

provided there is an accurate accounting of the doses ingested and their precise timing.

In general, the relative bioavailability between two formulations in a crossover study is a function of the ratio of their respective mean plasma concentrations at quasi- and nonsteady state. Appropriate correction factors may be introduced to compensate for the effects of dose, dosing sequence, half-life, sampling interval, and residuals. Each of these elements can be readily identified in the equations developed for each design variation.

Mathematical solutions in closed form have been derived for crossover studies in which: (a) the potency of the two treatments may be different, (b) time intervals between doses need not be uniform, (c) the daily dosage regimen for the two treatments may be different, (d) adjustments may be attempted for possible changes in half-life between treatments, (e) mean plasma concentrations may be sampled over any convenient dosing interval and need not be the same interval between treatment, (f) the elapsed time between the last dose of the first treatment and the first dose of the second treatment may be as long or as short as needed, (g) the dosage cycle of recurring sequences is not restricted to 24 hr and may differ between treatments, (h) treatment periods may vary in length, and (i) dosing sequences may be completely random, *i.e.*, acyclic.

Most of these design alternatives are equally applicable to comparisons at steady state. There is, however, one important differ-

ence. With the proposed method, steady-state plasma levels are inferred and, therefore, need not be experimentally attained. In this way, even though the dosing sequence may be acyclical, the corresponding steady state can be inferred by depicting the entire sequence as a single cycle repeated indefinitely. That is to say, steady state is more useful as a concept than an experimental reality.

The number of imaginable permutations in the design of bioavailability studies is limitless. Undoubtedly, many more will surface with time. No attempt has been made to consider any but the simplest of situations in the present discussion. Hopefully, sufficient details have been provided to permit useful extensions to more complex experimental designs.

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Correlation of Phase Inversion Temperature with Kinetics of Globule Coalescence for Emulsions Stabilized by a Polyoxyethylene Alkyl Ether

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Abstract □ The phase inversion temperatures, globule coalescence rates, and long-term stability of oil-in-water emulsions stabilized by polyoxyethylene 4 cetyl ether were measured. Addition of sodium chloride to the aqueous phase depressed the phase inversion temperatures of the emulsions and the cloud point of the surfactant. Linear correlations were obtained between phase inversion temperature and cloud point and also between phase inversion temperature and the logarithm of the globule coalescence rate at constant temperature. This latter finding is consistent with a theory of emulsion type based upon the kinetics of coalescence. The programmed viscometric technique of determining inversion revealed the presence of a liquid crystalline phase below 35°, which contributes significantly to emulsion stability.

Keyphrases □ Emulsions—phase inversion temperature correlated with kinetics of globule coalescence, long-term stability measured, polyoxyethylene alkyl ether as stabilizer □ Polyoxyethylene alkyl ether—as stabilizer in emulsions □ Phase inversion temperature—emulsions, correlated with kinetics of globule coalescence □ Stability, long term—emulsions, polyoxyethylene alkyl ether as stabilizer

When oil-in-water emulsions stabilized by nonionic surfactants are heated, they may invert because of the decreased water solubility of the emulsifiers. This phenomenon has been examined extensively (1–3), particularly the relation between phase inversion temperature and formulation variables such as the hydrophilic-lipophilic balance (HLB) of the emulsifier, the nature and volume of the oil phase, and the

presence of additives. The correlations obtained between emulsion stability and phase inversion temperature generally have been of a qualitative nature.

The work described here was concerned with the quantitative relationship between the phase inversion temperature and the rates of globule coalescence of a series of emulsions stabilized by a single nonionic surfactant. The relation of these parameters to long-term storage also was investigated. Emulsions with differing inversion temperatures and stabilities were produced by adding an electrolyte to their aqueous phases. The data were interpreted in terms of a quantitative kinetic theory for predicting emulsion type (4).

EXPERIMENTAL

Materials—The water was twice distilled from a quartz glass still. Liquid paraffin¹ was of BP quality. The surfactant², a commercial sample of polyoxyethylene cetyl ether containing an average of four ethylene oxide units (polyoxyethylene 4 cetyl ether), was used without purification (HLB 8.6). Sodium chloride³ and glycerol³ were analytical grade reagents.

Preparation of Emulsions—Oil-in-water emulsions were pre-

¹ Vestan grade A350, Fina S.A. Brussels, Belgium.

² Texofor A4, batch G964, Glovers Chemicals Ltd., Leeds, England.

³ B.D.H. Chemicals Ltd., Poole, United Kingdom.

pared containing 27% (w/w) liquid paraffin, 3% (w/w) polyoxyethylene 4 cetyl ether, and 0.1–10.0% (w/w) sodium chloride.

