PRESERVATIVE   DISTRIBUTION   IN   EMULSIONS

by

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ABSTRACT

Until recently, evaluation of the effectiveness of preservatives in emulsified systems has depended largely on time-consuming microbiological techniques. Mathematical models have now been developed which enable the amount of preservative necessary for adequate preservation to be calculated. Determination of the physico-chemical parameters for these models is again a time-consuming process, especially where complex emulsions are involved. In the present work a three-chambered dialysis method has been investigated. Using this method it is possible to determine the concentration of preservative in the various phases of an emulsion and thus the total concentration required for adequate preservation.

Various factors affecting the distribution of preservatives between oil and water and the interaction between preservatives and surfactant are discussed. These factors are then related to the problem of the distribution of a preservative in an oil in water emulsion system. Methodology used to evaluate the various physico-chemical parameters is reviewed and equations for representing the results are discussed.

The distribution of benzoic acid between peanut oil and water and mineral oil and water systems was studied over a wide concentration range. The interaction of benzoic acid with aqueous solutions of the nonionic surfactant cetomacrogol was studied using solubility and equilibrium dialysis techniques. The interaction of various other preservatives with aqueous solutions of the nonionic surfactant cetomacrogol was examined. A comparison was made of various methods of expressing this

interaction. It is suggested that the Scatchard equation is the most satisfactory equation for describing the binding data. Binding parameters determined from a Scatchard plot in the concentration range of free preservative appropriate for antimicrobial activity were used to calculate the total concentration of preservative required in the surfactant solution.

A three-chambered dialysis cell was used to estimate the distribution of benzoic acid between the oil phase and the aqueous phase of oil in water emulsions containing peanut oil or mineral oil. The method also differentiates between preservative bound, or solubilized, by the surfactant and free in the aqueous phase. The distribution data was plotted on a three-dimensional graph from which the total concentration of preservative needed to provide a given free concentration in the aqueous phase can be determined. Results from the dialysis method agree closely with those calculated using mathematical models for preservative distribution. Hence the three-chambered dialysis method provides a relatively simple direct method of determining the required preservative concentration without recourse to mathematical models.

Supervisor.

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ACKNOWLEDGMENTS

To my sister

Kauser Ishrat

# I    INTRODUCTION

The deterioration of creams and emulsions due to microbial contamination is responsible for industrial losses (Baker, 1959) and may present a clinical hazard to the user (Kallings, et al., 1965, 1966).  Adequate preservation is therefore essential from both the pharmaceutical (Bean, 1967) and clinical (Savin, 1967; Evans, 1970) point of view.

The microbiological activity of preservatives in heterogeneous systems, such as emulsions, is much more complex than in simple aqueous systems.  The antimicrobial activity of preservative in an emulsified system is less than the same amount of preservative in an aqueous system (Wedderburn, 1964).  An understanding of the factors controlling the effectiveness of preservatives in emulsified systems can only be achieved through a thorough study of the various physical, chemical and microbiological parameters governing the distribution and antimicrobial activity of preservatives in various phases of an emulsion.

At the present time, assessment of the ability of preservatives to prevent microbial invasion of emulsified products depends largely on empirical tests involving inoculation of the finished product, and examination during a prolonged period of storage (Wedderburn, 1964).  These methods are laborious, time consuming and are mainly qualitative in nature.  No information is obtained with regard to the mechanism of inactivation of the preservative and the concentrations of preservative in various phases of the emulsion.

Much work has been done on the distribution and antimicrobial

activity of preservatives in oil-water (Bean, et al., 1965) and surfactant-water (Wedderburn, 1964) systems. In these systems the antimicrobial activity depends mainly on the concentration of preservative in the aqueous phase. Preservative partitioned into the oil phase, or bound with the surfactant is biologically inactive. However until recently few quantitative studies had been made of the distribution and antimicrobial activity of preservatives in emulsified systems. A few authors (Kostenbauder, 1962; Garrett, 1966; Bean, et al., 1969; Patel and Romanowski, 1970) have derived mathematical equations, using physico-chemical parameters governing the distribution of preservative in oil-water systems and the interaction of preservatives with surfactants, to predict the quantity of preservative required in an emulsion in order to achieve adequate preservation. These equations have been derived on the assumption that the antimicrobial activity of a preservative in an emulsion is mainly a function of the minimum-inhibitory or minimum-lethal concentration of preservative in the aqueous phase. It is assumed that preservative partitioned into the oil phase or solubilized by the surfactant micelle is biologically inactive. Agreement was found between predictions made using these equations and the results obtained from microbiological studies (Bean, et al., 1969; Patel and Romanowski, 1970). Determination of the necessary physico-chemical parameters in these equations is a lengthy process, particularly if, as is usual in most pharmaceutical and cosmetic-emulsions, there is more than one type of oil or macro-

molecule present. Moreover even a slight change in the formulation of the emulsion necessitates a re-evaluation of the various terms. From practical viewpoint it would be advantageous 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. In the present investigation a three-chambered dialysis method has been used to study the distribution of the preservative, benzoic acid, in oil-water-surfactant systems. Results obtained, using the dialysis method, are compared with those obtained using a mathematical equation and experimentally determined physico-chemical parameters.

## II   LITERATURE   SURVEY

### A.   Distribution of Preservatives in Oil-Water Systems

(a)   Factors affecting antimicrobial activity of preservatives in oil-
water systems.

