{2+}
Ps aeruginosa (NCTC 1999)	2.4 mg g^{-1}	0.15 mg g^{-1}
Ps aeruginosa (recent isolate)	1.5 mg g^{-1}	0.12 mg g^{-1}

contains 36 ppm more Mg^{2+} ions than the media growing the sensitive culture. This is a very minor difference and unless one is aware of the problem it can be completely overlooked. Yet the penalites for doing so may be to reject a worthwhile antibacterial agent. This phenomenon has been repeated with every strain of *Ps aeruginosa* examined within the author's laboratory, including new isolates from patients suffering from *Pseudomonas* infections.

Whilst these results are obtained in a synthetic medium, in which careful control can be maintained over each of the ingredients, an identical situation can be achieved with the more complex nutrient media normally employed in bacteriological laboratories, simply by removing certain ions by the use of ion exchange columns and then replacing them at the required levels. Moreover, differences in the resistance of *Pseudomonas aeruginosa* cultures grown in different complex culture media (e.g. Oxoid nutrient

broth and Oxoid nutrient broth No. 2) to PCMX and other halogenated phenols can be explained in part by the magnesium content of the media. The effect, however, is more complex and phosphate ions have been shown to influence still further the resistance of the culture.

If *Pseudomonas aeruginosa* is transferred from a sensitive to a resistant type media, the culture becomes resistant within a few divisions and *vice versa*. The implications of these findings are important because they indicate that culture of this bacterium in the laboratory, even over a short period of time, can modify the resistance of the bacterium to such an extent that it cannot be related to the resistance of the organism in its natural state.

The influence of the growth medium on resistance is not confined to *Pseudomonas aeruginosa*. The incorporation of either glucose or glycerol into the medium used for the growth of *Staphylococcus aureus* also causes an increase in the resistance of the organism to a number of antibacterial agents which are lypophilic in nature (2, 3). This increase in resistance can be related to an increase in the lipid content of the outer surfaces of *S. aureus*. Whether staphylococci and micrococci, which are present on the skin, possess substantial quantities of lipid in their outer coat has not yet been established and so the question must remain unanswered as to whether glucose should or should not be added to the media used for the growth of micrococci and staphylococci for testing antimicrobial products.

Other, less well defined, factors also have a bearing on the resistance of staphylococci grown in the laboratory. Thus, filtering the growth media through filter paper during its preparation can have a profound effect on the subsequent culture dependent upon the type and grade of filter paper used *(Table II)*.

The difference in resistance probably arises from the absorption of certain minor components of the growth media on to the paper, but the difference highlights the influence that even minor components of a growth medium can exert on the resistance of a bacterium.

Table II. Sensitivity of *Staphylococcus aureus* to PCMX when grown in FDA Broth filtered through different types of filter paper

Filter paper	Concentration of PCMX required to kill culture in 10 min but not in 5 min
Green 803	0.45 mg g^{-1}
Whatmans No. 41	3.0 mg g^{-1}

The above observations are important for another reason also. The resistance or sensitivity shown by the bacterium is not universal to all antimicrobial agents, but is specific to certain types or groups of agent. Failure to recognize that such factors are imposing a possible unnatural resistance or sensitivity on a bacterial culture can lead to the selection of an agent which, whilst performing well in laboratory tests, can perform less satisfactorily in practice. Several factors have been highlighted here, but the question remains how many more, so far unrecognized, factors are influencing the resistance of the cultures used in the laboratory?

Age of culture

Once a factor influencing resistance is established steps can be taken to control it. For example, the age of the culture often has a bearing on the resistance of an organism, and it is generally reported that older cultures are more resistant than younger ones. However, this is by no means true and the reverse may be equally true for certain bacteria. Thus, the individual worker must decide, very often quite arbitrarily, whether to use a young or old culture. Either way he cannot be certain whether the resistance is related to the resistance existing in practice.

Numbers of bacteria used in test system

It is well known that the number of organisms used in a test may considerably affect the results obtained, particularly if the antibacterial agent is one which is highly active by virtue of a large proportion of the molecules binding to the active site. In laboratory testing less emphasis is usually placed on using realistic numbers of bacteria, more emphasis being placed on using a standard number, somewhat higher than those known to occur naturally. It is believed that the excess numbers may offset to some extent the exclusion from laboratory tests of other inactivating factors known to occur in practice. Eighteen to twenty-four hour cultures grown in a nutrient broth are often used on the assumption that the numbers of bacteria from such a culture remain fairly constant. This assumption is, however, only correct if the culture is treated in an identical manner each time it is grown. For instance, a 24-h static culture of a typical test bacterium grown for the testing of an antiseptic will produce approximately 2×10^8 organisms/g.

However, if this tube is removed from the incubator 3 or 4 h before it is used and gently shaken to examine the growth the numbers of bacteria may increase to 5×10^9 or greater by the time the culture is ready to use. As oxygen is often the rate limiting factor in a static culture containing 10 ml of broth in a tube of $\frac{3}{8}$–$\frac{1}{2}$ in diameter, the gentle shaking action has the effect of aerating the medium and permitting further growth to take place. Thus an unsuspecting operator may unwittingly alter the performance of a product merely by excessive keenness.

pH

Quite apart from the resistance of the bacterium other aspects may also play an important part in defining the activity of the product as measured in the laboratory.

The pH of conventional culture medium used for growth of bacteria usually ensures that testing is carried out around pH 7 or thereabouts unless the product is sufficiently buffered at either lower or higher pH to effect a change in the pH of the culture medium, or alternatively unless steps are taken to buffer the test medium to some alternative pH. A test carried out

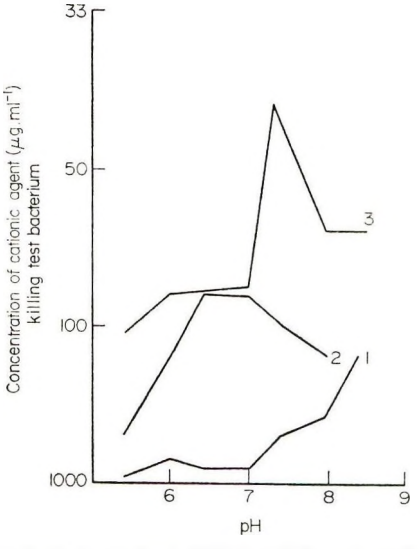

Fig. 1. The relationship between bactericidal activity of a cationic antimicrobial agent and the pH of the test medium. 1, *Staphylococcus aureus.* 2, *Pseudomonas aeruginosa.* 3, *Escherichia coli.*

at pH 7 may give a false impression of activity for cosmetics, the extent of which will depend on the pH profile of the antimicrobial agent incorporated into the product. A pH closer to that of the skin is to be preferred.

Cationic substances in particular show a marked diminution of activity at acid pH as may be seen in *Fig. 1* which shows the relationship between the pH and activity of a cationic antibacterial agent.

Between the range pH 5 and pH 8 this compound suffers as much as a four-fold reduction in activity when examined by a laboratory test.

A similar pH dependence may be seen if the compound is tested on skin which has been artificially infected with a series of test bacterium *(Table III)*.

Table III. Influence of pH on the performance of a cationic agent (500 μg ml^{-1}) when skin is artificially infected with a series of test bacterium

pH of skin	Mean percentage reduction in bacteria achieved 5 min after application of culture. Cationic agent and buffer applied to skin 1 h previous before addition of bacteria		
	S. aureus	*E. coli*	*Ps aeruginosa*
4.5	34	14	5
6.5–7.5	87	97	88

Even products which are designed to maintain the acid mantle of the skin may show, particularly upon dilution, a wide variation between the pH appertaining at the skin site and in the laboratory test tube as is indicated in *Table IV*. For those products whose activity is not materially affected by pH the difference of two units is probably insignificant, but with other products based upon antimicrobial agents which are pH sensitive a difference of two units may have a significant bearing on the results obtained.

Table IV. Influence of culture on pH of product

Product tested	pH of solution before addition of culture	pH of dilution after addition of culture	pH of dilution when applied to skin
Neat	5.7	5.7	5.9
1 : 3	6.0	6.3	5.7
1 : 10	6.2	6.3	5.4
1 : 100	6.7	7.0	5.0

pH of culture = 7.2. pH of skin = 5.0.

Effect of substances present on the skin

Currently laboratory evaluation of a product suffers from the disadvantage that it cannot take account of all the substances present on the skin which might influence the performance of the antimicrobial agent. Organic excretions from the skin can be demonstrated, in laboratory tests, to inactivate many antibacterial agents, yet these same antimicrobial agents when applied to the skin exert an antibacterial effect. The reasons for this are simple; they relate to the relative quantities of the excreted material. The quantity of substances such as sebum, fatty acids, proteins and soap, etc. present on the skin are relatively small, and because of this the antibacterial agent is able to act competitively between these substances and the bacterial cells. In most laboratory tests in which attempts are made to represent the situation appertaining on the skin, material representing organic excretions is usually used in excess and moreover is often added to the product prior to the addition of the bacterial culture. This latter action in itself can have a profound effect as may be seen from *Table V* in which PCMX is shown to be eight times more active when 10% blood is added along with the culture rather than prior to the addition of the culture.

Table V. Concentration of PCMX killing *Staphylococcus aureus* in the presence of 10% blood when the blood is added before the culture and with the culture

	Concentration of PCMX required to kill 0.5 ml 24 h culture added to 5 ml of PCMX solution plus 0.5 ml blood
Before the culture	4.8 mg ml^{-1}
With the culture	0.6 mg ml^{-1}

Although the quantity and quality of organic excretions on the skin may differ from person to person and in the individual on different sites and at different times it is necessary to establish these limits so that these limits may be applied in time to both the laboratory and simulated 'in-use' tests.

