Table II—Critical Surface Tensions

Material	γ_c Hydrocarbon Series	γ_c Diluted Surfactant Series[a]	γ_c Polyoxyethylene Octylphenols	Literature Value
Polytef	19.2	19.5	17.9	18.5 (Ref. 1)
Paraffin	—	—	24.9	25.5 (Ref. 3)

[a] Polyoxyethylene (5) octylphenol solutions.

were achieved by dilution. Figure 1 shows the relationship between surface tension and solution concentration for this surfactant, which has the shortest hydrophilic group among the octylphenol derivatives. Also included in Fig. 1 are similar data for polyoxyethylene (40) octylphenol, which has the longest hydrophilic group.

The third liquid series contained eight octylphenol derivatives of uniform concentration. From the results in Fig. 1, 2.5×10^{-3} *M* was selected as the concentration to be used because, at that value, small variations in concentration would have a negligible influence on surface tension. Properties of the surfactants in this series are presented in Table I.

The fourth liquid series consisted of some polyethylene glycol monostearates. Only three with sufficient water solubility were available. Figure 2 is a plot of surface tension as a function of concentration for two members of this surfactant family. All solutions for contact angle measurement contained 0.15% (w/v) of the monostearates; γ_L values for polyethylene glycol 600, 1000, and 4000 were 35.3, 39.7, and 44.2 dynes/cm, respectively.

Figure 3 is a Zisman plot for hydrocarbon liquids on polytef. The critical surface tension determined from this plot, 19.2 dynes/cm, is in good agreement with the literature value of 18.5 dynes/cm (1). Results with polytef using the second liquid series, diluted solutions of polyoxyethylene (5) octylphenol, are presented in Fig. 4. The curvature this plot exhibits interfered with the required extrapolation, but γ_c was estimated to be 19.5 dynes/cm.

Contact angles of the two surfactant series on polytef are shown in Fig. 5. A critical surface tension value for the polyethylene glycol stearates was not determined because of the long extrapolation required, but it is evident that results with both sets of surfactants were similar. The plot of $\cos\theta$ *versus* γ_L is linear. The same is true for contact angles on paraffin (Fig. 6).

The critical surface tension values obtained on polytef and paraffin are summarized in Table II. In both cases, γ_c values derived from the use of surfactant solutions are quite close to those found using pure nonpolar liquids. Values of critical surface tension are not independent of the properties of the group of liquids used in their determination (2). The agreement in γ_c found with surfactant solutions and hydrocarbon liquids supports the notion that surfactant solutions above the CMC behave as though the liquid surface were a hydrocarbon (4). In the presence of a low energy surface, the adsorbed surfactant film seems to maintain its orientation and arrangement.

The use of a family of surfactants has several advantages over dilution of a single compound to vary surface tension. Diluted surfactant solutions invariably lead to curved Zisman plots, which make extrapolation to γ_c difficult. This may be because dilution below the CMC causes the solution to lose its compact surface film, and the surface tension acquires a larger polar component. Use of a series of surfactants allows maintenance of the surface film in each solution and leads to linear plots (Figs. 5 and 6).

Furthermore, if adsorption of the surfactant molecules by the solid material or a component of the apparatus should occur, the concentration of surfactant in a highly dilute solution may be changed sufficiently to affect the surface tension in the liquid drop whose contact angle is being measured. Thus, the actual surface tension of the liquid would be different from that ascribed to it. Because concentrations of surfactant solutions are well above the CMC for each member of a surfactant family, some adsorption of surfactant would not cause this problem.

To summarize, critical surface tension values for polytef and paraffin determined using a family of solutions containing nonionic surfactants agree well with reported values. This liquid series provides a reasonable approach to the measurement of γ_c values for solid drugs that are soluble in organic liquids but relatively insoluble in water.

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Critical Surface Tensions of Pharmaceutical Solids

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Abstract □ Advancing contact angles measured on compacts of several drugs by the sessile drop method and also by penetration through a column of drug granules were used to find the critical surface tension of the drugs. After liquid was delivered at a very slow rate, the contact angle of sessile drops decreased with time, but use of a consistent method of timing always led to the same value for critical surface tension. Results from penetration studies and work on compacts were in agreement, provided that the surfaces of the compacts were smooth and highly reflective. Critical surface tension of the six drugs, three analgesics and three sulfonamides, ranged from 31 to 33 dynes/cm. The critical surface tension of mixtures of phenacetin and microcrystalline cellulose was not a linear function of the relative surface fractions of the two materials. If the surface contained 25% or more of phenacetin, the critical surface tension barely differed from that of pure phenacetin.

Keyphrases □ Surface tensions, critical—various pharmaceutical solids analysis, by sessile drop method □ Surfactants, nonionic—used to determine surface tensions of various pharmaceutical solids □ Solids—surface tensions □ Contact angles—wetting of pharmaceutical solids

Wetting of a solid by a liquid is an important step in several pharmaceutical processes including the preparation of suspensions (1) and the adhesion of film coatings to tablets (2). The dissolution rate of powdered phenacetin was sensitive to the surface tension of the dissolution medium because of differences in wetting (3).