A preservative added to an oil-water mixture partitions between the
two phases (Hibbott and Monks, 1961; Bean, Richard and Thomas, 1962).
The antimicrobial activity is mainly a function of the availability, or
thermodynamic activity, of a biologically effective concentration of
preservative in the aqueous phase, and not the total amount added, i.e.,
preservative in the oil phase is biologically inactive (Wolffhugel and
Von Knorre, 1881; Clark, 1939, Gershenfeld and Brillhart, 1939; Atkins,
1950; Bean, Richard and Thomas, 1962; Bean and Heman-Ackah, 1964; Bean,
Heman-Ackah and Thomas, 1965).   The availability of preservative in the
aqueous phase is controlled by various factors:

1. Dissociation constant $(K_a)$ of preservative and pH
   of aqueous phase.
2. Oil-water partition coefficient $(K_w^o)$.
3. Oil-water ratio (q).
4. Interfacial factor.
5. Temperature.
6. Effect of additives.

(1) <u>Dissociation constant of preservative and pH of aqueous phase</u>:
Weak acid preservatives are generally most effective in their undissoci-
ated form (Rahn and Conn, 1940; Bandelin, 1958), the equilibrium between
undissociated acid and anion being a function of pH levels. It can be
observed that 60 times as much benzoic acid is required at pH 6 as at
pH 3 to achieve equivalent antimicrobial activity.

Table 1

Proportions of Benzoic Acid Undissociated at
Various pH Values (Kostenbauder, 1962)

| pH | Undissociated Benzoic Acid % |
|----|----|
| 2 | 99.4 |
| 3 | 94.3 |
| 4 | 62.5 |
| 5 | 13.7 |
| 6 | 1.6 |
| $K_a = 6.3 \times 10^5$ | $pK_a = 4.2$ |

If the minimum inhibitory concentration of undissociated acid is
known, the total concentration of acid required in the aqueous phase can
be calculated from the following equation (Kostenbauder, 1962):

$$\text{Total required preservative} = \frac{\left(\begin{array}{l}\text{Inhibitory Concentration}\\ \text{of undissociated acid}\end{array}\right)(H+) + K_a}{(H+)}$$

A close relation is generally found between pH and antimicrobial activity of weak acid preservatives (Rahn and Conn, 1944; Wolf and Westveer, 1950; Simon, 1952; Von Schelhorn, 1952; Albert, 1957; Bandelin, 1958; Bell, et al., 1959; de Navaree, 1959; Enterikin, 1961). Since the dissociation constant of different preservatives varies, their behaviour in different pH conditions also varies. Some are inactivated by small increases in pH while others are not influenced at all. When the pH of the environment is below $pK_a$, changes of pH are of little consequence but as the pH is increased above the $pK_a$, higher concentrations are required to produce a standard response (Simon, 1952). In the case of benzoic acid a constant concentration of undissociated molecules does not produce the same response at different pH levels. This indicates that anions are also slightly toxic (Evans and Dunbar, 1965; Anderson and Cho, 1967).

(2) Oil-water partition coefficient: Partitioning has significant effect on the availability of preservative in the aqueous phase (Husa and Radin, 1932; Atkins, 1950; Allawala and Riegelman, 1953; Garrett and Woods, 1953; and others). When the preservative is more soluble in oil than in water enough must be added to an oil-water system to obtain a sufficient concentration in aqueous phase, i.e., the concentration of preservative in the aqueous phase is controlled by the overall concentration of preservative (Hibbott and Monks, 1961; Bean, Heman-Ackah and Thomas, 1965). A complicating factor is the concentration of preserva-

tive in oil phase which is always related to that of the aqueous phase, and may behave as a reservoir for the latter (Bean, Heman-Ackah and Thomas, 1965).

(3) <u>Oil-water ratio</u>: The concentration of preservative in the aqueous phase is not controlled by the partition coefficient alone, but by the interaction between the partition coefficient and the phase volume ratio (Bennett, 1962; Bean and Heman-Ackah, 1964; Bean, Heman-Ackah and Thomas, 1965; Bean, Konning and Malcolm, 1969). Table 2 shows the influence of partition coefficient and phase volume ratio on the concentration of preservative in aqueous and oil phase of a two phase system. When the partition coefficient is less than one, the majority of the preservative is in the aqueous phase and an increase in the oil-water ratio increases the aqueous phase concentration. When the partition coefficient is greater than one, most of the preservative is in the oil phase and an increase in the oil-water ratio reduces the concentration of preservative in the aqueous phase. When partition coefficient is equal to one, changing the oil-water ratio has no effect on the concentration of the preservative in either phase. Thus when selecting a compound for study as a possible preservative for a product, both the partition coefficient and the oil-water ratio must be considered.

(4) <u>Interfacial factor</u>: Bean, et al., (1965) has shown that the bactericidal activity of a given concentration of preservative in simple

## Table 2

Influence of Partition Coefficient and Phase-Volume Ratio on Concentration of
Preservative in Aqueous and Oil Phase of a Two-Phase System

| | $K_W^O$ at $25^O$ | Oil/Water ratio | | 0.2 | 1.0 | 2.5 | 5.0 | 10.0 |
|---|---|---|---|---|---|---|---|---|
| 0.4% w/v phenol in liquid paraffin/water dispersions | 0.067 | Preservative in oil | % | 0.031 | 0.050 | 0.080 | 0.080 | 0.176 |
| | | Preservative in water | % | 0.474 | 0.750 | 1.199 | 1.799 | 2.636 |
| 1% hypothetical preservative | 1.000 | Preservative in oil | % | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | | Preservative in water | % | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| 4.0% w/v chlorocresol in peanut oil/water dispersions | 116.7 | Preservative in oil | % | 22.96 | 7.93 | 5.60 | 4.79 | 4.40 |
| | | Preservative in water | % | 0.197 | 0.068 | 0.048 | 0.0411 | 0.038 |

aqueous solutions is less than the bactericidal activity of same concentration of preservative in the aqueous phase of an oil-water mixture. As the ratio of oil-water increases, the bactericidal activity of the preservative increases considerably. It was suggested that preservative molecules are adsorbed at the oil-water interface, with the polar portion of the molecule projecting into the aqueous phase, and the nonpolar portion projecting into the oil phase. In this way the concentration of preservative at the interface is higher than the bulk aqueous phase. When bacteria are added to such a system they are also adsorbed at the oil-water interface, but bacteria being heavier (diameter in microns) than preservative molecules (diameter in $\overset{o}{A}$) penetrate the aqueous phase more deeply than do the preservative molecules. It is therefore probable that part of the bacterial surface at the interface is in contact with higher concentration of preservative, but this is certainly not true for the whole cell. If the bacteria were strongly adsorbed at the interface, as have been shown by Kamakaka (1956), the preservative adsorbed at interface would have been much more effective than observed in these studies.

