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Journal of the Society of Cosmetic Chemists

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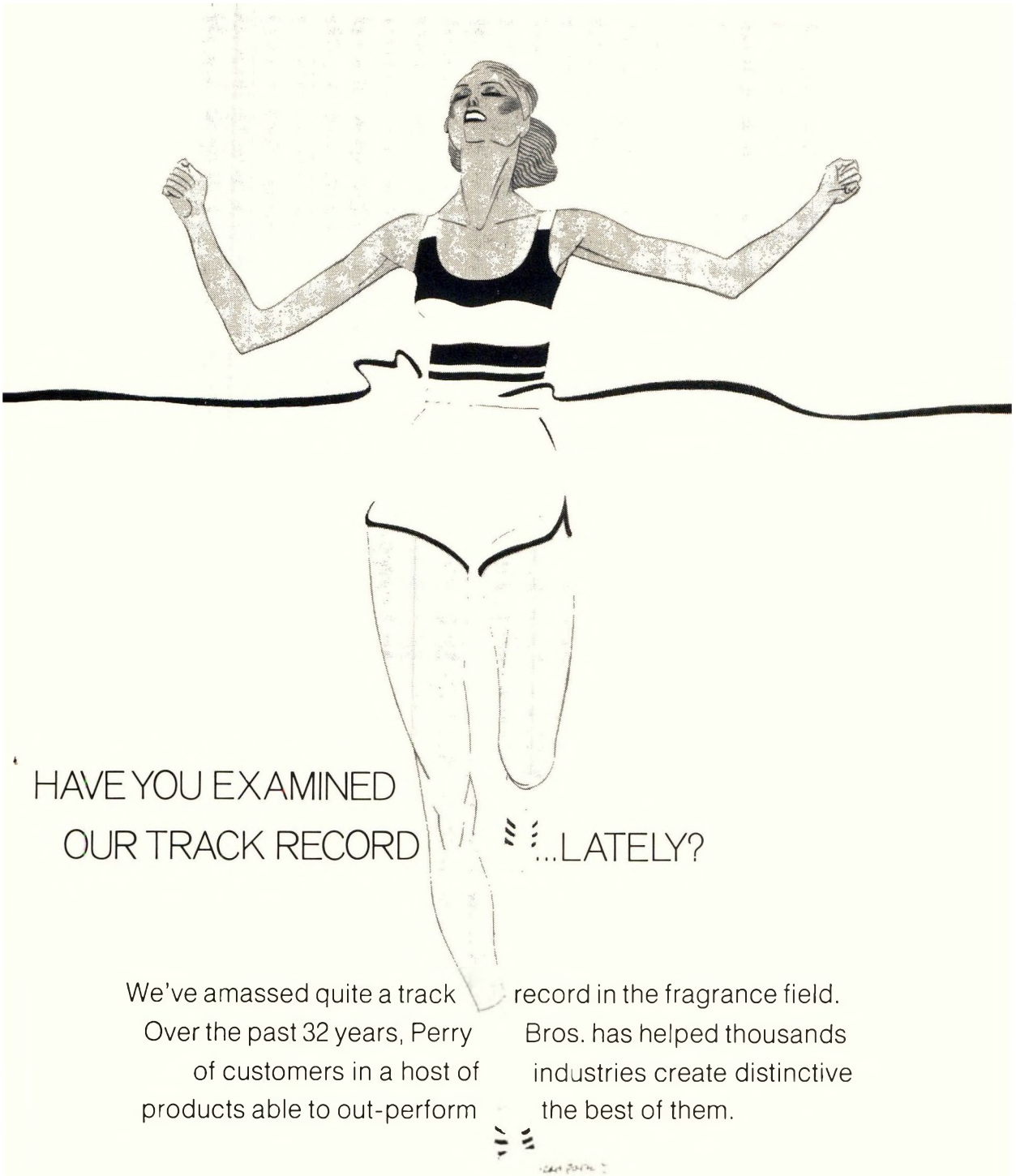

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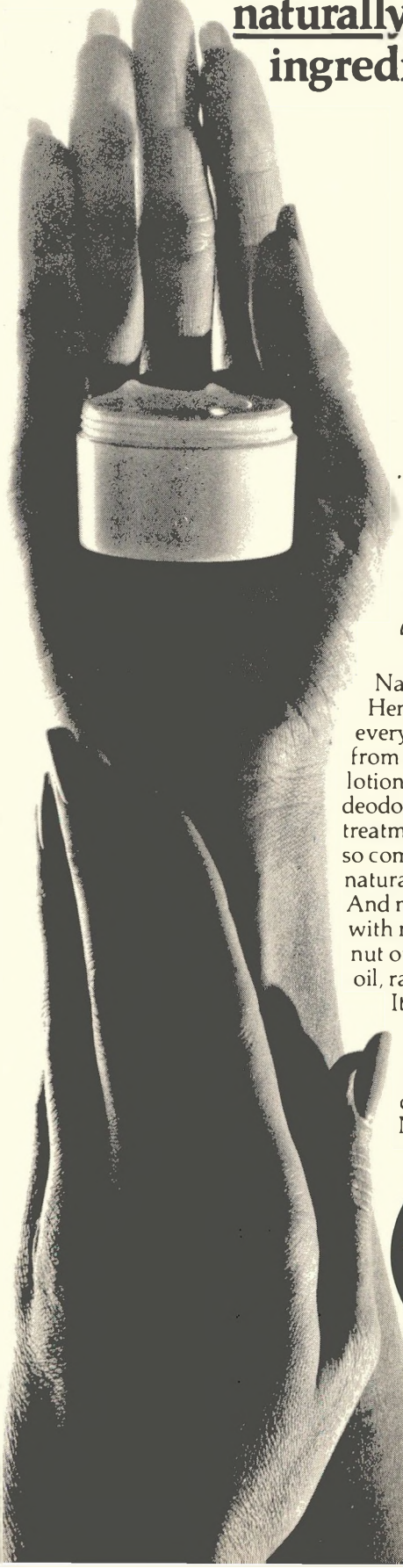

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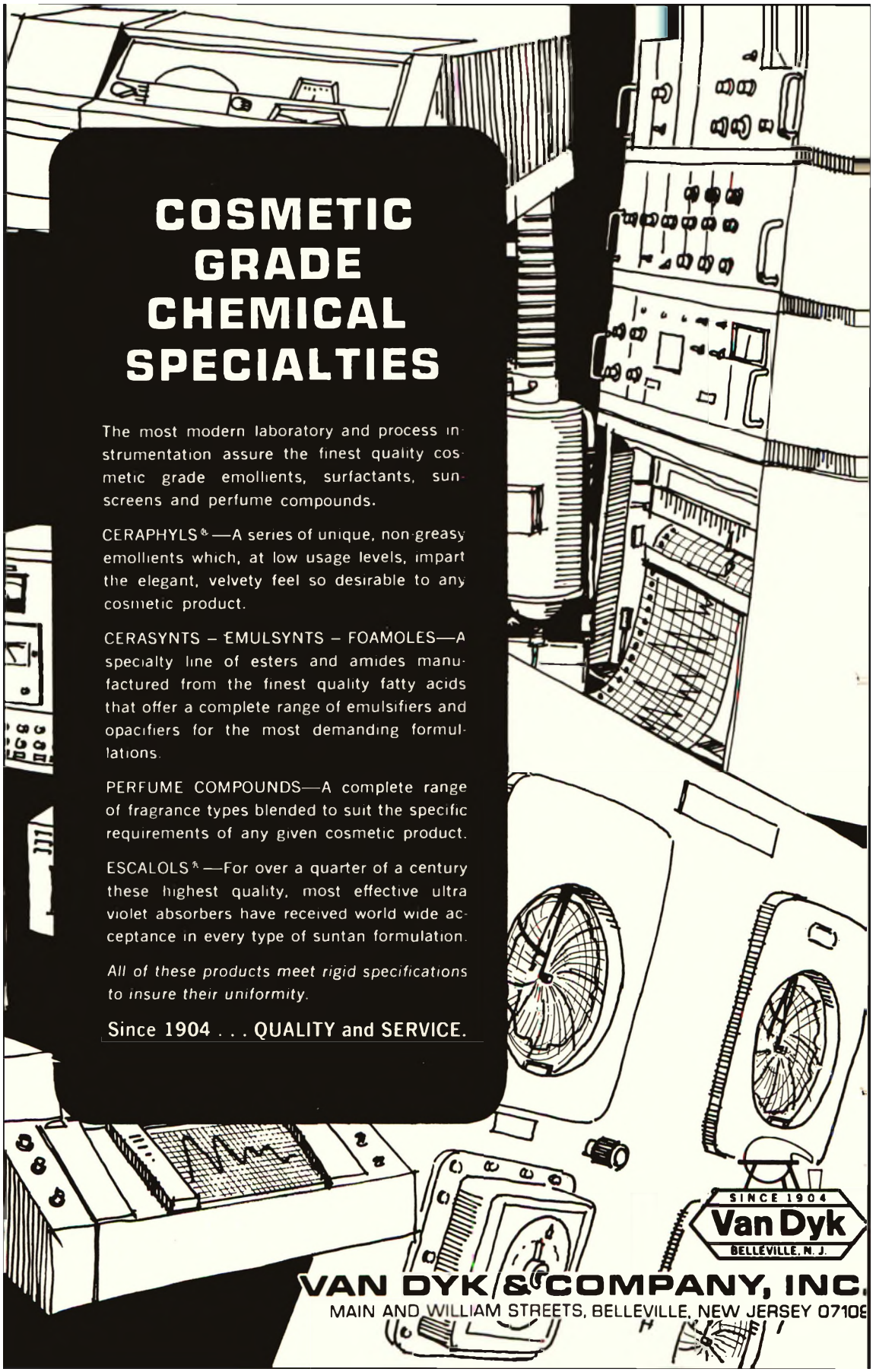

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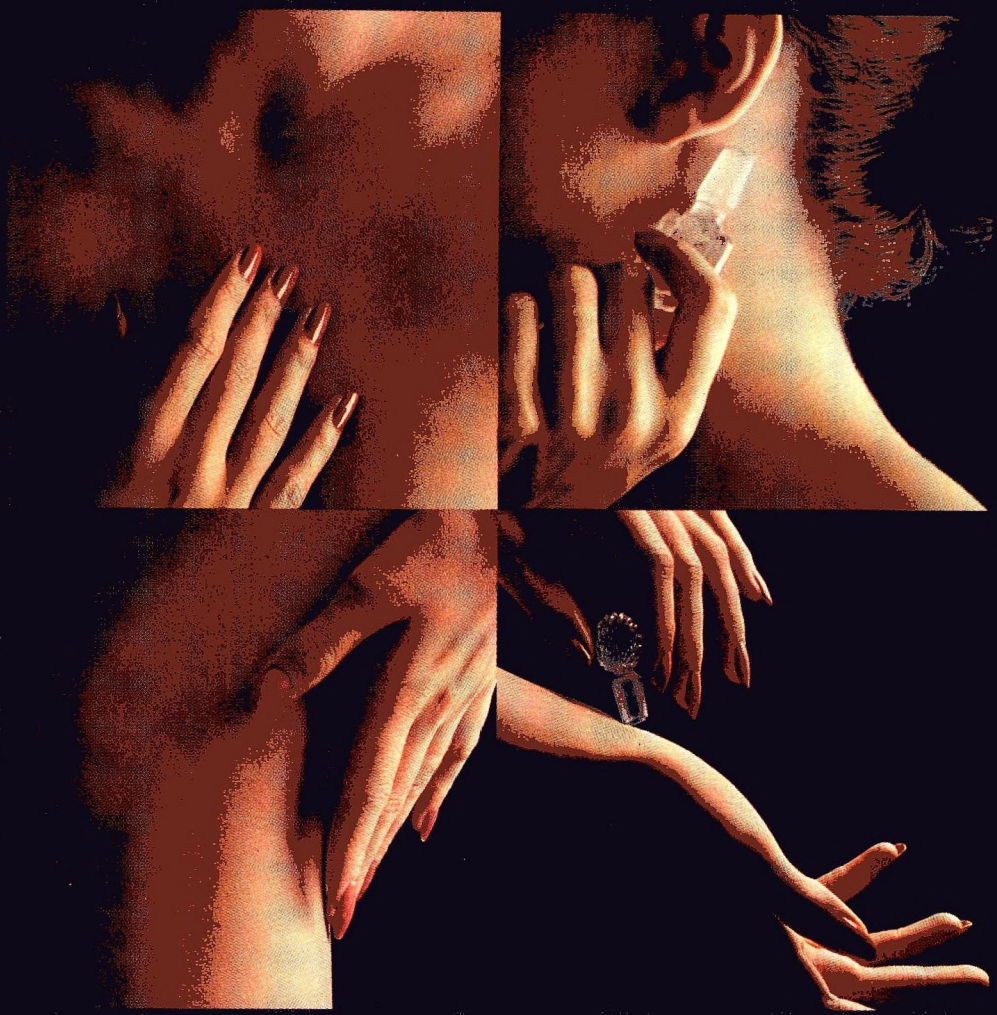

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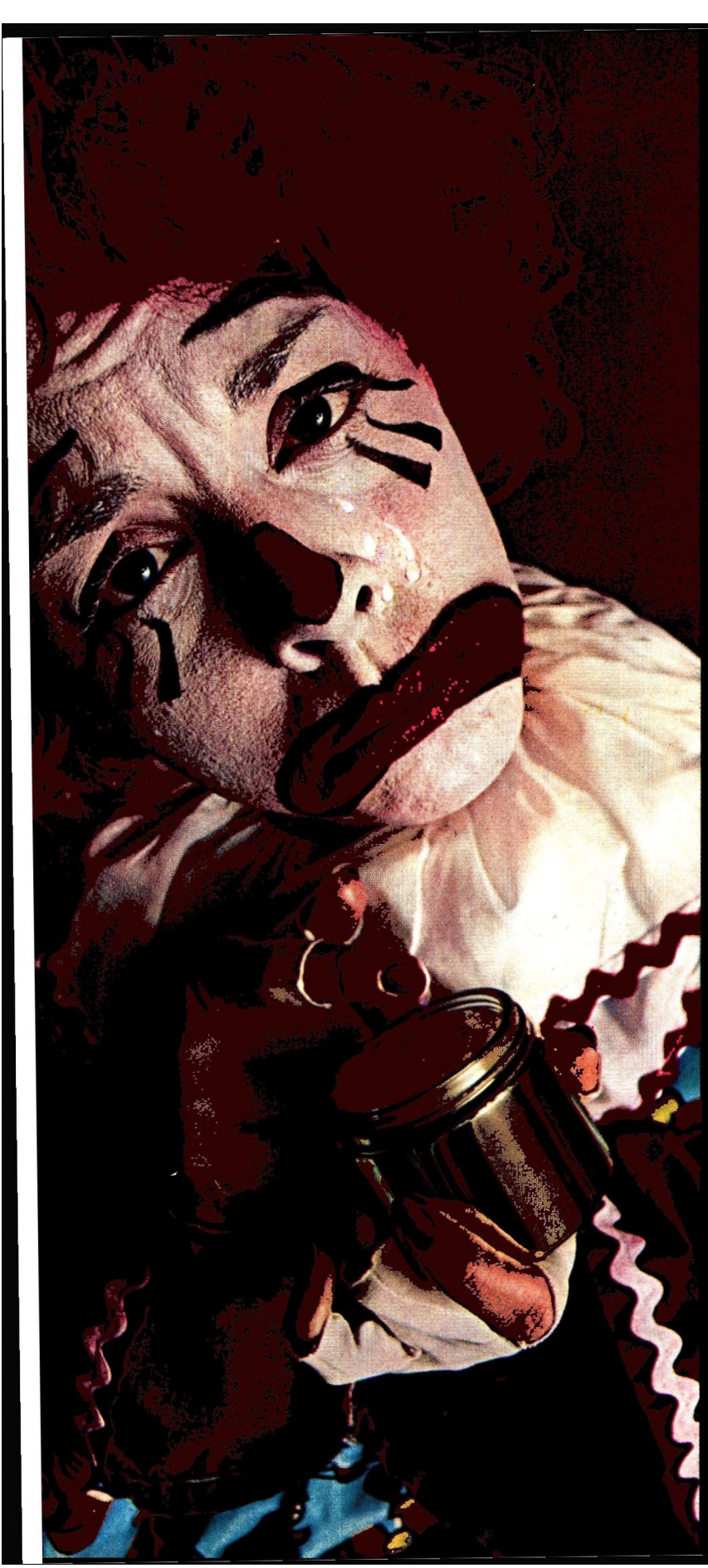

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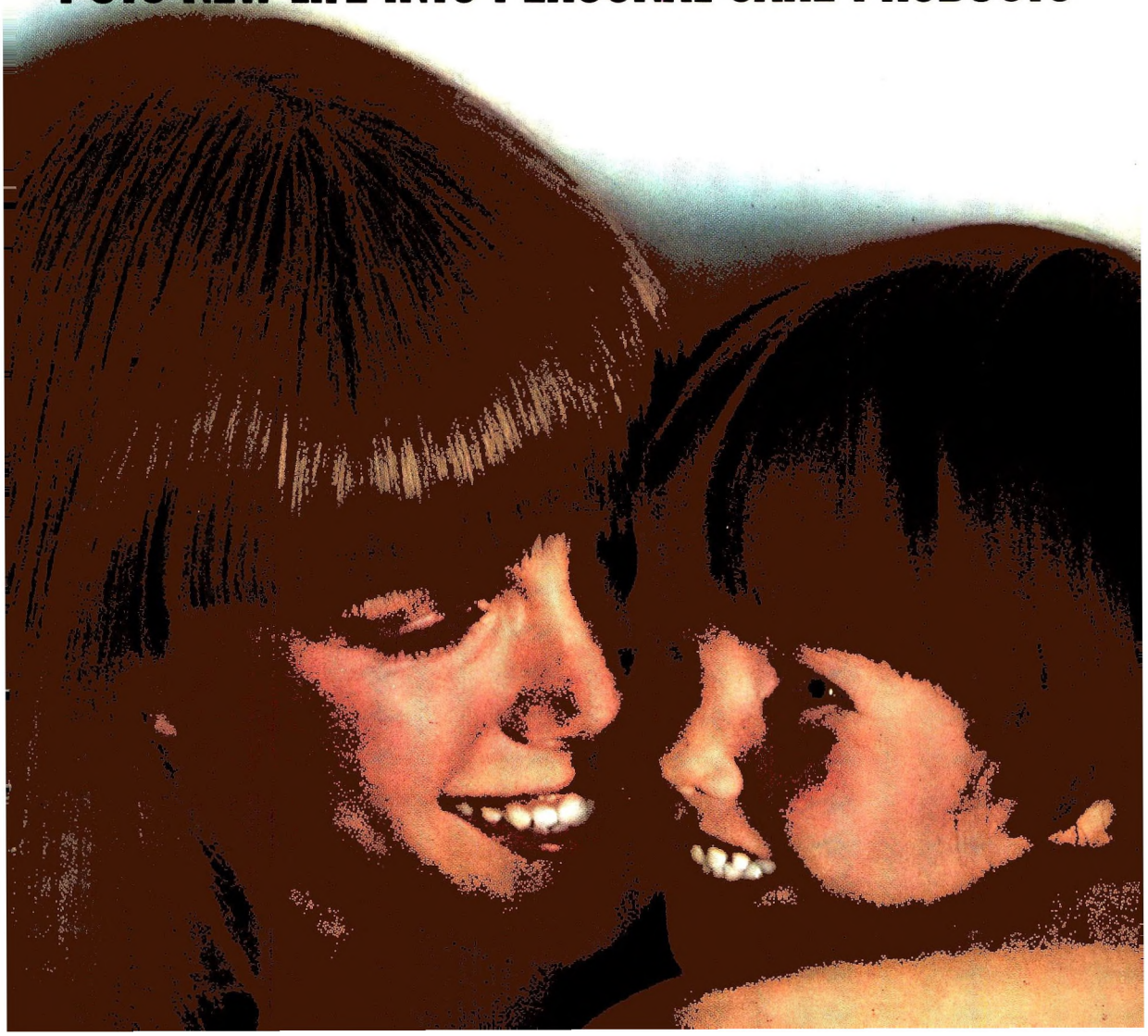

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- Recipient of SCC Scholarship will present paper
- Medal Award Dinner Dance

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Dr. Peter Sgaramella

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.

Thermodynamics and physical properties of a lyotropic mesophase (liquid crystal) and micellar solution of an ionic amphiphile: Arthur R. Mlodozeniec. *Journal of the Society of Cosmetic Chemists* **29**, 659 (November 1978)

Synopsis—Concentrated aqueous surfactant solutions can be phase examined to determine the extent of anisotropy present under varying thermodynamic conditions. This study describes the physical properties of nafoxidine hydrochloride, an ionic amphiphile which exhibits both thermotropic and lyotropic mesomorphism. Micelles, ordered fluids and liquid crystals were detected in stepwise aggregation as the kinetics of micellization was investigated during a drug development program.

Utilizing microscopic and thermal analysis, the phase behavior of a concentrated amphiphile system was studied. Turbidimetric and nephelometric data were utilized to determine the presence of "middle" (nematic), viscous isotropic and "neat" (smectic) mesophases. At lower concentrations, the micelle-monomer equilibrium was investigated utilizing classical spectrophotometric and surface activity measurements.

Thermodynamically stable phases have been individually identified which form emulsions, gels and lyogels. The solubility characteristics of a drug, cosmetic or toiletry product may be obscured if the molecule exhibits the lyotropic mesophase and micellar behavior of a surfactant. These are important to the cosmetic chemist who is charged with developing a stable product.

Permanent waving: utilization of the post-yield slope as a formulation parameter: David W. Cannell and Linn E. Carothers. *Journal of the Society of Cosmetic Chemists* 29, 685 (November 1978)

Synopsis—The stress-strain curve for keratin had previously been utilized to assess the action of cosmetics on the hair. The post-yield slope of this curve has been correlated with changes occurring to the disulfide bonding under permanent waving conditions. The evaluation of this mechanical parameter can be related to the time of processing, rate of penetration of mercaptan and the level of disulfide cleavage. It can further be utilized to assess the effects of formulation variables such as concentration, pH, structure of the mercaptan and formulation additives. The ease of this technique can save the chemist considerable time in optimizing a permanent wave formulation.

Strukturuntersuchungen von salben 1. mitteilung: röntgenstrukturuntersuchungen an der hydrophilen salbe DAB 7: C. Führer, H. Junginger and S. Friberg. *Journal of the Society of Cosmetic Chemists* **29**, 703 (November 1978)

Synopsis—In order to characterize the crystalline state of Hydrophilic Ointment DAB 7 as well as of its components, X-ray studies (using Kiessig's low angle technique and goniometric diffractometry) have been carried out. Both cetyl and stearyl alcohols crystallize in a mixture of β 0- and γ 4-polymorphs. Depending on the conditions of crystallization one or the other polymorphic phase is in excess. The mixture of these two alcohols, cetostearyl alcohol, forms mixed crystals with a uniform Bragg's distance, which is between the two β 0-polymorphic forms of the single components. On the other hand sodium cetyl sulfate and sodium stearyl sulfate crystallize as separate entities from their mixtures. The predominant species in Emulsifying Wax (90% cetostearyl alcohol and 10% sodium cetyl sulfate and sodium stearyl sulfate) are mixed crystals, which consists mainly of cetyl alcohol and sodium cetyl sulfate and partly of stearyl alcohol. In addition, mixed crystals of stearyl alcohol and sodium stearyl sulfate are found in small amounts. In Hydrophilic Ointment DAB 7 both types of mixed crystals form a framework of gel in which the white soft paraffin and the liquid components of the white petrolatum are immobilized either mechanically or by capillary attraction. Since the wide angle interferences are nearly identical, it may be assumed that the paraffinic carbon chains of all components of the ointment can form similar orthorhombic subcells.

Characterizing aluminum-skin interaction by an electrometric technique: David T. Floyd. *Journal of the Society of Cosmetic Chemists* **29,** 717 (November 1978)

Synopsis—Observed differences in the antiperspirant behavior of aluminum chlorohydroxide and aluminum chloride have been attributed to differences in their interaction with skin. The literature contains many references to methods for measuring the interactions of exogenous materials with skin. The electrical properties of skin have been used successfully as a means by which to describe this effect and it was thought appropriate to investigate this approach with respect to aluminum salts. Instrumentation and techniques for measuring the electrical impedance of excised epidermal membrane were developed. The effects of two aluminum salt antiperspirants on the impedance of guinea pig stratum corneum were measured. Aluminum chlorohydroxide reduced the impedance five times more than aluminum chloride. The results are in agreement with reported skin sorption behavior for these salts and with their antiperspirant activities in vivo. The hypothesis that antiperspirancy is based in part on antiperspirant/skin interaction is supported by the present study. The ELECTROMETRIC method described herein was found to be a viable TECHNIQUE for studying these interactions.

Structure-function relationships of surfactants as antimicrobial agents: Jon J. Kabara. *Journal of the Society of Cosmetic Chemists* **29,** 733 (November 1978)

Synopsis—structure-function relationships of various classes of surfactants as antimicrobial agents have been reviewed. It was concluded that while polar groups of the biocide tend to predict activity against a given genera, the chain length of the lipophilic group determines the most active member of the chemical class. In general, cationic surfactants are more active than anionic and nonionic agents. Optimum chain length for activity is between 10–16 carbon atoms. Gram (−) and yeast organisms are affected by the lower chain members while gram (×) organisms are affected by the longer chain surfactants. Nonionics, which in the past were considered not to have antimicrobial activity, were shown to be active when the mono-esters were formed from lauric acid.

Because of this new property, nonionics, particularly monolaurin (Lauricidin®), may be useful germicides in addition to their surface active properties. Their nontoxic and low irritation properties make them ideal candidates for cosmetic and toiletry formulations.

J. Soc. Cosmet. Chem., 29, 659–683 (November 1978)

Thermodynamics and physical properties of a lyotropic mesophase (liquid crystal) and micellar solution of an ionic amphiphile

ARTHUR R. MLODOZENIEC *Hoffmann-La Roche Inc., Nutley, NJ 07110.*

Received April 25, 1978. Presented at Annual Scientific Seminar, Society of Cosmetic Chemists, May 1978, Chicago, Illinois.

Synopsis

Concentrated aqueous surfactant solutions can be phase examined to determine the extent of anisotropy present under varying THERMODYNAMIC conditions. This study describes the PHYSICAL PROPERTIES of nafoxidine hydrochloride, an IONIC AMPHIPHILE which exhibits both thermotropic and lyotropic mesomorphism. Micelles, ordered fluids and liquid crystals were detected in stepwise aggregation as the kinetics of MICELLIZATION was investigated during a drug development program.

Utilizing microscopic and thermal analysis, the phase behavior of a concentrated amphiphile system was studied. Turbidimetric and nephelometric data were utilized to determine the presence of "middle" (nematic), viscous isotropic and "neat" (smectic) mesophases. At lower concentrations, the micelle-monomer equilibrium was investigated utilizing classical spectrophotometric and surface activity measurements.

Thermodynamically stable phases have been individually identified which form emulsions, gels and lyogels. The solubility characteristics of a drug, cosmetic or toiletry product may be obscured if the molecule exhibits the LYOTROPIC MESOPHASE and micellar behavior of a surfactant. These are important to the cosmetic chemist who is charged with developing a stable product.

INTRODUCTION

Often in the development of a cosmetic or pharmaceutical product formulation, the interaction of a major ingredient with water represents the most critical performance characteristic of the product. Occasionally, the compound is surface active and possesses some very unique physicochemical properties which may result in anomalous behavior in various aqueous systems. An example of such a case is nafoxidine hydrochloride, shown in Figure 1. Investigations of colloidal solutions of this drug have shown that the properties of aqueous systems of the drug exhibit a more or less abrupt change at critical values of concentration.

At lower concentrations the drug behaves as an ideal solution. Higher concentrations lead to micellar behavior and lyotropic mesophases. Nafoxidine hydrochloride may also be classified as a weak surfactant, although its solubilizing action does not

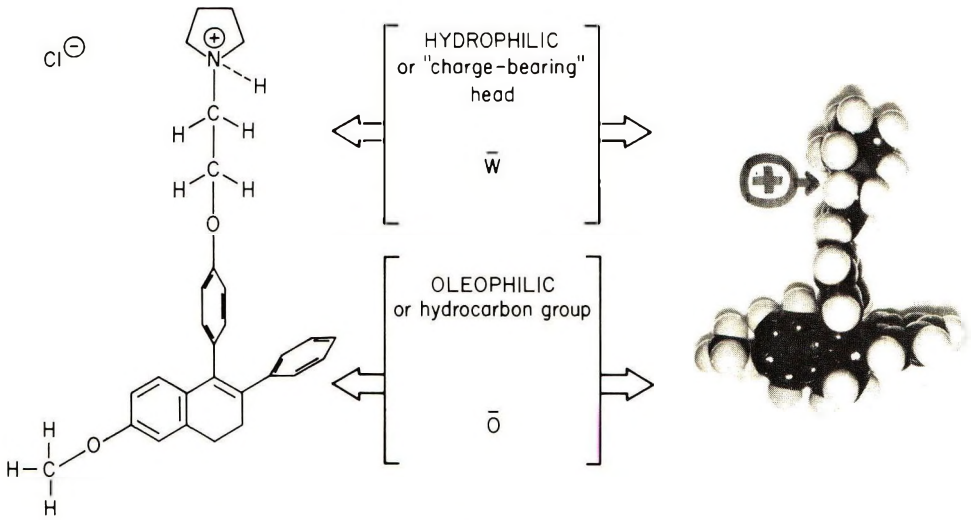

Figure 1. The surfactant cation of nafoxidine hydrochloride may be represented schematically as shown above. The conventional formula on the left is drawn to compare to the Hirschfelder model shown photographed on the right. Amphiphiles are commonly divided into regions of hydrophilic ($\bar{w}$) and of oleophilic ($\bar{o}$) characters which are linked by amphiphilic ($\bar{c}$) layers.

meet the usual criteria for such specific activity. It is a long-chained aromatic (diphenyl dihydronaphthalene) derivative, the oleophilic portion, $\bar{o}$, of a cyclic amine salt (pyrrolidine), the hydrophilic portion, $\bar{w}$, as described in Figure 1. It is essentially water soluble only in acidic solution. The salt ionizes to form the long-chain cation and simple chloride anion. Since the concentration of protonated amine cation is dependent on the ionic equilibrium between free amine and the charged amine, a striking pH dependency is expected and has been found experimentally.