As described previously (4), the critical surface tension, γ_c, of a solid is a measure of its wettability and is related

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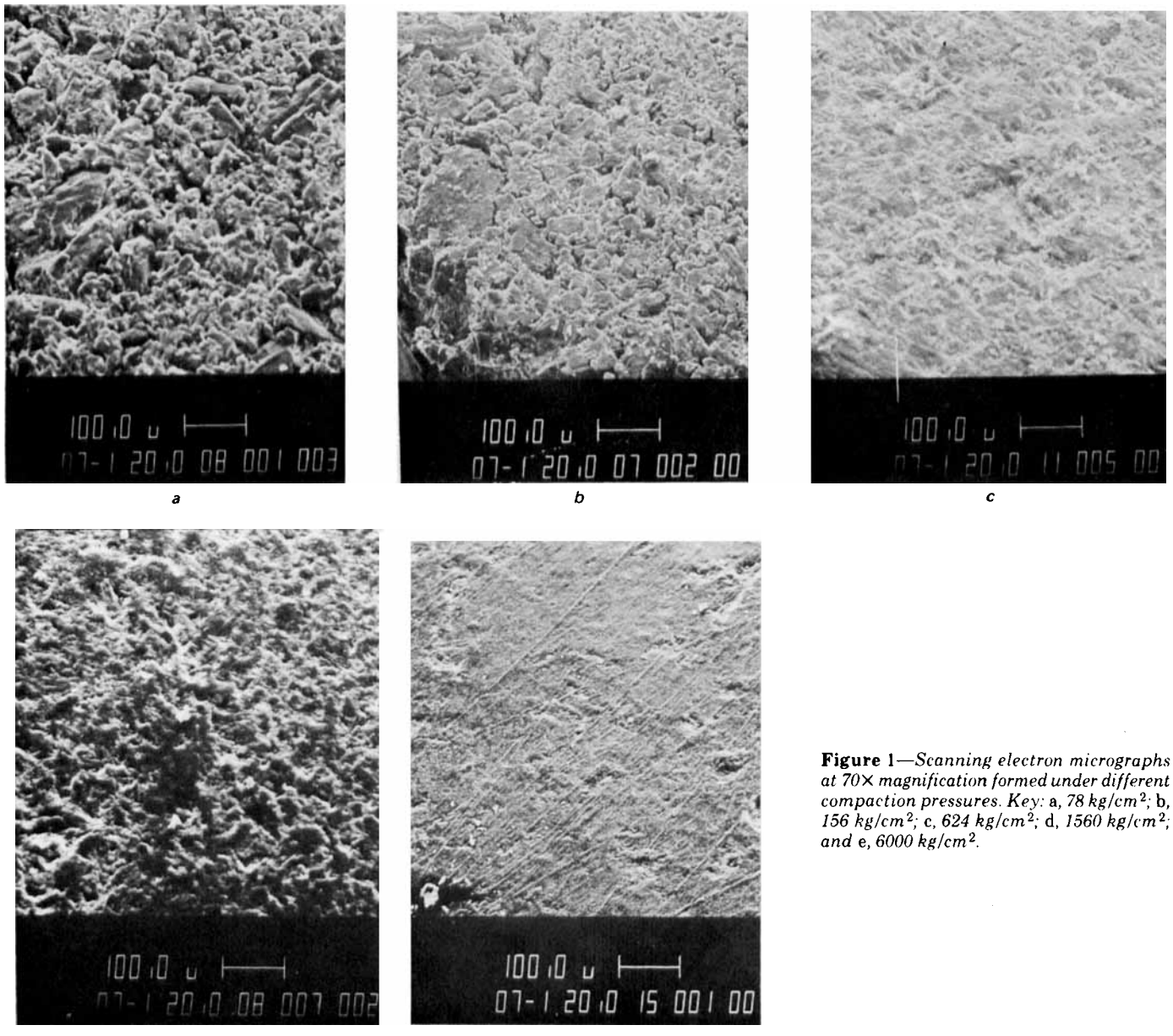

a b c d e

Figure 1—*Scanning electron micrographs at 70× magnification formed under different compaction pressures. Key:* a, *78 kg/cm²*; b, *156 kg/cm²*; c, *624 kg/cm²*; d, *1560 kg/cm²*; *and* e, *6000 kg/cm²*.

to solid surface energy (5–7). If nonpolar liquids are used for the contact angle measurements, γ_c is equal to the dispersion force component of the surface energy of the solid (6). While much work has been done on polymeric solids, comparatively few studies have been concerned with low molecular weight materials (8). Other studies dealt with keratin and skin (9–11).

BACKGROUND

The critical surface tension of aspirin and effects of adjuvants were studied using methanol–water and 1-butanol–formamide solutions to measure the contact angles (12). Contact angles of water and methylene iodide were determined on the surfaces of a number of substances (13). In experiments of this type, contact angles, θ, are used to gain an understanding of the forces acting at the interfaces. The known properties of the liquids are used as tools to explore the nature of the solid surface.

Various approaches have been used to measure contact angles on powdered pharmaceutical solids. Direct angle measurement with a goniometer was used after compression of the powder to obtain a smooth surface (13). The maximum height of a liquid drop on a compact of powder saturated with that liquid was measured (14). Contact angles were also determined from the penetration rate of liquids through the solid material (15–17).

With the sessile drop technique, smooth surfaces yield the most reliable measurements. Surface roughness causes deviation of the measured contact angle from its true value and increases the difference in magnitude between advancing and receding angles (18). Dissolution of the solid can lead to changes in the physical characteristics of the surface and also in the surface tension of the liquid. Both effects lead to biased contact angles. Penetration of the liquid into the solid can cause difficulties as well.