The surfactant was dissolved in the oil by warming to 40°. The sodium chloride was dissolved in the water, and the appropriate quantity of the oil-surfactant mixture was added with continuous stirring. The coarse emulsion that formed was immediately homogenized⁴. Each emulsion was stored at room temperature for a minimum of 4 days to allow equilibration of the surfactant between the two phases and was rehomogenized before use.

Phase Inversion Temperatures—The phase inversion temperatures of emulsions were determined by the programmed temperature rise viscometric technique (5). An emulsion was continuously sheared in a cone and plate viscometer⁵ at 266 sec⁻¹ while the plate was heated at 0.5° min⁻¹. The torque output from the viscometer was displayed on an X-Y recorder against the amplified voltage signal from a thermocouple embedded in the plate.

The viscometer was fitted with a vapor hood to prevent evaporation of the aqueous phase of the emulsion. The gap width between the cone and the plate (average value 0.05 mm) was controlled with an automatic gap-setting unit. Inversion was detected as a pronounced increase in the apparent viscosity when the oil became the continuous phase of the emulsion. Four determinations were carried out on each emulsion, and the mean value was recorded (range ±0.5°).

Phase inversion temperatures were also determined by monitoring the specific conductivity⁶ and temperature⁷ of 60 ml of emulsion that was continuously agitated at 100 rpm with a small propeller stirrer. The emulsion was heated at a controlled rate of 0.5° min⁻¹. Inversion was detected as a rapid fall in specific conductivity below that of the aqueous phase used to prepare the emulsion. The results quoted are the means of four determinations.

Cloud Points—The cloud points of 0.05% (w/w) aqueous solutions of the surfactant containing 0.1–15.0% (w/w) sodium chloride were determined. Each solution was placed in a water-jacketed 2-cm path length spectrophotometer cell fitted with a thermocouple. A tungsten light source and phototransistor circuit were used to monitor the turbidity of the solution while it was heated at 0.5° min⁻¹.

The amplified phototransistor and thermocouple signals were displayed on an X-Y recorder, and the cloud point was detected as a sharp increase in the turbidity of the solution. Four determinations were carried out on each solution, and the mean value was recorded.

Globule Coalescence—Emulsion stability was assessed by measuring the change in the number of oil globules per milliliter with time (6). Samples of each emulsion were diluted 1 in 500 by volume with 40% (v/v) aqueous glycerol warmed to 35°. An aliquot was mounted in a 100- μ m depth counting chamber⁸ and allowed to stand for 1 hr at room temperature.

Subsequently, photomicrographs⁹ were taken of five fields at $\times 800$ magnification; negatives were enlarged¹⁰ to give an overall magnification of $\times 1600$. The globules in each field were counted, and the number of globules per milliliter of emulsion was calculated. At various time intervals, four samples were taken from each emulsion and the mean value for the 20 fields was calculated.

Creaming Rates—Emulsion samples were stored in 10-ml stoppered graduated measuring cylinders at 20° for up to 18 months. At 2-week intervals, the degrees of creaming and of separation of oil phase were assessed.

RESULTS AND DISCUSSION

Data for an emulsion containing 0.1% (w/w) sodium chloride obtained by the two methods of measuring phase inversion temperature are shown in Fig. 1. The apparent viscosity fell as the temperature was increased from 22°, and this fall was most marked at approximately 35°. Above 40°, the viscosity was essentially constant

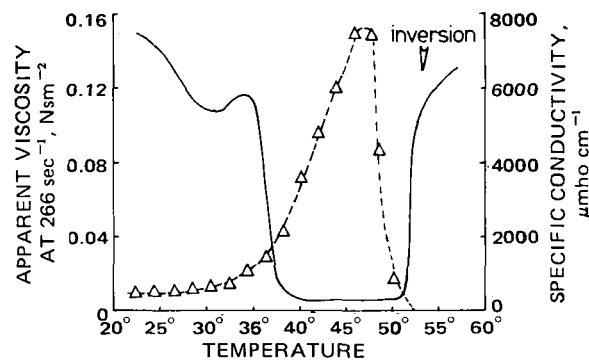


Figure 1—Relationship between apparent viscosity and temperature (—) and between conductivity and temperature (---) for an emulsion containing 0.1% (w/w) sodium chloride.

until inversion occurred with a rapid rise in viscosity. The discontinuity at 35° occurred in emulsions containing 0.1–6.0% (w/w) sodium chloride. Emulsions with higher sodium chloride contents inverted below this temperature.

