Vol. 28 No. 5 May 1977

Journal of the Society of Cosmetic Chemists

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VOLUME 28 • NUMBER 5

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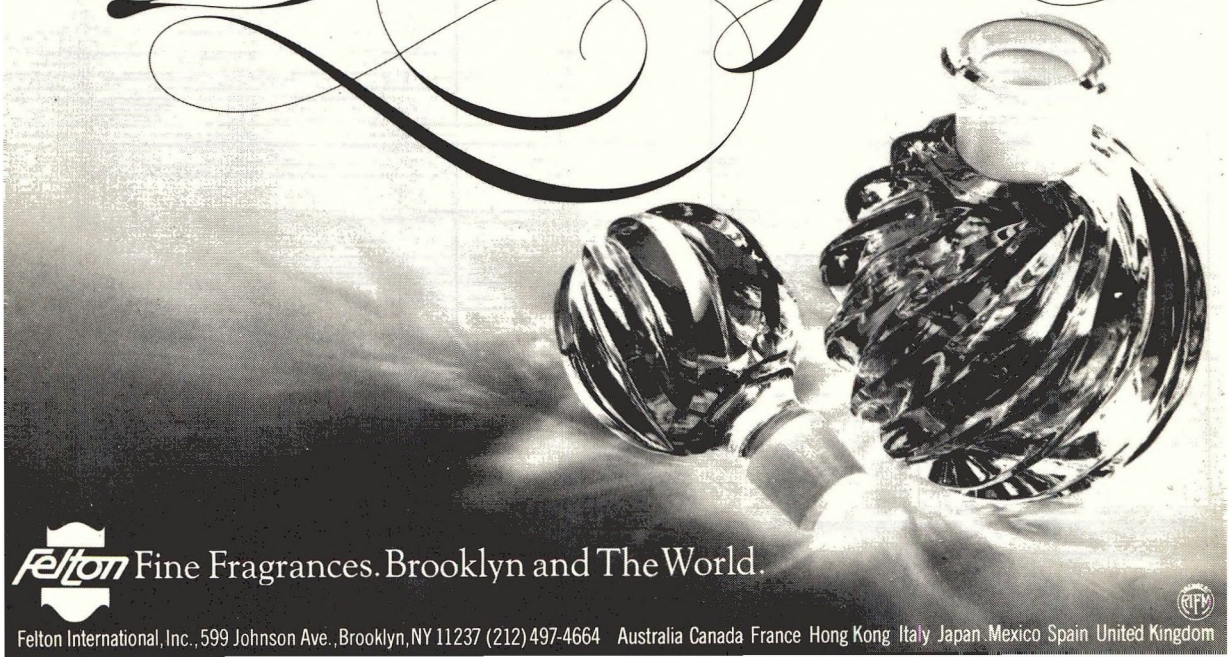

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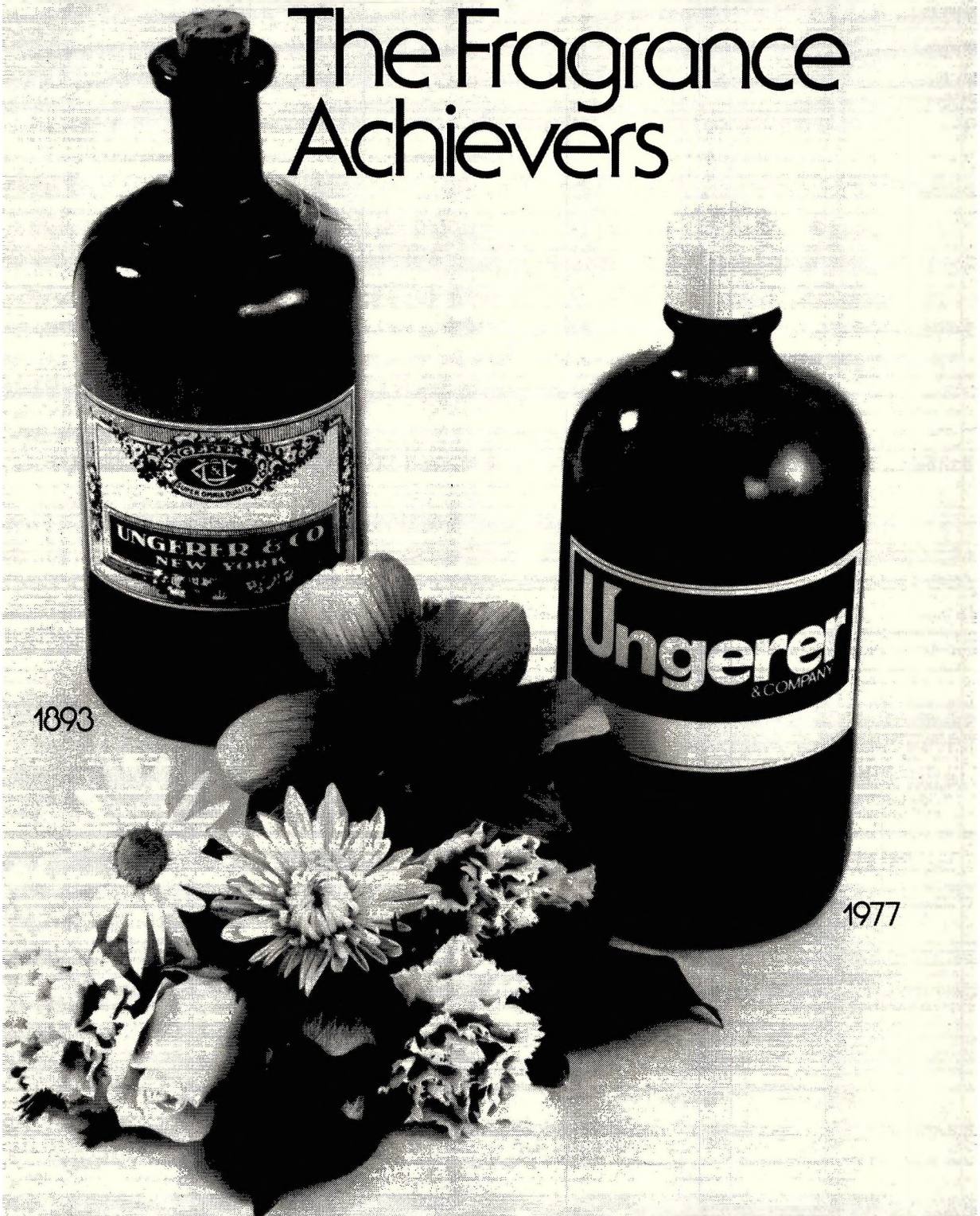

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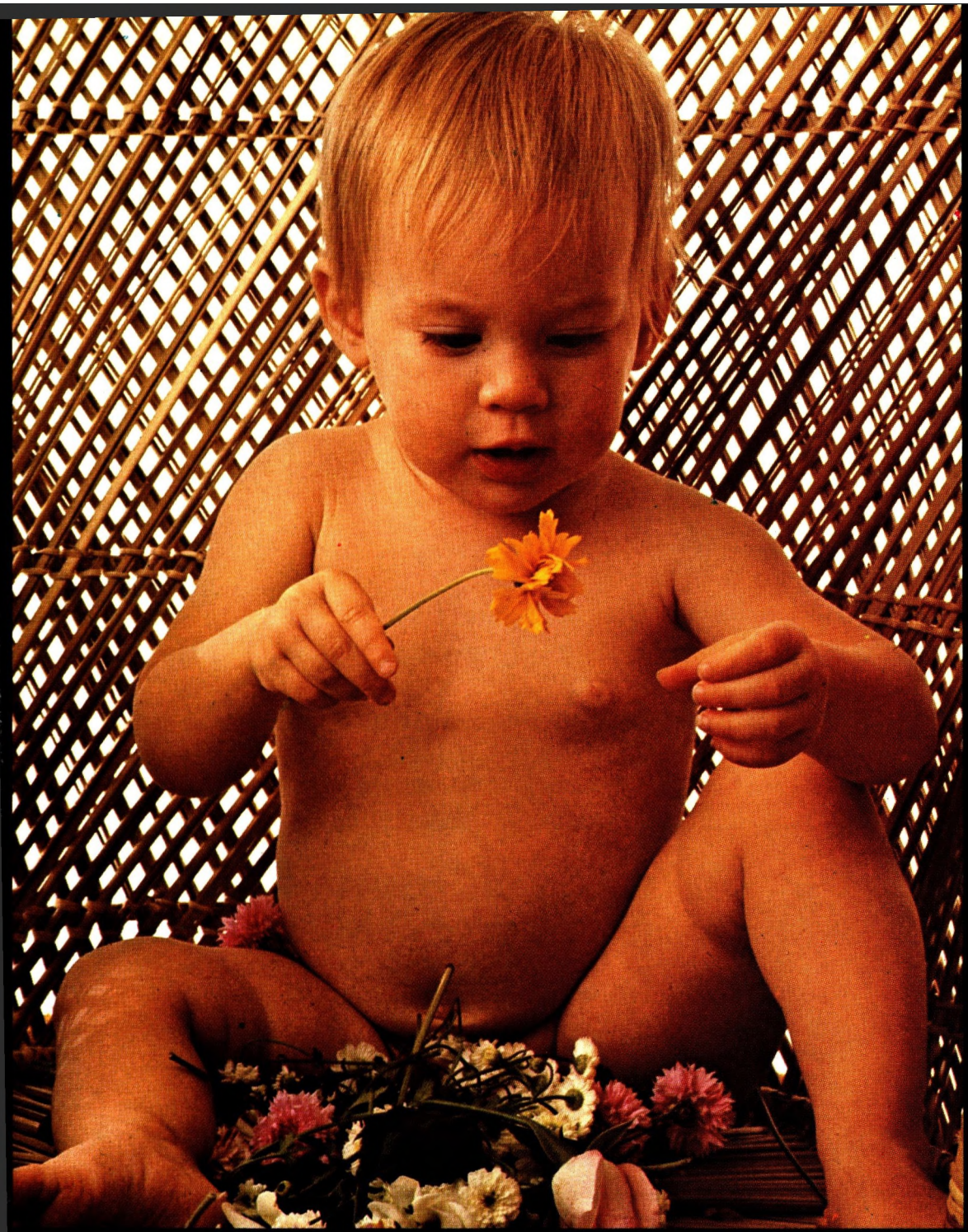

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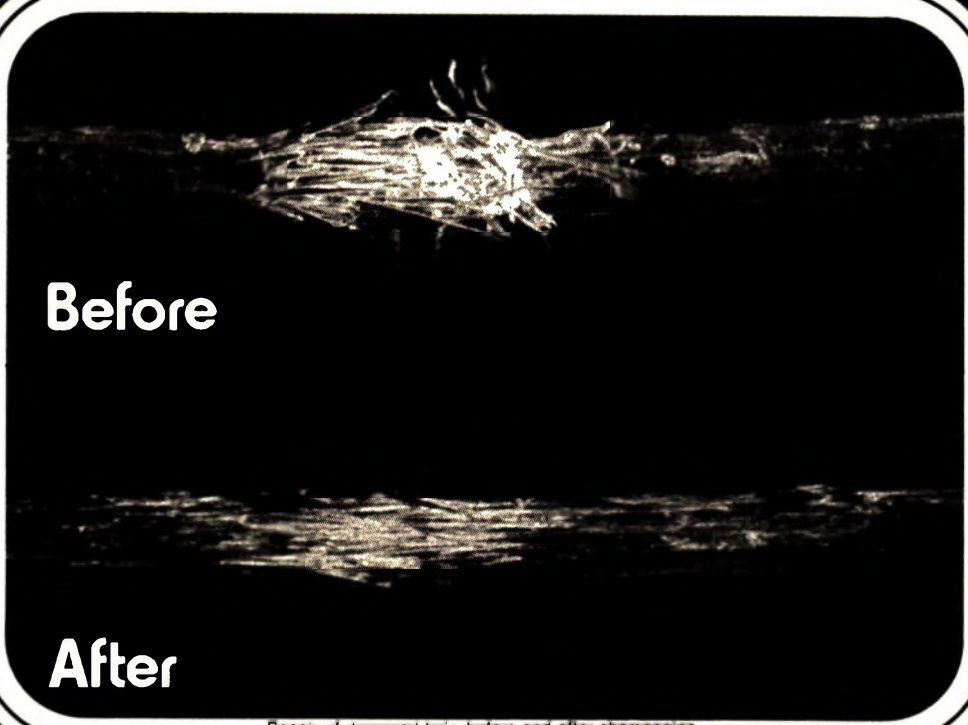

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SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 3 × 5 index cards for reference, without mutilating the pages of the Journal.

A method for appraising the stinging capacity of topically applied substances: Peter J. Frosch and Albert M. Kligman. *Journal of the Society of Cosmetic Chemists* **28,** 197 (May 1977)

Synopsis—Substances, which cause sustained stinging, can be recognized by application to the nasolabial folds and cheeks during profuse sweating. The test was carried out on preselected individuals in whom a susceptibility to stinging has been demonstrated by exposure to 5 per cent aqueous lactic acid. Stinging proneness is greater in females than in males, and in whites more so than in blacks, and especially in light complexioned persons who tan poorly. Stinging is mainly perceived on the face.

Stinging and irritancy were not strictly correlated. Most primary irritants do not cause stinging. Weak irritants may sting badly.

Skin surface lipids: Max Gloor and Holger Kohler. *Journal of the Society of Cosmetic Chemists* **28,** 211 (May 1977)

Synopsis—Skin surface lipids were obtained from symmetrical sites on the foreheads of 33 healthy subjects by the frosted glass and by the paper absorption methods. The specimens were then analyzed by thin layer chromatography. The results show that, due to the sorptive effect of the filter paper, the filter paper method removes more lipids from the excretory ducts of the sebaceous glands than from the epidermal lipids. The frosted glass method is recommended for qualitative lipid sampling because the paper absorption method is affected by several other variables. These include the extent to which horny layer lipids and especially those from the excretory ducts of the sebaceous glands are included in the sample subjected to analysis.

Observations on female scalp hair population, distribution, and diameter: Ellyn M. Cottington, Roy H. Kissinger, and William S. Tolgyesi. *Journal of the Society of Cosmetic Chemists* **28,** 219 (May 1977)

Synopsis—Scalp fiber population density, grouping, and diameter were studied on a panel of 20 women ranging in age from 24 to 59. The average number of fibers per square cm was above 200, which can be translated to about 170,000 fibers for a full head of hair. The fibers grow in a variety of groupings with wide individual variations. The average fiber diameter for the group was about 70 μm, with individuals ranging from under 60 to about 90 μm. The influence of the fiber number and diameter on the bulky appearance of the hair is often masked by other characteristics such as waviness and hair care practices.

Application of the theory of hydrophobic bonds to hair treatments: Kathleen E. Hall and Leszek J. Wolfram. *Journal of the Society of Cosmetic Chemists* **28,** 231 (May 1977)

Synopsis—A novel technique of hair treatment via introduction of nonpolar residues into hair structure in hydroalcoholic media is described. Hair modified in this manner exhibits greatly enhanced settability and high set retention, even at high levels of ambient humidity. The setting behavior can be manipulated by utilizing the differential swelling response of treated hair to water and aqueous alcohols.

Some aspects of the stratum corneum-organic solvent system: A. F. El-Shimi and H. M. Princen. *Journal of the Society of Cosmetic Chemists* **28,** 243 (May 1977)

Synopsis—A limited study of the sorption, desorption, and diffusion behavior of some organic vapors (benzene and toluene) in intact stratum corneum has been carried out, and the results compared to water vapor. The effect of treating the corneum in a mixture of organic solvents (chloroform-methanol) followed by water extraction, on the water vapor sorption, desorption and diffusion characteristics has been assessed. Scanning electron microscopy (SEM), was also used to examine the structural changes in the corneum resulting from the organic solvents-water treatment.

The shape of the benzene and toluene sorption isotherms was found to be compatible with type II in the BET classification. The diffusion process of the organic vapors in the corneum is much faster than that of water vapor. An increase by 3 orders of magnitude in the value of D_i (intrinsic diffusion coefficient) has been observed as the concentration of organic vapor increases in the corneum.

Sequential treatment of guinea pig corneum in organic solvent and water resulted in a marked decrease in the water vapor sorption capacity in the high humidity range. The initial portions of the water vapor sorption isotherms on the treated and intact corneum are the same (up to about 50 per cent RH). The diffusion of water vapor in the treated corneum is 10 times as fast as in the intact corneum.

Thin-layer chromatography (TLC) of redox reaction products of oxidative hair dyes: Mukund J. Shah. *Journal of the Society of Cosmetic Chemists* **28,** 259 (May 1977)

Synopsis—Oxidative or permanent hair dyes are based on alkaline peroxide oxidation of phenylenediamine (PDA) or related aromatic amines. These amines, when oxidized alone or in combination with other phenolic and aromatic amino compounds (couplers), yield a mixture of colored oxidation products. This paper describes the use of thin-layer chromatography (TLC) for the qualitative analysis of these complex mixtures of oxidation products. Effects of variables including the nature of adsorbency, layer thickness, water content, development of chromatograms, and sample application techniques are presented. Scopes and limitations of chromatography for the isolation and the identification of these dyes are discussed.

Wettability of keratin fiber surfaces: K. Y. Kamath, C. J. Dansizer, and H.-D. Weigmann. *Journal of the Society of Cosmetic Chemists* **28,** 273 (May 1977)

Synopsis—The wetting of hair fibers by water has been measured using the Wilhelmy balance technique developed at Textile Research Institute Princeton, N.J. specifically for fibrous materials. The data for selected and treated hair samples suggest cause-effect relationships between water wettability of the fiber surface and mechanical weathering, and chemical fiber damage. Critical surface tension of the hair fiber surface has been determined with water-butanol mixtures using the same technique. Furthermore, dispersion and nondispersion contributions to the surface free energy of the fiber have been evaluated by measuring wettabilities against a polar and nonpolar liquid. The results indicate that the molecular processes occurring at the interface between the keratin fiber surface and a liquid have considerable effect on the surface free energy of the fibers.

Studies of water-in-oil (w/o) emulsion stabilized with amino acids or their salts: Yoshimaru Kumano, Shin Nakamura, Saddaki Tahara, and Saburo Ohta. *Journal of the Society of Cosmetic Chemists* **28,** 285 (May 1977)

Synopsis—Water-in-oil (w/o) emulsions stabilized by using gels formed between surfactants and aqueous solutions of amino acids were studied. The gel can only be obtained with a fluid surfactant which has lipophilic properties and a specific orderly lamellar structure and amino acids or their salts which are readily soluble in water.

By dispersing these gels into the oil phase and then adding the water phase, extremely stable w/o emulsions with wide ranges of water content were obtained. This type of emulsification was termed the "gel-emulsification method" by the authors. When this new technology was applied to the preparation of cosmetics, products with outstanding characteristics were obtained.

The function of the amino acids in the emulsification were investigated by using physico-chemical methods such as X-ray analysis, nuclear magnetic resonance (NMR), heat of solution, electron microscopy (EM), and measurement of the water content solubilized in the surfactant phase. It may be concluded that the amino acids are effective in forming a tight surface atmosphere around the water particles and in preventing coalescence of water particles by strong hydration effect of the amino acids, thus stabilizing the w/o emulsion.

J. Soc. Cosmet. Chem., 28, 197–209 (May 1977)

A method for appraising the stinging capacity of topically applied substances

PETER J. FROSCH and ALBERT M. KLIGMAN *Duhring Laboratories, Department of Dermatology, University of Pennsylvania, 3500 Market Street, Philadelphia, Pennsylvania 19104.*

Received April 13, 1976.

Synopsis

SUBSTANCES, which cause SUSTAINED STINGING, can be recognized by APPLICATION to the NASOLABIAL FOLDS and CHEEKS during PROFUSE SWEATING. The test was carried out on preselected individuals in whom a susceptibility to stinging had been demonstrated by exposure to 5 per cent aqueous lactic acid. Stinging proneness is greater in females than in males, and in whites more so than in blacks, and especially in light complexioned persons who tan poorly. Stinging is mainly perceived on the face.

Stinging and irritancy were not strictly correlated. Most primary irritants do not cause stinging. Weak irritants may sting badly.

INTRODUCTION

Producers of medicaments, cosmetics, and toiletries are attuned to the necessity of certifying the safety of their products. Generally speaking, adequate test procedures have been developed for assessing the likelihood of toxicity from irritation, contact sensitization, and photosensitization.

Nonetheless, products which pass these tests and which fulfill the purposes for which they were designed may still be unacceptable. The consumer will reject even effective formulations if disagreeable sensations arise after application. In this paper, we are concerned with a special type of subjective discomfort, namely, delayed stinging or smarting from topical agents applied to the skin. In contrast to substances like alcohol which cause immediate but transient stinging, those that induce delayed and sustained stinging are not so easily recognized. This adverse effect may not come to light until after the product has been in widespread use.

Our focus is on substances which begin to "sting" or "burn" within a minute or two. The discomfort intensifies over the next 5 to 10 min and may become so severe that frenetic attempts are made at removal. Intense stinging generally abates within about 15 min. Signs of irritation—redness, scaling, edema—do not develop.

It is this sustained, crescendo type stinging that we undertook to study. An embarrassing experience precipitated our research. Although, it had been previously noted (1) that amyl-dimethyl-p-aminobenzoic acid (ADP)* could cause stinging, our premarketing tests of a sunscreen containing this ingredient repeatedly failed to disclose sustained stinging even under thermal stress. It was only after widespread sale that complaints were made by a small proportion of users. In our opinion, the prevalence of disagreeable stinging from this product is less than that reported by Parrish *et al.* (2) Were this not so, the extensive field testing, which was done in hot climates, would have revealed this adverse reaction much earlier.

We are not the first to become conscious of the need to ascertain undesirable subjective reactions. Armstrong and his coworkers applied test substances to the base of cantharidin blisters in order to appraise their capacity to cause pain (3). This test is not relevant for materials that provoke stinging on normal skin. Laden (4) clearly perceived the problem and attempted to develop appropriate methodologies for detecting stinging potentiality.

He immersed the abraded tails of rats in test solutions and determined the time required for the rat to flick its tail out of the solution. He emphasized the variability of the results though useful information could be secured. For human testing, Laden applied test materials to scotch-tape stripped skin, obtaining reasonable agreement with the animal data. Again this procedure mainly measures pain (or does not clearly discriminate between pain and stinging); its applicability to stinging from application to normal skin is uncertain. Laden made the important observation that stinging and irritation could not be correlated. For example, substances such as citric and acetic acids caused intense stinging but scored quite low in tests of primary irritancy.

Recently, Shanahan and Ward (5) described an interesting animal model for estimating the stinging capacity of shampoos. They injected mice intraperitoneally and scored the intensity of the ensuing writhing. The results correlated well with human eye sting tests. Again, stinging and irritancy were not found to parallel each other. Some shampoos which proved to be nonirritating by the Draize rabbit eye test caused intense stinging.

MATERIALS AND METHODS

I. SELECTION OF SUBJECTS

We gradually came to appreciate that only a small proportion of persons would show a stinging response to ADP. Our first task then was to develop a screen to locate "stingers," persons who had this peculiar susceptibility. The following procedure proved reliable. A volunteer was brought to a state of profuse sweating in an environmental chamber at 110°F and 80 per cent relative humidity (R.H.). Then a 5 per cent aqueous solution of lactic acid was rubbed briskly over the nasolabial fold and cheek.

*Padimate, Eclipse, G. S. Herbert Co.

Those who experience sharp stinging for at least 3 to 5 min are identified as stingers. An alternative screening test, which can be carried out at room temperature, involves the application of 2 ml of 90 per cent aqueous dimethylsulfoxide (DMSO) in a small glass cup on the cheek for 5 min. This produces intense burning in stingers; however, the disadvantages of DMSO are disqualifying. The substance smells and tastes badly, not to mention that a solid tender wheal and persistent erythema develop. By contrast, lactic acid produces no visible changes.

II. STINGING ASSAY

This is carried out on groups of 5 to 10 stingers, depending on the degree of accuracy required. The subject enters the environmental chamber (110°F, 80 per cent R.H.) and after the face has begun to sweat copiously (generally after 15 min) a liberal amount of the test material on a cotton swab is thoroughly rubbed over the nasolabial fold and cheek. Stinging is evaluated immediately (10 sec) and at 2.5, 5.0, and 8.0 min on a 4-point scale: 0 = no stinging; 1 = slight; 2 = moderate; and 3 = severe. The time sequence was formed empirically. Some substances cause slight to severe stinging immediately after application with disappearance of the sensation within 5 to 30 sec. Delayed stinging is generally not preceded by a transient phase and usually becomes evident within a minute or two. The delayed stinging score for an individual is the mean of the three readings at 2.5, 5.0, and 8.0 min. We arbitrarily regard substances with average scores falling between 0.4 and 1.0 as having slight stinging potential. The range 1.1 to 2.0 signifies moderate stinging, and 2.1 to 3.0 signifies severe stinging.

A second agent can be simultaneously evaluated on the opposite cheek. Within limits, a series of agents can be evaluated during 1 sweating period.

EXPERIMENTAL OBSERVATIONS

I. PREVALENCE OF STINGERS

Thirty young adult student volunteers (20 whites, 10 blacks, equally divided as to sex) were evaluated using both lactic acid and DMSO probes. Both tests gave concordant results.

There were 4 stingers among the whites, only one of whom was a male. Only 1 black, again a female, exhibited stinging. With lactic acid stinging began after a few seconds and in 4 of 5 subjects persisted strongly for about another 5 min, subsiding slowly over the next 10. DMSO produced peak stinging within 3 min; this remained at high intensity for another 10.

The nonstingers did not perceive significant stinging with either test. It is worth noting that DMSO induced considerably less whealing and erythema in the latter. All 5 stingers thought they had unusually "sensitive" facial skin having had past trouble with cosmetics, soaps, etc. Three had a history of atopy (but not atopic dermatitis). The white stingers had light complexions, sun-burned easily, and tanned poorly. All 5 blushed easily.

The sample is too small for any but the most tentative judgments. Females seem to be more susceptible. It remains to be shown whether this is a true sex difference or

Table I
Comparison Between Stingers and Nonstingers

Agent	Lactic Acid Positive					Lactic Acid Negative				
	Subject	Score				Subject	Score			
		10 sec	2.5 min	5.0 min	8.0 min		10 sec	2.5 min	5.0 min	8.0 min
5 per cent	1	1	3	3	0	1	0	0	0	0
lactic	2	0	1	2	1	2	0	0	0	0
acid	3	3	2	2	1	3	0	1	0	0
(Water)	4	1	2	2	1	4	0	0	0	0
	5	2	1	1	0	5	0	0	0	0
	Mean ± S.E.	1.4 ± 0.51	1.8 ± 0.37	2.0 ± 0.32	0.6 ± 0.24	Mean ± S.E.	0	0.2 ± 0.2	0	0
5 per cent	1	2	2	3	3	1	1	0	0	0
arnyl-	2	0	2	3	2	2	0	0	0	0
dimethyl	3	0	2	2	1	3	0	0	0	0
paba	4	1	1	2	0	4	1	0	0	0
(ethanol)	5	0	2	1	0	5	0	0	0	0
	Mean ± S.E.	0.6 ± 0.40	1.8 ± 0.20	2.2 ± 0.37	1.2 ± 0.58		0.4 ± 0.24	0	0	0
50 per cent	1	2	1	0	0	1	1	0	0	0
dimethyl	2	3	3	2	1	2	1	0	0	0
phthalate	3	3	1	1	0	3	0	0	0	0
(ethanol)	4	2	1	1	0	4	1	1	0	0
	5	1	2	2	0	5	0	0	0	0
	Mean ± S.E.	2.2 ± 0.37	1.6 ± 0.40	1.2 ± 0.37	0.2 ± 0.20		0.6 ± 0.24	0.2 ± 0.2	0	0
Propylene	1	3	3	3	2	1	0	0	0	0
glycol	2	2	1	1	0	2	0	0	0	0
	3	1	1	1	0	3	1	0	0	0
	4	2	2	1	0	4	0	0	0	0
	5	1	1	0	0	5	1	0	0	0
		1.8 ± 0.37	1.6 ± 0.40	1.2 ± 0.49	0.4 ± 0.4		0.4 ± 0.24	0	0	0
5 per cent	1	1	3	1	0	1	0	2	1	0
phosphoric	2	1	2	3	2	2	0	2	0	0
acid	3	0	3	3	1	3	0	1	0	0
(water)	4	2	3	2	0	4	1	2	1	0
	5	3	3	3	2	5	1	0	0	0
	Mean ± S.E.	1.4 ± 0.51	2.8 ± 0.20	2.4 ± 0.40	1.0 ± 0.45		0.4 ± 0.24	1.4 ± 0.40	0.4 ± 0.24	0

merely a reflection of the fact that more females had light complexions. Weigand and Mershon (6) evaluated tear gas (*o*-chlorobenzylidene malononitrile) for irritancy and stinging in various body regions of black and white subjects. They too found that on the cheek and retroauricular areas, stinging was weaker in blacks and appeared later.

II. COMPARISON BETWEEN "STINGERS" AND "NONSTINGERS"

ADP (5 per cent), dimethylphthalate (50 per cent in ethanol), propylene glycol (neat), and phosphoric acid (5 per cent) were applied during sweating to 5 lactic acid-positive and 5 lactic acid-negative persons, "stingers" and "nonstingers," respectively. The results are given in Table I.

Every stinger experienced moderate to severe stinging with all 4 test agents while stinging was virtually absent in the lactic acid-negative group, except for reduced reactions to phosphoric acid at 2 to 5 min. Five per cent ADP mainly produced delayed stinging, while 50 per cent dimethylphthalate caused strong transient stinging as well.

III. INFLUENCE OF SWEATING

A 5 member panel was tested with 5 per cent ADP in ethanol at room temperature. Tests on separate occasions were conducted at 5, 15, and 30 min after entering the environmental chamber.

Stinging did not occur at room temperature. However, stinging was already weakly evident when the application was made 5 min after entering the chamber, before sweating was at full flow. After 15 min, all 5 subjects experienced intense stinging at the 5-min reading. Rinsing the face with water without leaving the chamber was not followed by an immediate decrease in intensity; stinging persisted at the same level for about 10 min, fading to insignificance about 20 min after removal.

IV. ANHIDROSIS

The critical role of sweating was further demonstrated by testing skin that has been rendered anhidrotic. Sweating was completely suppressed on 1 cheek of 3 stingers by applying an occlusive patch of 5 per cent aqueous aluminum chloride hexahydrate for 24 h. Three h after removal of the patch, a 5 per cent ethanolic solution of ADP was applied to both cheeks after the subjects had been sweating for 15 min.

Stinging was markedly reduced on the anhidrotic side; the score was never greater than 1 compared to 3 on the sweating side.

V. MULTIPLE APPLICATIONS VERSUS 1 APPLICATION

We compared the intensity of stinging with 1 versus 5 applications of 5 per cent lactic acid and 5 per cent ADP in separate groups of 3 stingers each. The test material was applied to 1 side every 5 min for a total of 5 applications starting after the subject had been sweating for 10 min. The opposite side received 1 application at the time of the fifth application to the opposite cheek.

The results were most dramatic with lactic acid. The intensity of stinging increased with each application producing almost intolerable discomfort in all 3 subjects. These were hardy volunteers and it was only by dint of much persuasion that the test was completed. Indeed, superficial erosions subsequently developed in 2 of the 3. Signs of irritation have never been observed with 1 application.

The same pattern of progressive intensification of stinging was observed with ADP, but the severity was less.

VI. PRIOR DAMAGE

A. *Sunburn:* Using fluorescent sunlamp tubes* 2 MEDs were given to 1 cheek of 3 stingers. The next day stinging was assessed on both sides by applying 5 per cent ethanolic ADP after 15 min of sweating.

Stinging began earlier and was considerably more intense on the sunburned side in all 3 subjects. An inflammatory reaction thus accentuates the response.

B. *Chemical irritation:* A 5 per cent aqueous solution of a quaternary ammonium compound† was applied to 1 cheek by occlusive patches for 10 min twice daily for 2 days. Five per cent ADP was applied to both cheeks of 3 sweating stingers one day later when the treated skin showed moderate erythema and tiny pustules.

Stinging was sharply accentuated on irritated skin, emerging more rapidly and becoming very intense after a few minutes.

VII. STINGING ON STRIPPED SKIN

A. *Lactic acid:* The cheeks of 3 nonstingers were scotch-tape stripped to the glistening layer while half that number of strippings was made on the other side. After the subjects had been sweating for 15 min, 5 per cent lactic acid was applied to both cheeks.

Stinging appeared immediately and in high intensity on the completely stripped side. Stinging was less on the partially stripped opposite side, but was nonetheless appreciable. The duration on stripped skin was shorter than on the normal skin of "stingers." The stinging on completely stripped skin was very sharp upon application, but faded within 2.5 min. This result indicates that all persons will experience stinging after removal of the horny layer barrier.

In another test, 5 per cent lactic acid was applied to the stripped skin of the nonsweating back of 3 stingers and 3 nonstingers. Stinging, indeed pain, was equally intense in both groups immediately after application, declining rapidly within a few minutes. Discrimination is lost on stripped back skin.

B. *ADP:* A panel of 3 nonstingers was scotch-tape stripped to the glistening layer on 1 cheek and 1 side of the upper back. Five per cent ADP was applied to both normal and

*Westinghouse FS 20.
†Hyamine 3500, Rohm & Haas Co., Spring House, PA 19477.

stripped skin. The first test was carried out on the backs without sweating and on the cheeks after 15 min of sweating.

Stinging did not occur on normal skin. On the stripped sites of the back, as well as the cheek intense immediate stinging developed, disappearing within about a minute. Stripping practically eliminates the distinction between stingers and nonstingers. It should also be noted that almost as severe stinging occurred after application of the vehicle, 95 per cent alcohol. It had become evident by this time that the difference between stingers and nonstingers was merely quantitative. To test this idea, 5, 10, and 15 per cent ADP was applied on separate occasions to the profusely sweating cheeks of 3 stingers and 3 nonstingers. The stingers experienced sharp discomfort with 5 per cent ADP; the severity and duration of stinging increased progressively with the 10 and 15 per cent concentrations. With the latter, the distress was not appreciably reduced by leaving the chamber.

As expected, 5 per cent ADP had no effect on nonstingers. All 3, however, experienced slight stinging with 10 per cent, while with 15 per cent, 2 of the 3 had moderate stinging.

It is clear, then, that stingers merely have lower thresholds. When the stimulus is great enough, even nonstingers will experience mild to moderate stinging. We have repeatedly observed this phenomenon with other drugs at higher concentrations.

VIII. REGIONAL DIFFERENCES

Five per cent ADP was used on 3 stingers to compare the intensity of stinging in the following regions: central cheek, nasolabial fold, forehead, chin, infraorbital and retroauricular regions, axilla, antecubital fossa, upper back, and scalp. The applications were made after the subjects had been sweating for 15 min.

Clear-cut stinging was restricted to the face, being most pronounced on the nasolabial fold, followed by the cheek, chin, infraorbital, and retroauricular region. The forehead reaction was marginal. Stinging was not perceived on the scalp, back, arm, and axilla. These findings conform to those of Weigand and Mershon (6) using tear gas *o*-chlorobenzylidene malononitrile (CS) as the stinging agent. Stinging is predominantly a facial phenomenon.

IX. CORRELATION OF STINGING WITH IRRITANCY

A. *α-hydroxy acids:* The test agents were: lactic acid, pyruvic acid, tartaric acid, and glycolic acid. Five per cent aqueous solutions were evaluated in 3 stingers at different sweating sessions.

Comparative irritancy was determined by 24-h occlusive patch tests on the forearms with 5 and 15 per cent concentrations of the respective acids on the same subjects. The intensity of the reaction was scored on a 0 to 3 scale (0 = no erythema; 1 = slight; 2 = moderate; and 3 = severe). The rank order of descending irritancy was: pyruvic > glycolic > tartaric > lactic.

The first 2 produced severe erythema and vesiculation with the 15 per cent concentration. The rank order was the same in stinging tests; the correlation was thus very good despite the small size of the sample.

pH does not account for these differences (range 1.7 to 2.0). Laden (4) also found that acids of the same pH had quite different stinging capacities. Following his precedent, we compared equimolar (0.3 *N*) solutions (concentration percentages 2.25 to 2.70). The rank order of stinging was identical, although, stinging tended to be rather weak at these lower concentrations.

B. *Esters of p-aminobenzoic acid:* The test agents were glyceryl p-aminobenzoic acid (GP-Escalol 106), (ADP-Escalol 506), and octyl-dimethyl-paba (OCP-Escalol 507). Five per cent solutions in ethanol were tested for stinging in the usual manner on 3 subjects.

Five per cent solutions were evaluated for irritancy by occlusive patch tests on the forearm after criss–cross scarification of the skin with a 27-gauge needle. Applications were made daily for 3 consecutive days under cutaneous occlusion.

As usual, ADP induced sharp stinging. The other 2 esters lacked this property completely. GP caused modest redness while ADP and ODP were completely innocuous on sacrified skin.

So, in this instance, a stinging ester (ADP) was found to be nonirritating, while an irritating one (GP) was nonstinging.

C. *Metallic antiperspirants:* The test agents were aluminum chloride hexahydrate, aluminum chlohydroxide, aluminum bromhydroxide (basic aluminum bromide), and zirconium hydroxychloride. Thirty per cent aqueous solutions of these were evaluated for irritancy by 24-h occlusive patch tests on forearm skin. This same concentration was also used in stinging tests performed on 3 stingers. The rank order of irritancy was: aluminum chloride > zirconium hydroxychloride > aluminum chlorhydroxide equals basic aluminum bromide.

The last two caused no skin reaction, whereas the first two produced erythema and small pustules, aluminum chloride being somewhat more severe.

As with hydroxy acids, stinging capacity paralleled irritancy. Aluminum chloride caused slight stinging, followed closely by zirconium hydroxychloride, while the other 2 salts lacked this property altogether.

D. *Effect of strong irritants:* A 5 per cent aqueous solution of sodium lauryl sulfate (SLS) produced an intense dermatitis in a 24-h patch test on the forearm of 3 stingers, still this same solution did not induce stinging in any sweating subject when applied to the face.