Design of tests

Design of test, be it a laboratory test, simulated 'in-use' test or 'in-use' test, is very important. However, it is often impossible to predict beforehand some of the factors which might be involved.

In designing antiseptics it is desirable that the working strength solution should give a 100% reduction in bacteria. Consequently, methods like the Rideal Walker Test, Chick-Martin Test and the A.O.A.C. Phenol Coefficient Tests (suitably modified to take account of organisms which are currently a problem) are used in the evaluation of the product. It is usual on the basis of these results to fix the 'in-use' concentration of the product, allowing, of course, a considerable safety margin and then to check this both in simulated and in 'in-use' tests.

In designing a hair shampoo for which claims can be made about the reduction of bacteria on the hair it would seem logical to carry out a similar type of test using a species likely to be representative of the most prominent bacterium to be encountered in practice.

A simple test was carried out on a number of possible formulations in which the extent to which the product could be diluted and yet still kill

Table VI. Comparison of performance between a formulation containing a number of different antibacterial agents (a) by a simple laboratory procedure and (b) by an 'in-use' test

Product	(a) Degree by which shampoo may be diluted and yet still kill *Staph. aureus* in 10 min but not in 5 min	(b) Average reduction in colony forming units from switches of hair measured before and immediately after shampooing	
		Week 1	Week 2
Shampoo base plus 0.2% *Bronopol**	1 : 30	98%	89%
Shampoo base plus zinc *Omadine*	1 : 200	93%	88%
Shampoo base plus 0.2% *Bronopol*+ 0.25% hexachlorophane B.P.	1 : 10	92%	87%
Shampoo base plus 0.2% *Bronopol*+ 0.25% *Fentichlor*†	Less than 1 : 5	80%	82%
Shampoo base plus 1.6% Iodophor	Less than 1 : 5	75%	81%
Shampoo base (no germicide)	Less than 1 : 5	35%	—
Water	—	5%	—

* *Bronopol*—The Boots Co. Limited, Nottingham.

† *Fentichlor*—The Cocker Chemical Co., Oswaldtwistle, Lancashire.

Staphylococcus aureus in 10 min but not in 5 min was determined. The results from this test are compared with the percentage reduction obtained from an actual 'in-use' trial in which the number of bacteria recovered from adjacent switches of hair immediately prior to and immediately after shampooing were determined *(Table VI)*.

There is little correlation between the laboratory test and performance in practice. From the laboratory test it would be assumed that the base containing zinc *omadine** would perform significantly better than the other products, but from the 'in-use' test this is not the case. The laboratory test is inadequate since it measures only the concentration of product giving approximately a 99.997% or greater kill at a time between 5 and 10 min under idealized conditions. It gives no information as to the activity of the 'in-use' dilution which is the important concentration.

Measurement of the antimicrobial activity of bath products by laboratory methods and in-use methods

The importance of working with the use dilution may be seen when comparing the activities of several possible bath additive products containing different antimicrobial agents.

By applying the simple laboratory method used above, to determine the extent to which the products can be diluted and yet kill *Staphylococcus albus* in 10 min but not in 5 min, it is assumed that the product based upon PCMX would perform better than either a product based upon cationic agents or upon *Vespedol*† *(Table III)*. The 'in-use' results, however, do not bear out these findings. As with the previous example, the use-dilution has not been studied and moreover only a percentage kill of the order of 99.997% or greater has been measured. There is need to know the relationship between a variety of dilutions and percentage kill (*Table VII*).

When the percentage kill for each of the antimicrobial agents in the bath additive products is plotted as a function of the dilution a totally different picture emerges *(Fig. 2)*.

Vespedol, whilst requiring a relatively high concentration to give a 99.997% kill, reduces the number of bacteria to below 99% at a very low concentration, 'the slope of the graph between 99 and 100% being almost horizontal. The cationic substance shows a similar but less pronounced

* Olin Corporation.

† Registered Trade Mark, Reckitt & Colman.

Table VII. Comparison of antimicrobial activity of three bath additive products in a simple laboratory test and in an 'in-use' test

Product	Laboratory test	'In-use' test	
	Extent to which the product may be diluted yet kill *S. albus* in 10 min but not in 5 min	Percent reduction in numbers of bacteria recovered from skin and bath water after using product compared with soap and water control	
		Skin	Bath water
Bath additive with PCMX	1 : 1500	53 (26–72)	63 (62–65)
Bath additive with QAC	1 : 800	69 (51–89)	78 (55–90)
Bath additive with *Vespedol*	1 : 140	81 (76–87)	95 (90-99)

Figures in brackets represent maximum and minimum reduction.

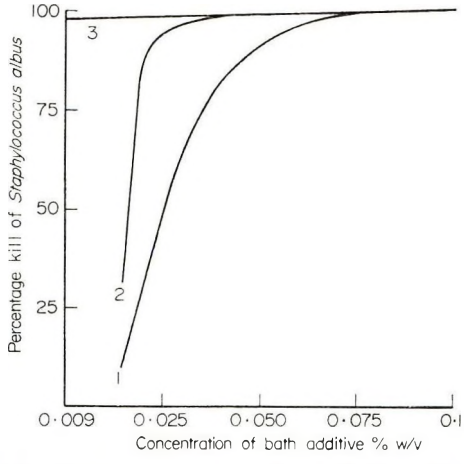

Figure 2. The relationship between concentration of bath additive and percentage kill of *Staphylococcus albus* at 40°C as determined by a simple laboratory procedure. 1, Bath additive with PCMX. 2, Bath additive with QAC. 3, Bath additive with *Vespedol*.

effect, whilst PCMX shows a curve more typical of a bactericidal agent. Abstraction of the percentage kills achieved by a dilution of 1 : 4400 (the concentration used in the 'in-use' test) indicate that the product based upon *Vespedol* is likely to be the most active, followed by the product based upon the cationic agent and in turn by the PCMX based product *(Table VIII)*.

Despite the good agreement with the 'in-use' results the laboratory test belies the 'in-use' performance to some extent, indicating a slightly greater activity for *Vespedol* and a considerably weaker activity for PCMX than occurs in practice. The slightly higher figure for *Vespedol* can probably be

Table VIII. Percentage kill given by bath additives at a dilution of 1 : 4400 as abstracted from results obtained in a laboratory test designed to measure percentage kills at different dilutions

Product	Percentage kill of *Staph. albus* at 1 : 4400
Bath additive with PCMX	25%
Bath additive with cationic substance	80%
Bath additive with *Vespedol*	99.97%

accounted for by the idealized situation exemplified by the laboratory test, but similar results would be expected for the other two products. This, however, is not the case and with PCMX it is difficult to account for the better performance in practice. Certainly, within the laboratory test, no account is taken of the effect of substances present on the skin, nor the accumulation, if any, of the antibacterial agent on the skin. Measurements indicate that this is very low and, moreover, such a situation would not account for the better performance exhibited by PCMX in the bath water as well as on the skin. It must be noted that the laboratory results are based upon a single strain of *Staphylococcus albus*, whereas the skin is host to a number of differing micrococcal species, as well as other bacterial species including diphtheroids.

The majority of normal skin flora when grown in the laboratory differ little in resistance to any one antimicrobial agent, although resistance to different antimicrobial agents varies considerably. However, it is not known how resistance of the various organisms actually on the skin differ in respect to an individual agent or how this resistance matches the resistance of laboratory cultures. Neither is the effect known of the influence of mixed populations on the overall resistance of the bacterial species present on the skin. There is some indication from laboratory tests that mixed populations are less resistant than the individual bacterial species making up the mixed population, but the information to date is rather scant. Such differences may well account for the discrepancy between the two sets of results.

Axillae odour and bacterial numbers

Axillae odour is according to Shelley, Hurley and Nichols (4) and other workers, the result of bacterial action on apocrine sweat. Consequently,

many deodorants make use of antibacterial agents as a means of reducing bacterial breakdown of the apocrine sweat with a view to reducing odour level.

In order to establish whether a significant reduction in axillary odour could be correlated with the antibacterial action of a product, a number of volunteers were asked to wash under the axilla with plain soap and water. The axilla were then covered with a pad which was removed 6 h later and assessed for odour by six assessors using a rating method on a 5-point scale. At the 6 h period the axilla were also sampled for bacterial flora by use of a modified glass cylinder method introduced by Story (5).

After 5 days each volunteer was supplied with a deodorant based upon hexachlorophane and asked to repeat the experiment, this time applying the deodorant to only one of the axilla after washing both axilla with plain soap and water. The remaining axilla served as a control. *Table IX* shows the results obtained for each subject. The log ratio of the bacterial count for the control axilla to that for the test axilla was taken as a measure of efficacy. The significance of this ratio was measured against the ratio for the control situation in which an antibacterial agent was applied to neither axilla. Similar criteria were applied to the odour level.