The choice of a suitable series of liquids may also present difficulties. The liquids should exhibit poor solvent power for the solid and a broad range of surface tension values. Previous work in this laboratory showed that homologous nonionic surfactants have suitable properties for contact angle work (4). Each surfactant is used to prepare a single solution well above its critical micelle concentration. In this way, the liquid surface consists of an oriented layer of surfactant molecules, making the surface similar to that of a hydrocarbon. Experiments with polytef and paraffin (4) showed that this liquid series could be used to obtain reliable values of critical surface tension.

In this study, critical surface tensions of several pharmaceutical solids were obtained using the nonionic surfactant series described. The poor solubility of hydrophobic drugs in these surfactant solutions minimizes problems due to dissolution during contact angle measurement. The

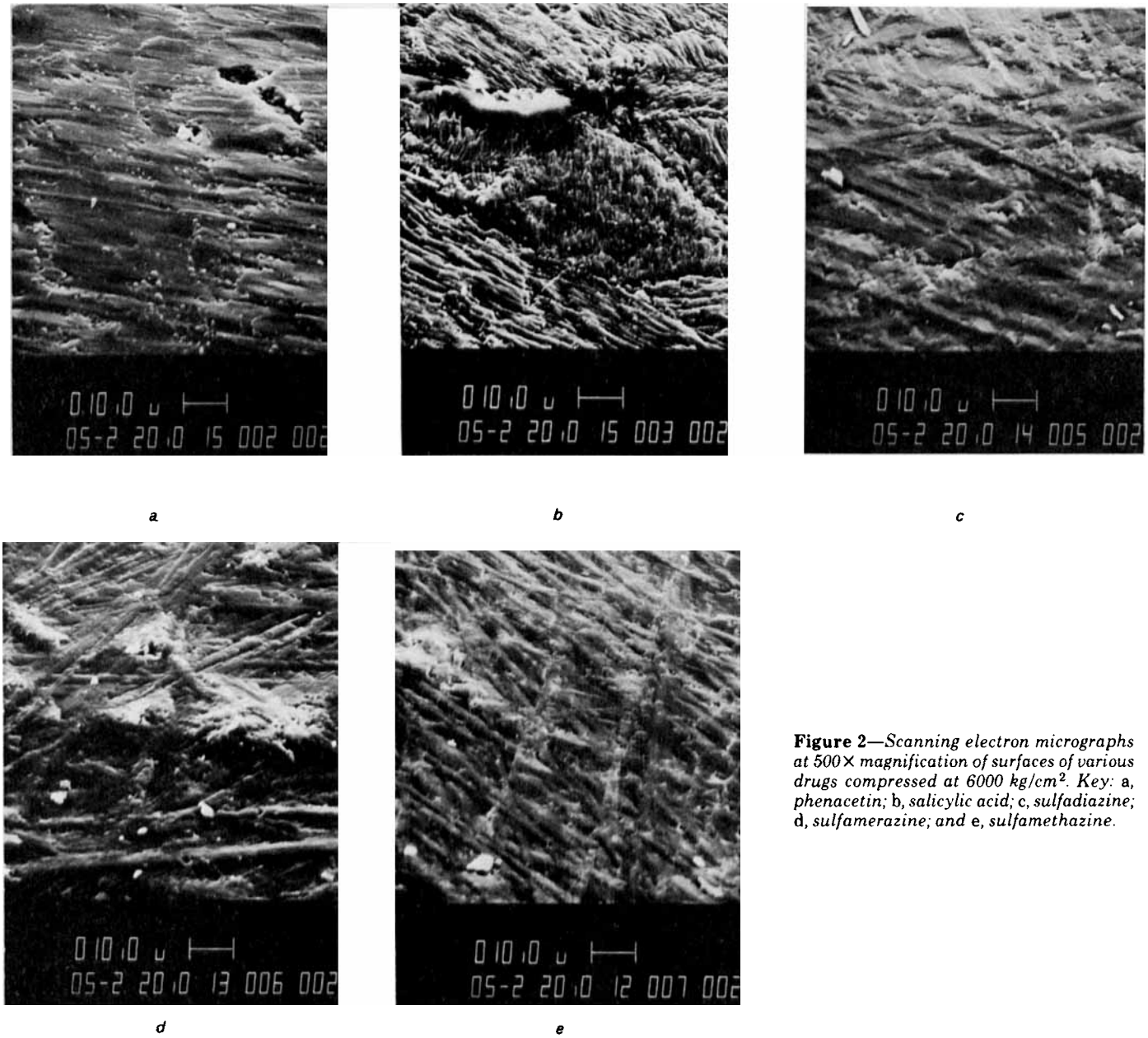

Figure 2—*Scanning electron micrographs at 500× magnification of surfaces of various drugs compressed at 6000 kg/cm². Key:* a, *phenacetin;* b, *salicylic acid;* c, *sulfadiazine;* d, *sulfamerazine; and* e, *sulfamethazine.*

sessile drop method and a penetration rate method were compared. The effects of surface heterogeneity in mixtures of phenacetin and microcrystalline cellulose on contact angles and critical surface tensions were also evaluated.

EXPERIMENTAL

Materials—Aspirin[1], phenacetin[1], salicylic acid[2], sulfadiazine[3], sulfamerazine[3], sulfamethazine[3], and microcrystalline cellulose[4] were the solid drugs studied. The nonionic polyoxyethylene octylphenol series tested previously (4) was used because of its numerous members with adequate water solubility and the relatively wide range of surface tension encompassed. Water was double distilled in an all-glass still. Other materials were reagent or USP grade.