Undiluted kerosene caused blisters in a 24-h patch test on the same 3 persons, but was utterly without stinging potentiality.

A 5 per cent solution of Hyamine 3500 produced a marked irritant reaction by a 24 hr occlusive patch test. It, too, failed to elicit stinging. It is thus apparent that strong irritants may be completely free of stinging capacity.

X. EFFECT OF VEHICLES

ADP was evaluated in 5 stingers using 5 per cent concentrations in the following vehicles: ethanol, hydrophilic ointment USP, carbowax USP, ethanol : propylene glycol (1:1) and vanishing cream USP.

The ethanol:propylene glycol solution stood out above all the others in stinging capacity, followed closely by ethanol. The onset and intensity of stinging was markedly reduced by vanishing cream. Stinging was virtually abolished with carbowax and hydrophilic ointment. Thus, solutions are more likely to cause stinging than creams and ointments. The effects are no doubt dependent on release from the vehicle.

XI. DOSE RESPONSES

ADP was tested at 1.0, 3.0, and 5.0 per cent concentrations in ethanol in 3 stingers. The 1 per cent concentration did not cause any stinging. Stinging was mild with 3 per cent in all 3 subjects. The 5 per cent solution produced intense stinging as usual; in addition, stinging came on earlier. Thus, clear-cut differences can be detected over a rather narrow concentration range.

XII. ASSAY OF MATERIALS OF DERMATOLOGIC INTEREST

Stinging tests were carried out on panels of 5 stingers with a variety of familiar substances. Stinging was not observed with 3 per cent hydrogen peroxide, 5 per cent ammonium hydroxide, saturated salt solution, vanishing cream USP, hydrophilic ointment USP, carbowax Ointment USP, "pool chlorine," and a variety of steroid creams and gels, 1 per cent aqueous neomycin sulfate, 5 per cent p-aminobenzoic acid in hydro-alcoholic vehicle*, 0.2 per cent Uvinul 539 in ethanol (2-ethylhexyl-2-cyano-3-3-diphenyl-acrylate), 8 per cent homosalate†. All the materials in Table II induced delayed stinging to varying degrees. These have been sorted into 3 groups (slight, moderate, and severe).

The following agents caused transient but not delayed stinging: methanol, ethanol, 5 per cent concentrations of ascorbic, acetic, citric and sorbic acids, retinoic acid.‡

DISCUSSION

The essential features of our stinging test are: (1) selection of volunteers who exhibit sharp stinging to 5 per cent lactic acid and (2) application of the test agent to the nasolabial fold after sweating has been induced. The results are highly repeatable; there is sufficient sensitivity to permit substances to be accurately rated as mild, moderate, or severe.

*Pre-Sun, Westwood Pharmaceuticals, Inc., Buffalo, N.Y. 14213.

†Coppertone Cream, Plough Inc., Memphis, TN 38151.

‡Retin A solution, 0.05 per cent, Johnson & Johnson Co., East Brunswick, N.J.

Table II
Agents That Induce Stinging

Agent	Concentration Per cent	pH	Vehicle	Severity
Benzene	1.0	7.5	Ethanol	Slight
Phenol	1.0	5.9	Ethanol	Slight
Salicylic acid	5.0	2.4	Ethanol	Slight
Resorcinol	5.0	6.4	Water	Slight
Phosphoric acid	1.0	2.1	Water	Slight
Sodium carbonate	15.0	11.2	Water	Moderate
Trisodium phosphate	5.0	12.0	Water	Moderate
Propylene glycol[a]	Neat	5.3		Moderate
Propylene carbonate[a]	Neat	8.05		Moderate
Propylene glycol diacetate[a]	Neat	3.8		Moderate
Dimethylacetamide	Neat	7.8		Moderate
Dimethylformamide	Neat	10.2		Moderate
Dimethylsulfoxide	Neat	14.0		Moderate
Diethyltoluamide[a] (Deet)	50.0	8.8	Ethanol	Moderate
Dimethyl phthalate[a]	50.0	4.0	Ethanol	Moderate
2-Ethyl-1, 3-hexanediol[a] (Rutgers 612)	50.0	10.5	Ethanol	Moderate
Benzoyl peroxide lotion[b]	5.0	9.9	Grease-free washable lotion base	Moderate
Benzoyl peroxide gel[c]	10.0	14.0	Polyoxyethylene lauryl ether gel	Moderate
Crude coal tar[a]	5.0	10.0	Dimethylformamide	Severe
Phosphoric acid	3.3 (1/3 mol)	1.9	Water	Severe
Hydrochloric acid[a]	1.2 (1/3 mol)	1.3	Water	Severe
Sodium hydroxide	1.3 (1/3 mol)	13.0	Water	Severe
2-ethoxyethyl p-methoxy-cinnamate[d]	2.0	7.4	Ethanol	Severe

[a]Immediate transient stinging as well as delayed type.
[b]Benoxyl 5 lotion, Stiefel Labs., Inc.
[c]Desquam-X 10 gel, Westwood Pharmaceuticals, Inc.
[d]Giv-Tan F®, Givaudan Corp.

The stinging phenomenon is peculiar to the face, particularly the nasolabial folds and cheeks, the latter being a little less sensitive. Our explanation for this localization relates to the high permeability of the nasolabial region (as determined by visible responses to vasoactive drugs), the high density of appendages (hair follicles and sweat glands), which can serve as penetration shunts into the dermis, and, not least, the elaborate sensory nerve network. In man, every vellus hair follicle is associated with specialized nerve endings; these along with the abundant dermal nerve network on the face confer an exceptional sensitivity to touch and pain.

Stinging seems to be a variant of pain and develops quickly after appropriate stimulation of sensory nerves. However, toxic and irritating chemicals, which can badly

damage skin, often lack the capacity to induce stinging. On the other hand, substances that are nonirritating may possess striking stinging capabilities.

As a class, acids tend to cause strong stinging. The differences in stinging potentiality among acids are not dependent on pH, but are probably related to their diffusional characteristics, the more troublesome ones penetrating more rapidly.

Likewise, strongly alkaline substances such as sodium carbonate, trisodium phosphate, and sodium hydroxide can induce marked stinging. Acids (below pH 2.0) and bases (above pH 11.0) probably excite nerve endings directly. The strong buffering capacity of the skin doubtlessly limits the damage from a single application.

While there is generally no clear correlation between irritancy and stinging, their properties may parallel each other within a specific class of substances, *viz.*, metallic antiperspirants and α-hydroxy acids.

That excitation of nerve endings is central to the stinging phenomenon is suggested by a number of observations. The intensity increases with each additional application of non-irritating materials. Hypertonic solutions do not cause stinging, but can cause its revocation after a stinging episode. For example, stinging will reappear when a saturated solution of sodium chloride is applied after the stinging induced by ADP or lactic acid has abated. Moreover, inflamed skin is far more prone toward stinging, even when the horny layer barrier is intact as in freshly sunburned skin. Of course, in chemically damaged skin the nerve endings are not only more excitable but increased permeability enables increased diffusion.

Stinging is perceived with 5 per cent ADP and 5 per cent lactic acid only when the subject is sweating; the intensity of the sensation is proportional to the amount and duration of sweating. The longer a person sweats, the easier and faster stinging can be elicited. In all probability, the principal role of sweating is to hydrate the skin, thus greatly increasing its permeability. It is well known that the flux of substances increases in moistened skin. Another is the associated increase in skin temperature, heightening the sensitivity of nerve endings. We found that stinging could be induced even at room temperature if a wet compress was applied to the cheek for 20 to 30 min before exposure to 5 per cent lactic acid. However, the intensity and duration of the stinging was always much less than with induced sweating. Then, too, diffusion through sweat-filled ducts promotes transport to dermal nerves. The sharp decrease in stinging on cheeks rendered anhidrotic by aluminum chloride is further evidence of the role of hydration.

Although, we have emphasized preselection of subjects, it is perfectly clear that stinging can be elicited in anyone by assuring access to nerve endings. Persons who do not sting, when lactic acid is applied to the normal cheek, will do so if the horny layer barrier is removed, partially or completely by scotch-tape stripping. Apparently, the peculiarity of "stingers" is more permeable skin. As a practical point, persons who are not stinging-prone may experience appreciable stinging if the skin has become inflamed through chemical or physical insults; for example, sunburned skin is considerably more reactive. We have preliminary evidence that the facial skin of stingers is more permeable. We have already mentioned that DMSO produced more intense wheeling and erythema in stingers. It is our impression that stingers have more permeable skin everywhere.

At first glance, stripping might seem to offer the alternative of screening substances on areas other than the face and without the requirement of sweating. Unfortunately, this is not a feasible alternative. ADP, for example, did not elicit typical stinging in stripped skin of the back and was not different from its vehicle ethanol. Moreover, stinging in stripped skin was evanescent and not at all discriminating. Many irritating substances, which do not cause facial stinging, will produce pain and stinging on stripped skin.

GUIDELINES FOR PERFORMING STINGING TESTS

SUBJECTS

Stingers are likely to be white females with light complexions who give histories of easy sunburning. They frequently complain of having sensitive skin and of having trouble with cosmetics and soaps. The lesser susceptibility of blacks is doubtless due to their possession of a more effective barrier, the horny layer being both more dense and with a greater number of cell layers (7).

THERMAL STIMULATION

The facial sauna* is a useful substitute for an environmental chamber; the latter can hardly be viewed as standard equipment.

The subject places the face directly into the steam stream for at least 15 min or until sweating is brisk. The steam exposure must be continued for another 10 min after application of the test agent. We obtained comparable results with a variety of stinging formulations in a panel tested with both the chamber and the sauna. The intensity of stinging was a little less with the sauna.

REPEATED TESTING

If no stinging develops in 5 min, one can wipe off the substance with a wet towel and apply a new test material. This can be repeated at least twice on each cheek, allowing for the evaluation of 6 agents in a single session. The limiting factor in "no response" testing is the time that subjects can stay in the thermal chamber. They are generally wrung out by 45 min.

On the other hand, a positive response increases susceptibility to stinging. To avoid false positive readings, no further testing should be conducted on that cheek.

We have never observed tachyphylaxis over the short term, that is, unresponsiveness induced by repeated testing. However, we have witnessed the development of considerable resistance in 2 of 5 stingers, who were regularly used twice weekly for several months. One subject became virtually a nonstinger after 3 months of testing with stinging materials though irritation was never observed. This induced refratoriness may be a subliminal form of "hardening" that occurs with prolonged exposure to irritants. In any event, subjects who are used repeatedly, should be monitored from

*Facial Beautifying Mist, Model 60. Schick Electric, Inc., Lancaster, PA.

time to time for retention of sensitivity to lactic acid. Susceptibility returns after a rest period of several weeks.

We have recently developed a simpler method of selecting stingers for recruitment of test panels. Thermally induced sweating is not required. The application of 10 per cent lactic acid to the face will elicit stinging which is almost equivalent to 5 per cent acid on sweating skin. The same is true for 15 per cent ethanolic ADP. These higher concentrations have no effect on nonstingers. Let it be clearly noted, however, that tests of unknown materials must be carried out on sweating facial skin of preselected stingers. It should also be noted that substances with a high capacity to induce stinging can do so in the absence of sweating.

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Über den Einfluß der Materialgewinnung auf die Zusammensetzung der Hautoberflächenlipide

Vergleichende analytische Untersuchungen der Mattglasmethode mit der Papierabsorptionsmethode

MAX GLOOR und HOLGER KOHLER*

Synopsis — Skin surface lipids were obtained from symmetrical sites on the foreheads of 33 healthy subjects by the frosted glass and by the paper absorption methods. The specimens were then analyzed by thin layer chromatography. The results show that, due to the sorptive effect of the filter paper, the filter paper method removes more lipids from the excretory ducts of the sebaceous glands than from the epidermal lipids. The frosted glass method is recommended for qualitative lipid sampling because the paper absorption method is affected by several other variables. These include the extent to which horny layer lipids and especially those from the excretory ducts of the sebaceous glands are included in the sample subjected to analysis.

SCHÄFER und KUHN-BUSSIUS (17, 18) haben eine Methode zur quantitativen Bestimmung der Lipidmenge auf der Hautoberfläche angegeben, die darauf beruht, daß die Lichtdurchlässigkeit von Mattglas durch Lipide verstärkt wird. In der vorliegenden Publikation wird erstmals über die Verwendung der Mattglasmethode zur Materialgewinnung für qualitative Lipidanalysen berichtet und das Ergebnis mit dem der Materialgewinnung durch das Papierabsorptionsverfahren verglichen.

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Material und Methode

Untersucht wurden 33 männliche Versuchspersonen im Alter von 12-15 Jahren, die sämtlich frei von Hauterkrankungen waren. Die Lipidgewinnung erfolgte an zwei symmetrischen Stellen an der Stirn, wobei jeweils auf einer Seite die Mattglasmethode und auf der anderen Seite die Papierabsorptionsmethode benutzt wurde. Bei der Hälfte der Versuchspersonen wendeten wir rechts die Mattglasmethode, bei der anderen Hälfte rechts die Papierabsorptionsmethode an. Das verwendete Mattglas war identisch mit dem, welches bei der quantitativen Bestimmungsmethode gebraucht wird. Als absorbierendes Papier benutzten wir ein Zigarettenpapier.* Die Auflagedauer betrug in beiden Fällen 15 Minuten. Das Auflagegewicht war bei beiden Methoden 1 kg. Zwischen dem Mattglas bzw. Papier und dem Auflagegewicht befand sich immer eine Lage Schaumgummi. Die Lipidgewinnung erfolgte durch Extraktion des Mattglases bzw. des Papiers mit Äther.

Die Analyse der Lipide wurde dünnschichtchromatographisch vorgenommen. Wir benutzten die Mikroobjektträgermethode nach VAN GENT (9) in einer von uns beschriebenen Modifikation (12). Auf diese Weise konnten folgende Lipidfraktionen isoliert werden: Freies Cholesterin, freie Fettsäuren, Triglyceride, Wachs- und Cholesterinester, Squalen und Paraffine. In diesen Fraktionen können kleine Mengen anderer Substanzen mit entsprechendem Rf-Wert enthalten sein. Statistisch verglichen wurden die durchschnittlichen Meßwertdifferenzen zwischen den beiden verschiedenen Lipidsammelmethoden mit dem hypothetischen Mittelwert 0 über den WILCOXON-Test für Paardifferenzen, wobei die prozentualen Anteile der einzelnen Lipidfraktionen an dem Lipidgemisch bewertet wurden.

Ergebnisse

Die Tabelle zeigt die durchschnittlichen prozentualen Werte der einzelnen Lipidfraktionen bei Zugrundelegung der Mattglasmethode und der Papierabsorptionsmethode. Als signifikant erwies sich die höhere Menge des freien Cholesterin bei der Materialgewinnung mit der Mattglasmethode ($\alpha = 1\%$). Nicht signifikant, jedoch tendentiell erkennbar war der höhere Squalenanteil bei der Materialgewinnung mit der Papierabsorptionsmethode. Auffallend waren weiter die niederen Prozentsätze der freien

* Sup-air®; Hersteller: Sté Job, Paris.

	Mattglasmethode	Papierabsorptions-methode
Freies Cholesterin	9,45 (2,12)	8,17 (3,17)
Freie Fettsäuren	6,72 (2,91)	7,04 (2,73)
Triglyceride	48,75 (4,61)	49,42 (5,28)
Wachs- und Cholesterinester	24,69 (3,13)	24,33 (2,85)
Squalen	10,29 (2,67)	11,05 (3,02)

Tabelle

Durchschnittliche prozentuale Anteile der einzelnen Lipidfraktionen an den Hautoberflächenlipiden bei Materialgewinnung mit der Mattglasmethode bzw. der Papierabsorptionsmethode. In Klammern finden sich die Standardabweichungen. Die Paraffine bleiben wegen ihres meist exogenen Ursprungs unberücksichtigt.

Fettsäuren und die hohen der Triglyceride bei beiden Methoden. Diese sind zumindest teilweise durch die große Zahl sehr junger Versuchspersonen in dem untersuchten Kollektiv zu erklären (10).

Diskussion

Analysen der Zusammensetzung der Hautoberflächenlipide haben in den letzten Jahren zunehmend an Bedeutung gewonnen, weil vor allem die dünnschicht- und gaschromatographischen Verfahren verbessert wurden.

Ein wichtiger Gesichtspunkt bei der Bewertung der Ergebnisse ist die Art der Materialgewinnung. In der Vergangenheit wurden vorzugsweise folgende Methoden angewendet:

1. Die direkte Extraktion der Hautoberflächenlipide mit einem Fettlösungsmittel, die ursprünglich von CARRIÉ (1) und EMANUEL (8) angegeben wurde.

2. Die Papierabsorptionsmethode, die in der Regel nach den Angaben von STRAUSS und POCHI (20) bzw. CUNLIFFE und SHUSTER (6) durchgeführt wird. Dabei wird ein absorbierendes Papier in Kontakt mit der Haut gebracht, das anschließend mit einem Fettlösungsmittel extrahiert wird.
3. Die Schwammethode, die ursprünglich von SCHNUR und GOLDFARB (19) beschrieben wurde. Dabei werden die Lipide von der Haut mit einem Schwamm entfernt, der zuvor in einem Fettlösungsmittel getränkt worden war. Diese Methode wird z. Z. u. a. von CUNLIFFE (3) angewendet.

All diesen Methoden ist gemeinsam, daß nicht nur Lipide der Hautoberfläche erfaßt werden, sondern auch die der Hornschicht und Lipide aus den Ausführungsgängen der Talgdrüsen. In welchem Maß dies der Fall ist, hängt von vielen Parametern ab, die stark variieren können.

Der Anteil der Hornschichtlipide ist wahrscheinlich von der Struktur (2) und dem Wassergehalt (22) der Hornschicht abhängig. Die Menge der Lipide aus den Ausführungsgängen der Talgdrüsen ist umso größer je weiter der Talgdrüsenausführungsgang ist, dessen Weite von Körperstelle zu Körperstelle (23) und wahrscheinlich von Mensch zu Mensch variiert. Eine Aufquellung der Keratine, z. B. nach heißen Bädern oder nach Anwendung von Okklusivfolien, führt zu einer Engerstellung. Andererseits hat die Dehydratation, z. B. nach Anwendung alkoholischer Lösungen oder Lösungsmitteln, keine signifikante Erweiterung zur Folge (5, 16, 22). Bekannt ist außerdem, daß der weibliche Zyklus die Weite der Talgdrüsenausführungsgänge beeinflußt (21). Schließlich führt eine Hyperkeratose des Talgdrüsenausführungsganges, die der Comedonenbildung zu Grunde liegt, zu einem vollständigen oder partiellem Verschluß (15).

SCHÄFER und KUHN-BUSSIUS (17, 18) haben eine Methode zur quantitativen Lipidbestimmung beschrieben, die darauf beruht, daß die Lichtdurchlässigkeit von Mattglas durch Lipide verstärkt wird. In der uns zugänglichen Literatur fanden wir keinen Hinweis darauf, daß die Mattglasmethode zur *Materialgewinnung* für analytische Zwecke verwendet wurde. Die Anwendung dieser Methode zur Materialgewinnung hat den Vorteil, daß ausschließlich die Hautoberflächenlipide erfaßt werden und kein Sogeffekt auf die Hornschicht und die Talgdrüsenausführungsgänge entsteht. So können zahlreiche Parameter ausgeschlossen werden, die bei den bisher üblichen Methoden die Zusammensetzung des zu analysierenden Lipidgemisches beeinflussen können. Die Mattglasmethode erscheint somit als die optimale Methode der Materialgewinnung, wobei allerdings einschränkend hinzugefügt werden muß, daß sie in der

Regel nur an der Stirn ohne Schwierigkeiten anwendbar ist, da an anderen Körperstellen die so gewonnenen Lipidmengen meist nicht für die chromatographischen Untersuchungen ausreichen.

CUNLIFFE et al. (4) sowie JOSEPHS et al. (14) haben gezeigt, daß mit der direkten Extraktionsmethode mehr epidermale und weniger Talgdrüsenlipide gefunden werden als mit der Papierabsorptionsmethode. Die Schwammethode führt zu ähnlichen Ergebnissen wie die direkte Extraktionsmethode (4). Außerdem finden sich bei der direkten Extraktionsmethode und der Schwammethode mehr freie Fettsäuren und weniger Triglyceride als bei der Papierabsorptionsmethode (4, 14). Die vorliegenden Untersuchungen zeigen, daß sich bei der Materialgewinnung mit der Mattglasmethode größere Anteile an freiem Cholesterin nachweisen lassen, als bei der Materialgewinnung mit der Papierabsorptionsmethode. Bezüglich des Squalen fanden sich tendentiell umgekehrte Verhältnisse, die allerdings nicht statistisch belegt werden konnten. Bei den freien Fettsäuren und den Triglyceriden fanden wir keine eindeutigen Unterschiede.

Diese Ergebnisse müssen dahingehend interpretiert werden, daß mit der Mattglasmethode mehr epidermale und weniger Talgdrüsenlipide erfaßt werden als mit der Papierabsorptionsmethode, da das freie Cholesterin vor allem ein Bestandteil der epidermalen Lipide und das Squalen überwiegend ein Bestandteil der Talgdrüsenlipide ist (11, 12, 13). Es kann also angenommen werden, daß sich der Sogeffekt bei der Papierabsorptionsmethode mehr auf die Lipide des Talgdrüsenausführungsgangs als auf die epidermalen auswirkt. Das Ausmaß des Sogeffektes ist abhängig von der Papierqualität (7).

Zusammenfassung

Bei 33 gesunden Versuchspersonen wurden die Hautoberflächenlipide der Stirn an symmetrischen Stellen mit der Mattglasmethode und der Papierabsorptionsmethode gewonnen und dünnschichtchromatographisch analysiert. Die Untersuchungen zeigen, daß sich bei der Papierabsorptionsmethode der Sogeffekt des Papiers dahingehend auswirkt, daß mehr Lipide aus den Ausführungsgängen der Talgdrüsen als epidermale in dem gewonnenen Material enthalten sind. Die Mattglasmethode wird als Lipidsammelmethode empfohlen, da bei den herkömmlichen Sammelmethoden zahlreiche Parameter Einfluß darauf nehmen, in welchem Maß Hornschichtlipide und vor allem Lipide der Talgdrüsenausführungsgänge in dem zu analysierendem Material enthalten sind.

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Observations on female scalp hair population, distribution, and diameter

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Synopsis

SCALP FIBER POPULATION DENSITY, GROUPING, and DIAMETER were studied on a panel of 20 women ranging in age from 24 to 59. The average number of fibers per square cm was above 200, which can be translated to about 170,000 fibers for a full head of hair. The fibers grow in a variety of groupings with wide individual variations. The average fiber diameter for the group was about 70 μm, with individuals ranging from under 60 to about 90 μm. The influence of the fiber number and diameter on the bulky appearance of the hair is often masked by other characteristics such as waviness and hair care practices.

INTRODUCTION

The hair fiber characteristics which determine most cosmetically important hair mass mechanical behavior are: population density, diameter, moduli, shape, and fiber–fiber interactions (1). Fiber length, not being an intrinsic characteristic, is excluded from this list, even though it influences certain properties such as combing (2). Most cosmetic products and processes perform their functions with regard to hair mass mechanical behavior in the dry state by modifying the last 2 groups of parameters. The first 3 groups of parameters, even if partly or completely outside the influence of cosmetic products, are of interest to the cosmetic industry because of their influence on hair behavior. This paper is aimed at collecting and interpreting data on scalp fiber population and diameter of adult women.

The traditionally accepted value for the fiber population of a full head of adult hair is about 100,000 (3, 4, 5). The mean fiber diameters for groups of individuals range from 60 to 100 μm (6, 7, 8, 9) with individual fiber values from 25 to above 100 μm having been reported (10, 11, 12, 13, 14, 15, 16). We wanted to check the validity of the accepted mean values, investigate variations between individuals, establish ranges of values for single heads, and determine correlations, if any existed, among different characteristics.

EXPERIMENTAL

Twenty women employees of the Gillette Research Laboratory were used for this study. They ranged in age from 24 to 59 years, with 17 of them between 24 and 42. The three oldest panelists were Chinese; all others were Caucasians. Within the Caucasian group, the hair color ranged from light blond for 2 panelists to a nearly black, dark brown for 2 panelists. One panelist had reddish hair, but most had hair color in the brown range. The ancestry of the Caucasians—when known—varied from Scandinavian to Mediterranian. All 3 Orientals were born in China. A minimum of 12 cm hair length and the absence of Negroid-type curliness were the only criteria for the selection of the panelists. These restrictions were necessary because of the counting technique used.

FIBER COUNTING TERMINOLOGY

Simple hair: one hair fiber emerges from a follicle orifice. Compound fibers: 2 or more fibers emerge from a follicular opening without a contiguous epidermal or stratum corneum bridge between them, as seen at 45-fold magnification. Single hair: a simple fiber whose nearest neighbor is set at more than about 10 fiber diameters distance. Grouped hairs: all compound hairs and those simple hairs which had neighbors not more than 3 fiber diameters away. Site: the area occupied by a single hair or by grouped hairs.

SCALP AREA

Counting was carried out on the left temporal area of each panelist just under the vertex anterior and in front of the vertex posterior, according to the area designation of Moretti (17). On 8 of the panelists, the counting was repeated on the symmetrical right temporal area.

COUNTING METHOD

Both counting and fiber collection were carried out in November and December, 1975. A template with a 1.00 cm^2 opening was placed on the head of the panelists within 24 h of the last shampoo. With the help of a hooked needle, all fibers emerging from the skin within the 1.00 cm^2 opening were pulled through the frame while they were being viewed through a stereo microscope at 45-fold linear magnification. All the fibers were bent in one direction and counting started at the opposite end of the square, scanning along the skin line row by row. Each fiber counted was pulled over the opposite side of the opening with the hooked needle and held there by hand and tape. All data and observations were called out by the observer and tape recorded for later transcription, because both hands of the observer were occupied during the process and the subject had to remain still. Only "terminal" fibers were counted. In our method, a fiber was classified as terminal if it was at least about 2 cm long (18) and was similar in diameter to the long fibers. The following characteristics were noted: total number of fibers, total number of sites, number of fibers within each single site, grouping versus compounding for each fiber, relative geometric arrangement of the sites within the area, and the relative geometric arrangement of the fibers within sites.

FIBER COLLECTION FOR DIAMETER DETERMINATION

Small areas, in the general location where fiber counting had been carried out, were randomly preselected. All fibers within this area were cut off at the skin line. About 30 fibers were collected from each individual. Compound and group fibers were marked and kept separately from others to allow evaluation of intragroup and intracompound variations. The collected fibers were cleaned with an ether rinse, and dried at room temperature. All further treatment and measurements were carried out at 70°F and 65 per cent RH. The fibers were individually cut to an exact length of 5.00 cm in a straightened configuration with the aid of a special instrument (19). The 5.00 cm length was cut starting about 1 cm from the original skin line of the fiber and, therefore, encompassed the last 4 to 6 months of growth. The 5 cm long fiber segments were individually weighed on an electrobalance to the nearest 0.1 μg. The idealized average cylindrical diameter was calculated from the weight of each fiber according to the following:

$$D = 20\sqrt{W/(3.14 \times l \times d)}$$

where D equals diameter in micrometers; W equals weight in micrograms; l equals length in centimeters = 5; and d equals density of fiber in g/cm^3 = 1.3. Correlations among different measured values were calculated according to a standard statistical program package supplied to our computer.

RESULTS AND DISCUSSION

HAIR COUNT

A large proportion of the scalp fibers grow in groups. The fibers within a group are closely spaced, but the distance between neighboring groups is considerable, about 1 mm. In order to minimize errors originating from the significant intergroup distance, the fibers were counted over a relatively large area—1 cm^2. This was in contrast to some earlier work (10, 11, 12, 13, 14, 15, 16), where the fiber number was determined in 2 mm squares. The results are shown in Table I.

The age of the subjects ranged from 24 to 59, but only 3 participants were over 42. Since both age and racial origin have been reported to influence some of the measured characteristics (10, 11, 13, 16, 20, 21, 22), the average values are given both with and without the inclusion of the Chinese panelists. On the basis of the present work, it cannot be determined whether the differences seen between the 2 averages were due to racial or age factors. The number of fibers/cm^2 ranges from 95 to 295 with a mean of 211. Subject number 1—with the extremely low fiber count—shows signs of diffuse allopecia, associated with age, which was confirmed by photographs of her going back a few decades. The next 4 individuals with fiber counts under 160 fibers/cm^2 include 2 over 45 years of age (Chinese) and one of Spanish origin (number 4) with very dark hair. Number 3 has light brown hair and, according to her account, never had had more hair. The remainder of the panel had more than 190 fibers/cm^2. The correlation between decreasing hair count with increasing age is rather weak; 0.601. This does not negate earlier findings that both hair and follicle numbers decrease with age (13, 16, 22). Our study did not include extreme ages, and even within the range, 85 per cent of

Table I
Fiber and Site Count on Left Side of Head

Subject	Age	Number of Fibers/ cm^2	Number of Sites/ cm^2	Average Number of Fibers/ Site
1	59[a]	95	48	2.0
2	48[a]	131	58	2.3
3	29	145	67	2.2
4	37	153	86	1.8
5	47[a]	159	64	2.5
6	30	195	72	2.7
7	36	196	84	2.3
8	36	205	76	2.7
9	27	206	88	2.3
10	42	208	105	2.0
11	24	227	87	2.6
12	26	231	88	2.6
13	33	233	82	2.8
14	42	243	110	2.2
15	31	245	98	2.5
16	31	250	110	2.3
17	31	251	108	2.3
18	36	275	101	2.7
19	37	279	98	2.8
20	28	295	90	3.3
Average[b]	35.5	211	86	2.4
Average[c]	32.7	226	91	2.5

[a]These subjects were Chinese, all others apparently Caucasians.
[b]Average for the 20 subjects.
[c]Average for the 17 Caucasian subjects.

the participants were between 24 and 42. If the 3 oldest individuals are left out, the correlation between age and hair count approaches zero, indicating that this age group is rather homogeneous, or at least the individual variability—probably due to inherited factors—is a stronger parameter than age for this sample size. The values of the average fiber count/cm^2—211 and 226—are noteworthy in the respect that they are practically identical with those given by Barman (10, 11, 13, 15, 16), although, different from some others cited by Giacometti (22).

A full head of adult hair is commonly stated to contain about 100,000 (3, 5) to about 120,000 (4) fibers. According to Behrman (23), the average scalp area for an adult is in the range of 775 cm^2. Using this figure, the fiber density would only be 129 fibers/cm^2 for a 100,000 fiber adult head of hair. Only 1 individual in our panel was below this number. With the presently obtained fiber densities of 211 and 226 fibers/cm^2, the full head of hair should comprise 164,000 and 175,000 fibers, respectively. Although the densities were determined only at a specific site on all heads, this site is neither an outstandingly dense hair growth area nor are the differences among different scalp areas very large on adult heads (13, 14, 15). Therefore, we believe the estimate for the average fiber content of a full head of hair should be raised to the 160,000 to 180,000 range for women in the age group of 25 to 50. The commonly used low figures probably originate from the works of Stelwagon and Pinkus (24) quoted and even misquoted later (25, 26).

Table II
Fiber and Site Count on Right Side of Head

Subject	Number of Fibers/ cm^2	Number of Sites/ cm^2	Average Number of Fibers/ Site
2	175	68	2.6
3	169	83	2.0
4	142	81	1.7
10	270	105	2.6
12	208	73	2.8
14	182	83	2.2
15	239	100	2.4
18	203	70	2.9
Average	199	85	2.4
Average[a]	204	89	2.3

[a]Left side of head for same group of subjects, derived from values in Table I.

Hair is known to grow in groups on the skin of lower mammals (27), primates (28), and on certain body areas of man (20, 21, 22, 29, 30) including the scalp. Within the site of such groups, each fiber is at most a few diameters away from its neighbors, while the sites are well separated from each other. The site count, obtained together with the fiber count, is shown in Table I. It is seen that the site count ranges from about 50 to just over 100 per cm^2. Based on the average values, the centerpoints of such sites are more than 1 mm distant from their neighbors. The average number of fibers per site ranges from 1.8 to 3.3, but 90 per cent of the participants were between 2 and 3. The number of sites showed a reasonably good positive correlation (0.800) with the total number of fibers. However, the correlation with age was even weaker (-0.518) than that of the total number of fibers. The results of a second measurement on the right side of the head of 8 individuals are given in Table II.

It is seen that sizeable differences can exist in both fiber and site count on the 2 sides of the head, even though they were symmetrically positioned. The variation is random—neither side is preferred—as is shown by the close correspondence of the averages for the two sides. These facts indicate that, while single counting for a panel is probably satisfactory, it may be misleading for any one individual.

FIBER DISTRIBUTION BY GROUP SIZE

The term "group" is used here to describe 2 or more fibers within a single site, whether they are separated by stratum corneum (simple fibers) or issue from a common follicle orifice (compound fiber) or contain a mixture of the 2 forms. The average number of fibers in a group varied between 1.8 and 3.3 for our panelists, as shown in Table I. The actual distribution of the fibers according to group size varied widely among the individuals. The results are given in Table III. The proportion of single fibers ranged from 1 to 23 per cent within the panel of 20. On some subjects, the largest groups contained only 4 fibers while on others 8. Even an identical maximum group size—as seen on subjects 1, 4, 5 and 17—does not ensure a similar distribution curve within the range. On the average, two-thirds of all fibers were in groups of 2 and 3, slightly more in the latter category. When the group frequency is expressed as a function of size, the results

Table III
Fiber Distribution According to Group Size

Subject	Per Cent of Fibers in Groups of:							
	1	2	3	4	5	6	7	8
1	14.7	46.3	34.7	4.2	—	—	—	—
2	6.4	31.4	42.3	12.8	3.2	3.8	—	—
3	6.1	62.6	25.8	2.5	3.1	—	—	—
4	23.4	47.5	26.4	2.7	—	—	—	—
5	1.3	44.0	39.6	15.1	—	—	—	—
6	2.6	30.8	35.4	22.6	5.1	—	3.6	—
7	7.1	41.8	32.1	10.2	5.1	—	3.6	—
8	4.9	19.5	46.8	21.5	7.3	—	—	—
9	5.8	44.7	33.5	5.8	7.3	2.9	—	—
10	11.9	28.5	37.7	16.7	5.2	—	—	—
11	5.7	30.0	30.4	17.6	13.2	2.6	—	—
12	2.1	34.6	39.0	22.5	2.2	—	—	—
13	4.7	15.4	43.8	24.0	4.3	7.7	—	—
14	11.8	31.1	46.6	9.4	1.2	—	—	—
15	8.7	26.4	41.5	14.0	4.1	3.7	1.4	—
16	8.4	41.6	28.8	19.2	2.0	—	—	—
17	8.4	34.3	38.2	19.1	—	—	—	—
18	4.2	25.5	31.4	18.4	13.6	3.8	1.5	1.7
19	5.0	19.3	32.3	4.4	12.5	6.4	—	—
20	6.1	6.8	26.4	23.0	15.2	10.2	9.5	2.7
Average[a]	7.5	33.1	35.6	15.3	5.2	2.1	1.0	0.2
Average[b]	7.5	31.8	35.1	16.1	6.0	2.2	1.2	0.3

[a] Average for the 20 subjects.
[b] Average for the 17 Caucasian subjects.

are somewhat different. Sites with 1, 2, 3, and 4 fibers occur at 18.2, 40.7, 28.7, and 9.2 per cent frequency, respectively, while groups with 5 or more fibers at 3.3 per cent. This is in slight disagreement with some published results (31), where the group of 3 fibers was found to dominate. The proportion of single fibers is much lower than found earlier (30). Within our panel, no correlation of any significance was found between group size distribution on one hand and age, fiber population density, or site population density on the other. This is in contradiction to the conclusions of Oberste-Lehn (20, 21), who claimed strong correlations between group distribution and age, although on the basis of smaller sample size. In our view, all available studies—including the present one—used too narrow a data base to allow generalized conclusions in this area. Even if strong correlations were obtained from a much larger study, the averages could not be applied to individuals because of the very large variability of the distribution pattern.

The relative positions of fibers in groups showed no specific geometric patterns in agreement with earlier findings (28).

