Dialysis Method for Determining Preservative Distribution in Emulsions

S. J. A. KAZMI and A. G. MITCHELL

Abstract □ A three-chambered dialysis cell was used to estimate the distribution of preservative between the oil phase and the aqueous phase of an emulsion. The method also differentiated between preservative that was bound, or solubilized, by the surfactant and preservative that was free in the aqueous phase. The distribution data were plotted on a three-dimensional graph from which the total concentration of preservative needed to provide a given free concentration in the aqueous phase could be determined. Results from the dialysis method were compared with those calculated, using mathematical models for preservative distribution.

Keyphrases □ Preservative distribution, emulsions—determination, dialysis method □ Dialysis—preservative distribution determination, emulsions, comparison with mathematical models

Garrett (1) developed a basic integrated model for the evaluation of preservative action in complex disperse systems. Bean *et al.* (2) and Patel and Romanowski (3) correlated simplified forms of the equation with the observed antimicrobial activity of preservatives in oil-in-water emulsions. The equation may be expressed:

$$[D] = [D_f]\{1 + nK[M]/(1 + K[D_f]) + K_w^oQ\}/(Q + 1) \quad \text{(Eq. 1)}$$

where:

- $[D]$ = total concentration of preservative
- $[D_f]$ = concentration of free preservative in the aqueous phase; for an acid preservative, $[D_f] = [D_f'](1 + ka/[H^+])$, where $[D_f']$ is the concentration of unionized preservative in the aqueous phase and ka is the ionization constant
- $[M]$ = concentration of surfactant, n = maximum number of independent binding sites on the surfactant, and K = association constant for the binding of a molecule of preservative to one of these sites
- K_w^o = observed pH-dependent, concentration-dependent oil–water partition coefficient; $K_w^o = K_w'^o/(1 + ka/[H^+])$, where $K_w'^o$ is the pH-independent partition coefficient
- Q = oil–water phase volume ratio = V_o/V_w, where V_o and V_w are the volumes of the oil and aqueous phases, respectively.

In the equation of Bean *et al.* (2) and Patel and Romanowski (3), the term $1 + nK[M]/(1 + K[D_f])$ was replaced by R, where $R = 1 + nK[M]$; that is, for a given macromolecule concentration, the R value, or binding or solubilization constant, was assumed to be independent of $[D_f]$. As discussed elsewhere, this is true only in special cases (4).

Determination of the necessary factors in Eq. 1 is a lengthy process, particularly if, as is usual in most pharmaceutical and cosmetic emulsions, more than one type of macromolecule is present. Moreover, even a slight change in the formulation of the emulsion necessitates a reevaluation of the various terms. From a practical viewpoint, it would be of advantage to have a direct method to measure the amount of preservative in each "phase" of the emulsion and, hence, the total concentration required to provide the desired concentration in the aqueous phase. Garrett (1) suggested an ultracentrifuge technique. In this article, a three-chambered dialysis method is described. Results obtained using the dialysis method are compared with predictions made with Eq. 1, using experimentally determined physicochemical parameters.

EXPERIMENTAL

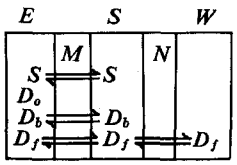
Reagents and Solutions—Peanut oil[1] and mineral oil[2] were used as the disperse phase in emulsions stabilized with the nonionic surfactant cetomacrogol 1000 BPC[3]. Analytical quality benzoic acid was used as the preservative. Aqueous solutions were made in citrate–phosphate buffer, pH 3.0 and ionic strength 0.2. Oil–water partition coefficients, the interaction between the preservative and the surfactant, and the distribution of preservative in emulsions were all studied at 30 ± 0.1° in a thermostatically controlled water-bath fitted with a tumbling device.