The cation of nafoxidine hydrochloride is surface active. The chloride ion is the counter-ion or gegenion and is important as an electrolyte in colloidal solutions of the drug. The presence of other additives, especially electrolytes, greatly influences the solubility and phase behavior of the drug in aqueous media. If a hot saturated solution of the compound is cooled, the deposition of liquid crystalline states is observed. Alcoholic solutions of the drug do not show anisotropic behavior and a high degree of hydration appears to be a necessary prerequisite for the occurrence of the lyotropic mesophases.

Phase examination of aqueous systems of nafoxidine hydrochloride reveals the presence of thermodynamically stable phases other than the mobile isotropic solutions (monomer and micellar). The dissolution of the drug follows a typical profile:

$$\text{Crystal} \xrightarrow[H_2O]{} \text{Neat Phase (Smectic)} \xrightarrow[H_2O]{} \text{Middle Phase (Nematic)} \xrightarrow[H_2O]{} \text{True Solution}$$

The kinetics of molecular association in the reverse reaction of the above dissolution scheme is quite complex. Micellar aggregation in dilute concentrations of the drug can be followed at lower temperatures (25 and 37°C). However stepwise aggregation of the drug through all the phases could not be observed. The lyotropic mesophase system for aqueous solutions of amphiphilic compounds used by Luzzati (1) and Winsor (2) were employed to describe nafoxidine hydrochloride behavior.

In cases where the kinetics of association controlled the order of appearance of a succession of phases, intermediate or conjugate two-phase systems can be observed with increasing concentration. Such systems led to emulsions, gels and lyogels. These phases show remarkable thermodynamic stability, demonstrating reproducible phase behavior for over 10 years at room temperature. These properties are important to the cosmetic formulator who is charged with developing a stable product.

EXPERIMENTAL

A. MATERIALS

The drug used in these studies was nafoxidine hydrochloride ($C_{29}H_{32}O_2NCl$), pyrrolidine, 1-{2-[*p*-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]ethyl}-hydrochloride, which was prepared for pharmaceutical use by chemical and physical property profile. Specifications for the drug showed reproducible X-ray diffraction, NMR and other spectroscopic confirmation, elemental analysis and physical data. The melting point of the drug ranged from 169–189°C, depending on the description of various thermal transition temperatures. These shall be discussed under "results." Observed simple solubility (obtained from the turbidity change) of the drug was approximately 1.4 mg/ml at 37°C, and the observed intrinsic dissolution rate was approximately 4 mg/cm^2/hr. The rate is determined by a standardized procedure using 3/8" (9.5 mm) pellet of a compound mounted by means of a polyethylene holder in the center of a 4-fluid-ounce, (120-ml) amber, oval bottle attached to a wheel rotating at 6 rpm. The

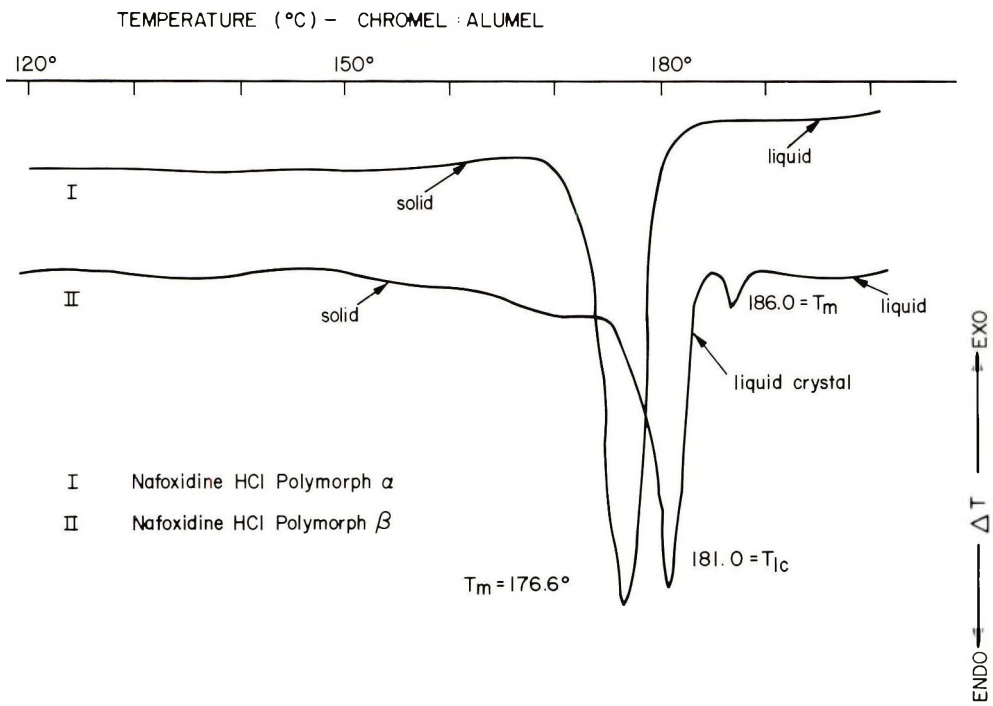

Figure 2. DTA thermogram of two polymorphic forms of nafoxidine hydrochloride. Polymorph β shows a crystal-mesomorphic (smectic) transition, T_{lc} followed by a mesomorphic-isotropic transition, T_m.

rate is that rate where the % saturation of the solution is 0 to ca. 10%. The UV spectrum shows a peak at $\lambda = 303$ mμ with an $\epsilon = 17{,}150$. Beer's law is not followed when water is the solvent. For purposes of colloidal investigations, only profiled lots were used in the studies reported.

The water used was all-glass, double-distilled deionized and had a specific conductance of 0.5-1.0 $\times 10^{-6}$ ohm^{-1} cm^{-1}. Surface tension studies additionally were performed on CO_2-free water to minimize aging effects.

B. INVESTIGATIONAL STUDIES

1. *THERMAL ANALYSIS OF CRYSTALLINE NAFOXIDINE HYDROCHLORIDE* was performed by DTA and hot-stage microscopy. The Dupont Differential Thermal Analyzer Model 900 was used to generate thermograms of each lot of drug. A typical thermogram is shown in Figure 2 for two different polymorphic forms of the drug. Thermograms were typically run at heating rates of 10°C/min under a nitrogen atmosphere on a sample size of 1 mm × 3.5 mm tube-containing volume. Glass beads were the reference. Form α was used to describe the lower melting polymorph. Form β was used to describe the polymorph which showed thermotropic mesomorphic behavior. A Kofler-Thomas hot stage mounted on a Leitz microscope equipped with 43X and 10X objectives and crossed polarizers was used for microscopic thermal analysis. A heat baffle and glass bridge were used to minimize thermal fluctuations. Aqueous

Table I
Surface Tension Measurements

Nafoxidine HCl Concentration			Measured Surface Tension (γ)			
			25°C		37°C	
mol/l	mg/ml = C	Log C	t_0	t_x	t_0	t_x
1.08×10^{-1}	50.0	1.7	53.3[a]		51.6	
2.17×10^{-2}	10.0	1.0	51.6	49.8	49.4	49.6
1.08×10^{-2}	5.0	0.7	49.5	49.8	49.8	49.3
6.49×10^{-3}	3.0	0.5	50.4[a]		48.7	
4.33×10^{-3}	2.0	0.3	49.9		47.5	46.2
3.25×10^{-3}	1.5	0.2	49.8[a]		47.3	
2.17×10^{-3}	1.0	0.0	48.8	49.2	44.4	44.3
1.62×10^{-3}	0.75	−0.1	48.5[a]		46.6	
1.08×10^{-3}	0.50	−0.3	50.0	50.7	50.1	49.4
6.49×10^{-4}	0.30	−0.5	53.7[a]		52.0	
4.33×10^{-4}	0.20	−0.7	52.9	57.4	60.8	54.5
2.17×10^{-4}	0.10	−1.0	57.2		59.6	58.5
1.08×10^{-4}	0.05	−1.3	58.9		61.9	
4.33×10^{-5}	0.02	−1.7	60.4	59.2	64.0	
2.17×10^{-5}	0.01	−2.0	68.2	68.3	67.0	
1.08×10^{-5}	0.005	−2.3	67.2		66.8	
4.33×10^{-6}	0.002	−2.7	68.5		69.0	
2.17×10^{-6}	0.001	−3.0	69.3			

[a] These solutions were prepared and measured at a later time to permit an evaluation of several intermediate values of interest.

t_x for 25°C was 1–3 days and t_x for 37°C was 19 days.

t_0 represents initial values.

systems were analyzed microscopically as well using a special depressed slide with micro cover glasses.

2. *SURFACE TENSION STUDIES* were done by the ring method on the Du Noüy Tensiometer as commonly described. A platinum-iridium ring was used. For nafoxidine hydrochloride solutions, the ring method is excellent because it can be used even if time effects are involved. Surface tension values can change with time because the composition of a new and an aged surface are different. Thus dynamic γ (nonequilibrium) values of very fresh surfaces of nafoxidine hydrochloride solutions can differ from their equilibrium γ values. It should be borne in mind that the rupture of the surface in the ring method does not give an equilibrium value for surface tension. However the method is so rapid that it can be used for measuring γ of rapidly aging systems. The concentrations employed are shown in Table I. Samples were prepared by dilution from an initial stock solution of 50 mg/ml. Samples were also equilibrated at the desired temperatures, 25 or 37°C, in a controlled water bath ±1°C. A jacketed sample holder was used in all determinations to control the temperature to 25.0 or 37.0°C (±0.5°). The results listed in Table I are averages of triplicate determinations. The data are plotted in Figure 3 (25°C) and Figure 4 (37°C).

3. *MOLECULAR WEIGHT DETERMINATIONS BY OSMOMETRY* were done on the Mechrolab Inc. Vapor Pressure Osmometer, Model 301. The principle involves an isothermal distillation. The Vapor Pressure Osmometer measures the difference in temperature between a drop of pure solvent and a drop of solution of sample, each suspended on separate thermistor beads. A known sample is also run as a standard in the

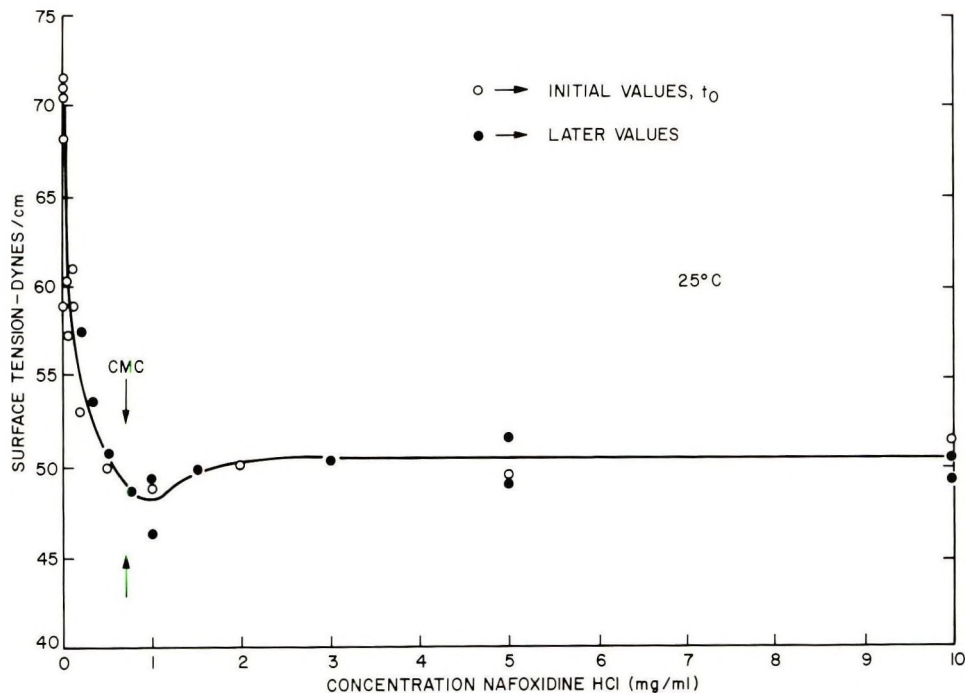

Figure 3. Surface tension-concentration curve for nafoxidine hydrochloride at 25°C showing critical micelle concentration minimum at 0.70 mg/ml.

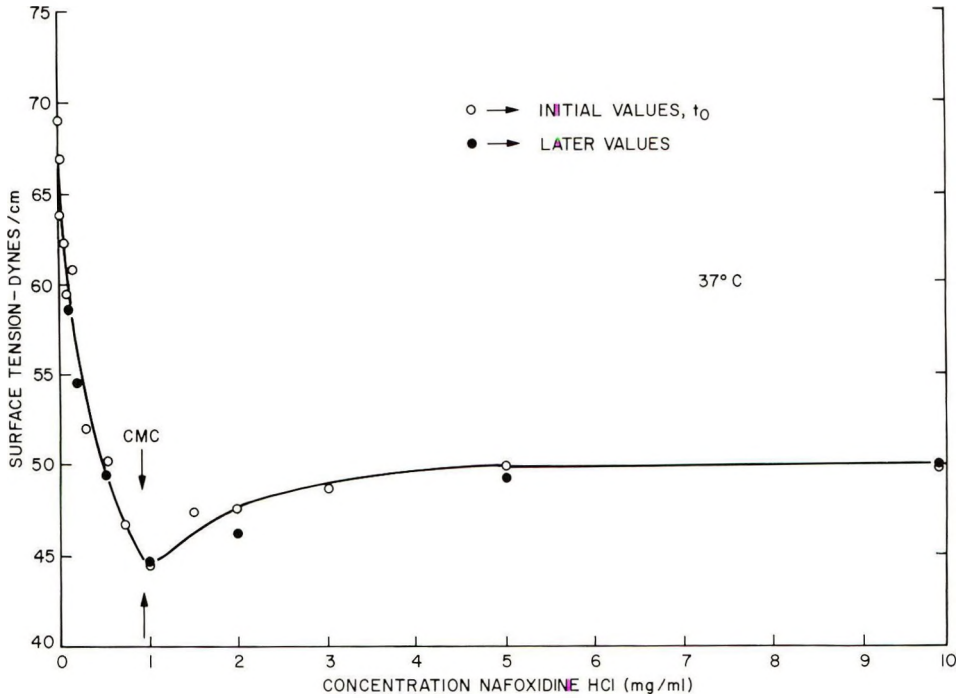

Figure 4. Surface tension-concentration curve for nafoxidine hydrochloride at 37°C showing critical micelle concentration minimum at 0.83 mg/ml.

same solvent. Thus in these studies the solvent was water and the standard was sucrose. The difference in temperature between solvent and solution after vapor equilibrium is a colligative effect. This temperature difference is detected on a thermistor bridge circuit by measuring electrical resistance. The measured resistances for a series of aqueous solutions of nafoxidine HCl at different concentrations at 37°C are shown in Table II and plotted in Figure 5.

Since nafoxidine HCl is an electrolyte and dissociates in water to form chloride ion and drug cation, at infinite dilution it will have a theoretical apparent molecular weight

Table II
Vapor Pressure Measurements by Thermoelectric Osmometry

Conc. mg/ml	Nafoxidine HCl Molarity	Resistance Ohms	Conc. Sucrose Molarity	Apparent[a] Mol Weight of Nafoxidine HCl
0.01	2.16×10^{-5}	0	0	0
0.088	1.91×10^{-4}	0.10	1.5×10^{-3}	59
0.460	9.94×10^{-4}	0.10	1.5×10^{-3}	307
1.012	2.19×10^{-3}	0.30	4.3×10^{-3}	235
2.012	4.54×10^{-3}	0.50	7.3×10^{-3}	276
4.959	1.07×10^{-2}	0.55	8×10^{-3}	621
9.984	2.16×10^{-2}	0.45	6.5×10^{-3}	1536
		0.70	1.0×10^{-2}	
		1.40	2.0×10^{-2}	

[a] Actual molecular weight of nafoxidine HCl is 462.

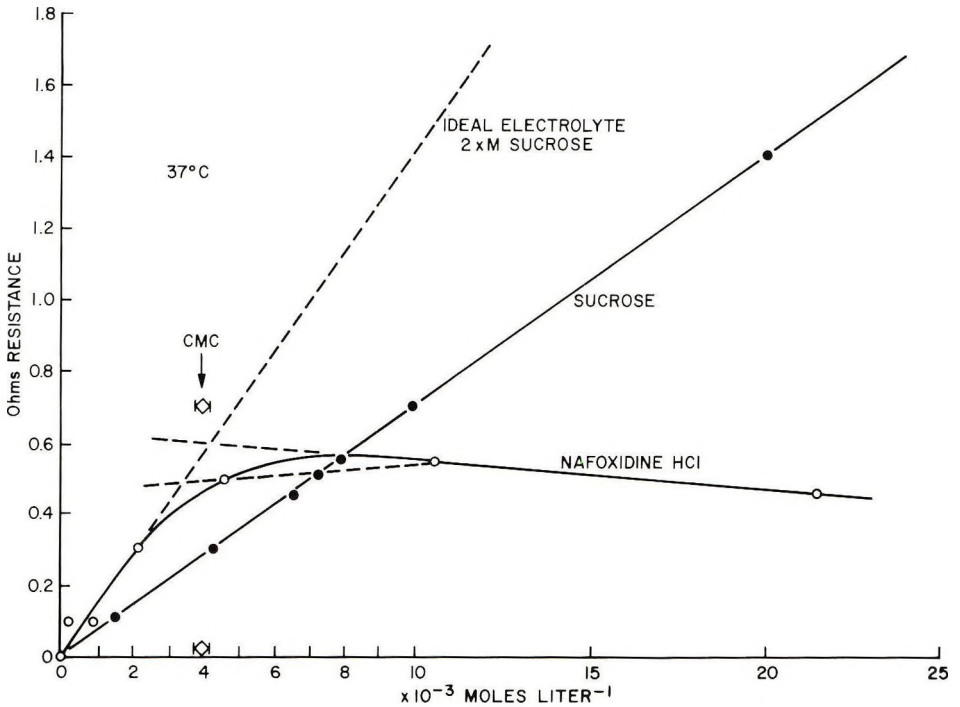

Figure 5. Molecular weight determination by thermoelectric osmometry of nafoxidine hydrochloride and sucrose standard at 37°C, showing a cmc value at 4×10^{-3} mol l^{-1} for the apparent levelling effect. Adjusting for ionic dissociation, the cmc range is then near 2×10^{-1} mol or 0.92 mg/ml.

equal to one-half of the true molecular weight. Thus the apparent molecular weight value as a function of concentration will vary, depending on the activity coefficient. If other effects, such as dissociation of cation to free amine, decomposition, or association such as micellization are involved, further variation and departure from ideality will occur. Sucrose is relatively well behaved and gives a response which is typical for singularly dispersed, monomolecular species. Completely ionized substances, which are not aggregated or associated, should also give a linear response of resistance with changing concentration. However, in many cases of ionizing substances in aqueous solutions, departure from ideality is common.

4. *LIGHT SCATTERING MEASUREMENTS* were all performed at 25°C because of the difficulty in adapting the commercial instruments to temperature control at other ranges. It is recognized that turbidimetric measurement of a solution at only one temperature is not sufficient to determine a solubilization end point (because turbidity can arise from either micellar solubilization limits or cloud point depression). Nephelometric measurements, however, were more carefully controlled at 25°C, providing a data obtainable span with a much broader range especially at lower monomer populations where turbidity by spectrophotometry is quite inaccurate.

Three methods of measuring turbidity were utilized. The first method measured the ratio of intensity of the scattered light, the Tyndall light, to that of the incident light by absolute or direct measurement of the Tyndall ratio. The second method measured the same effect as the first, but compared the intensities to a standard of known concentra-

tion (nephelos standard). The third method measured the ratio of intensity of the light transmitted through the solution to that of the incident light. The first two methods (tyndallmeter and nephelometer) are more sensitive to extreme dilutions while the turbidimeter is good at middle and higher concentrations.

a. *Tyndallmetric measurements* were made on a Coleman Model 14 absolute angular light scattering photometer using green light at $\lambda = 546$ mμ radiation. The photometer was calibrated against the opal glass diffusor supplied with the instrument. The temperature of the cell chamber and solutions were equilibrated and monitored by use of a surface temperature probe to maintain 25 ± 0.1°C. The results are plotted in Figure 6.

b. *Nephelometric measurements* were performed on the Coleman Model 7 Photo Nephelometer utilizing a concentrated-filament bulb yielding radiation at $\lambda = 700$ mμ (Model 7-500 exciter lamp suitably filtered). Coleman Certified Nephelos Standards were used in this operation so that haze readings were recorded in arbitrary Coleman Nephelos Units spanning the region from absolute clarity to the zones of visible turbidity. The temperature was controlled as in the tyndallmeter readings. The nephelos scale extends from 0-130 units and it is within that range that is found the most precise linearity in the relationship between Tyndall-light intensity and colloid concentration. The use of the nephelos standards permitted a proportional reduction of the response of the instrument to the standard of known value which gave results in arbitrary units as fractions of the true Standard Nephelos value. This made possible the

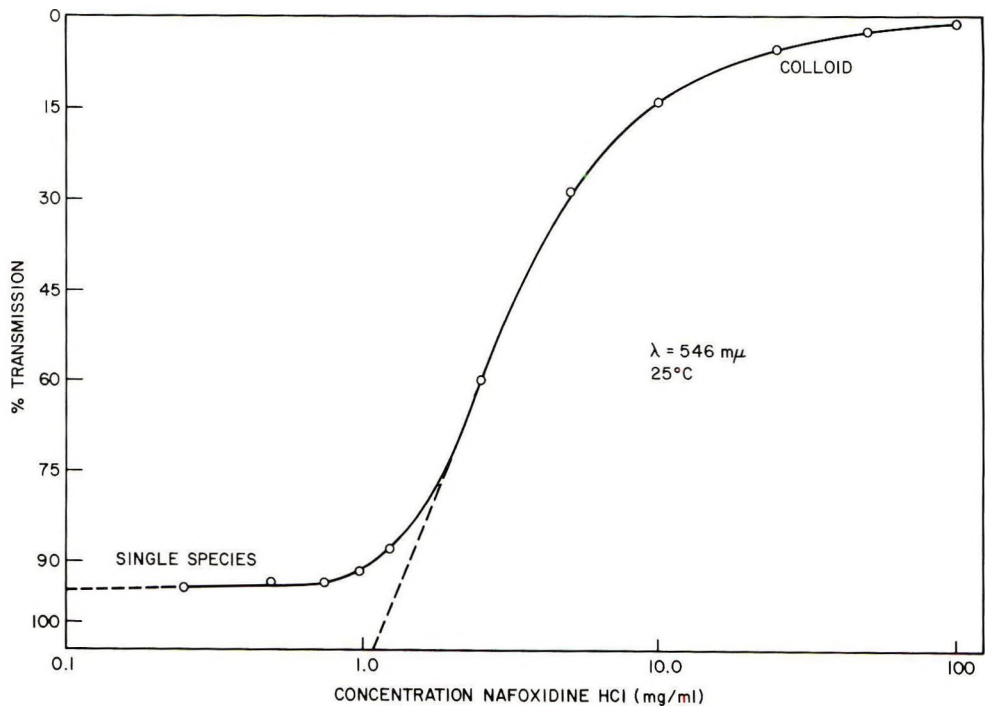

Figure 6. Absolute angular photometric light-scattering curve for nafoxidine hydrochloride at 25°C showing tyndallmetric cmc value extrapolated at 1.2 mg/ml.

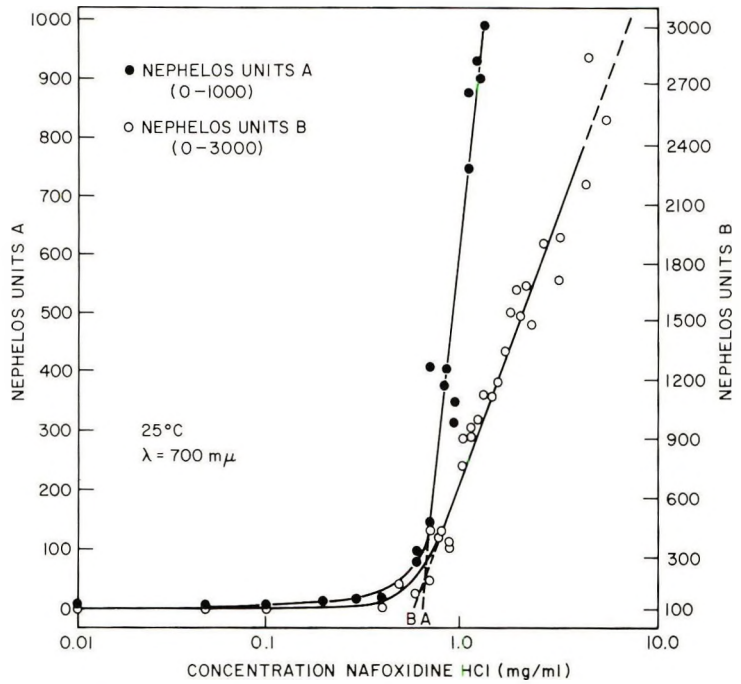

Figure 7. Nephelometric determination of aqueous solution of nafoxidine hydrochloride at 25°C plotting nephelos units vs. log concentration. The formation of haze is observed as an increase in light scattered. The extrapolated value of 0% transmission (light scattered) at 0 nephelos units for A and B units is 0.68 mg/ml and 0.75 mg/ml, corresponding to observed cmc values.

measurement of more turbid systems. Two series of nephelos units, A and B, were employed to extend the reference range from 0-1000 units and 0-3000 units, respectively. The readings of nafoxidine hydrochloride were extremely temperature dependent and care was needed to avoid thermal body contact or prolonged reading in the instrument. Data are shown in Figure 7.

c. *Turbidimetric measurements* were performed on an Aminco-Bowman Model Spectrophotometer at $\lambda = 700$ mμ measuring at an angle of 90°. Cuvettes with a 1-cm path were thermally controlled by jacketting the sample chamber and circulating water controlled at 25 ± 0.1°C. Because of the wide concentration range followed, a log-log plot was used to record the data shown in Figure 8. Deviations may be expected at high concentrations because of two reasons: part of the scattered light is scattered again, some of it re-entering the transmitted beam, or, as the viscosity of the turbid colloid increases, air bubbles become entrapped within the cuvette, creating a distorted signal.

5. *PHASE EXAMINATIONS WITH THE POLARIZING MICROSCOPE* were made on a Leitz Ortholux instrument equipped with a Bertrand lens, a 10X eyepiece and a hot stage. Microscopic textures were defined and identified utilizing phase classifications described by Rosevear (3) and Winsor (2,4). Thermotropic phase changes were observed on the crystalline nafoxidine HCl at heating rates of 1°/min in the transition regions. A small sample of the drug was heated on a microscope slide until the crystals passed to the isotropic liquid. The mesophase or mesophases were seen to pass across

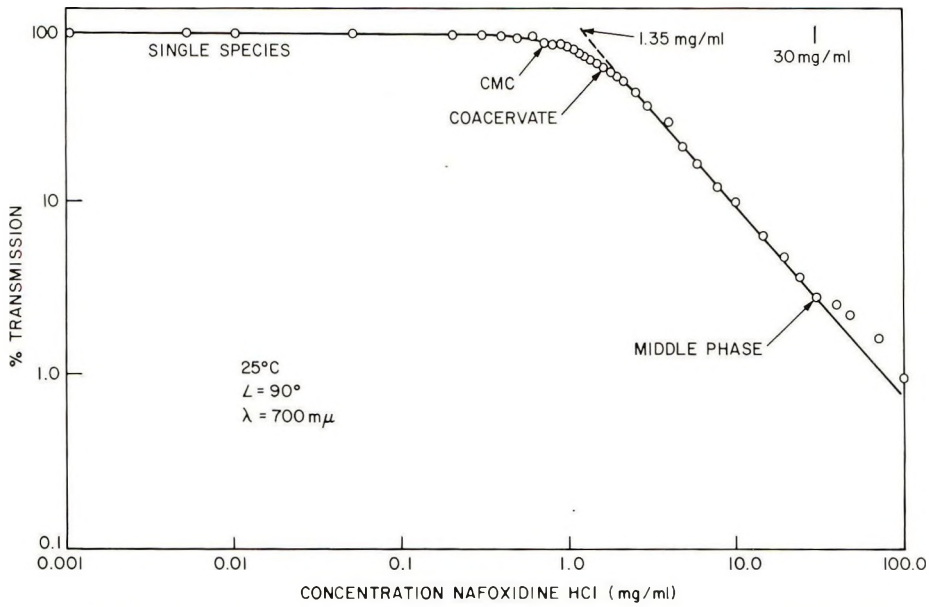

Figure 8. Log-log plot of turbidimetric data for aqueous solutions of nafoxidine hydrochloride at 25°C showing observed cmc values at 0.7 mg/ml. An extrapolated value of 1.35 mg/ml for a second critical concentration represents the onset of turbidity for a phase change (coacervate) which represents the appearance of the middle phase. The log turbidimetric behavior shows a departure from linearity above 30 mg/ml.

the field of view as successive, well defined, opaque regions. The solid-mesomorph, mesomorph-mesomorph, and mesomorph-isotropic solution changes were observed across the preparation as distinct fronts. All mesomorphic phases were found to be birefringent (doubly refractive) as well as showing uniaxial and positive optical signs. These are characteristic of both neat (smectic) and middle (nematic) liquid crystal textures. Figure 9 shows a micrograph taken under a 10X objective, displaying a focal conic texture with fanlike terrace fronts. Occasionally oily streaks can be observed in this turbid, viscous state. This phase was identified as a smectic mesophase.

Lyotropic mesophase transitions were observed under controlled temperature conditions at 25 and 37°C. For other temperatures, rapid heating rates of 10°C/min were used to minimize evaporation and may have led to slightly higher temperatures in recording a phase change. Three lyotropic phases of nafoxidine HCl observed at 25°C are shown in Figures 10, 11 and 12. These same phases were also observed at 37°C although evaporation represented a problem. By drying the micellar solutions at high temperature (25°C) and 37°C, each system was seen to pass through a sequence of mesomorphic phases. These observations were used to construct a phase diagram, shown in Figure 13. This phase diagram was constructed for phases which were observed by various methods listed in Table III. At higher temperatures above 37°C, only a few birefringent textures could be detected (too weak to be photographed). Additionally, the viscous isotropic region usually contained a conjugate solution which separated into a clear isotropic phase combined with either the middle or neat phase. The appearance of a homogeneous isotropic solutions was used to judge the transition points for this phase.