(5) <u>Effect of temperature</u>: Temperature influences the activity of preservatives in oil-water dispersions in a complex manner (Bean and Heman-Ackah, 1965; Bean, Heman-Ackah and Thomas, 1965). Temperatures above $50^o$ cause the death of vegetative cells by protein coagulation, enzyme inactivation, or both. In addition there are indirect effects of

temperature on: (i) the oil-water partition coefficient (Bean and Heman-Ackah, 1963); (ii) the oil-water interfacial activity which diminishes with rise in temperature (Heman-Ackah, 1965); (iii) the velocity of bactericidal action which increases with temperature (Madsen and Nyman, 1907; Chick, 1908; Phelps, 1911).

(6) Effect of additives: Additives such as propylene glycol, glycerin, etc., are often included in pharmaceutical and cosmetic emul-sions as humectants. These additives often bring about increased solu-bility of preservatives in the aqueous phase that results in reduction of oil-water partition coefficient (Hibbott and Monks, 1961; Anderson and Cho, 1967). It has been suggested (Hibbott and Monks, 1961) that this makes more preservative available in the aqueous phase with conse-quent increase in antimicrobial activity. On the other hand Anderson and Cho (1967) showed a reduction in preservative activity with the addition of glycerin. They suggested that although the inclusion of glycerin re-duces the oil-water partition coefficient, it also reduces the availa-bility of the preservative to microbial biophase. Consequently a higher concentration is required. In addition to reducing the oil-water parti-tion coefficient, Barr and Tice (1957a, b) found that glycerin and sor-bitol supplement each other in their inhibition of both bacteria and moulds. This effect was attributed to an osmotic effect of the high concentrations of the humectants. Propylene glycol, however, appeared to have a specific inhibitory effect in addition to its effect on the osmotic

pressure of aqueous solutions. It was concluded that propylene glycol

would have a significant and useful preservative effect. de Navarre

(1962) has found propylene glycol to be a reliable preservative at 16%

v/v in many cosmetic products and states its antimicrobial properties

to be three or four times that of the equivalent amount of glycerin.

(b)  Representation of distribution data.

The distribution of preservatives between oil-water systems can be

represented by the Nernst Equation (Nernst, 1891).

$$K_W^O = \frac{[Do]}{[Df]} \qquad (Eq.1)$$

Where $K_W^O$ is the distribution or partition coefficient; [Do], the con-

centration of preservative in the oil phase; [Df], the concentration

of preservative in the aqueous phase.

The ratio, $K_W^O$, is constant only for ideal solutions and is most

closely approximated when (a) the preservative neither dissociates

(ionizes) nor associates in either phase, (b) the preservative concen-

trations are approximately equal to activities, and (c) the two phases

are completely immiscible (Reese, et al., 1964).

When the preservative is monomeric in the oil phase, but ionizes

in the aqueous phase, then the ratio of concentration of preservative

in the oil phase to the concentration of unionized preservative in the

aqueous phase will be constant.

$$\begin{array}{|c c|}
\hline
[HA]_0 & \\
\Big\uparrow\Big\downarrow & \text{Oil} \\
\hline
& ka \\
[HA]_W \rightleftharpoons [H^+] + [A^-]_W & \text{Water} \\
\hline
\end{array}$$

$$K_{dw}^0 \quad = \quad \frac{[HA]_0}{[HA]_W} \qquad \text{(Eq. 2)}$$
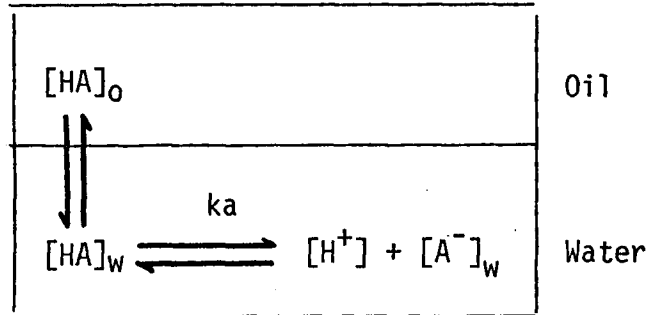
Where $K_{dw}^0$ is the distribution coefficient for monomer; $[HA]_0$, is the concentration of monomer in the oil phase; $[HA]_W$, the concentration of monomer in the aqueous phase.

In case of acid preservatives, such as benzoic acid and sorbic acid, the degree of ionization in the aqueous phase is a function of the pH of the aqueous phase and the ionization constant, ka, of the preservative. This can be expressed

$$ka \quad = \quad \frac{[H^+] \ [A^-]_W}{[HA]_W} \qquad \text{(Eq. 3)}$$

$$\therefore \quad [A^-]_W \quad = \quad \frac{ka \ [HA]_W}{[H^+]} \qquad \text{(Eq. 4)}$$

Where $[H^+]$ is the hydrogen ion concentration in the aqueous phase.

