combination of charge and molecular refraction indexes could extend greatly the applicability range of solubility-related correlation analyses such as have been reported for aromatic substances (8, 9). Since this approach is atom centered rather than bond centered, it appears highly suitable for arriving at molecular descriptors for pattern recognition studies (10, 11).

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Rheological and Drug Release Properties of Oil Gels Containing Colloidal Silicon Dioxide

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Abstract ☐ The rheological properties of oil gels prepared by dispersing colloidal silica in *n*-dodecane and 1-dodecanol were examined. The differences in gel strength using these two media were accounted for by the difference in the extent of hydrogen bond formation between the silanol groups on the silica surface. The incorporation of methyl salicylate further modified the rheological properties of the gels. The drug was capable of hydrogen bonding with silanol groups in the *n*-dodecane gels, which increase gel strength at low concentrations; at high concentrations, the drug acted as a plasticizer. In 1-dodecanol systems, the drug acted solely as a plasticizer. Adsorption studies showed that methyl salicylate was adsorbed only on the silica particles in the *n*-dodecane medium. Interaction of the drug with the silanol groups in the *n*-dodecane systems did not appear to effect methyl salicylate release from the gels.

Keyphrases ☐ Gels—colloidal silica in *n*-dodecane and 1-dodecanol, rheological analysis, hydrogen bonding, methyl salicylate incorporation and release ☐ Colloidal silica—in *n*-dodecane and 1-dodecanol gels, rheological analysis, hydrogen bonding, methyl salicylate incorporation and release ☐ *n*-Dodecane—gels, colloidal silica, rheological analysis, methyl salicylate incorporation and release ☐ 1-Dodecanol—gels, colloidal silica, rheological analysis, methyl salicylate incorporation and release ☐ Methyl salicylate—gels, colloidal silica, *n*-dodecane, 1-dodecanol, rheological analysis

Colloidal silicon dioxide (fumed silica), produced by the vapor-phase hydrolysis of silicon tetrachloride, is used widely in the pharmaceutical industry as a binder and glidant in tablets and as a suspending agent and viscosity modifier in suspensions, ointments, and suppositories. Its use as a viscosity modifier is largely attributable to the ability of the very small silica particles to form a network structure throughout the medium by interparticle hydrogen bonding *via* the silanol groups on the silica surface. In addition to these particle interactions, there is possible bonding between the silanol groups and other components that are also capable of hydrogen bond formation. A detailed investigation of this type of interaction was published (1).

Modification of the magnitude of the interparticle interactions and production of ointment bases of different consistencies are possible by selecting, as dispersion media, oils that differ from each other in hydrogen bonding abil-

ity. Incorporation of a drug capable of hydrogen bonding into such bases might be expected to influence not only their rheological characteristics but also their drug release properties.

The object of this work was to quantify some of these phenomena by incorporating methyl salicylate into model gel systems prepared by dispersing fumed silica in the nonhydrogen bonding *n*-dodecane and the hydrogen bonding analog 1-dodecanol. The influences of silica and drug concentration on rheological and release properties are reported.

EXPERIMENTAL

Materials—Specially pure (99%) 1-dodecanol[1], laboratory reagent grade *n*-dodecane[1] and BP quality methyl salicylate[2] were used as received. Colloidal silicon dioxide[3] was dried for 1 hr at 150° and stored in a desiccator prior to use.

Gel Preparation—The requisite amount of continuous phase, containing drug where appropriate, was added to the silica, and the system was sonicated[4] for 30 sec to obtain a uniform dispersion. The gel was transferred to a glass container, sealed, and stored at 40°.

Rheological Properties—Dynamic rheological properties were measured using a modified Weissenberg rheogoniometer[5] in conjunction with a digital transfer function analyzer[6]. This rheogoniometer is one of the few commercially available instruments with facilities for both oscillatory (dynamic) and continuous shear measurements. The viscoelastic properties of materials may be evaluated using the oscillatory technique, and its application to pharmaceutical semisolid systems was described previously (2, 3).