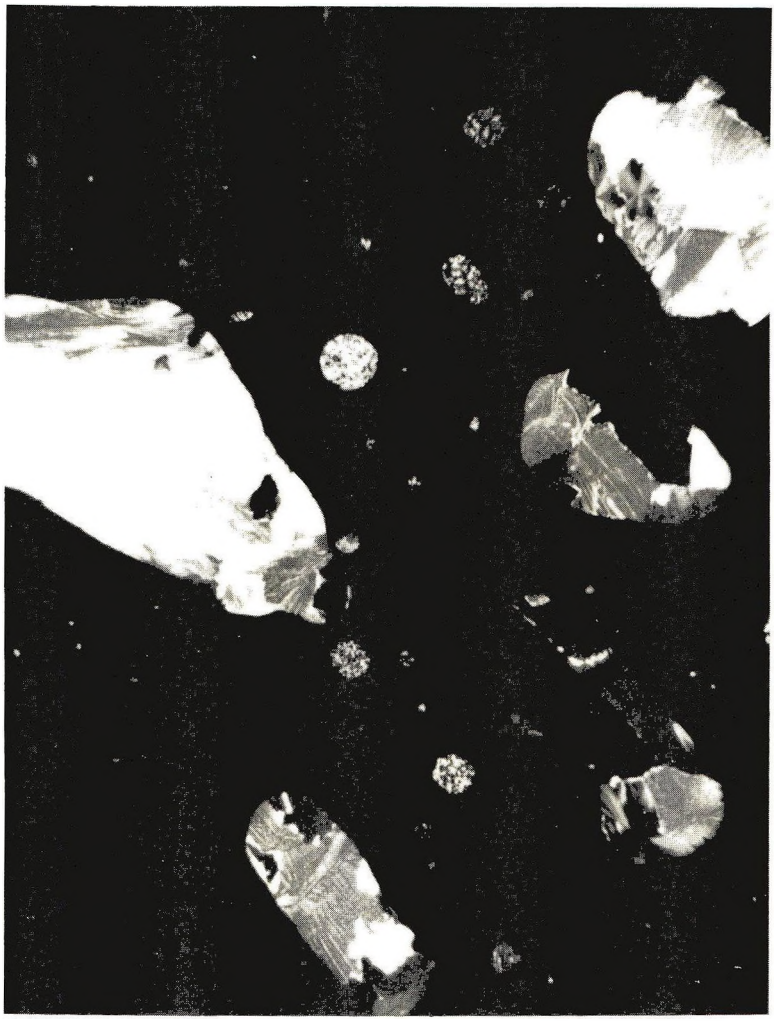

Figure 9. Thermotropic liquid crystal micrograph showing smectic mesophase with birefringent liquid layer at 10X objective under crossed Nicol prisms observed at approximately 183°C.

The microscopic texture of the middle phase was easiest to detect (its presence is shown on the phase diagram of Figure 13 by using X as data points). The middle phase was characterized by its threaded texture. It appeared as a turbid liquid with regions of birefringence occurring in various sequences such that the surface-active material itself, rather than water, was the continuous medium. The thread-like texture, also called fibrous, has a very mobile state. The typical birefringence with characteristic striations is shown in the Figure 10 micrograph.

At depressed temperatures, a heterogeneous separation of the micellar solution was observed in which a soap curd formed. The temperature at which the solution became opaque was observed to be approximately 12°C for several of the dilute systems. This temperature was regarded as the Krafft point. This point can be regarded as the melting point of the hydrated nafoxidine hydrochloride, above which the surfactant dis-

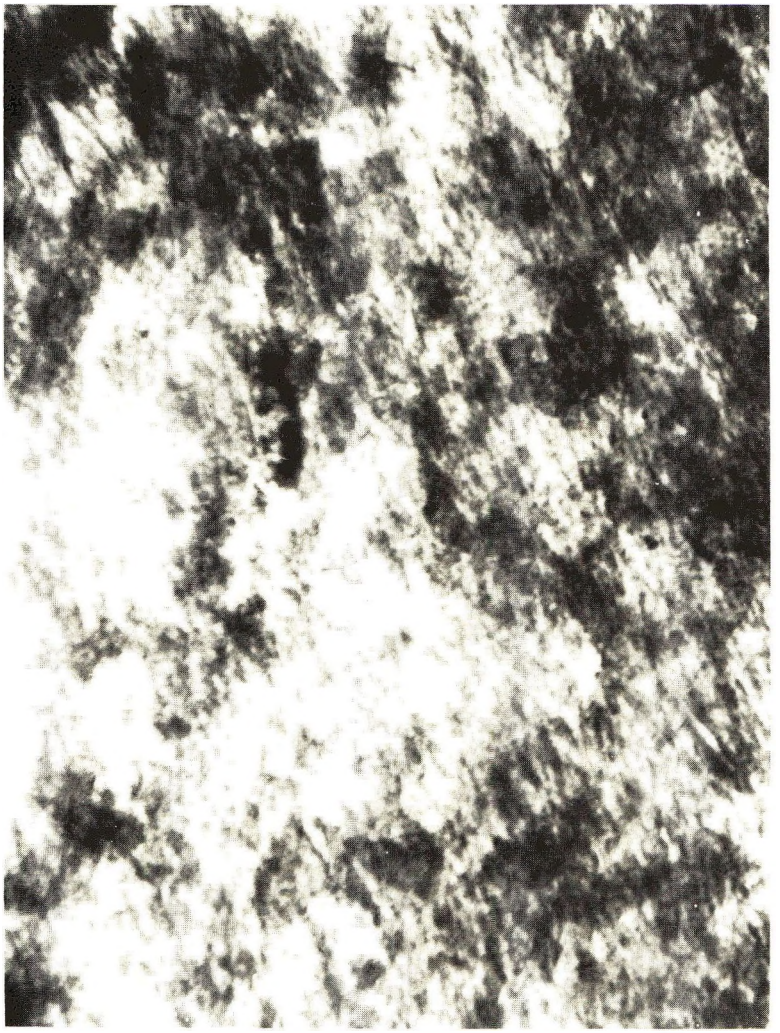

Figure 10. Micrograph of lyotropic mesophase of aqueous nafoxidine hydrochloride (approximately 10–30%) showing birefringent liquid layer at 43X objective under crossed Nicol prisms observed at 25°C.

perses in solution as micelles. This transition could not be observed at higher concentrations where the liquid was more viscous and turbid. The Krafft point can also be explained as the temperature at which the solubility of the hydrated crystalline drug reaches the cmc (critical micelle concentration) for micelle formation. These transition phases were indistinguishable by polarizing microscopy at 12°C.

The viscous isotropic phase could be separated from its conjugate solutions by centrifugation, but it was not physically stable alone at room temperature and usually developed neat phase as evaporation occurred.

The polarizing microscope can only show textures which suggest a distribution of the molecular orientation, as contrasted with the distribution of molecules. The high degree of orientation observed in the lyotropic mesophases described above has been

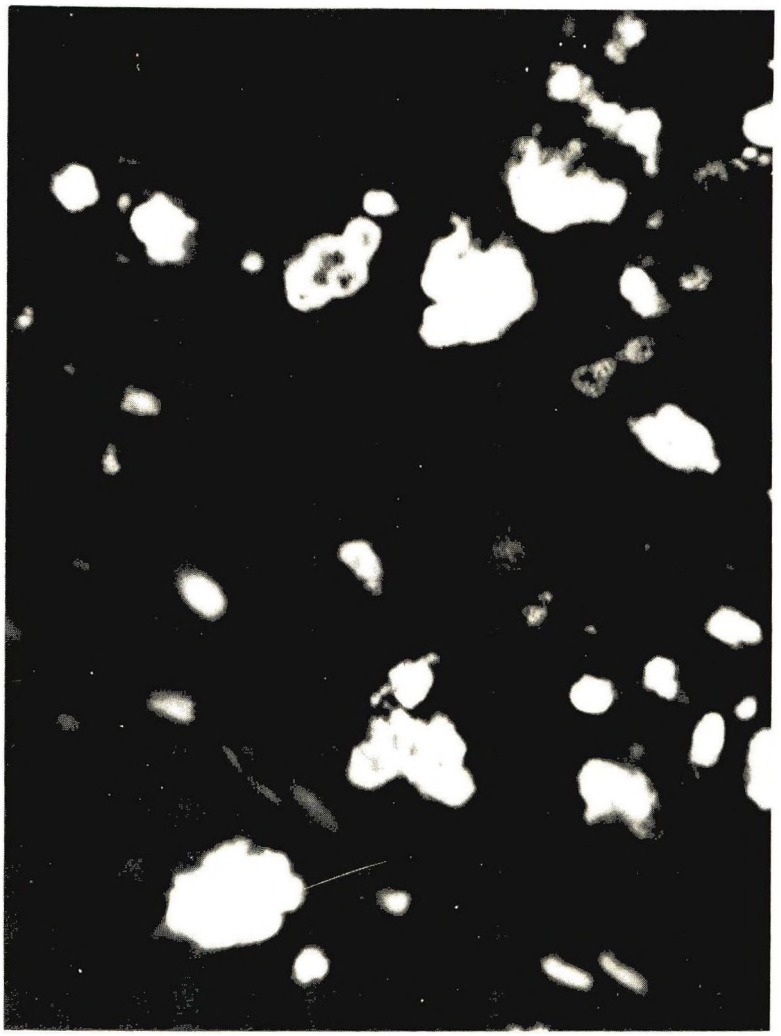

Figure 11. Micrograph of lyotropic mesophase of aqueous nafoxidine hydrochloride (approximately 70–90%) showing focal conic and spherulite texture with birefringent liquid layer at 43X objective under crossed Nicol prisms observed at 25°C.

structurally analyzed by X-ray and NMR investigations and has been described by many workers (see ref 5 and 6 for reviews). Rosevear (7) has also reviewed his microscopic texture classification in a Society of Cosmetic Chemists seminar paper.

RESULTS AND DISCUSSION

Nafoxidine hydrochloride exhibits both thermotropic and lyotropic mesomorphism. The drug is known to be polymorphic from X-ray diffraction and infrared evidence. Thermal analysis of the β polymorphic form shows that the compound undergoes a transition to a liquid crystal phase identified as smectic by optical characteristics. This solid form can be investigated in aqueous systems for various phase changes either by

Figure 12. Micrograph of concentrated viscous turbid lyotropic mesophase of aqueous nafoxidine hydrochloride (>95%) showing microcrystalline hexagonal isolates in rod-like forms in a focal conic textured birefringent liquid layer at 43X objective under crossed Nicol prisms at 25°C.

diluting concentrated phases of the drug or by concentrating micellar solutions through evaporation. These lyotropic mechanisms are of special interest at 25°C and at 37°C because these represent ambient storage conditions at room temperature and physiological temperature, respectively. The 37°C behavior is most often studied in pharmaceutical formulations for evaluating solubility and dissolution rate behavior of drugs and dosage forms.

Intermicellar Equilibria have been shown for various amphiphilic compounds in a scheme described by Winsor (2) and are depicted for nafoxidine hydrochloride in Figure 14. The formation of micelles from monomers can be assumed to follow a mechanism in which

$$n[Naf^+]+m[Cl^-] \overset{Km}{\rightleftharpoons} [Naf_nCl_m]^{n-m}$$

where n is the aggregation number (Cl) is the gegenion, (Naf^+) is the cation and Km is the cicellar equilibrium constant such that

$$Km = \frac{[\text{cations}]^n[\text{gegenion}]^m}{[\text{micelles}]} = \frac{(aNaf^+)^n(aCl^-)^m}{(aNaf_nCl_m)}$$

Thus micellization can be described by the law of mass action. Micelle formation is a thermodynamically reversible process and the micellar solution obeys the phase rule

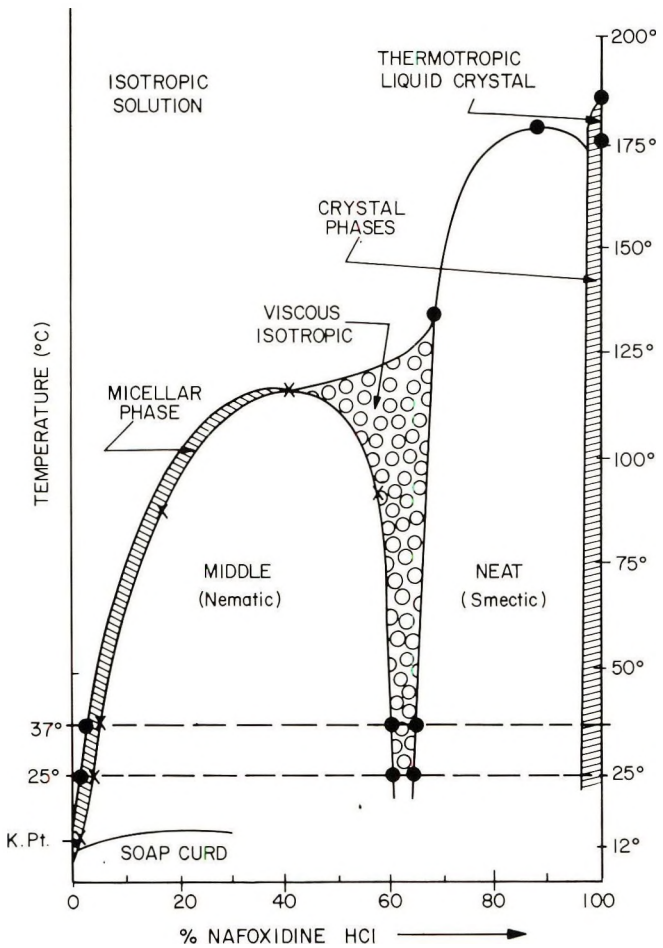

Figure 13. Binary phase diagram of nafoxidine hydrochloride in water as a function of mol % of drug in water. Phases observed at 25°C and 37°C isotherms (shown as dotted tie lines) are, sequentially: isotropic solution, micellar phase, middle (nematic) phase, viscous isotropic phase, neat (smectic) phase and crystalline solid. A Krafft point is estimated at approximately 12°C for dilute phases where a soap curd appears. The shaded micellar phase and shaded hydrated crystal phase are estimated from both thermal and spectrophotometric data. The speckled viscous isotropic phase is observed only in heterogeneous systems of conjugate solutions involving either middle or neat phases with an isotropic gel phase. Data points marked X represent the presence of the middle phase with other phases.

Table III
Phase Examinations of Nafoxidine HCl/Water Systems

Nafoxidine HCl Concentration mg/ml	mol %	Temperature, °C		Method of Observation[a]	Phase(s) Observed
1.0	2×10^{-3}	12	X	PM	Soap Curd
0.7	1.5×10^{-3}	25	O	Surface Tension, Nephelometry	Micellar
1.35	2.7×10^{-3}	25	X	Turbidimetry	Middle
	60	25	O	PM	Viscous Isotropic[b]
	65	25	O	PM	Neat[c]
1.0	2×10^{-3}	37	O	Surface Tension, Vapor Pressure	Micellar
2.0	4×10^{-3}	37	X	PM	Middle
	60	37	O	PM	Viscous Isotropic[b]
	65	37	O	PM	Neat[c]
	18	85	X	HSM	Middle
	60	88	X	HSM	Viscous Isotropic[b]
	40	113	X	HSM	Middle
	70	134	O	HSM	Neat[c]
	100	176	O	HSM, DTA	Smectic
	90	178	O	HSM	Neat
	100	186	O	HSM, DTA	Crystal

[a] Data points showing X always showed the presence of the mobile turbid middle phase (nematic). PM represents polarizing microscopy, HSM represents hot stage microscopy, and DTA is differential thermal analysis.

[b] Conjugate solutions of the middle turbid phase were observed with the clear isotropic gel.

[c] A conjugate solution or lyogel was observed composed of the neat (smectic) phase with the clear isotropic gel.

for heterogeneous equilibrium. For nafoxidine hydrochloride, micelles are formed because the state in which the hydrocarbon groups ($\bar{o}$) are aggregated possesses a lower energy than that in which they are surrounded by water molecules. Although the energy due to repulsion of the ionic heads ($\bar{w}$) increases in the process of micelle formation, the former effect is expected to control the reaction and, hence, micelle formation is an energy effect.

The aggregation of nafoxidine cations to form micelles obviously causes a new situation regarding the distribution of the chloride gegenions in solution. The ionic heads forming a charged layer on the external surface of the micelle (shown as a Hartley spherical micelle in Figure 14) may be expected to exert a high electrostatic field in the neighborhood of the micelles and therefore to effect the distribution of the chloride counterions around them. The resulting interaction between the micelles and gegenions leads to a lowering of the free charges of the micelles because of an extensive counterion binding. This will affect micellar growth and stability.

In this spherical micelle forming step (shown above in Figure 14), two major energy considerations are involved. Firstly, a transfer of the non-polar $\bar{c}$ region of the long chain ion from the aqueous environment to the micelle interior takes place with a lowering in free energy. Secondly, an increase in free energy also occurs due to the aggregation of the long chains in the micelle interior. The interior of the micelle is here designated as $\bar{o}$ because the new hydrocarbon core of this specific spherical micelle

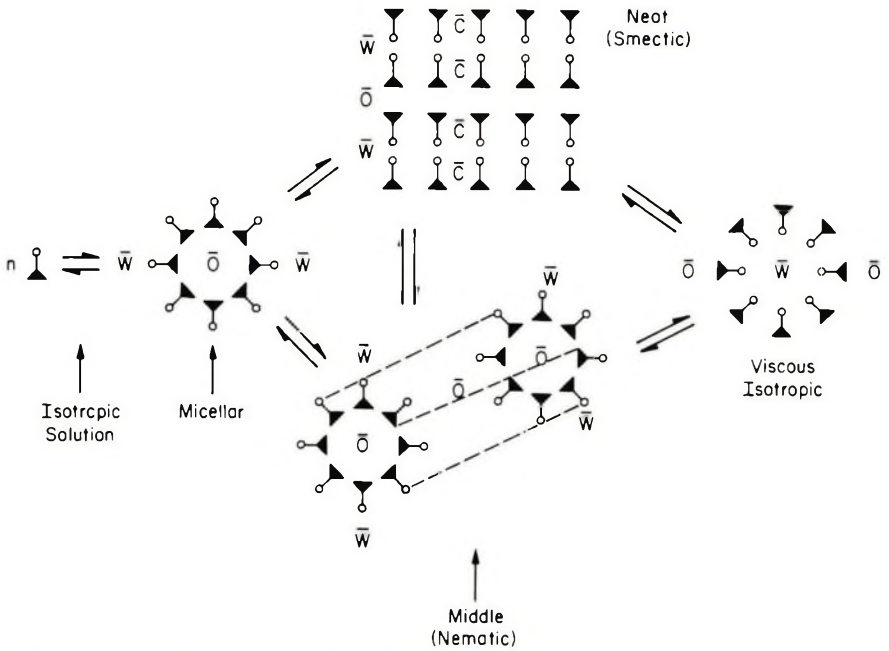

Figure 14. Intermicellar equilibrium and associated phase changes shown by nafoxidine hydrochloride shown as an amphiphile with
$\bar{w}$ hydrophilic section of micelle
$\bar{c}$ amphiphilic section of micelle
$\bar{o}$ oleophilic section of micelle as described in Figure 1
This scheme is described by Winsor (2) to include conjugate solutions at equilibrium mixtures between the phase changes. Anisotropic gel phase may be considered as a lamellar intermediate between the microemulsions of oil-in-water and water-in-oil forms.

renders a fluid nature to this region characteristic of an organic layer. The model shown in Figure 14 may be reversed to show a spherical micelle formed from an amphiphile in an oil or organic hydrocarbon environment. In such a case, the core of the micelle would be a $\overline{w}$ layer. This is shown as a viscous isotropic aggregate in Figure 14.

The micelles are thus, in the case shown, formed when the energy released by aggregating the hydrocarbon portions of the amphiphilic nafoxidine monomer is great enough to overcome the electrical repulsion among the ionic groups and balance the entropy decrease associated with micelle formation. Micelle formation and stability are dependent on temperature and concentration as well as the influence of additives. Micelles can be formed by several classes of amphiphiles, typically cationic, anionic and nonionic surfactants. Each will be capable of forming a liquid crystal phase in subsequent binary or ternary (i.e., emulsion) systems.

A theory of micelle stability based on hydrophobic bonding has been described by Scheraga and co-workers (8) who viewed the physical picture of a micelle as a spherical aggregate of hydrocarbon tails, the polar heads on the surface with entangled tails inside. Using a free valence approach and also considering a micelle as a lattice, they conclude that stable micelles require not only that the free energy per molecule be a minimum at some large degree of aggregation, but also that this minimum free energy should be smaller than that of the monodispersed amphiphile. The theory is related to

the excess turbidity which results with increased concentration of the amphilile above the cmc. This would explain the changes observed in Figure 8. Poland and Seheraga interpret this as evidence of an increase in the most probable micelle size.

The thermodynamics of micelle formation have been reviewed by Hall and Pethica (9). Their treatment is a refinement of the standard treatment of mass action vs. phase-separation models inasmuch as they introduce the small-system thermodynamic approach as applied to mixed micelles. The mass-action model has often been used successfully to predict cmc values. However at higher surfactant concentrations and in multicomponent or solubilized systems this model becomes limited in application. Since micelles are not arranged in a fixed stoichiometry, a multi-species approach to micellar equilibria has also been used in the treatment of micelles as reaction products of surfactants and water. The occurrence of lyotropic mesophase domains in amphiphile-water systems is not readily explained by the mass action model.

The phase separation model for micelle formation considers the cmc as the maximum concentration of molecular dispersion of the surfactant. Micelles are treated as a separate phase, often the justification based on ultra-filtration studies and the fact that the concentration of the surfactant monomer appears constant above the cmc. However apparent phase heterogeneity and electro-inequality (especially for surface layers of charged micelles, ionic surfactants) make a strict thermodynamic treatment of micelles as a phase somewhat difficult. This criticism of the phase model, which altogether is much better for concentrated surfactant systems than the mass-action model, has been the limiting reason for a strict thermodynamic approach to micellization through an entire concentration profile.

The treatment of micelle formation by small-system thermodynamics considers a general macroscopic system of multicomponent solutions and multicomponent micelles. Hall and Pethica (9) also consider the thermodynamics of mixed small systems and regard their overall treatment as chiefly speculative, awaiting experimental verification. In general, this small-system approach to micellization is very similar to the phase-separation model except that the solvent is considered to be in excess and therefore ignored. The assumption is therefore made that the small systems are so dilute in the solvent that they do not interact. For assimilation of this treatment into systems where lyotropic mesophases occur, however, several difficulties arise.

Micelles are in kinetic as well as thermodynamic equilibrium with the molecularly dispersed surfactant. It is generally accepted that micelles can occur well below the cmc and also that micelles cannot exist alone as a phase above the cmc without the presence of monomers in solution. The lamellar micelle in particular (shown as a McBain micelle in part of the neat phase of Figure 14), though often represented to the contrary, depends very critically on the presence of a solution phase for its "sandwich" or bimolecular leaflet structure. Micellization and demicellization occur throughout a surfactant solvent system at all concentration levels. Thus, depending on the overall concentration of the amphiphile in the entire system, it is best to refer to the most probable micellar size as the best representation of the structure. Most probably a statistical concentration of many micelles and micellar fragments (trimer, tetramers, etc.) occur in equilibrium with the recognized micellar size and structure.

The existence of a liquid crystal mesophase in these micellar systems helps in understanding the various anomalous phase characteristics. Being a mesophase thermodynamically, its structure is found to be uniform and homogeneous throughout its

domain under specified temperature and concentration conditions. As such, either the phase-separation model or the small-system thermodynamic approach offers the same convenience for lyotropic mesomorphism in concentrated surfactant systems. Fewer discrepancies due to solvent interplay, micellar size distribution and multicomponent participation (mixed micelles) are associated with accepting the lyotropic mesophase as a separate phase.

The origin of the ambiguous term liquid crystal originated because traditionally these compounds were discovered by their unique optical properties. Thus, while they behaved as solids optically, the compounds had the flow properties of liquids. The optical properties of lyotropic mesophases are still a principal means of identifying the phase transformations and texture. Rosevear (7), in a previous presentation before the Society of Cosmetic Chemists, described the liquid-crystal texture of surfactant mesophases.

Critical Micelle Concentrations were measured in these investigations by several methods. A comparison of observed cmc values is listed in Table IV. The methods selected are commonly employed for cmc determinations; they were, however, especially significant because of their pharmaceutical implication. Both surface activity (as it may affect drug solubility, dissolution and solubilization) and optical properties (as they affect product appearance, stability and spectrophotometric measurements of product performance, i.e., dissolution) are significant physical properties which are designed and controlled in product formulation. A reasonable agreement was found for cmc values determined by the various methods. Tyndallmetric values at 25°C appear a little higher (1.2 mg/ml) than the other methods. This may be explained by the high light-scattering shown by the isotropic solution itself (observed to be about 95% transmission in Figure 6).

The surface tension plots in Figures 3 and 4 show a minimum commonly encountered in such cmc determinations. The descending portion of the curve below the cmc can be explained by impurity adsorbed by surface while the ascending portion of the minimum after the cmc and before the plateau is explained by impurity being solubilized by the micelles. The ring method itself may be a contributor to this minimum. In the measurement of γ values, the true minimum equilibrium value may not be reached. This is evident in the aged-solution values shown in Table I and Figures 3 and 4.

Table IV
Observed Critical Concentrations for Nafoxidine HCl Aqueous Solutions

CMC Value Measured, mg/ml	Temperature, °C	Method
0.70	25	Surface Tension
0.83	37	Surface Tension
0.92	37	Vapor Pressure
1.2	25	Tyndallmetric
0.75	25	Nephelometric A
0.68	25	Nephelometric B
0.7	25	Turbidimetric
1.35[a]	25	Turbidimetric

[a] Represents a second critical concentration in which anisotropicity is observed and a phase change appears.

The temperature dependence of surface tension has been described (10) as resulting from the influence of two factors. One is the vapor pressure of the substance; the γ falls as the saturated vapor pressure rises. The other factor is the increase in the thermal motion of molecules in the liquid phase which leads to increased intermolecular distance; the surface tension falls as the temperature rises. This was observed in solutions of nafoxidine hydrochloride.

The *Ionic Equilibrium of the Amine Hydrochloride* plays a role in the micellar equilibrium. The observed solubility of total nafoxidine species can be represented by the following scheme.

$$\begin{array}{ccc} [\text{Naf} \cdot \text{HCl}]_{\text{solid}} & & [\text{Naf}]_{\text{solid}} \\ K_s \downarrow\uparrow & & \downarrow\uparrow \ K_s. \\ [\text{Cl}^-] + [\text{Naf} \cdot \text{H}^+] & \underset{[\text{HOH}]}{\overset{K_a}{\rightleftharpoons}} & [\text{Naf}]_{\text{base}} + [\text{H}_3\overset{+}{\text{O}}] \\ K_m \downarrow\uparrow & & \downarrow\uparrow \\ [\text{n Naf} \cdot \text{H}^+] & \rightleftharpoons & [\text{Mixed Micelle}] \end{array}$$

where $[\text{Naf}]_{\text{total}} = [\text{Naf}]_{\text{base}} + [\text{Naf} \cdot \text{H}^+] + [\text{n Naf} \cdot \text{H}^+]$

The observed pH of a saturated solution of nafoxidine hydrochloride was 4.6. This can be calculated for appropriate pKa values at 25°C to give an apparent solubility value of 0.7 mg/ml. This value coincides with the measured cmc values. For a given molarity, the concentration of free nafoxidine base present in the solution may be affected by back hydrolysis and as such may exert some effect on the drug solubility (micellar properties). Buffering to a suitable pH range may diminish the extent of free-base contribution. The solubility of free nafoxidine base in water is calculated to be 0.011 mg/ml at 25°C. The free base may act as an impurity in the nafoxidine hydrochloride solutions (micellar and otherwise).

The *Micellar Molecular Weight* was seen to increase at a second critical concentration as observed in the light scattering studies. This is shown in the equilibrium diagram in Figure 14 as the conversion to the middle phase. The micellar units of the phase, consisting of amphiphilic nafoxidine molecules associated in a fluid, reform into parallel cylindrical threads with the external polar groups ($\overline{\text{w}}$) surrounded by water. This liquid crystalline middle phase formed many conjugate solutions for nafoxidine hydrochloride. Winsor (2) has discussed this equilibrium in detail. The turbidimetric data shown plotted in Figure 8 show the observed phase change which represented the onset of visible turbidity, seen in micrograph, Figure 11, of the middle (nematic) phase. The texture of this phase has been described by Rosevear (3) and its structure was determined by Luzzati (1). It is formed by a set of indefinitely long cylinders, regular, two dimensional hexagonal array, and separated from one another by water.

The onset of turbidity may be interpreted as a cloud point (not in the terms of nonionic surfactants that experience an increase in micellar weight with increased temperature). This can be justified inasmuch as a phase separation occurs to form a coacervate in this region. This was described by Langmuir (11) as unipolar coacervation when two kinds of micelles were mixed. Phase separation in surfactant solutions normally occurs on heating for nonionic and on cooling for ionic surfactants. Thus the Krafft point ob-

served for nafoxidine hydrochloride corresponds to the classical case for ionic amphiphiles. Light scattering studies for cationic micelles that form coacervates have previously been reported (12) for systems with both zero and low electrolyte added.

The *Neat (Smectic) Phase* of nafoxidine hydrochloride is observed only in very high concentrations of drug. The kinetics of formation of this phase are such that it was not easily observed in systems in which the crystalline drug was only partially hydrated. This phase could be produced by concentrating the viscous isotropic gel phase by evaporation or by cooling a supersaturated isotropic solution of drug. Micrographs in Figures 12 and 13 showed the texture of phases prepared by cooling to 25°C.

Due to the kinetics of phase formation of the neat phase, there is some uncertainty associated with the phase boundaries for this phase, shown in Figure 9. This phase could not be produced in the concentration range below 65 mol % by the cooling method. Similarly, the stepwise aggregation of micelles in the evaporation method did not produce a neat phase that stayed anisotropic above 125°C. For this reason the kinetics of phase formation complicates the phase diagram, and is reflected in the multiple equilibria scheme in Figure 14 by showing various possible phase transformations between the three lyotropic mesophases observed.