The observed pH dependent distribution coefficient, $K_W^0$, is given by:

$$K_w^O = \frac{[Do]}{[Df]} = \frac{[HA]_O}{[HA]_W + [A^-]_W} \qquad \text{(Eq. 5)}$$

Where [Do], the concentration of preservative in the oil phase, [Df], the concentration of preservative in the aqueous phase; $[HA]_O$, the concentration of monomer in the oil phase, $[HA]_W$, the concentration of monomer in the aqueous phase; and $[A^-]_W$, the concentration of anion in the aqueous phase.

Substitute $[A^-]_W$ from Eq. 4 in Eq. 5 and rearrange

$$K_w^O = \frac{[HA]_O}{[HA]_W + \dfrac{ka\ [HA]_W}{[H^+]}} \qquad \text{(Eq. 6a)}$$

or

$$K_w^O = \frac{[HA]_O}{[HA]_W} + \frac{1}{1 + \dfrac{ka}{[H^+]}} \qquad \text{(Eq. 6b)}$$

or

$$K_w^O = Kdw^O + \frac{1}{1 + \dfrac{ka}{[H^+]}} \qquad \text{(Eq. 6c)}$$

A plot of $K_w^O$ versus $1/(1 + ka/[H^+])$ will give a straight line with a slope equal to $Kdw^O$, the monomer distribution coefficient.

Rearranging Eq. 6c in double reciprocal form gives

$$\frac{1}{K_w^O} = \frac{1}{Kdw^O} \cdot 1 + \frac{ka}{[H^+]} \qquad \text{(Eq. 7a)}$$

or

$$\frac{1}{K_w^o} = \frac{1}{K_{dw}^o} + \frac{ka}{K_{dw}^o} \cdot \frac{1}{[H^+]} \qquad \text{(Eq. 7b)}$$

A plot of $1/K_w^o$ versus $1/[H^+]$ gives a straight line with a slope of $ka/K_{dw}^o$ and intercept equal to $1/K_{dw}^o$. Thus from equations 6 & 7, the monomer distribution coefficient can be calculated from the values of the observed pH dependent, concentration dependent distribution coefficient, $K_w^o$, obtained over a range of hydrogenion concentrations.

An equation similar to equations 6 & 7 has been derived by Garrett and Woods (1953) to determine the monomer distribution coefficient when preservative ionizes in the aqueous phase.

$$\frac{ka + [H^+]}{[Df]} = \frac{q\,K_{dw}^o + 1}{[D]} \cdot [H^+] + \frac{ka}{[D]} \qquad \text{(Eq. 8)}$$

Where $q$, the oil-water ratio; [D], the total concentration of preservative in oil-water system.

A plot of $\dfrac{ka + [H^+]}{[Df]}$ against $[H^+]$ yields a straight line with a slope, $(q\,K_{dw}^o + 1)/[D]$ and intercept, $ka/[D]$. The monomer distribution coefficient can thus be calculated from slope and intercept over the range of hydrogenion concentration considered, as illustrated below:

$$m = \frac{(q\,K_{dw}^o + 1)}{[D]} = \text{slope} \qquad \text{(Eq. 9a)}$$

or

$$q\,K_{dw}^o = m\,[D] - 1 \qquad \text{(Eq. 9b)}$$

or

$$K_{dw}^{o} = \frac{m[D] - 1}{q} \qquad \text{(Eq. 9c)}$$

$$C = \frac{ka}{[D]} = \text{Intercept} \qquad \text{(Eq. 10a)}$$

or

$$[D] = \frac{ka}{C} \qquad \text{(Eq. 10b)}$$

Substituting the value of [D] in Eq. 9c

$$K_{dw}^{o} = \frac{m\ ka}{q\ c} - 1 \qquad \text{(Eq. 11a)}$$

or

$$K_{dw}^{o} = \frac{m\ ka - q\ c}{q\ c} \qquad \text{(Eq. 11b)}$$

Thus from Eq. 8, if pH of the aqueous solution and the concentration of preservative in the aqueous phase, [Df], are known the monomer distribution coefficient can be calculated. Unlike equations 6 & 7 there is no need to calculate the observed pH dependent, concentration dependent distribution coefficient.

Alternatively, the monomer distribution coefficient, $K_{dw}^{o}$, can be obtained according to Eq. 2 by analysis of the oil phase and aqueous phase at a pH sufficiently low to ensure that the preservative exists completely in the unionized form.

When the preservative is monomeric in the aqueous phase, but associates to one species of m - mer in the oil phase, three equilibrium constants are considered:

$$k' = \frac{[HA]_{om}}{[HA]_0^m} \qquad \text{(Eq. 12)}$$

$$K_{dw}^0 = \frac{[HA]_0}{[HA]_w} \qquad \text{(Eq. 2)}$$
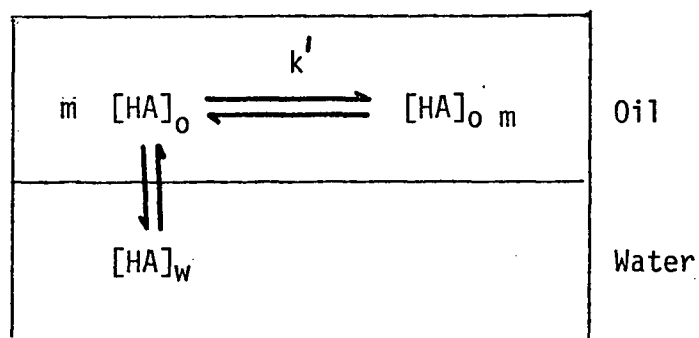
$$K_w^{'0} = \frac{[HA]_0 + m\,[HA]_{om}}{[HA]_w} \qquad \text{(Eq. 13)}$$

Where $K_w^0$, the pH independent, concentration dependent distribution co-efficient; $k$ , the association equilibrium constant for monomer and m - mer.

