free fraction between the hyperlipidemic patients and the healthy volunteers can be accounted for by slightly higher α_1-acid glycoprotein concentrations in the former group.

It appears, therefore, that the important binding proteins for quinidine are α_1-acid glycoprotein and albumin. Using the mean binding parameters for these proteins (Fig. 1), one can predict that at least a 50% decrease in albumin concentration would be necessary to obtain a 20% increase in quinidine free fraction. Clinically significant changes in quinidine binding are more likely to be due to changes in α_1-acid glycoprotein concentration, since serum levels of this protein may be increased in patients suffering from a number of common diseases.

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Simple, Rapid Method for Comparing the Self-Emulsifiability of Hydrocarbon Oils

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Received October 25, 1982, from the **Department of Pharmaceutics, University of Ife, Ile-Ife, Nigeria* and the ‡*Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3, U.K.* Accepted for publication May 12, 1983.

Abstract □ A simple and rapid method for comparing the degree of self-emulsifiability of different hydrocarbon oils is described. The method involved measurement of the intensity of light scattered at an angle of 31° to the incident radiation by the sample. The extent or degree of self-emulsification of selected hydrocarbon oils was observed to be affected by the nature of the oil as well as by the type and concentration of the surfactants employed. The method is useful when screening surfactant–hydrocarbon oil combinations as potential vehicles for drugs in the pharmaceutical industry or herbicides and pesticides for agricultural purposes.

Keyphrases □ Emulsions—surfactant-hydrocarbon oil, self-emulsifiability, measurement by laser nephelometry □ Laser nephelometry—self-emulsifiability, surfactant-hydrocarbon oil mixtures □ Hydrocarbon oils—self-emulsifiability, effect of added surfactants, measurement by laser nephelometry

Self-emulsifiable oils (also known as emulsifiable concentrates), widely used in the chemical and allied industries because they readily form emulsions without the need for powerful or sophisticated emulsification equipment, serve as vehicles for herbicides and pesticides and are used for cutting and rolling metals into thin sheets. They are also employed as lubricants in the textile industry and are currently being used for the recovery and processing of crude oils.

Self-emulsifiable oils have some potential applications in the drug industry. Solutions of drugs in oils have been administered to patients in soft gelatin capsules since the early part of the 19th century. In its modern form, this dosage is claimed to be advantageous since the accuracy, stability, and patient convenience is greater than that for the corresponding tableted form of a given drug. These oily solutions readily emulsify when released into the aqueous environment of the stomach. The generation of a large surface area means an optimum condition for extraction and absorption of the drug. The main requirement would be a suitable combination of nontoxic surfactants in bland oils.

It has been reported that the oil droplets in self-emulsifiable systems can be very small, ~1 μm (1). Measurement of droplet sizes in the submicrometer range has proved to be very tedious and/or time consuming (2). It is anticipated that the laser nephelometer[1], when used judiciously, can reduce the number of problems associated with particle size characterization in dispersed systems.

THEORETICAL SECTION

Several theories of light scattering by small particles (3–5) have appeared in the literature since the pioneering work of Rayleigh (6, 7) in this field. Recently, Bagchi and Vold (8) have commented on the limitations of some of these theories. In the present work, it is assumed that the basic equation

[1] Hyland Laser Nephelometer PDQ; Travenol Laboratories.

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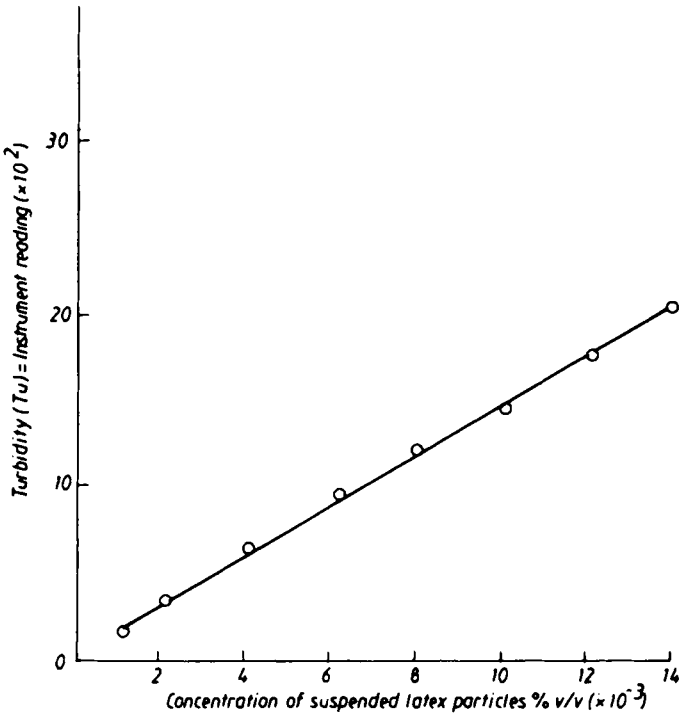