The *Viscous Isotropic Mesophase* for nafoxidine hydrochloride always appeared with the presence of either the middle or neat phases. If centrifuged, the neat phase always settled down as the dense pearlescent bottom layer. A scheme for lyotropic paracrystalline phases different from the scheme in Figure 14 has been proposed by Small and Bourges (13), in which they describe the transition to a cubic liquid-crystal phase as the viscous isotropic phase. This phase is described as a face-centered cubic structure with three-dimensional long-range ordering. It was observed in the stepwise hydration of the crystalline solid as intermediate between the neat and middle phases. Small and Bourges report that under the polarizing microscope this phase appears isotropic, but, if bubbles are trapped within it, they are angularly deformed and do not become spherical with time. This was not observed for the viscous isotropic phase of nafoxidine hydrochloride.

APPLICATIONS TO FORMULATION

STRUCTURAL CONSIDERATIONS

Having established that nafoxidine hydrochloride is an association colloid when in aqueous solution, the pronounced surface activity and micellar aggregation observed are not entirely surprising when the structure of the molecule is considered. The cmc ranges observed are typical for ionic surfactants. The effects of chemical structure or micellar and liquid crystal behavior have been reviewed by Usoltseva and Chistyakov (14). These authors give a detailed description of the effect of aromatic moieties, especially those joined in the *para*-position and the tendency for form mesophases.

The cosmetic chemist should be aware that factors other than impurity can lead to departures for the usual, well defined solid-isotropic liquid transition or solubility-turbidity phenomena. Sometimes supposedly impure pharmaceutical or cosmetic ingredients are apt to be discarded as inseparable mixtures simply because they exhibit lyotropic or thermotropic mesomorphism with which the formulators are not familiar. The reformulation of components based upon results of stability studies often dictates

the selection of costly ingredients, which may have been influenced by micellar or lyotropic mesomorphic behavior. Thus drug, flavor, odor or color changes may be associated with liquid-crystal phase transitions. This is especially true if we rely on optical methods for routine analysis (spectrophotometric, microscopic, light-scattering, etc.)

FACTORS AFFECTING THE CMC VALUES

The implications of the existence of a cmc range for nafoxidine hydrochloride aqueous solutions allows us to predict many physical-chemical properties. At concentrations above the cmc the solution is non-ideal. It should be emphasized, however, that the critical concentration is not a unique concentration value but a *concentration range* within which the constitution of the amphipathic solute in solution changes from the molecular (or ionic) disperse state to an equilibrium between molecules (or ions) and aggregates.

It is important to distinguish between (a) those *factors which influence the cmc* of nafoxidine hydrochloride solutions and (b) those properties of nafoxidine hydrochloride solutions which are a consequence of micellar behavior in the subsequent physical, chemical and/or biological systems investigated, i.e., *properties affected by the cmc.*

The cmc ranges determined in the studies described were for essentially simple binary systems of drug in water. The micellar behavior for ternary or more complicated systems becomes quite complex. Mention is only made here of the most common factors which can affect cmc values, namely, (a) the presence of additives such as (1) salts (gegenions), (2) polar compounds, (3) insoluble materials or (4) other surfactants; (b) the effect of pH; and (c) the temperature dependency. These effects are discussed by Shinoda and Sjoblom (15) in greater detail; however, the qualitative effect of these factors is important to recognize. Special emphasis is made in recognizing that nafoxidine hydrochloride solutions involve an ionic surfactant, and, as such, one must be careful not to assume that the typical behaviors of nonionic surface-active agents can be expected in these systems.

Salts lower the cmc values of ionic surfactants. However the salt effect is not governed by the principle of ionic strength or the Debye-Hiickel relationships. The depression of the cmc depends only on the concentration of ions bearing a charge opposite of that of the surface-active ions (i.e., anions). The nature and concentration of ions of the same charge (cations) are without effect. Correspondingly smaller cmc's for nafoxidine hydrochloride solutions would be observed for bivalent gegenions than for univalent additives. Long-chain alcohols, amines and similar compounds containing a polar group can produce a considerable lowering of the cmc. The effect is increased both by the concentration of the polar additive and by increasing the chain length (i.e., C_4OH lower the cmc more than C_2OH).

Insoluble materials such as hydrocarbons or any other material which can be solubilized in the interior of the nafoxidine hydrochloride micelle can cause an increase in the micellar size and thus lower the critical concentration. The interplay of the hydrophilic and oleophilic (may be here considered as hydrophobic) portions of the amphipathic nafoxidine ion have a great effect on the cmc. The solubility of the ion-pair (counterion + charged polar head of the amphipathic nafoxidine cation) in the surface

region of the micelle is probably a very important factor in determining the size of the micelle and hence its cmc.

The selection of aerosol components in cosmetic formulations including a mesomorphic compound may be affected by the above additives. The vapor pressure, spray pattern and concentrate/propellant ratio will depend on the surface activity and phase behavior of the major active ingredient. The addition of salts or alcohols will determine the stability and shelf-life of the product.

SIGNIFICANCE OF MICELLAR AND MESOMORPHIC PROPERTIES

The importance of the micellar behavior of nafoxidine hydrochloride can be subject to considerably varied interpretation. The presence of a cmc can manifest itself in a wide variety of physiochemical properties, many of which have been described in this report. It is pertinent here to mention those properties affected by micellar behavior which may significantly influence the use of drug, cosmetic or toiletry products. Shinoda describes the following properties as being critically affected by micellization: (a) solubilization, (b) surface tension, (c) partial molal volume, (d) refractive index, (e) light scattering, (f) colligative properties, (g) solubility, (h) electromotive force, (i) diffusion, (j) viscosity, (k) dialysis, (l) ultraviolet and infrared absorption and 10 or more other physicochemical phenomena. All are based on a non-ideal or varied behavior of the surfactant at concentrations above the cmc.

The physical and chemical methods employed in preparing the drug and the dosage form were of particular interest to us in the design of the most effective formulation of nafoxidine hydrochloride. In addition, any analytical procedure employed to aid us in dealing with the drug in its varied systems must be subjected to a possible involvement of micellar behavior. Having recognized such a contribution in several of the systems investigated in product development has greatly facilitated both an understanding of the phenomena observed and the selection of appropriate methods to study and develop suitable formulations.

The growing popularity of cosmetic hair preparations in transparent form has stimulated the development of clear gels. Emulsions, microemulsions and lyogels have been made from micellar and mesomorphic ingredients. The viscoelastic properties of lyotropic mesophases have been utilized to capture rheological cosmetic acceptance based on ease of application (feel) as well as detergent properties in shampoos and soaps. The color and fragrance of such products may be stabilized by concentrating the ingredient(s), often very expensive components, into a mesomorphic phase which is very thermodynamically stable.

The clear gel formulation has also been used for lipsticks, skin fresheners and antiperspirants, as well as hair products. While most of these products involve microemulsions which are not mesophases, the use of surfactants in high concentration offers special solubilization opportunities to incorporate other ingredients into the gel formulation (especially perfuming).

The optical properties of nafoxidine hydrochloride mesophases displayed pearlescent appearances which offer not only an acceptable cosmetic property but can also be used to overcome the problem of discoloration. Surfactant systems can be formulated to incorporate vanillin, eugenol, indole and other labile additives. While the pearlescent ef-

fect of mesophases is not as intense as that of bismuth oxychloride or guanine (common pearlescent additives in nail polishes, lipsticks and mascara), they are usually more stable.

CONCLUSIONS

The micellar properties of colloidal solutions of nafoxidine hydrochloride represent a very unique and complex system of drug in aqueous media. A detailed knowledge of the theory explaining some of the phenomena observed in formulating the drug has proven to be of invaluable assistance in developing an improved product and/or in understanding and predicting the physicochemical properties involved. To assume that the information gained is entirely or partly applicable to the successful development of a drug product recognizes only a part of the effort. Such a system, in which the active principle of the dosage form is a highly surface-active material possessing a cmc range in the concentration range of interest, represents a selected pharmaceutical product which is not too common. On the average, it has been assumed that about 1 in every 200 compounds is liquid crystalline. Although this is not a high occurrence, the phenomena can occur over a wide range of molecular types and can pose several opportunities as well as problems.

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Permanent waving: utilization of the post-yield slope as a formulation parameter

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Synopsis

The stress-strain curve for keratin had previously been utilized to assess the action of cosmetics on the hair. The POST-YIELD SLOPE of this curve has been correlated with changes occurring to the disulfide bonding under PERMANENT WAVING conditions. The evaluation of this mechanical PARAMETER can be related to the time of processing, rate of penetration of mercaptan and the level of disulfide cleavage. It can further be utilized to assess the effects of formulation variables such as concentration, pH, structure of the mercaptan and formulation additives. The ease of this technique can save the chemist considerable time in optimizing a PERMANENT WAVE FORMULATION.

INTRODUCTION

The stress-strain relationship for keratin fibers has been widely studied and the various regions of the stress-strain curve correlated with changes in the molecular conformation (1). When a hair fiber is stretched at a constant rate of elongation, three distinct regions appear, as shown in Figure 1. In the Hookean region of the curve (AB), the stress is linearly related to the strain until an extension of approximately 2%, when the fiber begins to yield as the crystalline α-helices begin to unfold. This yield process continues until about 30% extension where the stress rapidly increases during the so-called post-yield region (CD) until the fiber breaks at 40–50% extension.

While the details of changes in molecular conformation in the post-yield region are still under debate, it is generally accepted that the disulfide-rich matrix surrounding the microfibrils is becoming involved. The high disulfide content and thus the high degree of cross-linking in this amorphous matrix are resisting further extensions with a subsequent increase in stress. Many workers have investigated keratins which have been partially reduced and alkylated to obtain varying disulfide levels, and shown that the modulus (the slope) of the post-yield region decreases with decreased disulfide content (2–4).

The change in mechanical properties of human hair has often been utilized to assess the effect of varying cosmetic treatments on the fiber, particularly cosmetic treatments that

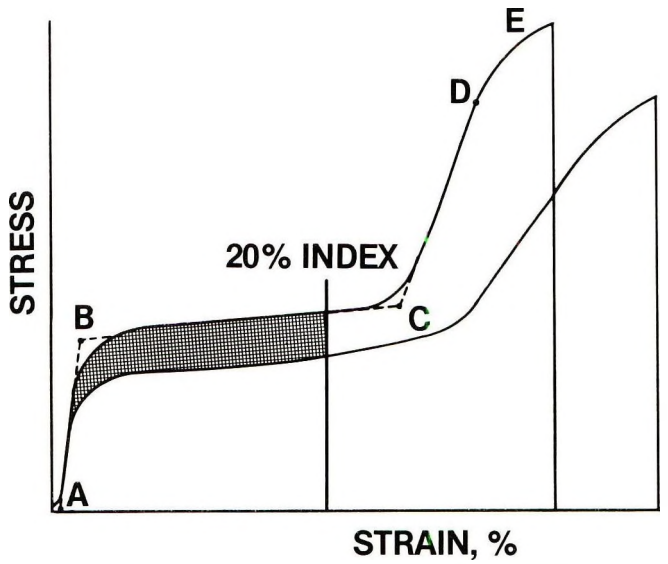

Figure 1. Typical stress-strain curve of untreated and permanent waved hair.

are known to involve bond breakage within the keratin structure, such as permanent waving, bleaching and straightening. A commonly reported parameter is the so-called 20% Index, or I_{20}, which is a measure of the ratio of the work required to stretch the fiber to 20% extension after a treatment to that before the treatment was given (5). If this ratio is 1.0, then the fiber was unaffected by the process; the lower the value of the ratio, the more effect the process has had on the fiber properties. For example, a typical alkaline permanent wave will reduce this value to about 0.8 (6), which is a reduction in the toughness of the fiber by about 20%. The lower stress-strain curve of Figure 1 is typical for a hair fiber which has been permanent waved, as compared to the normal curve. The shaded area in Figure 1 represents the difference in work of extension between the treated and untreated hair.

While such measurements are valuable to the chemist to assess the overall effect of a given process on the toughness of the fiber, they are "after-the-fact." It would be valuable to be able to correlate such changes to variations between cosmetic formulations themselves with the goal of optimizing the formulation from a performance and economic point of view while maintaining the hair's initial mechanical properties.

Hamburger and Morgan (7), in 1952, were the first workers to note the decrease in post-yield slope (PYS) as hair fibers remained in contact with typical permanent wave solutions. While no attempt was made to study various formulation variables, nor did the conditions of the experiments simulate the conditions of permanent waving, they demonstrated that the post-yield slope could be linearly correlated with the time of immersion of the hair in the waving solution. Subsequent investigations by Herrmann (8) showed that the rate-limiting step in the reaction between hair keratin and alkaline thioglycolate was diffusion of the mercaptan into the hair fiber. The time of diffusion was strongly dependent upon pH and temperature, and varied for different mercaptans.

The coupling of these observations led us to investigate the effect of typical permanent wave formulation variables on the slope of the post-yield region. The formulating

chemist is concerned with the variables of mercaptan concentration, pH, temperature and additives. At the same time, the ultimate user wants efficient and rapid processing. The post-yield slope can be used to assess the effect of formulation variables under actual waving conditions and can be correlated to molecular changes within the fiber, such as the degree of disulfide bond cleavage. The results of these investigations are reported herein.

EXPERIMENTAL

Hair samples were obtained from individual heads of known cosmetic history, or purchased as virgin European hair from DeMeo Brothers, New York, NY 10003. These samples were washed with a mild anionic commercial shampoo (Redken Laboratories, Inc., Canoga Park, CA 91303) and thoroughly rinsed with tap water.

For most experiments, 10 hair fibers were wrapped on single plastic mandrels (diameter 8 mm), saturated with the waving solutions, placed in plastic bags and treated for the desired length of time. For the reaction of hair keratin with sulfite, the procedures of Wolfram and Underwood (9) were duplicated. For the reaction of hair keratin with mercaptans to determine the degree of cleavage, the conditions of Haefele and Broge (6) were repeated.

Mercaptan compounds of known purity were commercially available and obtained as samples from either Halby Division, Argus Chemical Co., New Castle, DE 19720, or Evans Chemetics, Inc., Darien, CT 06820.

Solution concentrations throughout are based on the weight percent of active mercaptan species. All other chemicals were reagent grade.

Stress-strain curves for individual fibers were obtained at ambient temperature (20–25°C) and humidity (40–60% R.H.) utilizing the instrumentation described by Tyson and Curtis (10).[1] Post-yield slopes were obtained from the stress-strain curves by graphical interpolation. Reported slopes generally represent the average of 10 fiber determinations for each set of experimental conditions. In general the standard deviation was ± 10% of the determined value.

Penetration studies utilized the iodine decolorization technique as described by Herrmann (8).

DISCUSSION AND RESULTS

In order for the post-yield slope to be valuable in the assessment of formulation variables in permanent waving it must be sensitive to these variables under actual permanent waving conditions. The data of Hamburger and Morgan (7) had been obtained by immersing the hair fibers in containers of the waving lotion, which represents very high solution to fiber ratios. The treated hairs were rinsed and subjected to analysis. In permanent waving practice, the weight ratio of solution to fiber is typically between 0.6 and 1.2, with a value of 1.0 being most representative.

[1] A single hair fiber is horizontally suspended between a set of clamps. Force is applied by a constant speed motor which elongates the fiber at the rate of 1.5%/sec. Stress is monitored on the other end by a strain gauge transducer, while the stress-versus-strain graphs are plotted on an XY Recorder (Hewlett-Packard, Inc., Palo Alto, CA 94303).

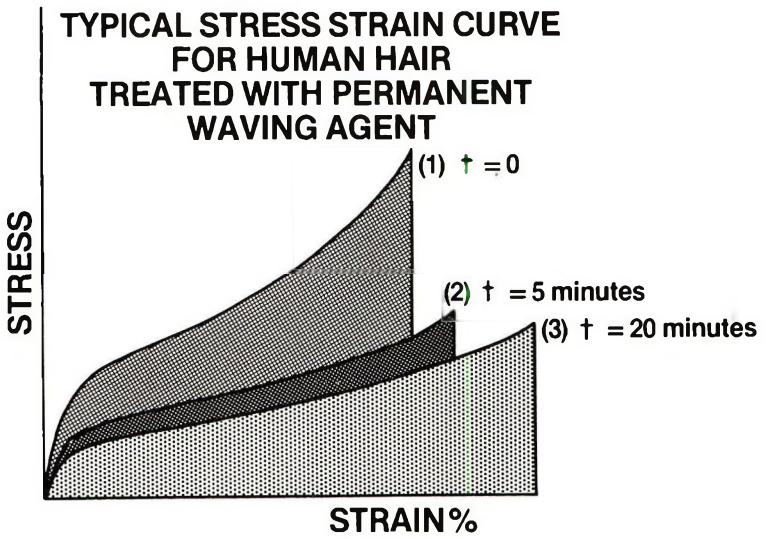

Figure 2. Typical stress-strain curves for human hair treated with permanent waving agent.

This was determined by actually weighing the combination of tresses and rods before and after they had been treated by different operators. Hairs were selected from these rods and the stress-strain curve obtained as a function of time. Indeed, the post-yield slope decreased with time of contact with the waving solution, and representative curves are shown in Figure 2. When the actual slopes are obtained graphically and plotted against time, a picture of the typical alkaline wave emerges from the initiation of waving with a rapid softening of the keratin structure to a reforming of the structure with bromate during the oxidation step. A typical alkaline wave is illustrated in Figure 3. Note the rapid drop in slope during the first 5 min as breakage of disulfide occurs. The slope values level off at 15 min, which approximately coincides with the time that the operator judges this sample to have a sufficient test curl. From the data presented by Herrmann (8), this is approximately the time for complete fiber penetration by thioglycolate at room temperature and pH 9.2. The slope value remains constant for times of at least 40 min, and this is not unexpected, since the reaction of disulfide with a mercaptan is an equilibrium process (9). Further cleavage of disulfide can be obtained by reapplication of mercaptan solution, increasing the temperature, or increasing the pH.

When bromate solution is applied, the slope rapidly returns to almost initial values as the disulfide bonds are reformed. The time of 5–10 min for the post-yield slope to reach a maximum value is generally accepted as the "neutralization" time in actual practice (11). The slope does not return to its initial value, and again this is expected since the efficiency of disulfide bond restoration is typically 80–90% with some cystine being lost to sulfur containing by-products such as cysteic acid (12).

Since it was our desire to focus our attention on the waving step and to conduct our investigation under actual waving conditions, the effect of external variables on the post-yield slope must be considered. The stress-strain curve of keratin is very sensitive to temperature and humidity, particularly in the Hookean and yield regions (13). While these variables can be readily controlled in the laboratory, they are difficult to control while obtaining salon samples.

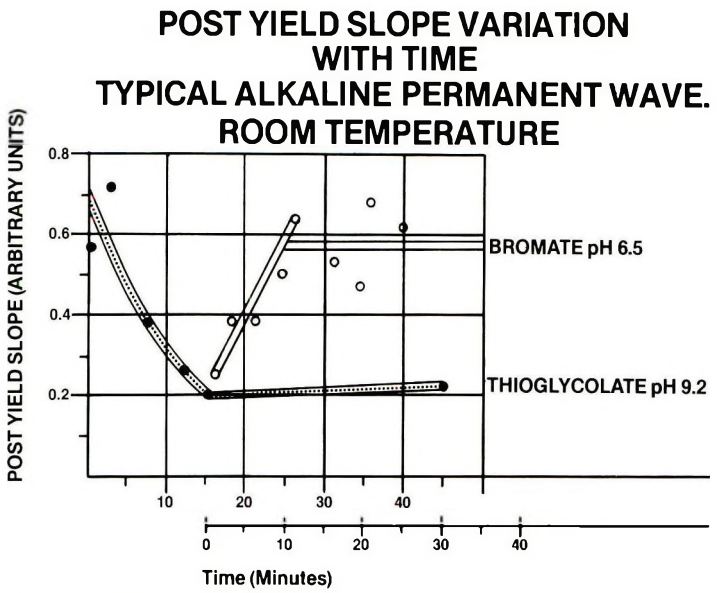

Figure 3. Variation of post-yield slope with time during typical alkaline wave.

Humidity variations have little effect on the post-yield slope. Hamburger and Morgan (7) have shown that there was little difference between the post-yield slope of a hair immersed in water and one conditioned at 65% R.H. This had also been shown for wool at 22°C over the relative humidity range of 0–100% (13). The post-yield slopes of all stress-strain curves for these conditions are roughly parallel. In actual experimental practice, no variations were observed for the relative humidity range of 40–60% commonly encountered in the salon. While the Hookean and yield regions involve extensive contributions from hydrogen bonds, the stiffness of the post-yield region depends upon disulfide crosslinks which are unaffected by water at room temperature (7).

The post-yield slope is strongly dependent upon temperature, and this has been shown for wool fibers in water over the range of 0–100°C (13). With increasing temperature, the post-yield slope decreases. It has been shown by Rebenfeld et al. (2) that untreated hairs undergo a transition at about 85°C where the disulfide bonds reach such a degree of instability that the disulfide-rich matrix shows a rapidly increasing flow component with increasing temperature. Partial reduction lowers this value to 66°C. While the slight variation in room temperature (20–25°C) does not affect the post-yield slope analysis, the contribution of temperature to results obtained with heat activated acid waves cannot be ignored.

Air oxidation of the reduced hair sample might conceivably be a third factor affecting the post-yield slope analysis. Since oxidation restores the disulfide bond, the post-yield slope would increase with increasing oxidation. In practice air oxidation is not an efficient process for the restoration of disulfide (7, 11, 12), and indeed samples were exposed to air overnight without an appreciable increase in post-yield slope.

Thus in practice these external variables do not make a significant contribution to the post-yield slope under waving conditions. The possible exception is the temperature contribution to matrix flow in heat activated acid waving.

THE EFFECT OF HAIR FIBER DIAMETER

It is well known to those who practice permanent waving that fiber diameter will affect the time of processing. Coarse hair is often described as "resistant" and may require higher strength waving solutions, or pre-softening with waving solution to decrease processing time.

The post-yield slope itself represents the stiffness modules of the post-yield region. While we have obtained the slope values by simple graphical interpolation and expressed the values in arbitrary units, we recognize that the slope is commonly expressed in dynes/cm^2—the stress divided by the cross-sectional area of the fiber. The variation of slope values with diameter can be minimized by selecting hair from a single individual, though of course there is a Gaussian distribution of fiber diameters even on an individual head. In general we averaged the slopes from 5–10 single hair samples for each set of experimental conditions, though in certain instances corrections were made for diameter to compare results between different hair sources. For a single head, the standard deviation for slope values was approximately ± 10%.

The change in arbitrary post-yield slope with fiber diameter and its effect upon the time of processing can be seen in Figure 4. A single mandrel was wrapped with 10 hairs, saturated with waving solution and placed in a plastic bag at 46°C for each time point. The waving solution was a commercial acid wave of pH 6.5. The initial arbitrary slope increases with increasing fiber diameter where the values of diameter represent the wide axis of the hair's elliptical cross-section. The time variation shown in this graph would suggest that the hairs of 64 and 95 μm would process in 10–15 min, while the 112 μm hair would require over 20 min if we use the same reasoning that we applied to the alkaline wave analysis in Figure 3.

We tested this result by evaluating the processing time for an acid wave on 49 heads in the salon and asking the operators to classify the hair according to the designations of

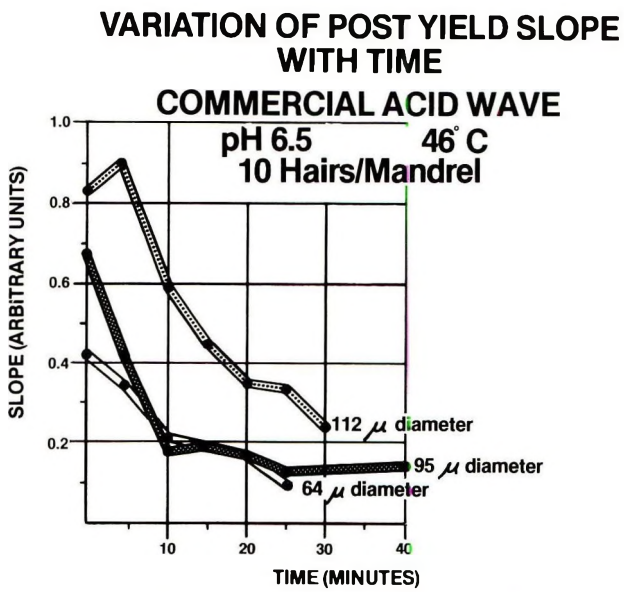

Figure 4. Variation of post-yield slope with time for hairs of varying diameter.

Table I
Commercial Acid Wave

Type of Hair (microns)	Time of Processing (minutes)
Fine (40–60)	18.0 ± 2.7
Medium (60–90)	16.6 ± 3.6
Coarse (90 and above)	23.6 ± 5.6

Salon Test at 50°C, n = 49 heads

Fine (40–60 μm), Medium (60–90 μm), and Coarse (90 μm or above). The average times are shown in Table I. In general the post-yield slope analysis was able to estimate the time of processing in the salon quite well with relatively few samples.

It is interesting to note that the diameter has relatively little effect on the time of processing up to an arbitrary point of 90 μm, but then the hair becomes "resistant" to penetration. This difference must reflect the different contributions of cuticle and cortex to the fiber's properties as the ratio of these morphological components changes with fiber diameter (14).

THE DEGREE OF CLEAVAGE OF DISULFIDE

With our initial success in determining the average time of permanent wave processing, it was of interest to attempt to utilize the post-yield-slope technique to estimate the

Table II
Equilibrium Cleavage of Disulfide By Sulfite

Sample No.	Final Solution pH	Temperature (°C)	Degree of Cleavage[a]	Post-Yield Slope[b]
1. $kSSk_0$ = 0.57m mol/g[c]	7.1	50	0.37	0.28
2.	7.1	50	0.37	0.32
3.	7.9	50	0.20	0.39
4.	8.5	50	0.10	0.48
5. $kSSk_0$ = 0.60m mol/g[c]	0.1	35	0.02	0.57
6.	2.0	35	0.31	0.27
7.	2.8	35	0.39	0.30
8.	3.6	35	0.44	0.26
9.	4.1	35	0.38	0.18
10.	4.7	35	0.42	0.17
11.	7.1	35	0.23	0.30
12.	9.2	35	0.07	0.50
13. Control (H_2O only)	6.0	35	0.00	0.60

Conditions: 1*M* Sodium Sulfite
100:1 Solution to Fiber Ratio
24 Hours Immersion

[a] The degree of cleavage represents $[(kSSk)_0 - (kSSk)_{eq.}]/(kSSk)_0$ as defined by Wolfram and Underwood (9).

[b] The post-yield slope is expressed in arbitrary units and represents the average of 10 hair fibers.

[c] The initial cystine values were determined by amino acid analysis in these laboratories as described by Gumprecht et al. (12).

degree of disulfide cleavage under waving conditions. Such measurements usually require a separate and often time-consuming chemical analysis (12).

Cleavage of disulfide by nucleophiles such as sulfite or mercaptide ion is an equilibrium process and has been treated in detail by Wolfram and Underwood (9). They determined the equilibrium disulfide cleavage values for human hair at 35°C in 1*M* sulfite over the pH range of 0–9. The solution to fiber ratio was 100:1. By repeating these conditions at various pH values it is possible to obtain disulfide cleavage values of 8–51%. Hair fibers can then be withdrawn from these solutions and the values of post-yield slope determined to obtain the correlation between slope and cleavage level. Cleavage values were determined by titration with salyrganic acid under varying experimental conditions and the post-yield slope values determined. These data are presented in Table II and Figure 5.

The values of the post-yield slope decrease with increasing cleavage of disulfide, and a good linear fit ($r = -0.94$) can be obtained over the range 0–50% cleavage. Crewther (4) has shown a linear relationship between the slope of the post-yield region and the disulfide content of S-methylated Lincoln wool between 0–75% cleavage. Above 75% the slope begins to drop rapidly and extrapolates to zero at zero disulfide content. For values above 40% cleavage by our technique, estimation of the slope graphically becomes too unreliable to extend the data to higher cleavage values. It is postulated that roughly 45% of the disulfide in the hair fiber is relatively inaccessible to sulfite under these experimental conditions (9), so higher cleavage levels were not investigated.

We attempted to improve the linear fit by correcting the measured slope values for diameter variations by normalizing them to the slope prior to treatment. The standardized slope thus represents the measured post-yield slope for the treated hair fiber divided by the measured post-yield slope for a length of the same fiber prior to treatment. For this series of experiments, the correlation was unchanged ($r = -0.94$). However the standardized slope is useful for comparing hairs of widely varying diameter as discussed in a following example.

If the slope values are independent of cleaving agent, then the data for sulfite can be extrapolated to cleavage by mercaptans under waving conditions. Haefele and Broge (6) determined the cysteine generated by disulfide cleavage with mercaptans of varying

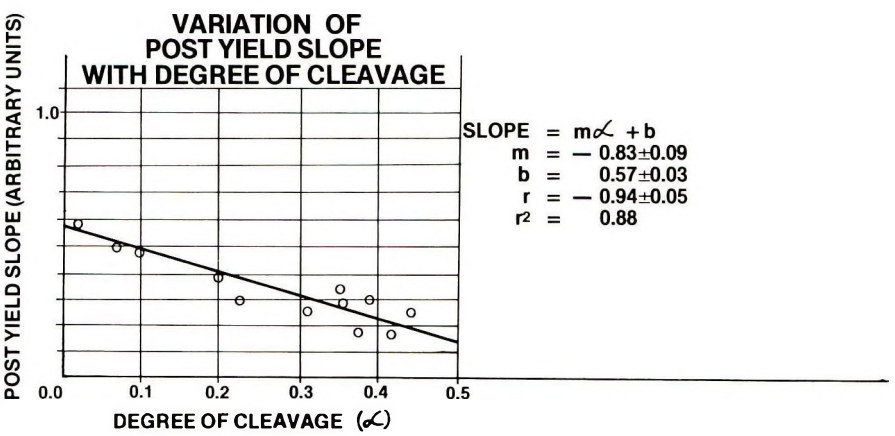

Figure 5. Variation of post-yield slope with degree of cleavage.

Table III
Comparison of Degree of Cleavage of Disulfide by Post-Yield Slope Analysis with Data of Haefele and Broge (6)

	pH of Mercaptan Solution	Standardized Slope	Degree of Cleavage	
			Slope	Haefele and Broge
1.	2.0	0.89	0.03	0.03
2.	5.0	0.63	0.22	0.10
3.	6.5	0.65	0.20	0.14
4.	8.0	0.46	0.34	0.31
5.	9.8	0.27	0.48	0.42

Conditions: 0.55 *N* Thioglycolic Acid
30:1 Solution to Hair Ratio
10 Minutes Immersion
Room Temperature

acidities utilizing a' heterogeneous iodine/thiosulfate titration of the hair samples. While blank values by this technique can be as high as 3.0% cysteine, it is useful for comparison purposes. The conditions of their experiment were repeated and the post-yield slopes determined. The degree of cleavage by standardized post-yield slope analysis is compared to the values extrapolated from the data of Haefele and Broge in Table III. These values assume that $(kssk)_0$ = 0.79m mol/g based on 5.05% sulfur in the hair.