Particle Size—The average particle size of the drugs was determined with a subsieve analyzer[5].

Preparation of Compacts—The solid material was loaded into a 1.27-cm die. A stainless steel block covered with aluminum foil previously cleaned with acetone was used to support the die. The lower punch was not used. Pressure was exerted by a manual press[6] through the upper punch to form the compact. Surfaces of representative compacts were examined by scanning electron microscopy. The specimens were mounted, coated with gold using a sputter coater, and scanned at 45° tilt under an accelerating voltage of 20 kv. Images were recorded on black and white film.

The effect of compaction pressure on the appearance of aspirin surfaces is shown in Fig. 1. Surface irregularities became fainter as the compaction pressure was raised. A standard value of 6000 kg/cm² was chosen, and all contact angle measurements by the sessile drop method were conducted on compacts formed at this pressure. Photomicrographs of other drug surfaces produced at this pressure are shown in Fig. 2.

All surfaces were judged by eye to be sufficiently smooth and reflective except salicylic acid, which exhibited channels. Compacts with smooth, shiny surfaces were used for contact angle measurement. For this purpose, the compacts were formed and retained in the die. Only the smooth lower surface was used.

Preparation of Solids for Penetration Study—Preliminary work showed that the use of powdered drug was unsatisfactory for penetration rate experiments. Anomalous results were obtained and were attributed to clogging of the frit by tiny particles. Consequently, granules were used in the experiments reported here.

The drugs studied by the penetration method, aspirin, phenacetin, and

[1] USP powder, Merck & Co., Rahway, N.J.
[2] Reagent powder, J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] USP powder, American Cyanamid Co., Pearl River, N.Y.
[4] Avicel Ph-102, Fisher Scientific Co., Pittsburgh, Pa.
[5] Fisher Scientific Co., Pittsburgh, Pa.

[6] Model 341-20, Loomis Co., Caldwell, N.J.

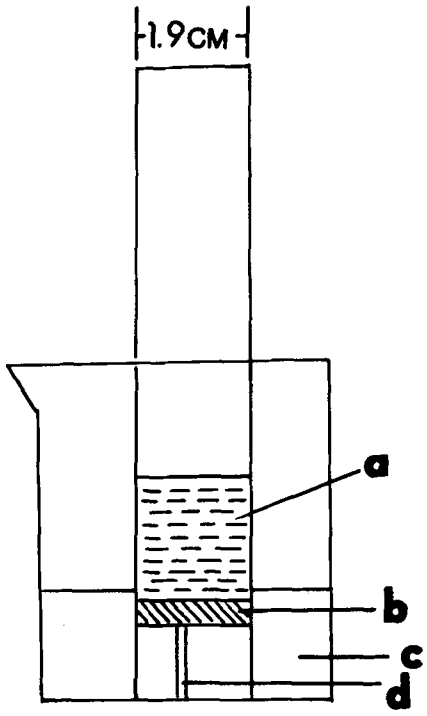

Figure 3—*Apparatus for penetration studies. Key:* a, *packed column in tube;* b, *glass frit;* c, *penetrating liquid; and* d, *slit in tube.*

salicylic acid, were granulated by adding 40 ml of acetone to 400 g of drug powder. Wet granules, obtained by extrusion through a 30-mesh sieve, were collected and dried in an oven for 1 hr with the ventilation air temperature at 26.7°. Dry granules that passed through a 20-mesh sieve but were retained on a 30-mesh sieve were collected and used.

Properties of Nonionic Surfactant Solutions—All of the polyoxyethylene octylphenols were used at 2.5×10^{-3} *M*. The surface tension of these solutions was reported (4). Solution viscosity was measured at 25° by a capillary viscometer[7]. Water was used as a standard. No kinetic energy correction was needed.

Contact Angle Measurements—For the sessile drop method, the instrumentation was the same as that previously employed (4). The total

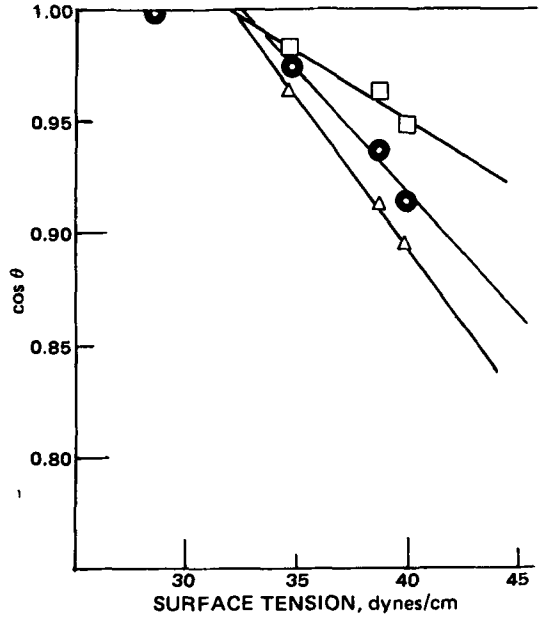

Figure 4—*Cosine of contact angle on sulfamerazine as a function of surface tension. Key:* △, *dynamic angle;* ○, *static angle; and* □, *angle after 5 min.*

[7] Cannon-Fenske.