Figure 1—*Turbidity as a function on concentration of suspended latex particles. Nominal (manufacturer's) particle diamter = 0.24 μm.*

of Rayleigh (6) given by:

$$I_s(\theta) = \frac{9\pi NV^2}{2d^2\lambda^4} \cdot \frac{(n_i^2 - n_{ii}^2)}{(n_i^2 + 2n_{ii}^2)} \cdot (1 + \cos^2\theta) I_0 \qquad \text{(Eq. 1)}$$

is applicable. In this equation, $I_s(\theta)$ is the intensity of light scattered at angle θ, N is the number of particles in volume V, d is the distance between the measuring unit and the sample, n_i and n_{ii} are the refractive indices of the dispersed and continuous phases, respectively, and λ is the wavelength of the incident light of intensity I_0.

Lee and Groves (9) have shown that with the laser nephelometer, some factors (such as d and θ) may be regarded as constant, due to the design of the instrument, and they represented the measured intensity of scattered light by:

$$I_s(\theta) = fNV \cdot fn \cdot f(n_i - n_{ii}) \qquad \text{(Eq. 2)}$$

V is related to φ (*i.e.*, the volume fraction of the dispersed phase). The measured turbidity, Tu, is:

$$\text{Tu} = \frac{I_s(\theta)}{(n_i - n_{ii})\varphi} = f(\text{mean particle size}) \qquad \text{(Eq. 3)}$$

EXPERIMENTAL SECTION

Monosize latex[2] particles were used as received. High purity *n*-hydrocarbon[3] oils and a hydrocarbon mixture (I)[4] were purified further by repeated percolation through beds of fuller's earth[5] until their physical properties agreed closely with literature values. Surfactant mixture II[6] was used at a 1:1 ratio; surfactant mixture III[7] was used as 1:1 and 3:2 mixtures. Water was triple distilled from an all glass apparatus (surface tension, $\gamma a/w = 71.9$ mNm^{-1} at 25°C).

The laser nephelometer[1] utilizes a low-powered (0.5 mW) neon laser (λ = 632.8 nm) and an arbitrary scale of 0–200 to indicate increasing scattering intensity or turbidity, and was operated according to the manufacturer's manual (15).

[2] Rhône-Poulenc Polymeres, Aubervilliers, France.
[3] British Drug Houses, Poole, England.
[4] Dobane JN; Shell Chemicals. It is a mixture of aromatic (15%), naphtheneic (14%), and paraffinic (44%) hydrocarbons (10).
[5] Adsorption grade; Hopkin and Williams, Romford, England.
[6] Mixture of a water-soluble, phosphated nonylphenol ethoxylate (11) and an oil-soluble, phosphated fatty alcohol ethoxylate (12); Lankro Chemicals, Manchester, England.
[7] Mixture of Arylan PWS [a straight-chain, water-soluble dedecyl benzene sulfonate derivative (13)] and Ethylan D254 [an oil-soluble fatty alcohol ethoxylate (14)]; Lankro Chemicals, Manchester, England.

Table I—Composition of Emulsifiable Oils

Total Conc. of Surfactants[a], % w/w	Conc. of Hydrocarbon Oil, % w/w
10	90
20	80
30	70
40	60
50	50

[a] Mixture II was used as a 1:1 ratio; mixture III was either a 1:1 or a 3:2 ratio.

Serial dilutions of suspensions of the latex particles in water were made. Emulsifiable oils were prepared by adding the hydrocarbon oils to the surfactant mixtures; all preparations were made on a weight basis, as indicated in Table I. Emulsions were prepared by adding 1 mL of the emulsifiable oil to 99 mL of water in a 100-mL graduated cylinder, which was then gently inverted until the dispersion was homogeneous. Serial dilutions of the emulsions were then made.