SIMPLE AND COMPOUND FIBERS

It has long been known that 2 or more fibers can emerge from a single follicular opening at the epidermal level on some parts of the body, including the scalp. In the litera-

Table IV
Fiber Distribution According to Compound Size

Subject	Per Cent of Fibers			
	Simple	2	3	4
1	36.9	50.5	12.6	—
2	90.2	9.8	—	—
3	48.0	48.3	3.7	—
4	63.7	31.2	5.1	—
5	54.7	34.0	11.3	—
6	94.9	5.1	—	—
7	80.6	19.4	—	—
8	96.1	3.9	—	—
9	73.8	26.2	—	—
10	80.5	18.8	0.6	—
11	74.5	25.5	—	—
12	51.3	46.0	2.7	—
13	40.8	42.1	15.4	1.7
14	73.9	24.0	2.1	—
15	59.1	32.6	7.4	0.8
16	67.6	28.8	3.6	—
17	56.6	37.4	6.0	—
18	63.0	31.4	5.6	—
19	65.6	28.0	6.4	—
20	57.3	27.1	14.2	1.4
Average[a]	66.5	28.5	4.8	0.2
Average[b]	67.5	28.0	4.3	0.2

[a] Average for the 20 subjects.
[b] Average for the 17 Caucasian subjects.

ture, these fibers have alternately been called "bunched" (20, 21) or "compound" (30). We follow the latter designation. The common opening for multiple fibers does not necessarily indicate a single papillary body. The literature refers to the convergence of follicular tubes in the epidermis (32). Our results, based on the total fiber number and not on the number of follicular openings, are given in Table IV. On the average, two-thirds of the fibers emerge in the simple form. Compound fibers with more than 2 members account for only 5 per cent. When the accounting is made on the basis of the number of follicular openings, the distribution is shifted even more toward the simple fibers; 80.7 per cent. Follicles with 2, 3, and 4 compound fibers were present at 17.3, 1.9, and less than 0.1 per cent, respectively. The present results significantly differ from earlier ones (20, 21, 30). This is probably due to the poor definition of what can be counted as a compound fiber. If the magnification during viewing is low, a thin but contiguous epidermal wall may not be seen between closely spaced but separately exiting fibers. On the other hand, loose skin debris may be positioned between real compound fibers unless the scalp is freshly cleaned and these then may be counted as simple fibers. In spite of these uncertainties, the categorical statement of Oberste-Lehn (20, 21) that compound fibers with 4 members appear only after age 50 cannot be accepted. The only 3 individuals with compounds of 4 fibers were 28, 31, and 33 years of age. No significant correlations were found between the number and size of compound hairs on one hand and age, number of fibers and sites, or group distribution on the

Table V
Fiber Diameter

Subject	Average Diameter	Standard Deviation	Range of Fiber Diameter		Diameter Ratio Maximum/Minimum
	μm				
1	82.9	4.8	88.9	72.4	1.23
2	79.6	8.6	92.4	58.9	1.57
3	59.6	5.9	68.8	45.3	1.52
4	70.1	4.4	79.4	63.2	1.26
5	96.5	7.4	111.3	86.6	1.29
6	58.9	7.8	75.1	44.0	1.71
7	68.8	8.9	85.5	52.4	1.63
8	61.4	10.4	77.0	45.6	1.69
9	80.6	6.9	96.5	66.7	1.45
10	75.2	5.2	86.0	61.7	1.39
11	67.4	10.0	82.3	42.4	1.94
12	84.9	6.9	96.9	73.8	1.31
13	71.4	7.2	90.8	58.7	1.55
14	69.4	7.8	86.9	53.0	1.64
15	75.2	8.8	89.8	51.1	1.76
16	70.8	10.0	99.8	53.3	1.87
17	68.4	5.4	80.5	57.2	1.41
18	71.6	5.5	81.2	54.0	1.50
19	68.6	8.1	78.2	37.6	2.08
20	67.0	9.3	83.0	52.4	1.58
Average[a]	73.4				
Average[b]	71.1				
Average[c]	86.3				

[a] Average for the 20 subjects.
[b] Average for the 17 Caucasian subjects.
[c] Average for the 3 Oriental subjects.

other in any combination of these factors. Again, the extreme individual variability needs to be pointed out. Subject 8 had nearly exclusively simple hairs, while for others more than half the fibers were compounded.

FIBER DIAMETER

Most literature references provide only mean diameters for a given population. According to these, Caucasian hair diameters average less than 60 μm (8), more than 60 μm (9), about 77 μm (6), and 79 μm (7). For Oriental hair, the averages range from about 85 to 100 μm (6, 7, 9). Barman and coworkers (10, 11, 12, 13, 14, 15, 16) segmented the hair population by size, but only by crude categories: about 100, about 50, and 25 μm. Our interest centered more on the variations among individuals and among fibers on the same head. The results, concerning the calculated "idealized" diameters, are given in Table V.

The mean diameter for the Caucasian group was 71 μm, which is within the range of earlier data. This is well differentiated from the 86 μm average value of the Orientals.

The average diameters within the Caucasian group range from just under 60 to 85 μm in a natural distribution pattern. The correlation between mean diameter and age is only 0.45 for the whole group; that is, of no significance. With the exclusion of the Orientals, the correlation approaches zero. Therefore, the age, at least between the midtwenties and early forties, is not a governing factor for hair diameter within a somewhat varied population. Nor was the diameter found to correlate with the number of fibers, sites, or any specific group and compound size. The variation in fiber size on individual heads is shown in Table V. The standard deviation ranges from 5 to 17 per cent, but independently from any other characteristics. The range of absolute diameter of individual fibers is rather wide; from 37 to 111 μm, and it would certainly increase with larger data bases. Nonetheless, the findings of Barman and coworkers (10, 11, 12, 13, 14, 15, 16) of 10 to 15 per cent fiber content in the 25 μm range cannot be substantiated with the present results, as we found only one fiber below 40 μm. The occurrence of fibers below 40 μm diameter is very low in commercially available human hair as well when determined both by weight and microscopic methods. Even in wool, a 25 μm fiber is classified as medium fine. The range of diameters on individual panelists, as expressed by the thick–thin ratio, is more restricted. On some subjects the fibers are closely homogeneous with a ratio of less than 1.3. Only in one case did the value exceed 2.0. This thick–thin ratio shows a weak positive correlation (0.49) with fiber population density and similarly weak, but negative correlation with mean fiber diameter and age of the subjects. However, the exclusion of the Orientals from the data base eliminates all significance from these already weak correlations.

VISUAL APPEARANCE OF THE HAIR

The hair of the panelists differed in a number of characteristics—length, curl, style, cosmetic modifications, and product use—in addition to the measured fiber population density and diameter values. Due to this large number of variables, strict conclusions cannot be drawn concerning the visual bulkiness of hair as a function of the measured parameters on the basis of 20 panelists. Nonetheless, the indications were clear that the fiber diameter had a stronger influence on the visible bulk and elevation of the hair over the head than the population density. Even the curl level and hair setting seemed to be more important factors than the fiber number. Only when a number of these characteristics—low density and diameter with straight configuration—happened to combine, was the total hair mass judged to be very small and weak by visual methods, as in the case of subject 3. When the hair was long—waist length—and very straight, as for Subject 19, a somewhat similar judgment was arrived at, in spite of the very high fiber number and average diameter. On the other hand, wavy hair—not necessarily natural wave—with average to high diameter can show up with high bulk even at low to very low fiber densities, as were seen on panelists 1, 2, 4, and 5. Subject 12 showed the highest bulkiness with naturally strongly waved thick hair, even though the density was only average and the hair reached below shoulder level.

CONCLUSIONS

Based on the population density, the number of fibers on a full adult head of hair may have to be revised upward by a considerable amount to 150,000 to 200,000. The indi-

vidual variability in fiber number, grouping and diameter is very great and no correlations of statistical significance were found for any of these characteristics. Nor were these characteristics related to age, at least within the age range studied. Deficiency in fiber number or even in diameter can be overcome by other characteristics to provide a hair mass with the visual impression of bulk and fullness.

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Application of the theory of hydrophobic bonds to hair treatments

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Synopsis

A novel technique of HAIR TREATMENT via introduction of nonpolar residues into hair structure in HYDROALCOHOLIC MEDIA is described. Hair modified in this manner exhibits greatly ENHANCED SETTABILITY and HIGH SET RETENTION, even at high levels of ambient humidity. The setting behavior can be manipulated by utilizing the differential swelling response of treated hair to water and aqueous alcohols.

I. INTRODUCTION

Conformational stability of a protein, and thus, its response to external mechanical or chemical forces, depends on the type and number of stabilizing bonds present within the protein structure. In the case of α-keratin, this stability is primarily derived from covalent crosslinking by cystine and intrachain hydrogen bonding. Some contribution also comes from the electrostatic interaction of basic and acidic sidechains, as well as from the hydrophobic bonding of nonpolar residues such as proline, leucine, and valine (1). However, the contribution of the latter is small, and in the intact fiber, the covalent and polar interactions greatly overshadow the nonpolar ones.

In an earlier work reported by Harris (2), it was shown that the wet mechanical properties of reduced keratin fibers could be restored without crosslinking by alkylating the fibers with high molecular weight monohalides. Alkylation with alkyl halides of low molecular weight produced permanently weakened fibers. Successful mechanical recovery of the alkylated fibers was ascribed to the interaction of secondary forces, arising from the high molecular weight residues incorporated into the fiber structure during alkylation. In this respect, it is of particular interest to note that the introduction of apolar residues creates an environment favoring the formation of hydrophobic bonds, and that the strength of these bonds depends on the size and shape of the introduced alkyl groups.

The term "hydrophobic interaction" describes the tendency of nonpolar groups to associate in aqueous solution. This interaction results in an increased ordering of the water molecules into a quasi-crystalline structure in which there is improved hydrogen bonding surrounding the nonpolar groups. These hydrophobic regions are disrupted in nonpolar solvents because stronger solute-solvent interactions are thermodynamically favored. Thus, a unique property of the hydrophobic bond is its dependence on water for its existence.

The important practical point raised by these considerations is the utility of this type of bonding for setting hair. If the hydrophobic bonds could resist the swelling pressure generated within the keratin which is exposed to high humidity, then the set-conformation would be maintained and a novel process for hair manipulation would be feasible.

An investigation of the properties of S-alkylated keratin was therefore undertaken, and this report is an account of such study.

II. EXPERIMENTAL

A. MATERIALS AND METHODS

1. *Reagents:* The chemicals utilized in this study were commercially available American Chemical Society grade reagents and were used without further purification.

2. *Caucasian hair:* Brown Caucasian hair as supplied* was used without further cleansing.

3. *Mechanical properties:* The mechanical properties of hair were determined on a table model Instron.† The fibers were mounted on plastic tabs at 2 in. guage length, equilibrated under the desired conditions, and stretched to break at a rate of 1 in./min. The broken ends were conditioned at 65 per cent RH, cut off the tabs, weighed, and the denier of the tested fibers calculated.

In some cases, the calibration technique (3) was used to follow the change in the fiber performance. Intact fibers were mounted as above, equilibrated in the desired solvent, and then stretched to 30 per cent extension at a rate of 1 in./min, using the table model Instron. After a 24-h relaxation period in water, the calibrated fibers were given the proposed chemical treatment. The ratio of the energy required to stretch the fibers (30 per cent extension) the second time to that required initially, was expressed as the 30 per cent index.

4. *Amino acid analysis:* Hair/wool samples (~10 mg) were hydrolyzed at 105°C for 24 h in 6 *N* HCl followed by lyophilization for removal of HCl. The hydrolyzates were analyzed for cystine on a Phoenix‡ model M-7800 Micro Analyzer.

5. *Liquid retention measurements:* The swelling of hair was determined by the liquid retention technique as described by Valco and Barnett (4). This involved measuring

*De Meo Brothers, New York, N.Y.
†Instron Corp., Canton, MA.
‡Phoenix Instrument Co.

the liquid retained by the hair after a 30 min equilibration in water or other specified solvent.

6. *Setting:* One gram (7 in.) tresses were set on one-half in. rollers with water or aqueous alcohol as specified in the text. The set tresses were allowed to dry overnight at ambient temperature and humidity. After removing the tress from the roller, the hair was combed, being careful to maintain the alignment of the hair fibers.

The set stability of treated hair was assessed by measuring the hanging length of the tresses after various relaxation times, while exposing them to maintained conditions of humidity and temperature (85 per cent RH, 85°F).

B. RESULTS AND DISCUSSION

1. *The reaction of reduced hair with alkyl iodides:* Earlier investigations conducted by Harris (2) on wool suggested that the wet mechanical properties of reduced wool could be restored following alkylation with long chain alkyl halides. Both the magnitude of the restorative effect and the simplicity of the alkylation step suggested this approach as being particularly attractive for application to hair. An attempt was, therefore, made to evaluate the efficacy of the alkylation reaction.

Calibrated hair fibers were treated with 0.25 *N* potassium thioglycolate at pH 5 (3 h at 50°C, 25:1 bath ratio) to cleave approximately 50 per cent of the disulfide bonds. Samples of the reduced fibers were then alkylated with 0.02 *M* alkylating agent suspended in 0.1 *M* pH 8 phosphate buffer utilizing 100:1 bath ratio. The alkyl halides used as blocking agents were methyl, hexyl, and decyl iodides, respectively. After 20 h, at 35°C, the fibers were thoroughly rinsed with running tap water; and dried. Bulk samples were treated simultaneously in order to determine the weight changes following alkylation. A small weight increase (1.9 per cent) was observed only in the case of the sample treated with decyl iodide. This weight increase corresponded to less than 20 per cent yield of the alkylation reaction.

The alkylation treatment also had a negligible effect on the mechanical properties of the reduced hair (Table I). These results were in sharp contrast with the data reported for wool by Harris (2). To ascertain whether the reactivity of the substrate (hair versus wool) contributes to these large differences in behavior, it was decided to re-examine the reaction system using wool fibers. The reduction-alkylation cycle was run under conditions identical to those described by Harris.

2. *Alkylation of wool with alkyl iodides:* New Zealand wool samples were reduced with 0.2 *N* potassium thioglycolate at ph 4.5 for 21 h at 35°C (25:1 bath ratio). The subsequent alkylation was performed using 0.02 *M* alkylating agent, methyl, hexyl, or decyl iodides suspended in 1 *M*, pH 8 phosphate buffer, 35°C at 100:1 bath ratio. The alkylation proceeded very slowly in the presence of the longer chain halides, as was evident by the persistence of thioglycolic acid after 18 h reaction time. To insure that an excess of alkylation agent was present, fresh solutions of the hexyl and decyl iodides, respectively, were added to the wool samples, and the alkylation continued for an additional 7 h. These reactions were monitored by measuring the weight changes, as well as examining the properties of the treated wool.

Table I
30 Per Cent Indices of Hair Samples Following Treatment with Alkyl Iodides

Treatment	30 Per Cent Index
Intact	0.98
Reduced	0.54
Reduced—CH_3I	0.66
Reduced—$C_6H_{13}I$	0.66
Reduced—$C_{10}H_{21}I$	0.64

Table II
30 Per Cent Indices of Wool Samples Following Treatment with Alkyl Iodides

Treatment	30 Per Cent Index
Intact	1.02
Reduced—CH_3I	0.63
Reduced—$C_6H_{13}I$	0.85
Reduced—$C_{10}H_{21}I$	0.92

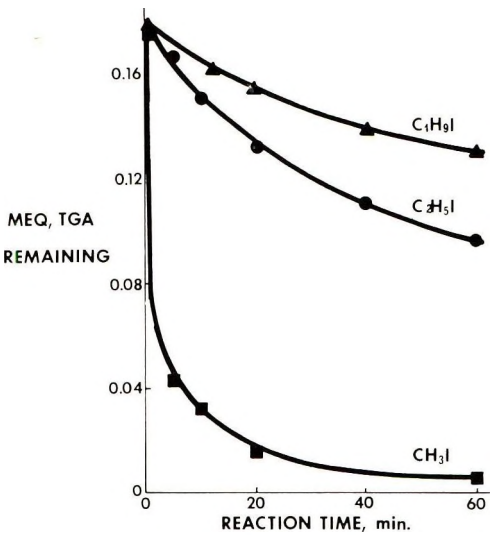


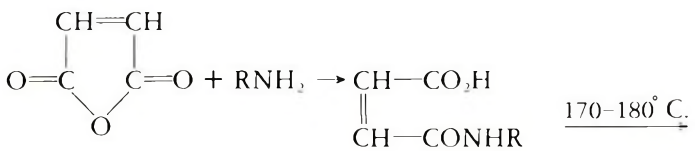
Figure 1. Reactivity of thioglycolic acid with methyl, ethyl and butyl iodides in 40 per cent ethanol, pH 9 with respect to time

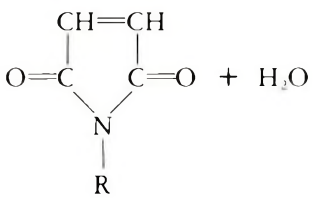
The alkylation of the reduced wool with hexyl and decyl iodides resulted in weight gains of 1.7 and 2.5 per cent, respectively. It was obvious that the extent of alkylation was again low. On the basis of the weight gain, only 0.18 to 0.20 m moles at most, of SH per gram of wool had been alkylated. Yet, the mechanical performance of the alkylated wool (Table II) conformed with the earlier data published by Harris. There was a steady improvement in the mechanical recovery of the fibers with the increasing

chain length of the alkylating agent. However, this unexpected discrepancy between the weight gain values and the 30 per cent work indices was resolved satisfactorily by the amino acid analyses of the treated wools. The cystine contents of both hexyl iodide and decyl iodide treated samples were almost identical with those of the untreated unreduced wool (860 μmol/g). The methyl iodide alkylated sample had a cystine content of 470 μmol/g. It is, thus, obvious that the mechanical recovery of the alkylated wool fibers had been brought about by reformation of the keratin disulfide and not by the residue reinforcement effect. The mechanism of the disulfide rebuilding is not yet known. Most likely, the alkyl iodides undergo some secondary reactions involving formation of iodine, which acts as an oxidant for the protein sulfhydryl. This secondary reaction is unimportant in the case of methyl iodide, which reacts with mercaptans very rapidly. An increase in chain length of the alkyl group causes a precipitous drop in the rate of the alkylation reaction (Fig. 1), and thus, may set a stage for the secondary process.

3. *Synthesis of N-alkyl maleimides:* A more dependable method for introducing apolar residues into the keratin was clearly required. N-ethyl maleimide is often used as a standard blocking agent for protein sulfhydryl, and it was thought that its higher homologues might be of value in this respect.

Although, the N-alkyl maleimides are not commercially available, they were easily prepared by pyrolysis of the corresponding N-alkyl maleamic acids (5, 6). N-hexyl, N-heptyl, and N-dodecyl maleamic acids were prepared by reacting maleic anhydride with the appropriate amine in glacial acetic acid. The acids were isolated in good yields (*ca.* 85 per cent) as white crystalline solids and pyrolyzed without further purification. The properties of the maleamic acids and the corresponding maleimides are given in Table III. The low yields of final product (26 per cent) were due to a concurrent polymerization reaction leading to a resinous by product. In the course of our work, a one step synthesis was also utilized for the preparation of N-alkyl (aryl) maleimides. The overall yield continued, however, to be low (~30 per cent). The overall reaction is shown below





4. *Reduction of hair with dithiothreitol (DTT):* DTT was used as an alternate reductant in our studies. This reagent (8) causes a specific and symmetric scission of the

Table III
Properties of N-Alkyl Maleamic and N-Alkyl Maleimides

Derivative	Maleamic Acid[b] Melting Point, °C	Maleimide[b] Melting Point, °C
Hexyl	78°	125° @ 5.5 mm[a]
Heptyl	75–77°	43–44°
Dodecyl	—	56–59°
Benzyl	140–142°	73–75°

[a]Boiling point.
[b]Observed melting points were within the range reported by Coleman *et al* (7).

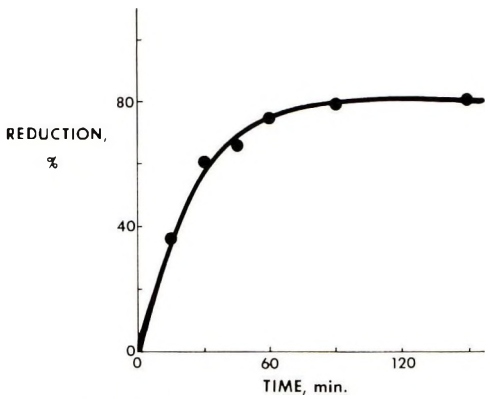


Figure 2. Effect of time on reduction of hair by 0.1 *M* DTT at self-pH, 35 C

disulfide bonds without producing any byproducts such as mixed disulfides. Its efficacy as a reductant allowed us to perform the reduction swiftly at neutral pH and a temperature of 35°C.

Hair samples were treated with unbuffered solutions of 0.1 *M* DTT (self-pH, 5.4) at 25:1 bath ratio, 35°C for various times. Levels of reduction were calculated on the basis of the SH content determined via mersalyl acid titration (9). These data agreed with the reduction levels determined from residual disulfide analyses (via amino acid analysis) after cyanoethylation of the free SH groups. A plot of the reduction levels against time is shown in Fig. 2.

5. *Alkylation of hair with N-alkyl maleimides:* N-hexyl maleimide was used to alkylate reduced hair containing 0.6 m moles of SH per gram of hair. The extent of reaction was monitored by determining the residual SH following the alkylation. Under the conditions employed, the alkylation was complete within 2 h. The alkylation was carried out in 20 per cent n-propanol/0.04 *M* phosphate buffer, pH 7 at 35°C, 100:1 bath ratio; under these conditions the extent of hydrolysis of the N-alkyl maleimides is negligible.

In using the next higher homolog, i.e., N-heptyl maleimide, the alkylation reaction could also be completed within 2 h. Following the alkylation with N-ethyl maleimide,

Table IV
Effect of Time on the Alkylation of Hair with N-Ethyl and N-Hexyl Maleimide[a]

Time, Minutes	Degree of Alkylation, Per Cent	
	N-Ethyl Maleimide	N-Hexyl Maleimide
30	75	82
60	81	94
120	86	100

[a][Maleimide] = 0.01 *M*; Solvent = 20 per cent n-propanol/0.04 *M* phosphate (pH 7) buffer; temperature = 35°C; bath Ratio = 100:1.

approximately 15 per cent of the SH remained unreacted after 2 h, while a corresponding sample of hair treated with the N-hexyl maleimide contained no residual SH (Table IV). This finding was in accord with the data presented by Heitz (6), who determined the second-order rate constants for the binding of N-heptyl maleimide to yeast alcohol dehydrogenase. There, it was shown that the reaction rate for the N-heptyl maleimide reaction was approximately 8.4 times that observed with N-ethyl maleimide. This was somewhat unexpected, since a chainlength effect was not observed in the reactions of these maleimides with cysteine and glutathione (6). We observed similar enhancement of the rate of alkylation in the case of reduced hair. Following reduction with thioglycolic acid, the sample was rinsed only briefly prior to the alkylation, and thus, residual thioglycolic acid remained in the fiber. At the end of a 24-h treatment, the cysteinyl residues were completely blocked by N-heptyl maleimide, while free thioglycolic acid was still detected in the alkylating solution. This observation and the previous results on the alkylation of reduced keratin indicate that an increase in the alkyl sidechain of the maleimide leads to faster rates of alkylation in spite of the unfavorable diffusion factor. Such an enhancement in the reactivity may be tentatively ascribed to the interaction between the alkyl sidechain of the reactant and the nonpolar residues of the keratin, which apparently provide an effective hydrophobic environment for the combined cystine.

6. *Swelling properties of the alkylated hair:* The extent of internal modification of keratin often can be readily assessed from the change in the swelling characteristics of this protein. Thus, fission of the disulfide bonds is accompanied by an increase in water imbibition, which is almost directly proportional to the number of crosslinks severed. According to our hypothesis of hydrophobic modification of hair, the introduction of apolar residues should compensate for at least some of the disulfide bond breakdown. A strong support for this view was obtained from the liquid retention measurements of reduced and reduced-alkylated hair (Table V). The reduction treatment alone causes a large increase in swelling in both water and aqueous alcohol. Alkylation of reduced hair with methyl iodide or N-ethyl maleimide slightly intensifies the swelling. This increment in hydration is probably caused by the elimination of weak hydrogen bonding involving the sulfur hydrogen and the apparent inability of the methyl or N-ethyl derivatives to establish any specific interactions with the environment. On the other hand, alkylation with either N-hexyl or N-heptyl maleimides brings about significant decrease in hydration. Obviously, the introduction of hydrophobic residues can impart

TABLE V
Effect of Apolar Residues on the Swelling Properties of Hair

Per Cent Reduction	Alkylating Agent	Per Cent Swelling[a] in: Water	50 Per Cent Methanol	50 Per Cent Ethanol	20 Per Cent Propanol	50 Per Cent Propanol
0	None	31.4	n.d.[b]	n.d.[b]	31.6	32.2
45	None	39.9	35.8	37.4	42.6	45.2
45	Methyl iodide	47.2	42.2	46.2	47.8	49.2
45	N-ethyl maleimide	45.3	41.0	45.5	48.6	51.1
45	N-hexyl maleimide	33.9	33.7	37.8	39.5	46.3
45	N-heptyl maleimide	34.8	33.6	37.7	40.9	46.4
82	N-hexyl maleimide	31.0	n.d.[b]	n.d.[b]	44.9	56.5
82	N-dodecyl maleimide	28.2	n.d.[b]	31.9	36.4	47.5
82	N-benzyl maleimide	31.5	n.d.[b]	34.1	35.4	46.1

[a]Calculated on the treated weight of the fiber.
[b]Not determined.

substantial conformational stability to the reduced keratin structure and successfully resist the swelling pressure.

This conformational stability is, of course, lost in aqueous alcohols, where the hydrophobic interactions between the apolar residues present in hair are prevented. Such a differential response to aqueous solvents offers a unique way of hair manipulation, particularly with regard to setting.

7. *Mechanical properties:* Swelling data have shown that the imbibition of water by keratin can be restricted by hydrophobic modification of the fiber. Although, the precise nature of the intermolecular arrangement remains a subject of controversy (10, 11), the stabilization of hydrophobic bonds by water is not disputed. The strength of the hydrophobic bond is represented by the tendency of nonpolar groups to adhere to one another. The free energy of this process has been assessed (12), and in the case of the interaction of 2 methyl groups, was found to be -0.73 kcal/mole for alkyl sidechains. The increment in the free energy of binding was in the order of -0.37 kcal/mole per CH_2 group. The overall contribution of these hydrophobic crosslinks to the stabilization of the keratin structure will depend on the size, shape, and number of the introduced apolar residues.

Some further insight on these hydrophobic interactions was obtained from a study of the mechanical properties. It is well known that the wet strength of intact hair bears a linear relationship to the cystine content over a wide range of reduction levels (13). Using this linear relationship as a guide, a preliminary assessment of the stabilization effect arising from hydrophobic interactions was obtained from the mechanical behavior

Table VI
Yield Stress of Hair Alkylated with N-Hexyl Maleimide

Reduction Level, Per Cent	Yield Stress, g/denier in:					
	Water		20 Per Cent Propanol		50 Per Cent Propanol	
	Calculated[a]	Observation	Calculated[a]	Observation	Calculated[a]	Observation
0	0.42	0.42	0.36	0.36	0.36	0.36
31	0.28	0.40	0.25	0.30	0.25	0.27
42	0.22	0.32	0.21	0.17	0.21	0.14
82	0.08	0.27	0.06	0.11	0.06	0.10

[a]Calculated yield stress = (intact hair yield stress) (100-per cent reduction/100).

of alkylated fibers (Table VI). The alkylation was performed at 3 reduction levels using N-hexyl maleimide as the alkylating agent.

If one relies on the fact that the percent reduction in work to stretch a reduced fiber is directly proportional to the extent of reduction, then it is evident that the alkylated fibers do not exhibit such a loss of strength. In water, the formation of hydrophobic bonds, via interaction of the hexyl residues, results in significant stabilization of the keratin structure. Although, some repairing effect was anticipated, the extent of the stabilization and particularly the resistance of the treated fibers to the external stresses was unexpected. Even with the maximum overlap of the apolar sidechains introduced in the alkylation step, the average strength of the newly formed bonds would not exceed 5 kcal. This is only a fraction of the energy loss which accompanies the breakdown of cystine crosslinks (~50 kcal/mol). It is apparent that the hydrophobic interactions which accompany the blocking of cysteine residues are very intensive, although, a possibility of cooperative multichain hydrogen bonding in a hydrophobic environment cannot be excluded.

8. *Setting properties of alkylated hair:* The alkylation of reduced hair limits the swelling of hair in water. On the other hand, such hair can be readily deformed in alcoholic media. An attempt was made to utilize this change in swelling characteristic for the setting purposes. Thus, the hair was swollen in 50 per cent propanol and set on rollers. In the presence of alcohol, the alkylated hair is very pliable and moldable and conforms readily to the desired configuration. After setting the hair, the alcohol was removed by rinsing with water. Removal of the alcohol leads to the formation of hydrophobic crosslinks, which stabilize the new (set) configuration. Hair alkylated with N-heptyl maleimide retained the set in liquid H_2O, while a tress alkylated with N-ethyl maleimide straightened within 1 to 2 min. This demonstrates that a water-resistant set can be attained by the introduction of apolar residues, providing that a sufficiently long sidechain is used. A 7-carbon alkyl chain appears to fulfill this requirement.

The set stability of tresses was also assessed by the conventional manner. This involves the exposure of set tresses to controlled conditions of humidity and temperature and measuring the extent of relaxation with respect to time. Following the reduction, the tresses were rinsed with water and then alkylated with N-heptyl maleimide for 2 h. The setting was performed with 50 per cent propanol; after thorough rinsing with water the

Table VII
Set Stability of Hair Following Alkylation with N-Heptyl Maleimide

Sample	Hanging Lengths (cm)[a] After Following Relaxation Time (min): 0	5	7.5	10	15	30	150
Intact (H_2O set)	2	7.5	11	12	13	13	13
Intact	2	5.0	7	8	10	11	12
Reduced/heptyl maleimide	2.5	3.5	4	4	4.5	5.0	6

[a]Measured at 84 per cent RH, 85°F.

Table VIII
Effect of Reduction Level on the Set Stability of Alkylated Hair

Treatment	Hanging Lengths (cm) at Relaxation Times (min): 3	6	9	12	20	45
Intact (H_2O)	2	3	5.5	8	10	11
Intact	2.5	3	3.5	5.5	8.5	10.5
15′ Reduction/heptyl maleimide	2.5	5.0	6	7.5	9	10
30′ Reduction/heptyl maleimide	2	3	3.5	4	5	6.5
60′ Reduction/heptyl maleimide	2	2.5	3.0	3.5	4	5.0

tresses were air dried overnight on the rollers (alternatively, the set hair was dried with a hand held dryer). The set stability is shown in Table VII.

The alkylation results in excellent set stability during a prolonged exposure to conditions of high humidity. Additional experiments indicated that increasing the size of the alkyl substituent above C_7 offered no benefit.

Optimal interaction of the hydrophobic sidechains or, indeed, the mobility of such chains, may not be possible in the case of the dodecyl residues because of steric factors. Thus, the maximal stabilization of the keratin would not be attained. It is of interest to note that the best results have been obtained with hair alkylated either with heptyl or with benzyl maleimides.

The effect of alkylation on the set stability (measured at 84 per cent RH, 85°F) was determined at several reduction levels (Table VIII). It appears that alkylation of reduced hair having less than 50 per cent cleavage does not greatly improve the setting properties.

An important consideration in the reduction-alkylation process is the respective bond energies of the system. As discussed earlier, disulfide bonds represent approximately 50 kcal/mol of stabilization energy. The introduction of two apolar residues (heptyl sidechain) for the blocking of a reduced disulfide will contribute only about 3 kcal/mol of binding energy. At low reduction levels, the introduction of a few hydrophobic crosslinks cannot compete with the residual disulfides which significantly contribute to the overall setting characteristics of intact hair. At very high reduction levels, only a

few restraining disulfide crosslinks remain, thus, the stabilizing influence of the hydrophobic crosslinks becomes more prevalent in maintaining the native structure of the keratin.

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Some aspects of the stratum corneum-organic solvent system

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Synopsis

A limited study of the SORPTION, DESORPTION, and DIFFUSION behavior of some ORGANIC VAPORS (benzene and toluene) in intact STRATUM CORNEUM has been carried out, and the results compared to WATER VAPOR. The effect of treating the corneum in a mixture of ORGANIC SOLVENTS (chloroform-methanol) followed by water extraction, on the water vapor sorption, desorption and diffusion characteristics has been assessed. Scanning electron microscopy (SEM), was also used to examine the structural changes in the corneum resulting from the organic solvents-water treatment.

The shape of the benzene and toluene sorption ISOTHERMS was found to be compatible with type II in the BET classification. The diffusion process of the organic vapors in the corneum is much faster than that of water vapor. An increase by 3 orders of magnitude in the value of D_i (intrinsic diffusion coefficient) has been observed as the concentration of organic vapor increases in the corneum.

Sequential treatment of GUINEA PIG corneum in organic solvent and water resulted in a marked decrease in the water vapor sorption capacity in the high humidity range. The initial portions of the water vapor sorption isotherms on the treated and intact corneum are the same (up to about 50 per cent RH). The diffusion of water vapor in the treated corneum is 10 times as fast as in the intact corneum.

INTRODUCTION

The hydration of stratum corneum has been the subject of a number of investigations (1–5). We have recently examined in detail the sorption, desorption, and diffusion behavior of water vapor in a number of keratinous materials including stratum corneum obtained from excised human skin, guinea pig and neonatal rat corneum, and human hair (6–7).

In this paper, we present the results of a limited investigation into the sorption, desorption, and diffusion characteristics of benzene and toluene vapors in human stratum corneum, and compare these to the results obtained with water vapor. We have also examined the effect of solvent damage (chloroform/methanol 2:1) on the water vapor sorption and diffusion in guinea pig stratum corneum in an attempt to identify the role of the physical structure of the corneum.

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Reviews of previous work on the permeability of skin to organic materials have been presented by Scheuplein and Blank (8) and Tregear (9). Scheuplein and Blank (8) report some data on the sorption and diffusion behavior of a series of *n*-alkanes and alcohols in stratum corneum. King and Cassie (10) studied the vapor sorption and diffusion of methanol in horn keratin and wool and of ethanol in wool.

Acetone, ether, hexane, and other common solvents were found to damage the skin, but the most effective solvents were found to have both polar and nonpolar character and the most potent of all appear to be mixed solvents like chloroform-methanol (2:1) and ether-ethanol (10:1) (1, 4, 11, 12).

In our work, we have assessed the effect of treating guinea pig corneum in the chloroform-methanol mixture in two ways: (1) comparison of the water vapor sorption and diffusion characteristics in a treated and untreated sample; and (2) examination of the treated corneum in the scanning electron microscope (SEM).

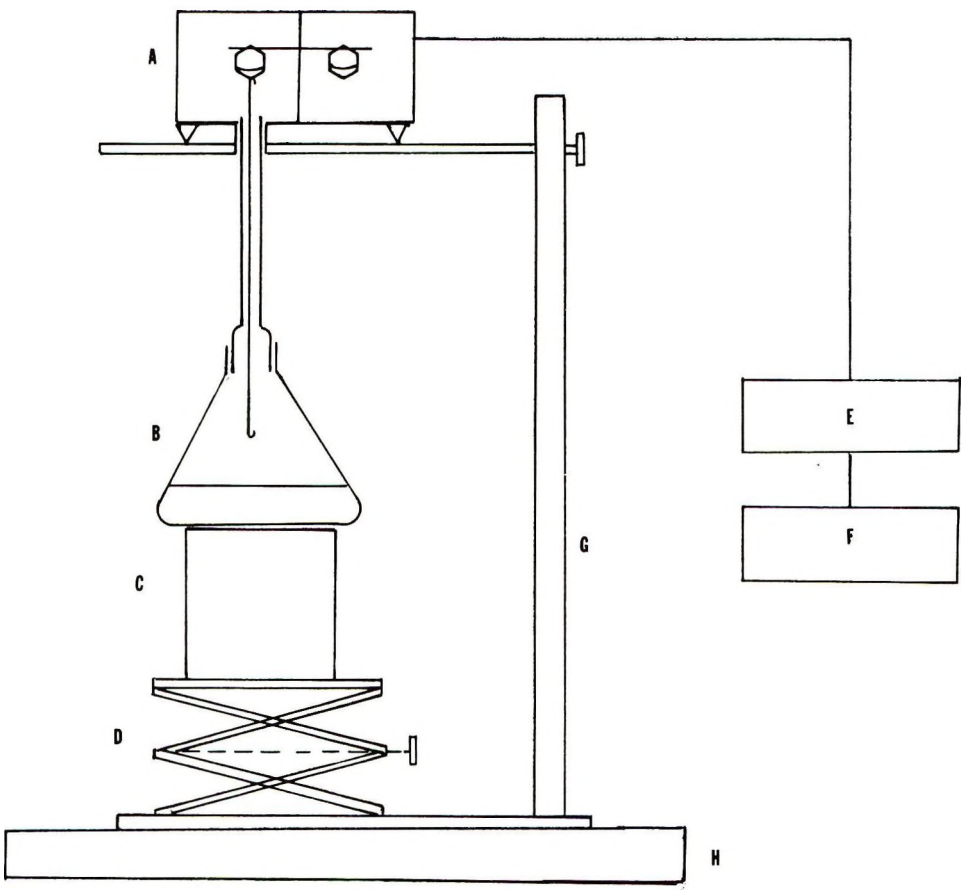

Figure 1. Gravimetric technique for vapor sorption and desorption

EXPERIMENTAL

The gravimetric technique used to study benzene and toluene vapor sorption or desorption is shown in Fig. 1. The method employs a recording microbalance and stirred organic solutions to generate the required relative vapor pressure. The corneum sample was hung on a very thin glass rod, which was suspended from the microbalance terminal (A) into a 500 ml flask containing the organic vapor system (B). The distance between the sample and the organic solution was maintained at about 1 in. To eliminate draughts, the glass rod was shielded by a glass tube, the upper end of which fitted tightly into the base of the microbalance, while the lower end was connected to a ground glass joint. The flask was placed on a small magnetic stirrer (C), which gently rotated a small magnetic bar placed in the solution. The magnetic stirrer was placed on a regular lab jack (D), which could be conveniently raised or lowered to allow quick change of flasks containing solutions of varying vapor pressures. The microbalance was placed on a specially constructed base which was secured to a stand (G). The whole setup was placed on antivibration base (H) in a constant temperature and humidity room (23°C, 55 per cent RH). In Fig. 1, E and F depict the balance control of a Beckman*-microbalance L-600 and a chart recorder, respectively. This method was also used to examine the sorption, desorption, and diffusion of water vapor in keratins using saturated salt solutions. The salt solutions used to generate the required relative humidities at 23°C are as follows:

Lithium chloride	11.5 per cent
Potassium acetate	21.5 per cent
Sodium iodide	38.0 per cent
Sodium dichromate	54.0 per cent
Copper chloride	68.0 per cent
Ammonium chloride	78.0 per cent
Potassium chromate	87.0 per cent
Ammonium dihydrogen phosphate	93.0 per cent

A flask containing drierite provided zero humidity. The partial pressure P of the organic solvents was controlled by using solutions of benzene or toluene in hexadecane, the latter being essentially nonvolatile. According to Raoult's law, assuming ideality

$$P = P^0 X$$

where P^0 is the vapor pressure of pure benzene or toluene and X is the mole fraction of benzene or toluene in the hexadecane.

The sorption experiments were carried out in the relative vapor pressure range from about 0.3 to 0.94. Lower relative vapor pressure conditions were employed in a number of cases. The stratum corneum samples were initially dried over Drierite to obtain a base weight. The experimental approach was as follows: after drying out the corneum, it was exposed to the organic vapor atmosphere of a given relative vapor

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pressure and the kinetics of vapor sorption was monitored continuously until equilibrium was achieved. At this point, desorption was started by replacing the organic solvent system with activated charcoal or Drierite. Both drying systems were found to be equally effective. Experiments were carried out at 23 and 32°C. Measurements at the higher temperature were carried out using the technique described in (6).