Parallel plate geometry (platen radius of 3.75 cm) was used, and all gels were tested over the frequency range of 0.01–25.0 Hz using two torsion strips (Nos. 6 and 7) to minimize problems associated with natural resonance (4). The measurement temperature, as monitored by a thermocouple system[7] embedded in the top platen, was 37 ± 0.5°. Samples were
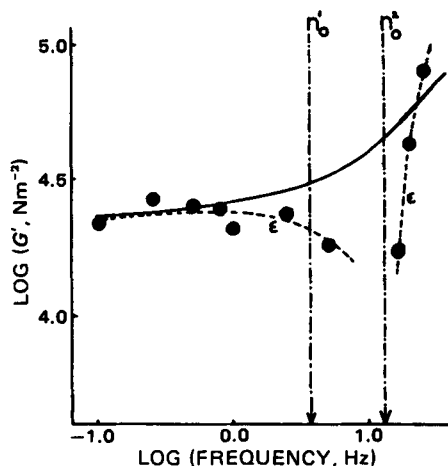
Figure 1—*Frequency dependence of log G′ for n-dodecane gel containing 8% (w/w) silica at 37°. The lines labeled ε represent the lower bounds of error due to the discontinuity in the data produced as the test frequency passed through the natural frequency of the rheogoniometer; n_0^1 is the resonance frequency for torsion strip No. 6, and n_0^2 is that for torsion strip No. 7.*

equilibrated for 30 min between the platens prior to measurement. For the 1-dodecanol gels, the gap width between platens was maintained at 0.05 ± 0.002 cm.

The analysis of Walters and Kemp (5) was used to evaluate the inertia parameter, α^2. The inertia parameter of the system is related to the complex dynamic viscosity of the test material, η^*, in the following manner:

$$\alpha^2 = \frac{-i\omega\rho}{\eta^*} \qquad \text{(Eq. 1)}$$

where i is the square root of -1, ω is the frequency of sinusoidal oscillation, and ρ is the density of the test sample.

For a linear viscoelastic system undergoing small amplitude oscillations, η^* is expressed as a combination of real and imaginary parts:

$$\eta^* = \eta^1 - \frac{iG'}{\omega} \qquad \text{(Eq. 2)}$$

where η^1 is the dynamic viscosity and G' is termed the real component of the complex shear modulus, G^*.

Once the inertia parameter has been determined, all test material parameters can be evaluated. Equation 2 can be transformed to give:

$$G^* = G' + iG'' \qquad \text{(Eq. 3)}$$

since the imaginary component, G'', of the complex shear modulus is given by:

$$G'' = \eta^1\omega \qquad \text{(Eq. 4)}$$

and G', the real component, describes the elastic properties of the system, and G'', the imaginary component, describes the viscous properties.

With parallel plate geometry, the upper platen is constrained by the torsion strip and the lower platen is oscillated with a frequency of ω Hz
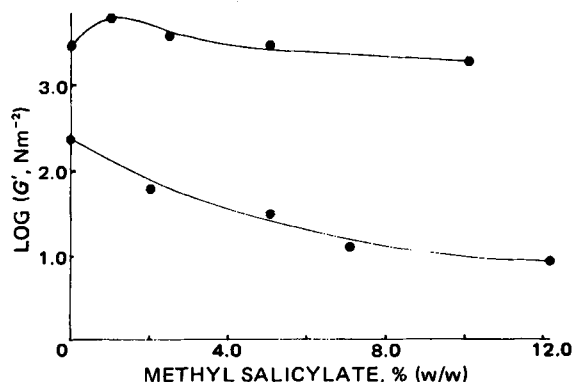

Figure 2—*Variation of G′ with methyl salicylate concentration for gels containing 8% (w/w) silica at 37°. Oscillation frequency was 0.25 Hz. Key: ●, n-dodecane; and ●, 1-dodecanol.*
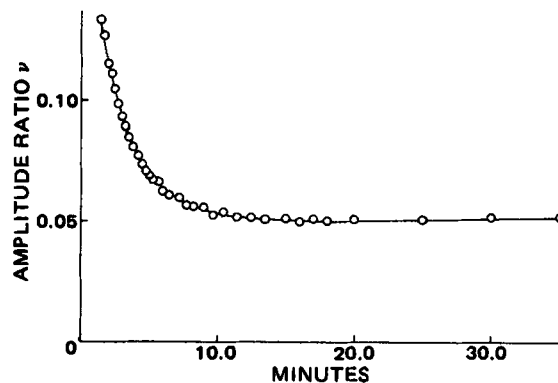