Oil–Water Partition Coefficients—Equal volumes of solutions of benzoic acid in oil and aqueous buffer were pipeted into glass-stoppered cylinders and agitated by a wrist-action shaker for about 1 hr. at room temperature. The cylinders were then tumbled in the water bath until equilibrium was reached (about 7 days). The aqueous phase was separated by centrifugation, and the benzoic acid concentration was analyzed. The concentration of benzoic acid in the oil phase was calculated by difference.

Two-Chambered Dialysis Cell—The interaction between benzoic acid and cetomacrogol was studied using equilibrium dialysis cells made according to the design of Patel and Foss (5). The two chambers of the cell were separated by a nylon membrane[4]. Details of the method and the results were described previously (4).

An emulsion containing various amounts of benzoic acid was pipeted into one compartment of a dialysis cell fitted with a nylon membrane. An aqueous buffer containing various amounts of benzoic acid was pipeted into the other compartment. A few glass beads were added to each compartment to ensure continuous mixing. The cells were equilibrated (about 4 days), and the concentration of benzoic acid in the aqueous compartment was analyzed.

Three-Chambered Dialysis Cell—The two-chambered dialysis cell was enlarged by the addition of an extra spacer in the center (Scheme I). A Millipore VS membrane[5] was used between compartments *E* and *S* and a nylon membrane between compartments *S* and *W*. The Millipore membrane is permeable to surfactant and benzoic acid but not to oil, while the nylon membrane is permeable to the benzoic acid only. Twenty milliliters of emulsion was pipeted into compartment *E*, 20 ml. of cetomacrogol solution was pipeted into compartment *S*, and 20 ml. of aqueous buffer solution was pipeted into compartment *W*. Known amounts of benzoic acid were



Scheme I—Three-chambered dialysis cell. Key: E, *emulsion;* S, *surfactant;* W, *water;* M, *Millipore VS membrane;* N, *nylon membrane;* D, *preservative;* D_o, *preservative in oil phase;* D_b, *preservative bound to surfactant; and* D_f, *free preservative in the aqueous phase*

[1] Planters Peanut Oil, Standard Brands Ltd., Canada.
[2] Primol 355, Imperial Oil Ltd., Canada.
[3] Glovers Chemicals Ltd., England.
[4] Capran 77C, Allied Chemical Corp., Morristown, N. J.
[5] Millipore Ltd., Montreal, Canada.

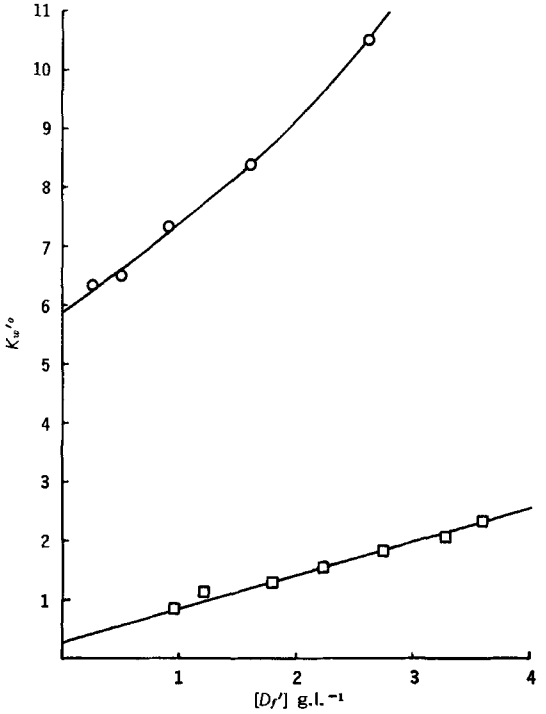


Figure 1—*pH-independent partition coefficient of benzoic acid* versus *the aqueous concentration of unionized benzoic acid. Key:* O, *peanut oil; and* □, *mineral oil.*

included in each chamber to accelerate equilibrium. The concentration of cetomacrogol in S was the same as used in the emulsion. Glass beads were added to each chamber, and the cell was tumbled until equilibrium was reached (about 7 days). The concentration of benzoic acid was determined in compartments S and W, and compartment S was analyzed for cetomacrogol.