Stratum corneum was prepared according to the procedure suggested by Kligman and Christopher (13) and described in detail in (14). The treatment of the stratum corneum in organic solvents was conducted as follows: a piece of guinea pig stratum corneum was placed between 2 small pieces of saran gauze held in a specially constructed Teflon®* frame. The frame was placed in a beaker containing the chloroform-methanol mixture (2:1), maintained at 40°C, for 30 min with gentle agitation. This was followed by immersing the frame in a beaker containing distilled water maintained at the same temperature for another 30 min. The corneum was then taken out and dried at room temperature before storing it in the refrigerator. Although, no quantitative estimate was made, it was observed that the above described treatment brought about a substantial decrease in the dry weight of the corneum (~ 30 to 50). The treated corneum was examined for its water vapor sorption and diffusion properties, which were compared to data on intact (untreated) guinea pig corneum, obtained earlier.

In order to acquire some information on possible structural changes in the corneum samples, a preliminary SEM examination was conducted. The sample preparation for the SEM was as follows. Pieces of the stratum corneum were mounted on stubs using transfer tape. The stubs were then placed into a vacuum evaporator and coated with 5 nm of carbon followed by 30 nm of 80:20 gold/palladium from two angles.

DISCUSSION

Quantitative analysis of equilibrium sorption isotherms of water vapor in keratin has been described in detail (6). Briefly, a number of theories and equations were employed, including the BET and D'Arcy–Watt equations, the Flory–Huggins polymer solution theory, and Zimm's clustering function. In general, the water vapor sorption isotherms on excised skin and hair were found to fit the D'Arcy-Watt eq. (16).

$$W = \sum_{i=0}^{I} \frac{A_i B_i (P/P_0)}{1 + B_i (P/P_0)} + C(P/P_0) + \frac{DE(P/P_0)}{1 - E(P/P_0)} \qquad (1)$$

where W is the weight of sorbate adsorbed by 1 g of sorbent; $A_i = mn_i/N$ is the number of primary sites of type i, multiplied by the molecular weight of sorbate and divided by Avogadro's number N; B_i is a constant which is a measure of the attraction of the sites for the sorbate; I is the number of different types of sorption sites for primary adsorption described by a Langmuir isotherm; C is a constant for the linear ap-

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proximation to Langmuir adsorption on specific sites; and D and E are constants describing secondary adsorption processes. P/P_0 is the relative vapor pressure (relative humidity) of the sorbate.

The results of the analysis indicate that in the low relative humidity range (0 to 30 per cent RH) sorption of water vapor occurs on reactive sites. Multilayer formation, accompanied by extensive clustering, occurs at the high relative humidity end of the sorption isotherm. Clustering was evaluated according to Zimm's Eq. (17):

$$\frac{G_{AA}}{V_A} = -\phi_B \left[\frac{\partial(a_A/\phi_A)}{\partial a_A}\right]_{PT}^{-1} \tag{2}$$

where G_{AA} is the cluster integral for water molecules; and V_A and a_A denote, respectively, the partial molar volume and the activity (relative humidity) of water. ϕ_A and ϕ_B are the volume fractions of components water and keratin.

Examination of the sorption/desorption isotherms showed that hysteresis is not a general phenomena in keratins, and was observed in a number of samples only. In general, the sorption isotherm was shown to exhibit the equilibrium properties required for thermodynamic treatment.

A background review of the mathematics of diffusion, and of the application of the principles developed by Crank and coworkers to the problem of water vapor diffusion in swelling keratins (for example, stratum corneum and human hair), has been given in (7). Determination of the diffusion coefficient is based on accurate measurements of the kinetics of vapor sorption or desorption in a small sheet of stratum corneum according to the following equation:

$$\frac{Mt}{M\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} e^{-D(2n+1)^2\pi^2 t/4l^2} \tag{3}$$

where M_t denotes the total amount of diffusing substance which has entered the sheet at time t, and M_∞ the corresponding quantity after infinite time. Equation (3) is the exact solution of Fick's basic diffusion equation, assuming a constant D, for the boundary conditions

$$C = C_0 \quad 0 < X < l \qquad t = 0$$
$$C = C_1 \quad X = 0 \text{ and } X = l \qquad t > 0$$

where C_0 is the initial concentration of water in the sheet when the surfaces ($X = 0$ and $X = l$) are exposed to a constant concentration C_1 of vapor. The boundary conditions describe sorption or desorption depending on the values of C_0 and C_1.

A simple method based on eq. (3) for the determination of a mean value $\bar{D}$ (when D is not a constant) has been suggested by Crank (18). The value of t/l^2 for which $M_t/M_\infty =$

1/2 (designated as $(t/l^2)_{1/2}$ or simply half-time) is determined experimentally according to the relationship

$$\overline{D} = \frac{0.04919}{(t/l^2)_{1/2}} \tag{4}$$

More accurate values for $\overline{D}$ are obtained when the average of sorption $\overline{D}_s$ and desorption $\overline{D}_d$ is taken. Our findings indicate that the diffusion properties of the water vapor-stratum corneum system can be characterized by a concentration-dependent diffusion coefficient. A detailed analysis of the behavior of the diffusion coefficient as a function of the water content in stratum corneum shows that in highly swelling samples, a maximum is observed in the relationship between the mean diffusion coefficient ($\overline{D}$) and water content. If the mean diffusion coefficient is corrected for swelling (intrinsic diffusion coefficient [D_i])

$$D_i = \frac{\overline{D}}{(1 - V)^3} \tag{5}$$

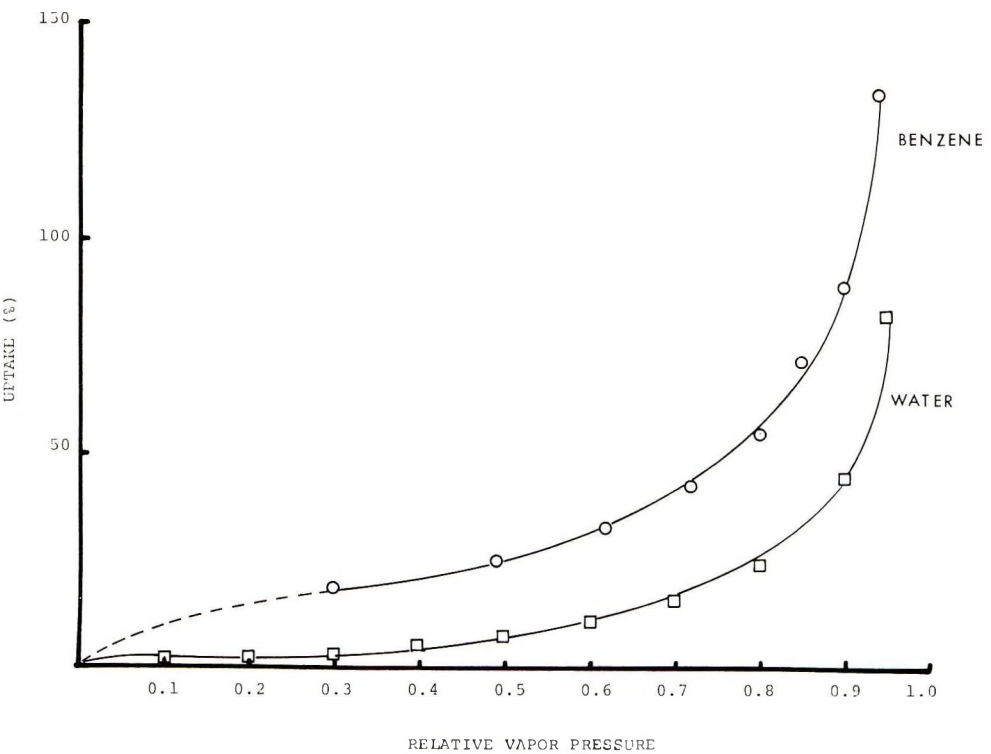

Figure 2. Sorption isotherms of benzene and water vapor on female stratum corneum, age 20, at 23°C

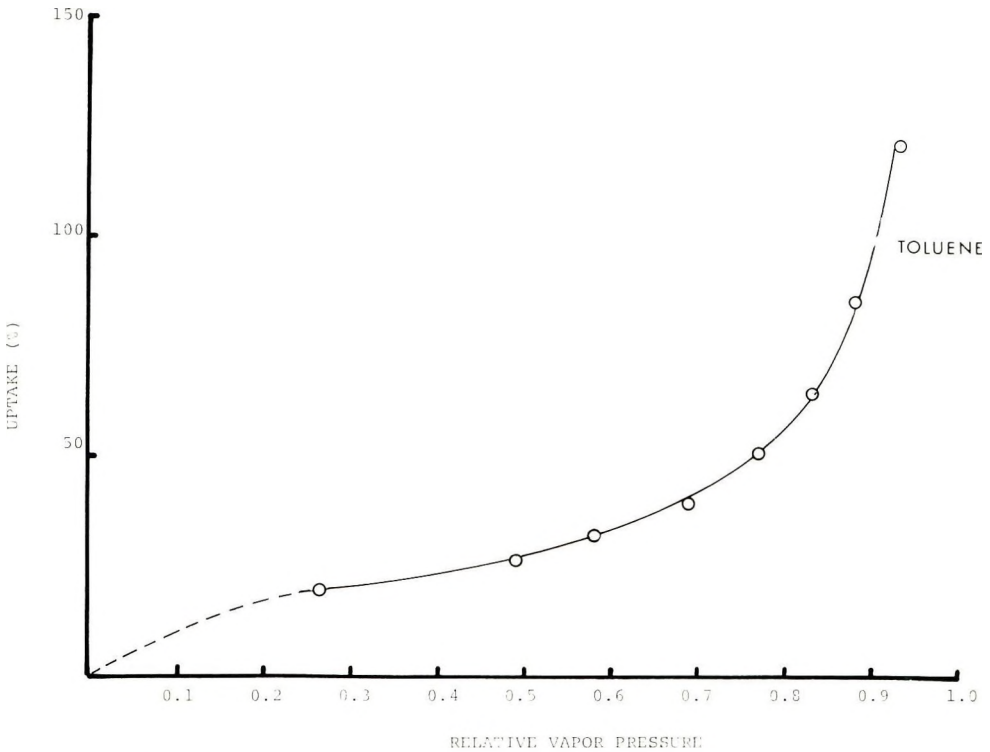

Figure 3. Sorption isotherm of toluene vapor on female stratum corneum, age 20, at 23°C

where V is the volume fraction of water, it was found that the diffusion coefficient usually shows a continuous increase with increase in water content. It was thought of interest to extend our investigations to some organic vapor systems to improve our understanding of the physical and chemical factors associated with sorptive and barrier properties of the stratum corneum.

The benzene and toluene vapor sorption isotherms of female stratum corneum (age 20), are shown in Figs. 2 and 3. The uncertainty in the uptake values at the high end of the isotherms is quite high (±15 per cent at the highest relative vapor pressure); nevertheless, it is clear that the sorption isotherms are of type II according to the BET classification. Similar sorption isotherms were obtained for other human corneum samples and the results generally indicate a higher sorption capacity for the organic vapor. However, if the uptake values are expressed in moles rather than the conventional weight increase in grams per 100 gm corneum, it was found that the organic vapor (benzene, toluene) sorption values were generally higher than those obtained for water up to about 0.5 relative vapor pressure then progressively fell below the water uptake values. These preliminary findings seem to indicate that there are more binding sites available for benzene or toulene in the corneum samples examined in the vapor range where the sorption process is presumed to follow a Langmuir model. At higher relative vapor pressure, formation of multilayers and clusters of sorbate molecules may occur, hence no binding between the sorbate molecules and the substrate. These data seem to

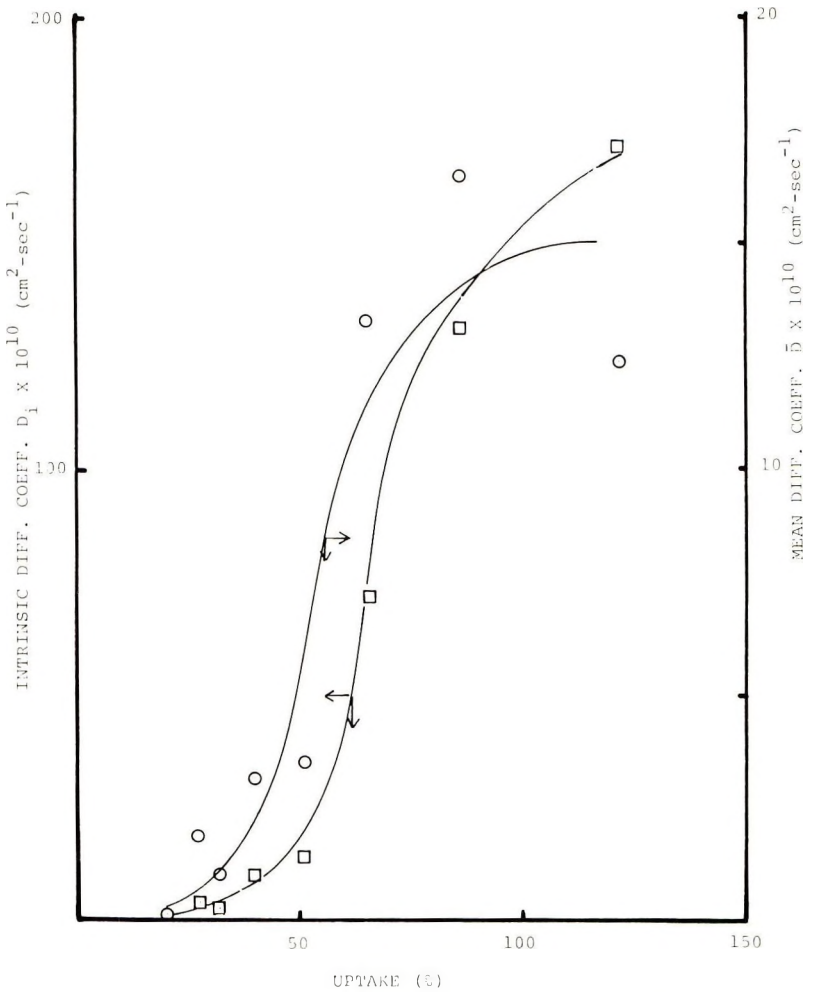

Figure 4. (□) intrinsic (D_i); and (○) mean ($\bar{D}$) diffusion coefficient of toluene vapor in stratum corneum (female, age 20) as a function of concentration, at 23°C

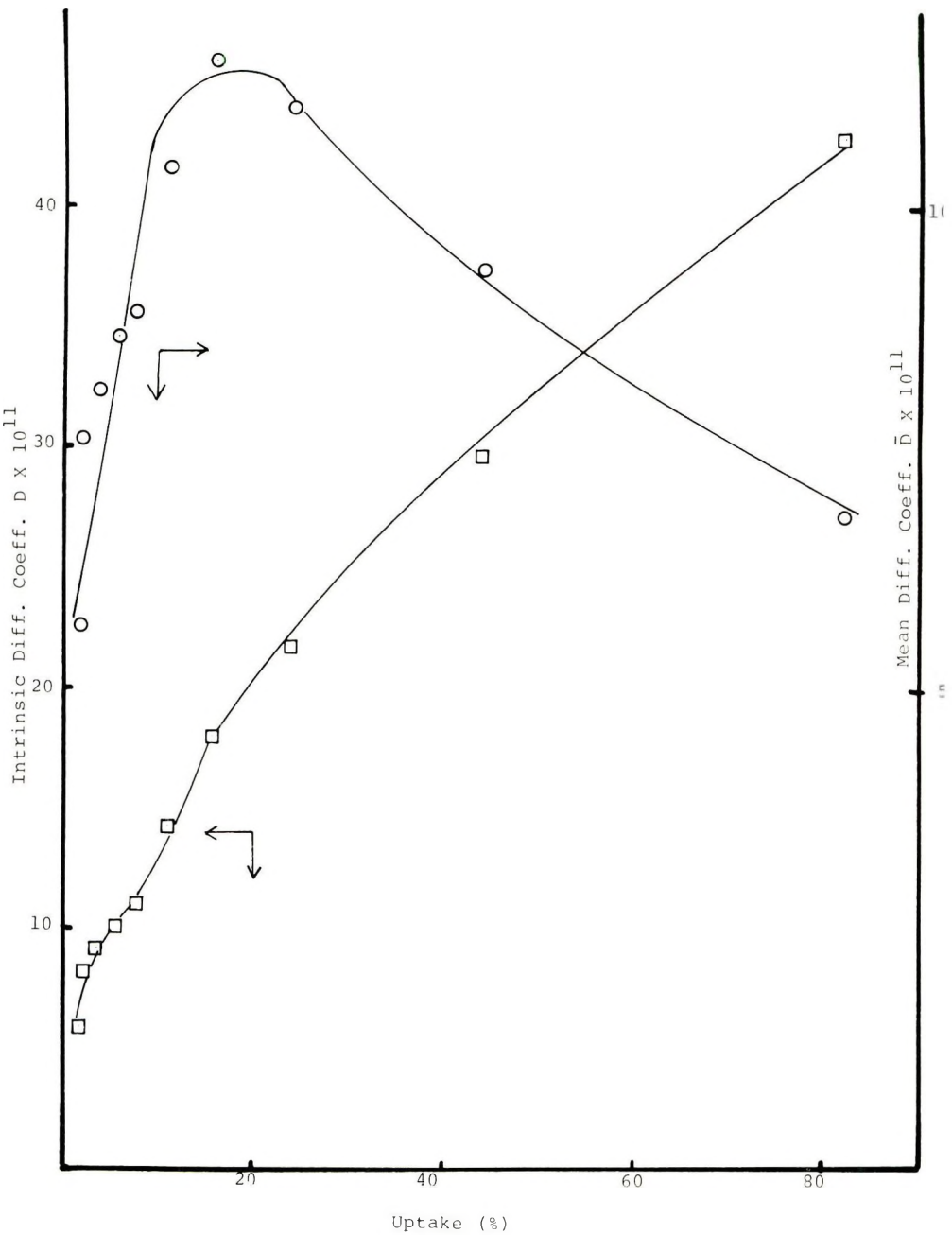

Figure 5. (□) intrinsic (D_i); and (○) mean ($\bar{D}$) diffusion coefficient of water vapor in stratum corneum (female, age 20) as a function of concentration at 32°C

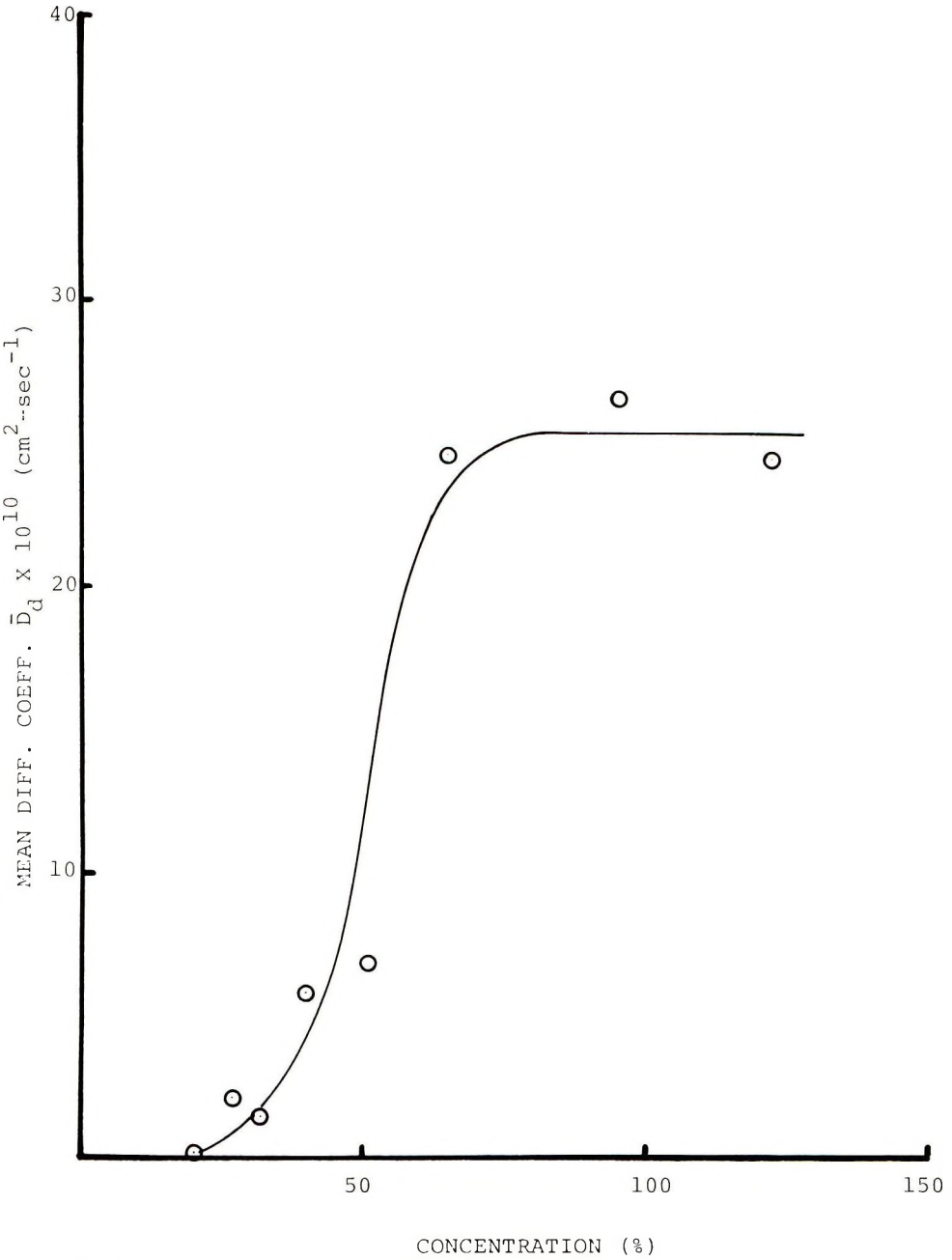

Figure 6. Mean desorption diffusion coefficient ($\bar{D}_d$) of toluene vapor in stratum corneum (female, age 20) as a function of concentration, at 23°C

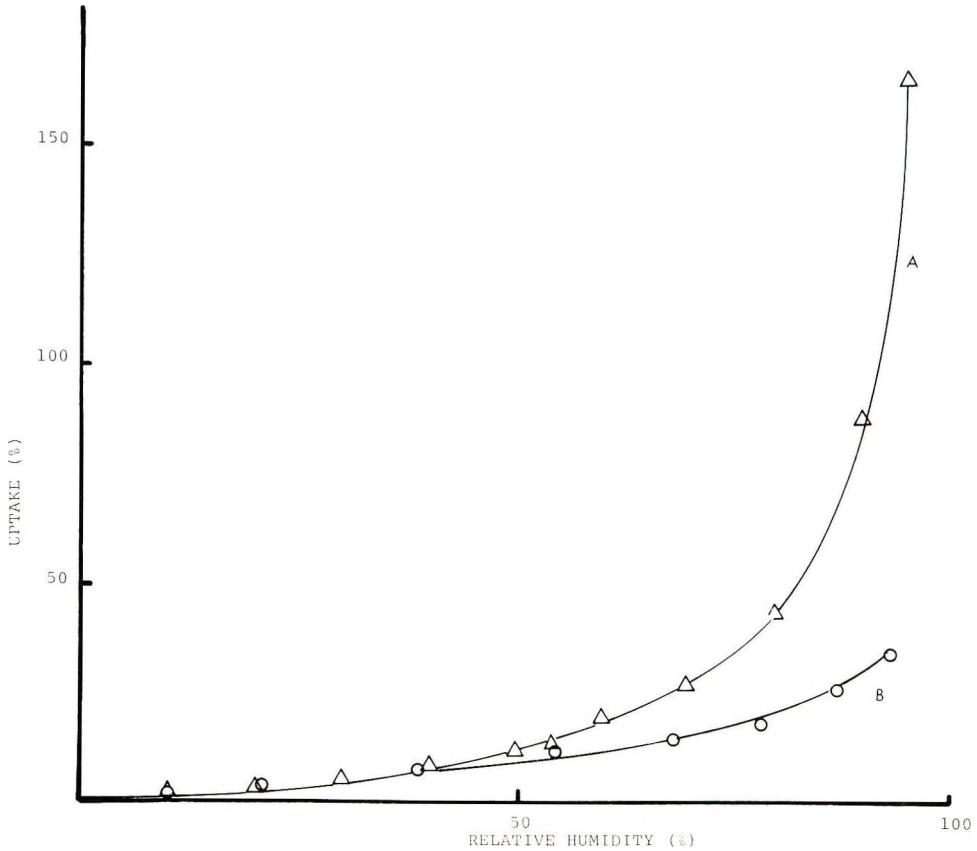

Figure 7. Sorption isotherm of water vapor in untreated (curve A) and organic solvent-water treated (curve B) stratum corneum (guinea pig), at 32°C

confirm our earlier conclusions, based on the wetting behavior of human stratum corneum and hoof keratin, that these keratinous materials are, on balance, hydrophobic in nature (15).

The diffusion process of toluene vapor in the stratum corneum is also quite interesting. The graphs of the intrinsic and mean diffusion coefficients as a function of concentration are shown in Fig. 4. It can be seen that, as the concentration of toluene in the stratum corneum increases, the diffusion coefficient (D_i or $\overline{D}$) increases by three orders of magnitude in the range of relative vapor pressure examined. In the case of water vapor sorption, the observed increase of D with C was one order of magnitude at most, as shown in Fig. 5. Starting from the high concentration end, the desorption process is marked by a constant mean diffusion coefficient $\overline{D}_d$ as shown in Fig. 6; this is followed by a sharp decrease in the value of $\overline{D}_d$. The constancy in the $\overline{D}_d$ values can be interpreted as reflecting the evaporation process of the "free" toluene in the corneum, and the sharp decrease in the diffusion coefficient marks the start of desorption from high energy binding sites.

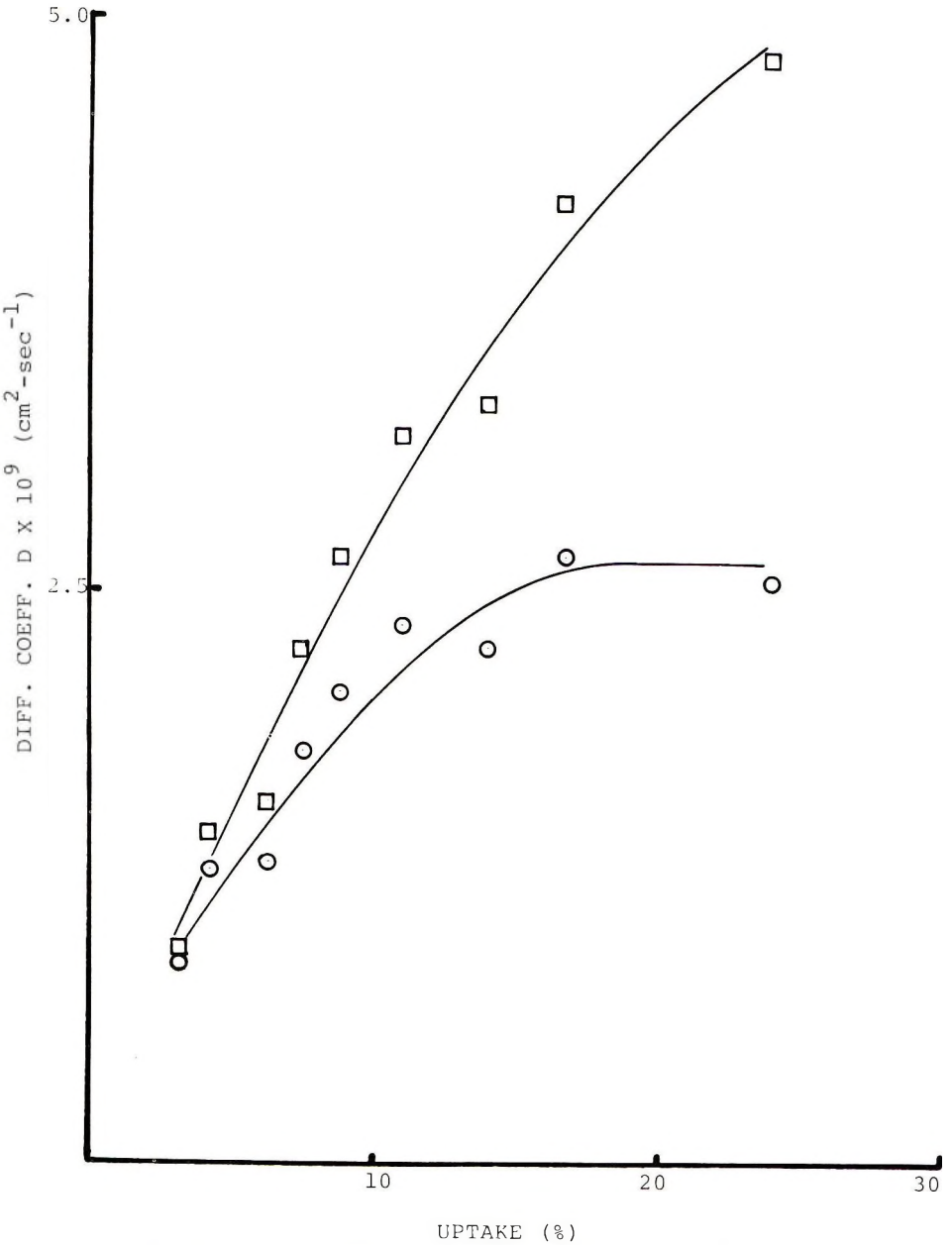

Figure 8. (□) intrinsic and (○) mean diffusion coefficient of water vapor in organic solvent-water treated guinea pig corneum, at 32°C

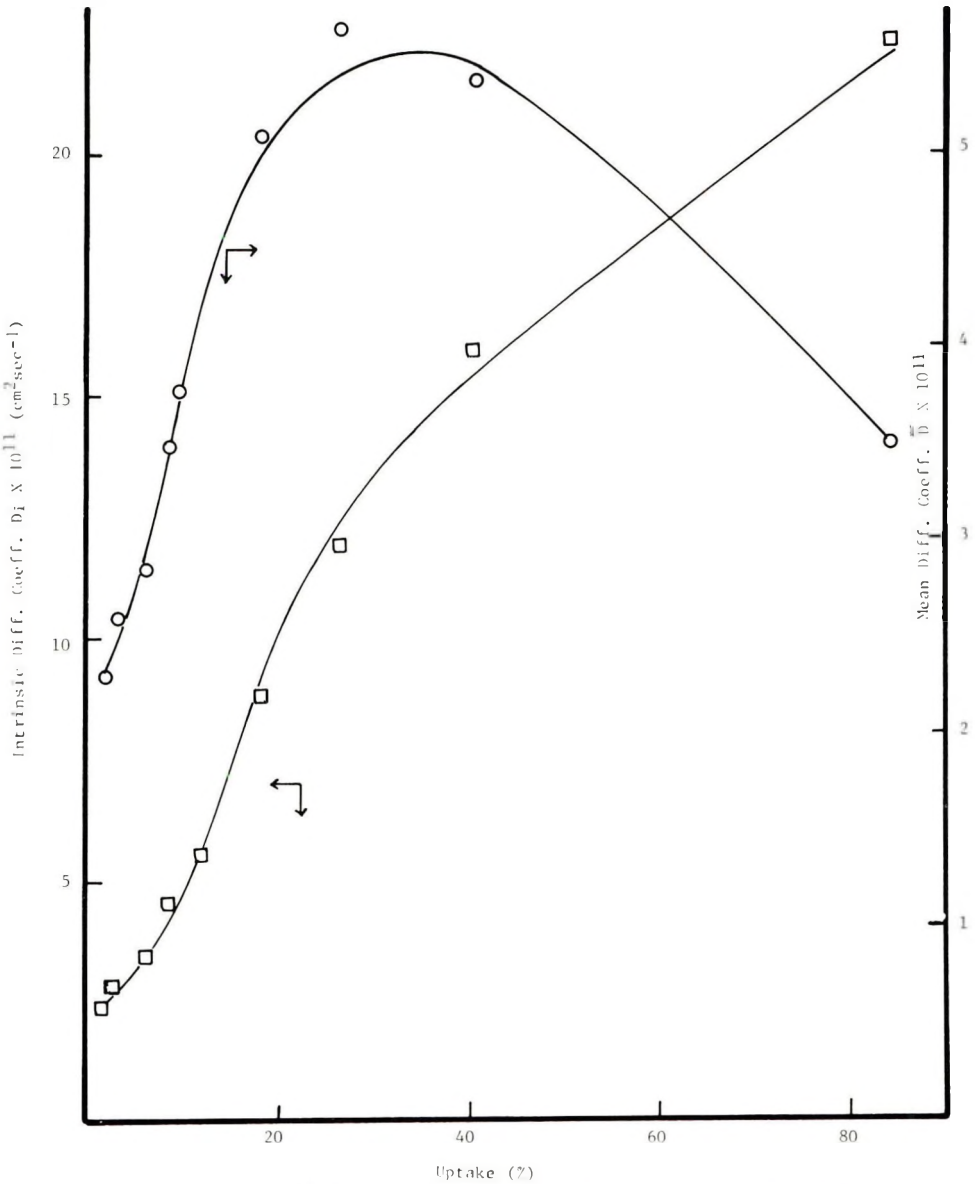

Figure 9. D_i and $\bar{D}$ of water vapor in untreated guinea pig corneum at 32°C

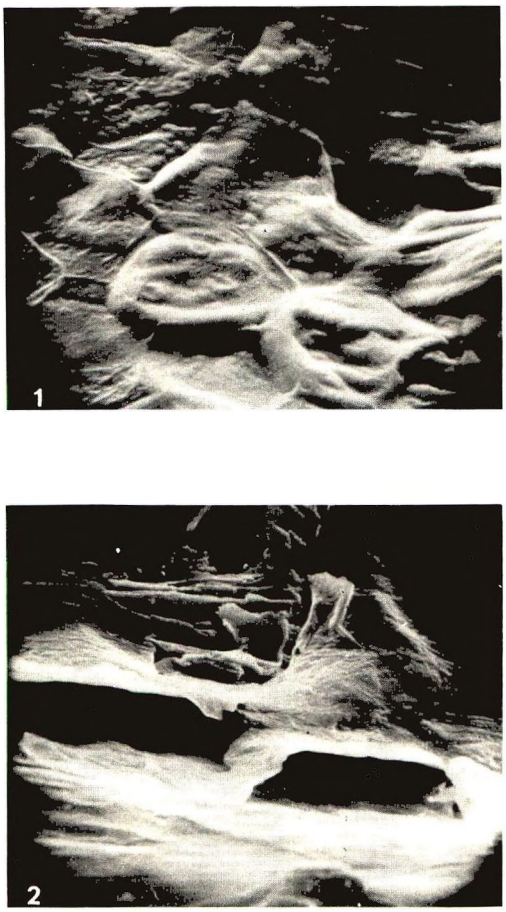

Figure 10. (1) Untreated guinea pig corneum, 200 ×, (2) guinea pig corneum treated in 2:1 chloroform-methanol mixture, followed by extraction in distilled water, 200 ×

The effect on the water vapor sorption isotherm of treating guinea pig corneum in a 2:1 chloroform-methanol mixture, followed by extraction in distilled water, is shown in Fig. 7. Curve A depicts the water vapor sorption isotherm for intact (untreated) corneum, and curve B depicts the effect of the organic solvents-water treatment. Similar results for this system have previously been reported by Singer and Vinson (4). It is seen that the initial portions of the isotherms up to about 50 per cent RH almost coincide, but the two curves markedly diverge beyond 70 per cent RH. A total number of 4 water vapor sorption-desorption isotherms were obtained on different corneum pieces from the same guinea pig sample, treated in the organic solvents and water. The reproducibility of the data points on the isotherms was within 1 per cent of the mean. The substantial decrease in the water vapor sorptive capacity of the treated guinea pig corneum in the higher humidity range indicates a marked change in the corneum structure which affects primarily the formation of multilayers. The extraction of some materials and, perhaps, disruption of molecular bonds could conceivably result in a more open

matrix structure and, hence, decrease the possibility of multilayer formation. It is also expected that the diffusion of water vapor molecules would be faster in such a modified structure. This is evidenced by the diffusion coefficient-concentration relationship, shown in Fig. 8 as the intrinsic diffusion coefficient D_i and mean diffusion coefficient $\bar{D}$ against percentage water vapor uptake. An average increase of an order of magnitude in the value of D is observed as a result of the solvent treatment. Compare Fig. 9 which shows data for the untreated corneum. The effect of treatment was also assessed with the help of SEM. Figure 10 (1) and (2) provide a comparison between intact (untreated) and treated guinea pig corneum. The effects of the organic solvent treatment are quite apparent in (2), as judged by the extent of cleavage and disruption of the cellular matrix.

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Thin-layer chromatography (TLC) of redox reaction products of oxidative hair dyes

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Synopsis

OXIDATIVE or permanent HAIR DYES are based on ALKALINE PEROXIDE OXIDATION of PHENYLENEDIAMINE (PDA) or related AROMATIC AMINES. These amines, when oxidized alone or in combination with other phenolic and aromatic amino compounds (couplers), yield a mixture of colored oxidation products. This paper describes the use of thin-layer chromatography (TLC) for the qualitative analysis of these complex mixtures of oxidation products. Effects of variables including the nature of adsorbency, layer thickness, water content, development of chromatograms, and sample application techniques are presented. Scopes and limitations of chromatography for the isolation and the identification of these dyes are discussed.

INTRODUCTION

Permanent hair dyes, which are presently available commercially, are mostly oxidative dyes which contain 2 main ingredients. One ingredient is a dye precursor, while the other ingredient is a developer or oxidizer, usually hydrogen peroxide. The dyeing process involves mixing the dye precursors with the dye developer in an alkaline medium generally around pH 9 to 10. The dye precursors and oxidizer diffuse in the hair fibers where chemical reactions leading to color development take place inside the hair fiber.