Figure 3—*Variation of amplitude ratio, ν, with time for 8% (w/w) silica in 1-dodecanol containing 12% (w/w) methyl salicylate at 37°. Oscillation frequency was 0.25 Hz.*

and amplitude θ_2. The platens are coupled through the sample under test; its rheological properties result in a phase lag, C, between the constrained and driven platens. In addition, the constrained platen oscillates with an amplitude of θ_1. The experimentally measured parameters for the sample were thus the amplitude ratio, ν, (θ_1/θ_2), and the phase lag, C, which were used to calculate α^2 using an iterative procedure, since:

$$\frac{\exp{(iC)}}{\nu} = \cos{\alpha h} + S\alpha h \sin{\alpha h} \qquad \text{(Eq. 5)}$$

where h was the gap between the platens and S was defined by the test geometry.

Subsequently, α was used to calculate G' and G'' using Eqs. 1–3.

Methyl Salicylate Adsorption—Methyl salicylate solutions ranging in concentration from 0.008 to 0.04 mg/ml were prepared in n-dodecane and 1-dodecanol. Ten milliliters of each solution was equilibrated with 0.1 g of silica in glass-stoppered vessels for 24 hr at 37°. The supernatant solutions were subsequently centrifuged[8] to remove the residual silica and assayed for equilibrium methyl salicylate content by UV spectrophotometry[9] using the 308-nm absorption maximum.

Drug Release from Gels—Approximately 10 ml of each gel was placed in a Perspex holder and covered with a cellophane membrane[10] so that there was complete contact between the gel and the 20.43 cm^2 of membrane area. The holder was placed at the bottom of a 1-liter, flat-bottom, glass reaction vessel containing 500 ml of pH 2.0 Clark and Lub buffer (6) at 37°. A stainless steel paddle stirrer was placed in the surface of the medium and rotated at 75 rpm. Aliquots were removed at 5-min intervals and assayed spectrophotometrically[9] for methyl salicylate with the absorption maximum at 238 nm.

Methyl Salicylate Partition Coefficients—Five-milliliter volumes of 1-mg/ml solutions of methyl salicylate in n-dodecane and 1-dodecanol were equilibrated with 5-ml volumes of pH 2.0 buffer at 37° for 1 week. Drug concentrations in organic and aqueous phases were determined spectrophotometrically[9] with the absorption maximum at 308 nm. The mean values of four determinations were used to calculate the partition coefficient (organic phase–pH 2.0 buffer).

RESULTS AND DISCUSSION

Preliminary experiments established that the gel properties were critically dependent on the preparation method; for this reason, the dispersion technique using an ultrasonic bath was used.

The n-dodecane gels exhibited a linear viscoelastic response under the test conditions. At any given silica concentration, G' increased slightly with frequency. Specimen data for a gel containing 8% (w/w) silica are shown in Fig. 1. The discontinuity was due to the test frequency passing through the natural frequency range of the rheogoniometer, n_0 (7). At any given frequency, G' increased as the silica concentration increased from 6 to 11% (w/w); G'' was lower than G' under all experimental conditions. These results are characteristic of the plateau region of viscoelastic response where there is reversible elastic deformation of the gel structure. Figure 2 shows the influence of methyl salicylate concentration on G' for n-dodecane gels containing 8% (w/w) silica. There was an initial

[8] Junior centrifuge, Gallenkamp Ltd., London, England.
[9] Unicam SP1800 UV spectrophotometer, Pye-Unicam Ltd., Cambridge, England.
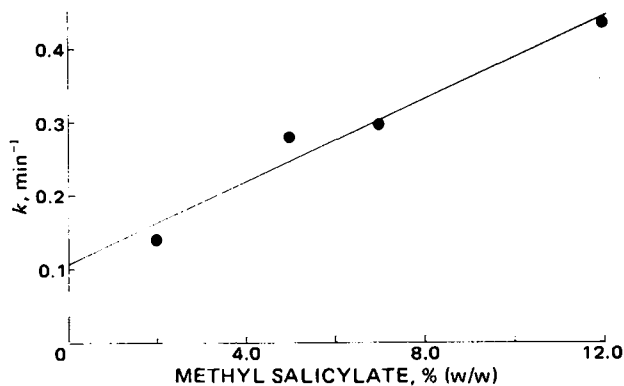[10] Visking tubing, Scientific Instrument Centre, London, England.