In general the post-yield slope gives slightly higher cleavage values than the titration technique, though agreement is good with the exception of the value for pH 5.0. Considering the number of assumptions that are made in this comparison, the agreement is very good indeed.

Since the assumption that disulfide cleavage could be estimated by comparison against a standard slope vs. cleavage plot seemed valid, the technique was applied to patrons having waves in the salon. Random hair lengths were obtained before perming, and the average post-yield slope determined. The same procedure was followed at the time the operator judged the test curl sufficient. An averaged standardized slope was obtained by dividing the average perm slope value by that prior to treatment. The degree of cleavage could then be estimated from the previously determined correlation. These data are presented in Table IV.

The average degree of disulfide cleavage for this wave under salon conditions was 28 ± 9% as estimated by post-yield slope analysis. The range of the cleavage values is 13–41%. This can be compared with the data of Gumprecht et al. (12), who determined the per cent reduction for salon waving by tagging the cysteine generated by cleavage and analyzing the hair by amino acid analysis. Both radioactive and "cold" tags were used. Cysteine was assayed as C^{14}—carboxymethyl cysteine utilizing labeled iodoacetic acid as the hot tag, and as S-aminoethyl-cysteine utilizing ethyleneimine as the cold tag. The values by the hot tag method for the degree of cleavage were 22–45%, and 20–36% by the cold tag methods. The post-yield slope determination yields cleavage values that compare well to those obtained by the more laborious tagging techniques and can be used to estimate the degree of cleavage of disulfide under actual waving conditions.

Table IV
The Degree of Cleavage of Disulfide by a Commercial Acid Wave Under Salon Waving Conditions

Patron No.	Standardized Slope	Degree of Cleavage
1	0.54	0.28
2	0.39	0.39
3	0.40	0.38
4	0.54	0.28
5	0.53	0.29
6	0.39	0.39
7	0.64	0.21
8	0.60	0.24
9	0.75	0.13
10	0.69	0.17
11	0.50	0.31
12	0.41	0.38
13	0.37	0.41
14	0.59	0.25
15	0.75	0.13
16	0.64	0.21
Average	0.55 ± 0.13	0.28 ± 0.09

THE EFFECT OF MERCAPTAN CONCENTRATION

Since we were convinced that the analysis of the post-yield slope could be used to assess waving parameters, we turned our attention to formulation variables. The equilibrium cleavage of disulfide by mercaptan has been theoretically treated by Wolfram and Underwood (9). The degree cleavage of disulfide by mercaptans depends upon the total concentration (C) of mercatan plus mercaptide anion and varies with $C^{1/2}$ or $C^{2/3}$ depending whether eq 1 or eq 2 is dominant.

$$kSSk + RS^- \rightleftharpoons kSSR + kS^- \quad (1)$$

$$kSSk + 2RS^- \rightleftharpoons 2kS^- + RSSR \quad (2)$$

This concentration dependence assumes that the degree of cleavage is small and that the system is at equilibrium. While such conditions may be approached for alkaline thioglycolate preparations which rapidly penetrate the fiber (8), equilibrium cleavage values are more slowly attained under acid conditions. In practice, the formulating chemist attempts to maximize curl formation while minimizing the processing time and the cost. Our data suggested that the time at which the post-yield slope reached a minimum value correlated with the time of test curl formation. Accordingly, the time of processing could be preselected and the effect of concentration on the post-yield slope investigated by preparing a series of solutions, applying them to hair wrapped on small mandrels and determining the post-yield slope values. Such a technique can give a great deal of information about the formulation without the need for curl evaluation of many separate trial formulas.

The results of a typical experiment are presented in Figure 6. In this case the mercaptan was glyceryl monothioglycolate (glyceryl thioglycolate) (16), the pH 6.5, the temperature 50°C and the time of treatment was preselected to be 20 min. The shape of this curve is typical of others that we have investigated. The degree of cleavage increases (or the post-yield slope decreases) with increasing mercaptan concentration

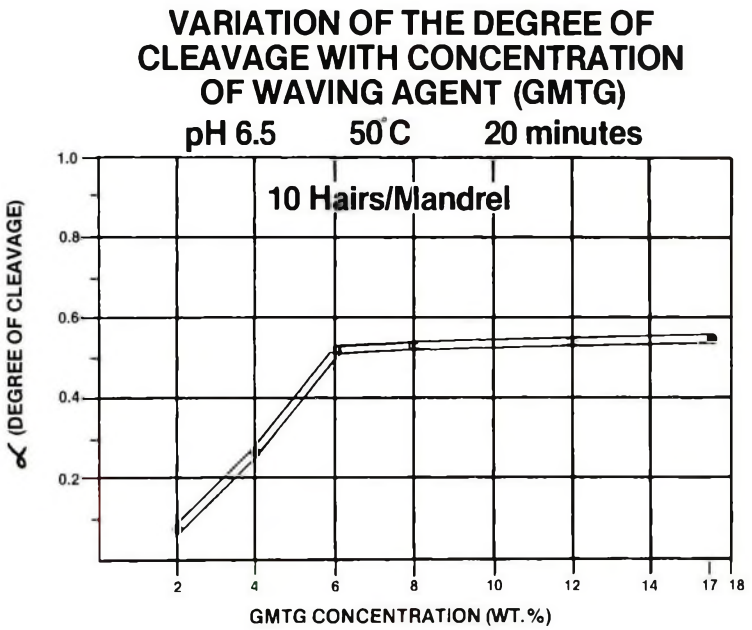

Figure 6. Variation of degree of cleavage with concentration of waving agent.

until it reaches a maximum value. Concentrations above this value do not further affect the cleavage level. In the example presented, concentrations above 6–8% are not advantageous if we accept a 20-min processing time.

This of course implies that for concentrations above 6–8% the disulfide is at its equilibrium cleavage level for this set of conditions and that the fiber is completely penetrated by mercaptan. It also suggests that roughly 45% of the disulfide in the keratin fiber is inaccessible to mercaptan since maximum cleavage is approximately 55%. This has also been suggested for the reaction of sulfite with the keratin disulfide (9).

In an independent series of experiments, the degree of penetration of glyceryl monothioglycolate was determined utilizing the iodine decoloring technique as reported by Herrmann (8). The results (shown in Table V) are in complete agreement with the concentration data. Concentrations of glyceryl monothioglycolate above 6% completely penetrate the hair in 20 min or less. The higher the concentration of mercaptan, the faster the penetration. The post-yield slope reaches a minimum at the time of com-

Table V
Time of Penetration of Glyceryl Monothioglycolate as a Function of Concentration

Mercaptan Concentration (%)	Time of Penetration (Minutes)	
	T 50%	T 100%
2	19	50
6	11.5	20
12	7	12

Conditions: pH = 6.5
Temperature = 50°C

plete penetration and this is approximately the time at which the operator judges a sufficient test curl. This interpretation is in agreement with Herrmann (8) who showed that the stress at 2% extension decayed linearly with the area of the fiber penetrated by ethanolamine thioglycolate at pH 9.3. It further suggests that the same rate-determining step applies to both acid and alkaline waving, though this remains to be verified by thorough study.

THE EFFECT OF WAVING pH

The effect of pH on the reaction between mercaptan and keratin disulfide has been widely studied. For a given mercaptan species at constant concentration, the equilibrium degree of cleavage of disulfide is governed by the sulfhydryl pKa of the mercaptan relative to the pKa of cysteine in the keratin fiber. This value has been found to be 9.8 (9).

If the mercaptan sulfhydryl is more acidic than cysteine ($pK_{RSH} < pK_{kSH}$), equilibrium disulfide cleavage increases with decreasing solution pH. If the mercaptan is less acidic than cysteine ($pK_{RSH} > pK_{kSH}$), the equilibrium cleavage increases with increasing pH.

The pK of glyceryl monothioglycolate is approximately 7.8, so that cleavage should be favored by low pH mercaptan solutions. Again it is convenient to preselect a time and a concentration and vary the pH independently. A typical experiment with 17% glyceryl monothioglycolate at 50°C and 20 min is shown in Figure 7.

The cleavage values increase as the pH is lowered from 9.5 and reaches a maximum at about pH 4.5. Lowering the pH further results in a decrease in cleavage levels. This is

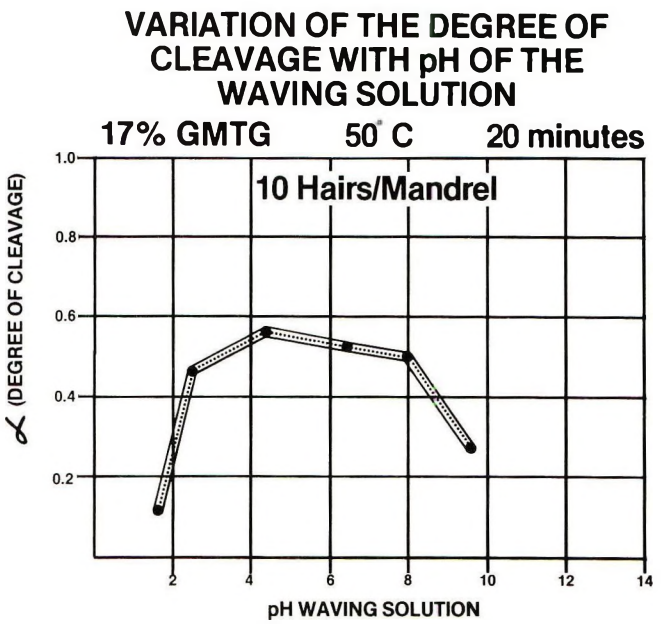

Figure 7. Variation of degree of cleavage with pH of waving solution.

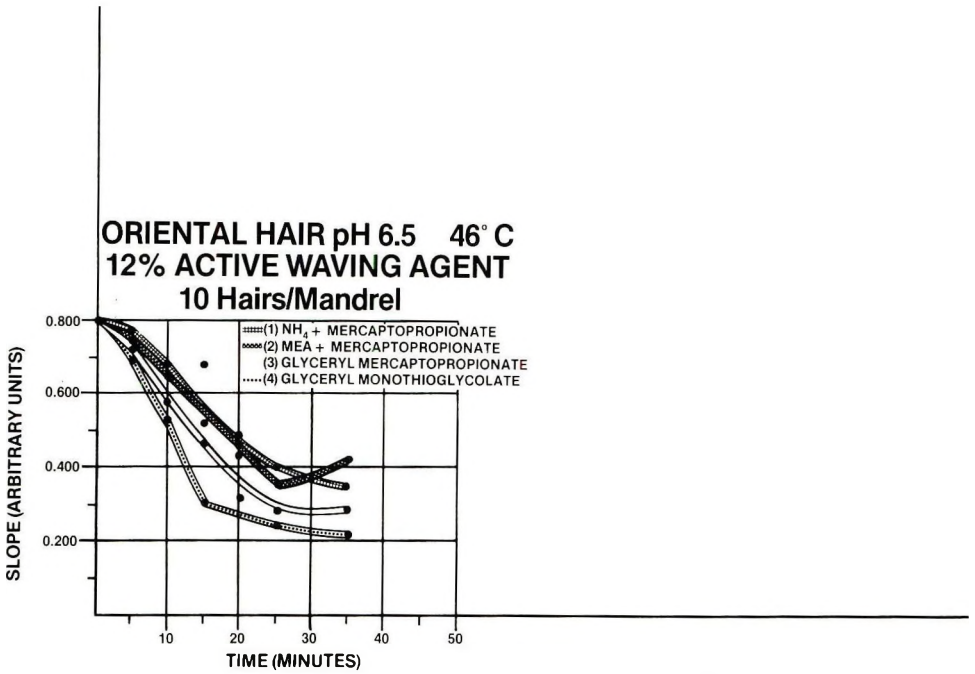

Figure 8. Variation of post-yield slope with time for different mercaptans.

not contrary to theory since equilibrium may not be attained in 20 min at these lower pH values, even at 17% GMTG concentration.

For mercaptans of similar acidities, other parameters such as ionic nature and molecular size can become important. A comparison of four commercially available mercaptans on Oriental hair is presented in Figure 8.

The ammonium salt and the monoethanolamine salt of mercaptopropionic acid show almost identical behavior at pH 6.5 and 46°C. Both are slower than the ester, glyceryl mercaptopropionate. The pKa of mercaptopropionic acid is 10.2, while the pKa of glyceryl mercaptopropionate can be estimated at 7.8 (15). Herrmann (8) explains the variation in penetration rates between mercaptans as the result of the electrostatic interactions between ionizable groups in the mercaptan's structure and ionizable groups in the keratin fiber. For compounds such as glyceryl mercaptopropionate which contain no ionizable groups other than sulfhydryl, penetration is rapid at low and neutral pH values (6.5). However the mercaptopropionate salts have an ionizable carboxylate group which is negatively charged at pH 6.5 as is the net charge on the keratin fiber. Electrostatic repulsion between these negative charges reduces the rate of fiber penetration. Herrmann found that the slowest rates of penetration for thioacids occurred around pH 6.

While glyceryl thioglycolate has a pKa comparable to glyceryl mercaptopriopionate and contains no other ionizable groups, it is a lower molecular weight than the propionate and causes a faster decay in the post-yield slope. These results further support the dependence of the rate of decay of post-yield slope on the rate of penetration.

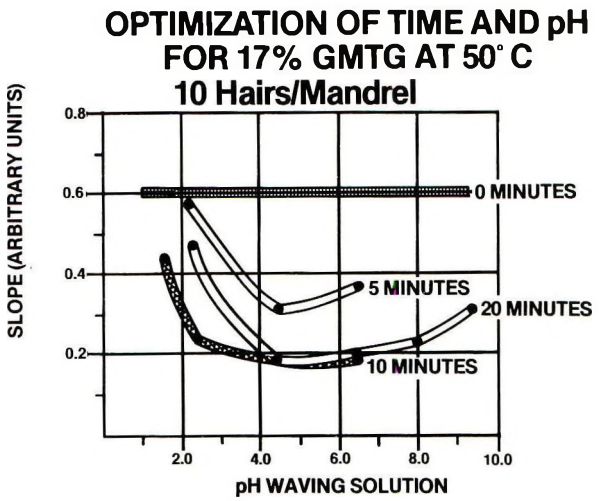

Figure 9. Optimization of time and pH for glyceryl monothioglycolate.

OPTIMIZING THE WAVING PARAMETERS

The experiments illustrated in the above examples can be accomplished quickly and the formulation will rapidly target a few sets of conditions before beginning curl analysis or any salon testing. As an example, the data for 17% glyceryl monothioglycolate at three different times over the pH range of 2–9 is combined in Figure 9.

Since we are trying to achieve a minimum post-yield slope in minimum time, this data would suggest that 17% glyceryl monothioglycolate would yield satisfactory softening

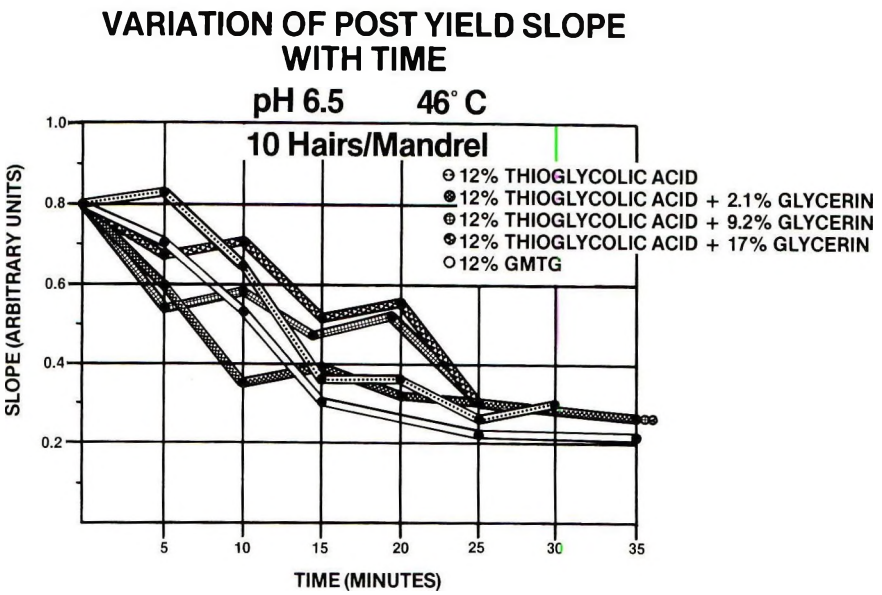

Figure 10. Variation of post-yield slope with time at different levels of glycerin/ATG.

in 10 min between pH 4 and 6.5. This has been verified on both hair tresses in our laboratories and on patrons in our test salon.

THE EFFECT OF FORMULATION ADDITIVES

The completed permanent wave is generally not just a mercaptan solution. Additives are commonly used to increase the tightness of the curl, further decrease the processing time, or to improve the feel of the permed hair. Any such additives can have an effect on the rate of permanent wave processing.

Glyceryl monothioglycolate solutions are mixed at the time of processing since this ester hydrolyzes in water to yield glycerin and thioglycolic acid. If the hydrolysis were rapid, then the same waving results should be obtained from equivalent amounts of glycerin and thioglycolic acid. This hydrolysis is of course not instantaneous. As an example, consider the addition of glycerin to a waving solution of 12% thioglycolic acid which has been adjusted with ammonia to pH 6.5 and compared to thioglycolate itself and glyceryl monothioglycolate as shown in Figure 10.

While thioglycolic acid at 12% processes slowly under these conditions, and glyceryl monothioglycolate at 12% processes only slightly faster, the addition of glycerin to the formula at levels greater than 2% retards the decay of the past-yield slope with time. The overall rate for slope decay decreases with increasing glycerin concentration. The 9.2% glycerin level is approximately equimolar with 12% thioglycolic acid and the results differ from those obtained with glyceryl monothioglycolate, which further supports the fact that hydrolysis is not appreciable during the time of waving. It is likely

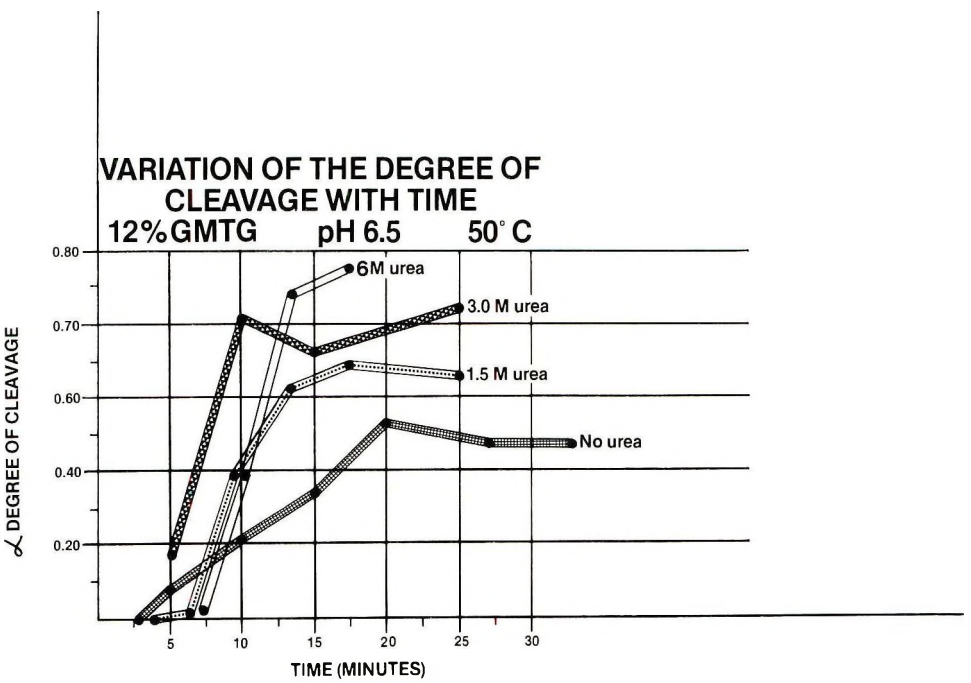

Figure 11. Variation of degree of cleavage with time at different levels of urea/GMTG.

that the effect of glycerin is to alter the pentration rate of the mercaptan into the fiber by altering fiber swelling under these conditions.

As a second example, consider the effect of the addition of urea to a 12% glycerol monothioglycolate soluton. Urea is known to alter the secondary and tertiary structure of proteins, predominantly through hydrogen bond breakage. This would conceivably open the structure of the fiber to more rapid penetration of waving agent. This experiment was conducted for three levels of urea as shown in Figure 11.

While all levels of urea increased the rate of post-yield slope decay relative to 12% glyceryl monothioglycolate (approximately 50% cleavage in 20 min), they all also increased the equilibrium cleavage value (62% for 1.5*M* urea to 78% for 6*M* urea). This suggests that the effect of urea has been to open areas of the fiber that were previously inaccessible to the mercaptan. While this at first might suggest more effective perming, high degrees of keratin disulfide cleavage are generally damaging; and when tresses were examined utilizing these solutions, the results were entirely unsatisfactory.

The effect of additives on the rate of perming and degree of cleavage can be assessed by post-yield slope analysis though the ultimate evaluation of other important perming parameters must be done by conventional methods to evaluate cosmetic appeal and hair damage.

CONCLUSIONS

The post-yield slope evaluation can provide the formulator with important information about formula parameters. We have demonstrated its use in estimating the time of processing, rate of penetration and degree of cleavage. We have further presented examples showing the effects of mercaptan structure, mercaptan concentration, pH of waving and formulation additives.

The ultimate evaluation of a permanent wave depends upon the degree of curling that is achieved, its lasting qualities and the cosmetic appeal of the hair. These factors are best observed by more conventional methods of evaluation. The evaluation method that has been presented can be used to guide the formulation through early formulation steps with a minimum of laborious tress evaluation.

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J. Soc. Cosmet. Chem., 29, 703–716 (November 1978)

Strukturuntersuchungen von Salben

1. Mitteilung: Röntgenstrukturuntersuchungen an der Hydrophilen Salbe DAB 7

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Synopsis — In order to characterize the crystalline state of Hydrophilic Ointment DAB 7 as well as of its components, X-ray studies (using Kiessig's low angle technique and goniometric diffractometry) have been carried out. Both cetyl and stearyl alcohols crystallize in a mixture of β-and γ-polymorphs. Depending on the conditions of crystallization one or the other polymorphic phase is in excess. The mixture of these two alcohols, cetostearyl alcohol, forms mixed crystals with a uniform Bragg's distance, wich is between the two β-polymorphic forms of the single components. On the other hand sodium cetyl sulfate and sodium stearyl sulfate crystallize as separate entities from their mixtures. The predominant species in Emulsifying Wax (90% cetostearyl alcohol and 10% sodium cetyl sulfate and sodium stearyl sulfate) are mixed crystals, which consists mainly of cetyl alcohol and sodium cetyl sulfate and partly of stearyl alcohol. In addition, mixed crystals of stearyl alcohol and sodium stearyl sulfate are found in small amounts. In Hydrophilic Ointment DAB 7 both types of mixed crystals form a framework of gel in which the white soft paraffin and the liquid components of the white petrolatum are immobilized either mechanically or by capillary attraction. Since the wide angle interferences are nearly identical, it may be assumed that the paraffinic carbon chains of all components of the ointment can form similar orthorhombic subcells.

1. Einleitung

Zum Nachweis des Gelcharakters bei Salben konnte von Führer (1) bei erstarrten Schmelzen von Cetylalkohol und flüssigem Paraffin aufgezeigt werden, daß diese Gemische ausgedehnte, stark vernetzte Kristallisate bilden, die eindeutig für eine Gelnatur dieser Modellsysteme sprechen. Anhand von polarisationsmikroskopischen Untersuchungen konnten

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sowohl bei diesen Systemen als auch bei echten Salben kristalline Bereiche als solche erkannt werden. Diese erlauben jedoch wegen ihrer kleinen Abmessungen und den unscharfen Übergängen in die weniger kristallinen bzw. amorphen Bezirke keine weitergehenden Informationsmöglichkeiten über die Feinstruktur ihres kristallinen Aufbaus. Um Aufschlüsse über die Verteilung der verschiedenen Komponenten einer Salbenrezeptur am Aufbau des Gelgerüstes und der darin immobilisierten flüssigen Bestandteile bzw. der Anordnung der einzelnen Moleküle zueinander zu erhalten, bietet sich die Röntgenstrukturanalyse an. Über die Ergebnisse dieser Untersuchungen am Beispiel der Hydrophilen Salbe des DAB 7 soll hier berichtet werden.

2. Material und Methoden

Die Hydrophile Salbe des DAB 7 besteht aus 30 Teilen Emulgierendem Cetylstearylalkohol, 35 Teilen dickflüssigem Paraffin und 35 Teilen weißem Vaselin. Der Emulgierende Cetylstearylalkohol selbst setzt sich zu gleichen Teilen aus Cetyl- bzw. Stearylalkohol zusammen und enthält zu 10 Prozent die Natriumsalze der Schwefelsäureester dieser beiden Komponenten. Für die Untersuchungen sowohl der Ausgangssubstanzen als auch der entsprechenden Mischungen wurden Materialien in der Qualität des DAB 7 verwendet. Da kolloide Strukturen entscheidend vom Reinheitsgrad der Substanzen abhängen können, wurden bewußt keine speziell gereinigten Produkte eingesetzt, um für die Hydrophile Salbe repräsentative Systeme zu bearbeiten.

Aufnahmetechnik nach Kiessig (2)

Die Untersuchungen wurden mit Hilfe von Kiessig-Kammem für den Kleinwinkelbereich und den nahen Weitwinkelbereich durchgeführt. Die wiedergegebenen Aufnahmen wurden mit einem Objekt-Filmabstand von 100 bzw. 200 mm gewonnen. Aufnahmen mit größeren Objekt-Filmabständen führten zu keinen weiteren Ergebnissen. Die Proben wurden nach dem Aufschmelzen in eine Probenkapillare eingesaugt, die dann durch Abschmelzen beiderseitig verschlossen wurde. Die Untersuchungen erfolgten im Vakuum.

Röntgengenerator: Müller-Mikro 111; Strahlung Cu K α, Nickelfilter. Beschleunigungsspannung: 40 kV; Anodenstrom: 20 mA.

Die Expositionszeiten lagen je nach Intensität der Interferenzen und in Abhängigkeit von der Kammerlänge zwischen 3 und 8 Tagen.

Aufnahmetechnik mit dem Vertikalgoniometer

Die Proben wurden in dünner Schicht auf eine Glasplatte aufgeschmolzen.

Röntgengoniometer: Siemens Interferenz-Goniometer; Röntgengenerator: Siemens Kristalloflex IV; Beschleunigungsspannung: 20 kV; Anodenstrom: 20 mA; Strahlung: CU K α, Nickelfilter; Abtastgeschwindigkeit $^1/_4$°/min. Stat. Fehler: 2%, Impulsrate: 4 x 10^3 Imp./min.

3. Ergebnisse

Zunächst wurden die einzelnen Bestandteile der Salbe untersucht und diese anschließend stufenweise zum Aufbau der Hydrophilen Salbe (DAB 7) zusammengefügt.

3.1. Cetylalkohol

Die Untersuchungen von Müller und Shearer (3), Piper und Malkin (4), Clarkson und Malkin (5) sowie Tanaka und Mitarbeiter (6) ergaben, daß Fettalkohole ebenso wie Fettsäuren und Triglyzeride polymorphes Verhalten zeigen, d. h. bei gleicher chemischer Zusammensetzung verschiedene Kristallstrukturen ausbilden können. Die Fettalkohole zwischen Dodecanol und Eicosanol bilden kurz unterhalb ihrer Schmelzpunkte stabile Hochtemperaturmodifikationen aus, die als α-Form bezeichnet werden. Fettalkohole mit geradzahliger Kette von Kohlenstoff-Atomen sind in der Lage, zwei Tieftemperaturmodifikationen auszubilden. Bei der orthorhombischen β_O-Phase liegen dabei die Hydroxylgruppen in der gleichen Ebene wie die Kohlenstoffketten; bei den monoklinen γ-Phasen dagegen sind diese Ebenen zueinander geneigt (7). Theoretisch sind sowohl Cetylalkohol als auch die anderen genannten Fettalkohole in der Lage, abhängig von den Kristallisationsbedingungen in einer Vielzahl von Modifikationen zu kristallisieren. Bei gleichbleibender orthorhombischer Subzelle der Kohlenwasserstoffketten (Abbildung 1) wird diese Vielfalt durch Verschieben der einzelnen Ketten gegeneinander um definierte Winkel erreicht. Es muß angenommen werden, daß die freie Energie dabei ein Minium aufweist. Der Kettenneigungswinkel kann deshalb nicht beliebig sein, da sich nur bei ganz bestimmten Winkeln, die durch die Länge einer Methylen-Einheit oder deren Vielfachem vorgegeben sind, für eine Modifikation ein energetisch günstiger Zustand mit minimaler freier Energie des Systems einstellen wird. Dieser ist dann erreicht, wenn die

C-Atome nebeneinanderliegender paralleler Moleküle bei zur Kettenachse senkrechter Projektion auf die Nachbarkette genau zusammenfallen, wodurch der kürzeste Abstand zwischen zwei C-Atomen benachbarter Moleküle entsteht (8). Dabei ergeben sich monokline Elementarzellen mit verschiedenen diskreten Winkeln. Aus einer einheitlichen Subzelle der Paraffinkohlenwasserstoffketten der Fettalkohole können monokline β-Modifikationen mit entsprechender Neigung des Winkels zur kürzeren Achse Elementarzellen ebenso entstehen wie monokline β-Modifikationen mit diskreten Winkeln der c-Achse zur a-Achse. Cetylalkohol zeigt somit ebenso wie andere Fettalkohole ein polymorphes Verhalten, das dem der Polytypie einiger anorganischer Materialien sehr ähnlich ist (9).