The results in *Table IX* indicate only a low degree of correlation. Although as yet the reasons for this have not been pinpointed they

Table IX. Correlation between antimicrobial activity of a hexachlorophane based deodorant and its effect in reducing axillary odour

Subject	Antibacterial assessment		Deodorant assessment		Degree of correlation
	Change in log ratio	*t* test	Change in odour	*t* test	
1	4.0	NS	−1.1	*	−
2	7.57	**	−0.5	NS	−
3	−0.70	NS	+0.1	NS	+
4	11.53	***	−0.5	NS	−
5	6.99	**	−1.3	*	+
6	5.70	**	−0·8	NS	−
7	3.79	NS	−0.9	NS	+
8	9.49	***	−1.4	**	+
9	8.97	***	−1.9	*	+

*** The difference is significant at 0.1% level.
** The difference is significant at 1% level.
* The difference is significant at 5%.
NS The difference is not significant.

undoubtedly lie with the antibacterial assessment. The number of bacteria present on the axilla at the time of sampling is relatively unimportant. What is important is the number of bacteria present which are capable of breaking down apocrine sweat. Since hexachlorophane is used as an antibacterial agent it is probable that a number of the bacteria isolated from the axilla are in a state of bacteriostasis and only begin metabolic activity, growth and division once transferred to the agar plate. In order to assess whether this is the case, fairly frequent sampling of the area is necessary to determine whether growth is taking place. However, the very nature of sampling serves to deplete the bacteria already present and to dilute and neutralize the action of the antibacterial agent. Quite clearly a very sophisticated system of measuring the antibacterial effect is necessary, preferably in which the metabolic state of the organisms can be measured on site. In the meantime until such methods are available, by far the easiest method of measuring the efficacy of this type of product is to determine its control in odour production.

Assessment of products intended as antiseptics

The assessment of the antibacterial activity of four products intended for use as antiseptics, by a laboratory test, a simulated 'in-use' test and an 'in-use' test highlights the problems facing anyone trying to design a test.

Measurement of activity within the laboratory by the simple technique of determining how far the product can be diluted and yet still retain activity (loop sampling method), indicates that three out of the four products meet the requirements of a good antiseptic. The fourth does not reach the necessary level *(Table X)*.

However, when the activity is measured by a simulated 'in-use' test in which *Staphylococcus aureus* is painted onto the skin and allowed to dry before application of the antiseptic, subsequent sampling 5 min later reveals that the product failing the loop sampling method reduces the level of bacteria by 100%. An iodophor preparation brings about the same reduction, but the other two products produce a smaller percentage reduction. The situation becomes more complex because the 'in-use' test reveals that none of the products reduce the level of bacteria by 100%, but that the product failing the loop sampling method reduces the level by a mere 28%. The iodophor preparation which performs best in the simple laboratory test performs relatively weakly in the 'in-use' situation, whilst the

Table X. Comparison of the activity of four antiseptic solutions as measured (a) by a laboratory test, (b) by a simulated 'in-use' test and (c) by an 'in-use' test

Antiseptic solution	In-use concentration	Ratio of killing dilution to recommended 'in-use' dilution	Percentage reduction in bacteria artificially applied to forearm (simulated 'in-use' test)	Percentage reduction in bacteria on skin ('in-use' test)
Chloroxylenol B.P.	0.12%	7.5	92	77
Chlorhexidine B.P.	0.05%	1.7	72	68
Iodophor Prep.	1% avI_2	100	100	60
Thiomersal B.P.	0.1%	Product does not kill when tested neat	100	28

other two products show a somewhat better performance. PCMX gives the best result despite its relatively weaker performance than the iodophor in the laboratory test. Without doubt bacteria painted onto the skin are exposed to a greater extent to the antimicrobial agent than are the natural flora, and organisms painted on the skin may be more akin to the transient flora and this may account for the results achieved with this test. The 'in-use' test, employing the method of Verdon (6), was performed on the perineum (7) of women patients in labour and the predominant organisms isolated were *Staphylococcus epidermis* and cutaneous corynebacterium, which is indicative that resident rather than transient flora were involved in the 'in-use' test. The poorer performance in the 'in-use' test of the iodophor can probably be accounted for in terms of the deactivation of the germicide by skin secretions such as protein. Iodophors tend to release iodine relatively slowly and this may be neutralized almost as fast as it is released. Mercurial compounds are not noted for their bactericidal action, but give good bacteriostatic activity. The reason for the good performance of thiomersal in the simulated 'in-use' test is not yet understood.

Test for adequate preservation

Preservation is an important aspect of all formulation work and the simplest type of test to check for 'adequate' preservation is an insult test in which the product is challenged with a number of likely bacteria

which are considered as possible contaminants. This challenge can either be made with single organisms or mixed cultures, and, moreover, may, if required, be repeated at intervals. This procedure can fail, however, primarily because the resistance of the organisms to a preservative may be lower when grown in the laboratory than the resistance which can occur in practice. Since resistance to antimicrobial agents is often variable in many organisms it is necessary to ensure during preservation tests that organisms of a suitable resistance are used.

This can be achieved by growing the organism in a suitable medium to which has been added either the preservative or the product in low concentration. The method of Basset, Stokes and Thomas (8) which uses the above principle has worked well in establishing whether working strength solutions of antimicrobial agents are likely to be contaminated. The results from practical experience correlating 100% with the predictions from the Basset *et al.* method (8).

Conclusions

In comparing the relative merits of 'in-use' and laboratory methods no attempt has been made to review the various methods available; neither has any attempt been made to review the various methods by which bacteria may be recovered from the skin. Such topics are covered in a number of reviews (9–11). Instead the purpose of this paper has been to demonstrate that sufficient data is not yet available to enable laboratory tests to be designed which can represent sufficiently closely, in most cases, the situation appertaining in practice. 'In-use' testing is necessary in order that an insight in the various factors affecting performance may be studied, but 'in-use' testing by itself is incapable of doing this without some interplay from the laboratory tests.

At the present time, therefore, wherever possible both 'in-use' and laboratory testing should be carried out, but if resources restrict both types of evaluation it is far safer to apply 'in-use' tests rather than laboratory tests. The 'in-use' tests have, however, to be very carefully designed, taking into consideration the various factors which may influence the final conclusions reached.

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REFERENCES

(1) Lowbury, E. J. L., Lilly, H. A. and Bull, J. P. *Brit. Med. J.* **1** 1251 (1963).
(2) Hugo, W. B. and Franklin, I. *J. Gen. Microbiol.* **42** 365 (1968).
(3) Cowen, R. A. Unpublished observations.
(4) Shelley, W. B., Hurley, H. J. and Nichols, A. C. *Arch. Dermatol. Syph.* **68** 430 (1963).
(5) Story, P. *Brit. Med. J.* **2** 1128 (1952).
(6) Verdon, P. E. *J. Clin. Pathol.* **14** 91 (1961).
(7) Byatt, M. E. and Henderson, A. Personal communication.
(8) Bassett, D. C. J., Stokes, K. J. and Thomas, W. R. G. *Lancet* **i** 1188 (1970).
(9) Lowbury, E. J. L. in Maubach, H. I. and Hildick-Smith, G. *Skin bacteria and their role in infections* (1965) (McGraw-Hill, London).
(10) Gucklhorn, I. R., *Mfg. Chem.* **40** (6) 23; (7) 38; (8) 71; (9) 33; (10) 33; (11) 35; (12) 38 (1969). **41** (1) 42; (2) 30; (3) 26; (4) 34; (6) 44; (7) 51; (8) 28; (9) 82; (10) 49; (11) 48; (12) 50 (1970). **42** (1) 34; (2) 35 (1971).
(11) Selwyn, S. and Ellis, H. *Brit. Med. J.* **1** 136 (1972).

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The behaviour of perfumery ingredients in products

J. W. K. BURRELL*

Presented at the 2nd Joint Perfumery Symposium organized by the British Society of Perfumers and the Society of Cosmetic Chemists of Great Britain at Eastbourne on 7–9th May 1973

Synopsis—A new technique involving the GLC ANALYSIS of PERFUMERY INGREDIENTS directly from product bases has been developed. This technique has been used to study how materials behave in SOAPS and laundry powders after storage under various conditions.

INTRODUCTION

Perfumers and cosmetic chemists have for some time been interested in predicting how perfumes will behave in the various bases into which they are to be incorporated. Until now, the perfumer has had to resort to many hours of tedious trial and error to ensure a final pleasing effect and even then he could not be certain that the effect will hold constant over months of storage under possibly adverse conditions.

Apart from simple frustration and tedium, this situation presents problems of economics. It is of little value incorporating relatively expensive ingredients in a product if they contribute nothing to the odour over time because of incompatibility with the base or because they are lost by chemical reaction or evaporation. It therefore became imperative that reliable objective methods should be developed to study these problems of behaviour in greater detail.

*Proprietary Perfumes Ltd, Ashford, Kent.

History

Several workers have tried to develop instrumental analytical techniques to study the loss of perfumery ingredients, particularly with respect to soap. Sfiras and Demeilliers (1), for instance, developed a technique of analysing by glc the vapour surrounding unwrapped perfumed soap after storage under various conditions. Although some conclusions could be drawn from the data obtained, the authors were unable actually to observe what was taking place inside the soap itself.

One question they were trying to answer was which materials had the highest 'perfumery value'. Derivichian (2) had stated earlier that esters have higher perfumery values than alcohols. Sfiras and Demeilliers found from their experiments that the vapour from the soap contained higher proportions of esters (e.g. benzyl acetate) to alcohols than that which was found in the vapour from the perfuming liquid itself.

Whether this proves a higher 'perfumery value' for the esters is debatable. It could be argued that the higher proportion of esters in the vapour emitted by soap shows that these materials are lost to a greater extent and that therefore less is available for the physical transfer of perfume from the soap base to the skin. There has been conjecture that there is a virtually inexhaustible reservoir of perfume in the central core of a soap tablet and therefore the perfume ingredients are always available in their original proportion, but no concrete evidence was found to support this theory. It therefore became very desirable to discover exactly what happens inside the soap, by analysing samples directly.