Figure 4—*Variation of first-order rate constant for gel breakdown, k, with methyl salicylate concentration for 1-dodecanol gels containing 8% (w/w) silica at 37°. Oscillation frequency was 0.25 Hz.*

increase in G' with drug concentration to a maximum of ~2% (w/w) methyl salicylate, followed by a progressive decline thereafter.

In contrast, the 1-dodecanol gels exhibited nonlinear viscoelastic behavior to the extent that there was a progressive decrease in ν during the time of the rheological measurement. This behavior (Fig. 3) indicates irreversible shear breakdown of the gel network. Analysis of the ν-time data using Guggenheim's method (8) showed that the breakdown obeyed first-order kinetics. The irreversibility of the process was evident from the fact that a sheared sample left between the plates of the rheogoniometer for 15 hr only recovered to ~5% of its original ν value on re-shearing. The 1-dodecanol systems also exhibited syneresis when placed in the rheogoniometer at narrow gap widths.

To minimize this problem and to obtain comparative data, all samples were tested using the 0.05-cm gap. As a consequence of this behavior, it must be emphasized that G' data for the 8% (w/w) silica in 1-dodecanol gels (Fig. 2) have no absolute meaning since they are values calculated 1 min after shear. Nevertheless, the plot demonstrates that there was a progressive decrease in G' with increasing methyl salicylate concentration and no evidence of a maximum in the relationship.

The 1-dodecanol systems contained such an excess of hydroxyl groups relative to silanol groups that the probability of silanol–1-dodecanol interactions was higher than that of silanol–silanol interactions. This fact accounts for the much weaker network structure exhibited by these gels when compared with the n-dodecane systems. Methyl salicylate incorporated into the 1-dodecanol gels would act solely as a plasticizer and would reduce G', since it is not likely that any silanol groups would be available for hydrogen bond formation with the drug. The efficiency of the methyl salicylate molecule as a plasticizer is demonstrated by examining its effect on the kinetics of breakdown of the 1-dodecanol gel. The influence of the drug concentration on the first-order rate constant, k (Fig. 4), clearly demonstrates the disruptive effect of the drug on the interparticulate network structure.

In the n-dodecane systems, since there is no possibility of hydrogen bonding between silanol groups and the continuous phase, all silanol groups are available for interparticle or drug–particle interactions. Therefore, the initial increase in G' with methyl salicylate concentration
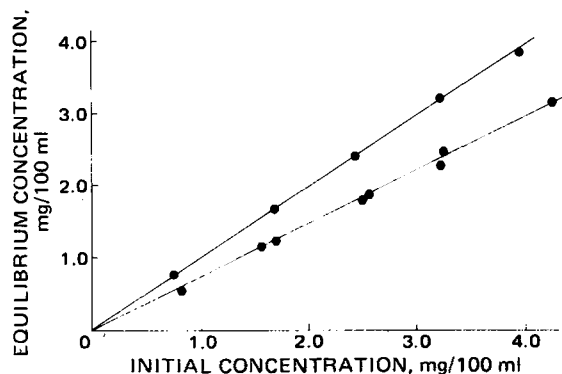

Figure 5—*Relation between initial and equilibrium methyl salicylate concentrations for systems containing 0.1 g of silica in 10 ml of n-dodecane (●) or 1-dodecanol (●) at 37°.*
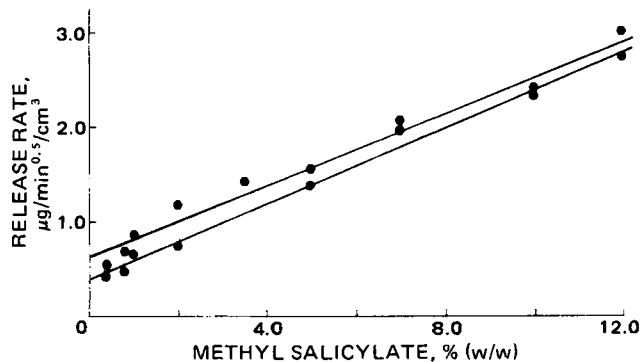
Figure 6—*Influence of continuous phase and methyl salicylate concentration on the release rate from gels containing 8% (w/w) silica at 37°. Key: ●, n-dodecane; and ●, 1-dodecanol.*