Untersuchungen über energetisch besonders günstige strukturelle Anordungsmöglichkeiten der Fettalkohole ergaben (10, 11), daß die β_0-Modifikation mit einer nahezu orthorhombischen Elementarzelle eine energetisch günstige Anordnungsmöglichkeit darstellt, da mit ihr ein Subzellenparameter c_S erhalten wird, der die Moleküle als spannungsfrei erscheinen läßt. Monokline β-Modifikationen müssen als energetisch ungünstige Formen angesehen werden. Aus ähnlichen Gründen wird als die stabilste γ-Modifikation der Fettalkohole die γ_4-Modifikation beschrieben (10).

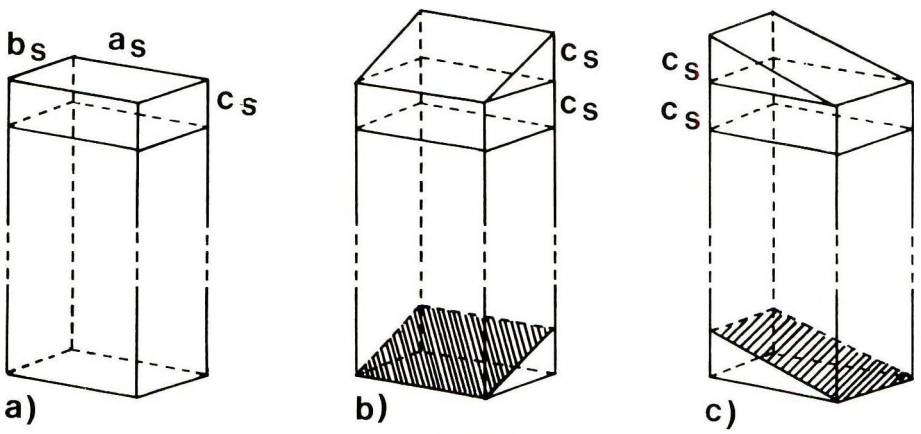

Abbildung 1

Schematische Darstellung der bei den Fettalkoholen möglichen Elementarzellen. Die verschiedenen Modifikationen entstehen durch Verschieben der Paraffinkohlenwasserstoffketten der Fettalkohole um diskrete Abstände, die dem Subzellenparameter C_S oder dessen Vielfachem entsprechen.

a) orthorhombische Modifikation

b) monokline β-Modifikation

c) monokline γ-Modifikation

Abbildung 2 zeigt die Kiessig-Aufnahme von handelsüblichem Cetylalkohol bei 100 bzw. 200 mm Objekt-Filmabstand. Die Abstände der inneren Interferenzringe, die den Interferenzen 1., 2. und 3. Ordnung der Netzebenenabstände entsprechen, die durch die doppelte Kettenlänge bei senkrechter Anordnung des Cetylalkoholmoleküls erhalten werden, ergeben einen Bragg'schen Abstand, der nahezu mit der c-Achse der in der Literatur beschriebenen Elementarzelle (10) übereinstimmt (s. Tabelle).

Die beiden starken Interferenzen am Bildrand der Aufnahme mit 100 mm Objekt-Filmabstand können nach Kohlhaas und Soremba (12) den Netzebenen 200 und 110 zugeordnet werden, die in ihrer Lage praktisch identisch mit denen der orthorhombischen Subzelle von langkettigen Paraffinen sind.

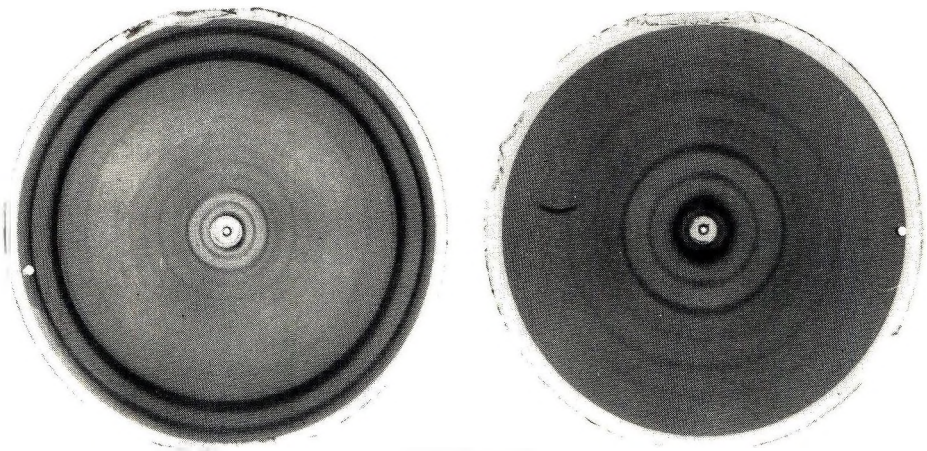

Abbildung 2

Kiessig-Aufnahme von Cetylalkohol mit 100 bzw. 200 mm Objekt-Filmabstand.

Abbildung 2 macht aber weiterhin deutlich, daß in geringem Umfang noch eine weitere Modifikation vorliegen muß, da in der Kiessig-Aufnahme noch schwach ausgebildete Interferenzmaxima vorhanden sind, die zu einem geringeren Bragg'schen Abstand führen. Die begleitende Modifikation konnte als γ_4-Modifikation identifiziert werden (s. Tabelle), die als stabilste γ-Modifikation beschrieben wird (10).

In erheblichem Umfang ist das Ausmaß der als dominierende Modifikation auftretenden Phase von der Präparationstechnik der Proben für die röntgenographischen Untersuchungen abhängig. Wird im Gegensatz zur Präparationstechnik für eine Kiessig-Aufnahme, bei der die Probe als Schmelze in eine Glaskapillare eingesaugt wird und anschließend

Tabelle

Identitätsperioden der Bragg'schen Abstände d_{001}. Die bei den eigenen Versuchen verwendeten Meßmethoden ergeben dabei Identitätsperioden, die der doppelten Länge der Moleküle entsprechen, während in den Literaturzitaten die vierfache Länge der Moleküle angegeben wird.

	d_{001} [Å]	d_{001} [Å] (Referenz)	
Cetylalkohol			
Kiessig-Aufnahme			
β_0-Modifikation	46,2 ± 0,1	90,85	(10)
begleitende γ_4-Modifikation	38,1 ± 0,1	74,4 ± 0,3	(7)
		74,9	(10)
Goniometerdiffraktogramm			
γ_4-Modifikation	37,4 ± 0,1	74,4 ± 0,3	(7)
		74,9	(10)
begleitende β_0-Modifikation	45,3 ± 0,1	90,85	(10)
Stearylalkohol			
β_0-Modifikation	51,5 ± 0,1	101,03	(10)
begleitende γ_4-Modifikation	41,4 ± 0,1	84,43	(10)
Cetylstearylalkohol	47,95 – 49,45		
Cetylschwefelsaures Natrium	48,2 ± 0,1	96,5	(16)
Stearylschwefelsaures Natrium	53,1 ± 0,1	103,04	(16)
Emulgierender Cetylstearylalkohol			
Hauptkomponente	48,5 ± 0,1		
Nebenkomponente	52,8 ± 0,1		
Hydrophile Salbe	48,5 ± 0,1		

erstarrt, der Cetylalkohol in dünner Schicht auf eine Glasplatte aufgeschmolzen und anschließend erstarren gelassen, so erhält man die γ_4-Modifikation als die vorherrschende Phase, während die β_0-Modifikation nur noch in geringem Umfang nachgewiesen werden kann. Abbildung 3 zeigt ein solches Goniometerdiffraktogramm. Die Interferenzmaxima bei kleinen ϑ-Winkeln ergeben einen Bragg'schen Abstand, der dem von Precht (10) beschriebenen d_{001}-Abstand der γ_4-Modifikation entspricht (Tabelle). Die bei $2\vartheta = 21{,}6°$ und $2\vartheta = 24{,}5°$ = auftretenden Interferenzen können hierbei wiederum entsprechend den von Kolhaas und Soremba (12) indizierten 110 und 200 Netzebenen der Elementarzelle von Paraffinkohlenwasserstoffen zugeordnet werden.

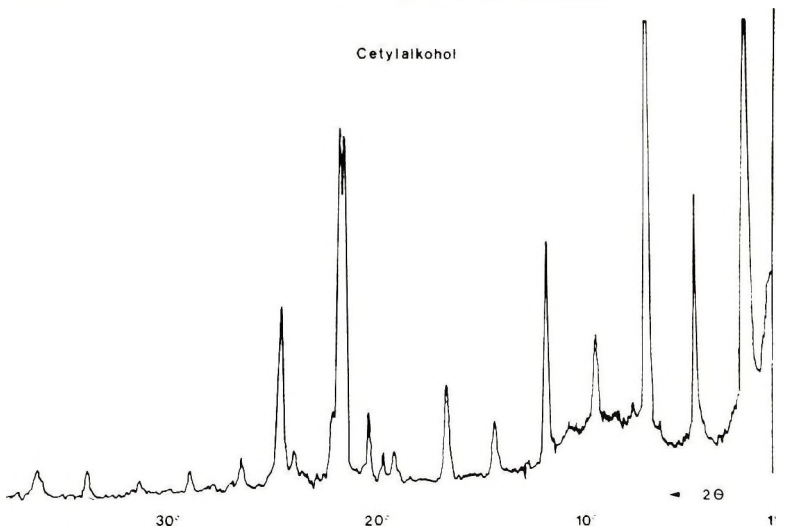

Abbildung 3

Goniometer-Diffraktogramm von Cetylalkohol.

3.2. Stearylalkohol

Bei handelsüblichem Stearylalkohol wurde ebenfalls in Abhängigkeit von der Präparationstechnik sowohl die β_0-Modifikation als auch die ϑ_4-Modifikation nachgewiesen. Reine Modifikationen konnten nicht erhalten werden. Die Ergebnisse sind in der Tabelle zusammengefaßt.

3.3. Cetylstearylalkohol (DAB 7)

Die in Abbildung 4 dargestellte Kiessig-Aufnahme zeigt das Interferenzmuster des Cetylstearylalkohols in 200 mm Objekt-Filmabstand. Dabei wird folgendes deutlich: Die Interferenzen der Bragg'schen Abstände

der beiden Alkohole im Kleinwinkelbereich treten nach ihrem Zusammenschmelzen nicht als Doppelringe auf, sondern haben einen einheitlichen Netzebenenabstand, der zwischen den Beugungsmaxima der β_0-Modifikationen beider Einzelkomponenten liegt. Demnach bilden Cetyl- und Stearylalkohol ein in sich homogenes Kristallisat aus unter statistischer Verteilung der beiden Komponenten. Untersuchungen verschiedener handelüblicher Chargen ergaben nie ein Aufspalten der Interferenzen, sondern in der resultierenden mittleren Kettenlänge des Kristallisates nur geringfügige Schwankungen, die von den Anteilen beider Komponenten in der jeweiligen Probe abhängig waren. Dies konnte auch durch Untersuchungen der Schmelzen mit wechselnder Zusammensetzung der reinen Einzelkomponenten Cetyl- und Stearylalkohol nachgewiesen werden. Diese Ergebnisse stehen im Einklang mit Untersuchungen von Heß, Kiessig und Philippoff (13, 14) bei Seifenmischkristallen und von Ott und Slagle (15) bei Mischpräparaten von Paraffinen unterschiedlicher Kettenlänge. Es wird angenommen, daß durch entsprechende Faltungen Korrekturen in der Länge der Kohlenwasserstoffkette vorkommen, die einen einheitlichen Abstand in der c-Achse ermöglichen. Da die orthorhombischen Subzellen beider Alkohole praktisch identisch sind, ändert sich die Lage der Weitwinkelreflexe im Mischkristallisat des Cetylstearylalkohols nicht.

Abbildung 4

Kiessig-Aufnahme von Cetylstearylalkohol mit 200 mm Objekt-Filmabstand.

Das Auftreten eines Bragg'schen Abstandes, der dem arithmetischen Mittel der γ_4-Modifikationen beider Alkohole entspricht, konnte bei keiner der untersuchten Proben nachgewiesen werden. Es ist somit

wahrscheinlich, daß bei den untersuchten Gemischen von Fettalkoholen verschiedener Kettenlänge das nahezu orthorhombische Gitter der β o-Modifikation den stabilsten kristallographischen Zustand des Systems darstellt.

3.4. Cetylstearylschwefelsaures Natrium (DAB 7)

Nach den Untersuchungen von Rawlings und Lingafelter (16) kristallisieren die von ihnen untersuchten Natrium-n-Alkylsulfate mit einer Kettenlänge zwischen C_8 und C_{18} in Abhängigkeit von den Kristallisationsbedingungen und der Zusammensetzung des Lösungsmittelgemisches in einer Vielzahl von Modifikationen aus, von denen die als α-Form beschriebene Phase die stabilste ist. Alle polymorphen Formen dieser Natrium-n-Alkylsulfate besitzen eine orthorhombische Elementarzelle, wobei wechselnde Mengen Wasser mit in das Kristallgitter eingebaut werden.

Die in unseren Untersuchungen mit der Kleinwinkeltechnik nach Kiessig erhaltenen Aufnahmen von Cetylstearylschwefelsaurem Natrium mit einem Objekt-Filmabstand von 100 bzw. 200 mm sind in Abbildung 5 dargestellt. Im Gegensatz zu dem Gemisch aus Cetyl- und Stearylalkohol bilden die entsprechenden Natriumsalze der Schwefelsäureester dieser Verbindungen kein Mischkristallisat aus. Die beiden kristallinen Komponenten müssen getrennt nebeneinander vorliegen, da ihre Bragg'schen Abstände genau differenziert werden können. Aufgrund der

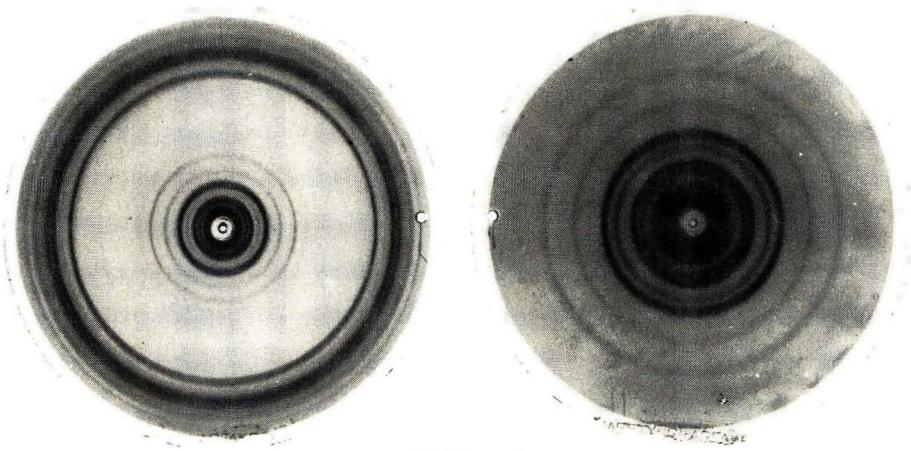

Abbildung 5

Cetylstearylschwefelsaures Natrium; Kiessig-Aufnahme mit 100 bzw. 200 mm Objekt-Filmabstand.

Intensitätsverteilung der Interferenzen ist es offensichtlich, daß bei dem untersuchten Präparat der kürzerkettige Ester dominiert. Während die in diesem Natrium-n-Alkylsulfatgemisch für die kürzerkettige Komponente ermittelten Bragg'schen Abstände gut mit den von Rawlings und Lingafelter (16) für das Natrium-Cetylsulfat korrelieren, ergibt sich keine Übereinstimmung mit den Daten für die längerkettige Komponente mit den von diesen Autoren publizierten Daten für die C_{18}-Komponente (Tabelle). Aufgrund von durchgeführten Berechnungen kann aber angenommen werden, daß die von diesen Autoren für die C_{18}-Komponente gemachten Angaben nicht korrekt sind.

3.5. Emulgierender Cetylstearylalkohol (DAB 7)

Im Emulgierenden Cetylstearylalkohol (DAB 7) liegen diese beiden Natrium-n-Alkylsulfat-Komponenten zwar nur zu 10 Prozent vor, dennoch sollte man bei einer getrennten Kristallisation von Cetylstearylalkohol und der Cetylstearylalkoholsulfate aus einer gemeinsamen Schmelze erwarten, daß der daraus resultierende Emulgierende Cetylstearylalkohol die Interferenzmerkmale der verschiedenen Komponenten trägt. Die Abbildung 6 zeigt die Kiessig-Aufnahmen von Emulgierendem Cetylstearylalkohol in 100 bzw. 200 mm Objekt-Filmabstand. Die Auswertung dieser Diffraktogramme ergibt im Kleinwinkelbereich für die Interferenzen hoher Intensität eine Periodizität der Bragg'schen Abstände von 48,5 ± 0,1 Å (Tabelle). Daraus ergibt sich, daß beim Erstarren der Schmelze von Emulgierendem Cetylstearylalkohol Cetylalkohol und ein Teil des Stearylalkohols zusammen mit dem Natrium-Cetylsulfat ein gemeinsames Mischkristallisat unter Ausbbildung eines einheitlichen Netzebenenabstandes liefern. Daneben können aber noch Interferenzen schwächerer Intensität nachgewiesen werden, die einen Bragg'schen Abstand von 52,8 ± 0,1 Å ergeben. Offensichtlich ist daher auch die C_{18}-Schwefelsäureesterkomponente in Verbindung mit einem Teil des Stearylalkohols in der Lage, ein gemeinsames Kristallisat unter Entwicklung eines einheitlichen Netzebenenabstandes auszubilden. Die beim Emulgierenden Cetylstearylalkohol gefundenen Netzebenenabstände liegen dabei stets innerhalb der für die Einzelkomponenten ermittelten Werte. Da die Weitwinkelinterferenzen für die 110 und 200 Netzebenen in ihrer Lage erhalten bleiben, ist anzunehmen, daß die beiden Natrium-n-Alkylsulfate mit den beiden Alkoholen ein gemeinsames Mischkristallisat ausbilden können, was durch den ähnlichen Aufbau der orthorhombischen Subzelle aller Komponenten begünstigt wird.

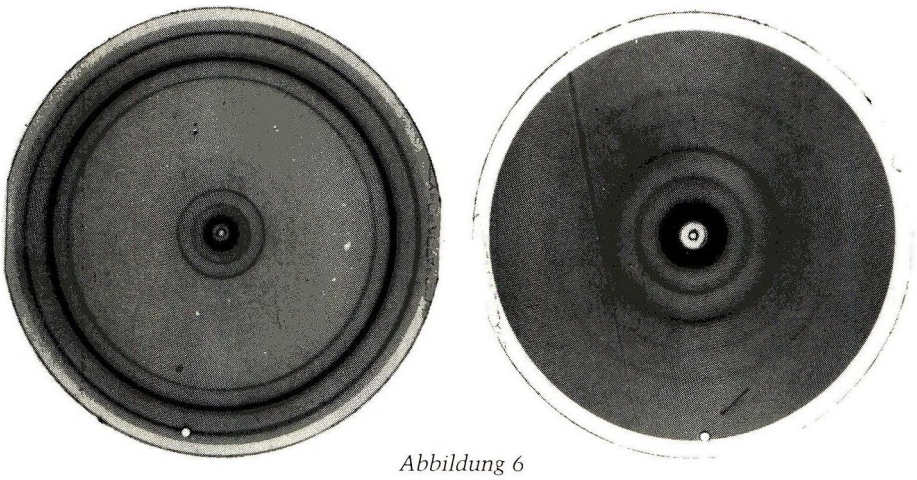

Abbildung 6

Emulgierender Cetylstearylalkohol; Kiessig-Aufnahme mit 100 bzw. 200 mm Objekt-Filmabstand.

3.6. Dickflüssiges Paraffin und Weißes Vaselin

Von den restlichen Bestandteilen der Hydrophilen Salbe (DAB 7) zeigt das dickflüssige Paraffin einen einzigen breiten, stark verwaschenen Halo, der auf eine gewisse bevorzugte Nahordnung der Moleküle hindeutet (Abbildung 7).

Das Weiße Vaselin zeigt im Weitwinkelbereich erwartungsgemäß die starken Interferenzen der 110 und 200 Ebenen der orthorhombischen Subzelle der Paraffinkohlenwasserstoffe. Die Interferenzen im Kleinwinkelbereich fehlen. Dies rührt entweder daher, daß aufgrund der breiten Molekulargewichtsverteilung ein Kristallaufbau vorhanden ist, der die Ausbildung scharfer diatroper Beugungsreflexe nicht zuläßt und somit ein Fransenmicellverband vorliegt, oder aber diese Basisreflexe sind so schwach, daß sie mit der verwendeten Strahlungsdosis noch nicht eindeutig nachgewiesen werden konnten (Abbildung 7).

3.7. Hydrophile Salbe (DAB 7)

Vergleicht man nun das Interferenzbild der Hydrophilen Salbe (DAB 7) (Abbildung 8) mit dem des Emulgierenden Cetylstearylalkohols (Abbildung 6), so wird offensichtlich, daß sich dieses Mischkristallisat auch in der Gesamtrezeptur behauptet, wobei bei der Salbe nur noch die stärksten Beugungsreflexe der kristallinen Bestandteile nachzuweisen sind. Die Kiessig-Aufnahme der Hydrophilen Salbe zeigt deutlich das Auftreten

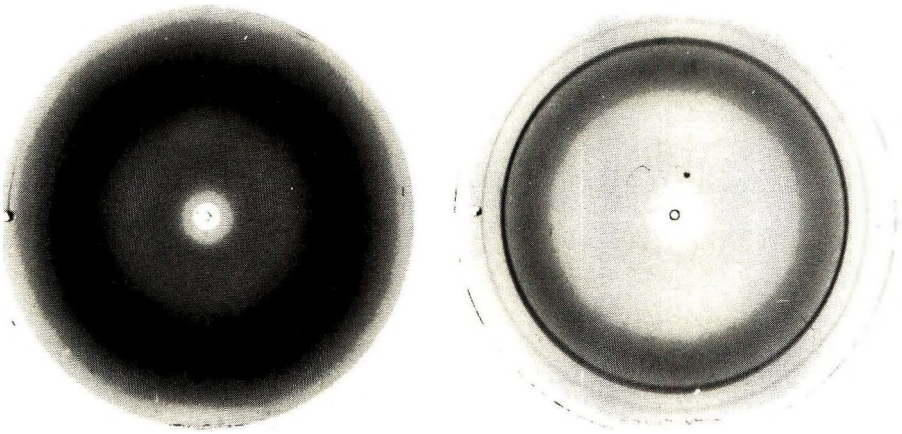

Abbildung 7

Kiessig-Aufnahme mit 100 mm Objekt-Filmabstand von Dickflüssigem Paraffin (links) und weißem Vaselin (rechts).

von amorphen Anteilen, die vor allem dem flüssigen Paraffin, teilweise auch dem in dem Vaselin enthaltenen niedermolekularen Anteil zugeordnet werden müssen. Ein Einbau unverzweigter Paraffinkohlenwasserstoffe entsprechender Kettenlänge in das Kristallisat des Emulgierenden Cetylstearylalkohols kann diskutiert werden, da die orthorhombischen Subzellen der Kohlenwasserstoffketten aller dieser Substanzen nahezu identisch sind.

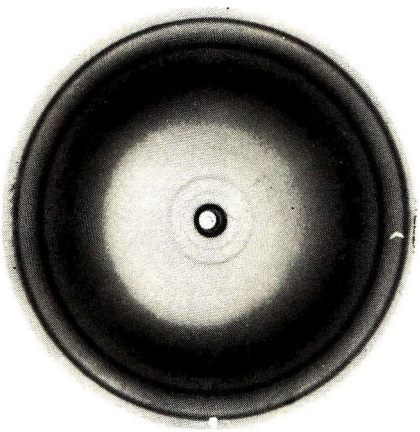

Abbildung 8

Kiessig-Aufnahme von Hydrophile Salbe (DAB 7) mit 100 mm Objekt-Filmabstand.

4. Diskussion

Die Ergebnisse deuten darauf hin, daß die Hydrophile Salbe (DAB 7) als Gemisch aufgefaßt werden muß, bei dem Vaselin und dickflüssiges Paraffin von dem konsistenzgebenden Mischkristallisat des Emulgierenden Cetylstearylalkohols durchzogen werden. Es ist anzunehmen, daß infolge seiner geringen Löslichkeit der Emulgierende Cetylstearylalkohol in den geschmolzenen Paraffinkohlenwasserstoffen beim Erstarren in einer fibrillären Struktur unter Ausbildung bestimmter bevorzugter Wachstumsrichtungen kristallisiert. Ein ausgeprägtes Sphärolithenwachstum wird durch die mechanische Bearbeitung der Salbe bei der Herstellung während der Rekristallisationsphase verhindert. Es resultiert somit ein feingliedriges dreidimensionales Netzwerk, das als Nebenvalenzgel aufzufassen ist, in dem die flüssigen Komponenten teils durch Lyosorption, teils durch mechanischen Einschluß oder Kapillarwirkung immobilisiert werden. Da das System Plastizität und Thixotropie aufweist, stellt die Hydrophile Salbe (DAB 7) ein Plastisches Gel dar und entspricht somit den von Münzel (17) entwickelten Vorstellungen über die Strukturen von Salben.

Zusammenfassung

Zur Charakterisierung der kristallinen Strukturen in der Hydrophilen Salbe (DAB 7) und ihrer Bestandteile wurden Röntgenuntersuchungen nach der Kiessig Kleinwinkeltechnik und dem Goniometerverfahren durchgeführt. Sowohl der Cetylalkohol als auch der Stearylalkohol sind dabei in der Lage, als Gemisch der β_0- und γ_4-Modifikationen zu kristallisieren, wobei in Abhängigkeit von den Kristallisationsbedingungen die eine oder andere polymorphe Form überwiegt. Das Gemisch der beiden Alkohole, der Cetylstearylalkohol, bildet ein Mischkristallisat aus mit einem einheitlichen Bragg'schen Abstand, der zwischen denen der beiden β_0-Modifikationen der Ausgangskomponenten liegt. Aus dem Gemisch Natriumcetylsulfat und Natriumstearylsulfat kristallisieren dagegen die Komponenten getrennt. Beim Emulgierenden Cetylstearylalkohol dominiert ein Mischkristallisat, das sich hauptsächlich aus Cetylalkohol, einem Teil des Stearylalkohols und dem Natriumcetylsulfat zusammensetzt. In geringem Umfang liegt daneben das Mischkristallisat aus Stearylalkohol und Natriumstearylsulfat vor. In der Hydrophilen Salbe (DAB 7) setzen sich diese Mischkristallisate als gerüstbildende Komponenten durch, in denen die flüssigen Bestandteile

des dickflüssigen Paraffins und des weißen Vaselins entweder mechanisch oder durch Kapillarwirkung immobilisiert sind. Aufgrund der nahezu identischen Lage der Weitwinkelreflexe kann angenommen werden, daß die Paraffinkohlenwasserstoffketten aller Bestandteile in der Lage sind, ähnliche orthorhombische Subzellen aufzubauen.

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Characterizing aluminum-skin interaction by an electrometric technique

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Synopsis

Observed differences in the antiperspirant behavior of ALUMINUM chlorohydroxide and aluminum chloride have been attributed to differences in their INTERACTION WITH SKIN. The literature contains many references to methods for measuring the interactions of exogenous materials with skin. The electrical properties of skin have been used successfully as a means by which to describe this effect and it was thought appropriate to investigate this approach with respect to aluminum salts. Instrumentation and techniques for measuring the electrical impedance of excised epidermal membrane were developed. The effects of two aluminum salt antiperspirants on the impedance of guinea pig stratum corneum were measured. Aluminum chlorohydroxide reduced the impedance five times more than aluminum chloride. The results are in agreement with reported skin sorption behavior for these salts and with their antiperspirant activities in vivo. The hypothesis that antiperspirancy is based in part on antiperspirant/skin interaction is supported by the present study. The ELECTROMETRIC method described herein was found to be a viable TECHNIQUE for studying these interactions.

INTRODUCTION

The antiperspirant profiles of aluminum chloride and aluminum chlorohydroxide have been studied extensively in recent years (1,2). These studies indicated that at low concentration (< 0.44M Al), aluminum chlorohydroxide exhibited greater antiperspirant activity, but as the concentration and/or the contact time was increased, the effect of the aluminum chloride became more intensified and surpassed that of aluminum chlorohydroxide. These differences in the bioavailability patterns of the two salts were suggestive of differences in their interactions with stratum corneum.

Dermatological literature describes the stratum corneum as a barrier which is quite resistant to penetration by exogenous agents (3–10). It has been demonstrated that this barrier function can be related to the electrical impedance properties of the skin (6,11–17).

A comprehensive review and discussion of the factors governing the passage of electricity across the skin have been written by Tregear (6), and evaluated by others (11, 13, 14, 18).

Current flow is determined by the migration of ions across the membrane and is thus related to the permeability of the membrane to the ions. Such relationships may be demonstrated by the Nernst-Planck flux equations and the Nernst-Einstein and Ussing's flux relations for ideal solutions (14, 19, 20), but can best be readily correlated with the analogy between molecular (Fick's Law) and electrical (Ohm's Law) transport. Fick's Law: (intergrated form applicable to steady state)

$$J_s = k_p \cdot \Delta C_s$$

where J_s, the amount of solute diffusing across an area of membrane per unit time is described by ΔC_s, the concentration difference across the membrane multiplied by k_p, the permeability constant. Ohm's Law:

$$I = VR^{-1}$$

where I, the current is equal to V, the potential difference multiplied by R^{-1}, the reciprocal of electrical resistance, a constant. If ions carry current across an epidermal membrane, then J_s and I are directly related in the proportionality,

$$k_p \sim R^{-1}$$

Therefore, by following changes in conductance (the reciprocal of R) of the skin, it is possible to follow changes in K_p of ions. This inverse relationship between electrical resistance and permeability constant strictly applies only to ions but, in the case of the stratum corneum, most probably extends to the permeability of any small, highly polar molecule as was shown by Dugard and Scheuplein (14) in their work with ionic surfactants.

MATERIALS

Stratum corneum membrane. Guinea pig stratum corneum was obtained from wax epilated animals. The stratum corneum membrane was separated by ammonia vapors from the tissue.

Sample preparation. The aluminum solutions used in this study were made from commercial 50% stocks (The Reheis Company, Berkeley Heights, N.J.). Solution containing 1% aluminum chloride (0.044*M* Al) was prepared in deionized distilled water at pH 3.42. The ionic strength of the solution was adjusted to 0.5*M* with sodium chloride. The control and rinse solution in this study was 0.5*M* sodium chloride.