Dye precursors contain 2 main ingredients: primary intermediates and couplers. Primary intermediates used in oxidative dyes are mainly ortho- and para-aromatic diamines or aminophenols, which are colorless, but, upon oxidation, give colored oxidation products. The most commonly used primary intermediates are p-phenylenediamine (PDA), p-toluenediamine (TDA), and p-aminophenols (PAP).

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Color couplers are compounds which produce little or no color when oxidized alone in the hair fiber, but which produce new colored species when used in the presence of primary intermediates. These color couplers include m-diamines, m-aminophenols, polyhydric phenols, etc. The most commonly used couplers are resorcinol (R) and 2,4 diaminoanisole (DAA), which when oxidized with p-phenylenediamine give green and blue colored products, respectively, along with many other minor products.

When studying these dyes, it is important to ascertain the primary intermediates and couplers used in a particular dye, and secondly, the colored oxidation products formed from the dye precursors with the dye developer, i.e., hydrogen peroxide. To study these aspects, improved methods are needed for the separation and identification of oxidative hair dyes.

Much work has been done in developing separation techniques to identify dye intermediates and other components present in oxidative hair dyes. These methods include the use of paper chromatography (PC) (1–6), gas chromatography (GC) (7–10), thin-layer chromatography (TLC) (11–19), and/or combinations of these chromatographic techniques (11,16), and other methods (20–23). In order to understand the mechanisms of the color-forming reaction, it is vital to identify the colored species produced during the hair dyeing process. Application of chromatography of the oxidation products of primary intermediates alone or in the presence of various couplers is essential. Chromatography separation of the oxidation products of oxidative hair dyes has been reported (24–31), which emphasizes the importance of understanding the redox reaction products of the dye intermediates.

In the field of separation methods, chromatography occupies a rather unique position, and TLC provides the best answer to this problem in many cases. This paper describes a comprehensive study of TLC of the oxidation products and their application to oxidative hair dye analysis. It presents the effect of 3 main factors of chromatographic separations, i.e., solutes (nature and amount); sorbents (quality and nature, thickness and uniformity, activation and sotrage); and solvent (quality and nature, vapor saturation) of the separation of oxidative dyes.

Also, an isolation procedure for the desired component of the oxidation mixture after their separation is shown. This paper should (a) serve as a background of information for those who would be utilizing this technique for identifying oxidation dye products; and (b) establish variables of this analytical method to provide the best separation technique for the multicomponent dye product.

EXPERIMENTAL AND DISCUSSIONS

The use of TLC for oxidative dyes presents 2 specific problems: first is the limited stability, and second is the problem of closely related structures of the oxidation products (Tables I, II). As mentioned previously, 2 of the primary ingredients are aromatic diamines and polyhydric phenols. The chemical transformation in the reaction system, once initiated, may continue indefinitely. Even the individual fractions isolated are self-reactive and/or react with each other and with foreign agents such as air, moisture, sunlight, proteins, etc.; which are manifested by several types of reactions in solid state or in solution.

Table I

RF Values of the Oxidation Products of p-Phenylenediamine and m-Methoxyphenol[a]

Band Color	Relative Intensity	RF X100
Redish Brown (origin)	Strong	0
Yellow Orange	Strong	12.05
Pink	Strong	17.64
Purple	Light	21.47
Yellow	Strong	26.17
Brown	Strong	29.70
Yellow	Medium	35.58
Green	Light	40.58
Orange	Medium	41.76

[a] 1:1 mixture with hydrogen peroxide oxidant and sodium carbonate base; Chloroform:Ethylacetate:Methanol (6:2:2) solvent system.

There is a great advantage in working with these oxidative dyes. Since the products are colored, it is easy to visualize the progress of the chromatography during development, to obtain useful information on the presence or absence of certain components, and even to make a rough estimate of their relative abundance.

Although, PDA yields a number of color components in its reaction with alkaline peroxide both alone and with the individual couplers, the dominant composite colors are a purplish-brown for PDA, a green for PDA-resorcinol, and a purplish-blue for PDA-diaminoanisole. The oxidative reaction of resorcinol and diaminoanisole alone or in combination produce negligible color compared with their coupling products with PDA. Self-coupling of PAP provides multicomponent products like other products, but gives a major yellow color. The binary coupling with resorcinol provides green color similar to that of PDA-resorcinol R, but with coupling with DAA the predominant product is a vivid red with blues, greens and relatively few brown components either in primary, or in polymeric products. Chemical structures of the major colors are shown in Fig. 1. With prolonged dye development time, all the above compounds increasingly convert to polymeric compositions of brown components, having a very low mobility on thin layer plates.

Table II

RF Values of the Oxidation Products of p-Aminophenol and Resorcinol[a]

Band Color	Relative Intensity	RF x100
Brown (origin)	Strong	0
Blue	Light	7.35
Pink	Strong	18.17
Green	Medium	26.47
Brownish Red	Medium	30.88
Brown	Medium	32.35
Red	Strong	37.64
Yellow	Strong	38.82

[a] 1:1 mixture with hydrogen peroxide oxidant and sodium carbonate base; Chloroform:Ethylacetate:Methanol (6:2:2) solvent system.

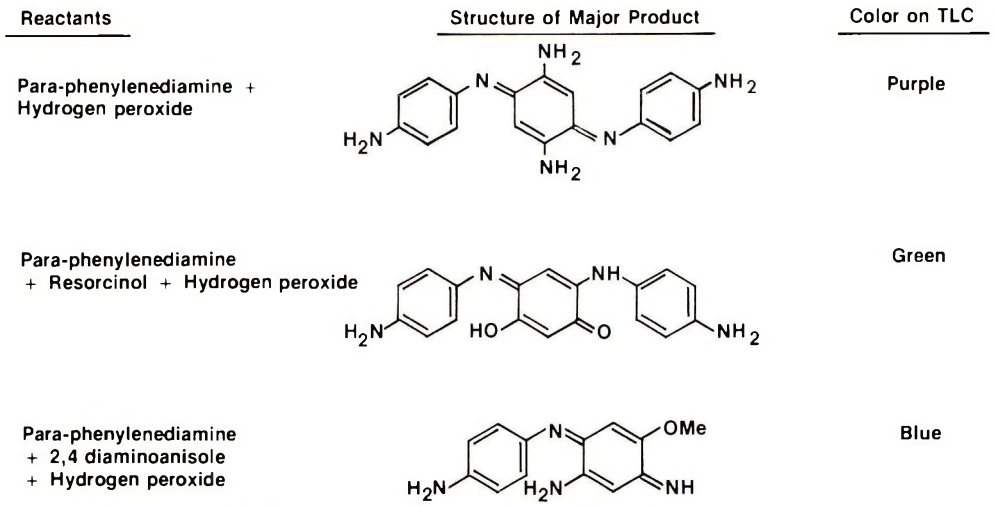

Figure 1. Structure and color of major oxidation products

Table III

THIN LAYER PLATES

No	Source	Adsorbent Coating	Inert Backing
1	Brinkman (Merck)	Slica Gel [a]	Glass plate
2	Mallinckrodt	Slica Gel	Glass fiber web
3	Eastman	Silica Gel	Polyethylene terephthalate
4	Anal. tech.	Alumina Acid	Glass plate
5	Anal. tech.	Alumina Neutral	Glass plate
6	Anal. tech.	Alumina Basic	Glass plate
7	Brinkman	Cellulose	Glass plate
8	Corning	Glass powder	Glass plate
9	Varied	Filter papers	None

[a] Analytical (.25 mm) and preparative (2 mm)

FACTORS OF CHROMATOGRAPHIC SEPARATIONS

Solutes: The nature of the solutes to be analyzed is important to the success of obtaining an effective separation. Upon oxidation, PDA and allied amines give a very complex mixture of products. This is because oxidation of PDA involves the formation of a highly reactive intermediate (32), which can undergo various reactions. Upon oxidation, PDA gives quinonedimine, which on hydrolysis, gives quinone-monoimine, and on further hydrolysis, results in the formation of benzoquinone (33).

Once formed, quinonediimine reacts rapidly with unreacted PDA and gives a major products known as Bandrowski's base. As has been shown before in oxidative dyes, there are may couplers, other than primary intermediates, which undergo competitive reaction with quinonediimine to give a mixture of products (34). These are mostly indophenols and indamines and can be bound through nitrogen or oxygen. These are main products. In addition to these, various forms of one ring, two ring or poly-ring structures can form (25). Thus, selectivity in the reaction is very low. Even when a single precursor is used, one always ends up with 3 to 4 major components, and at least 5 to 6 minor components. While the nature of the solute plays a role, the amount of solvent is also important. In many instances, inversion of the bands was found, with a change in the amount of solute, fast-moving bands becoming slow-moving and vice-versa.

Table IV

Resolution of p-Phenylenediamine and Resorcinol Oxidation Products on Silica Gel and Alumina TLC Plates[a]

Alumina plate[b]		Slica gel plate[b]	
Band color	distance removed, cm.[c]	Band color	distance removed, cm.[d]
Brown	0	Brown	0
Green	1.2	Blue	1.5
Yellow	1.9	Pink	4.0
Blue	3.0	Green	5.0
Yellow	4.5	Yellow	6.0
Pink	5.4	Orange Yellow	8.5
Red	6.7	Yellow	9.5
Yellow	7.1		

[a] Solvent system, Chloroform:Ethylacetate:Methanol (6:2:2)

[b] In the case of alumina plate the solvent front moved 17.0 cm, while in silica gel it moved 17.5 cm.

[c] Result of three development

[d] Result of single development

Adsorbent: In order to find out which adsorbent would best resolve the complicated mixtures, a number of different adsorbents with plastic or glass backing were tried as is shown in Table III.

In all analyses, commercially available thin-plates were used. For these studies, silica gel plates were the most useful in overall separation, up to 8 components were easily resolved in a single elution. Plates with a flexible inert phase, such as plastics or glass fiber web, never equaled the quality of resolution obtained on glass plates—even when identical adsorbent phases were used. Although Chroam AR sheets* carried the same

*Mallinckrodt Inc., St. Louis, MO.

Table V

Solvent Selection		
Dye Group	Properties	Solvent
1	Fast moving, low molecular wt. one or two ring structures	Ether: chloroform
2	Two or three ring structures	Chloroform: ethylacetate: methanol Chloroform: DMF
3	Immobile polymeric	Alcohol, DMF, DMSO

solid phase as the thin-layer plates, they did not have the sharpness of the resolution of the analytical plates. All of the components diffused much more, not only in the direction of the elution, but also perpendicular to it as well, showing channeling of the solvents along the fibers which form an isotroric web. This resulted in more extensive component spreading.

Alumina was less efficient in performing the total analysis of all product-compositions studies. In some instances, alumina gave much sharper or cleaner separations for specific components. Specificity was not a characteristic of pH, but of the adsorbent itself, and it was qualitative for most components in the form of no adsorption or extremely strong adsorption.

Development time was slightly reduced when a glass adsorbent was used, but there was poor resolution, especially for the fast-moving and slow-moving components. Good resolution was never achieved with cellulose solid phase either in the form of papers or TLC plate. Table IV shows the resolution of PDA and resorcinol oxidation products on silica gel and alumina plates. On silica gel plates, the product is well resolved and there is not much specificity of strongly adsorbed and nonadsorbed components. On alumina plates, the fast-moving pink and many other components stayed close to the origin.

Solvent: A difficult task in this study was the choice of a proper solvent or solvents, as no one system was found which resolved all components simultaneously. From the point of view of solvent requirement, all dye components can be divided into 3 groups (Table V).

The dyes belonging to Group 1 are low molecular weight, one-ring or two-ring structures (such as nitro aniline) and are compounds which are mostly orange, yellow, or red. These components are usually water soluble. This group of dyes can be separated by using chloroform and ether in different ratios.

The dyes in Group 2 include all the specific dye components, which are of primary importance for the shade and intensity of hair color. They are components containing 2 or 3 rings bound by nitrogen or oxygen. The elution system for the resolution of these components must contain significant amounts of highly polar and hydrophilic components such as dimethylformamide (DMF), dimethylsulfoxide (DMSO), and alcohols, as well as low-activity solvents, such as halogenated hydrocarbons.

In an arbitrary way, all dye components, which did not elute with the above solvent systems, were grouped as polymeric materials (Group 3). They are mostly immobile and can be resolved partially using DMF, DMSO, or methanol.

Oxidation products are sensitive to both acids and bases used in the solvent system and produce irreversible destruction of a number of components of the product. Their use in solvent systems was avoided.

Moisture: In the resolution of oxidative dyes, a trace amount of moisture is more detrimental for the reproducibility than other chromatographic separations. It was observed that the resolution of certain components can be eliminated completely by allowing moisture in the system through the plates or from the atmosphere.

Activation: Developed plates, on which oxidative dyes cannot be reactivated for a second elution in a similar fashion, because they undergo irreproducible changes or cannot be controlled. Dyes to be applied on the activated plate were dissolved in DMF, since the polymeric component of the dye had a limited solubility in other less polar solvent. When preparing a sample of a solvent, that solution should never be warmed, and fresh solution should always be prepared just before applying it to the plate.

Application to plate: With oxidative dyes, it is preferable to apply the products to the plate as a sharp band, rather than as a spot, for 2 reasons. One, the left-over unreacted dye precursors, which developed during the elution process, causes heavy trailing and interferes with the chromatography of an already formed oxidized product. Second, the oxidized products undergo secondary coupling reactions on silica gel, giving grayish-brown polymeric trailing. When the dye is applied as a band, visual identification of the components present in smaller amounts can be achieved.

Evaluation and documentation: Evaluation and documentation of the thin-layer plates should be done immediately after elution to record the presence or absence of components and the relative strength of color of individual components. This is required because 3 events may occur on the developed plate. One, a new color may form from untreated precursors; two, color may fade from the intensely colored products; and three, color may change. For example, green color changes to gray, blue changes to purple, and so on.

All of these processes can be slowed down, immediately following elution, if the plates are wrapped tightly to exclude air in polyethylene bags (preferably black) or are kept in a dark place.

ELUTION TECHNIQUES

Single elution: Single elution of the spotted plates should be done as soon as the spotting solvent has evaporated. A spotted plate cannot be dried at an elevated temperature or even at low temperature for very long. It should be developed immediately after drying.

If not completely removed, DMF moved all the components until it was sufficiently diluted by the weaker eluting solvent. Aging at room temperature resulted in a disproportionate loss of specific components and caused the formation of polymeric material. Complete drying caused the destruction of some components. So it was necessary to choose between complete drying and leaving spotting solvent on the plate. These studies indicated that it was harmful to leave some spotting solvent rather then to dry completely.

Two dimensional: Two-dimensional chromatography was most useful for compositions in which individual members differ qualitatively in their response to solvents, e.g., acidic and basic amino acids. This was not the case with oxidative dyes. Any increase in solvent activity increased the mobility of all the components. No solvent compositions was found which specifically favored slow-moving components.

Continuous elution: This method was used in order to obtain single components in sufficient amount for structural determination. Continuous elution was made in an apparatus* (Fig. 2). Oxidation product was applied as a band at the bottom of the plates. The lids on the developing tank were positioned to form slots 4 nm wide. Plates were allowed to stand in the solvent with the upper ends projecting into the free atmosphere. The solvent moved and evaporated at slot levels as a continuous process, so that all components, except the undesirable polymeric uneluted product, were redeposited at lid level as a narrow line. The plate was reversed so that the streak became the origin and developed again. Two separate functions can be achieved using this method. First, broad areas of sample can be converted into hairline streaks; thus, subsequent runs provide better separation. Second, the running length of a plate is multiplied many times, which enables even the most slow-moving fractions to be separated effectively.

Continuous elution was found to be more advantageous than the repeated elution technique. In addition to eliminating time-wasting multiple developments, it required no continuous attention. Furthermore, in repeated elution technique, the plate had to be dried after each step. During each exposure to air and humidity, some components undergo polymerization, which leaves a brown residue.

ISOLATION PROCEDURE

From the complex mixtures, a few components were isolated and purified for structural determination by spectroscopic methods. The procedure used is shown in Fig. 3.

The total dye mixture, obtained as a solid material after filtration, was applied as a band on a 2-mm preparative plate and developed a number of times. After maximum resolution was obtained, single component bands were scraped from the plate and were extracted with methanol at room temperature. This extract was centrifuged to remove silica gel and then filtered to remove any remaining silica. The extracts were evaporated immediately to reduce the extent of "polymerization," and then were purified by spotting on analytical plates. This process was repeated several times. Despite all precautions, single components after 3 elution and recovery cycles consistently showed the same nonmoving residue at the origin, demonstrating the chemical sensitivity of the dyes.

Although column chromatography can be used for the separation of the components in large amounts, trailing after the first few components was more severe than with thin-layer plates. For this reason, column chromatography was useful as a preliminary enrichment of components for subsequent thin-layer separation.

*Shandon Southern Instrument Co., Inc., 515 Broad Str, Sewickley, PA 15143. Cat. #SAB-2852.

Figure 2. Apparatus for continuous elution TLC

Figure 3. Isolation procedure

CONCLUSION

TLC of oxidative dyes is a less than perfect answer for the dye chemist because of problems associated with the chemical composition of the dyes. However, to date, TLC has been the single most effective method for providing pure components for structural determinations. It is the only standard analytical technique able to identify the composition of the already-formed dyes, simply because it indicates colors and the range of mobilities of the different product components whch can form from specific precursors. The chromatographic separation of the oxidative products will make it possible to deduce trends in the pattern of dye developments.

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Wettability of keratin fiber surfaces

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Synopsis

The WETTING of HAIR FIBERS by WATER has been measured using the Wilhelmy balance technique developed at Textile Research Institute Princeton, N. J. specifically for FIBROUS MATERIALS. The data for selected and treated hair samples suggest cause–effect relationships between WATER WETTABILITY of the FIBER SURFACE and MECHANICAL, WEATHERING, and CHEMICAL FIBER DAMAGE. Critical surface tension of the hair fiber surface has been determined with water-butanol mixtures using the same technique. Furthermore, dispersion and nondispersion contributions to the surface free energy of the fiber have been evaluated by measuring wettabilities against a polar and a nonpolar liquid. The results indicate that the molecular processes occurring at the interface between the keratin fiber surface and a liquid have considerable effect on the surface free energy of the fibers.

INTRODUCTION

Human hair is an important member of the keratin fiber group. Keratins are structural proteins, which occur widely in the vertebrate epidermis and its appendages. Unlike man-made fibers, human hair is cellular in structure, consisting of a central core called the cortex covered by a sheath of several layers of flattened cuticle cells. The cuticle cell itself consists of various layers, the endocuticle, exocuticle, and the a-layer, proceeding from the inside to the outside in that order. The thin outermost layer that forms a sheath around the cuticle cell is known as the epicuticle and is hydrophobic, whereas, the cortex is hydrophilic. Although, no definitive information is available about the composition of the epicuticle of hair fibers, King and Bradbury [1] have found that the epicuticle obtained from Merino wool consists of 78 per cent protein, 5 per cent lipid, and 4 per cent ash. Values may be of a similar order for hair. The hydrophobicity of the fiber surface may in part be due to the lipid content of the epicuticle.

The characteristic toughness and the insolubility of keratins in water is attributable to the presence of the sulfur-containing amino acid, cystine, which acts as a crosslinking agent. From the work of Wolfram and Lindemann [2] and Swift and Bews [3], it can be seen that the cuticle of hair contains more cystine than the cortex. Histochemical observations of cuticle cross sections by the latter authors show that most of the cystine is concentrated in the exocuticle and especially in the a-layers. This renders these outer

layers of the cuticle tough and somewhat brittle. Exposure to ultraviolet rays of the sun can degrade the cuticle material, giving rise to hydrophilic groups in the surface. Severe mechanical damage during washing and combing of hair can destroy the cuticle and expose the hydrophilic cortex. It would appear, therefore, that measurement of the water wettability of the fiber surface could provide useful semiquantitative information about the extent of damage undergone by the fiber.

Apart from the kinds of damage described above, various chemical treatments of hair for aesthetic purposes such as bleaching (oxidation) and waving (reduction) also degrade the cuticle by breaking disulfide bonds and generating hydrophilic groups in the surface. Again, changes in the wettability of the hair surface against water should be a good measure of the extent of oxidation or reduction in the surface regions of the fiber. However, it should be noted that such measurements do not give any information about the changes occurring within the bulk of the fiber.

Knowledge of the surface free energy of the fiber will be useful for the formulator of hair-care products, which are applied in the form of sprays, and are expected to spread spontaneously on the surface. The critical surface tension would be a useful measure of the surface free energy of the hair fiber surface. It should be noted, however, that the conventional method of Zisman [4] for determining the critical surface tension of solids, is of limited applicability in that it represents only the dispersion contribution to the surface free energy.

In the work presented here, an attempt has been made to understand the effect of weathering and mechanical damage on the wettability of the fiber surface against water. The same technique has been used to monitor oxidation and reduction reactions at the fiber surface. The role of dispersion and nondispersion contributions to the surface free energy have been evaluated, and it is hoped that this will lead to a better understanding of the processes occurring at hair-liquid interfaces.

THEORETICAL

The spreading of liquids on the surface of a solid is governed by the 3 interfacial tensions, γ_{SV}, γ_{SL}, and γ_{LV}, where the symbols S, L, and V stand for solid, liquid, and vapor, respectively. The relationship between these tensions when a liquid surface is in equilibrium contact with a solid surface is given by the Young-Dupré equation:

$$\gamma_{LV} \cos \theta = \gamma_{SV} - \gamma_{SL} \tag{1}$$

where θ is the contact angle. The term $\gamma_{LV} \cos \theta$ is often referred to as the wettability W of the surface.

Determination of the wettability of a fiber by the Wilhelmy balance principle involves the measurement of the force acting (upward or downward), depending on the contact angle) on a counterbalanced single fiber when contact with the liquid surface is established. Equations relevant to this situation have been developed by Miller and Young [5]. The vertical force acting on the fiber is given by

$$F_w = w - F_b \tag{2}$$

where w is the wetting force, F_w is the electrobalance force reading corrected for force

reading in air, and F_b is the buoyancy force on the fiber. The buoyancy force on the fiber is given by

$$F_b = lad \tag{3}$$

where l equals immersed length of the fiber, a equals area of the fiber cross section, and d equals density of the liquid. Substituting for F_b in equation (2) leads to equation (4):

$$F_w = w - lad \tag{4}$$

Therefore, a plot of F_w as a function of immersion depth l should give a straight line with slope $(-ad)$ and intercept w. Wettability W, defined as the wetting force per unit length of the wetted perimeter, is given by w/P, where P is the perimeter of the fiber, i.e.,

$$W = \frac{w}{P} = \gamma_{LV} \cos \theta \tag{5}$$

The interfacial tension between a solid and a liquid phase in equilibrium is given by

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - A \tag{6}$$

where A is the work of adhesion. Substituting eq. (1) in (6) gives

$$A = \gamma_{LV}(1 + \cos \theta) = \gamma_{LV} + W \tag{7}$$

It should be noted that the work of adhesion is physically more meaningful than either W or the contact angle, since it quantifies solid-liquid interactions in such a way that the attraction between a series of liquids and one or more solids can be compared directly. Therefore, some results have been expressed in terms of the work of adhesion instead of wettability or contact angle.

Equation (7) can be written in logarithmic form

$$\log(1 + \cos \theta) = \log A - \log \gamma_{LV} \tag{8}$$

According to Fowkes [6], if only dispersion interactions are involved, a plot of $\log(1 + \cos \theta)$ for a series of liquids on a given solid versus $\log \gamma_{LV}$ should be linear with a slope of -1, and the intercept on the γ_{LV} axis at log 2 ($\cos \theta = 1$) should give the so-called critical surface tension, a measure of the surface free energy of the solid. Conventional Zisman plots have been found to be nonlinear for the solid-liquid systems used in this work, suggesting that there are contributions from interactions other than dispersion forces.

Therefore, dispersion and polar contributions to surface free energy have been evaluated by the method of Wu [7]. This method uses the "reciprocal means" approach for the dispersion and polar contributions to the work of adhesion A in eq. (7), so that we obtain

$$\gamma_{LV} \cos \theta = -\gamma_{LV} + \frac{4\gamma_S^d \gamma_L^d}{\gamma_S^d + \gamma_L^d} + \frac{4\gamma_S^p \gamma_L^p}{\gamma_S^p + \gamma_L^p} \tag{9}$$

By measuring $\cos \theta$ (or, in our case, $\gamma_{LV} \cos \theta$) for a solid in 2 different liquids, 1 polar and the other nonpolar, 2 simultaneous equations are obtained, which can be solved

for γ_S^d and γ_S^p. Values of γ_L^d and γ_L^p for the 2 liquids must be known (for details see the original reference). Symbols "d" and "p" stand for dispersion and polar contributions, respectively.

EXPERIMENTAL

MATERIALS

European dark brown hair* has been used throughout this work. The hair was cleaned by extraction with methylene chloride followed by exhaustive rinsing with distilled water. All specimens were conditioned at 65 per cent RH and 70°F.

Water used in this work was distilled twice in an all-glass apparatus. For preparing water-butanol solutions analytical reagent grade n-butanol was used. These solutions were prepared according to the method of Shuyten *et al.* [8], and their surface tensions were measured by the standard capillary rise method. Fischer purified reagent grade methylene iodide was redistilled under vacuum (38°C, ~2 mm Hg). Hydrogen peroxide† for oxidation was electronic grade. Dithiothreitol used in reduction was obtained from Calbiochem.‡

WETTING FORCE MEASUREMENTS

The apparatus for the wetting force measurements has been described elsewhere [5]. It consists of an electrobalance and a microscope stage for raising and lowering the liquid level. The fiber is glued to a small wire hook mounted on the beam of the balance and counterbalanced in air. The liquid level is raised to immerse the fiber 1 mm at a time up to an immersed length of 5 mm. The fiber is then allowed to remain immersed in the liquid for ~15 min during which time the force on the electrobalance reaches a constant level. Advancing wetting forces at immersion depths of 1–5 mm and the force after it has reached a constant level are read from the chart. All measurements were made in an environment of 65 per cent RH and 70°F.

Perimeters of the fibers were determined microscopically. Values of the perimeters calculated from the major and minor axes of the elliptical cross section have been found to agree with the actual measured values within ~3 per cent.

Electron micrographs were made with a scanning electron microscope.** Fiber specimens were coated with gold prior to microscopic examination.

RESULTS AND DISCUSSION

WETTING BEHAVIOR OF HAIR-WATER SYSTEM

The recorder trace obtained in a typical measurement of wetting force is shown in Fig. 1. At the first contact with the water surface, an upward (negative) force is experienced

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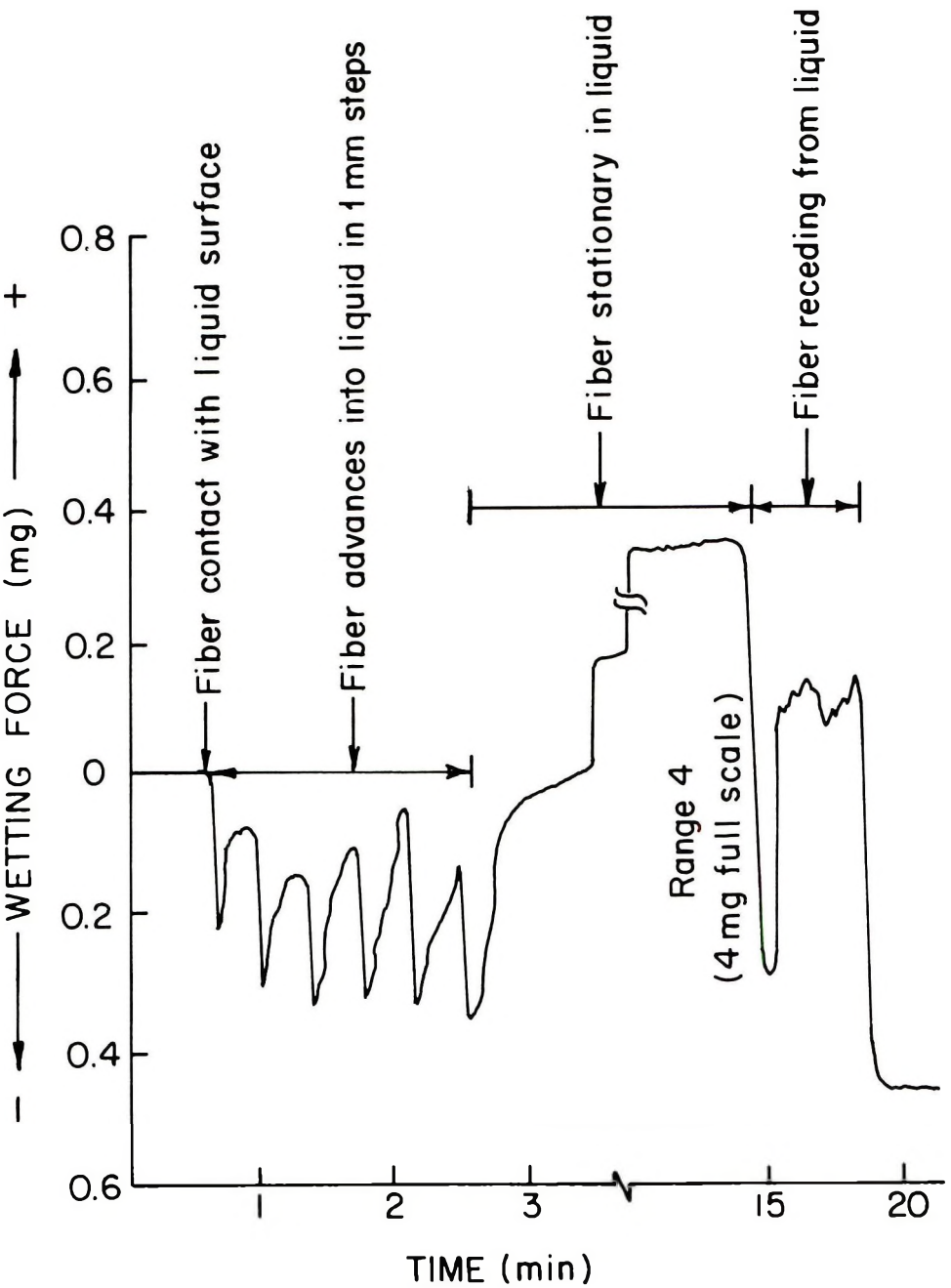


Figure 1. Wetting force curve (advancing and receding) for human hair fiber in water

by the fiber which quickly reduces with time. On further immersion of the fiber, the upward force on the fiber is reinstated (in fact it is slightly higher due to the additional buoyant force on the fiber). This pattern is observed during the immersion of each millimeter of the fiber up to a depth of 5 mm. When the fiber is left in the water, the upward force slowly decreases, and in most cases, the direction of the force changes to the downward (positive) direction and reaches an approximately constant value. This value is referred to as the "equilibrium" wetting force w_e. All the results pertaining to the so-called "equilibrium" condition are designated by the subscript 'e'. The advancing wetting force is calculated from the 5 force readings obtained during the immersion of millimeter lengths of the fiber by linear regression analysis using eq. (4). The intercept is the advancing wetting force w_a. Results pertaining to this condition are designated by the subscript 'a'.

The time-dependent change in the wetting force toward more positive values cannot be attributed to absorption of water into the fiber, because the amount absorbed is much too small to account for the difference between w_a and w_e. To understand the time dependence of the wetting behavior of hair fibers illustrated in Fig. 1, glass, nylon, and polypropylene fibers were examined [9], and qualitatively all of them exhibited a similar behavior, i.e., $w_e > w_a$. In the case of glass and nylon fibers, both w_e and w_a were positive, whereas, in the case of polypropylene both were negative. In the case of untreated hair, w_e was positive in most cases and w_a was negative. Even a platinum wire exhibited the same behavior. However, the difference between the two wetting forces ($w_e - w_a$) was much larger for hair and nylon fibers than for glass and polypropylene fibers and the platinum wire. This would suggest that the time-dependent increase in the wetting force occurring when the fiber is left in the water at a depth of 5 mm may at least partly be due to interaction of the liquid with the surface molecules of the solid; in the case of hair and nylon, hydrogen bond breaking can occur. It is possible that the lower wetting force w_a observed when a new interface is established between the solid and the liquid, is partly due to the high advancing velocity (~15 mm/min) and partly due to the lowering of the surface energy of the solid as a result of adsorption of molecules of water vapor during conditioning at 65 per cent RH. When such a conditioned solid is brought into contact with the liquid surface, adsorbed liquid molecules reorient at the solid-liquid interface reducing γ_{SL}, thus increasing the wettability of the solid surface. This phenomena has been observed by Shafrin and Zisman [10] in the case of glass at various relative humidities. In the case of polymers that interact with the liquid, however, a further increase in wettability would be caused by relaxation of polymer molecules in the surface regions and orientation of polar groups toward the liquid and nonpolar groups away from the liquid. This situation is strongly indicated in the wetting behavior of hair fiber against water.

EFFECT OF WEATHERING AND MECHANICAL DAMAGE

In the case of long hair fibers, the extent of weathering and mechanical damage is likely to be greater near the tip than near the root. Therefore, a 10-in. long hair fiber was cut into 6 sections and the wettability of each section was determined by immersing the end closest to the tip. The advancing and "equilibrium" contact angles calculated from measured wetting forces are reported in Table I. In this Table, A is the tip section (may not be the natural tip) and F is the root section. As can be seen, the tip end is more hy-

Table I
Contact Angles (Calculated) of Water Against Hair Measured Along the Length of the Fiber

Fiber section	θ_a(degrees)	θ_r(degrees)
A	72	64
B	73	68
C	67	68
D	99	81
E	101	76
F	103	89

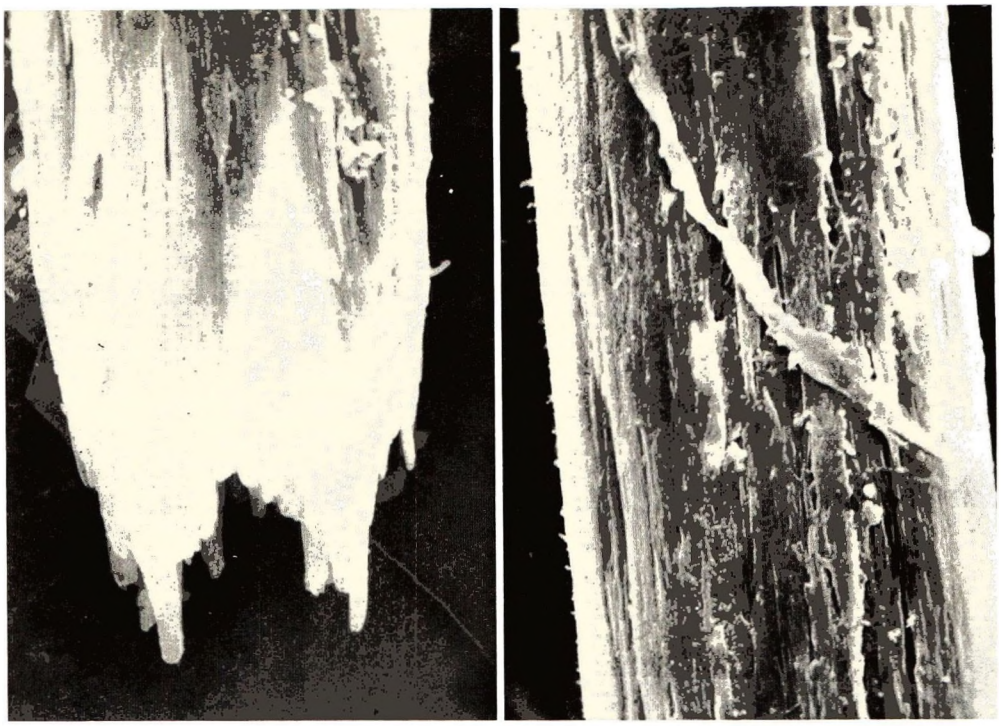
drophilic than the root end. This may be due to degradation of the protein by the ultraviolet rays of the sun or by environmental factors that generate hydrophilic groups at the surface, or it may be due to loss of cuticle by mechanical damage.

To establish the difference in the wetting behavior of the cortex and the cuticle, wettability of the tip (natural tips were chosen by viewing in the microscope) and the root ends of hair fibers were measured. Damaged tips with the cortex exposed yielded positive wetting forces, while the root ends, with cuticle intact, gave negative wetting forces. Scanning electron micrographs of one of these fibers are shown in Fig. 2 along with the measured advancing wetting forces.

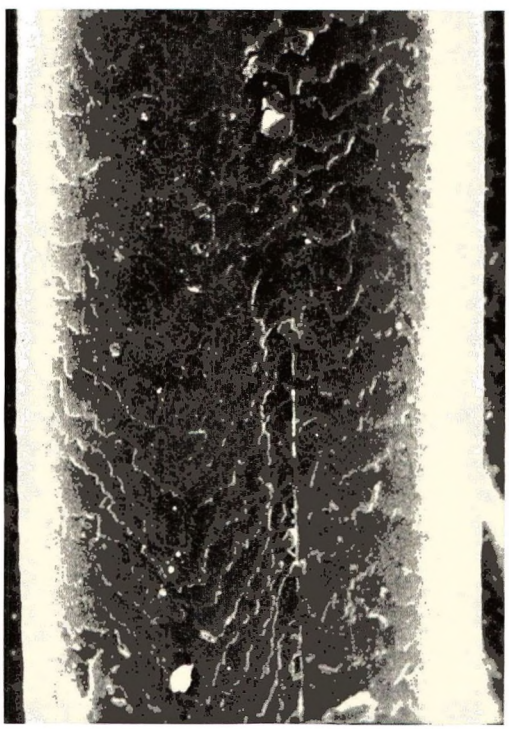
EFFECT OF CHEMICAL OXIDATION AND REDUCTION

Oxidation of hair samples was carried out with a 3 per cent solution of hydrogen peroxide adjusted to a pH of ~10 with 0.1 *N* ammonium hydroxide. Single fibers mounted on hooks were immersed in this solution for 2 min at a time followed by exhaustive rinsing with distilled water. Fibers were conditioned at 65 per cent RH and 70°F prior to measurement. Successive treatments of 2-min duration were carried out on the same fibers. Reduction was carried out in the same way using a 2.5×10^{-2} *M* solution of dithiothreitol, but the fibers were rinsed with deoxygenated distilled water.