Techniques of analysis

Some early work that was carried out in Unilever involved the use of uv spectroscopy as a method of assessing the behaviour of perfumery ingredients. The materials benzophenone and anisaldehyde were incorporated separately in soap and it was found when the ingredient was extracted with aqueous isopropanol that a reasonable uv spectrum of the ingredient could be obtained. Using this technique it was proved that for those materials studied, the rate of loss of the ingredients from an unwrapped bar of soap is determined by the rate of diffusion of those materials through the soap matrix. This was achieved by measuring the concentration of the ingredients at various points along the axes from the centre to the surface. It was found that the concentration of the ingredients was highest

at the centre and lowest near the surface. Had the rate of evaporation from the surface been the rate determining step, then of course the concentrations at points along the axis would have been identical.

For some ingredients that were studied, e.g. benzophenone, no measurable change in the concentration at the centre of the soap bar was observed after 24 weeks storage.

This technique had many restrictions and could not be applied generally to the study of all perfumery ingredients. Firstly, there are only a limited number of materials used in perfumery which have uv absorbing properties. Secondly, the preparation of each sample for analysis is time consuming; and, thirdly, the method does not identify possible breakdown products which may be either falsely identified as the original material or may be missed altogether. Any complex breakdown would of course be impossible to diagnose.

A more generally applicable technique was therefore required and glc appeared to be the most promising. Previous workers have used this technique, but they have all employed some cumbersome form of extraction procedure to isolate the perfume material prior to analysis. Although preliminary extraction techniques can be used for studies of a limited number of materials, it became desirable to develop a simpler, more straightforward system for studying a large number of perfumery materials under various storage conditions in different products. Such a straightforward system has been used by Proprietary Perfumes Limited for about 4000 analyses with good results.

The approach that was used was very simple. It involved placing small samples of the soap (or detergent) on the top of the glc column in the flash-heated zone, the idea being that the perfumery ingredients evaporate into the carrier gas, leaving the soap behind. Many who have tried this technique have found that the chromatogram is ruined by the presence of large peaks from the soap base, as shown in *Fig. 1*. This problem was overcome by utilizing a modified injection system. The sample is placed in a sample tube which is then inserted in the injection head; the latter is then flushed with carrier gas. Once the correct column inlet pressure is reached the sample tube is injected into the flash-heated zone of the glc column. After a short period the sample tube is removed.

In this way it was found possible to elute all of the perfumery ingredients from the sample without getting the undesired components from the base, some of which are probably produced by thermal breakdown. Complete elution of the ingredients was proved by the re-injection of the sample

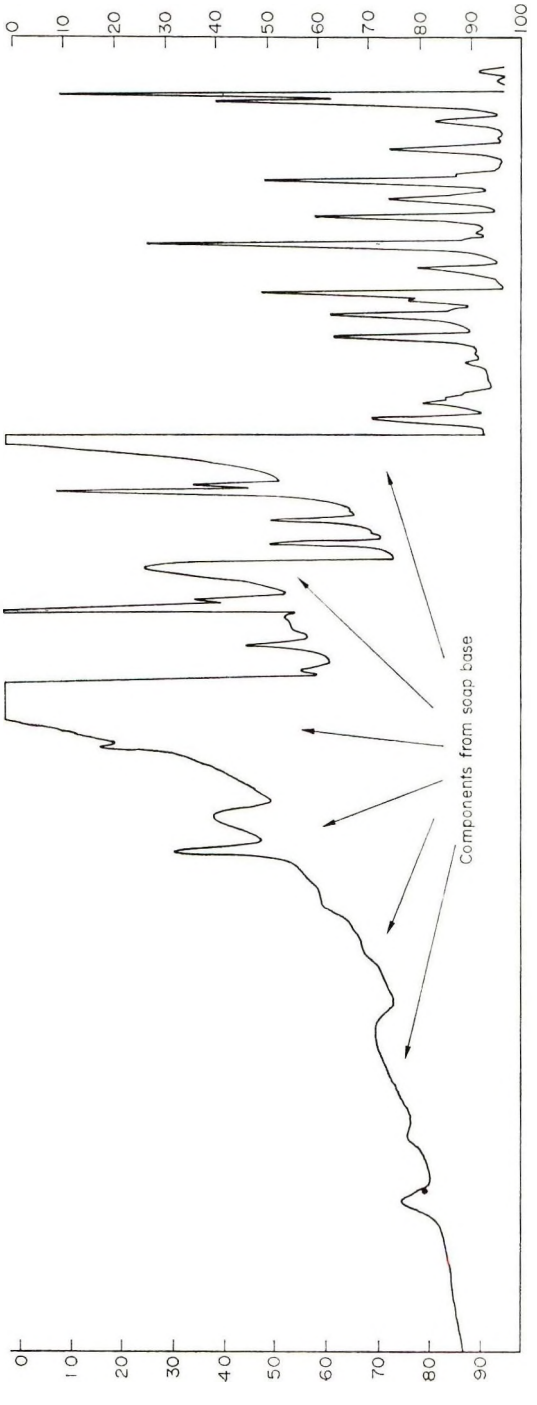

Figure 1. Chromatogram showing interference from soap base components.

when no residual traces of the ingredients could be found. Losses of the majority of perfumery materials during the weighing and sample loading procedure were minimal and a coefficient of variation of $\pm 7\%$ relative was obtained for the method when analysing a mixture of cineole, linalool, benzyl acetate, benzyl benzoate and diethyl phthalate in superfatted soap.

To carry out controlled storage tests it was necessary to use a stable reference material which would be incorporated into the product with the ingredient under investigation. It was also desirable that this reference material should be odourless so that parallel odour assessments could be made.

Diethyl phthalate (DEP) was selected as it is both odourless and stable in soap bars under the conditions of storage that were to be used in the test. The suitability of DEP was confirmed by incorporating a mixture of benzophenone (which had previously been shown to be stable by uv measurements) and diethyl phthalate in soap bars and analysing samples from the centre of each bar after various periods of storage.

The behaviour of each ingredient was studied in the following manner. A mixture of the ingredient and diethyl phthalate (ratio of about 1 : 1) was incorporated in superfatted soap at 0·2%; the soap was plodded into bars $3'' \times 1''$ and wrapped in standard glassine and glazed paper wrapping. The bars were then stored for both 12 and 24 weeks both at room temperature (*c.* 20°) and at 37°. Samples were taken from the centre of each bar and analysed by glc. Chromatograms were compared with those obtained from freshly prepared bars of perfumed soap (1 week old to allow equilibration of distribution). The incorporation and storage were arranged so that analyses could be carried out within a 24 h period.

Results

Table I gives the results obtained for some common perfumery materials. Although some results have been omitted, no general conclusions could be drawn as to relationships between behaviour and chemical class except in the case of formates, which all showed a tendency to hydrolyse and produce the parent alcohol. Low boiling materials generally were lost more readily than the higher boiling materials. Samples taken from the surface layers of the wrapped bars had the same composition as those taken from the centre, which proves that any loss other than by chemical breakdown is governed, in the case of the wrapped bar, by the rate at which the ingredient migrates through the wrapper and hence into the atmosphere. Apart

Table I

Material in soap	% material incorporated present after:			
	12 weeks		24 weeks	
	at RT	at 37°C	at RT	at 37°C
Hydrocarbons				
Diphenylmethane	95	67	82	61
Camphene	62	62	50	38
Limonene	85	41	81	17
Myrcene	100	20	50	10
α-Pinene	100	40	50	4
Alcohols				
n-Nonanol	100	100	100	100
cis-p-tert-Butylcyclohexanol	100	100	100	100
trans-p-tert-Butylcyclohexanol	100	100	100	93
α-Terpineol	100	100	100	90
Borneol	100	96	100	89
Benzyl alcohol	100	87	100	81
Caryophyllene alcohol	100	81	88	75
Linalool	100	78	100	53
cis-Hex-3-enol	86	53	46	35
trans-Hex-3-enol	88	56	49	37
Esters				
Citronellyl acetate	100	100	100	88
Bornyl acetate	100	88	83	83
cis-p-tert-Butylcyclohexyl acetate	100	100	100	89
trans-p-tert-Butylcyclohexyl acetate	100	100	100	78
Terpinyl acetate	97	97	85	82
Inonyl acetate	84	56	64	49
Methyl heptine carbonate	67	31	59	13
Benzyl acetate	64	41	49	0
Benzyl formate	0	0	0	0
Citronellyl formate	0	0	0	0
Trimethylhexyl formate	0	0	0	0
Linalyl formate	0	0	0	0
Phenylethyl formate	0	0	0	0
Aldehydes				
Amylcinnamaldehyde	100	100	100	100
Hydroxycitronellal	100	100	100	98
Undecenal	100	96	100	86
Cinnamaldehyde	70	76	63	60
cis Citral, neral	95	58	68	53
trans Citral, geranial	97	58	74	52
Hydratropic aldehyde	62	36	33	16
Benzaldehyde	0	0	0	0
Phenylacetaldehyde	0	0	0	0

Table I—continued

Material in soap	% material incorporated present after:			
	12 weeks		24 weeks	
	at RT	at 37°C	at RT	at 37°C
Ketones				
α-Ionone	100	100	100	100
Benzophenone	100	100	100	100
Jasmalone	100	93	100	89
Methyl nonyl ketone	83	75	79	64
Methyl hexyl ketone	40	20	14	0
Ethyl amyl ketone	0	0	0	0
Ethers				
Amyl benzyl ether	100	100	100	79
β-Naphthyl methyl ether	94	79	96	78
Anther	100	77	73	58
Rose oxide	83	52	65	35
p-Cresyl methyl ether	100	63	80	9
Phenylethyl methyl ether	53	44	25	9

from the formates only a few materials showed signs of chemical breakdown.