Sample tubes containing the dilute suspensions of the latex particles or the emulsions were placed, in turn, in the low-power laser beam, and the turbidity as indicated by the instrument reading was recorded after a scanning period

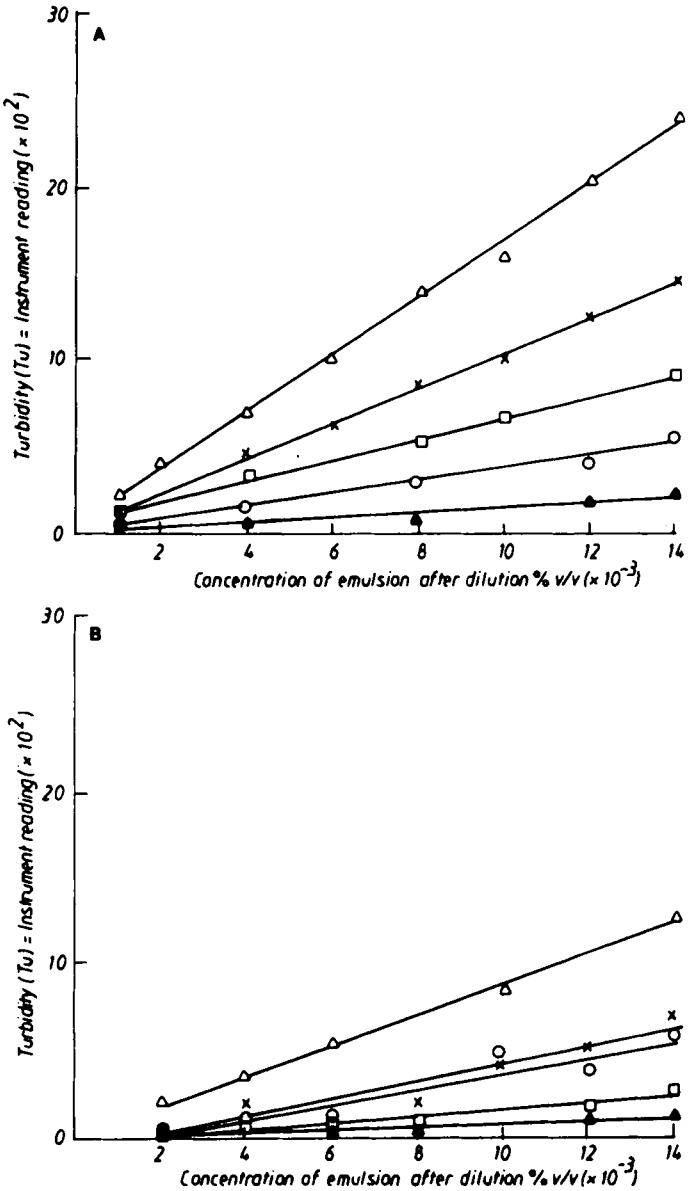

Figure 2—*Turbidity as a function of concentration of emulsions prepared from emulsifiable oils consisting of III (1:1) and I (A) and II (1:1) and n-hexane (B). Key: (□) 10% w/w; (△) 20% w/w; (×) 30% w/w; (○) 40% w/w; (▲) 50% w/w.*