The increase in wetting expressed as work of adhesion (see equation (7)) caused by both oxidation and reduction are shown in Fig. 3 as a function of treatment time. As expected, both oxidation and reduction increase the wettability of the surface. These increases are attributed to the generation of sulfonic acid groups in the case of oxidation and of thiol groups in the case of reduction, both of which are hydrophilic. The data presented here are inadequate for a comparison of the hydrophilic nature of the scission products and for the determination of the extent of disulfide cleavage in these 2 reactions. Wettability or work of adhesion are only indirect means of assessing the quantitative effects of these reactions. Oxidation of cystine by hydrogen peroxide is complicated by the reversible nature of several intermediate steps eventually leading to the formation of cysteic acid [11]. It is possible that under these conditions of oxidation cleavage of peptide linkages may also occur to some extent, which may account for the discontinuous nature of the "oxidation" curve in Fig. 3 after a 6-min oxidation time. Reaction of hydrogen peroxide with peptide bonds is known to occur in wool and silk at 60°C [12], and, though the reaction may not be extensive at room temperature, it may not be ruled out. It should be noted that wettability or work of adhesion is a measure of reactions occurring at the fiber surface only.



(A) (B)



(C)

Figure 2. SEMs of tip and root ends of an untreated human hair fiber: (A) tip end, 640 X, $F_W = 0.041$ mg; (B) ~0.5 mm from tip, 640 X; (C) toward the root end, 640 X, $F_W = 0.330$ mg

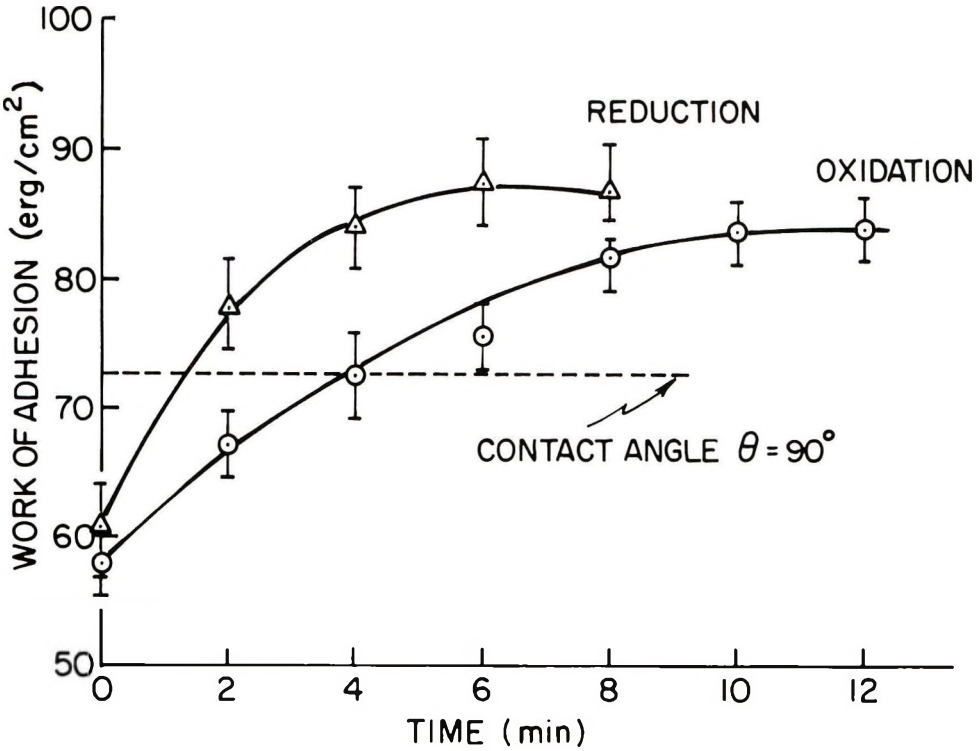


Figure 3. Effect of oxidation and reduction treatments on human hair fiber wettability

CRITICAL SURFACE TENSION OF HAIR

The determination of the critical surface tension of keratins in various forms using alcohol-water solutions has been achieved by several authors. A summary of these investigations has been given by El-Shimi and Goddard [13].

The method employed in this investigation of using n-butanol-water solutions is based on the work of Feldtman and McPhee [14] on wool. n-Butanol-water solutions covering a surface tension range from 37 to 72.5 dyn/cm were used. In earlier measurements, the direction of immersion into the liquid with respect to hair scales was ignored. Later, wettability was found to depend on the scale direction of immersion [9], and therefore, measurements were carried out in both scale directions. Both advancing and equilibrium wetting forces were utilized to evaluate the corresponding values of cos θ. A plot of cos θ_a versus γ_{LV} according to the Zisman approach [4], was found to be nonlinear, suggesting contributions from nondispersion interactions. A logarithmic plot of $(1 + \cos \theta)$ versus γ_{LV} according to equation (8) is shown in Fig. 4 for a single hair fiber. As expected, the plots for the advancing and the equilibrium measurements are both linear. The slope of the line and the value of γ_c (critical surface tension) at $(1 + \cos \theta)$ equals 2, i.e., at a contact angle of 0°, were calculated for each of 5 fibers by linear regression analysis, and the average values are reported in Table II. The γ_{ca} values are close to the γ_c of pure bulk water with respect to nonpolar liquids, which has been found to be ~22 dyn/cm [6]. This supports the idea that conditioning the hair fibers at 65 per cent RH might have led to the adsorption of a multimolecular

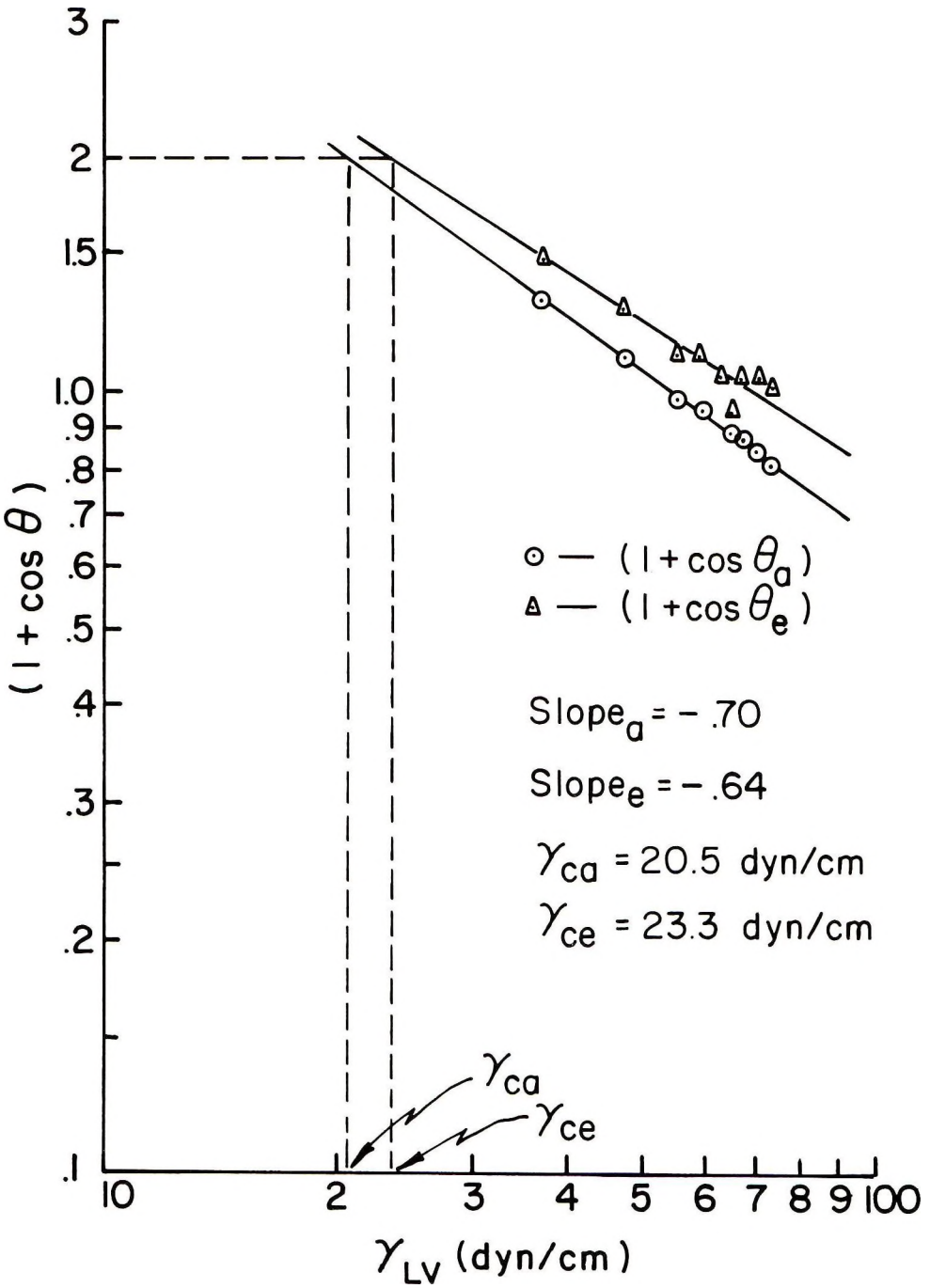


Figure 4. Variation of $(1 + \cos\theta)$ with γ_{LV} for 1 human hair fiber measured in against-scale direction

Table II
Critical Surface Tension of Hair Fibers

Scale direction	Advancing		"Equilibrium"	
	Slope	γ_{ca} (dyn/cm)	Slope	γ_{ce} (dyn/cm)
Ignored	-0.72 ± 0.03	19.4 ± 1.0	-0.67 ± 0.16	24.8 ± 3.9
Against scale	-0.75 ± 0.09	20.9 ± 2.4	-0.68 ± 0.11	26.0 ± 6.9
With scale	-0.78 ± 0.10	20.0 ± 2.0	-0.70 ± 0.11	24.9 ± 3.0

Note: Each entry is an interfiber average for 5 fibers reported at 95 per cent confidence level.

layer of water molecules, thus reducing the surface free energy of the keratin surface. The values of γ_{ce} obtained in this work are close to those reported by Alter and Cook [15], i.e., ~26 dyn/cm, although the variability is high. The measurement of γ_c using alcohol-water solutions is known to give a value of ~26 dyn/cm irrespective of the solid surface. This is attributed to the adsorption of alcohol molecules on the solid with the hydrocarbon chain oriented towards the liquid, so that all surfaces behave like hydrocarbon surfaces. Thus, the difference between γ_{ca} and γ_{ce} in our work may be due to the replacement of adsorbed water molecules by butanol molecules at the interface.

The observation that the slopes in Fig. 4 are greater than -1 (see also average values in Table II) suggests contributions from nondispersion interactions. According to Dann [16], polar interactions make a significant contribution to γ_c. Therefore, the γ_c values in Table II cannot represent the total surface free energy of the hair keratin. Alternative methods capable of evaluating both dispersion and nondispersion contributions to the surface free energy have to be used.

DISPERSION AND NONDISPERSION CONTRIBUTIONS TO γ_c OF HAIR

As has been mentioned earlier, such a method is based on the evaluation of cos θ in 2 different liquids, one polar, i.e., water (γ_L^d = 22.0 dyn/cm, γ_L^p = 50.5 dyn/cm), and the other nonpolar, i.e., methylene iodide (γ_L^d = 44.1 dyn/cm, γ_L^p = 6.7 dyn/cm). The values of cos θ, γ_{LV}, γ_L^d, and γ_L^p for each liquid is substituted in equation (9), resulting in 2 simultaneous equations with the unknowns, γ_s^d and γ_s^p (for details see El-Shimi and Goddard [13]). The equations can be solved graphically to obtain the values of the unknowns.

Such equations were obtained for advancing and "equilibrium" conditions by using the corresponding values of cos θ. The values of γ_s^d and γ_s^p obtained for both the above

Table III
Dispersion and Nondispersion Contributions to γ_c of Hair Fibers (dyn/cm)

Scale Direction	Advancing			"Equilibrium"		
	γ_s^d	γ_s^p	$\gamma_s^d + \gamma_s^p$	γ_s^d	γ_s^p	$\gamma_s^s + \gamma_s^p$
Against scale	24.8 ± 2.2	2.6 ± 1.3	26.8 ± 1.4	19.5 ± 1.9	11.5 ± 1.7	31.0 ± 1.6
With scale	23.9 ± 2.2	2.5 ± 1.5	26.5 ± 1.0	19.5 ± 2.4	10.0 ± 2.0	29.6 ± 2.2

Note: Each entry is an interfiber average for 10 fibers reported at 95 per cent confidence limit.

conditions are given in Table III. The nondispersion or polar contribution γ_s^p in the advancing mode is small compared to the dispersion contribution γ_s^d. In the "equilibrium" condition, when the fiber is left in the liquid for ~15 min, it is seen that γ_s^p increases considerably and γ_s^d decreases slightly, thus leading to a net increase in the total surface free energy. The increase seems to be due to the interaction of hair and water leading to hydrogen bond breaking and consequent orientation of the macromolecules in the surface regions, as was suggested earlier.

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Studies of water-in-oil (w/o) emulsion stabilized with amino acids or their salts

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Synopsis

WATER-IN-OIL (W/O) EMULSIONS STABILIZED by using gels formed between surfactants and aqueous solutions of AMINO ACIDS were studied. The gel can only be obtained with a fluid surfactant which has lipophilic properties and a specific orderly lamellar structure and amino acids or their salts which are readily soluble in water.

By dispersing these gels into the oil phase and then adding the water phase, extremely stable w/o emulsions with wide ranges of water content were obtained. This type of emulsification was termed the "gel-emulsification method" by the authors. When this new technology was applied to the preparation of cosmetics, products with outstanding characteristics were obtained.

The function of the amino acids in the emulsification were investigated by using physico-chemical methods such as X-ray analysis, nuclear magnetic resonance (NMR), heat of solution, electron microscopy (EM), and measurement of the water content solubilized in the surfactant phase. It may be concluded that the amino acids are effective in forming a tight surface atmosphere around the water particles and in preventing coalescence of water particles by strong hydration effect of the amino acids, thus stabilizing the w/o emulsion.

INTRODUCTION

Generally, w/o emulsions are said to be much more advantageous to human skin than an oil-in-water (o/w) emulsion. Gattefosse et al. (1,2) described the mechanism of application of w/o emulsions to the skin as follows.

The continuous fatty layer, in which minute droplets of water are distributed, is in contact with the epidermis and facilitates adhesion. After the water evaporates, the residual fatty phase of the emulsion on the skin is elastic and resistive, protecting the deeper layers of skin from dehydration and exaggerated hydration. Furthermore, other scientists (3) have also dealt on the properties of w/o emulsions of spreading well onto stratum corneum and aid in the prevention of chemical and natural attacks thereon, retarding moisture loss, which in turn helps to maintain flexibility.

Clar (4) has recently published results on skin impedance measurement that, in spite of the variation in the moisture of the atmosphere, when the w/o cream is applied, the moisture of the skin is preserved for some time.

However, oil-in-water (o/w) type emulsions have better consumer acceptance than the w/o type emulsion, despite the various benefits of the latter to the skin. This can be attributed to the difficulties of maintaining the stability of the w/o emulsion as well as the inferior feel during application. Generally, w/o emulsions are prepared by increasing the ratio as well as the viscosity of the outer phase (oil) in order to improve stability. This results in a product with a transparent and glaring appearance and with a greasy and oily feeling, which will not readily gain consumers' acceptance.

It is of great interest for cosmetic scientists to try to eliminate such defects from w/o emulsion (for example, the excessive addition of water caused separation (60 to 70 per cent)). The addition of oil-soluble polyvalent metallic soaps increased the stability of the w/o emulsions to some extent, but hardly altered the application defects.

From the above facts, the authors carried out a series of experiments to obtain w/o emulsions, which were designed to hold wide ranges of water ratio, a nongreasy feel, and still have good stability. As a result, it was possible to develop a new emulsification method, which the authors termed as the "gel-emulsification method."

The main points of this method are described as follows: By mixing an aqueous solution of amino acids or their salts with lipophilic surfactants having specific requirements in their chemical structure, a kind of gel, consisting of the surfactant in the continuous phase and an aqueous solution of amino acids or their salts in the dispersed phase, could be formed. In the following emulsification step, the gel was dispersed into the oil phase, and then the water phase was added into the mixture and emulsified. It was possible to obtain a stable w/o emulsion and/or cream having excellent characteristics with a wide range of water content.

The major characteristics of the creams obtained by this method were their excellent affinity and nongreasy feel to the skin which has never been achieved before with a w/o emulsion. Moreover, the surfactants such as the monoglycerides used in the creams prepared by this gel-emulsification method are highly safe materials found widely in nature and lipids. Furthermore, the amino acids used in this investigation are also found to be in the natural moisturizing factor (NMF) of the skin and safe enough to be used as food fortifiers for human nutritional purposes.

From this viewpoint, the creams obtained by the new method have great advantages over existing formulations, since they have been prepared from ingredients which have been proven to be physiologically safe for human beings to use.

The research findings will be discussed in 3 parts. First, it will be necessary to clarify the necessary requirements in the relationship between surfactants and amino acids in order to form the gels (which is characteristic in the new technology). Secondly, the details of the gel emulsification method, in which the gel is dispersed into the oil phase and water is added, will be discussed together with its characteristics. Finally, the examples of the practical application of the new technology to actual cosmetic formulations and their characteristics will also be explained. In addition, the similarity existing between the phenomena obtained in connection with amino acids and surfactants, and the spontaneous emulsifying phenomena on the skin will also be discussed to some extent.

EXPERIMENTAL

GEL FORMATION OBTAINED BETWEEN SURFACTANTS AND AMINO ACIDS

Materials: Most of the surfactants used in this study were commercially available. For example, Sunsoft® O-30B* (glycerol monooleate) was used as a standard surfactant for many of the experiments. Whenever necessary, those synthesized in the usual way or those fractionated by molecular distillation were used. The amino acids and their salts were of special reagent grade. Other reagents used were also of the same grade. Distilled water was used throughout the study.

METHOD

a. *Gel Formation:* Surfactants and amino acids or their salts, which were possible to form the gels, were classified by the following simple method. The surfactant was added to an aqueous solution of the amino acid or its salt at room temperature and stirred with a laboratory mixer. A gel was formed as shown in Fig. 1.

All of the gels obtained by this method were observed as to their electrical conductivity and their stability in hot water. Figure 2 illustrates this property and only those without electrical conductivity and insoluble in water were selected for further study.

b. *Other Measurements:* In order to investigate the various functions of the gels, the chemical structure of the surfactants, the structure and properties of the gels obtained, and the effects of amino acids, etc., were examined by the following methods:

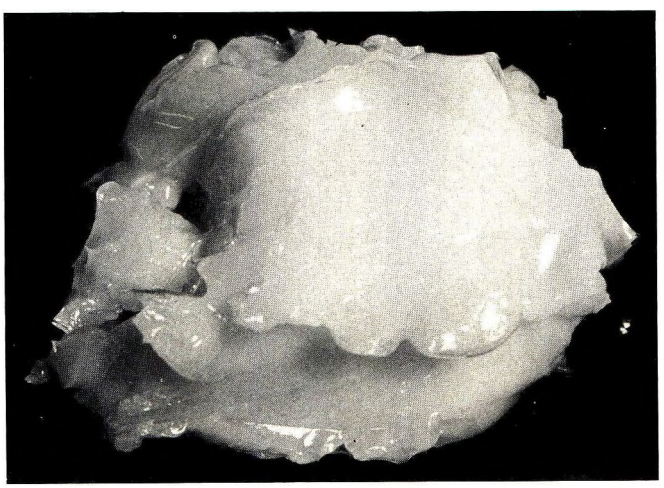

Figure 1. Example of gels prepared between surfactants and aqueous solution of amino acids

*Taiyo Kagaku Co., Ltd., 62 Akahori, Yokkaichi, Mie, Japan.

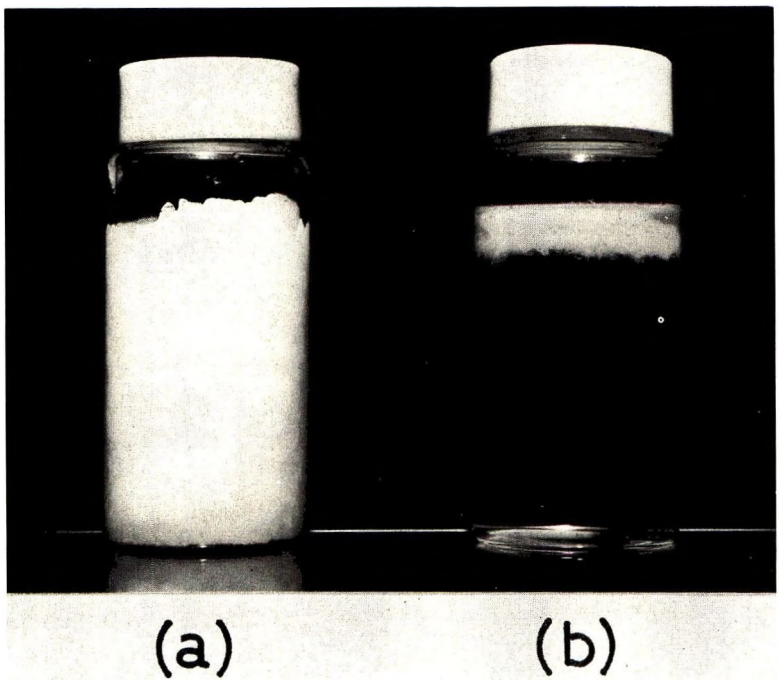

Figure 2. Stability of gels in hot water: (a) stable gel; (b) unstable gel

(1) *X-Ray Analysis:* Two types of X-ray diffractometers, Rotaflex* and JRX-12VA† were used to elucidate the structures of the surfactants and gels by means of small angle scanning and camera method at 25°C; Target; Cu.

(2) *Differential Thermal Analysis (DTA):* The gel sealed in aluminum cell was measured at 0 to 70°C, raising the temperature 2.5°C/min by a scanning type DSC.*

(3) *NMR:* Variation of chemical shift with proton in water of the aqueous solution to which various solutes were added, was measured by Hitachi‡ R-20 type NMR at 34°C.

(4) *Phase Inversion Temperature (PIT):* Influence of the addition of amino acids on the PIT was measured with Squalane—Beeswax—POE(6) oleyl alcohol ether 5 per cent (w/w)—water system (volume ratio = 0.6).

(5) *Heat of Solution:* A sealed ampule containing about 2 g of the surfactant, Sunsoft O-30B, was broken in 50 g aqueous solution of amino acid having various concentrations. Evolution of heat on mixing (cal/g) was measured by a twin type microcalorimeter** at 35°C.

(6) *Water Content Migration to the Surfactant Phase:* The amount of water migrating into the surfactant (Sunsoft O-30B) through an interface of the surfactant and an

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†Japan Electron Optics Laboratory, 1418, Nakagami, Akishimashi, Tokyo, Japan.
‡2 Shibanishikubo, Minatoku, Tokyo, Japan.
**Applied Electric Laboratory, 4-672 Sekimachi, Nerimaku, Tokyo, Japan.

aqueous solution of the amino acids was measured by a MK-A type Karl Fischer apparatus* at 35°C; measuring point: 1 cm above the interface (constant).

(7) *Optical Microscopy:* Emulsion particles were observed by means of phase-contrast and polarizing microscopies; magnification; 400×.

(8) *Electron Microscopy:* Specimens were exposed to the saturated osmium tetroxide atmosphere for 72 h at 4°C, then dehydrated with ethanol and finally embedded in epoxy resin Epon 812. The specimens were sectioned at 500 Å thickness by a LKB III type ultramicrotome equipped with a diamond knife and observed under the Hitachi HU-12A EM.

(c). *Inorganic–Organic Property Balance (IOB):* The authors make reference to this concept in order to correlate the results of the experiment. Fujita (5) proposed the idea of the inorganic–organic property as a tool for predicting the various properties of organic substances. From the physical properties, such as boiling point, refractive index, etc., he gave an empirically specific number to each inorganic and organic property which corresponded to each functional group. Those surfactants capable of forming gels with an aqueous solution of amino acids or their salts are shown in Table I. Table II

Table I
Classification of the Surfactants Applicable to Gel Formation

Surfactant		Appearance		X-Ray Diffraction	Spacing (Å)		
Trade Name	Common Name	RT	IOB	Pattern	d_1	d_2	d_2/d_1
Sunsoft O-30B[a]	Glycerol monooleate	L	0.39	C	33.8	70.7	2.09
Arlacel 186[b]	Glycerol monooleate	L	0.47	C	33.8	73.9	2.18
G-EIS	Glycerol monoisostearate	L	0.42	C	33.8	70.7	2.09
POEM O-72-D[c]	Diglycerol dioleate	L	0.66	C	32.3	67.7	2.10
DIG-EIS[d]	Diglycerol diisostearate	L	0.47	C	33.0	67.7	2.05
PE-EIS	Pentaerythritol diisostearate	L	0.53	C	31.5	67.7	2.15
Arlacel 83[b]	Sorbitan sesquioleate	L	0.63	C	34.3	73.9	2.15
Emalex[e] EG2854-ol	POE (2.4) sorbitol tetraoleate	L	0.49	C	33.8	70.7	2.09

L: Liquid; S: Solid, C: Clear; I: Indistinct; N: No peak.
[a]Taiyo Kagaku Co., Ltd. (62 Akahori, Yokkaichi, Mie, Japan).
[b]Kao Atlas Co., Ltd. (1-1 Kayaba, Nihonbashi, Chuoku, Tokyo, Japan).
[c]Riken Vitamin Oil Co., Ltd. (3-8-10 Nishikanda, Chiyodaku, Tokyo, Japan).
[d]Matsumoto Trading Co., Ltd. (3-1 Nihonbashihoncho, Chuoku, Tokyo, Japan).
[e]5 Nihon Emulsion Co., Ltd. (5-32-7 Minami, Koenji, Suginamiku, Tokyo, Japan).
[f]Surfactants synthesized by the authors.

*Kyoto Denki, 68 Kisyoinshinden, Minamiku, Kyoto, Japan.

Table II
Classification of the Surfactant Lacking the Function of Gel Formation

Surfactant		Appearance		X-Ray Diffraction	Spacing (Å)		
Trade Name	Common Name	RT	IOB	Pattern	d_1	d_2	d_2/d_1
G-di-nC_8[f]	Glycerol dioctanoate	L	0.58	I	24.2	(−)	(−)
G-tri-nC_8[f]	Glycerol trioctanoate	L	0.33	I	28.0	(−)	(−)
G-mono-brC_8[f]	Glycerol mono 2-ethylhexanoate	L	1.24	I	28.5	(−)	(−)
G-di-brC_8[f]	Glycerol di-2-ethylhexanoate	L	0.61	N	—	—	—
G-tri-brC_8[f]	Glycerol tri-2-ethylhexanoate	L	0.35	N	—	—	—
DIG-MO[d]	Diglycerol monooleate	L	0.80	I	38.2	86.6	2.27
DIG-TRO[d]	Diglycerol trioleate	L	0.26	I	29.3	(−)	(−)
DIG-TEO[d]	Diglycerol tetraoleate	L	0.17	I	27.7	(−)	(−)
TENOS®[a]	Glycerol monostearate	S	0.64	C	58.7	(−)	(−)
SPAN® 85[b]	Sorbitan trioleate	L	0.31	C	29.6	59.6	2.01
Nikkol®[g] GM-18IS	Batylalcohol monoisostearate	S	0.23	C	28.0	44.5	1.59
Hostaphat®[h] KO-300	Trioleyl phosphate	L	0.23	I	32.2	(−)	(−)
Emalex®[e] EG-O	Ethyleneglycol monooleate	L	0.40	I	30.5	(−)	(−)
EG-OPG-O[e]	Propyleneglycol monooleate	L	0.38	I	27.4	(−)	(−)
EG-O 300 dio[e]	Propyleneglycol 300 dioleate	L	0.54	I	(−)	(−)	(−)
EG-O 503[e]	POE(3)oleylethel	L	0.57	N	—	—	—
Nikkol®[g] MYO-2	POE(2)oleate	L	0.53	N	—	—	—
POEM®[c] O-105	POE(5)glycerol monooleate	L	1.03	N	—	—	—

[a]Taiyo Kagaku Co., Ltd. (62 Akahori, Yokkaichi, Mie, Japan).
[b]Kao Atlas Co., Ltd. (1-1 Kayaba, Nihonbashi, Chuoku, Tokyo, Japan).
[c]Riken Vitamin Oil Co., Ltd. (3-8-10 Nishikanda, Chiyodaku, Tokyo, Japan).
[d]Matsumoto Trading Co., Ltd. (3-1 Nihonbashihoncho, Chuoku, Tokyo, Japan).
[e]5 Nihon Emulsion Co., Ltd. (5-32-7 Minami, Koenji, Suginamiku, Tokyo, Japan).
[f]Nippon Fine Chemical Co., Ltd. (4-4-26 Honzanminami, Higashinodaku, Kobe, Japan).
[g]Nikko Chemicals Co., Ltd. (1-4-8 Bakurocho, Nihonbashi, Chuoku, Tokyo, Japan).
[h]Hoechst Dyestuffs & Chemicals Co., Ltd. (Frankfurt, Germany).

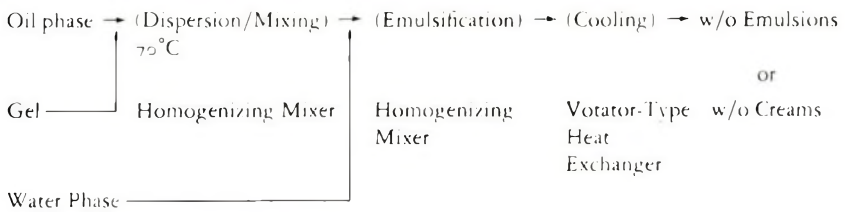
indicates those surfactants which did not produce a suitable gel. The various physical properties of the gel which were evaluated are shown in Figs. 3 to 17 and Tables III to IV.

GEL EMULSIFICATION METHOD

Materials: A standard gel was formulated with Sunsoft O-30B and an aqueous solution of monosodium L-glutamate monohydrate. Other surfactants used are shown in Table I. Squalane (special reagent grade) was used as the oil phase. For the formulation of the creams, materials readily available commercially were used.

METHOD

When the various premade gels were dispersed in the oil phase and emulsified by adding the water phase, excellent w/o emulsions were obtained. The emulsification method may be schematically shown as follows:



In order to compare the properties of these w/o emulsions and/or creams with those of the gels initially used, the following measurements were carried out.

a. *Hardness:* Hardness of the creams was measured at 25°C using a Curd Tension Meter.* The diameter of the needle was 8 mm and the load was 200 g.

b. *Viscosity:* Viscosity of the samples was measured using a B-type viscometer (at 30°C) and a Ferranti–Shirley cone and plate viscometer† (at 25°C, upper viscosity at the maximum rpm of 100 and a sweep time of 10 sec using M-cone).

c. *Emulsion Particles:* These were determined in a manner previously described.

d. *Stability:* The stability of the gels and w/o emulsions (or creams) stored for a month at 0°C, 25°C, 37°C was observed. These results are shown in Tables V and VI and Figs. 18 to 20.

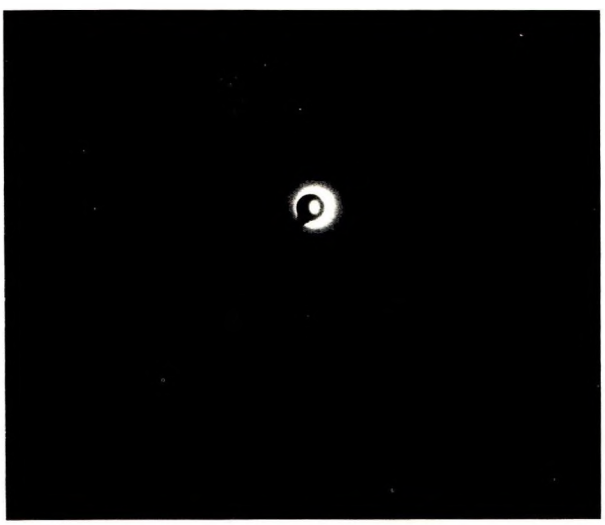


Figure 3. Typical small-angle diffraction pattern of surfactant, Sunsoft O-30B

*Iio Denki, 2-27 Yoyogi, Shibuyaku, Tokyo, Japan.
†Ferranti Ltd., Maston, Manchester 10.

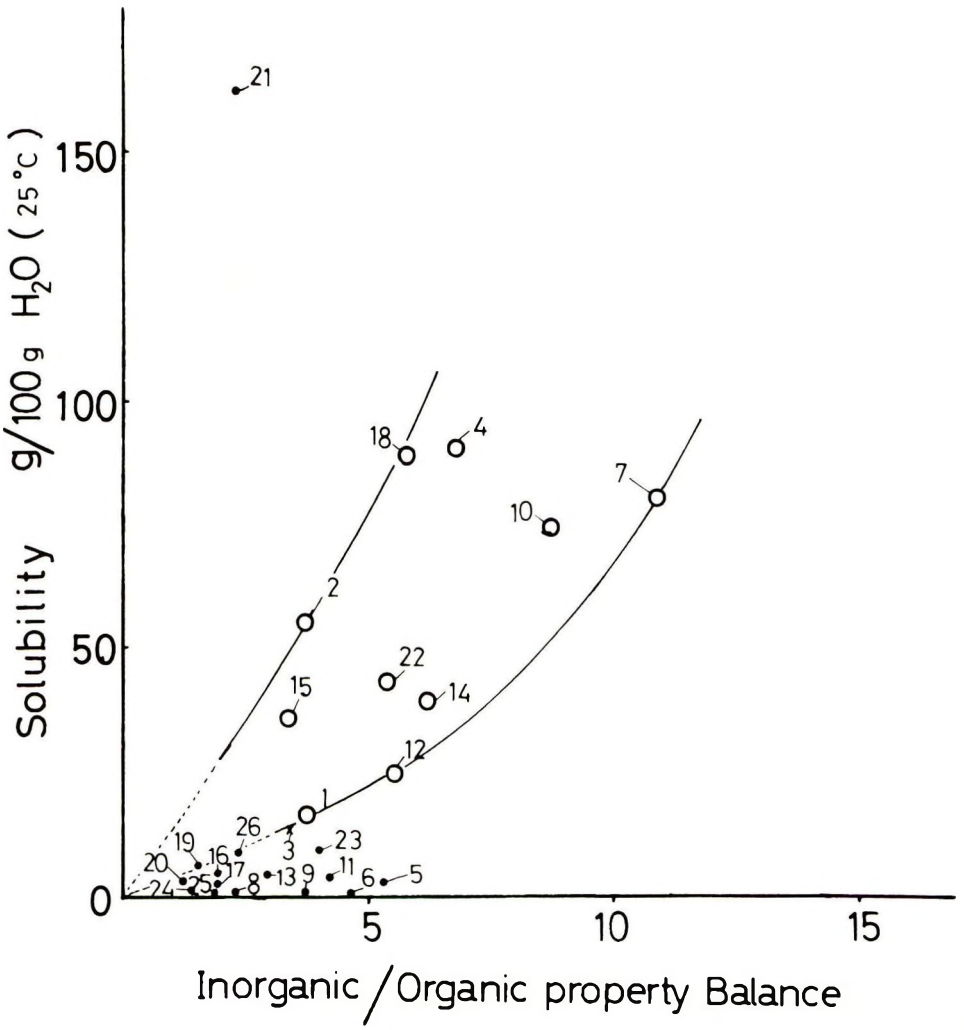


Figure 4. Relationship between solubility of amino acids or their salts and their inorganic/organic property balance

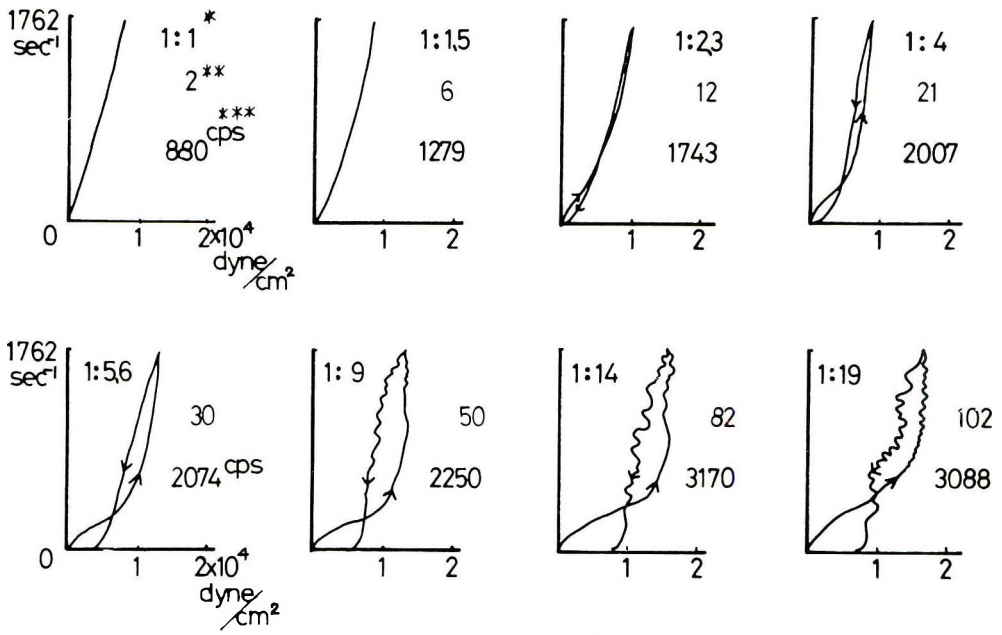


Figure 5. Rheological properties of gels obtained by combination of Sunsoft O-30B and 10 per cent aqueous solution of glycine: (*) Mixing ratio, (**) hardness (by curd tension meter), and (***) upper viscosity (by Ferranti-Shirley viscometer)

DISCUSSION OF RESULTS

The gels cannot be formed using any combination of surfactants and amino acids. To make the gelation possible, certain requirements in both surfactants and amino acids must not be neglected. Among the many surfactants tested, those capable of forming gels with aqueous solution of amino acids or their salts have been shown in Table I. It became evident from the results of the experiments, that the requirement common to the surfactants capable of forming gels were as follows.