Phenylacetaldehyde is so unstable that about 60% was lost 1 week after incorporation. Benzyl acetate, which has often been studied in this context in the past by others (3), is among those materials which show chemical instability, but the amount of benzyl alcohol produced does not account for all the loss and therefore evaporation must also be a contributory factor.

After the initial screening of individual materials it became interesting to examine some time-honoured beliefs that perfumers have held in connection with the behaviour of various ingredients and mixtures. For example, it has been widely believed that the behaviour of aldehydes can be improved by the addition of an alcohol, and in particular the corresponding alcohol, because of the supposed formation of hemiacetals. In order to test the validity of the argument, mixtures of the aldehyde, the corresponding alcohol and diethyl phthalate (in the ratio 1 : 1 : 1) were incorporated into soap bars and stored under the same conditions as described previously. *Table II* gives the results of the analyses and shows, within experimental error, that there is no difference in the behaviour of aldehydes with or without the alcohol.

Table II

Material in soap	% material incorporated present after			
	12 weeks		24 weeks	
	at RT	at 37°C	at RT	at 37°C
n-Octanal	80	35	30	4
n-Octanal (+ *n*-Octanol)	90	40	28	6
n-Nonanal	100	75	100	55
n-Nonanal (+ *n*-Nonanol)	100	7[illegible]	100	50
Citronellal	91	9[illegible]	33	59
Citronellal (+ Citronellol)	95	9[illegible]	80	57
Benzaldehyde	0	0	0	0
Benzaldehyde (+ Benzyl alcohol)	0	0	0	0
	1 week			
	at RT	at 37°C		
Phenylacetaldehyde	37	0		
Phenylacetaldehyde (+ Phenylethyl alcohol)	37	0		

Another belief which was investigated was that concerning 'fixation'. It has long been thought that the addition of high boiling materials reduces the loss of low boiling ingredients from perfumes. Although this has been well established for perfumes applied to the skin it has never been proved for perfumes in products. It was possible using this method of analysis to show that even when the addition of a fixative (*Hercolyn D**) was made at a level five times that of the perfumery material, no improvement in the retention of the material in the soap could be observed.

The lack of detectable formation of hemiacetals and the ineffectiveness of fixatives in soap are not surprising when one considers the vast differences in the rates of collision between the molecules of perfumery ingredients themselves and between the ingredient molecules and the soap base molecules. The number of collisions between individual perfumery ingredient molecules would be extremely low in soap as compared with those in the essence and therefore the effects of physical or chemical interaction between such molecules would be correspondingly small. Any supposedly beneficial effects of interactions that take place in the essence before incorporation and which are reversible, as in the cases of hemiacetal formation, and the physical interactions associated with 'fixatives', will be subsequently minimized after incorporation of the essence in the product base.

*Hercules Powder Co.

Table III

Material in laundry powder	% material incorporated present after			
	12 weeks		24 weeks	
	at RT	at 37°C/ 70% rh	at RT	at 37°C/ 70% rh
Hydrocarbons				
Diphenylmethane	62	24	72	33
Alcohols				
Phenylethyl dimethyl carbinol	100	100	100	95
Caryophyllene alcohol	100	100	100	88
Dodecanol	100	100	100	70
Decanol	100	84	92	67
Anisyl alcohol	64	40	45	19
Borneol	77	31	37	0
Benzyl alcohol	65	18	31	0
Linalool	38	8	27	0
Esters				
Musk oxalide	100	100	100	100
Benzyl benzoate	100	100	100	71
Gardocyclene	94	69	98	70
Ethyl cinnamate	59	67	63	38
Geranyl acetate	67	12	54	0
Citronellyl acetate	65	0	31	0
Benzyl acetate	18	0	13	0
Aldehydes				
Hexylcinnamaldehyde	100	100	100	100
Anisaldehyde	30	14	17	13
Hydroxycitronellal	80	40	75	0
cis Citral, neral	40	0	0	0
trans Citral, geranial	40	0	0	0
Cinnamaldehyde	28	0	0	0
Phenylacetaldehyde	20	0	7	0
Decanal	14	0	0	0
Ketones				
Versalide	100	100	100	100
Benzophenone	100	90	91	89
Celestolide	100	100	100	71
Methyl naphthyl ketone	100	100	100	71
α-Ionone	86	26	78	4
Jasmalone	92	20	72	0
Acetophenone	0	0	0	0
Ethers				
Methyl diphenyl ether	100	100	100	83
β-Naphthyl ethyl ether	93	76	97	66
Phenylethyl *n*-butyl ether	44	15	38	0
Phenylethyl amyl ether	35	0	23	0
Amyl benzyl ether	40	0	12	0

Apart from the study of the behaviour of ingredients in soap base, a similar detailed study has been carried out in laundry powders. The perfumery ingredients were incorporated individually with diethyl phthalate into a laundry powder (in this case *Persil Automatic*) at a level of 0·1%. The products were then stored in cartons for periods of both 3 and 6 months at both room temperature and 37°C/70% relative humidity. Again the incorporation and storage were so arranged as to enable the analysis to be made in a period of 24 h. The chromatograms obtained from the stored samples were compared with those from fresh samples kept in glass jars. *Table III* shows the behaviour of some common perfumery ingredients relative to diethyl phthalate.*

One can see from the table that many perfumery ingredients do not perform as well in packets of laundry powder as in wrapped soap bars. Once again similar conclusions were reached, namely that lower boiling materials are lost more readily than the higher boiling ones, and that there are no obvious links between chemical structures and stability.

*The results are relative for it has been shown that there is loss of diethyl phthalate under the most severe conditions of 24 weeks at 37°C and 70% rh amounting to about 20%.

Table IV

Material in laundry powder	% material incorporated present after			
	12 weeks		24 weeks	
	at RT	at 37°C/ 70% rh	at RT	at 37°C/ 70% rh
n-Decanal	14	0	0	0
n-Decanal (+ *n*-Decanol)	15	0	0	0
n-Undecenal	50	0	29	0
n-Undecenal (+ *n*-Undecenol)	41	0	22	0
n-Dodecanal	83	0	74	0
n-Dodecanal (+ *n*-Dodecanol)	80	0	68	0
Linalyl acetate	32	0	12	0
Linalyl acetate (+ *Hercolyn D*)	30	0	13	0
Citronellol	100	74	74	30
Citronellol (+ *Hercolyn D*)	94	61	74	33
Cyclamen aldehyde	81	0	59	0
Cyclamen aldehyde (+ *Hercolyn D*)	80	0	49	0
Phenylethyl amyl ether	35	0	23	0
Phenylethyl amyl ether (+ *Hercolyn D*	45	0	25	0
Jasmacyclene	78	14	59	11
Jasmacyclene (+ *Hercolyn D*)	85	18	59	14

Hemiacetal formation from aldehydes and the fixative effects of *Hercolyn D* were investigated for ingredients in laundry powder and once again these effects were not demonstrable, as shown by the results in *Table IV*.

These results showed that the majority of perfumery materials do not perform well in laundry powders stored in cartons, therefore a second test was undertaken in order to differentiate between the loss by evaporation and that by chemical instability. To achieve this objective the product containing the ingredient was stored in glass jars. The results of this test are given in *Table V*, which clearly demonstrates that in the case of this particular laundry powder the loss of ingredients observed in packets was caused mainly by evaporation.

Table V

Material in laundry powder		% material incorporated present after			
		12 weeks		24 weeks	
		at RT	at 37°C/ 70% rh	at RT	at 37°C/ 70% rh
Linalool	Carton	38	8	27	0
	Glass	100	100	100	98
Phenylethyl alcohol	Carton	66	66	52	17
	Glass	100	97	93	80
Benzyl acetate	Carton	18	0	13	0
	Glass	100	83	75	50
Citronellyl acetate	Carton	65	0	31	0
	Glass	100	100	100	86
Linalyl acetate	Carton	32	0	12	0
	Glass	100	100	100	100
Citral (*cis* and *trans*)	Carton	40	0	0	0
	Glass	100	100	100	92
Dihydrojasmone	Carton	92	20	72	0
	Glass	100	79	83	37
α-Ionone	Carton	86	26	78	4
	Glass	100	100	100	93
Phenylacetaldehyde	Carton	20	0	7	0
	Glass	100	100	100	100
Amyl benzyl ether	Carton	40	0	12	0
	Glass	100	100	100	75
Phenylethyl amyl ether	Carton	35	0	23	0
	Glass	100	100	100	93
Phenylethyl *n*-butyl ether	Carton	44	15	38	0
	Glass	100	100	100	100

Application of results

Clearly the fact that evaporation is a major cause of perfumery ingredient loss from laundry powders warrants investigations into the efficiency of various packaging materials. The loss of perfume can be as much as three times higher in the least suitable type of packet than some of the best packets used commercially, and the glc method can be of use in discovering the best package compromise in terms of effectiveness and cost.

Another area where the glc technique has found application is in market research. The ability to analyse complete perfumes directly from a product, which is possible by attaching the glc to a mass spectrometer, permits study of perfumery chemicals used in relation to market trends and fashions. This type of information is of immense value to a company in a competitive situation.

Perhaps the most valuable result of this study, however, is that it is now possible to create perfumes which are chemically stable and relatively unaffected by evaporation, even with completely new product base formulations. In these cases there are no historical data for the perfumers to call upon. More detailed investigations have shown that the materials in a complex perfume mixture behave in the same way as when ingredients are incorporated individually. Therefore it is possible to reduce the total number of analyses required by screening a large number of ingredients as a series of mixtures, thereby acquiring expert knowledge in a relatively short period.