(Fig. 2) can be attributed to the drug acting as a cross-linking agent, via hydrogen bonds, and extending the network structure. Since there is a finite number of silanol groups in the gels, a point will be reached where they will all be involved in particle–particle or drug–particle interactions; upon further addition, methyl salicylate will act as a plasticizer and will reduce G' in a similar manner to that seen with the 1-dodecanol gels. A crude estimate of the drug concentration needed to produce saturation of the available silanol groups can be made from a knowledge of the specific surface area of the silica, its concentration in the gel, and the number of silanol groups in a given surface area.

According to the manufacturer's data (9), on average there are three silanol groups/100 Å^2 of silica surface. For this silica sample, the specific surface calculated using the Brunauer, Emmett, and Teller equation for adsorption (10) was 300 m^2/g. If the gross assumptions are made that all silanol groups are available and that each methyl salicylate molecule can react with a silanol group, then all groups will have interacted when approximately 2% (w/w) methyl salicylate is present. In practice, the correspondence ratio between methyl salicylate and silanol groups would probably be less than 1:1, and this would reduce the saturation concentration. Despite the crudity of the calculation, the result is in agreement with the experimentally observed maximum in G'.

The ability of methyl salicylate to bond to silanol groups in n-dodecane gels but not in 1-dodecanol systems also was demonstrated by the results of the adsorption studies (Fig. 5). If no bonding occurred, there would be a linear relation between initial and equilibrium methyl salicylate concentrations with a slope of unity. Bond formation, with consequent absorption, would result in an initial linear relation of a slope less than unity. Least-squares regression analysis gave slope values of 0.986 for 1-dodecanol systems and 0.744 for n-dodecane systems.

The differing effects on gel structure produced by addition of methyl salicylate to the two systems should be reflected in the drug release data. Preliminary work showed no significant drug adsorption to the cellophane membrane in the drug release experiments. Linear relations were obtained on plotting amounts released per unit area against (time)$^{0.5}$ after steady-state conditions had been attained.

Figure 6 shows the relation between the slope values for such plots and drug concentrations for the two systems. The experimental scatter reflects the difficulty in obtaining high quality data in this particular experiment. For this reason, no attempt was made to calculate effective diffusion coefficients. Statistically, there was no significant difference in the slopes of the two lines ($p = 0.05$), and this result parallels the similarity of the oil–pH 2.0 buffer partition coefficients for the two oily media (193.3 for n-dodecane and 184.4 for 1-dodecanol).

The conclusion to be drawn is that the amount of methyl salicylate bound in the n-dodecane gels is small with respect to the total drug concentration in the gels available for diffusion. Nevertheless, if the drug incorporated had been of high potency, its ability to bond to the silanol groups might seriously have reduced its availability. Further work is in progress on such systems; the continuous phase hydrogen bonding capability will be varied in a systematic manner to permit an investigation of the interaction thermodynamics.

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ACKNOWLEDGMENTS

Quinazolines and 1,4-Benzodiazepines LXXXIX: Haptens Useful in Benzodiazepine Immunoassay Development

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Abstract □ The syntheses of some 1,4-benzodiazepines potentially useful as haptens are reported. These compounds are related to chlordiazepoxide, diazepam, nitrazepam, clonazepam, and some of their metabolites. The chemistry reported here is intended to support specific immunoassay development for these drugs.

Keyphrases □ Benzodiazepines—chemical synthesis, potentially useful haptens □ Tranquilizers—benzodiazepines, various, chemical synthesis, potentially useful haptens □ Haptens—benzodiazepines, chemical synthesis

Immunoassay (1, 2) is a powerful method for measuring drug levels in biological fluids. The extensive clinical use (3, 4) and continued development (4, 5) of benzodiazepines as a drug class make immunoassays for these compounds desirable. This paper reports some benzodiazepine hapten[1] syntheses. Many haptens reported here have been utilized (6–8) in the immunoassay development for clinically important benzodiazepines and their metabolites.