Rearranging Eq. 13 gives

$$K_w^{'0} = \frac{[HA]_0}{[HA]_w} + \frac{m\,[HA]_{om}}{[HA]_w} \qquad \text{(Eq. 14)}$$

or

$$K_w^{'0} = K_{dw}^0 + \frac{m\,[HA]_{om}}{[HA]_w} \qquad \text{(Eq. 15)}$$

Substituting the value of $[HA]_{om}$ from Eq. 12 into Eq. 15 gives

$$K_w^{'0} = K_{dw}^0 + \frac{m\,k'\,[HA]_0^m}{[HA]_w} \qquad \text{(Eq. 16a)}$$

or

$$K'^o_W = K^o_{dw} + m\,k'\,(K^o_{dw})^m\,[HA]^{m-1}_W \qquad (Eq.\ 16b)$$

If $[HA]_W = [D'_f]$ = concentration of unionized preservative in aqueous phase.

$$K'^o_W = K^o_{dw} + m\,k'\,(K^o_{dw})^m\,[D'_f]^{m-1} \qquad (Eq.\ 17)$$

The simplest case i.e. dimerization of preservative in oil phase, corresponds to m=2 and then Eq. 17 becomes

$$K'^o_W = K^o_{dw} + 2k'\,(K^o_{dw})^2\,[D'_f] \qquad (Eq.\ 18)$$

This linear relationship between $K'^o_W$ and $[D'_f]$ is the one used by Gross and Schwarz (1930), its form has since been deduced by other authors (Shikata, 1931; Philbrick, 1934; Moelwyn-Hughes, 1940; Davies and Hallam, 1956). In the case of dimerization, a plot of $K'^o_W$ against $[D'_f]$ gives a straight line extrapolating to $K^o_{dw}$ at $[D'_f]$ = o, and of slope $2k'\,(K^o_{dw})^2$, from which the association constant, k , is calculated. In cases other than m = 2, Eq. 17 leads to simple curves if $K'^o_W$ is plotted against $[D'_f]$, showing m - merization of preservative in the oil phase.

B.    Interaction of Preservatives with Nonionic Surfactants

This aspect has been the subject of numerous investigations and has been reviewed elsewhere (Allawala and Riegelman, 1953; Wedderburn, 1964; Evans and Dunbar, 1965; Elworthy, Florence and Macfarlane, 1968). The following is a brief account of the factors pertinent to an understanding of this problem.

(a)    Potentiation of preservative activity

Early observations of the inactivation and inhibition of germicides and preservatives were made in presence of ionic surfactants (Frobisher, 1927; Hampil, 1928; Ordal, et. al., 1941; Alexander Tomlison, 1949; Bean and Berry, 1950, 1951). Most of these workers found that low concentrations of surfactants (i.e. below critical micelle concentration) enhanced the effects of germicides, while higher concentrations led to varying degrees of inactivation. Alexander and Trim (1946) studied the effects of ionic surfactants on the penetration of hexylresorcinol into the Ascaris worm. Maximum penetration was found to occur at the critical micelle concentration (CMC) which corresponds to the maximum concentration of monomolecularly dispersed surfactant. Very little evidence is available to indicate the inactivation of preservatives by low concentrations of nonionic-surfactants, possibly because of the practical difficulties of working below the CMC. This falls approximately in the region of $10^{-4}$ to $10^{-6}$M for nonionics. Schoog (1957) reported that the activity of hexachlorophene was increased by concentrations of a polyoxyethylene lauryl ether up to $10^{-4}$M, but reduced by higher concentrations of the nonionic. Brown and Richards (1964) showed that the antibacterial activity of chlorhexi-

dine was enhanced by the presence of 0.02% polysorbate 80 but was reduced by o.05%.

The exact mechanism of potentiation of preservative activity by surfactants, below their CMC, is not well understood. It has been ascribed to the surface-active properties of the surfactant monomers. The surfactant monomers possibly help in the adsorption of preservative molecules at bacteria/water interface by reducing the interfacial tension.

(b)  Inactivation of preservative activity

Since their introduction about 20 years ago, nonionic surfactants have found ever increasing use in the preparation of solubilized and emulsified systems. Despite their many advantages in formulation, they have one serious disadvantage of suppressing or even inactivating the efficacy of added preservatives. Bolle and Mirimnoff (1950) were the first to point out the importance of this phenomenon and showed the suppression of antimicrobial activity of methyl p-hydroxybenzoate in the presence of several structurally different nonionic surfactants. They found that the surface-active sorbitan esters and polyoxyethylene sorbitan esters reduced the effect of methyl p-hydroxybenzoate, oxyquinoline sulfate and dioxydichlorodiphenylmethane; whereas the non surface-active Carbowax 1500, a polyethyleneglycol polymer, had no inactivating effect. Lawrence and Erlandson (1953) and Erlandson and Lawrence (1953) disclosed that Tweens and certain other nonionics reduced the germicidal effect of a number of phenolic compounds, in which there was a 2000 - 5000 fold re-

duction in activity. Barr and Tice (1957b) found that 5% Tween 20 rendered ineffective a large number of phenolic substances when tested at the concentrations at which they are normally used. Similar inactivations of commonly used preservatives in the presence of a number of nonionic surfactants have been observed by de Navarre and Bailey (1956) and de Navarre (1957). The preservatives studied included benzoic acid, sorbic acid and methyl p-hydroxybenzoate. Wedderburn (1958) assessed the effect of thirty-six different nonionics on twenty-six antimicrobial agents and found that although all the surface-active nonionics exerted some depressant effect, those which were not surface-active had negligible effects. The extent of the adverse effect was different for different combinations of nonionic surfactant and preservative, and appeared to be related not only to the hydrophile-lipophile balance of the surfactant but also to the chemical structure of the preservative. Blaug and Ahsan (1961b) examined the interaction of the methyl, ethyl, propyl and butyl p-hydroxybenzoates with several nonionic macromolecules and reported that p-hydroxybenzoates of higher molecular weights were influenced to a greater extent than those of lower molecular weight. In determining binding tendencies of the p-hydroxybenzoates with Tween 80 (polyoxyethylene sorbitan mono-oleate), Myrj 52 (polyoxyethylene monostearate), polyethylene glycol 4000, 6000 and Pluronic F - 68 (polyethylene polypropylene glycol), they confirmed that the hydrophile-lipophile balance strongly influenced the effect. Those compounds with greater lipophilic tendencies reduced the effectiveness more than the more hydrophilic macromolecules.