Observation of emulsions using a hot-stage microscope¹¹ with polarized light showed that the discontinuity at 35° coincided with the disappearance of a liquid crystalline phase from the aqueous continuous medium. This finding was confirmed by separation of the emulsion phases by ultracentrifugation¹² at 250,000 \times g, followed by storage at temperatures above and below 35°. The reason for the small "shoulder" in the apparent viscosity curve just below 35° is not known but might be due to structural changes in the liquid crystalline phase prior to its disappearance.

The specific conductivity initially showed an increase with temperature, which also was most marked at the point of disappearance of the liquid crystalline phase. This result is to be expected since the decrease in viscosity will result in increased ionic mobility. Subsequently, conductivity fell sharply as the emulsion began to invert. There was good agreement between the values of phase inversion temperature obtained by the two methods.

This agreement is also evident in Fig. 2, where the phase inversion temperature was plotted against the sodium chloride concentration. The temperature of inversion fell with an increase in the sodium chloride concentration in a curvilinear manner. Similar relationships were reported for emulsions stabilized by various non-ionic surfactants (3, 5). The variation in the cloud point of the emulsifier with the sodium chloride concentration was similar to that of the phase inversion temperature; in fact, a linear correlation was obtained between the inversion temperature and cloud point ($r = 0.995$).

Examples of globule coalescence data are plotted in Fig. 3. Results are for emulsions stored at 37° and show that there was a decrease in stability with an increasing sodium chloride concentration. It is evident that coalescence can be described by a first-order process; thus the slopes of the lines give the coalescence rate constants. The theory of coalescence developed by Van den Tempel (6) predicts this first-order relationship and has been found to hold for a number of emulsion systems (6, 7).

Figure 4 illustrates the effect of the liquid crystalline phase upon the rate of globule coalescence. The data, for an emulsion containing 2.0% (w/w) sodium chloride, show that the phase greatly enhanced emulsion stability. Above 35°, with disappearance of the phase, the rate of globule coalescence rapidly increased.

Linear correlations were obtained between the phase inversion temperature and the logarithm of globule coalescence rate constants at constant temperature for this emulsion series. Correlations were obtained both at 25 and 37° and thus exist in the presence and absence of the liquid crystalline phase, although the slope value at 37° is much greater (Fig. 5).

Davies (4) advanced a quantitative kinetic theory to account for the type of emulsion formed upon mixing oil and water. He considered that the relative rate constants for coalescence of oil globules in water (Rate 1) and water globules in oil (Rate 2) control emulsion type. For example, if Rate 2 is greater than Rate 1, the forma-

⁴ Omerod URF-1 hand homogenizer, Omerod Engineers Ltd., Rochdale, England.

⁵ Ferranti-Shirley, Ferranti Ltd., Moston, Manchester, England.

⁶ Type PE/1 solid-state conductivity meter, L.T.H. Electronic Ltd., Luton, England.

⁷ Type 1664 thermometer, Comark Electronic Ltd., Rustington, Sussex, England.

⁸ Improved Neubauer cell, Hawksley and Sons Ltd., Lancing, England.

⁹ Swift series 800 microscope fitted with Kodak Retina Reflex IV camera.

¹⁰ Focomat 1c enlarger, E. Leitz GMBH, Wetzlar, Germany.

¹¹ Mettler FP5/FP52, Mettler Instrument A.G., Zurich, Switzerland.

¹² Omega II, Heraeus-Christ GMBH, West Germany.

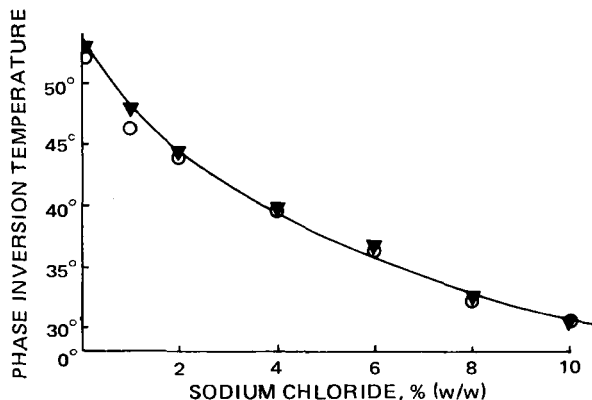


Figure 2—Relationship between phase inversion temperature and sodium chloride concentration. Key: ▼, phase inversion temperature from viscosity data; and ○, phase inversion temperature from conductivity data.

tion of an oil-in-water emulsion is favored. For inversion of an oil-in-water emulsion to occur, Rate 1 must become large relative to Rate 2.