Table I—Contact Angles of Polyoxyethylene Octylphenol Solutions on Sulfamerazine Compacts by the Sessile Drop Technique

Average Number of Oxyethylene Groups per Molecule	Surface Tension, dynes/cm	θ		
		Dynamic	Static	5 min
5	28.5	Spreads	Spreads	Spreads
9	30.0	Spreads	Spreads	Spreads
12.5	33.0	>10°	>10°	>10°
16	34.7	16°	13°	11°
30	38.8	24°	21°	16°
40	40.0	26°	24°	19°

volume of the drop was about 15 μl. The liquid was slowly placed on the surface (0.5–1.0 μl/sec), and the contact angle of the moving liquid boundary was measured directly. This measure is called the dynamic contact angle. Fifteen seconds following the final addition of liquid, a second measurement, the "static" contact angle (19), was made. A third measurement was made 5 min after the liquid application. Mean values of contact angle were calculated from at least three readings, each on a newly prepared solid surface. Values of θ were reproducible within ±3°.

The apparatus used for penetration rate measurements is shown in Fig. 3. A glass frit (with a pore size of 25–50 μm) was fused into the glass tube about 1.0 cm above the bottom. Two slits were cut in the glass from the frit to the bottom of the tube to permit liquid to reach the cylinder without trapping air bubbles. With aspirin, 2.40 g of the granules was loaded into the tube. The granules were packed to a constant height (1.7 cm) by dropping the tube 60 times from a height of 2.5 cm. Column preparation was the same for phenacetin and salicylic acid, except that 2.50 g of these materials was used.

A very small amount of amaranth was sprinkled on top of the packed column. The tube was then inserted into a 50-ml beaker containing 8.00 ml of the solution whose contact angle was to be measured, and the timer was started. Penetration of the liquid completely through the column caused the appearance of a red color at the center of the top of the column, and this color was used as the end-point. A single beaker was used for all experiments on a particular solid.

RESULTS AND DISCUSSION

The contact angles determined by direct observation on compacted powdered drugs depended on the time lapsed after application of the drop. Table I lists the various contact angles for sulfamarazine. Angles less than 10° were difficult to measure accurately and were not used in Zisman plots to determine critical surface tension.

The pattern seen in Table I was observed for all drugs. As observed previously (19), the dynamic angle was always the largest of the three

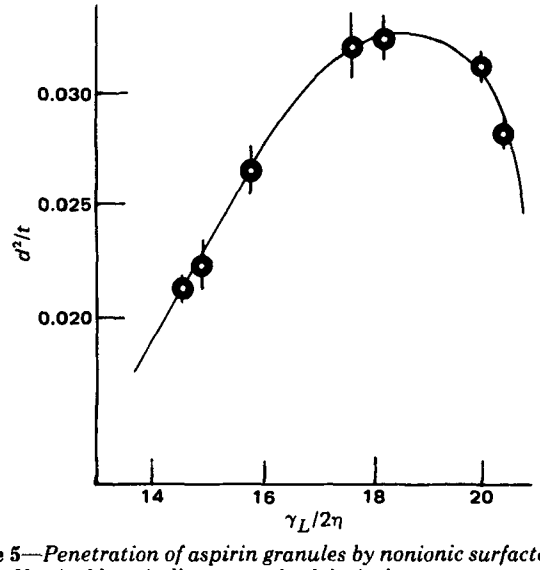

Figure 5—*Penetration of aspirin granules by nonionic surfactant solutions. Vertical bars indicate standard deviation.*

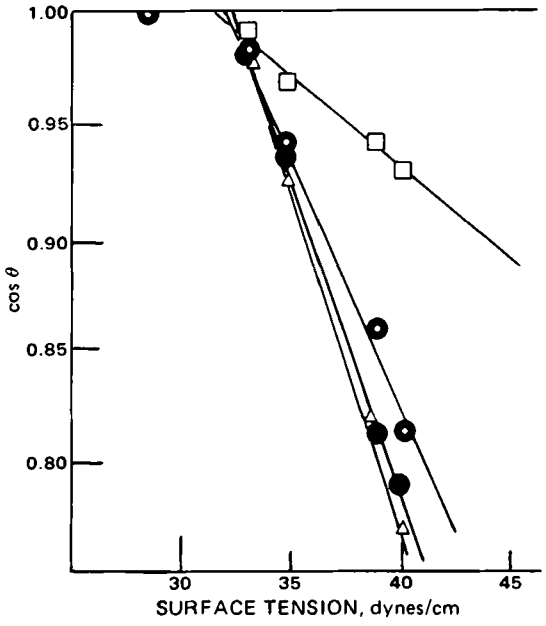

Figure 6—*Cosine of contact angle on aspirin as a function of liquid surface tension. Key: △, dynamic angle; ○, static angle; □, angle after 5 min; and ●, angle from penetration study.*

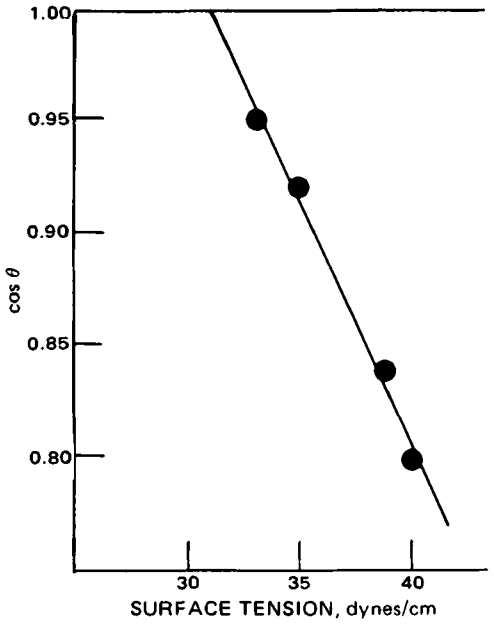

Figure 8—*Cosine of contact angle on salicylic acid as a function of liquid surface tension.*

measured values. The static angle, measured after 15 sec, was always somewhat smaller. The contact angle observed after 5 min was the smallest.