The chlorohydroxide solution was diluted to 0.5% (0.044*M* Al). The pH of this solution was 4.71 and its ionic strength was adjusted to 0.9*M* sodium chloride. The control and rinse solutions were 0.9*M* sodium chloride. The adjustment of ionic strength with sodium chloride was made to negate the effect of ionic differences on the impedance and to reduce the effect, if any, of Na^+ ions on one side of the membrane by ensuring Na^+ would be present in both sides of the membrane chamber.

Electrical impedance device. The impedance monitoring device was a modified half bridge circuit as shown in the photograph below (Figure 1). The circuit was powered by an audio generator (Heath Company, Benton Harbor, Michigan) adjusted to deliver 1.0V ac (rms) at 5.0 Hz. The resistance decade boxes and capacitance decade boxes both from (Heath Company, Benton Harbor, Michigan) were modified for ranges of 10 ohms to 10

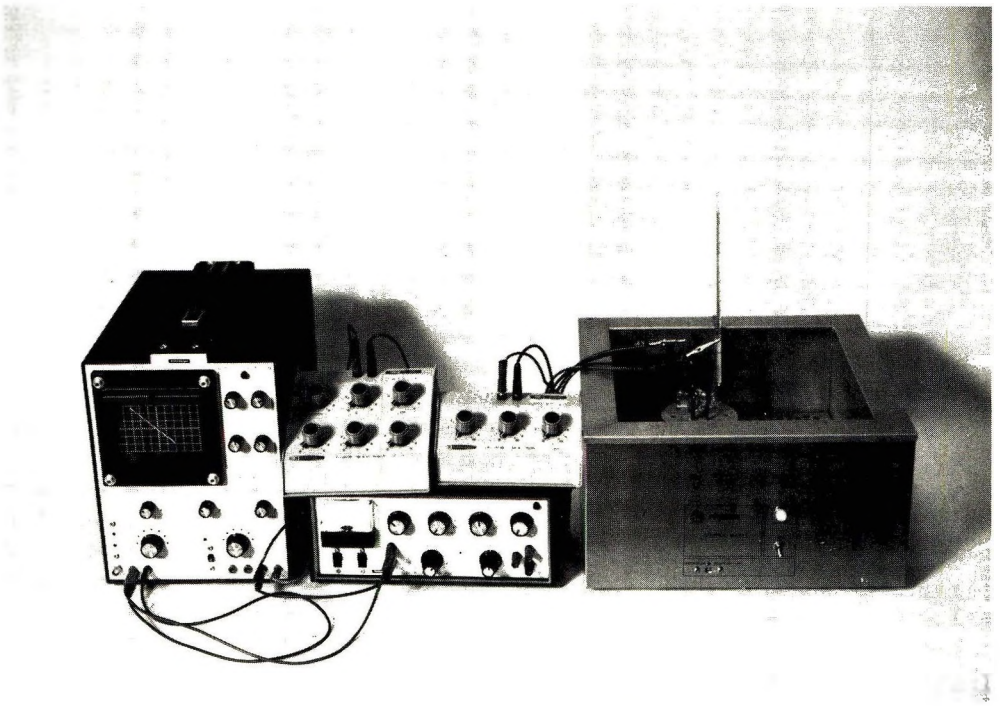

Figure 1. Electronic instrumentation.

Figure 2. Membrane diffusion cells.

megaohms; 0.001 μF to 100 μF and were adjusted to balance the respective resistance and capacitance properties of the system. The resultant balance was monitored on an oscilloscope. The oscilloscope monitored 1.0V Vdc and the horizontal and vertical amplifiers were matched.

Diffusion cells. Glass diffusion cells (Figure 2) were made from 15mm i.d. vacumm-tight, O-ring, glass joints. The joints were cut and sealed to hold 5.0ml and 3.0ml. Each cell had a side arm 5 cm long by 3 mm i.d. for the insertion of the stainless steel electrode. The cells were joined by a standard glass clamp and a type "M" O-ring.

METHOD

Differences in impedance between tissue samples, even from the same animal, have been noted by several workers (14,21–23). For this reason, the impedance of each section of excised membrane was measured prior to treatment. The measurement served to verify the integrity of the membrane as well as to establish a control value. The following regimen was used for each piece of excised stratum corneum and each solution of interest. A 2.5-cm square section of tissue was cut from a larger sheet of membrane and placed on a 4-cm square piece of fiberglass screening; the tissue was then covered by another piece of screening. The sandwiched membrane was placed in an uncovered weighing dish and dessicated over anhydrous calcium sulfate (10–20 mesh) for 24 hr to stabilize the tissue. The weighing dish was then removed and placed over water in another covered dessicator for 17 hr to hydrate the tissue to a standard value. The skin was then removed from the screening and placed between the diffusion cells. The O-rings were employed to tension mount the tissue in place. Standard glass joint clamps were used to hold the chambers together. The appropriate control solutions were then added to both sides of the cells. The electrodes were inserted into the appropriate sidearm and the system was placed in the 35°C waterbath. Impedance was then measured. An X-vs.-Y mode was used to compare and match the phase and magnitude of voltage across the membrane with those of the decade boxes. Balance was assumed when a 45° angle was observed in relation to the X-Y axis. From the decade units, values representing those of the membrane were obtained and the related impedance value, Z, in ohms, was calculated, using the formula:

$$Z = \left(R^2 + \frac{1}{(2\pi fC)}\right)^{1/2}$$

where R and C are the values observed on the resistance and capacitance decade boxes respectively, and f is the frequency of the alternating current. Thus, the ac conductance is the reciprocal of the calculated impedance, Z.

After the impedance had stabilized, the rinse solution was removed and the chambers rinsed six times with additional rinse solution. The test solution was then added to the 5.0-ml side of the chamber and the rinse solution added to the 3.0-ml side. A teflon stirring bar (3 × 9 mm) was added to the receptor side (control side) to disperse any ions which might have diffused through the membrane. The cells were reintroduced to the waterbath and placed on a support over a microsubmersible magnetic stirrer. The change in impedance, ΔZ, was monitored as a function of time until the impedance again stabilized. All test solutions were monitored for 5-6 hr. Twenty-four hour measurements showed that no additional major change in impedance had taken place. To compare the

effects of the various antiperspirant solutions on the impedance of skin, ΔZ values were plotted against time. The ΔZ values are the average Z ratios obtained per unit of time,

$$\Delta Z = \frac{\text{treatment Z}}{\text{control Z}} = \text{ratio}$$

RESULTS AND DISCUSSION

As a test of the instrumentation, the electrical impedance of several untreated excised stratum corneum sections was measured using the appropriate rinse solutions. As shown in Figure 3, a plot of Z values as function of time, using 0.09*M* sodium chloride, 0.05*M* sodium chloride and 0.05*M* acetate buffer, indicate that the impedance rate of change at 5 Hz is similar for all sections of stratum corneum. Further, stabilized values for Z (at 5.0 Hz) with guinea pig stratum corneum are closely related to Z values (at 5.0 Hz) given in the literature (11) for animal stratum corneum.

The difference in Z values noted in Figure 3 merely reflect the impedance differences due to salt and buffer concentrations. Aluminum chloride and aluminum chlorohydroxide solutions with an aluminum content of 0.044*M* were compared for their relative effects on the impedance of the excised epidermal membrane. The results are graphed as Z ratios per unit of time and are presented in Figure 4. The graph represents an average of five trials per test condition. Aluminum chlorohydroxide was shown to reduce the electrical impedance

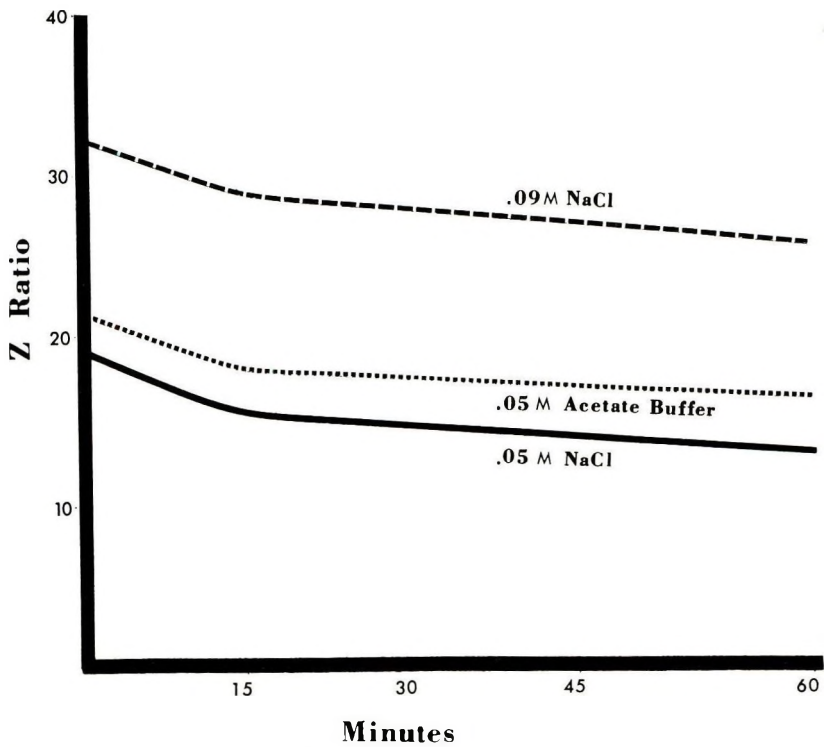

Figure 3. Impedance of stratum corneum (bathed in control solutions) as a function of time. Frequency fixed at 5Hz for all experiments.

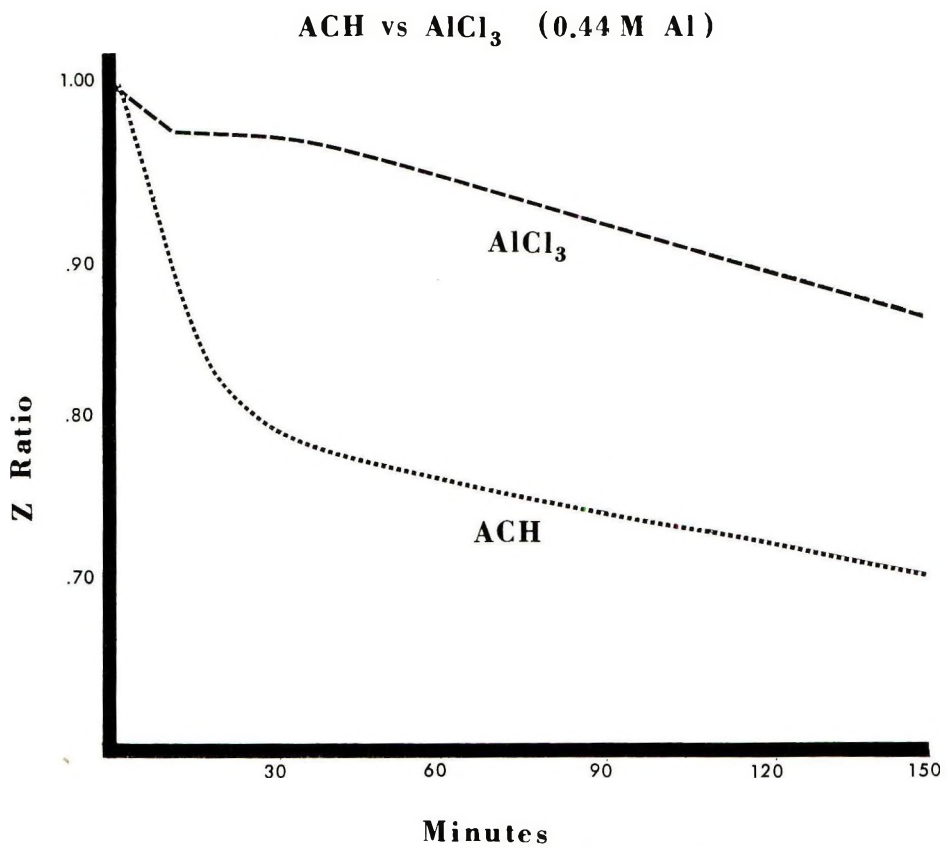

Figure 4. Impedance of aluminum salt treated membranes as a function of time.

of guinea pig stratum corneum by a factor of five when compared to aluminum chloride.

Studies from a number of authors (7, 22, 24–28) have established some of the fundamental physical and chemical characteristics required for epidermal penetration or sorption. Scheuplein (22) clearly showed that the barrier to skin permeability was the horny layer and that individual compounds show different permeability characteristics, dependent on their own particular properties of solubility and diffusion.

Allenby et al. (13) established by in vitro experiments that the impedance of skin is localized in the horny layer. At low frequencies the impedance approximates the direct current resistance and polarization is not a problem. Impedance in this case is largely determined by the resistance component of skin (11, 16) and this may be expected to correlate with ion mobility in the skin barrier. For the studies, an alternating current was used to measure the hypothetical sorption and/or permeation factors. The use of an alternating current overcomes problems of polarization of both membrane and electrodes by preventing increasing charge accumulation and the resultant falsely high, apparent resistance. However, the higher the alternating current frequency, the more readily current passes via capacitative channels which are not dependent on free ionic movement. Permeability and sorptive factors are thus better represented by the resistive current which dominates at low frequencies. Passage of any electric current at a voltage greater than 1–2 volts across human skin results in non-ohmic behavior indicative of damage to the stratum

corneum (14). Thus the low frequency, low voltage ac conductance can be employed to follow alterations in the barrier function of epidermal membranes during contact with various aluminum solutions.

The observed impedance of an epidermal membrane is dependent on the concentration and nature of the current-carrying ions. Thus molarity, ionic strength and pH are important variables in these measurements. Unfortunately when dealing with aluminum compounds, it is often difficult to achieve constant values for all of the above parameters. Thus, within an experiment, values were selectively assigned. In the experiments conducted, each membrane was balanced or stabilized for impedance and this measurement was used as a reference for the test.

The relationship of impedance values obtained to the type of excised membrane used has been reported (6, 11, 14, 29, 30). Tregear (6), using rabbit and guinea pig stratum corneum reported values of 10–20 kilaohms. In the studies reported here, our ohmic values varied on the controls from 12.6 to 31.9 kilaohms. Thus good correlation with those reported in the literature was observed.

Allenby et al. (13) extensively looked at the effects of both temperature and pH on the electrical impedance of skin. They found that between 25 and 60°C the impedance changes very little. Additionally, between the pH's of 5.0 and 8.0 little variation was noted. However dramatic reductions were noted at pH 2.0 and 10.0. In our experiments, a pH of 4.71 was maintained for the aluminum chlorohydroxide and the aluminum chloride solution was at pH 3.41. It should be noted that the major impedance change was observed with the aluminum chlorohydroxide at a pH value which is not as critical as the pH value of aluminum chloride in regard to impedance. Thus the differences observed in the role of these salts in altering skin impedance cannot be based merely on their self pH values but are based on their differences in their interaction with the tissue.

The rates of aluminum chloride and aluminum chlorohydroxide binding to skin has been reported by Putterman et al. (31). These authors found that aluminum chlorohydroxide bound to guinea pig stratum corneum at twice the rate of aluminum chloride. Similar findings for these salts were reported by Fitzgerald and Rand (32) using Sephadex G-25 as the sorption media. Subsequent work by Fitzgerald (33,34) has reaffirmed that aluminum chlorohydroxide binds more quickly than aluminum chloride. Recalling that the Z values for aluminum chloride and aluminum chlorohydroxide are based on R^2, the resistive function of the plot, the differences observed between the salts should be a square of the change due to the sorption. Thus the ΔZ for aluminum chloride at 30 min was approximately 5% and the corresponding ΔZ for aluminum chlorohydroxide was roughly 25%. The magnitude is not an exact square since reciprocal frequency and capacitive functions are reflected in the Z values. From analyzing the impedance data and relating this data to recently established sorption times and rates for aluminum chlorohydroxide and aluminum chloride, it now can be stated that the aluminum chlorohydroxide does sorb more quickly than aluminum chloride and that this is shown directly by impedance changes. It now becomes apparent that the onset of antiperspirant activity can be related to the parameter of sorption which can be measured by the impedance change.

SUMMARY AND CONCLUSIONS

Instrumentation for measuring the electrical conductance of guinea pig skin has been developed. The results were reproducible and in agreement with other studies using similar

tissues and common buffer systems. Sweat inhibition by aluminum can be related to some degree to tissue sorption and that relative sorption can now be measured as a function of impedance change on excised stratum corneum. Aluminum chlorohydroxide sorbs to skin more quickly than aluminum chloride and this is reflected in a five-fold greater effect on impedance. The skin impedance data for aluminum chloride and aluminum chlorohydroxide correlate well with dextran and guinea pig stratum corneum binding data as determined by gel filtration chromatography and morin fluorescence analysis. The electrometric technique discussed in this report can be used as a laboratory method for estimating the sorption and/or antiperspirant potential of ionic salts.

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Regulatory considerations concerning mutagenesis

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As the study of mutagenesis has developed over the past decade, our understanding of mutagenesis and related phenomena has undergone a rapid evolution that may have profound implications for public health. This rapid evolution is also compelling us to reconsider our methods of testing substances for their potential adverse effect on human health and their implications for regulatory decisions. Thus a series of questions have arisen for which society must find answers. I shall attempt to formulate several of the questions and to explain some of the scientific and regulatory considerations that determine the utility of presently available test systems for regulatory decision-making.

Society's concern about preventing cancer and genetic damage is readily understood in terms of public health, but in my judgment this concern may not always be properly directed. The chief question is not primarily whether mutagenesis assay systems are useful for identifying compounds that have the potential for causing genetic damage in future generations, although that is a major question in itself. Instead, the question is whether the results of mutagenesis testing alone can enable us to decide if a substance poses an appreciable risk for inducing cancer in humans.

The scope of that question covers many related issues. For example, are the biological phenomena associated with positive results in mutagenesis assay systems relevant to cancer induction in humans? My answer is both "yes" and "no, not necessarily." I answer "yes" because induction of mutations in the DNA of somatic cells appears to be associated with an important pathway in the cancer induction pattern in experimental animals by many chemical "carcinogens." Furthermore, certain human cancers appear to be associated with exposure (primarily occupational) to chemicals all of which have the properties associated with the quality of *carcinogenicity* and, by definition from the results of mutagenesis tests, of *mutagenicity* as well. Hence we could label some of these substances "carcinomutagens" or "mutacarcinogens." The current leaders of this school of thought include Drs. Ames and McCann. They have accomplished a great deal by expanding their system so that many compounds can be tested rapidly, at moderate cost, and with results that are reproducible by independent laboratories. Their test system (1) has many attractive features, including the capability of assessing mutagenic potency quantitatively over a very wide range. Furthermore, their system is

sensitive enough to detect biological phenomena associated with many (but not all) so-called chemical carcinogens. I have a favorable attitude toward the Ames system because I have been directly and indirectly associated with the development of mutagenic assay systems ever since the 1960's, even before I came to the Food and Drug Administration. The outstanding accomplishments of Drs. Ames and McCann represent considerable progress and improvement on bacterial systems which have also been the subject of research by others, including FDA workers, since the 1960's. FDA's activities in this field have been substantial and have included tests of many compounds, including some on the so-called GRAS list, by various systems as those systems have evolved over the past decade. As we entered the phase of testing in 1970, we encountered problems in interpreting the results from some of the systems. It was not always evident whether or not we were dealing with true positives. We have always encouraged and recommended mutagenesis testing, for we believe that it is helpful to know whether or not "positive findings" are associated with particular compounds, both for developers of products and for those primarily involved in public health.

The question then arises, why doesn't FDA *require* mutagenesis testing *now?* This leads to another question: Which test should be required for what effect, and will the results be used any differently than they are now used on a voluntary basis? The second question involves many issues.

In detecting *genetic* hazards, it would be helpful to measure the potency of a sample of mutagenic compounds in intact experimental mammals. This would provide a better correlation of in vivo effects inherited by succeeding generations. We could thus improve our assessment of genetic risk and our extrapolations from in vitro mutagenic test systems with high sensitivity such as the Ames system. However, the whole issue of forecasting the risk to future generations from mutagenic substances on the basis of our present testing capabilities is the subject of a "white paper" by a select committee functioning under the auspices of the DHEW Toxicology Coordinating Committee. Their report (2) may well serve as a foundation for the evolution of additional test requirements in the future.

In deciding whether or not a test such as the Ames test should be *required* by a Federal regulatory agency for controlling carcinogens, it must be recognized that results of a required test, both positive and negative, will have immediate regulatory significance in decision-making, either alone or in conjunction with other tests. If decisions are to be made on the basis of such tests, those tests must be able to stand up under legal scrutiny by the courts. In court, all relevant evidence will be considered and all relevant testimony by experts qualified by training and experience to assess toxicologic risk to human health is admissable. The following questions are fairly typical of those directed at chemists when they testify in court as to their findings.

1. Is the test *sensitive* in the sense of being capable of detecting the parameters of concern?

2. Is the test *specific* for detecting the parameters of concern (or are other qualities also detected as positive responses, necessitating another independent test to confirm the identity of the parameter being detected)?

3. Does the test *quantitatively* measure the parameters of concern and what is the range of error?

4. Is the test as performed *acceptable* by the current standards of good scientific practice?

My comments on those questions reflect current issues and important considerations, but some of my judgments will undoubtedly change as the data base improves.

1. SENSITIVITY

The Ames test is extremely sensitive. It will detect the majority of chemical carcinogens presently identified as such in our current data base, except for hormones, metals, and carcinogens dependent on physicochemical effect, such as asbestos. So far there is a strong statistical association with known carcinogens (3, 4). The test system has been greatly modified to improve its sensitivity to known carcinogens, such as the activation systems and the modification of the bacterial cell wall.

It must be recognized that the incidence of cancer in test animals or in man can be affected by many other factors that have not been (nor should be) identified as carcinogens. Some of these factors affect susceptibility to cancer, such as nutritional and metabolic factors important to the mammalian body's defense against toxicity, including cancer. Other modifying factors are more appropriately classified as tumor promoters. Examples are hormonally active substances which can stress target receptor cells; they may lead directly to adenocarcinomas or to certain epithelial changes, more properly called metaplasia, in which glandular or transitional epithelium is transformed to squamous, and a squamous cell or undifferentiated type of tumor can form. These factors are associated with overall patterns of cancer induction, but are not necessarily involved in causation or mechanism; latent viruses and their genomes may play a substantial part in the etiology of some cancers in humans. Thus reducing the number of mutacarcinogens in our environment will generally help to control cancer but will not solve the entire problem. The public cannot be protected against all or even most cancers by using a very sensitive test system to identify all "positive" compounds and then trying to reduce all human exposures to these positives to infinitesimal levels. Other Federal laws also affect regulatory decisions. A relevant statute known as the "Delaney Clause" prohibits the intentional addition to foods of a substance legally classified as a food additive if that substance induces cancer in animals or man or has been shown by appropriate tests to induce cancer. The question of whether the Ames test is an appropriate device to support the Delaney Clause should be considered in the context of statistical association with identified carcinogens. We should likewise question the relevance of in vivo long-term feeding experiments in rodents to the human situation.

2. SPECIFICITY

So far, the test system has been fairly specific for the substances tested in that mutagenic effects have been detected for many carcinogens. Thus the present designation of true positives is acceptable, but the question of false positives has not yet been answered satisfactorily. Most of the substances tested so far have been carcinogens; relatively few so-called noncarcinogens have yet been tested. Many more substances commonly accepted as safe for human exposure or consumption should be tested before a judgment is made. I did not say "commonly accepted as being noncarcinogenic," because, under our present in vivo bioassay system and current dosing regimens, some substances will register positive in an animal feeding experiment even though they are not carcinogens. Any factor—carcinogen, promoter, or modifier—can

register as positive in a feeding experiment if the right convergence of factors is present under the particular experimental circumstances. If the Ames test is less specific in actuality than some of its proponents now believe, a number of unexpected positives are likely to be identified in the near future. What do these positives mean? Are they more truly positives than bioassay positives, or are they less potent carcinogens for the intact mammal? And how should we extrapolate these results to humans? Some advocates propose that the Ames test should be used as a screening test and that all compounds found positive should then be tested by appropriate exposure to intact mammals in vivo. Others propose that compounds found positive by the Ames test should be withdrawn from use until they have undergone bioassay testing in mammals and have been demonstrated negative in two rodent species. Still others maintain that a compound which is Ames-positive and bioassay-negative merely proves that carcinogenic bioassay is insensitive either because of species-specific metabolism or the β error problem inherent in using practical sample sizes of 50 male and 50 female rodents per dose and controls. Obviously the term "screening test" can have many connotations, especially in regulatory judgments.

What a manufacturer chooses to do after voluntarily testing for mutagenesis and finding a positive result is still largely a matter of free choice. He can try to eliminate the positive component from his product, he can shift to an alternative compound, or he can undertake lifetime bioassays which are expensive and whose outcome is unpredictable. But in a strict regulatory approach, the number of false positives must be kept to an acceptable level. Undoubtedly, false positives occur in bioassays; that problem, however, was identified only after the carcinogenic bioassay system was accepted by most of the scientific community. Even then, some scientists had reservations about whether the bioassay protocols are a relevant model for human exposures that occur at substantially lower levels than those to which test animals are exposed.

In summary, the question of how false positives relate to the specificity of the Ames test can be resolved only by expanding the number of chemicals tested; however, it is essential not to select only those chemicals known to have the characteristics of carcinogens. The question of relation of false negatives to the specificity of the Ames test does not appear to be a significant problem. Many other criteria can be used to help determine whether a substance should be tested for carcinogenic activity.

3. QUANTITATIVE MEASUREMENT OF THE PARAMETERS OF CONCERN

In one sense, the ability of the Ames test to measure the strength of biological activity of the parameter of concern (reverse mutations) over an exceedingly broad range appears better than the ability of the intact animal assay to measure carcinogenesis. However, each test measures different—although probably related—parameters. The carcinogenesis bioassay measures the capability of a substance in a limited system to statistically affect the incidence of tumors in test animals. For true positives, it measures the ability of the substance to express its carcinogenic activity in the intact mammal. Thus penetration through defense mechanisms is involved, and the critical convergence of intrinsic capability (penetration to the target of opportunity and survival in activatable form) has resulted in a carcinogenic hit. In my judgment, dosage must be considered, as well as many parameters of classical toxicology that show the ability of a chemical to penetrate to the target after having survived various defense

mechanisms that tend to operate on classical dose response curves. Others believe that any exposure to a carcinogen, however small, presents a substantial risk to human health. The Ames test is sensitive enough to detect activities that may be involved in the causation of cancer, but it does not measure penetrability of the substance nor selective parameters such as biological resistance, which are important to evaluation of toxicity, including carcinogenicity.

4. SCIENTIFIC ACCEPTABILITY OF THE TEST

The Ames test is very acceptable at present for limited purposes, namely, decision-making on a voluntary basis with considerable latitude for exercising judgment. However, regulatory decision-making requires stricter criteria for applying judgment, and the answer ultimately must be a simple yes or no. At present there still is considerable controversy over the use of in vitro test systems for assessing risk to human health. Some of the controversy is legitimate. If the Ames test is to be used as a screening test, then what is the definitive test—in vivo carcinogenic bioassay? What happens if the latter test is negative? In how many rodent species and strains? How reliable is this assay system? How much evidence will be needed to make the chemical acceptable if such should be the true and correct response of society? Should the Ames test then be the definitive test? That would be a major decision that would also invoke consideration of changes in how the Delaney Clause is applied.

Many issues are involved that should be settled concurrently to the best of our ability as we continue to evaluate the potential utility of the Ames test for regulatory purposes. At present, if results of a carcinogenic bioassay were equivocal but selected mutagenicity tests were positive, I would consider that observation to be of regulatory importance. The Ames type of test has some regulatory use for evaluating which metabolites of a drug given to humans or to food-producing animals should receive attention if we are to protect the public against carcinogens. However, a decision-tree approach to regulation based solely on positive versus negative results of any single short-term test may cause confusion. For example, what happens if positive results are obtained with natural constituents of foods, spices and flavors? My major recommendation is that we devote more effort to expanding the number of substances tested during the next few years before making a final decision. We need further insight into the specificity of the test system to detect mutacarcinogens. It has been said that most chemical carcinogens have mutagenic activity. With some exceptions, I agree. However, a more relevant question is whether or not most mutagenically positive compounds are also carcinogenic in the sense of posing a substantial hazard to public health. What regulatory decision should be made about weak mutagens or weak carcinogens? We could, of course, make these decisions on a day-to-day basis, but it would be better to answer some of the pressing questions before we settle on such tests for regulatory decision-making. Otherwise the distinction between a screening test and a definitive test will rapidly disappear.

The Ames test and other similar tests have great utility for voluntary decision-making and may be directly useful for regulatory decision-making in the not too distant future. But a premature rush to regulatory judgment may create unanticipated regulatory difficulties and lead to a controversy with connotations similar to the Delaney Clause.

Moreover, a premature confrontation in a judicial setting may create legal decisions adverse to the appropriate regulatory significance of such test systems as the Ames test.

We in FDA recommend using mutagenic tests, including Ames-type tests, to evaluate products. These test systems have many advantages, and further experience with them may demonstrate direct regulatory utility. At present, some of the disadvantages, including the larger questions relating to society's policies about mutacarcinogens, should be addressed and resolved.

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Structure-function relationships of surfactants as antimicrobial agents

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Synopsis

STRUCTURE-FUNCTION RELATIONSHIPS of various classes of SURFACTANTS as ANTIMICROBIAL AGENTS have been reviewed. It was concluded that while polar groups of the biocide tend to predict activity against a given genera, the chain length of the lipophilic group determines the most active member of the chemical class. In general, cationic surfactants are more active than anionic and nonionic agents. Optimum chain length for activity is between 10–16 carbon atoms. Gram (−) and yeast organisms are affected by the lower chain members while gram (+) organisms are affected by the longer chain surfactants. Nonionics, which in the past were considered not to have antimicrobial activity, were shown to be active when the mono-esters were formed from lauric acid.

Because of this new property, nonionics, particularly monolaurin (Lauricidin™), may be useful germicides in addition to their surface active properties. Their nontoxic and low irritation properties make them ideal candidates for cosmetic and toiletry formulations.

INTRODUCTION

Surface active agents are defined as substances which alter the energy relationships at interfaces. Compounds displaying surface activity are characterized by containing hydrophilic (polar) and hydrophobic (hydrocarbon or nonpolar) groups. In our paper the term surfactant was chosen as descriptive of those compounds under discussion. Such terms as wetting agents, detergents or emulsifying agents should be reserved for surfactants denoting specific functions.