1. Fatty acid partial esters of polyhydric alcohol having at least 3 hydroxyl groups in 1 molecule;
2. inorganic-organic property balance (IOB) of the molecules were within the range of about 0.4 to 0.7;
3. the carbon number of the esterified fatty acids was within 16 to 18; and
4. must be liquid at room temperature.

As is evident from Table II, no gels were formed with the surfactants which do not meet the above requirements, such as glycol esters, low fatty acid glycerides, surfactants that are solid at room temperature, higher alcohol ethers, higher fatty acid esters, and ethylene oxide adducts.

Polyhydric alcohols, which can be the main core of the gel forming surfactants were known from other experiments to include glycerin, diglycerin, trimethyl ethane, trimethylol propane, pentaerythritol, sorbitan, and sorbitol. On the other hand, the fatty acid part of the surfactants includes, for example, oleic acid, isostearic acid, ricinolic

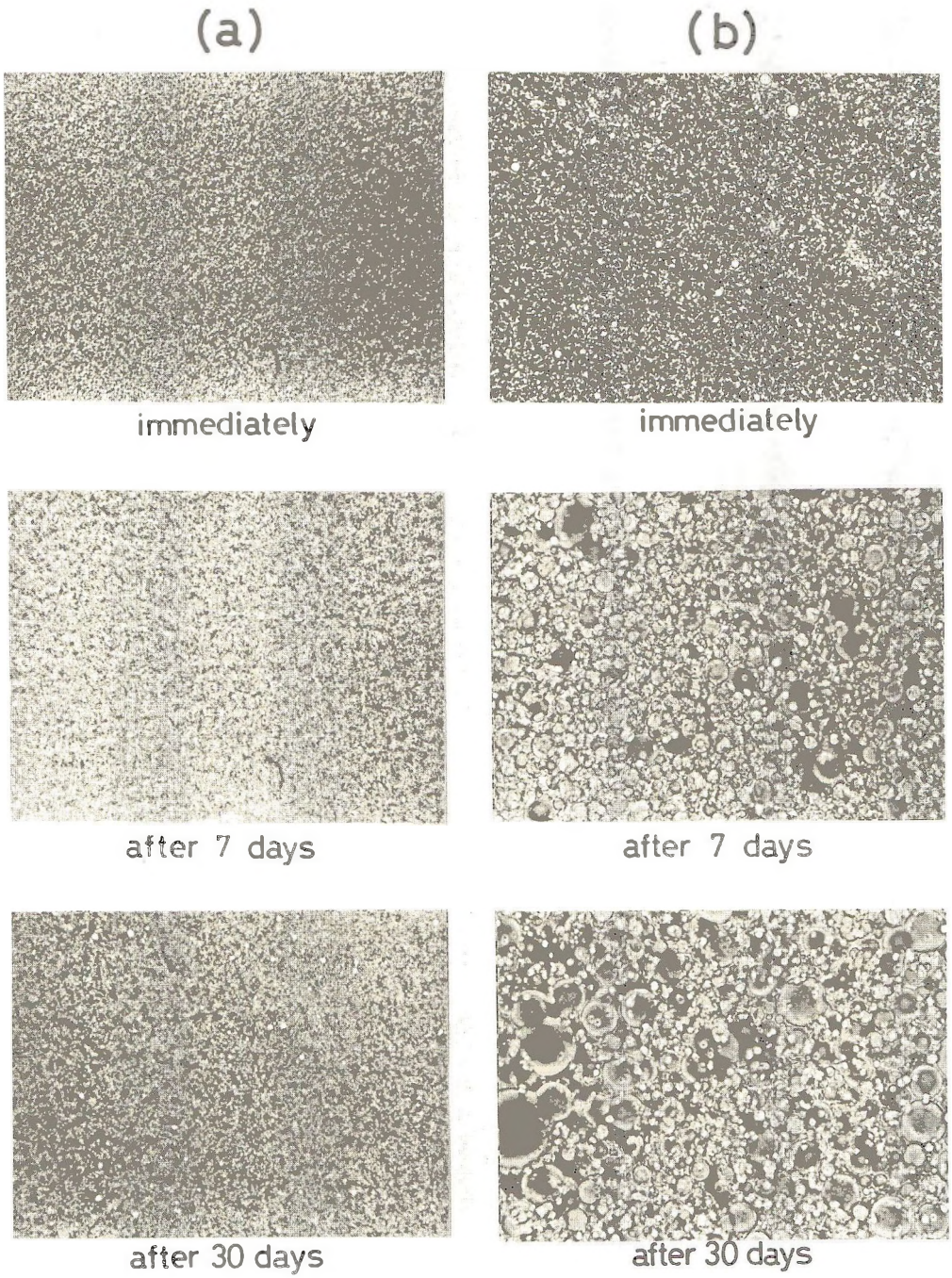


Figure 6. Change of water particles in gels with time at 25°C: (a) Sunsoft O-30B and 40 per cent aqueous solution of monosodium L-glutamate monohydrate system; (b) Sunsoft O-30B and water system

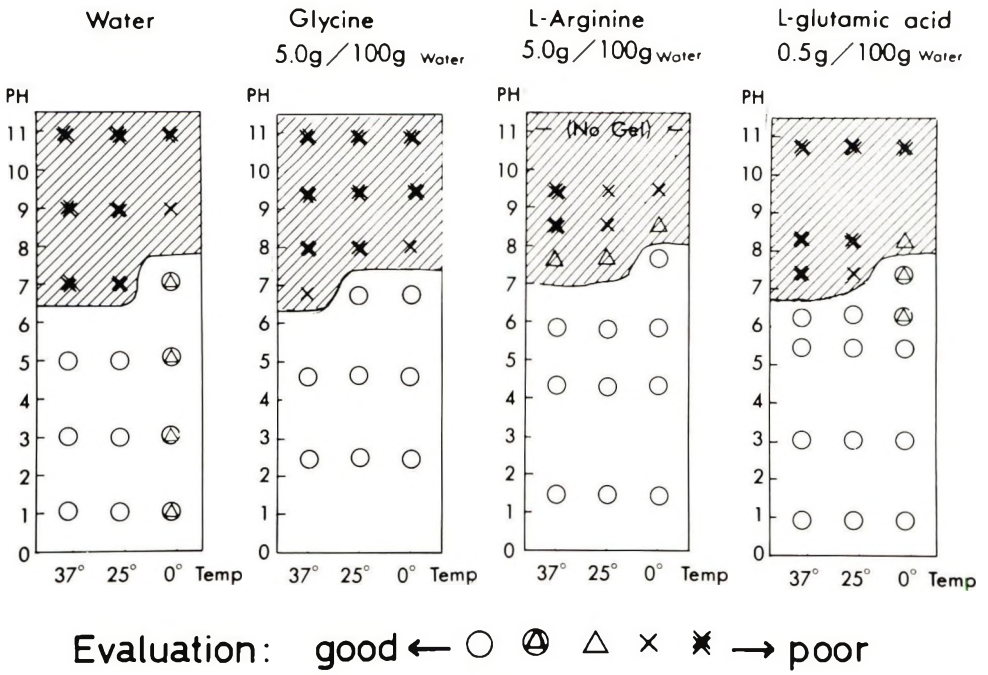


Figure 7. Effect of pH on stability of gels

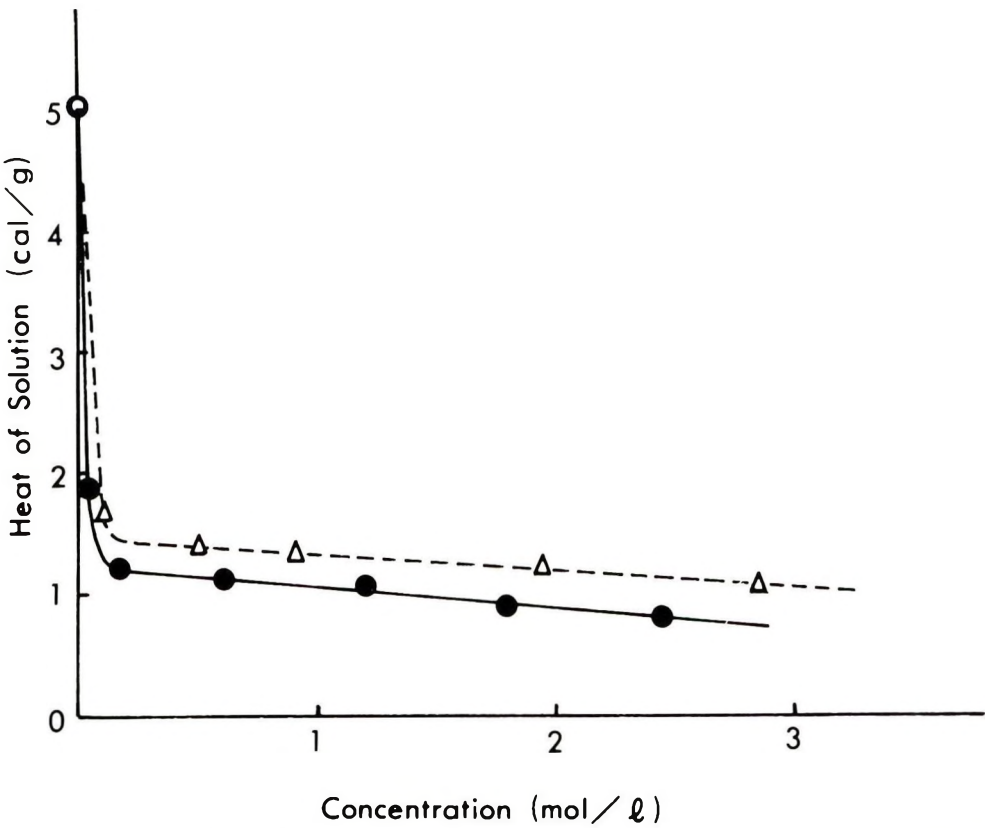


Figure 8. Heat of solution of surfactant to aqueous solution of amino acid or salt with various concentrations: (○) water, (△) L-serine, (●) Monosodium L-glutamate monohydrate

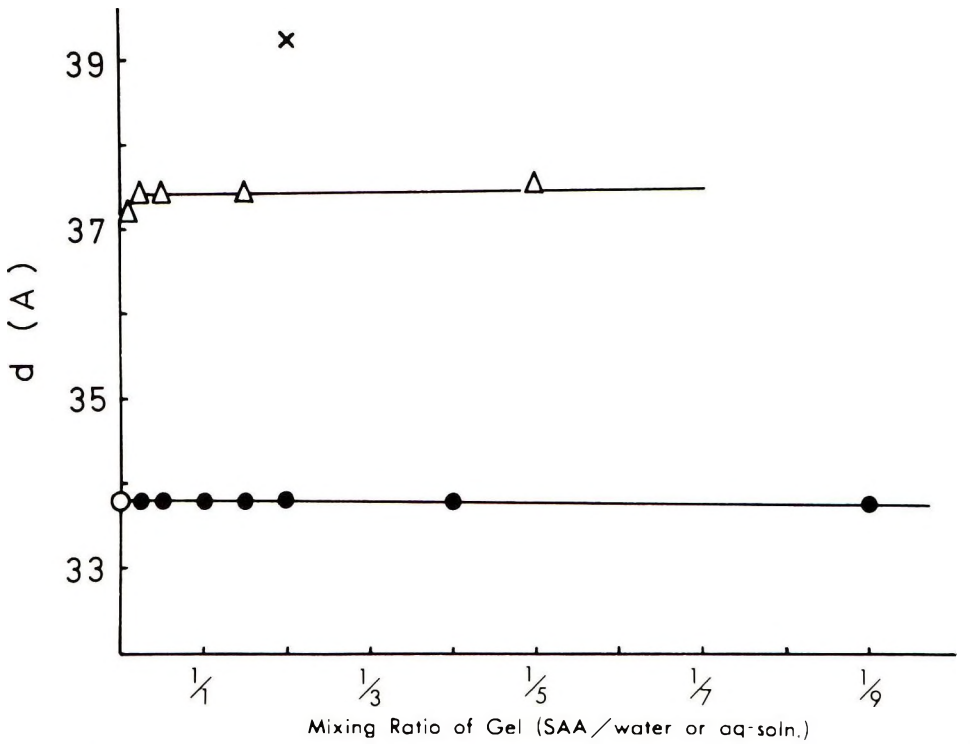


Figure 9. Comparison of spacings of surfactant in gels prepared by various combinations of water, monosodium L-glutamate monohydrate, and urea: (○) sunsoft O-30B itself, (△) water, (●) 40 per cent aqueous solution of monosodium L-glutamate monohydrate (×) 20 per cent aqueous solution of urea

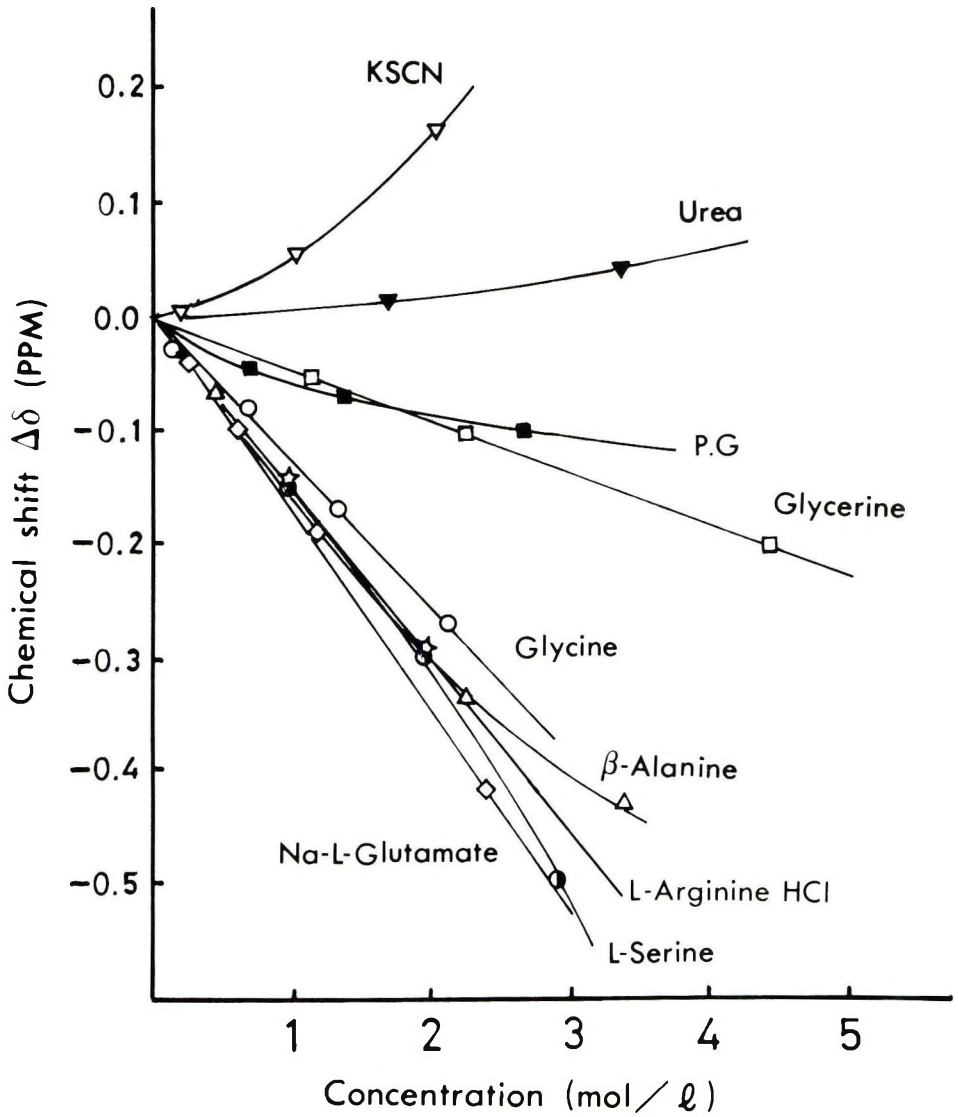


Figure 10. Chemical shift in proton of water for various aqueous solutions by nuclear magnetic resonance (NMR)

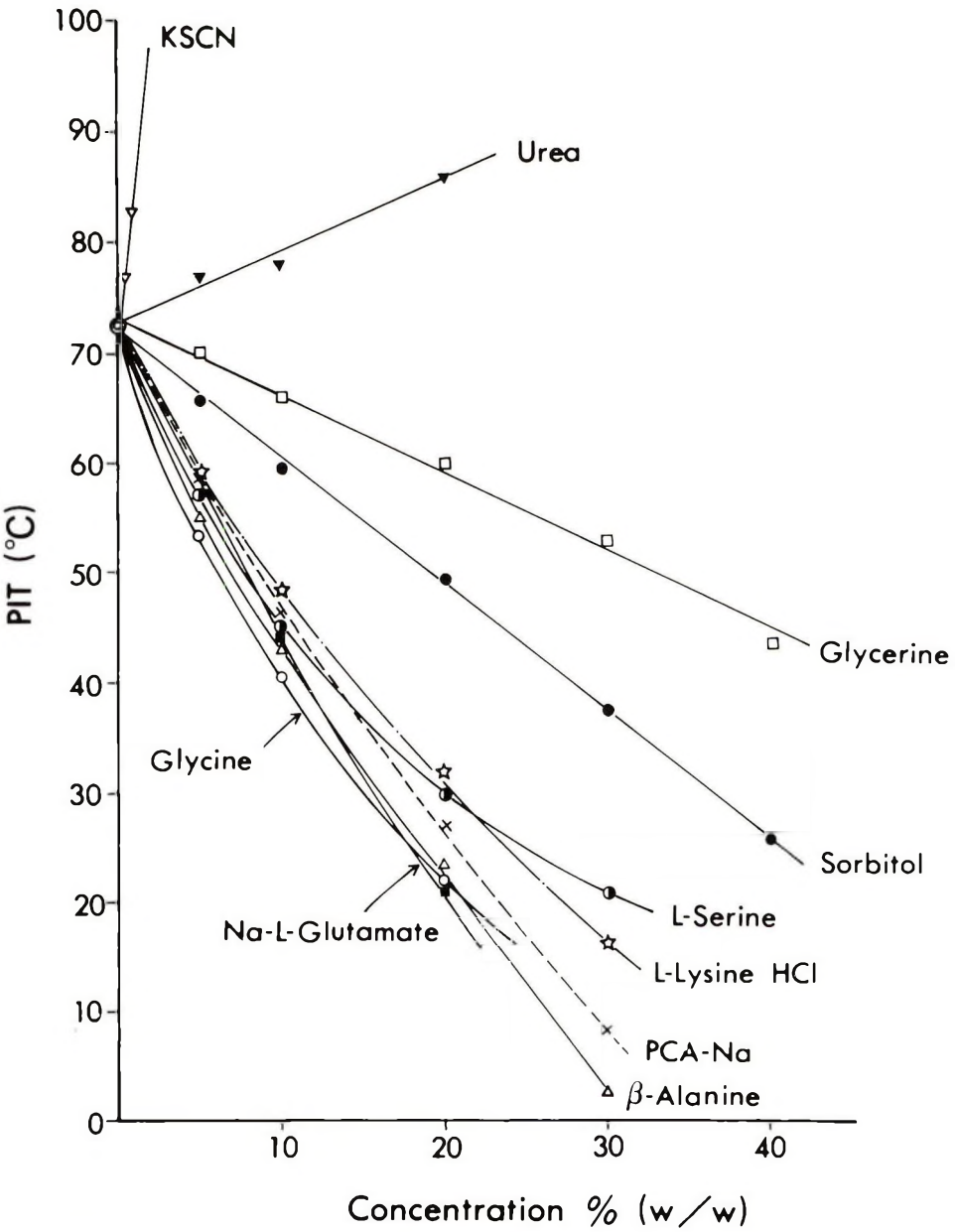


Figure 11. Effect of addition of amino acids or their salts to phase inversion temperature (PIT)

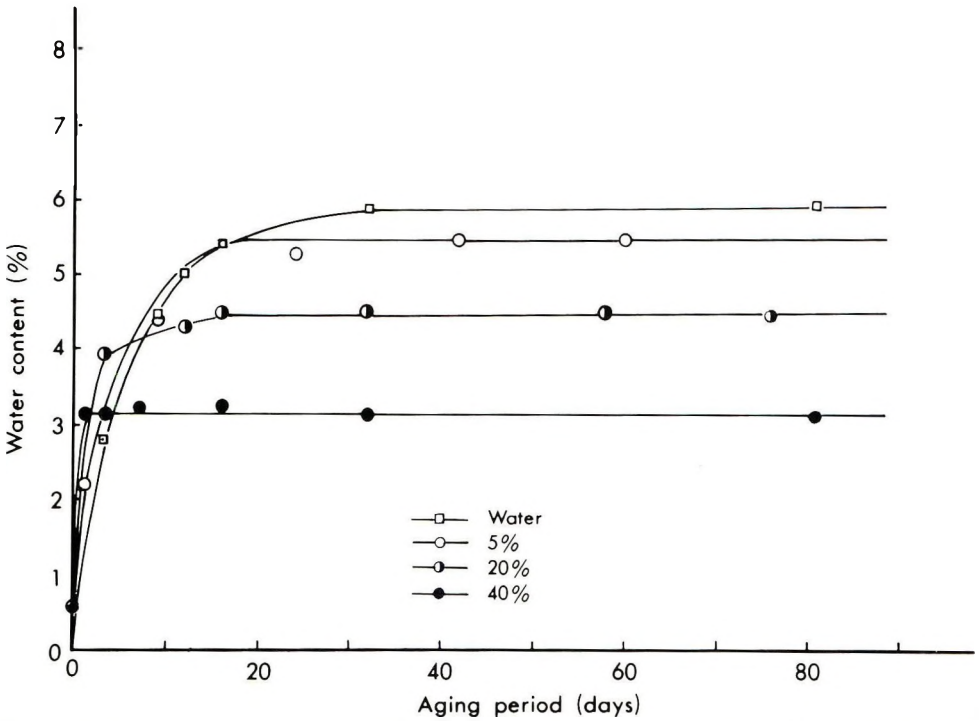


Figure 12. Change of water content migrating from water phase into surfactant phase (Sunsoft O-30B) with varied concentration of monosodium L-glutamate monohydrate with time

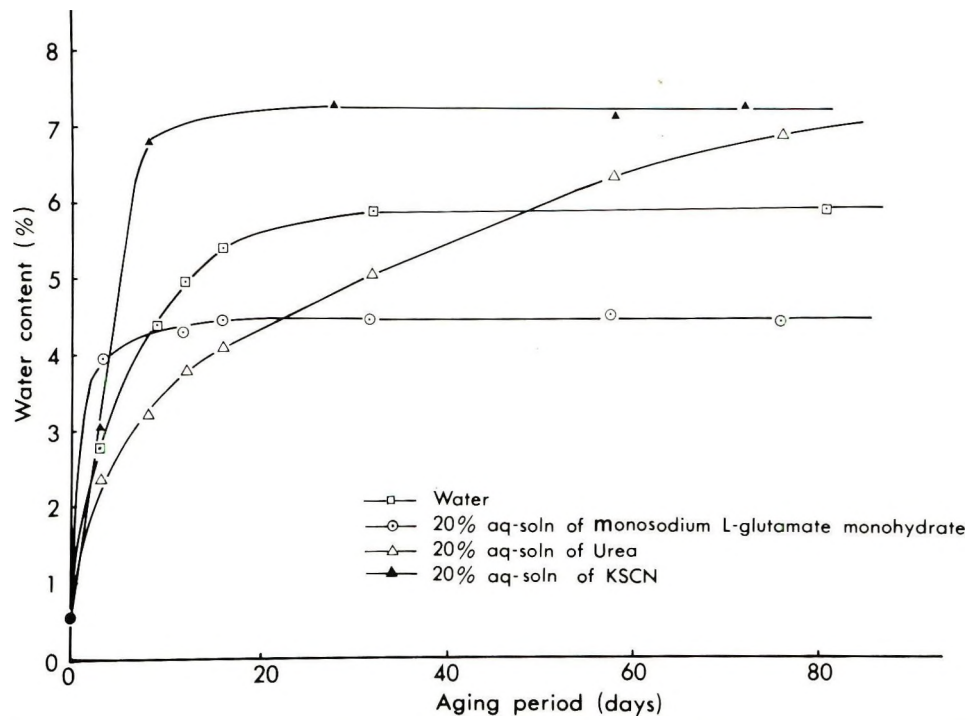


Figure 13. Change of water content, migrating from water phase into surfactant phase (Sunsoft O-30B) with varied solute with time

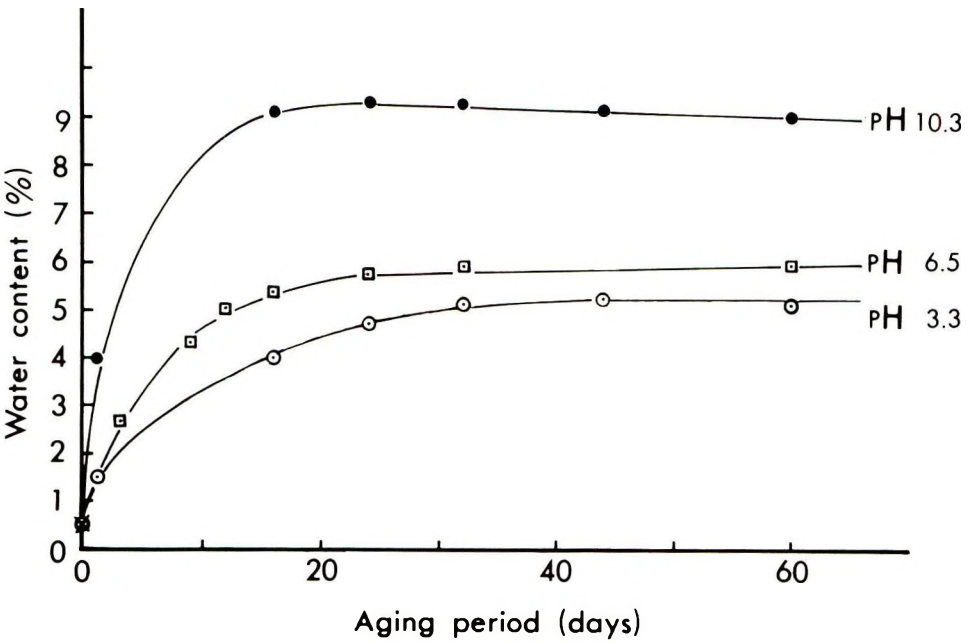


Figure 14. Change of water content, migrating from water phase into surfactant phase, with varied pH with time

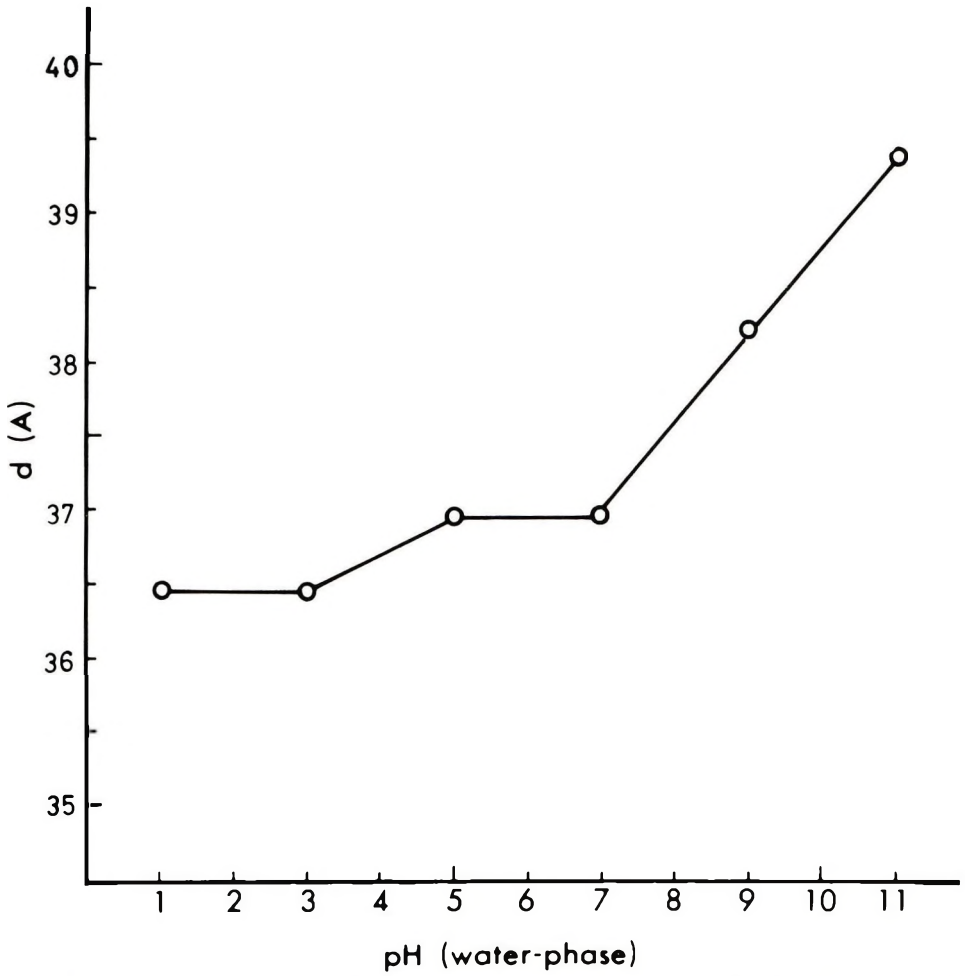


Figure 15. Effect of pH on spacings of surfactant in gel prepared between Sunsoft O-30B and water. Mixing ratio = 1:4

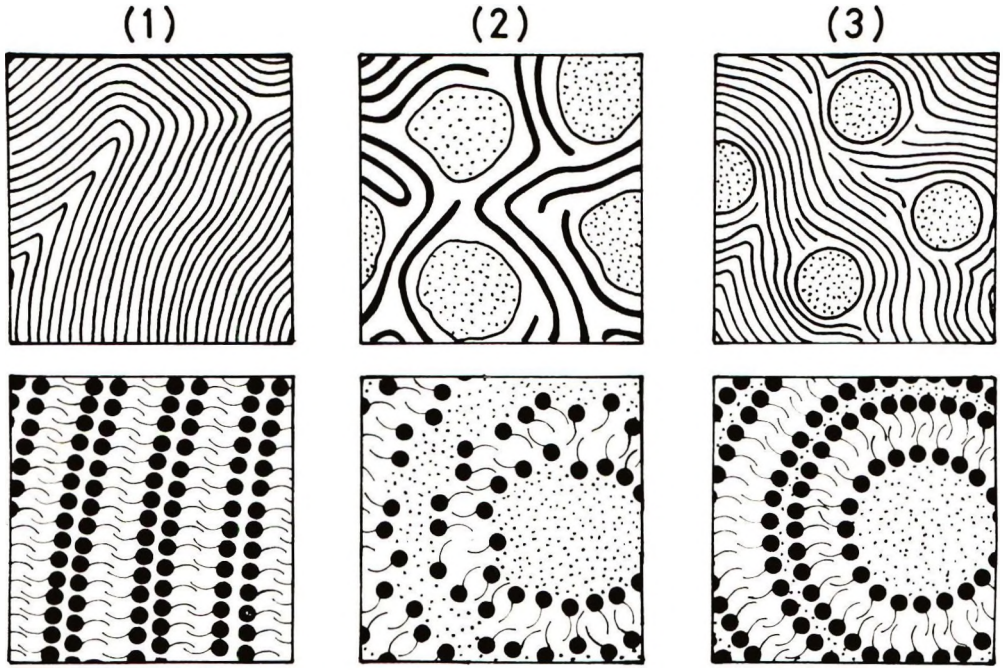


Figure 16. Structural model of surfactant and gel: (1) surfactant, (2) gel prepared between surfactant and water; (3) gel prepared between surfactant and aqueous solution of amino acid

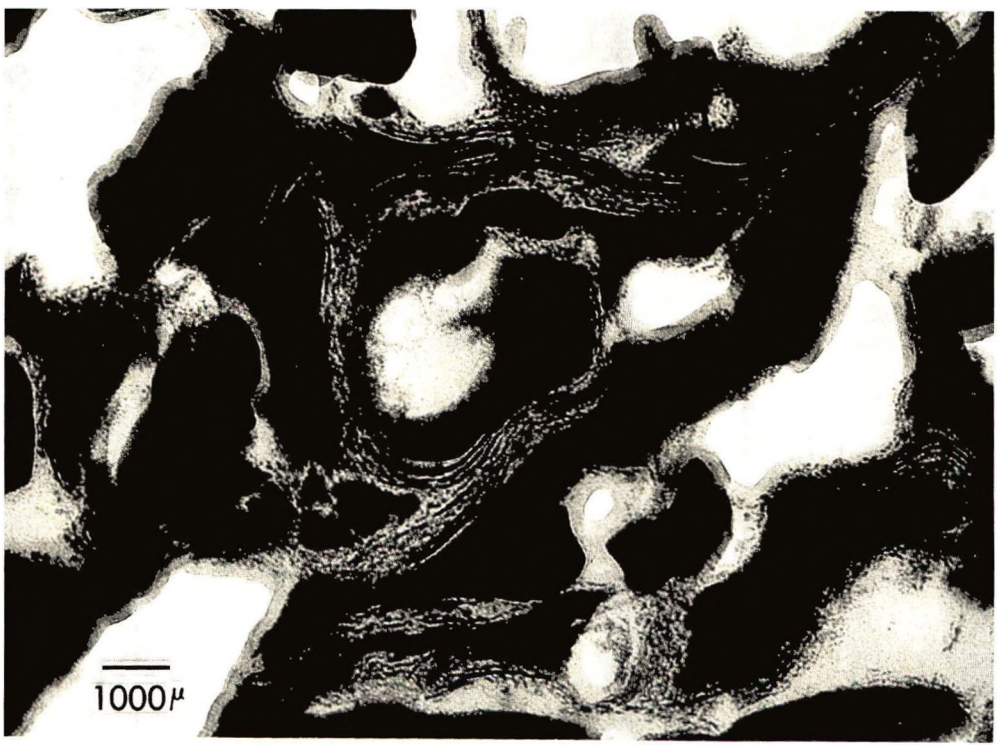


Figure 17. Gel structure by EM: magnification: 42000 x

Table III
Physical Properties of Amino Acids and their Salts

Number	L-Amino Acid and a Salt thereof	Solubility (6) g/100$_g$H$_2$O[25°C]	ΣN Inorganic	ΣN Organic	Gel Formation
1	L-Alanine	16.51	220	60	○
2	β-Alanine	55.50	220	60	○
3	L-Arginine	14.6 (20°C)	410	120	X
4	L-Arginine HCL	90.0	810	120	○
5	L-Asparagine H_2O	3.00	420	80	●
6	L-Aspartic acid	0.50	370	80	●
7	L-Asparate Na-H_2O	80.1	870	80	○
8	L-Cystine	0.011	240	100	●
9	L-Glutamic acid	0.84	370	100	●
10	L-Glutamate Na-H_2O	74.22	870	80	○
11	L-Glutamine	4.25	420	100	●
12	Glycine	24.99	220	40	○
13	L-Histidine	4.29	352	120	●
14	L-Histidine HCL-H_2O	39.0 (24°C)	752	120	○
15	Hydroxy L-proline	36.11	330	100	○
16	L-Isoleucine	4.12	220	110	●
17	L-Leucine	2.19	220	110	●
18	L-Lysine HCL	89.0	690	120	○
19	L-Methionine	5.92	230	140	●
20	L-Phenylalanine	2.97	231	180	●
21	L-Proline	162.3	230	100	●
22	L-Serine	43.0	320	60	○
23	L-Threonine	10.0	320	80	●
24	L-Tryptophan	1.14	321	220	●
25	L-Tyrosine	0.045	341	180	●
26	L-Valine	8.85	220	90	●

Evaluation: (○) good; (●) poor; (X) impossible.

Table IV
The Relationship of Water Content and Spacing to the Stability of Gel and W/O Cream

Test Items Gel's Aqueous Solution	Water Content (per cent)	Spacing (Å)	Stability Gel 1 day	Gel 7 days	Gel 30 days	W/O Cream 1 day	W/O Cream 7 days	W/O Cream 30 days
Water	5.93	37.6	△	x	X	△	△	x
5 per cent monosodium L-glutamate H_2O	5.49	37.1	○	◎	x	○	○	○
20 per cent monosodium L-glutamate H_2O	4.49	34.9	○	○	○	○	○	○
40 per cent monosodium L-glutamate H_2O	3.12	33.8	○	○	○	○	○	○
20 per cent potassium thiocyanate	7.32	40.4	x	X	X	△	x	X
20 per cent urea	6.91	39.2	X	X	X	X	X	X

Evaluation: good←○ ◎ △ x X→Poor

Table V
The Properties of the Creams Obtained from the Gels Prepared by the Addition of an Amino acid, and without an Amino acid

	Physical Properties of Cream						Stability of Cream					
	Viscosity		Hardness			Emulsion	After 1 Day			After 30 Days		
Number	70°C	30°C	0°C	25°C	37°C	Particles	0°C	25°C	37°C	0°C	25°C	37°C
	cps											
(1)[a]	18000	94400	42	11	6	(See Fig. 18(a))	○	○	○	○	○	○
(2)[b]	13900	101600	53	15	7		○	○	○	○	○	○
(3)[c]	8500	36000	12	3	1	(See Fig. 18(b))	△	◎	△	x	△	X
(4)[d]	5750	38400	15	4	1		◎	◎	△	△	△	x

Evaluation: good←○ ◎ △ x X→poor.
[a](1) 40 per cent aqueous solution of monosodium L-glutamate monohydrate ~ Sunsoft® O-30B.
[b](2) 40 per cent aqueous solution of monosodium L-glutamate monohydrate ~ POEM® O-72-D.
[c](3) Water ~ Sunsoft® O-30B.
[d](4) Water ~ POEM® O-72-D.