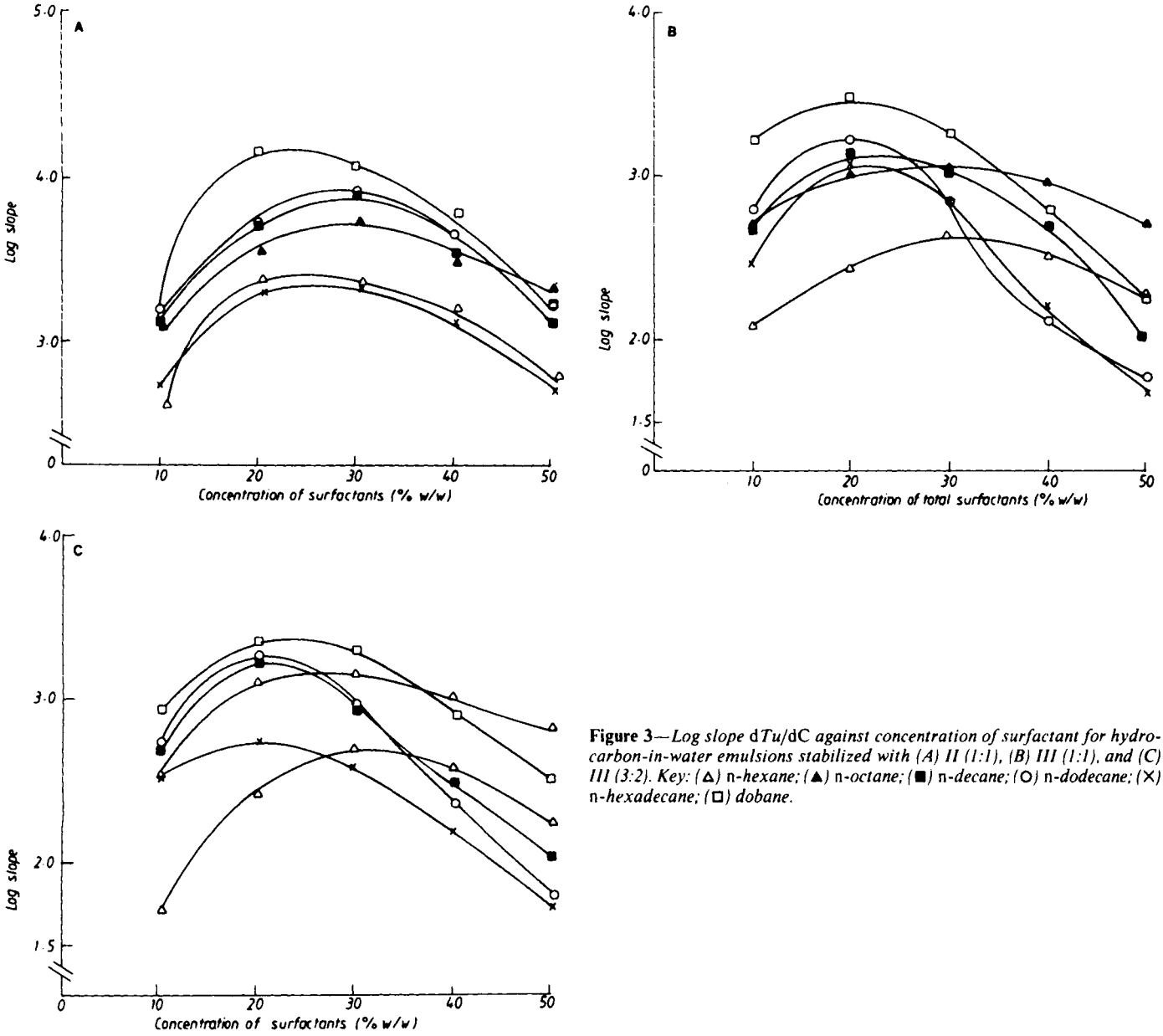

Figure 3—*Log slope* d*Tu*/d*C against concentration of surfactant for hydrocarbon-in-water emulsions stabilized with (A) II (1:1), (B) III (1:1), and (C) III (3:2). Key: (△) n-hexane; (▲) n-octane; (■) n-decane; (○) n-dodecane; (×) n-hexadecane; (□) dobane.*

of 5 s. At least 10 samples from each suspension or emulsion were analyzed and the results averaged.

RESULTS AND DISCUSSION

Graphs were plotted between turbidity (instrument reading) and concentration of latex particles or emulsions; representative results are shown in Figs. 1 and 2, respectively. The graphs were rectilinear within the range of concentrations examined. This observation conforms with Beer's law, which states that the decrease in intensity of radiation is related exponentially to the concentration of an absorbing substance in solution through which the radiation passes provided the light is monochromatic and the material homogenous.

To quantify the extent of emulsification of each hydrocarbon oil, slopes (*i.e.*, $d\mathrm{Tu}/dC$) were determined. The logarithms of the slopes (to accommodate the range) were then plotted against the concentration of surfactants in the emulsifiable oil (see Table I). Representative results[8] are shown in Fig. 3.

It can be seen that as the total concentration of the surfactants was increased, there was an increase in the value of the slope for each hydrocarbon oil until a maximum was reached, after which there was a decrease. The increase in slope (as a function of surfactant concentration) was probably the result of increased adsorption of surfactant molecules at the oil-water interface leading to an increase in the stabilization of the interfacial area until a maximum was reached. The fall in slope at higher concentrations of surfactants could be due to formation of associated structures with a corresponding decrease in translucency.

At any particular total concentration of surfactants, the numerical value of the slope (log $d\mathrm{Tu}/dC$) is a measure of the amount of light scattered by the system and, hence, a measure of its self-emulsifiability. Thus, from Fig. 3A it is seen that I is more readily emulsified by II (1:1) than *n*-hexane because at all concentrations of these emulsifiers, the curve for the former is above that of the latter. This observation is supported by recent work (16), which showed that droplets of *n*-hexane stabilized with equal weight ratios of these surfactants were larger and fewer than those of I.

When comparing the efficiency of II (1:1) with III (1:1) as an emulsifier for *n*-hexane, it can be said that the former is more efficient, since at each total concentration of surfactants the value of $d\mathrm{Tu}/dC$ with II (1:1) is higher than with III (1:1).