Other base applications

All of the work described so far has been concerned with two major products, i.e. soaps and laundry powders. However, the technique has wider application and it has been generally employed in our laboratories in the analysis of perfumes and flavours in other bases. For example, it is possible to study the behaviour of flavours in toothpaste bases, perfumes in haircreams and in talcum powders, and the technique has even been used as a method of analysing the essential oil of lavandin directly from a single floret. The technique can in fact be used whenever an analysis is required of any volatile material present in a relatively non-volatile base.

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REFERENCES

(1) Sfiras, J. and Demeilliers, A. Study by gas chromatography of odorant vapour emitted by a perfumed cake of soap. *Recherches* **14** 33 (1964).
(2) Dervichian, D. Role de la structure moleculaire dans la fixation des parfums dans le savon. *La France et ses Parfums* 324 (August 1961).
(3) Shiftan, E. and Feinsilver, M. Practical research of the art of perfumery. *Ann. N.Y. Acad. Sci.* **116** 692 (1964).

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN

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J. Soc. Cosmet. Chem. **25** 339–354 (1974)

Evaluation of skin bleach creams

K. V. CURRY*

Presented on 12th November 1973 in Nottingham, at the Symposium on 'Evaluation of Product Performance', organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The use of test animals to screen DEPIGMENTING CHEMICALS is described and an attempt is made to correlate these results with tests carried out on humans.

A comparison of various techniques used for measuring changes in SKIN LIGHTNESS showed that the method of visual assessment using a simple numerical scoring system was the simplest and gave the most statistically significant results.

Products based on hydroquinone or its derivatives, and designed to lighten the skin colour of the dark skinned races in Africa, Asia and the U.S.A., have been marketed for many years. A considerable amount of data on the depigmenting effects of the chemicals on black guinea pigs is available (1–3) and since the products containing these chemicals have a fairly high volume of sales one must assume that they have some degree of effectiveness on humans. However, although there have been reports in the literature of the treatment of specific problems of skin hyperpigmentation with hydroquinone compounds, no work on the quantitative evaluation of the depigmenting effect of skin lightening creams on coloured races has yet been published.

There are two closely related problems that are encountered in the selection of suitable depigmenting agents for skin lightening creams. The first is that of setting up a suitable test method for the rapid screening of large numbers of compounds in order to narrow them down to one or two with the required effects. Coupled with this is the problem of selecting a method to measure changes in skin colour produced by the test material (4).

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Preliminary screening tests

The classical method of measuring the depigmenting effect of chemicals is to use black-skinned guinea-pigs which do not pose very great problems in rearing, handling and testing. It is also generally agreed that the skin of the guinea-pig has enough similarity to human skin to make comparisons meaningful.

Many of our early screening tests were in fact carried out on black guinea-pigs and many compounds and combinations of compounds were examined for their depigmenting effects on these animals. However, the interpretation of the results of these tests was difficult since the degree of lightening could not be extrapolated to humans.

It was decided, therefore, to evaluate four creams containing various depigmenting agents first of all on black guinea-pigs, then on black pigs, and finally on a panel of humans. This was considered necessary in order to:

ascertain the usefulness of guinea-pigs as a screening technique by correlating the results with those obtained on humans;

determine the usefulness of black swine as an alternative test animal;

establish the best practical method for measuring changes in the lightness of skin.

Tests on black guinea-pigs

In this test four products were evaluated for their depigmenting effect on black-skinned guinea-pigs:

Product A, Product B } Commercially-available skin lightening creams

Product C, Product D — Experimental skin lightening creams

(Products C and D were simple water-continuous emulsions containing about 20% oil phase.)

The creams were applied daily for 26 days in a latin square design to the shaved backs of four black-skinned guinea-pigs.

During the course of the experiment a visual estimation of the lightening effects was made at intervals using a set of grey standards. These standards formed a photographic step wedge consisting of shades of grey and each step was numbered arbitrarily with increasing lightness from 1 to 7 (*Fig. 1*).

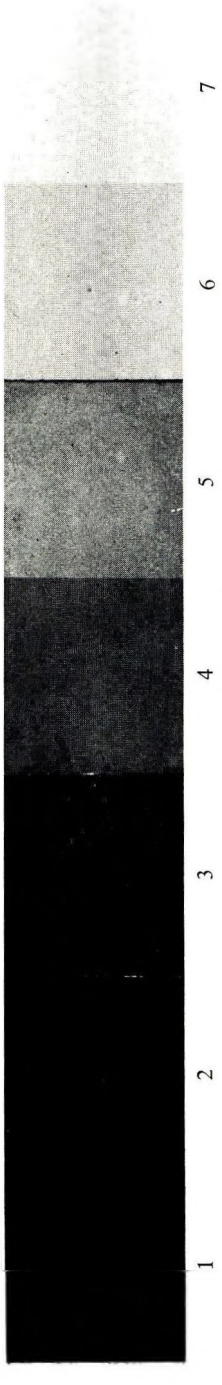


Figure 1. Photographic wedge.

(*Facing p.* 340)

Measurements of skin lightness were made by holding the wedge alongside the animal and noting the step numbers which most nearly matched each site.

The time course of depigmentation produced by the four products is shown in *Fig. 2* where each point is the mean of four observations. Product A was apparently the most effective of the products tested since it produced a more rapid and a greater degree of depigmentation than the other creams which were all similar in performance. It was noted however that this product (Product A) was also somewhat irritant, producing more scaliness than the other products and so giving a false impression of enhanced whiteness.

One noticeable feature of tests of this sort carried out on black guinea-pigs was that the maximum lightening effect was achieved after about 15 days.

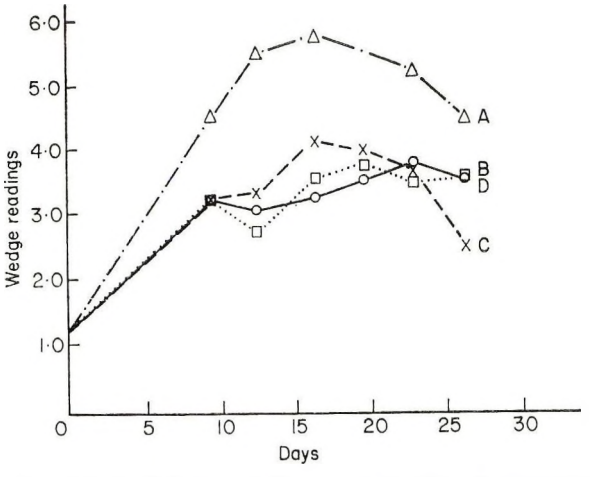


Figure 2. Time course of depigmentation on black-skinned guinea-pigs—wedge technique.

TESTS ON BLACK-SKINNED SWINE

Whilst the guinea-pig is a suitable animal for screening large numbers of compounds, it was considered advisable to obtain comparative data on at least one other black-skinned species other than man. It was not easy to find another suitable species since most animals with black hair have, in fact, white skin. The pig appeared to be a satisfactory choice for a number of reasons.

(1) The anatomy of the skin is similar to that of man.
(2) The hairs are more sparse than on the guinea-pig.
(3) A large area of skin is available for application of materials.

It should be borne in mind, however, that the physiology of pig skin may be completely different from that of human skin in that the barrier properties of the stratum corneum may be different, resulting in differing rates of penetration of active ingredients. The sensitivity of the melanocytes to skin depigmenting agents may also be different.

The animals used in this test were nine pure bred Wessex Saddlebacks (8 hogs and 1 gilt) and at the beginning of the experiment the pigs were shaved and the pigmented area of the back and flanks divided into sites about 5 cm square using a *Magic Marker*. Sites had to be frequently remarked during the experiment. The skin reflectance was measured using an *EEL* reflectometer which in this case was considered to be more useful than the stepped wedge since the colour of the pig skin was less grey than that of the guinea-pig. The instrument consisted of a reflectance head comprising a photocell and a light source connected to a *Unigalvo* 20 galvanometer. Reflection readings of the skin were expressed as a percentage of the reflectance of a standard $MgCO_3$ block (the reflectance of which was set at 100%). The creams were packed in tubes and applied daily for 38 days, 6 mm of cream being applied to the relevant site on the animal. Measurements of skin reflectance were made at weekly intervals, after washing the skin with water to remove traces of dirt and old cream.

The time course of depigmentation is shown in *Fig. 3*. These results are expressed as the change in reflectance of the skin compared to its initial reflectance before treatment. Positive values indicate lightening whilst darkening is shown by negative values. Each point on the graph is the mean of nine observations (one reading on each of nine pigs).

A statistical analysis of the total results (confined to the results recorded on day 38, that is, the final level of depigmentation reached), showed that there was no significant difference between any of the four products tested.

A comparison of *Fig. 2* and *Fig. 3* does show, however, that the depigmentation occurs more slowly on pigs than on guinea-pigs and it was considered highly likely that depigmentation would be slower still on humans.