The above studies have demonstrated qualitatively the failure of preservatives in the presence of nonionic surfactants. It is evident that the inactivation results from an association of the preservative molecules with the macromolecules, and many qualitative studies have been carried out to determine the nature and extent of the interactions which occur, from both a physical and microbiological point of view.

In these systems an equilibrium may be postulated (Kostenbauder, 1962; Garrett, 1966) of the form of Eq. 16, which for most of the interactions reported has been shown to be reversible. It is generally agreed

$$Preservative + Macromolecule \rightleftharpoons Preservative-Macromolecule$$
(Eq. 16)
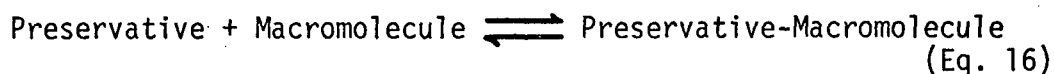
that the antimicrobial activity of such systems depends mainly on the concentration of unbound or free preservative (Wedderburn, 1964; Mitchell, 1964) rather than total concentration present. Recently Humphreys, Richardson and Rhodes (1968) showed that the antimicrobial activity of a concentration of preservative in aqueous solution was less than the same concentration of preservative in an aqueous phase of a surfactant solution. They concluded that antimicrobial activity is only a function of the concentration of preservative in the aqueous phase but some additional factors also control the extent of antimicrobial activity.

(c) Mechanism of inactivation of preservatives by nonionic surfactants

An examination of the literature reveals that the mechanism of interaction has been a subject of much debate, and the controversy has centred between two schools of thought. One maintains that the inter-

action between preservative and surfactant is due to complex formation, while the other believes that interaction is due to partitioning of preservative into surfactant micelles.

Higuchi and Lach (1954) reported the formation of hydrogen bonded complexes between polyethylene glycols and phenols and between polyethylene glycols and organic acids. Since most nonionic surfactants have polyethylene glycol chains, many authors (Guttman and Higuchi, 1956; Mulley and Metcalf, 1956; de Navarre, 1956, 1957; Barr and Tice, 1957b) have attributed both the solvent properties and inactivation of preservatives to complex formation. Evans (1964) showed that complex formation between surfactant monomer and preservative is unlikely and suggested that inactivation arises from solubilization of preservative within the surfactant micelles. Mulley (1964) collected evidence from a number of sources which indicates that the solubilization of a wide range of solutes in nonionic surfactants can be treated as a solution process within the hydrocarbon-like interior of the micelle. He considered that the data does not support suggestions that solubilization is controlled by more specific factors such as complex formation. Some workers (Evans and Dunbar, 1965; Wedderburn, 1964) suggested that since inactivation occurs with preservatives and surfactants of such diverse chemical structures, solubilization rather than specific complexing is a more probable explanation. Support for these authors is found in the fact that non-surface active macromolecules interact with preservatives to a much lesser

extent than surfactant molecules. Kostenbauder (1962) maintains that it is unnecessary to distinguish between the mechanism of complex formation and micellar solubilization and considers that solubilization and micelle formation itself fall within the broad scope of complex formation described by Higuchi and Lach (1954), because both processes obey the law of mass action. Micelles of nonionic surfactants appear to provide ideal conditions for association with preservatives. They afford the possibility for hydrogen bonding and solubilization within the hydrocarbon-like interior of the micelle. It seems likely that under suitable conditions both these mechanisms may operate simultaneously.

(d)   Possible sites for the interaction of preservatives in a surfactant micelle.

The site of incorporation of the solubilizate in a surfactant micelle is believed to be closely related to its chemical nature, as well as the chemical nature of the surfactant. In aqueous solution it is generally accepted that nonpolar solubilizates, e.g. aliphatic hydrocarbons are dissolved in the hydrocarbon core of the micelle (Fig. 1, (a)). Semipolar or polar solubilizates, e.g. fatty acids and alkanols, are taken up in what is often termed the palisade layer of the micelle, oriented with their hydrophobic moieties towards the centre of the micelle and their polar groups in its surface.

Riegelman, et. al. (1958) studied various aromatic compounds solubilized in aqueous solutions of potassium laurate, dodecyl-amine hydrochlo-
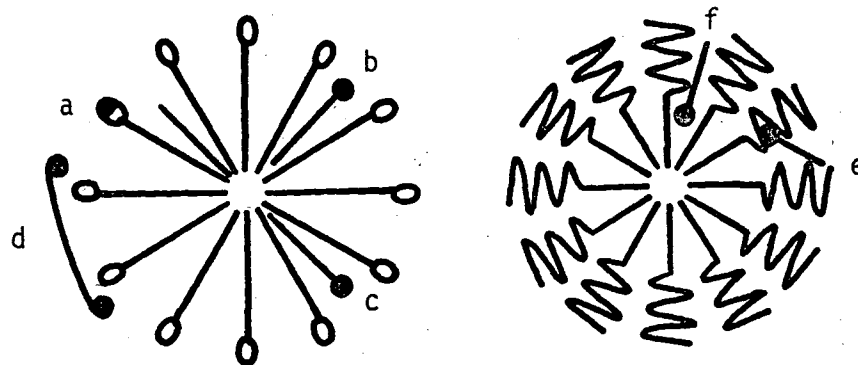
Fig. 1.  Possible sites of incorporation of solubilizate in a micelle
(Elworthy et. al., 1958):  (a) in the hydrocarbon core;  (b)
short penetration of the palisade layer;  (c) deep penetration
of the palisade layer;  (d) adsorption on the surface of the
micelle;  (e) in the polyoxyethylene shell of the micelle of
a nonionic surfactant;  (f) at the junction of hydrocarbon
core and polyoxyethylene chain of nonionic surfactant.  A,
micelle of ionic surfactant.  B, micelle of nonionic surfac-
tant.