Analytical Methods—Benzoic acid was analyzed spectrophotometrically at 273 nm., using a Coleman-Hitachi 124 spectrophotometer. Control experiments without the addition of benzoic acid were performed to provide blank solutions for use in the reference cell. Cetomacrogol was analyzed by the method of Crabb and Persinger (6). Preliminary experiments were carried out to determine the extent of binding of preservative and surfactant to the dialysis membranes. The values obtained were used to correct results from the interaction studies.

RESULTS AND DISCUSSION

Oil–Water Distribution Coefficient—Figure 1 shows the pH-independent partition coefficients, $K_w'^o$, plotted as a function of unionized benzoic acid concentration, $[D_f']$, in peanut oil and mineral oil. The distribution does not obey the simple partition law, and it is apparent that benzoic acid associates in both oils. According to the treatment of Gross and Schwarz (7), the equation of the line is:

$$K_w'^o = K_{dw}^o + mk'(K_{dw}^o)^m[D_f']^{m-1} \qquad \text{(Eq. 2)}$$

where K_{dw}^o is the distribution coefficient for the monomer, m is the number of molecules in an m-mer, and k' is the association equilibrium constant for monomer and m-mer. The linear relation between $K_w'^o$ and $[D_f']$ for mineral oil (Fig. 1) indicates dimerization of the benzoic acid, where $m = 2$, while the curve for peanut oil indicates m-merization in the oil phase. The intercept on the $K_w'^o$ axis at $[D_f'] = 0$ gives K_{dw}^o values of 5.84 and 0.23 for peanut oil and mineral oil, respectively. For substitution into Eq. 1, the pH-dependent observed partition coefficient, K_w^o, is more convenient than $K_w'^o$ or K_{dw}^o. Appropriate values of K_w^o were determined, therefore, from a plot of K_w^o against $[D_f]$.

Interaction between Preservative and Surfactant—Appropriate values of n and K for the interaction of benzoic acid with cetomacrogol were determined from a Scatchard plot of $[D_b]/[M][D_f]$ *versus* $[D_f]$, as described elsewhere (4), where $[D_b]$ is the concentration of preservative bound to the surfactant.

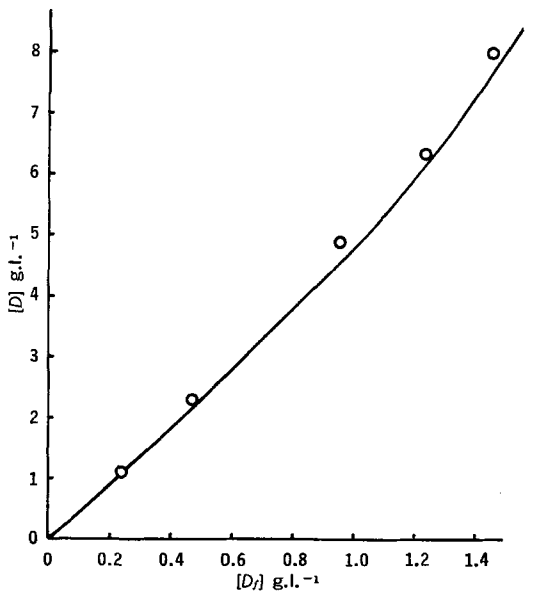


Figure 2—*Variation of free benzoic acid concentration,* $[D_f]$, *in the aqueous phase of an emulsion with total benzoic acid concentration,* [D], *for an o/w emulsion containing 50% v/v peanut oil emulsified with 4.0% w/v cetomacrogol. Key:* O, *observed* $[D_f]$; *curve calculated from Eq. 1.*

Estimation of Free Preservative Concentration in an Oil-in-Water Emulsion—Analysis of the concentration of benzoic acid in the aqueous compartment of the two-chambered dialysis cell enables the free benzoic acid concentration, $[D_f]$, to be determined. From $[D_f]$ and the total amount of benzoic acid added, $([D_o] + [D_b])$ can be determined, although it is not possible to separate these quantities. Figure 2 shows the total preservative concentration, $[D]$, plotted as a function of $[D_f]$.