The dynamic angle is based on a measurement made at the edge of a moving liquid boundary in contact with a fresh surface. After addition of liquid has ceased, evaporation and, possibly, penetration cause a decrease in drop volume that leads to a lowering of the contact angle. Under these conditions, the contact angle approaches that of a receding drop (20).

In Fig. 4, $\cos\theta$ for sulfamerazine is plotted against liquid surface tension. The critical surface tension is the surface tension required for the liquid just to spread on the solid. It is found by extrapolation of the curves to a value of $\cos\theta = 1$. Extrapolation of each set of points leads to the same value of critical surface tension for sulfamerazine. This pattern was observed for all solids.

In the penetration studies, there was no evidence of collapse or cracking of the granule columns. The penetration data were analyzed by the Washburn equation (15–17):

$$\frac{d^2}{t} = \frac{\bar{r}\gamma_L \cos\theta}{2\eta} \qquad \text{(Eq. 1)}$$

where d is the distance traveled by the penetrating liquid, t is the time required, $\bar{r}$ is the average pore radius, and η is the viscosity. In these experiments, d was always the same for a particular solid and t was experimentally determined for each liquid.

A typical penetration plot is shown in Fig. 5. Curves with this shape are obtained when the range of liquid surface tensions encompasses both wetting and nonwetting liquids. For the wetting liquids, $\cos\theta = 1$. According to Eq. 1, d^2/t should decrease as γ_L is decreased. This decrease accounts for the shape of the left side of Fig. 5. In this region, a plot of d^2/t *versus* $\gamma_L/2\eta$ is linear and permits determination of $\bar{r}$ from the slope. This plot did not go through the origin as predicted from Eq. 1. Instead, a

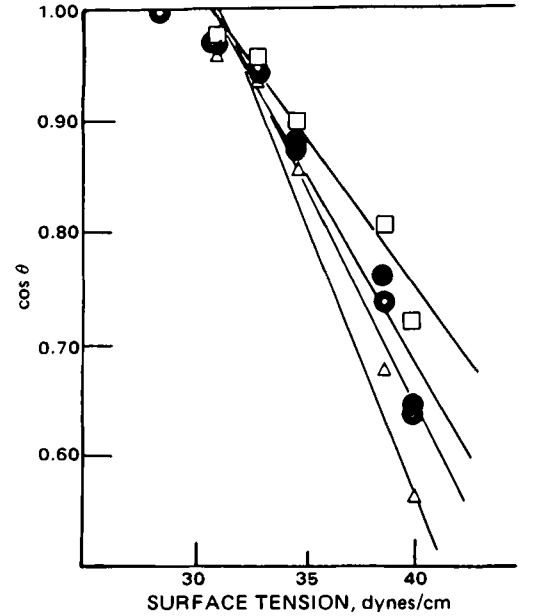

Figure 7—*Cosine of contact angle on phenacetin as a function of liquid surface tension. Key: △, dynamic angle; ○, static angle, □, angle after 5 min; and ●, angle from penetration study.*

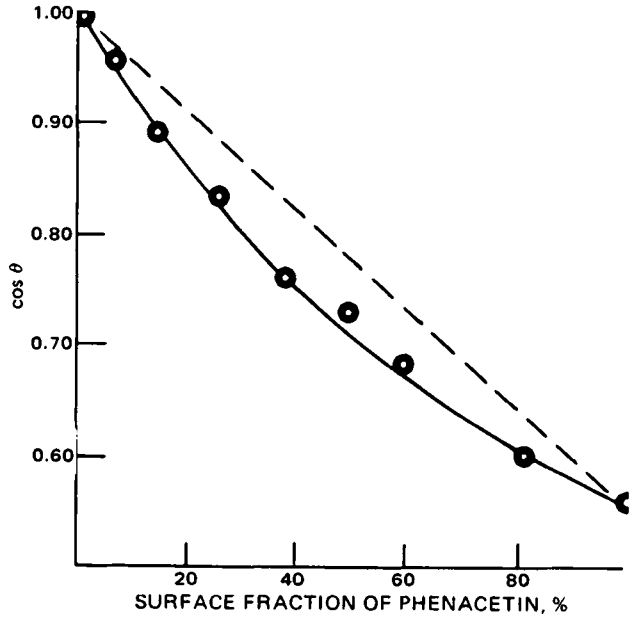

Figure 9—*Contact angles of polyoxyethylene (40) octylphenol solution on mixtures of phenacetin and microcrystalline cellulose. Points are experimental; the theoretical curve is from Eq. 5.*

Table II—Critical Surface Tension Values of Drugs

Substance	γ_c, dynes/cm
Sulfadiazine	33
Sulfamerazine	32
Sulfamethazine	31
Aspirin	32
Phenacetin	31
Salicylic acid	31

constant term, C, had to be added:

$$\frac{d^2}{t} = \frac{\bar{r}\gamma_L}{2\eta} + C \qquad \text{(Eq. 2)}$$

to correct for such effects as the resistance of the glass frit, the effect of the glass walls of the tube on the rate of liquid movement, and possible vapor adsorption on the unwetted granules (21). Equation 1 must be modified to yield:

$$\frac{d^2}{t} = \frac{\bar{r}\gamma_L \cos\theta}{2\eta} + C \qquad \text{(Eq. 3)}$$

Once $\bar{r}$ is known, cos θ for each nonwetting liquid can be calculated from Eq. 3.