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ride and a polyoxyethylene ether of lauryl alcohol and concluded that ethyl benzene was incorporated in the hydrocarbon portion of the micelle (a), O-nitroaniline was located on a position of short penetration of the palisade layer (b), azobenzene, naphthalene and anthracene were in a position of deep penetration of palisade layer (c), and dimethyl phthalate was adsorbed on the surface of the micelle (d), (Fig. 1).

Mulley and Metcalf (1956) suggested that Chloroxylenol solubilized in the micelles of cetomacrogol formed hydrogen bonds between phenolic hydroxyl groups and oxygen atoms of polyoxyethylene chains (Fig. 1 (e)).

Donbrow and Rhodes (1966) examining the solubilization of benzoic acid in cetomacrogol, favour the view that the position of the benzoic acid molecules in the micelle is at the junction of the hydrocarbon core and the polyoxyethylene chains (Fig. 1 (f)), with the benzene ring enclosed in the former and the carboxylic acid group protruding outwards. Such a position would still allow for hydrogen bonding between carboxyl group and the innermost ether oxygen.

(e)  Representation of interaction data.

The interaction between preservative and surfactant can be represented by the law of mass action.  Consider a simple situation where a macromolecule (M) combines with one molecule of preservative (D) to form a complex (DM), then at equilibrium

$$D \ + \ M \ \underset{}{\overset{K}{\rightleftharpoons}} \ DM \qquad \text{(Eq. 19)}$$

the association constant, K, is defined by the following equation:

$$K = \frac{[Db]}{[Mf][Df]} \qquad \text{(Eq. 20a)}$$

or

$$[Db] = K [Df][Mf] \qquad \text{(Eq. 20b)}$$

Where [Df], the concentration of free preservative; [Db], the concentration of preservative bound with macromolecule; [Mf], the concentration of free macromolecule. But

$$[M] = [Mf] + [Db] \qquad \text{(Eq. 21a)}$$

or

$$\text{Total concentration of macromolecule} = \text{Free concentration of macromolecule} + \text{Bound concentration of macromolecule}$$

or

$$[Mf] = [M] - [Db] \qquad \text{(Eq. 21b)}$$

Substitute the value of [Mf] in Eq. 20b and rearrange

$$[Db] = K [Df] \left\{ [M] - [Db] \right\} \qquad \text{(Eq. 22)}$$

$$\therefore \frac{[Db]}{[M]} = \frac{K [Df]}{1 + K [Df]} \qquad \text{(Eq. 23)}$$

If the macromolecule has n independent binding sites and each site has the same intrinsic affinity for preservative and is not influenced by its neighbours, the Eq. 20 is multiplied by 'n', then

$$r = \frac{[Db]}{[M]} = \frac{n K [Df]}{1 + K [Df]} \qquad \text{(Eq. 24)}$$

Where r represents molar ratio, i.e. the number of moles of preservative bound per mole of macromolecule. This ratio indicates the extent of

binding.

If all the monomers in a surfactant micelle behave independently and the interaction of preservative with the micelle does not change the micellar molecular weight, then Eq. 24 can be utilized to represent the interaction of preservative with surfactant micelles. In this case, r will give number of moles of preservative bound, [Db], per mole of micelle, [M]; n, the number of binding sites per micelle, and K, the association constant for interaction with micelle.

Rearrangement of Eq. 24 gives

$$[Db] = \frac{n\ K\ [M]\ [Df]}{1 + K\ [Df]} \qquad (Eq.\ 25)$$

C.   Distribution and Antimicrobial Activity

of Preservatives in Emulsified Systems.


Factors affecting the antimicrobial activity of preservatives in

emulsified systems are much more complex than simple aqueous systems.

Some notable reviews on the subject have appeared in the literature

(Tice and Barr, 1958; Kostenbauder, 1962; Bennett, 1962; de Navarre,

1962; Wedderburn, 1964).  Aoki, et. al. (1957) studied various factors

important in the preservation of hydrophilic ointment (U.S.P. XV; J.P.

VI) and similar emulsions by p-hydroxybenzoates.  They found that the

amount of ester required was dependent not only on the nature of the

ester but also on the nature of components in the oil and water phases.