The *EEL* proved to be a simple instrument to use although there were occasions when it did not seem to be differentiating markedly between sites that appeared visually to be of different lightness. This is probably because the eye measures the contrast between a specific site and its background and the observer can also ignore differences in surface texture such

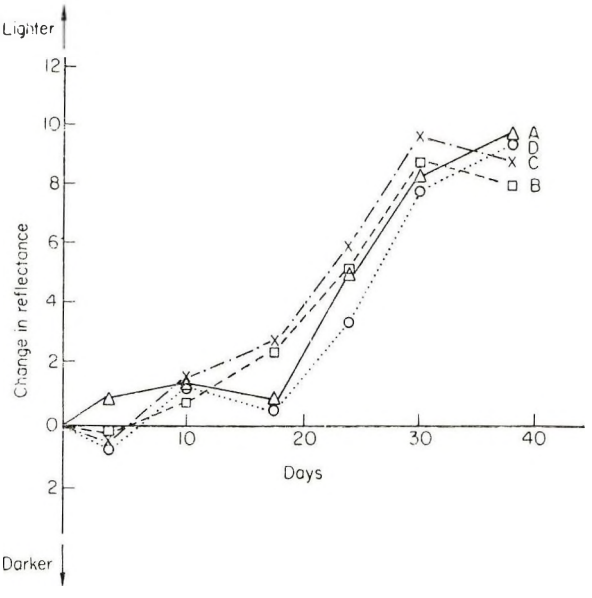


Figure 3. Time course of depigmentation on black-skinned swine—reflection technique.

as scaling due to irritation, and stubble, which may in fact contribute to the surface reflection measured by the instrument.

At this stage, therefore, we had two sets of rather conflicting data, neither of which was satisfactory. Although all creams had a marked depigmenting effect it was still not clear whether Product A was more effective than the others. It was felt at the time that the irritation of A may have affected both the wedge and reflectance values. It was at this stage that it was decided to measure the effectiveness of skin lightening creams on humans and for the time being to disregard the animal tests.

Tests on humans

In order to demonstrate the efficacy of skin lightening creams on coloured subjects and if possible to correlate any effects achieved with those shown on the experimental animals, the four products previously described (A, B, C, D) were tested. The opportunity was also taken to compare various techniques of measuring changes in the lightness of skin.

Test method

The test was carried out in Africa on a panel of 40 male volunteers. Most of the subjects were chosen because they had jobs which caused them to spend a considerable amount of their time out of doors, since it was suspected that strong sunlight might be playing some part in the efficiency of the bleach creams. The groups were matched on the basis of the reflectance values of the skin on the outside of the forearm. Each group used only one of the previously mentioned products during the whole test period.

Application of the creams

These creams were applied to four test sites on each person, namely the outside upper forearm and the outside of the wrist on each arm, by means of a cardboard template. In this way the central portion of the outside forearm could act as the control site for each arm. It was also hoped that the subjects would agree to apply the appropriate skin bleach cream product to their faces, but only a few were willing to do so. The cardboard template was located on the outside of the forearm by placing one end on the head of the ulna bone of the wrist. The presence of the template caused some difficulty during the rubbing in of the creams and it was found that the operators applying the cream became so adept in judging the area of application that the template was only used to locate the application area and then removed when the cream was rubbed in. The creams were applied twice daily during the working week (5 days) by skilled operators (the few subjects who agreed to use the cream on their faces were allowed to apply it themselves, but only to their faces). Approximately 0.25 g of cream was applied to each test site on each occasion.

Since the test lasted 8 weeks there was a total of 10 (subjects) $\times$ 40 (days) $\times$ 2 (applications) $\times$ 4 (test sites) = 3200 applications of each cream during this test.

Examination

The subjects were examined before the test began using a variety of subjective and objective evaluation techniques. In addition the panel members were examined by a consultant dermatologist to ensure that they were

suitable for the test (i.e. had no existing diseases or history of allergy/sensitization), and also during the test in order to detect any signs of primary irritation that might develop.

Evaluation techniques

Both subjective and objective methods for assessing the degree of skin lightening were used in this test. To avoid any bias, conscious or unconscious, in the subjective evaluations, it was decided to carry out the subjective evaluations before the objective measurements were made. In addition the panel members were examined in a completely random order which was dictated by their availability.

The evaluation techniques employed were the following.

Grey scale wedge assessment

The shade intensity of colours of the skin on both the test sites and control sites of the forearms were matched with the same set of numbered photographic standards as that used in the guinea-pig tests (*Fig. 1*).

Visual assessment

The colours of the treatment sites were visually compared with the control site on each arm and the degree of lightening was assessed using the following simple numerical scoring system:

Score	*Degree of Lightening*
0	No difference
+1	Slightly lighter
+2	Noticeably lighter
+3	Markedly lighter

Photographic assessment

Photographs were taken of the forearms and faces of all subjects initially and of the forearm on each examination. Final photographs were taken of the faces of a few subjects who agreed to apply the creams to their faces.

The photographs of the forearms were taken with a 'Startec' clinical camera fitted with a flash attachment and the forearms were photographed against a standard grey background. The background was used as an additional control when micro-densitometer traces were made of the test and

control sites of the forearms, since it was thought that differences in photographic developing conditions could lead to a wide variation in photographic image density. Photographs of the face were taken with a 35 mm camera. All the photographs were taken in colour.

Reflectance of skin

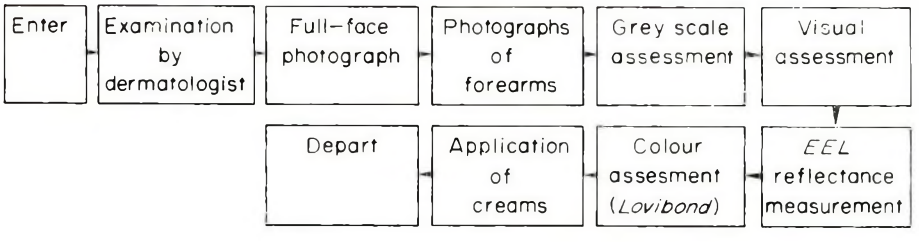
The reflectance of the skin on the test and control sites of the forearms and on the forehead of those subjects applying products to their face was measured using the *EEL* spectrophotometer. 'White Light' was used for the measurements, and the instrument was calibrated to give a reading of 50 units when using the grey tile standard. This instrument had been found to give quite good results in measuring lightening in test animals.

Colour of skin

The colour of the skin was measured with a reflectance *Lovibond* tintometer. The instrument consists essentially of a reflectance head to which are attached two light guides, one delivering the incident light and the other taking away the reflected light. The reflected light is transmitted to a monocular eyepiece where it constitutes half of the field of view while the other half is obtained from a separate source whose colour can be altered by the insertion of various coloured filters. The probe was placed on the skin surface and the colour of the skin matched by means of the red, blue and yellow filters. The colour of the skin was then designated in terms of combinations of these colours required to match the colour of the skin surface.

In all, therefore, there were six different types of assessments made on each subject on eight different sites on each formal examination after 0, 1, 2, 4 and 8 weeks.

A simple flow chart demonstrating the evaluation procedure is shown below.



Various difficulties were encountered with some of the evaluation techniques and these will be dealt with later. However, it was almost immediately apparent that the use of the *Lovibond* tintometer to measure the colour of the skin was a laborious and time-consuming exercise, and since it was found that it took approximately 15 min to carry out the colour measurements on the eight sites on an individual (i.e. 10 h for the whole panel), it was decided to confine this evaluation to only a few randomly-selected individuals.

Results

Grey Wedge Assessment technique

In previous tests carried out on black guinea-pigs this technique had been found to be very useful, and so it was chosen as an evaluation technique in Africa. However, none of the Africans on the panel were really black and ranged from dark red-brown to light brown and considerable difficulty was experienced by all assessors in matching the grey scale with the coloured skin.

The results were analysed statistically and are shown in graphical form in *Fig. 4.*

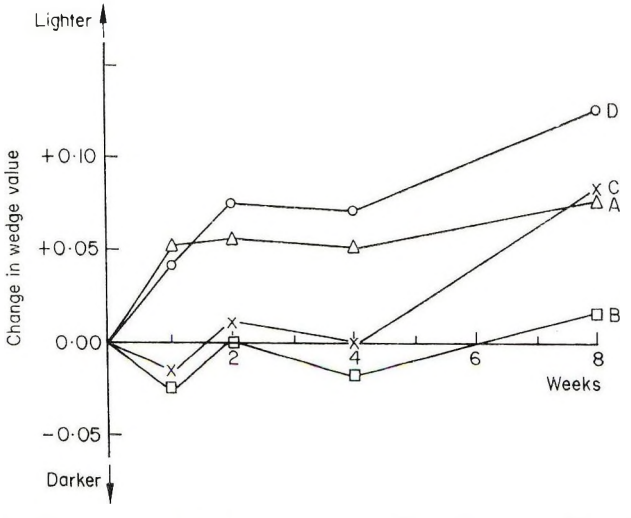


Figure 4. Time course of depigmentation on coloured human subjects—wedge technique.

The main conclusions from this test are as follows.

None of the products gave a statistically significant lightening effect after 4 weeks.

Only Product C and Product D gave a significant lightening effect after 8 weeks.

There was no significant difference between any of the products after 8 weeks.

The lack of statistical significance arises from the very wide scatter of results obtained with this technique, which is indicative of the difficulty of using the grey wedge on brown-coloured skin.

Visual assessment

This was an extremely simple technique to use and the scoring system is one that has been used successfully for many years. The results of this test which were statistically analysed are shown graphically in *Fig. 5*.