Zisman plots were constructed using contact angles from penetration studies and sessile drop measurement. With aspirin (Fig. 6), contact angles determined from penetration data fell between values of the dynamic and static angles of sessile drops. They were within 2° of the dynamic angles. All four sets of values may be extrapolated to yield very nearly the same critical surface tension, 32 dynes/cm. A value of 31 dynes/cm was reported for aspirin (12).

For phenacetin (Fig. 7), the contact angles from penetration experiments were nearly identical to the static angle, differing from the dynamic angles by about 5°. Again, the same critical surface tension was obtained from all extrapolations. Results for salicylic acid are shown in Fig. 8. Contact angles were calculated from the penetration data. Contact angle measurements on these surfaces of compacts by the sessile drop method always yielded angles considerably lower than those from the penetration study. This result may be attributed to the effect of surface roughness of the salicylic acid compacts noted by scanning electron microscopy. When the true contact angle is less than 90°, surface roughness reduces the apparent contact angle (18). Therefore, the angles measured on the compacts were too low and were not used.

Of the two techniques studied, the sessile drop method is faster and less tedious. The penetration method may be applied when compacts with smooth surfaces cannot be obtained.

The values of critical surface tension of the six solid materials investigated are summarized in Table II. They were all rather close to each other, ranging from 31 to 33 dynes/cm. Zisman (18) stated that methylene and phenyl groups in the surface would lead to γ_c values of 31–35. It is possible that γ_c is controlled largely by the hydrophobic groups in the molecule.

The critical surface tension can serve as a useful empirical guide in the selection of a wetting agent in aqueous suspension formulation. Complete wetting will occur spontaneously if the particular agent in the concentration chosen is capable of lowering the surface tension of the medium to correspond to γ_c. Reduction of surface tension much below γ_c has no advantage as far as wetting is concerned. Although the γ_c values reported in Table II were obtained using solutions of a single surfactant family, they are expected to apply with little error to other surfactant systems, provided specific adsorption effects are absent.

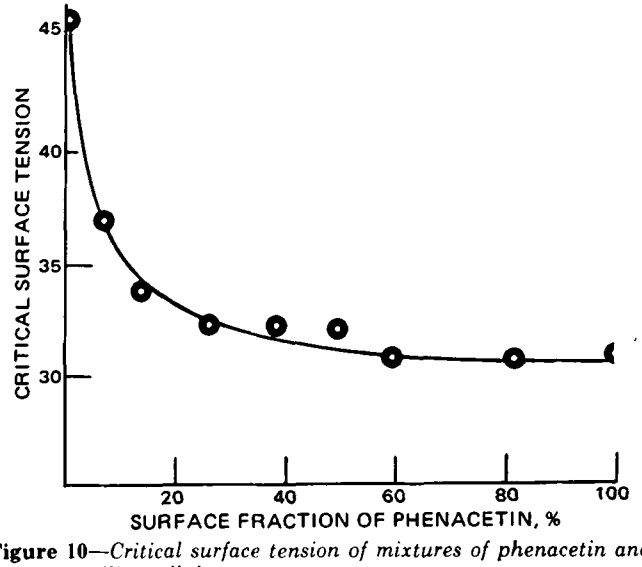

Figure 10—*Critical surface tension of mixtures of phenacetin and microcrystalline cellulose.*

A set of experiments was conducted on mixtures of microcrystalline cellulose and phenacetin. Microcrystalline cellulose was chosen because all surfactant solutions used as test liquids spread on this material. Phenacetin was chosen because, of the six drugs studied, the average particle size of phenacetin (12.0 μm) was closest to that of microcrystalline cellulose (9.7 μm). A series of mixtures of varying composition was prepared in 250-ml erlenmeyer flasks by extensive stirring with a glass rod. This mixing method was used to avoid comminution of the particles. Contact angle measurements by the sessile drop technique were complicated by the rapid penetration of liquid into the compact and disturbance of the surface geometry. Only the dynamic contact angle measurements are considered reliable.

The Cassie and Baxter equation (22) was employed to analyze the contact angles as a function of solid composition:

$$\cos\theta_{obs} = \sigma_P \cos\theta_P + \sigma_A \cos\theta_A \qquad \text{(Eq. 4)}$$

where σ_P and σ_A are the surface area fractions of phenacetin and microcrystalline cellulose, respectively; θ_P is the contact angle on pure phenacetin; θ_A is the contact angle on pure microcrystalline cellulose; and θ_{obs} is the contact angle on the mixture. Since $\sigma_P + \sigma_A = 1$ and cos $\theta_A = 1$ for all test liquids used, substitution in Eq. 4 and rearrangement yield:

$$\cos\theta_{obs} = 1 - \sigma_P(1 - \cos\theta_P) \qquad \text{(Eq. 5)}$$

In calculating area fractions for the mixtures, which were prepared on a weight/weight basis, it was assumed that the particles were spherical, that they were evenly distributed on the surface, and that the area fractions depended only on the relative particle numbers at the surface because the particle sizes were close. Equation 6 is the expression for σ_P:

$$\sigma_P = \frac{n_P 2\pi r_P^2}{n_P 2\pi r_P^2 + n_A 2\pi r_A^2} \qquad \text{(Eq. 6)}$$

where n_A and n_P are the surface particle numbers for microcrystalline cellulose and phenacetin, respectively; and r_A and r_P are the respective particle radii. The particle number, n, is defined by:

$$n = \frac{W}{\rho \frac{4}{3}\pi r^3} \qquad \text{(Eq. 7)}$$

where W is total weight and ρ is true particle density. Substituting Eq. 7 for n_A and n_P in Eq. 6 gives:

$$\sigma_P = \frac{W_P \rho_A r_A}{W_P \rho_A r_A + W_A \rho_P r_P} \qquad \text{(Eq. 8)}$$