According to Davies (4):

$$\text{Rate 1} = A_1 \exp [(-0.24\psi_0^2 - \theta \Sigma E_h)/RT] \quad (\text{Eq. 1})$$

where A_1 is a hydrodynamic collision factor (which takes into account the phase volume of oil and the viscosity of the continuous phase), ψ_0 is the electrical potential at the interface, θ is the fraction of interface covered by the surfactant, and ΣE_h is the total energy necessary to displace the water of hydration from the polar groupings of the surfactant prior to coalescence of the oil globules:

$$\text{Rate 2} = A_2 \exp (-600n\theta/RT) \quad (\text{Eq. 2})$$

where A_2 is a constant accounting for hydrodynamic factors, and n is the number of methylene groups in each surfactant hydrocarbon chain.

For emulsions stabilized by nonionic surfactants, the main stabilizing factors appear to be the viscosity and/or elasticity of the interfacial film and, most importantly, hindrance to close approach of the globules by the helical conformations of the hydrated polyoxyethylene chains. It is debatable, although unlikely, as to whether the surfactant contributes to the charge on the emulsion globules (8) and to the electrical forces of the repulsion. An increase in the temperature of the emulsion results in disruption of the hydrogen bonds linking water molecules to the ether oxygens of the polyoxyethylene chains. It follows that the value of ΣE_h falls with a rise in temperature, and this phenomenon is the major contribution to the increase in Rate 1. Provided the increase in Rate 2 is not significant relative to Rate 1, then, as has been shown in these experiments, inversion of an oil-in-water emulsion occurs.

Addition of sodium chloride to the emulsions also dehydrates

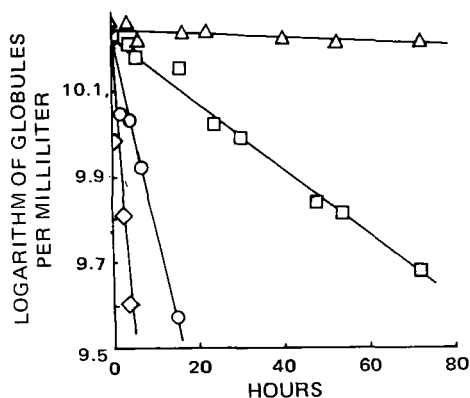


Figure 3—Globule coalescence data for emulsions stored at 37°. Key [percent (w/w) sodium chloride]: ▲, 0.1; ○, 2.0; □, 1.0; and ◇, 4.0%.

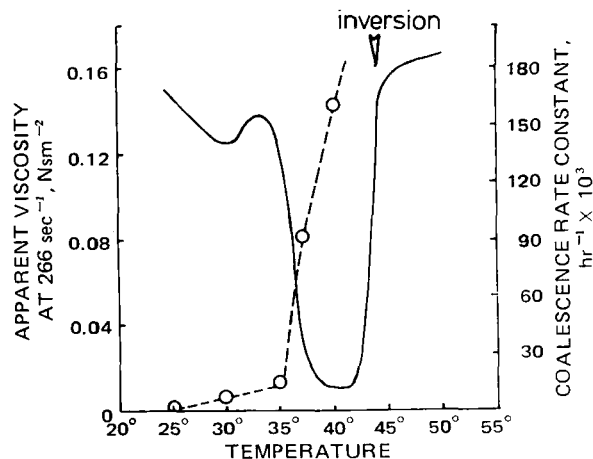


Figure 4—Relationship between apparent viscosity and temperature (—) and between coalescence rate constant and temperature (---) for emulsions containing 2.0% (w/w) sodium chloride.

the polyoxyethylene chains (3) and reduces ΣE_h . The electrolyte compresses the electrical double layer around the oil droplets and lowers ψ_0 as well, although, as already indicated, the electrical repulsion forces do not appear to play a significant role in stabilizing such emulsions (7). The net result should be a further increase in Rate 1 with an increasing sodium chloride content and a lowering of the temperature at which inversion occurs. The present experimental findings are in agreement with this hypothesis.

Equation 1 indicates that there is an inverse relationship between the logarithm of Rate 1 and ΣE_h . As Davies (4) pointed out, the cloud point temperature is related to the energy of dehydration of the surfactant; the present work with polyoxyethylene 4 cetyl ether found a linear correlation between the phase inversion temperature and the cloud point. Therefore, provided the electrical potential, ψ_0 , makes little contribution to emulsion stability and the experimentally determined globule coalescence rate constants are a measure of Rate 1, the observed correlation between the phase inversion temperature and the logarithm of coalescence rate constant at constant temperature (Fig. 5) is consistent with the Davies hypothesis.