Distribution of Preservative between the Oil, Surfactant, and Aqueous "Phases" of an Oil-in-Water Emulsion—Analysis of compartments W and S of the three-chambered dialysis cell after

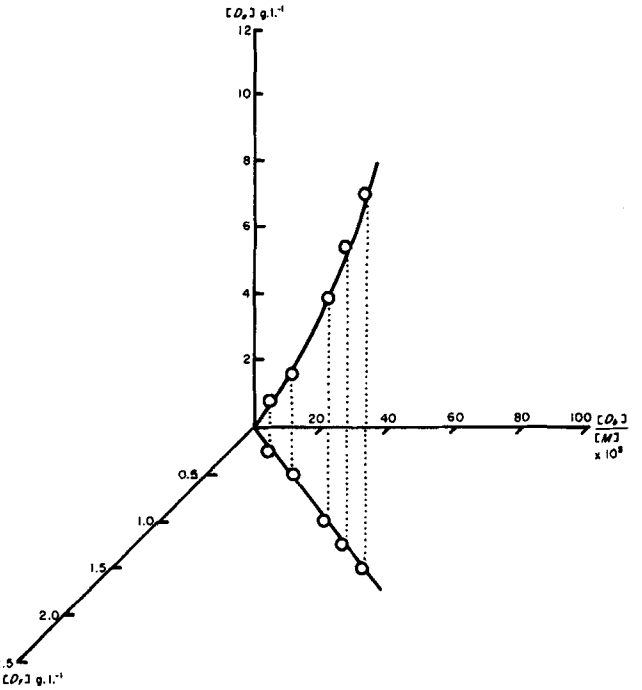


Figure 3—*Three-dimensional graph of the distribution of benzoic acid in an o/w peanut oil emulsion stabilized with cetomacrogol. Key:* O, *experimental values; curves calculated using Eq. 1.*

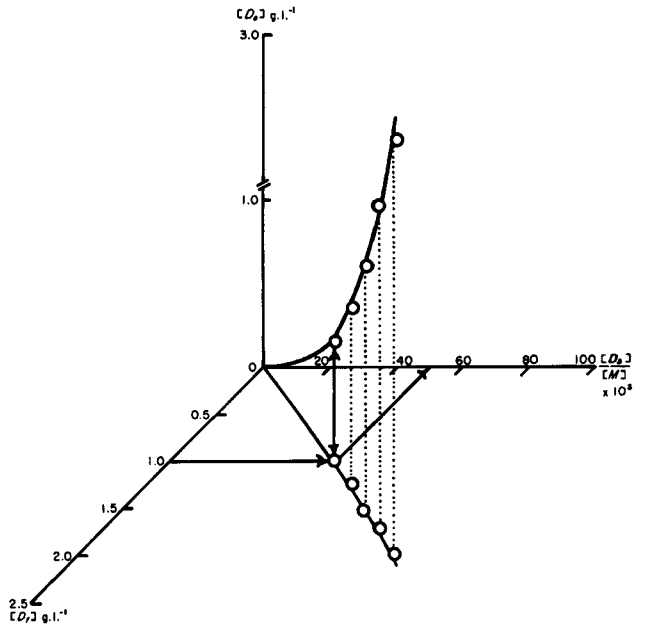


Figure 4—*Three-dimensional graph of the distribution of benzoic acid in an o/w mineral oil emulsion stabilized with cetomacrogol. Key: O, experimental values; curves calculated using Eq. 1.*

equilibrium gives $[D_f]$ and $([D_f] + [D_b])$, respectively, and, hence, $[D_b]$. From these terms and the amount of preservative added initially, $[D_o]$ can be obtained. Figures 3 and 4 show three-dimensional plots of $[D_o]$, $[D_b]/[M]$, and $[D_f]$ for the distribution of benzoic acid between peanut oil–cetomacrogol–aqueous buffer and mineral oil–cetomacrogol–aqueous buffer, respectively. There is close agreement between the experimentally determined values and the curves predicted using the oil–water distribution coefficient, K_w^o, and the preservative–surfactant binding constants, n and K.