Contact angles of polyoxyethylene (40) octylphenol on mixtures are shown in Fig. 9. Included is the curve based on the equation of Cassie and Baxter (22) (Eq. 5). Agreement is poor. Wetting of the solid mixture is apparently dominated by the nonwetting component and is not as good as that predicted by the equation. This result was typical and is similar to the pattern found in the wetting of aspirin–dicalcium phosphate dihydrate mixtures by water (14).

Critical surface tension is plotted against phenacetin surface fraction in Fig. 10. If the surface contained 25% or more of phenacetin, the critical surface tension barely differed from that of pure phenacetin. Wetting of the mixture is largely controlled by the component most resistant to wetting.

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Fluorometric Determination of Clobazam, a 1,5-Benzodiazepine, in Human Plasma

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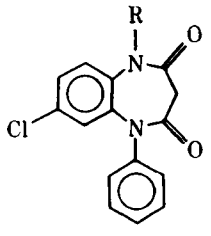
Abstract □ A fluorometric procedure for clobazam, a 1,5-benzodiazepine, based on a fluorophore formed upon irradiation of the drug using short wavelength UV light (254 nm) for 35 min is presented. Fluorescence is linear over a 100–6400-ng/ml range using excitation and emission wavelengths of 350 and 400 nm, respectively. Application of the method to the determination of clobazam in spiked human plasma samples revealed that the drug can be determined at nanogram per milliliter levels with an accuracy of 1–5%. The procedure is specific for clobazam in samples containing its major plasma metabolite, *N*-desmethylclobazam, and also in samples containing 1,4-benzodiazepines and other selected drugs. A plasma level–time profile after oral administration of a single 40-mg dose of clobazam to a healthy adult male is also illustrated.

Keyphrases □ Clobazam—fluorometric determination in human plasma □ Benzodiazepines—fluorometric determination of clobazam in human plasma □ Tranquilizers—clobazam, fluorometric determination in human plasma □ Fluorometry—analysis, clobazam in human plasma

Clobazam (I) [7-chloro-1-methyl-5-phenyl-1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)-dione] is a new antianxiety agent currently under clinical investigation. It has demonstrated relatively low sedation potential (1, 2) and relatively limited effects on normal levels of human performance[1] (3, 4). An effective therapeutic dose of 20–40 mg/day has been suggested (4).



I: R = CH_3
II: R = H

[1] J. A. Kotzan, T. E. Needham, I. L. Honigberg, J. J. Vallner, J. T. Stewart, W. J. Brown, and H. W. Jun, presented at the APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1978.

Table I—Typical Calibration Data for Clobazam in Human Plasma

Initial Concentration, ng/ml	Fluorescence Intensity[a]	Slope	Intercept	$r \pm s_{y \cdot x}$ [b]
150	4.05 ± 0.07	0.0258	0.2525	0.9998 ± 0.0906
300	8.00 ± 0.01			
600	15.75 ± 0.21			

[a] Based on duplicate samples. [b] s is the standard error of estimate of y (fluorescence intensity) on x (concentration).

This paper presents a fluorometric procedure for the analysis of clobazam in human plasma. The method allows the determination of drug in the presence of *N*-desmethylclobazam (II), its major plasma metabolite, which has also been reported to possess psychosedative and anticonvulsant activities with low toxicity (5). The utilization of fluorescence offers nanogram per milliliter sensitivity along with suitable reproducibility and accuracy.

EXPERIMENTAL

Apparatus and Reagents—Fluorescence measurements were obtained using a spectrophotofluorometer[2] equipped with a corrected spectra accessory and operated in the true emission mode. Excitation and emission slits were set at 7 and 4 nm, respectively, and sample sensitivity for all measurements was set at 1.

Clobazam powder[3] was used for the preparation of a stock solution (1 μg/ml) in plasma. This solution was prepared by the addition of 0.25 ml of an ethanolic clobazam solution (200 μg/ml) to a 50-ml volumetric flask, followed by the addition of the blank human plasma[4] to volume. *N*-Desmethylclobazam, mp 305–307°, was synthesized according to the procedure of Rossi *et al.* (6). All other chemicals were the highest purity available from commercial sources and were utilized as received.

Determination of Calibration Curve—Quantities of 0.15, 0.30, and 0.60 ml of the clobazam stock solution in plasma were each placed in 15-ml ground-glass stoppered centrifuge tubes, followed by the addition of blank human plasma to make 1 ml. The solution was mixed[5] for 1 min.

[2] Model MPF-4, Perkin-Elmer, Norwalk, Conn.
[3] Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876.
[4] Obtained from a local hospital blood bank.
[5] Vortex-Genie mixer, Scientific Industries, Bohemia, N.Y.

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