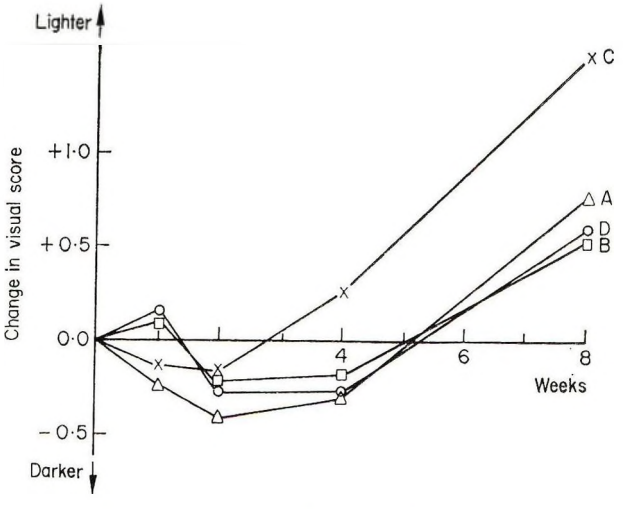


Figure 5. Time course of depigmentation on coloured human subjects—visual technique.

The main conclusions were as follows.

None of the products had a significant lightening effect at the end of 4 weeks.

All products had a significant lightening effect at the end of 8 weeks.

Product C produced a significantly better lightening effect than the other three products, which were equivalent, after 8 weeks.

The one interesting feature of *Fig. 5* is that it shows that the effect of Product C on the skin was to produce an effect which was between 'slightly lighter' to 'noticeably lighter' than the control area after eight weeks. The other three products gave effects that were much less than 'slightly lighter'.

Photographic assessment

Forearms

Visual examination of these photographs showed that there would be obvious difficulties in carrying out traces with the microdensitometer. This was due to the presence of very strong highlights on various parts of the arm arising from the use of a flash attachment and the different curvatures on control and test sites. After careful consideration it was decided not to carry out microdensitometer traces on forearms.

Faces

The same sort of difficulty due to highlights was also experienced on the photographs of subjects' faces before and after the test and therefore no microdensitometer traces were carried out on these photographs.

It was disappointing that the photographic technique did not yield any useful information, but this was probably due to the fact that the lightening effects were much smaller than anticipated.

Colour of skin (Lovibond)

As mentioned previously this was a slow and laborious method for measuring the skin colour and only a few subjects were evaluated with this technique. An examination of the results of this assessment did not reveal any pattern of change of colour either with regard to the product used or the length of treatment.

Reflectance of skin (EEL *spectrophotometer*)

This direct measurement made on the forearms (and foreheads where applicable) was a very simple technique to carry out. The only experimental

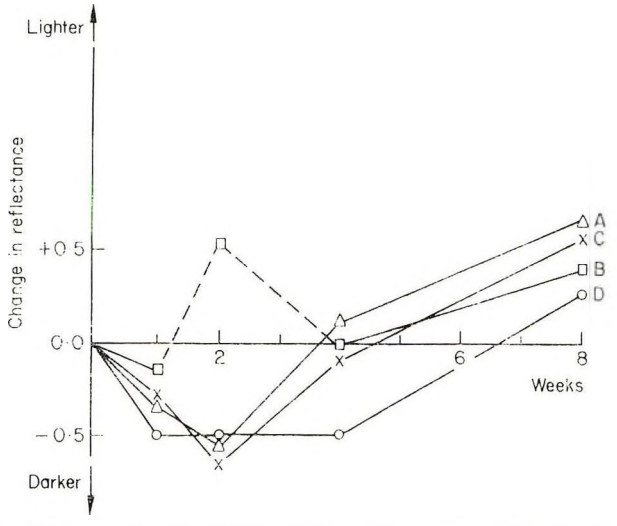


Figure 6. Time course of depigmentation of human subjects—reflectance technique.

difficulty observed was in a slow drift of the galvanometer but this was overcome by frequent recalibration with the standard grey tile. The results are shown in graphical form in *Fig. 6.*

A statistical analysis of the results of this test showed the following.

None of the products produced a significant lightening effect after 4 weeks.

Only Product A had a significant lightening effect after 8 weeks.

There was no significant difference between any of the four products after 8 weeks.

Product B did give a significant lightening effect after 2 weeks but this appears to be an anomalous result.

The lack of statistical significance is perhaps again indicative of the difficulties associated with instrumental measurements on non-homogeneous surfaces. The skin surface on the arms of quite a few subjects showed considerable scarring due to previous wounding of the skin (both deliberate tribal cuts and accidental wounding), and since the area of skin evaluated by the spectrophotometric head was only about 1 cm^2 this gave rise to considerable variation in reflectance readings owing to slight differences in positioning the reflector head. However, the results are still useful since they also indicate that appreciable lightening effects are not obtained until between 6 and 8 weeks. The high reflectance values for Product A in this test

may be due to the irritation properties of this product causing 'white scaling' on the skin surface which would increase the reflectance measurement. This effect has been noted previously in animal tests.

Because of the large variation in initial skin colour of the subjects it was decided to analyse the test data to determine whether the products had greater or less lightening effect on dark skins than on light coloured skins. The panel was split into three groups for the purpose of statistical analysis.

Group 1 Those giving a skin reflectance of less than 28 units.
Group 2 Those giving a skin reflectance of between 28 and 32 units.
Group 3 Those giving a skin reflectance of more than 32 units.

The statistical analysis was carried out on Group 1 who were arbitrarily classified as 'dark' and also on Group 3 who were classified as 'light'.

The test did in fact reveal that the effects of the various products were more apparent on the subjects with 'dark' skin than on those with 'light' skin. Unlike the results from the total panel, the dark-skinned subjects all showed significant lightening effects after eight weeks whereas none of the light-skinned panel showed any significant lightening effects (*Fig. 7*).

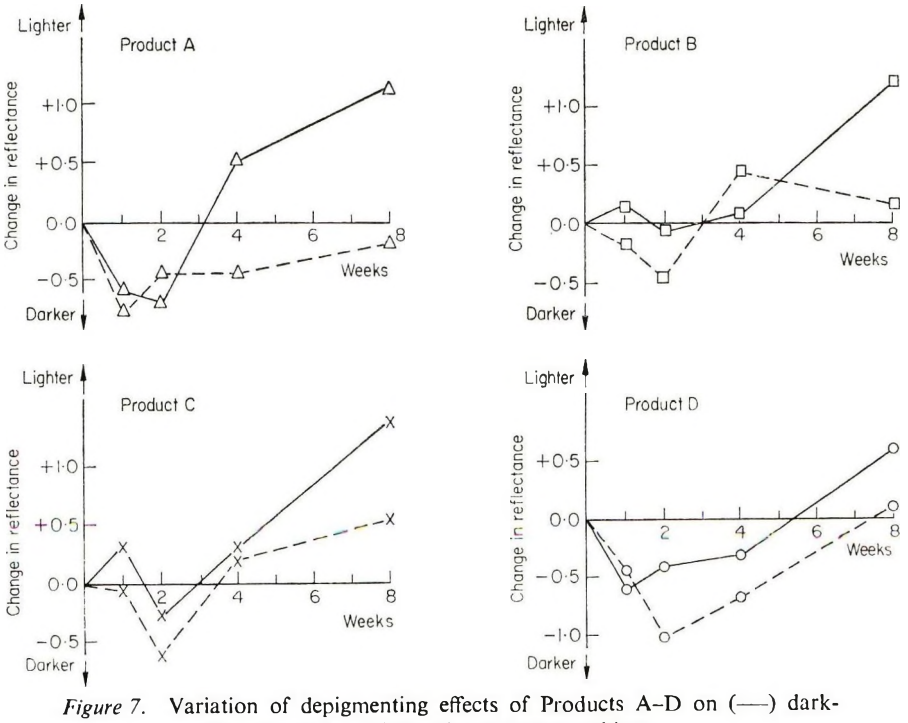


Figure 7. Variation of depigmenting effects of Products A–D on (——) dark-skinned and (- - -) light-skinned human subjects.

Although the three groups were arbitrarily chosen, and the perception of greater or lesser lightening effects on groups may change depending on the group boundaries chosen, it is still nevertheless apparent that clinical tests carried out on light-skinned subjects would need larger panels and longer testing periods to show significant differences.

Discussion

Of the various techniques used to measure lightening of the skin, only three gave results that were capable of being analysed. These techniques were (i) the grey scale; (ii) *EEL* reflectance technique; (iii) comparative visual assessment. A comparative summary of the results obtained with these three techniques is shown below.

	Grey wedge	Visual	Reflectance (*EEL*)
Lightening effect after 4 weeks	None	None	None
Lightening effect after 8 weeks	Only products C and D were effective	All products effective	Only product A effective
Differences between products	None	C better than ABD	None

It is apparent that the only technique capable of clearly showing the depigmenting effects of all the skin lightening creams is that based on comparative visual assessment. This is probably due to the fact that the eye can compensate for odd blemishes, scaling due to irritation and can also integrate the lightening effect over the total area of skin treated.

The technique is extremely simple to use, and the scatter of results compared to the other techniques is smaller which gives a greater degree of statistical significance. In addition since it is a visual technique, it is giving a measurement of effectiveness of the products which is directly related to the way in which users judge the effectiveness of skin lightening creams.

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REFERENCES

(1) Beehan, S. S., Pathak, M. A., Hori Y. and Fitzpatrick, T. B. Depigmentation of the skin with 4-ispropylcatechol, mercaptoamines and other compounds. *J. Invest. Dermatol.* **50** 103 (1968).

(2) Frank, E., Pathak, M. A., Szabo, G. and Fitzpatrick, T. B. Selective action of mercaptoethylamines on melanocytes in mammalian skin. *Arch. Dermatol.* **97** 465 (1968).

(3) Gellin, G. A., Possick, P. A. and Perone, V. B. Depigmentation from 4-tertiary butyl catechol—an experimental study. *J. Invest. Dermatol.* **55** 190 (1970).

(4) Gibson, I. M. Measurement of skin colour *in vivo*. *J. Soc. Cosmet. Chem.* **22** 725 (1971).

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