Before 1930 the lack of success in the search for active antibacterial agents had slowly formed the philosophy that bacteria could never be expected to respond to chemotherapy. The discovery of antimicrobial sulfonilamides, first observed by Trefouels in Fourneau's laboratory in France in 1935, gave impetus to test other classes of chemicals (1). The recognition and study of the germicidal action of certain surfactants stimulated widespread interest in the possible bactericidal potential of this class of chemicals. For early review of the subject, the reader is referred to Glassman (2). A recent edition of a 1958 treatise is also highly recommended even though the new text is without extensive revision (3). I will limit the scope of my discussion to include only

those germicides which are aliphatic surfactants. My reason for concentrating on aliphatic rather than aromatic surfactants is their lower toxicity. In general, the lipophilic group of the aliphatic surfactant is metabolized to carbon dioxide and water.

Since 1966, our goal has been to discover a highly effective and relatively nontoxic germicide. This review is a summary of these efforts. Our findings combined with others have pointed to certain structure-function (antimicrobial) relationships that may have universal application in the germicide field.

CHEMICAL STRUCTURE—GENERAL CONSIDERATIONS

Compounds exhibiting surface activity are characterized by an appropriate structural balance between one or more water-attracting groups and one or more water-repellent groups. In light of my discussion, the hydrophobic group may be a hydrocarbon chain, branched or straight. Both saturated and unsaturated chains must be considered. The kind, geometric form (*cis, trans*) and position of unsaturation on antimicrobial properties also must be studied.

It is generally recognized that the antimicrobial property of an aliphatic surfactant is dependent on chain length. This relationship is complex since it varies in a nonlinear fashion and is somewhat dependent on the specific class of organism tested. This nonspecific drug action is best understood in terms of Ferguson's principle (4). This principle is modified to include the statement that on ascending a homologous series, although the potency should increase, equipotent concentrations should require increasing thermodynamic concentrations and beyond one particular member the series should become less active. More simply, the antimicrobial affect of an aliphatic surfactant becomes optimal at some specific chain length. This optimum length will vary depending upon the polar group and test organism used.

Whether *n* or *iso*branched fatty acids are more active is controversial. Recent studies indicate that branched-chain acids, like those of the straight-chain acids, are specific with regard to the test organism. There appears to be little overall difference in bactericidal effect which can be ascribed to branching (5).

With a given chain length, the position of the hydrophilic group(s) is an important variable in determining surface properties and biological activity. The kind, geometric isomer and position of unsaturation can influence biological activity. In general, the acetylenic containing fatty acids are more active than the ethylenic members. In the ethylenic series, the *cis* form is more effective against microorganism than the *trans* form.

The polar or hydrophilic portion of the surfactant determines its class. Among the various classes of surfactants, the cationic and, more particularly, the quaternary ammonium compounds have high commercial application. The cationic compounds are by far the most effective wide-spectrum germicides (6). This series can kill or inhibit growth of organisms over a rather wide pH range. The anionic surfactants are frequently active only against gram (+) and yeast organisms and are rarely effective against gram (−) strains. Their action is less rapid than the cationics and is more susceptible to changes in the pH of the system. The nonionic surfactants are not generally considered to be germicidal. However our own research on this group has indicated otherwise. Suffice to say that esters of polyhydric alcohols, amides and aminimides are nonionic surfactants but still have good germicidal properties, as will be discussed later.

CHEMICAL STRUCTURE VS. ANTIMICROBIAL ACTIVITY

As noted in prior publication, chain length is one of the more important variables relating chemical structure to antimicrobial activity (7). For anionic-saturated compounds, the optimum length is 12 carbons (Table I). This statement is true for gram (+) organisms alone since compounds active against yeast are generally one or two carbons shorter in length. Fatty acids with six carbons or less, are active against gram (−) organisms. While the esterification of a fatty acid to a monohydric alcohol leads to an inactive ester, esterification to a polyhydric alcohol forms an active biocide (8). Interestingly enough, the size of the polar group has little effect on the chain length optimum. Glycerol, as well as polyglycerol (*tri, hexa* and *decaglycerol*), derivatives seem to have lauric acid as the most important acyl fatty acid. The bulkier hydrophilic groups seem to impart a narrower spectrum of antimicrobial activity to the surfactant structure.

Indeed, polar groups direct action towards specific organisms while the hydrocarbon chain determines over-all activity of the compound. This is noted in comparing surfactant activity against gram (−) strains. In these cases cationic agents are active against most organisms while anionic and nonionic materials (esters, amides and minimides) have narrower germicidal activities. Except where noted for fatty acids and their esters, amines, amides and aminimides reach optimal biocidal activity with chain lengths of C_{14}—C_{16} (9, 10). In the case of aminimides, chemical agents with rather diverse polar groups, all were active at a chain length of C_{16} (11).

All of these studies emphasize the priority of the hydrocarbon chain as compared to the polar group in determining surfactant biocidal activity against a given species.

Whether unsaturation was important to biological activity was greatly dependent upon the length of alkyl chain. This fact has not been stressed in earlier reports. Unsaturated fatty acids with chain length of C_{12} or lower were generally less active than the saturated derivative. Unsaturated fatty acids with chain length of C_{14} to C_{18} were more ac-

Table I
Minimal Inhibitory Concentrations of Saturated and Unsaturated Fatty Acids[a]

	Pneumococci	Streptococcus Group A	Streptococcus betahemolytic non-A	Candida	S. Aureus
Caproic	NI	NI	NI	NI	NI
Caprilic	NI	NI	NI	NI	NI
Capric	1.45	1.45	2.9	2.9	2.9
Lauric	0.062	0.124	0.249	2.49	2.49
Myristic	0.218	0.547	2.18	4.37	4.37
Myristoleic	0.110	0.110	0.110	0.552	0.441
Palmitic	0.48	3.9	3.9	NI	NI
Palmitoleic	0.024	0.098	0.049	0.491	0.983
Stearic	NI	NI	NI	NI	NI
Oleic	NI	1.77	NI	NI	NI
Elaidic	NI	NI	NI	NI	NI
Linoleic	0.044	0.089	0.089	0.455	NI
Linolenic	0.179	0.35	0.35	NI	1.79
Linolelaidic	NI	NI	NI	NI	NI
Arachidonic	NI	NI	NI	NI	NI

[a] Results are given in mM. NI = not inhibitory at the concentrations tested (1.0 mg/ml or 3 to 6.0 m*M*).

tive than the saturated compound. The unsaturation contributes the most biological activity to the longer chain fatty acid (Table I). Whether or not the position of unsaturation was important to biocidal activity follows this same trend, i.e., the position of unsaturation had no influence on $C_{11:1}$ fatty acids activity (12), some importance to $C_{12:1}$ derivatives and reached maximum effect in $C_{18:1}$ compounds (13). This was true whether unsaturation was ethylenic or acetylenic (Figures 1 and 2). In general the acetylenic derivatives were slightly more active than ethylenic isomers. In the $C_{12:1}$ series the most active isomer was the Δ^{10} $C_{12:1}$, while in the $C_{18:1}$ series the Δ^2, Δ^7 and Δ^8 were more inhibitory to group A Streptococcus than were the other $C_{18:1}$ acids.

The addition of a second ethylenic bond to $C_{18:1}$ further increases the biocidal activity (13). In contrast to mono-unsaturated fatty acids, the addition of a second double bond increases the activity of the fatty acid but without concern to specific positions of the ethylenic bond. The addition of a third ethylenic bond, as in linolenic acid, made the fatty acid less active.

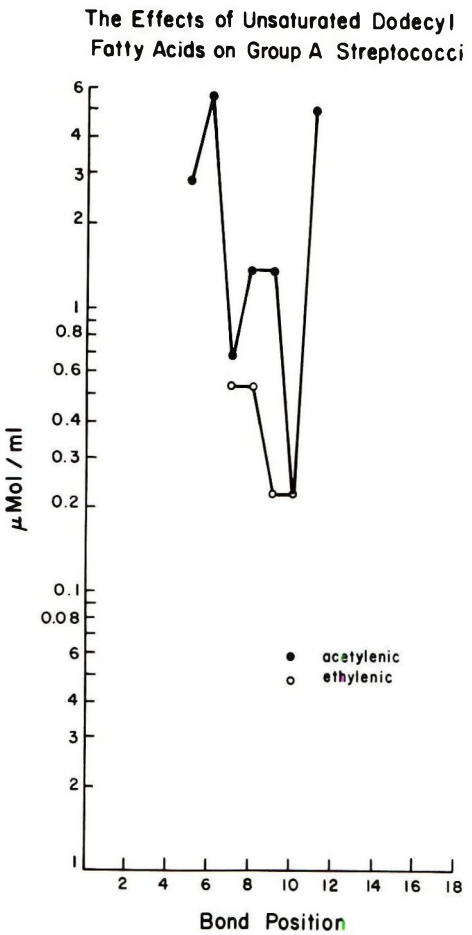


Figure 1. The minimum inhibitory concentration (MIC) values for unsaturated lauric acid derivatives are presented. All compounds are *less* toxic than lauric acid (MIC = 0.12 mM).

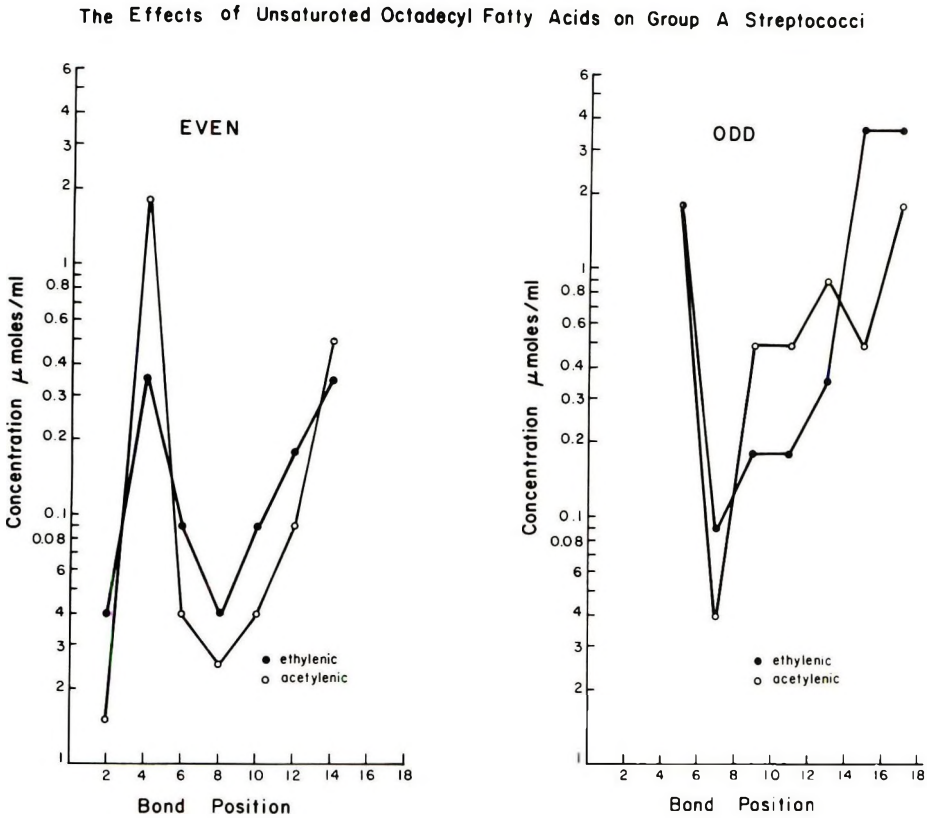


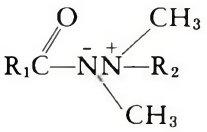
Figure 2. The minimum inhibitory concentration (MIC) of $C_{18:1}$ acids. MIC value for $C_{18:0}$ is 3.52 mM and greater than $C_{18:1}$ derivatives.

Similar experiments involving other unsaturated surfactants have not been carried out. In those few instances wher comparisons are possible, the oleic derivatives are more active than stearic derivatives (9, 14).

While the lipophilic (hydrocarbon) portion of the surfactant is important to biological function in general, the polar group also contributes to biocidal activity. In our early studies, I was interested in the question: what modifications of the carboxyl group are possible and still retain or improve antimicrobial activity?

The answer to that question lead us to examine over 500 compounds before finding monolaurin (Lauricidin™) (15, 16). In our initial studies, I was interested in the chemical reduction of the carboxyl group to aldehyde and alcohol. In general, the order of increasing antimicrobial activity was COOH < CHO < C—OH (7). While the reduction of the carboxyl group lead to a more active species, the oxidation of lauric acid to the dicarboxylic acid produced a less active compound. Wyss et al. (17) reported a similar finding. The esterification of the fatty acid with a mono-hydric alcohol produced an inactive ester. In contrast, esterification carried out with a polyhydric alcohol and yielding a monoester lead to biocidal compounds more active than the parent acid (8). This distinction of monoester formation is extremely important since di and tri esters were less active (8).

Other modifications of the carboxyl group were studied. The formation of a hydroxamic acid derivative lead to an inactive specie, while amide and substituted amide species retained or increased their biological activity (10). Aminimides, a hybrid between amides and amines, represent a new class of surfactants (18). These compounds were particularly effective against yeast and fungi while possessing weak antibacterial activity. Of special note was the fact that aminimides,



are effective when R_1 or R_2 was C_{14}—C_{16} in length (10, 11). This again emphasizes the importance of chain length over polar structure. The final change in surfactant structure studied was the structure-function activity of amine compounds. Amines possess wide spectrum activity and are particularly active against gram (−) organisms (Figure 3). From this figure two observations can readily be made and reinforce previous conclusions. Where agents are tested against the gram (−) genera, lower chain (C_{10}—C_{13}) derivatives are active. Alkyl amines with chain length between C_{12}—C_{18} were more active against gram (+) organisms. From these and other studies I have concluded that all

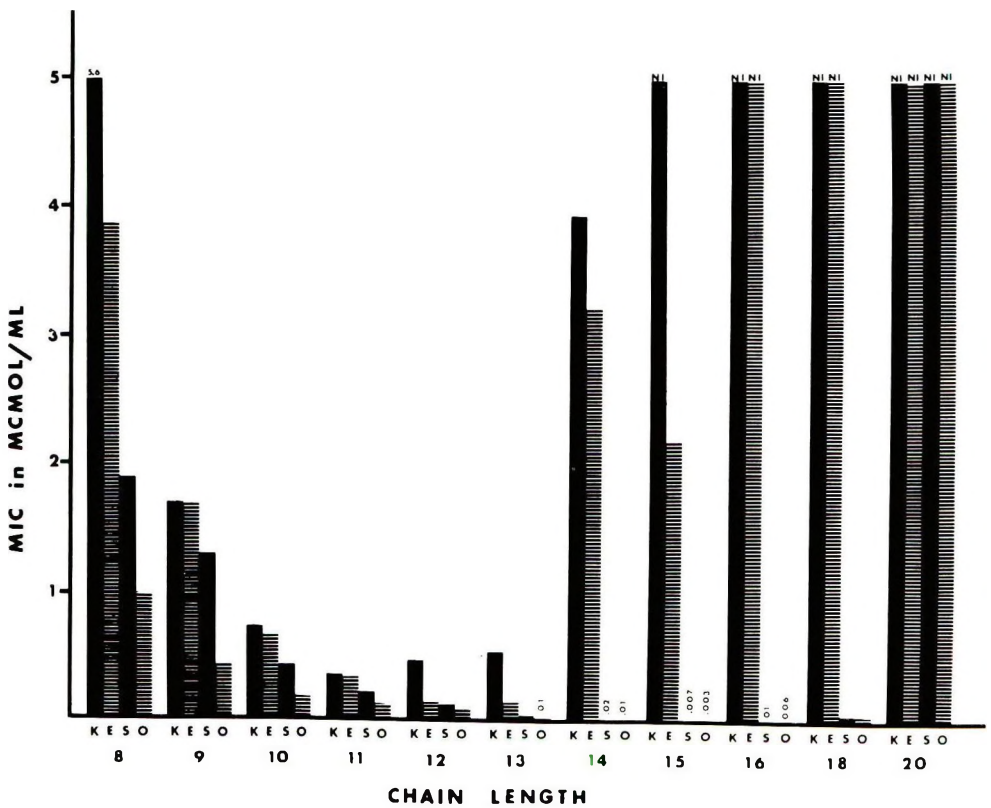


Figure 3. Effect of chain lengthening on amine activity tested against Klebsiella-Enterobacter sp. (K), *E. coli* (E), *S. aureus* (S) and *S. pyogenes* group A (O).

basic compounds, whether aliphatic or aromatic and when active, will show effect on gram (−) organisms.

To summarize, acidic or neutral compounds (amides, aminimides or esters) are active against gram (+) and yeast organisms while amine compounds characteristically enjoy wide spectrum biocidal activity.

ANTIMICROBIAL ACTIVITY VS. HUMAN TOXICITY

The aspect of toxicity and irritation and surfactant biological activity while representing two separate functional properties are today of great concern, especially to the cosmetic chemist. Regardless of the practicality of the zero-risk concept, regulatory agencies have challenged the cosmetic chemist to seek better and safer alternative chemicals. Because all cells (microorganisms) are not alike, it may be possible to design compounds which are effective against microorganisms but not man. All cells are unique and they should be expected to react differently, quantitatively if not qualitatively, to chemical agents. This uniqueness resides primarily in the cellular walls and/or membranes. Indeed it may be the membrane (wall) that not only gives the cell its unique character but also controls its metabolic function. The above argument needs no supporting references for acceptance. Despite this recognition, most drugs have been designed to affect the interior of the cell rather than the membrane. It is my hypothesis that drugs, and surfactants in particular, can be made to act more selectively if their action is directed to certain cells or classes of cells. This hypothesis, conceived during my work with aliphatic surfactants, has now reached fruition.

The best example of this can be found in our study of unsaturated fatty acids. Oleic acid is considered a growth factor for mammalian cells (19). Jenkin et al. (20) found that while Δ^3 18:1 was inhibitory for kidney cells and the Δ^2, Δ^7 and Δ^8 18:1 isomers were growth stimulators, Kabara et al. (13) reported these latter isomers to be lethal against *group A Streptococcus*. This is the first example that I am aware of where a compound such as the Δ^2 $C_{18:1}$ derivative can be a growth stimulator for a mammalian cell and an inhibitory agent for a microorganism. Such specificity can only stimulate the imagination to look for other examples.

Among the surfactants there is a distinct trend towards higher toxicity for aromatic compounds as compared to lipophilic groups, which are aliphatic and natural in origin. Aliphatic surfactants offer germicidal activity which are reasonably high and safe since their biotransformation products—water, carbon dioxide and ammonia or trimethyl amine—are rather innocuous. From a toxicity point of view, the cationic surfactants, while representing the most active biocides, are also the most toxic. The oral LD_{50} toxicity of these basic compounds is between 50–500 mg/kg, the amide and aminimides between 1–3 g/kg, anionic compounds 2–8 g/kg, while the nonionic surfactants, the least toxic, have values between 5–50 g/kg.

The above generalization on agent toxicity suggests that nonionic surfactants deserve greater attention in the future as compared to the past. Past experience, however, has suggested that nonionics have little antimicrobial activity. Our research has demonstrated that this conclusion was based on nonionic compounds composed of mixed acids and representing mixed esters. Our laboratory data with purified surfactants reveal that mono-esters of polyhydric alcohols and esterified with lauric

acid are biologically active (15). The most active of these mono-esters is monolaurin (Lauricidin, > 90% mono-ester), which is considered GRAS material. While the parent compound has useful but limited antimicrobial activity, monolaurin has been successfully compounded with other food-grade materials to yield products with wide spectrum activity and a short killing time (16). I'm sure other successful nontoxic formulations will be developed in the future.

The other toxicological aspect of surfactants of particular concern to the cosmetic formulator is skin irritation (contact dermititis). Lipophilic chains (C_8 and C_{10}) in saturated soaps are highly irritating while C_{12} chain length is less irritating and C_{14} to C_{18} are the blandest. Thus, among soaps, short chains and high alkalinity are conducive to irritation. Commonly, long, straight chain surfactants are less irritating than short, branched chain products. Again, as with toxicity, the quaternaries are, in general, much more irritating than the anionics or nonionics. The nonquaternary amine salts tend to be at least as irritating as the quaternaries and possibly even more hazardous.

In terms of eye irritation, the U.S. Food and Drug Administration laboratories have found a wide variation among individual surfactants and surfactant mixtures. As a general statement, the nonionics are least injurious, the cationics the most injurious and the anionics as intermediate.

SUMMARY

I have, thus, presented some structure-function associations which relate to cationic, anionic, amphoteric and nonionic surfactants as biocides. Of the large number of fatty acid surfactants used, the nonionics appear to be the most attractive. Based on their low index of toxicity and irritability and derived from renewable resources, nonionic surfactants offer real advantages to the cosmetic chemist. Contrary to the art for other nonionics, the high antimicrobial properties of the lauryl monoesters are unusual in that they can impart "medicated" or preservative quality to cosmetic formulations without added toxicity.

With the current rage of "returning to nature," these fatty acid derivatives can rightly claim to be composed of "all natural ingredients." Current research indicates their usefulness as food-grade preservatives, anticariogenic agents and in topical antimicrobial products (15, 16). As petro chemicals become less available and toxicology, rather than high germicidal property, becomes a greater consideration, I predict a wider and more diversified use for nonionic surfactants, particularly lauryl derivatives, as antimicrobial agents in foods, cosmetics and toiletry formulations.

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Book Review

THE FRAGRANCE AND FLAVOR INDUSTRY, Wayne E. Dorland and James A. Rogers, Jr., Wayne E. Dorland Company, Box 264, Mendham, New Jersey, 1977, XV + 444 pages. Price $30.00 + $2.00 for postage and handling.

This most comprehensive text dealing with the fragrance and flavor industry consists of 18 chapters covering almost every aspect of this subject. The authors have succeeded in presenting the subject material in a most interesting and enlightening manner.

Chapter I educates the reader about the nature of fragrances and flavor while Chapter II indicates the role of a flavor and fragrance in the success of a product. Many examples of this are given and the authors recount many of the success stories about products we all are familiar with. It was of particular interest to this reviewer to read about the start of the "Wrigley" empire and the success which followed. The same can be said of products such as "Coca-Cola," "Yardley's Lavender," "Shulton's Old Spice" and many others. Chapter III gives the reader an insight into how the fragrances and flavors are developed by quoting many of the individuals in the industry. Definitions, classification of both flavors and fragrances, and natural and synthetic raw materials are covered in Chapters IV through VIII in a most comprehensive manner. Some of the most important properties of each material is covered.

The history and anatomy of the industry (Chapters IX and X) make worthwhile reading material since it gives the reader a personal insight into the fragrance and flavor industry. The history is covered by giving a brief review of the accomplishments of different individuals and the role they played in developing specific fragrance and flavor companies. Most of the present-day companies are included along with the names of the individuals associated with them.

The newcomer to the industry will enjoy reading Chapter XI since it deals with production processes and equipment. It was interesting and educational to read about the "enfleurage process," "distillation," "solvent extraction," "expression" and the other methods used to produce these materials. It gives much of the necessary background information to the formulator of cosmetic products which will enable one to better utilize these products.

The scientific side of the industry has also been covered in Chapter XII which has been authored by Dr. Ernst Theimer. He covers in detail the composition of various materials and the basic chemistry involved. The remainder of the chapter is devoted to the use of instrumental analysis for the identification of each component. The use of gas chromatog-

raphy for perfumes and fragrances is covered in great detail. Various spectrometers including UV, IR and mass spectrometer are included along with methods which will utilize a computer hookup. The use of the "nose" as an analytical instrument is *not* forgotten and its use is indicated.

The final chapters include a discussion of quality control (Chapter XIII), product safety and regulations (Chapter XIV), and a description of various associations related to essential oils and the perfume and fragrance industry (Chapter XV). It is interesting to note that our own SCC is included along with a description of our activities. Chapter XVI reviews the many journals, magazines and books concerned with this industry and is entitled, "We Share Our Knowledge." It is only fitting that one of the final chapters be concerned with "People" and the important role they play in the industry. The training necessary to develop suitable skills to function properly in the industry is covered. The authors also include job descriptions for people in the industry along with suitable qualification. The final chapter is devoted to some statistics of the industry.

This reviewer has had a great difficulty in putting this book aside rather than reading it from "cover to cover" in one sitting. The authors are to be congratulated on this accomplishment since this book represents a "must" for anyone concerned with any aspect of the development of cosmetic products and any other product containing a fragrance or flavor. It should be required reading for all newcomers to the industry. Not only is this book an "encyclopedia of fact," but it makes reading enjoyable while one is learning. I recommend that it be on the literature shelf of every laboratory or office.—JOHN J. SCIARRA—Arnold & Marie Schwartz College of Pharmacy and Health Sciences.

SYNTHETIC DETERGENTS, 6th Edition, A. Davidsohn and B. M. Milwidsky, Halsted Press, New York 1978 VIII + 265 pages. Price $25.00.

In the preface, the authors state that "this is a work by practical men for practical men" and, in general, they have accomplished this objective quite well. This book is intended primarily for the manufacturing chemist in the detergent field and it should be very useful for that purpose. However, for cosmetic chemists and others working with surfactants, it could be of inestimable value to know how these detergents are prepared, what methods are used in their quality control, and their applications. A knowledge of all phases of production of raw materials one uses in cosmetic and pharmaceutical development can be very useful in tracing down troublesome problems in stability, component interation and packaging integrity.

The first chapter deals with the development of the detergent industry and is brief but comprehensive. Next the authors deal with principle groups of synthetic detergents—anionics, cationics, and non-ionics and their constituent subgroups. Again brevity is the rule, but not at the expense of accuracy and comprehension. Such things as basic structural formulas, common methods of preparation, and salient characteristics of each group are catalogued. This chapter is followed by one dealing with inorganic components of detergents, builders and other additives covering such groups as phosphates, silicates, carbonates, oxygen-releasing materials and sundry inorganic builders. A whole chapter is devoted to sundry organic builders such as anti-redeposition agents, thickening agents, optical brighteners, chelating agents, hydrotropes, enzymes, bacteriostats, amines and solvents. Chapters five and six deal with the synthesis and manufacture

of detergents and this is handled in a succinct and informative way. These chapters are followed by one on application and formulation of detergents where various helpful starting formulas for various types of finished products are presented. Most of these deal with straightforward detergent applications; however, there is a section on toilet preparations such as shampoos, bubble baths and household and cosmetic soaps.

The final chapter, which deals with a brief exposition of analytical methods used in the detergent field, is most noteworthy for a schematic procedure for the analysis of detergents.

In summary, this is an excellent book dealing with the practical aspects of the preparation, use and characterization of synthetic detergents and is intended primarily for technologists in this field. Its use would be limited for most cosmetic chemists except for the reasons cited previously.—ROBERT MARCHISOTTO—Bioservices Information Service, Philadelphia, Pa.

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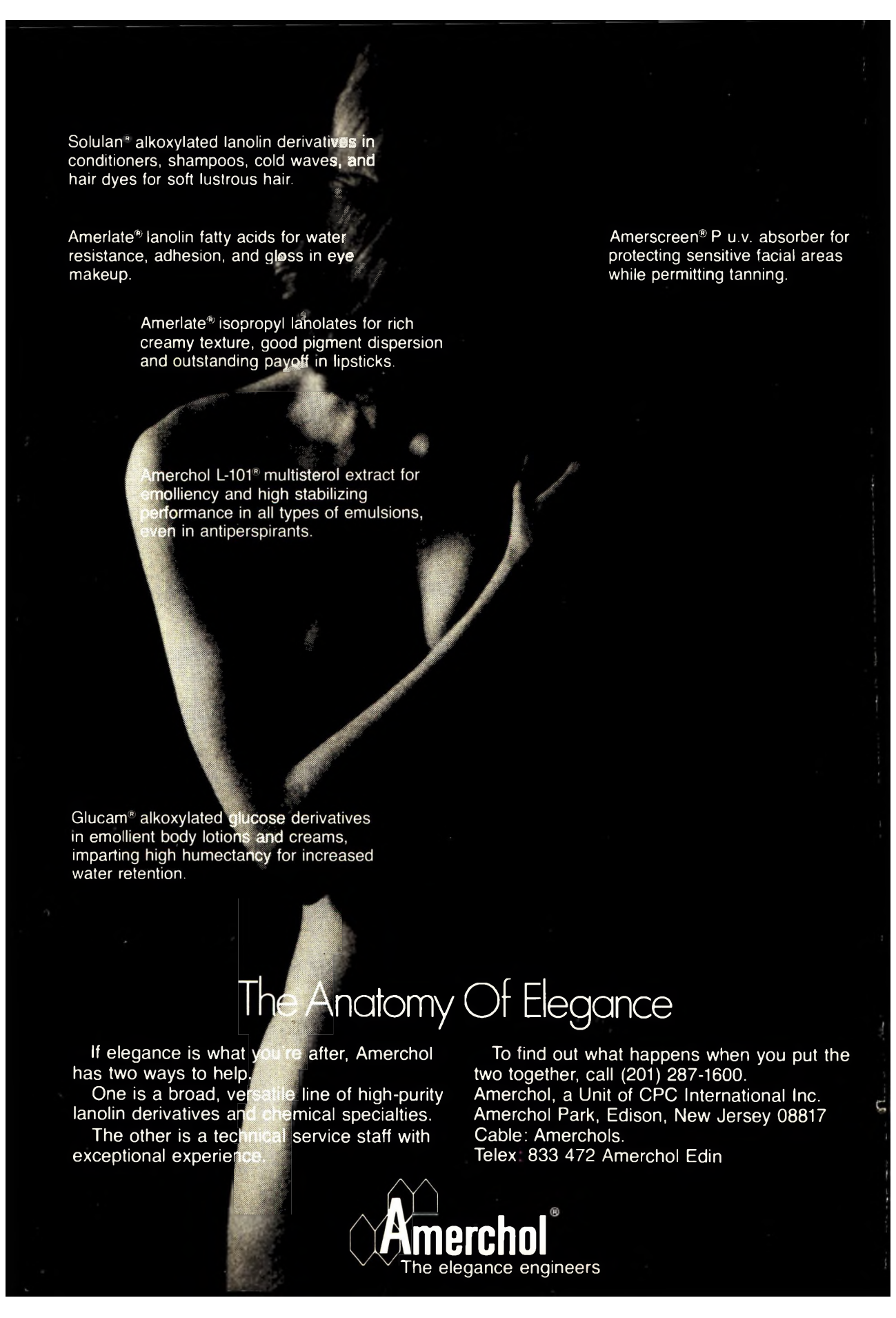

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