The preservation of an emulsion requires that there must be a minimum inhibitory concentration of free preservative in the aqueous phase. The total amount of preservative required in the emulsion to provide a minimum inhibitory concentration of free preservative in the aqueous phase can be calculated from Eq. 1, where $[D_f]$ is the minimum inhibitory concentration. Determination of the various physicochemical parameters for substitution into Eq. 1 is a time-consuming process, however. Agreement between the predicted and observed values shows that the three-chambered dialysis technique provides a relatively simple method by which the total concentration of preservative necessary can be estimated. The first step is to construct three-dimensional calibration curves using the values of $[D_o]$, $[D_b]/[M]$, and $[D_f]$ obtained by dialyzing the emulsion containing varying known concentrations of preservative in the three-chambered cell. The graph is entered at a $[D_f]$ value corresponding to the minimum inhibitory concentration, and the corresponding values of $[D_b]/[M]$ and $[D_o]$ are determined. These terms are then used to calculate the total concentration of preservative necessary.

The procedure is illustrated using the mineral oil emulsion, containing benzoic acid as preservative (Fig. 4). Let the minimum inhibitory concentration = 1.0 g. l.$^{-1}$ = $[D_f]$ (8). The corresponding value of $[D_b]/[M]$ = 0.05. The corresponding value of $[D_o]$ = 0.75 g. l.$^{-1}$.

The total preservative concentration, $[D]$, is given by:

$$[D] = \{([D_f] + [D_b])V_w + [D_o]V_o\}/1000 \text{ g. .}^{-1} \quad \text{(Eq. 3)}$$

For an emulsion containing 4% cetomacrogol with an oil–water ratio of 1.0:

$$[D] = [(1.0 + 0.05 \times 40)500 + 0.75 \times 500]/1000 = 1.88 \text{ g. l.}^{-1} \quad \text{(Eq. 4)}$$

For a given oil–water–surfactant system, the three-dimensional calibration curves are theoretically independent of the oil–water ratio and the surfactant concentration. This was confirmed experimentally for oil–water ratios from 0.18 to 1.0 and for cetomacrogol concentrations from 1 to 4%.

In addition to the simple emulsions used in this work, the method should be applicable to more complex emulsions in which the existence of liquid crystalline phases and the presence of reversed micelles in the oil phase (9) would make the use of a mathematical model difficult or impossible.

The total preservative concentration can also be determined from a calibration curve of $[D]$ *versus* $[D_f]$ (Fig. 2), constructed using the two-chambered dialysis technique. However, separate calibration curves would be required for each oil–water ratio and for each surfactant concentration. Moreover, the three-chambered dialysis method provides more information because it differentiates between preservative in the oil phase and preservative associated with the surfactant. The effect of modification to the formulation on the distribution of preservative between the oil, surfactant, and water "phases" of the emulsion can, therefore, be readily assessed.

In an emulsion, some surfactant is adsorbed at the oil–water interface and, depending on its oil solubility, some partitions into the oil phase. Both factors reduce the amount of surfactant available for interaction with the preservative and affect the oil–water partition coefficient. Analysis of compartment S of the three-chambered dialysis cell for surfactant enables the distribution of surfactant between the oil and aqueous phases to be estimated. In this work, no change could be detected in the amount of cetomacrogol in compartment S after equilibration, which indicates that little surfactant was lost from the aqueous phase by adsorption or partition. This observation was supported by the close agreement between the predicted and observed values of $[D_b]$.

Where appreciable amounts of surfactant are adsorbed or partitioned into the oil, determination of the parameters necessary for substitution into Eq. 1 becomes difficult. This problem is avoided by using the three-chambered dialysis technique which permits direct observations to be made of the preservative distribution under conditions existing in the actual emulsion.

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