The synthetic compounds are presented in three groups. Where possible, the numerical sequence reflects the synthetic sequence. The end-products, XII–XXIV, in Group A are related to diazepam and metabolites of diazepam and chlordiazepoxide. Compounds XXXI–XXXVI in Group B are related to chlordiazepoxide. Group C contains compounds derived from nitrazepam, clonazepam, and their metabolites.

The synthetic methods are known either in the general art or in the special benzodiazepine chemistry described elsewhere (5, 9, 10). 5-Chloro-3-(4-hydroxyphenyl)-2,1-benzisoxazole (I) (11) and the corresponding 3-(4-aminophenyl) analog II (12–14) were the crucial starting materials for compounds in Groups A and B. All other compounds reported are new except for the following: IV (11, 15), VII (16), XXIV (6), XXV (17), XXXVIII (18), XXXVII (19), and XL (20). The preparation of VII, XXIV, and XL, however, is reported for the first time.

EXPERIMENTAL[2]

3-(4-Aminophenyl)-5-chloroanthranil (II) (12–14)—To a mixture of 100 g (0.662 mole) of o-nitrobenzaldehyde and 160 g (1.05 mole) of phosphorus oxychloride was added dropwise, with stirring, 100 g (1.08 moles) of aniline while the temperature was kept below 30°. After 3 hr at room temperature, the solution was heated at 75° for 18 hr and at 90° for 3 hr. (The reaction becomes exothermic when heated.)

The mixture was cooled, 200 ml of ethanol and 200 ml of concentrated hydrochloric acid were added, and the solution was heated to reflux for 3 hr with stirring. On cooling, the precipitate was collected and washed with acetone, resuspended in dilute ammonium hydroxide for 1 hr, and collected again. After crystallization from ethanol, 70 g (86%) of II was obtained. Recrystallization from ethanol gave orange rods, mp 208–211°.

4'-(5-Chloro-3-anthranilyl)-2,2,2-trifluoroacetanilide (III)—A mixture of 70 g (0.286 mole) of II, 75 g (0.357 mole) of trifluoroacetic anhydride, and 1 liter of tetrahydrofuran was heated to reflux for 30 min. After concentration to a small volume, ether (300 ml) was added. The solution was filtered to give 60 g of product and then was concentrated to give an additional 5 g. The filtrate was evaporated, and the residue was stirred with cold aqueous potassium carbonate solution and filtered.

The solid thus collected was dissolved in tetrahydrofuran and treated with charcoal. After filtration and concentration to a small volume, ether was added. A 10-g precipitate of III was collected to give a total yield of 75 g (77%). A sample recrystallized from tetrahydrofuran–hexane gave pale-yellow rods, mp 251–254°; IR (KBr): 3310 (NH) and 1705 (C=O) cm^{-1}.

Anal.—Calc. for $C_{15}H_8ClF_3N_2O_2$: C, 52.88; H, 2.37; N, 8.22. Found: C, 52.85; H, 2.32; N, 8.27.

2-Amino-5-chloro-4'-hydroxybenzophenone (IV) (11, 15)—To a solution of 83.7 g (0.34 mole) of 5-chloro-3-(4-hydroxyphenyl)-2,1-benzisoxazole (I) (11) in 1500 ml of acetic acid was added 45 g of iron filings. The mixture was stirred and heated on the steam bath for 20 min. Every 30 min, an additional 20 g of iron filings and 100 ml of water were added for 2.5 hr. After 30 min more, the reaction mixture was filtered while hot.

The collected precipitate was heated with acetic acid and filtered. The combined filtrates were diluted with ice water to precipitate 39.8 g (47%) of IV, mp 170–175°. Recrystallization from methanol–water gave yellow rods, mp 173–178°.

2-Amino-5-chloro-4'-(2,2,2-trifluoroacetamido)benzophenone

[1] Haptens are defined (1) as antigens that are coupled to larger molecules, usually proteins, to provoke an antibody response.