There was also a qualitative relationship between the phase inversion temperature and the degree of creaming and cracking of the emulsions on long-term storage at 20°. Figure 6 shows this relationship in the form of a plot of phase inversion temperature against the volume fraction of oil, water, and cream after 200 days of storage. It is evident that the lower the inversion temperature, the greater was the degree of separation of the phases. Nevertheless, these emulsions were not grossly unstable, since there was evi-

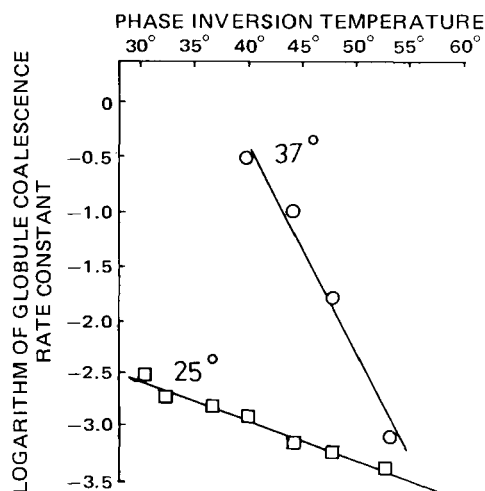


Figure 5—Relationship between phase inversion temperature and logarithm of globule coalescence rate constant for emulsions stored at 25 and 37°.

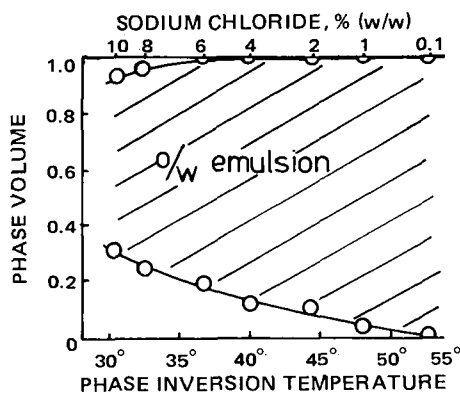


Figure 6—Relationship between phase inversion temperature and volume fraction of oil, cream, and water after storage at 20° for 200 days.

dence of cracking after 1 year of storage only with sodium chloride concentrations above 6.0% (w/w).

Shinoda and Saito (9) concluded that for satisfactory shelflife of emulsions stabilized by nonionic surfactants, the phase inversion temperature should be 25–70° above the storage temperature. Parkinson and Sherman (10) further developed this concept by examining the use of phase inversion temperature as an accelerated method for predicting emulsion stability. The relationships among the HLB of mixed emulsifier systems, the phase inversion temperatures, and the globule coalescence rates were investigated. The inversion temperatures were all greater than 85°, and a qualitative relationship was observed between the coalescence rate and the phase inversion temperature.

In comparison with the data of Shinoda and Saito (9) and Parkinson and Sherman (10), the inversion temperatures determined in the present work were considerably lower (ranging from 53.0 to 30.5°); one might be tempted to conclude that this result indicated unstable formulations. However, as with all accelerated test procedures, the primary requirement is that the stress applied should speed up, but not alter, the mechanism of deterioration operating at ambient temperatures. This work showed that the viscous liquid crystalline phase that disappears above approximately 35° has a considerable stabilizing influence on the emulsions. Although phase inversion temperature is a valuable method for ranking the emulsions in order of relative stability, its general applicability for predicting long-term stability is limited when phase changes occur on heating.

SUMMARY

1. The phase inversion temperatures of oil-in-water emulsions stabilized by polyoxyethylene 4 cetyl ether were determined by viscometric and conductimetric methods, and there was good agreement between the results. Addition of sodium chloride to the aqueous phases of the emulsions produced a lowering of the phase inversion temperature, and this result correlated with the fall in the cloud point of an aqueous solution of the surfactant.

2. The viscometric technique detected the presence of a liquid crystalline phase below approximately 35°.

3. Globule coalescence followed first-order kinetics. The liquid crystalline phase greatly influenced the rate of coalescence.

4. A linear correlation existed between the phase inversion temperature and the logarithm of the globule coalescence rate constant at a given temperature. This correlation was observed both in the presence and absence of the liquid crystalline phase. The finding was interpreted in terms of a quantitative kinetic theory of emulsion type.

5. A qualitative relationship was obtained between the degree of creaming and cracking of the emulsions on long-term storage and the phase inversion temperature.

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