Table VI
The Relationship of the X-Ray Diffraction Patterns of the Surfactants to their Gel-Forming Function and the Properties of W/O Emulsions using the Gels

Surfactant			W/O Emulsion's	
Name	X-Ray Diffraction Pattern	Gel-Formation	Stability (RT)	Viscosity (30°C)
Sunsoft® O-30B[a]	Clear	○	○	94400[Hz]
DIG-EIS[b]	Clear	○	○	112800
Emalex® EG-O[c]	Indistinct	x	X (two-phase separations)	—
Emalex® 300dio[c]	Indistinct	x	x	1750
Emalex® 503[c]	No peak	x	x	750
Nikkol® MIO-2[d]	No peak	x	X (two-phase separations)	—

[a]Taiyo Kagaku Co., Ltd. (62 Akahori, Yokkaichi, Mie, Japan).
[b]Matsumoto Trading Co., Ltd. (3-1 Nihonbashihoncho, Chuoku, Tokyo, Japan).
[c]5 Nihon Emulsion Co., Ltd. (5-32-7 Minami, Koenji, Suginamiku, Tokyo, Japan).
[d]Nikko Chemicals Co., Ltd. (1-4-8 Bakurocho, Nihonbashi, Chuoku, Tokyo, Japan).

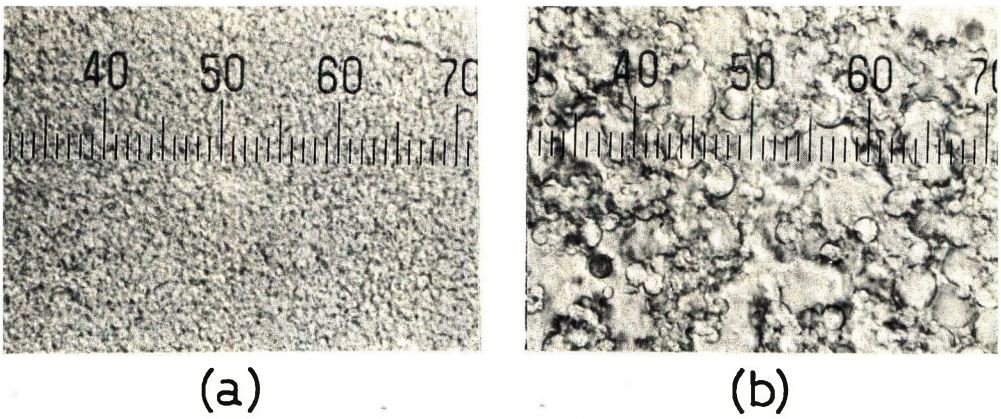


Figure 18. Comparison of emulsion particles of creams: division, 2.5μm

acid, linolic acid, and isohexadecanoic acid. These surfactants are supplied in the form of a mixture of partial esters. For example, when those with 3 hydroxyl groups were used, a mixture comprised mainly of monoester was preferred, and when those with 4 hydroxyl groups were used, a mixture comprising of diester gave the better results. On the other hand, even within the above mentioned range, those in which the hydroxyl groups were completely esterified, failed to form gels. Although, those surfactants which formed gels did not contain water, all of them gave 2 clearly discernible diffraction lines in the small angle region as seen in Fig. 3. As is seen in Table I, the spacings of the surfactants appeared at approximately 33 and 70 Å, and the ratio was about 1:2. From these facts, it was considered that, although, they were liquids, these surfactants possessed very orderly lamellar structures by themselves. On the other hand, almost all of those surfactants lacking the function of gel formation did not have a clear structure, as is shown in Table II. The X-ray diffraction patterns were indistinct or completely lacking even though they were clear; the spacings were either too wide or too narrow. Such results of X-ray analysis were identified as having a close correlation to gel formation.

As a result, it can be concluded that, in order to form gels, the corresponding surfactants are required to have at least a high orderly lamellar structure.

Contrary to the restriction of the surfactants used, almost all of the amino acids and their salts formed gels. As shown in Table III, nearly all of the monoamino-monocarboxylic acids (neutral amino acid) with an isoelectric point in the weak acidic range, such as monosodium salts of monoamino-dicarboxylic acid (acidic amino acid) and monohydrochlorides of diamino-monocarboxylic acid (basic amino acid) were capable of forming gels. Those readily soluble in water were very effective, such as glycine, L-alanine, B-alanine, hydroxy L-proline, L-serine, monosodium L-glutamate monohydrate, monopotassium L-glutamate monohydrate, monosodium L-aspartate monohydrate, monopotassium L-aspartate dihydrate, L-lysine monohydrochloride L-arginine monohydrochloride, and L-histidine monohydrochloride. The D- and DL stereoisomers also gave good gel formations.

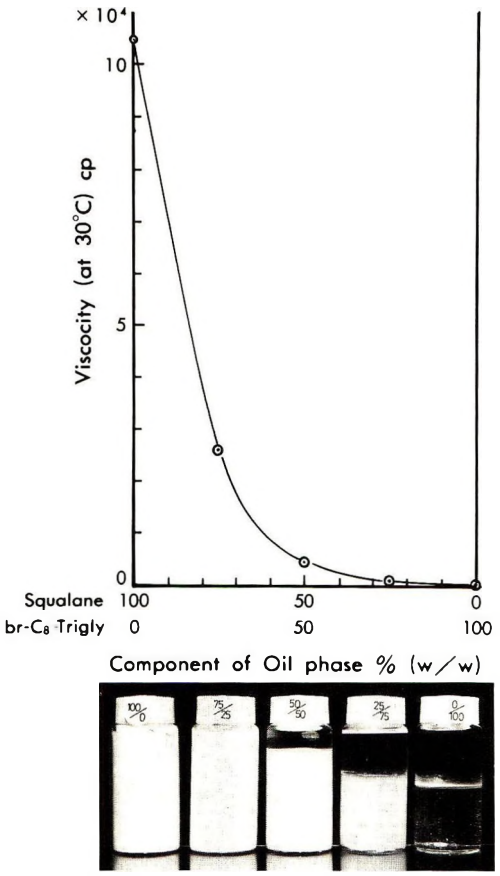


Figure 19. Influence of a component in oil phase on stability of o/w emulsions

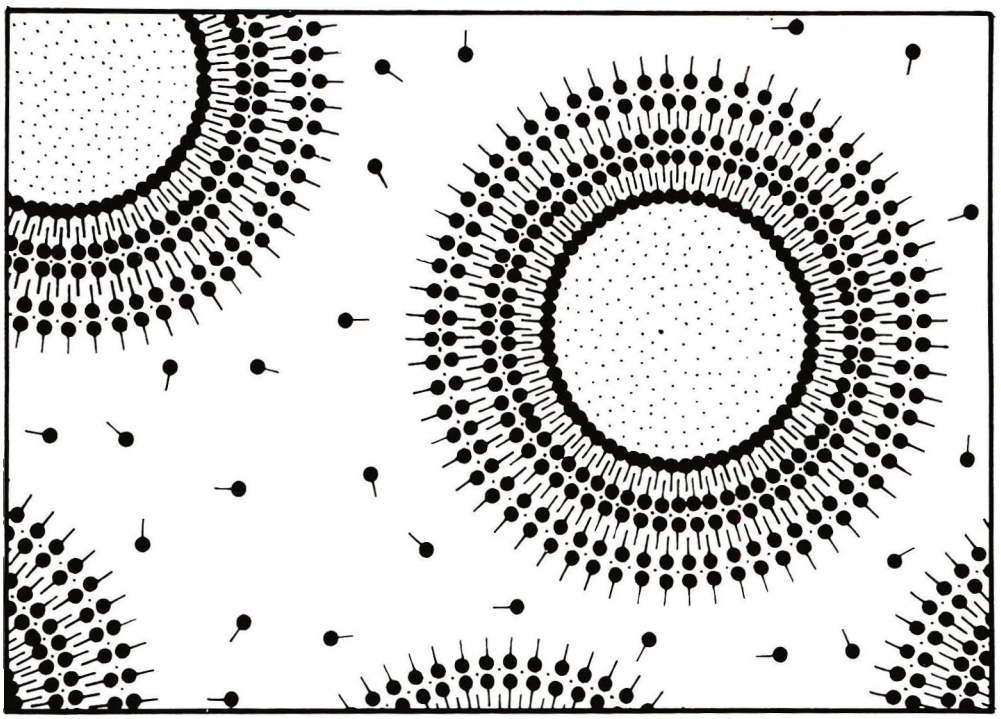


Figure 20. Model of emulsion obtained by gel-emulsification method

The fact that in the case of acidic amino acids and basic amino acids, the best gels were obtained only when the monosodium salts or monohydrochlorides, respectively, was considered to be closely related to the fact that the molecules were in the highest possible polyvalent state (III) among the 4 in the aqueous solution as is shown below.

(I)	(II)	(III)	(IV)
CO_2H	CO_2H	COO^-	COO^-
$H_3N^+-Glu-CO_2H$ ⇌	$H_3N-Glu-COO^-$ ⇌	$H_3N-Glu-COO^-$ ⇌	$H_2N-Glu-COO^-$
pK'_3 2.2	3.2	4.3	9.7

In the case of neutral amino acids, the isoelectric range was also found to be the most suitable for the formation of the gels.

When one note the relationship between the solubility (6) and IOB as is shown in Fig. 4, it becomes apparent that the higher the solubilities in water the better the results obtained. The IOB is within the range of about 4 to 10. With acylated amino acids and their higher alcohol esters, there were no gel formations.

Various gels were obtained by a variety of combinations between amino acids and surfactants, such as their kinds and mixing ratio. The higher the concentration of amino acid, and it's mixing ratio to the surfactant and shearing stress the more stable was the gel obtained. In this case, the hardness and viscoelasticity of the gels increased and the gels became transparent. On the contrary, when the concentration and mixing ratio became lower, the gel appeared milky white and fluid. Figure 5 shows the rheological properties of the gels obtained between Sunsoft O-30B and a 10 per cent aqueous so-

lution of glycine. From the rheological properties, the previously mentioned results can also be explained. In this case, a maximum of 19 times the amount of glycine aqueous solution by weight to the surfactant was contained as the inner phase. When a 40 per cent aqueous solution of monosodium L-glutamate monohydrate was used, a maximum of 46 times was obtained. A gel in the metastable state lasting only for several hours was obtained in the absence of an amino acid as in the case of a system containing only surfactant and water. The water particles in the gel soon coalesced and the gel finally separated into 2 phases. The changes of water particles with time in these two cases are compared in Fig. 6.

It can be seen that the gels obtained by the addition of amino acids were very stable with no coalescence of droplets. The effect of pH on the gels was also examined. Figure 7 shows the stability of the gels obtained from buffer solution and that with a fixed amount of glycine (neutral amino acid, pI equals 5.97) L-arginine (basic amino acid, pI equals 11.15), L-glutamic acid (acidic amino acid, pI equals 3.22). It was apparent from the diagrams, that in all of cases, the gels were not stable in a higher pH range, but stable at the lower pH, i.e., the acid side. Gelation was poor in the range where the pH was extremely low and became better on the weakly acid side at 4.0 to 6.5. Stability also depended on the storage temperature. As the temperature increased, the stability shifted to the lower pH range.

As will be described later, the hydration power of the amino acids is considered to relate to the formation of stable gel, and it may be determined depending upon the ionic state of the amino acids.

From the fact that the aqueous solution of the amino acids and the above mentioned surfactants are combined to form gels having very high viscosity and showing no coalescence of particles of the aqueous solution, it is presumed that some specific interaction exists between the amino acid and surfactant at the interface. In order to confirm the possible existence of this interaction, first, the structure of the gel was investigated by the measurement of the heat of solution, X-ray analysis, DTA, and microscopy.

Figure 8 shows the results of the heat of solution at 35°C when the surfactant, Sunsoft O-30B, was mixed and dissolved into an aqueous solution of the amino acids. In the case of water only, exothermic heat of about 5 cal/g was measured. Should it be assumed that a new complex is formed by the addition of amino acid, an increase of exothermic heat higher than in the case of water will be expected and the curve should go upward.

However, as shown in the figure, in both the case of L-serine and monosodium L-glutamate monohydrate, the heat of solution decreased rapidly even with a minor increase of concentration. This indicates that the solubility of surfactant to amino acid solution decreases more than that to water by the possible reduction of the interaction between amino acid and surfactant and also confirms the fact that there are no new complex formations taking place.

Subsequently, as a result of X-ray analysis of the gels, which is the most common method, two diffraction lines were found in the small angle. It corresponded completely with the X-ray diffraction pattern of the surfactant itself as is previously described and was finally confirmed as that of the lamellar structure of the surfactants *per se*. However, it could not be confirmed that the formation of a new structure having new spacings between amino acid and surfactant was formed. The same results were

obtained by DTA and optical microscopic observations. From Fig. 6, it can be said that the gel itself is considered to be the same emulsion system as an ordinary w/o emulsion in which the particles of aqueous solution are dispersed in the lipophilic surfactant phase. It can be readily understood, as is shown in Fig. 5, that fluidity, high viscosity, and hardness of the gel are due to the increasing volume ratio and interfacial area. In the gels obtained by the addition of aqueous solution of amino acids, the interfacial films are very strong and stable so that the particles do not coalesce even though the inner phase is increased. These phenomena owe much to the functions of the amino acid. From these facts, the authors studied the influence of the amino acids on the change of the lamellar structure possessed in common with the surfactants used.

Figure 9 indicates the comparison between the spacings of stable gels with aqueous solutions of amino acid and those of unstable gels with water only and urea. Sunsoft O-30B, which was used as the surfactant in the experiments, had spacings of 33.8 Å. In the case of an aqueous solution of amino acid, even though the mixing ratio of the gel was altered, the original spacing of the surfactant did not change. However, in the case of water, it increased to 37.6 Å. Such change had been determined in either case at an early stage, regardless of the increased mixing ratio. It may be considered that the amino acid results in weakening the interaction between the water and the surfactant; that is, the amino acid decreases the solubility of the surfactant in the water phase and also prevents the solubilization of water into the hydrophilic parts of the lamellar structure, therefore, it seems to prevent the change in the original structure of the surfactant. It was found that urea further increased the spacing than in the case of water. Urea formed a gel with difficulty and though formed, was immediately destroyed and inverted to the o/w type.

The explanation of the results of NMR measurement are shown in Fig. 10 and shows how the amino acids or their salts interact with water. A negative sign Δd indicates a shift of proton in water to the lower magnetic field, and a positive sign means a shift to the higher magnetic field. The amino acids allows the proton in water to shift to the lower magnetic field. The gradient was sharp, hence, the effect was much greater even in lower concentration. On the contrary, potassium thiocyanate (KSCN) and urea shifted the proton to the higher magnetic field. In other words, these facts suggest a change in the physical properties of water. An amino acid, which shifts the proton to the lower magnetic field, indicates a condition in which the affinity of water to the amino acid is very strong and results in a decrease of free water. Therefore, these amino acids decreased the solubility of surfactant, which previously existed in water or prevented the solubilization of water in the hydrophilic part of the surfactant. These concepts are supported by the data obtained in the heat of solution shown in Fig. 8.

Furthermore, since the amino acid will expel the surfactant from the water phase, it becomes evident that a w/o type emulsion is the easier one to obtain. It is presumed that there is a decrease in the PIT with the increase concentration of the amino acids. This hypothesis was confirmed by the results of the PIT as is shown in Fig. 11. The amino acids decreased the PIT, and conversely, KSCN and urea increased the PIT. Such a trend in the PIT corresponded perfectly with the results of the NMR analysis. From this view point, it can be readily understood how the amino acid changes the physical properties of water and decreases the interaction with the surfactant. In order to investigate how much water causes a change of structure when solubilized in the hydrophilic part of the surfactant, the authors compared the changes in the amount of water migrat-

ing into the surfactant phase with that of the spacings. Figs. 12 and 13 show the changes in the water content caused by its migration into the surfactant phase with a lapse in time.

In the case of water only, the migration to the surfactant phase was as much as 6 per cent. In case of monosodium L-glutamate monohydrate, the amount of migration decreased with an increase in concentration. This corresponds well with the decreasing trend of affinity between the surfactant and water. The water content became constant rapidly and did not increase over a prolonged period.

The relationship of this water content to the spacings and the stability of the gels is indicated in Table IV. As is evident from this Table, with the increase of water migrating to the surfactant, the spacing becomes much wider. The spacing obtained with 40 per cent aqueous solution of monosodium L-glutamate monohydrate was exactly the same as that of the surfactant itself (Sunsoft O-30B). No changes in the structure of the surfactant was observed when the solubilized water was less than 3 per cent. The larger the variation of the spacings, the poorer the gel formation became.

The effect of pH on the stability of the gel can also be explained by the variation of water content and the spacings as shown in Figs. 14 and 15. As the pH of water increased, the water solubilized in the surfactant increased, and at the same time the spacings also increased rapidly.

From the above results and discussions, it is possible to draw the structural model indicating the mechanism for the stabilization of the gels by the amino acids as is shown in Fig. 16. The upper models indicate the overall view, and the lower models show a magnified view. The surfactant, though it is in the liquid state, has a lamellar structure as seen in view (1) of Fig. 16. In the case of the aqueous solution in the absence of amino acids, a large amount of water is solubilized in the hydrophilic part of the surfactant having an orderly form and its original structure becomes disordered and loosened due to the widening of its spacings. Under such conditions, water particles coalesce easily, which indicates instability. The same explanation can be made in the case of KSCN and urea, which change the structure of the surfactant. This is shown in view (2) of Fig. 16. View (3) of Fig. 16 indicates the case when amino acid is added. Since the amount of water to be solubilized in the hydrophilic part of the surfactant is limited and does not induce any change of structure, a concentrated and tight interfacial atmosphere is established around the particles. As a result, coalescence of the particles hardly occurs; thus, maintaining stability. As an important fact to support such a structural model, we succeeded in taking the photograph of the gel by EM (as is seen in Fig. 17). The sample shown was the gel obtained with Sunsoft O-30B and a 40 per cent aqueous solution of monosodium L-glutamate monohydrate mixed in the ratio of 1:4. Apparently, the water particles surrounded by the surfactant phase in the lamellar structure can be seen.

GEL-EMULSIFICATION METHOD

The relationship between the stability of the gels and that of w/o emulsions made by using the gels has also been noted. Hardness of the gels was influenced by the shear stress given at the time of preparation. When the hardness of the gels was changed, the

hardness of the w/o cream using such gels also changed depending on the former change.

As was previously described, the gel obtained by Sunsoft O-30B and the amino acid was stable; however, it is necessary to observe the changes in the cream when an unstable gel is used. Table V and Fig. 18 indicate the results obtained when an unstable gel, such as obtained between Sunsoft O-30B and water in the absence of an amino acid, was used. In comparing these results with the case where stable gels were used in the preparation of creams, remarkable differences were recognized as to viscosity, hardness, stability, and size of the emulsion particles. Table VI indicates the relationship of the X-ray diffraction patterns of the surfactants to its function in gel-formation and the properties of the w/o emulsions using these gels. As is evident from this Table, it is readily understood that the surfactants having clear X-ray diffraction patterns can readily form gels and the creams obtained were stable while those having indistinct X-Ray diffraction patterns did not form gels and produced unstable creams.

These facts can also be confirmed by other studies. As was mentioned previously, the amino acid functions to prevent the expansion of the spacings of the surfactant when an aqueous solution of amino acid is added to the surfactant phase. It was also found that amino acids possessing this property produced a good stable gel. Furthermore, the relationship between these properties and the stability of w/o emulsions were also studied. The results are shown in Table VII. There is a correlation between the stability of the gels and the stability of w/o emulsions; namely, the better the stability of the gels, the better the w/o emulsion obtained. Furthermore, the stability of the gels, which have been studied on the standard sample by using squalane as the main constituent of the oil, must be changed with the polarity of the oil. Figure 19 shows the influence of a component in the oil phase on the stability of the w/o emulsion. When squalane only was used as the oil phase, the destruction of the gel was not observed and the resulting w/o emulsion had a high viscosity of greater than 100,000 cps, but as the mixing ratio of glycerol tri-2-ethylhexanoate became higher, the viscosity dropped rapidly and finally, no emulsification occurred which was accompanied by a total destruction of the gel in the oil phase. In a nonpolar oil, such as squalane, there was no structural change of the surfactant; however, in a polar oil, such as glycerol tri-2-ethylhexanoate, the structure disappeared completely. Therefore, as the polar components in the oil phase increased, the emulsion became increasingly unstable. Thus, in the gel-emulsification method, it is advisable to use nonpolar oils and waxes in the oil phase, such as, squalane, liquid paraffin, and microcrystalline wax, etc. These facts have been proven to be identical in practical formulations of cosmetic creams as well as in the simple systems studied.

In the gel-emulsification method, the drawings of the emulsion when the oil is added can be assumed to be as is shown in Fig. 20. In the case of nonpolar oils, the water particles surrounded by several layers of surfactant disperse in the oil phase. On the contrary, in the case of polar oils, it may be assumed that the orientation of the surfactant is loosened and its adsorption at the interface are hindered, resulting in an unstable emulsion.

The above results may be summarized as follows. By initially forming a stable gel and maintaining conditions to maintain gel stability, a stable w/o emulsion having high vis-

Table VII
Example of w/o Type Emollient Cream

Squalane	25.0 parts
Ceresine	3.0
Beeswax	1.5
Lanolin	0.5
Petrolatum	6.0
w/o gel	20.0
Propylene glycol	5.0
Water	39.0
Perfume	proper amount
Antiseptics	proper amount

cosity and uniform particles may be obtained. On the other hand, when unstable gels are used (or under conditions inducive to gel destruction), unstable w/o emulsions and creams result. In other words, there is a correlation existing between the properties of the gel used and the stability of the final emulsion obtained.

APPLICATION TO COSMETICS

As mentioned above, the authors elucidated the requirements for surfactants that formed stable gel and the function of the amino acid contributing to the stability of the emulsions. It was noted therein that the characteristics of the gel used played an important role, and that the better the gel used in the formulation, the better the emulsions or creams produced were. We will discuss some of the advantages obtained by the application of this emulsification technique in the preparation of cosmetic base together with the characteristics of the finished products. Several creams were prepared by the gel-emulsification method and used as a base for cosmetics.

From the measured values and stability of the prepared creams by this method, we noted many advantages with this emulsifying method and products. For example, any type of cream with required values may be readily selected by referring to the component ratio, and a stable cream may be obtained by adding a definite quantity of the gel to cover the wide ranges of volume ratio. Even those at the range of extremely biased volume ratio where another surfactant such as soaps are used to adjust HLB for stabilizing the system can also be used.

Furthermore, from the evaluation of the application tests, we were able to know the suitable formulation ranges for 3 types of creams such as the cleansing, massage, and emollient types, respectively. It was especially interesting to note that suitable formulations for emollient creams were concentrated in the ranges, wherein there was a fairly large content of water contrary to the former 2 creams. By using these new techniques and know-how in the formulation of cosmetics, the authors were able to prepare various types of bases for cosmetics. An example of the emollient cream is shown in Table VII. This method may be applied not only to cosmetic creams, but also to stick type emulsion products with extremely low water content and also to different pharmaceutical preparations. The characteristics of the cosmetic bases prepared by using this method were their ready adaptation to the skin, their excellent spreadability and moisturizing effect with hardly any greasy feel and glaring appearance, which were

common to conventional w/o type cosmetics. In addition, the products were highly safe for skin application. These many advantageous characteristics of the creams may be attributed to the phenomena suggesting the role of NMF. The stability of these products were excellent and had long shelf life, even at elevated temperature together with less dependability of hardness on aging and temperature.

It is well known that the NMF content in stratum corneum is high and has water absorbing and water holding properties. It is said that the chapping of the epidermis in the winter and the dryness of the aged skin are due to insufficient secretion of sebum and NMF. In view of this fact, it is quite natural and reasonable to supply the deficiency of NMF with cosmetics. NMF is said to be found mostly at the boundary below the stratum corneum and is found also in stratum granulosum and sweat. Sixteen free amino acids have been identified such as serine with more than 30 per cent, glutamic acid, proline, etc. (7). It has been proven that mono-, di- and triglycerides of fatty acids also exist in sebum. Sebum flows readily and spreads on the surface of skin after reaching the hair follicles and is then mixed with sweat and eventually forms a skin surface lipid film which is a w/o type emulsion. It is easily inverted to o/w type emulsion under the condition when the ratio of sweat becomes high. But, this is reversible and the type emulsion is determined depending upon the physiological condition of the skin surface (w/o$\rightleftharpoons$o/w) (8).

We can find an interesting similarity between the natural emulsification phenomena on the skin surface and that of the gel-emulsification method developed by the authors. As mentioned before, in our emulsification method, monoglyceride, and aqueous solution of amino acid which correspond to sebum and NMF, respectively, were mixed and a w/o type emulsion was formed. On the contrary, urea and uric acid, which are found in sweat broke down such w/o emulsions and inverted to that of the o/w type. Furthermore, when the gel was kept in water, it expanded remarkably. This property is similar to semipermeability of the lipid film. As the amino acids in the gels retard moisture loss and maintain moisture by their strong hydration effect, it is considered that cosmetic bases utilizing amino acids play an important role in maintaining the flexibility and elasticity of the skin. The effect of products formulated with amino acids on the skin are now under investigation by the authors from the view point of viscoelasticity of stratum corneum and percutaneous absorption of amino acids using radioisotope.

CONCLUSION

The w/o emulsions stabilized with amino acids or their salts were investigated. The following may be concluded.

1. Stable w/o type emulsions which have the capacity to contain wide ranges of water ratio, were obtained by using the gels prepared in a given combination of the amino acids and their salts with lipophilic surfactants having high orderly lamellar structures.
2. The authors applied this technology to the formulation of cosmetics such as creams, stick type cosmetics, foundations, etc.
3. The outstanding characteristics of the products obtained by using this method are their refreshing, smoothing, ready adaptation to the skin and moisturizing effects

with hardly any greasy feel and glaring appearance which are common to w/o type emulsion.

4. It is important that such surfactants to be used in these w/o emulsions have lamellar structures.
5. Amino acids are very effective in forming lamellar structure and contribute to preserve the tight interfacial atmosphere concentrating the surfactant at the interface. As a result, coalescence of water particles is prevented and stabilization of the w/o emulsion is achieved.
6. The stabilization mechanism of the w/o emulsions using the new method was elucidated through surface chemical investigation synthetically by means of X-Ray analysis, heat of solution, NMR, electron microscopy, etc.

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Book Reviews

THE CHEMISTRY AND MANUFACTURE OF COSMETICS, Vol. III., 2nd Ed., by Maison G. de Navarre. Continental Press, Florida, published and revised 1975.

This series in which this book is a part, has been the "Bible" of reference for the industry since its inception in 1945. Mr. de Navarre was the first President of the Society of Cosmetic Chemists and was also Editor of the *"Journal of the Society of Cosmetic Chemists,"* and President of Beauty Counselor,' and is now Editorial Director of *"Cosmetics and Perfumery,"* and President R & D of Vanda Beauty Counselor. In his rich background, he has acquired vast knowledge, which is reflected in this excellent series. He has assembled a distinguished panel of contributors to this series.

The contents covers important cosmetic topics such as: emollients; HLB system; hypoallergenic cosmetics; preservation; moisturizers; sunscreens and sun tanning; creams; masks; dental products; bath products; herbal cosmetics, etc., plus trade names, suppliers, and excellent indices.

I thought the chapter entitled "Theory of Sunscreens and Suntanning," by D. S. Berger, was well written and informative. Of special interest are the numerous formulae interspered throughout the text, beneficial to embryonic cosmetic chemists. Many chapters were written by Mr. de Navarre himself.

This new edition is not only a book I highly recommend, but I also feel that this is a reference book no cosmetic library should be without.—C. J. DEZEIH—Aloe Creme Laboratories, Inc.

KOSMETIK—JAHRBUCH 1977. Verlag fur chemische Industrie H. Ziolkowsky Kg. 423 pages. Price 34 DM ($13.60).

This cosmetic year book is a new venture by the German Organization Publisher for the Chemical Industry, of Augsburg, Bavaria, which also publishes a "Yearbook for the Practitioner," and "Soaps, Oils, Fats, Waxes." Thus, establishing the credentials of the "author," 3 questions about this publication should be asked. What does this publication attempt to accomplish? Does it succeed? Is it of value to workers in the area of cosmetic chemistry outside the country of origin?

The goal the publishers have set for themselves is quite encompassing and broad. They conceive of it as a practical handbook for the cosmetic expert in his practice; to be an aid in his daily work in the total field of cosmetics. To get an idea of the extent of the undertaking, it would be useful to list the titles of some of the 24 chapters in this book: recipies, per-

fumery, technology, quality control, microbiology, toxicology, packaging, analysis, applied and natural cosmetics, marketing, new preparations, and patents. These subjects, of course, are of importance and interest across national boundaries. Of more local significance, would be the chapters dealing with regulations and laws and one listing industrial services (e. g., suppliers of packaging materials and machinery). One section lists the names and address of societies, institutions and related organizations (besides the expected German and other European Societies, it was interesting to see the Society of Cosmetic Chemists and the American Society of Perfumers listed as well as such institutions as the American Society for Testing Materials and the Indian Standards Institution). A perusal of the book by the reviewer appears to substantiate the publishers intent to have this book give an overview of recent developments and present tendencies, which the journal literature and certain texts may only touch on tangentially. The statement that much is to be found here, which otherwise would be sought in vain, is probably pretentious, although, it is certainly conveniently gathered and organized in this annual. Thus, the answer to the second and third questions posed before is yes in both instances.

The most extensive (and to this reviewer, most fascinating) chapter is the 100-page collection of formulae ranging from both preparations (shampoos, douches, bubble baths) to vitamin-containing cosmetics—twelve categories in all.

Textual material, where it exists, is concisely written and readable. The book is laced with advertisements, both in color and black-and-white, which does not, however, detract from the overall good impression.

The book should be useful in any cosmetic laboratory or plant for all personnel involved in cosmetics be it chemical, technological, marketing, or legal. In these days of spiraling costs, even the reasonable price is appealing.—DR. ALEX GRINGAUZ—Arnold and Marie Schwartz College of Pharmacy and Health Sciences

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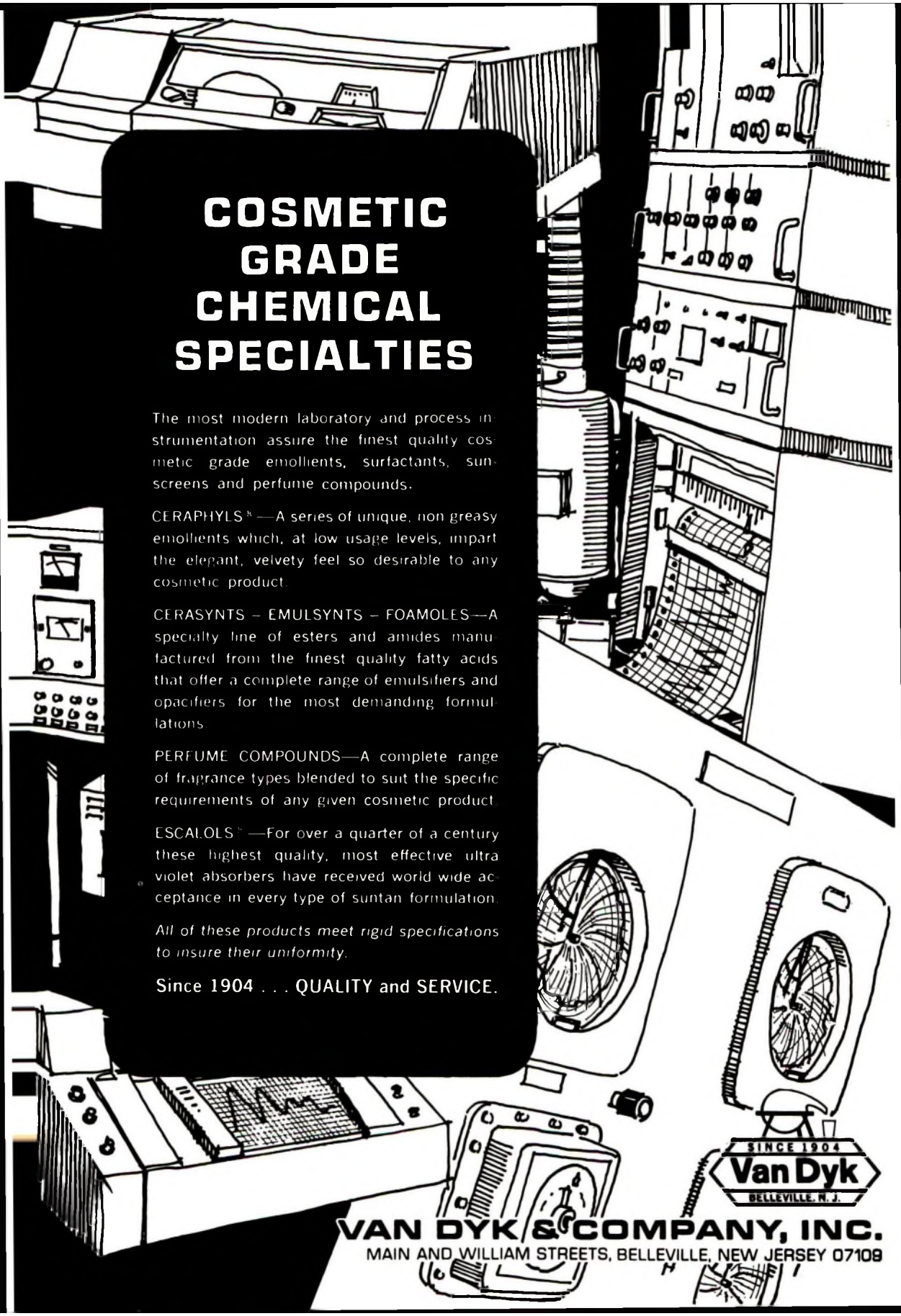

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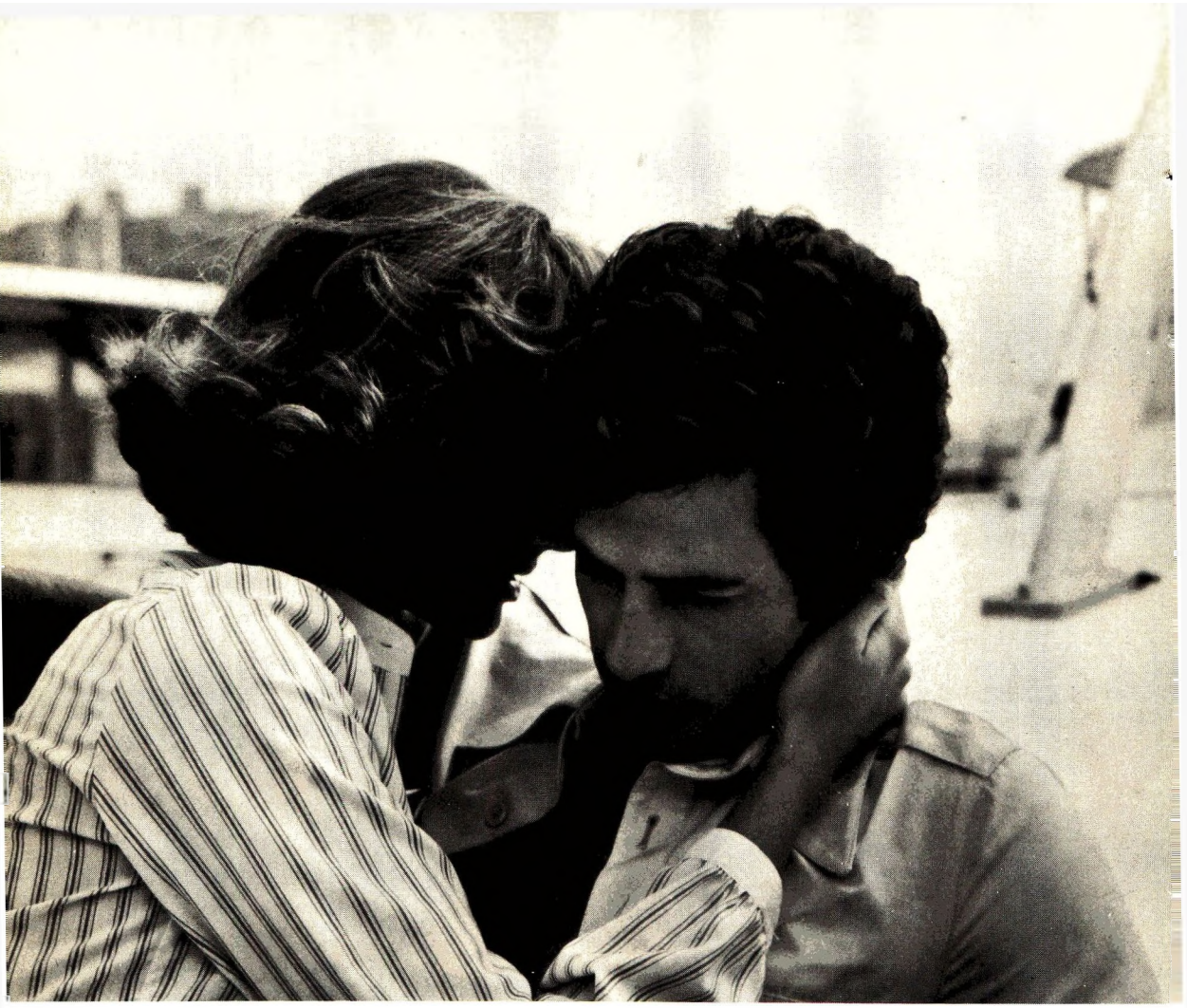

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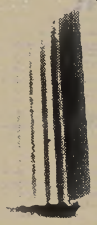

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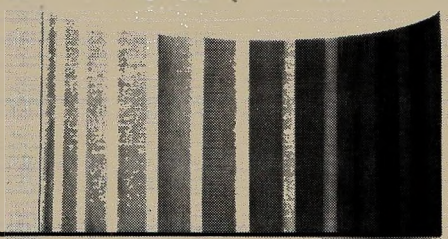

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