CONCLUSIONS

The objective in any formulation exercise should be to achieve a maximum value of $d\mathrm{Tu}/dC$ at the lowest convenient total concentration of surfactants. The present equipment and technique should prove suitable for comparing the relative self-emulsifiabilities of different oil-surfactant combinations.

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Analytical Methods for the Determination of Sulindac and Metabolites in Plasma, Urine, Bile, and Gastric Fluid by Liquid Chromatography Using Ultraviolet Detection

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Received March 15, 1983 from the *Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.* Accepted for publication October 17, 1983.

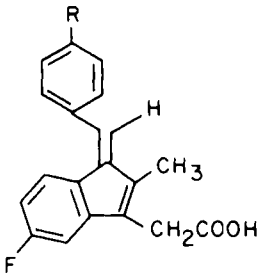
Abstract □ A high-performance liquid chromatographic method using a linear elution gradient has been developed for the analysis of sulindac, sulindac sulfone, and sulindac sulfide in plasma, urine, bile, and gastric fluid. The methodology uses reverse-phase, radial compression chromatography with gradient elution, and UV detection. Sulindac and its metabolites in plasma can be quantitated at 0.25 μg/mL with a mean *CV* of 6.0 ● 2.9%; urine, bile, and gastric fluid (0.5 μg/mL) yield a mean *CV* of 5.5 ± 1.9%.

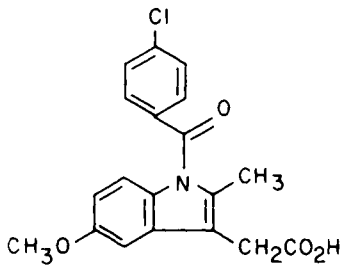
Keyphrases □ Sulindac—metabolites, liquid chromatography with UV detection, human plasma, urine, bile, and gastric fluid □ Liquid chromatography—sulindac and its metabolites, human plasma, urine, bile and gastric fluid

Sulindac (*cis*-5-fluoro-2-methyl-1-[*p*-(methylsulfinyl)-benzylidene]indene-3-acetic acid; I) is an anti-inflammatory drug with analgesic and antipyretic properties. Metabolites include sulindac sulfide (II) the active species (I), and sulindac sulfone (III). Sulindac and its metabolites are detected in plasma, while sulindac, sulindac sulfone, and their respective conjugates are the major constituents excreted in urine (2).

Previous methods of analysis include an isotope dilution radioimmunoassay (3), a computerized mass spectral assay (4), and a high-performance liquid chromatographic assay (HPLC) (5), and appear to be restricted to serum or plasma. A stepwise isocratic HPLC assay has been recently reported (6) for plasma and urine determination; however, it involves tedious extraction procedures and is not free of interfering substances.

Preliminary investigations using the Dusci and Hackett method (5) led to incomplete resolution of sulindac and its metabolites from endogenous substances. Modifying this method with gradient elution afforded an assay that is applicable for measuring total levels of sulindac and its metabolites in plasma, urine, bile, and gastric fluid, and one that appears to be free from interfering substances.





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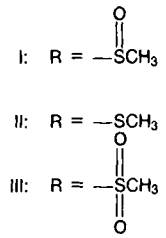

EXPERIMENTAL SECTION

Apparatus—The chromatography was performed on HPLC equipment which included two solvent delivery systems[1], an auto sampler[2], a fixed-wavelength absorbance detector with a 340-nm filter[3], and a solvent programmer[4]. The absorbance responses were recorded by a computing integrator[5] (attenuation 16 mV full scale; chart speed 0.5 cm/min).

Materials—Sulindac[6], sulindac sulfone[6], sulindac sulfide[6], and indomethacin[6] (IV, internal standard) were used as received (7, 8); acetonitrile,

[1] Model 6000A Solvent Delivery Systems; Waters Associates.
[2] WISP 710B auto sampler; Waters Associates.
[3] Model 440 Absorbance Detector; Waters Associates.
[4] Model 660 Solvent Programmer; Waters Associates.
[5] SP4100 Computing Integrator; Spectra Physics.
[6] Merck Sharp & Dohme Research Laboratories. Purity for sulindac and metabolites was determined by HPLC and elemental analysis. Sulindac sulfide: calc. for $C_{20}H_{17}FO_2S$: C, 70.6; H, 5.00. Found: C, 71.0; H, 4.68. Sulindac sulfone: calc. for $C_{20}H_{17}FO_4S$: C, 64.5; H, 4.57. Found: C, 64.3; H, 4.48. Sulindac: calc. for $C_{20}H_{17}FO_3S$: C, 67.4; H, 4.81. Found: C, 67.4; H, 4.72.

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