Later on Matsumoto and Aoki (1962) found that the degree of inactivation

of p-hydroxybenzoates were dependent upon the relationship between the

properties of the ester, surfactant and the oily substance to be incor-

porated in the emulsion.  Oily substances, such as isopropyl myristate,

olive oil, lauryl alcohol, xylene and dibutyl phthalate, considerably

altered the activity of the esters.  Propyl p-hydroxybenzoate was found

to be more subject to inactivation than the methyl ester.  It was sugges-

ted that the propyl p-hydroxybenzoate was inactivated by ready solubili-

zation into the lipophilic part of the micelle.  Kostenbauder (1962) de-

rived an equation which involved the use of the oil-water partition co-

efficient and preservative-macromolecule binding data.  The equation

permitted a prediction to be made of the amount of preservative required

in an emulsion to provide a concentration of preservative in the aqueous

phase to inhibit microbial growth.  Later on Garrett (1966) developed a

more comprehensive mathematical model, which quantified all the various factors responsible for the inactivation of preservatives in heterogeneous systems. Anderson and Cho (1967) reported on the distribution and activity of benzoic acid in oil-water systems emulsified with 0.1% polyoxyethylene lauryl ether (Brij 35). The antifungal activity of benzoic acid was related to its concentration in the aqueous phase. Bean, Konning and Malcolm (1969) suggested a mathematical equation to calculate the concentration of preservative required for an emulsion which would achieve adequate preservation. They found a close agreement between the predictions made by the equation and the results obtained from microbiological studies. From the results the authors concluded that activity of preservatives in emulsified systems was related to the concentration free in the aqueous phase. Recently Patel and Romanowski (1970) verified Kostenbauder's equation (1962) by an in-vitro microbiological procedure. They showed that the fungistatic activity of methyl and propyl p-hydroxybenzoates in emulsified systems was primarily a function of the free paraben concentration in the aqueous phase.

D.  Prediction of Total Preservative Concentration
    Required in an Emulsion.

When a preservative is added to a simple emulsion consisting of an
oil, water and surfactant, part of it is partitioned into the oil phase
and part is complexed or solubilized within the surfactant micelles.
The rest of the preservative remains in the aqueous phase.

It has been shown that the amount of preservative partitioned into
the oil phase, or bound to the surfactant micelles has little antimicro-
bial activity.  The antimicrobial activity depends mainly on the concen-
tration of preservative in the aqueous phase (Anderson and Cho, 1967;
Bean, Konning and Malcolm, 1969; Patel and Romanowski, 1970).

Thus from the physico-chemical parameters governing the distribu-
tion of preservatives in oil-water systems and the binding of preserva-
tive with surfactant micelles it should be possible to calculate the
total concentration of preservative required in an emulsion to provide
a concentration of preservative in the aqueous phase sufficient to inhi-
bit microbial growth.

The total amount of preservative in an emulsion, $W$, is given by

$$W = [Dt] Vw + [Do] Vo \qquad \text{(Eq. 26)}$$

Where $[Dt]$, the total concentration of preservative in aqueous phase;
$[Do]$, the concentration of preservative in oil phase; $Vw$, the volume of
aqueous phase; $Vo$, the volume of oil phase.    But

$$W = [D] (Vo + Vw)$$

and

$$[Dt] = [Df] + [Db]$$

Where [D], the total concentration of preservative in emulsion; [Df], the concentration of free preservative in the aqueous phase and [Db], the concentration of bound preservative in the aqueous phase.

Substitute the values of W and [Dt] in Eq. 26 and rearrange

$$[D] (Vo + Vw) = \left[ [Df] + [Db] \right] Vw + [Do] Vo \quad \text{(Eq. 27)}$$

$$\therefore [D] = \left\{ \left[ [Df] + [Db] \right] Vw + [Do] Vo \right\} / (Vo + Vw) \quad \text{(Eq. 28)}$$

But

$$[Db] = n K [M] [Df] / (1 + K [Df]) \quad \text{(Eq. 25)}$$

and

$$(Do] = K_W^O [Df] \quad \text{(Eq. 5)}$$

$$q = Vo/Vw = \text{oil : water ratio}$$

$$\therefore Vo = q Vw$$

Substitute the values of [Db], [Do] and Vo in Eq. 28 and rearrange.

$$[D] = \left\{ \left[ [Df] + n K [M] [Df] / (1 + K [Df]) \right] Vw + K_W^O [Df] q Vw \right\} / (q Vw + Vw) \quad \text{(Eq. 29a)}$$

or

$$[D] = \left\{ [Df] Vw \left[ 1 + n K [M] / (1 + K [Df]) + K_W^O q \right] \right\} / \left\{ Vw (q + 1) \right\} \quad \text{(Eq. 29b)}$$

or

$$[D] = \left\{ [Df] \left[ 1 + n K [M] / (1 + K [Df]) + K_W^O q \right] \right\} / (q + 1) \quad \text{(Eq. 30)}$$

For an acid preservative:

$$[Df] = [D_f'] (1 + ka/[H^+])$$

Where [D_f'], the concentration of unionized preservative in the aqueous

phase; ka, the ionization constant; [H$^+$]. the hydrogen ion concentra-
tion

and

$$K_W^O = [Do]/[Df] = [Do] / \left\{ [D_f'] (1 + ka/[H^+]) \right\}$$

$$\therefore K_W^O (1 + ka/[H^+]) = [Do]/[D_f'] = K_W^{'O}$$

Where $K_W^{'O}$, the pH independent, concentration dependent distribution co-
efficient.

Substituting the values of $K_W^O$ and [Df] in Eq. 30 gives

$$[D] = \left\{ [D_f'] (1 + ka/[H^+]) \left[ 1 + n K [M] / (1 + K [D_f'] \right. \right.$$
$$\left. \left. (1 + ka/[H^+])) + K_W^O q/(1 + ka/[H^+]) \right] \right\} /$$
$$(q + 1) \qquad\qquad (Eq. 31)$$

Equations similar to 30 and 31 have been derived by other authors:

(a) Kostenbauder (1962); Patel and Romanowski (1970).

$$W = [Df] \left\{ R Vw + K_W^O Vo \right\} \qquad (Eq. 32)$$

and for acid preservative,

$$W = [Df] \left\{ \left[ R + ka/[H^+] \right] Vw + K_W^O Vo \right\}$$
$$(Eq. 33)$$

(b) Garrett (1966)

$$[D] = [D_f'] \cdot f1 \cdot f2 \cdot f3 = [D_f'] \left\{ 1 + \sum_{i=1}^{m} ni [Ml] / \right.$$
$$\left. \left[ K1 + [D_f'] \left( 1 + ka/[H^+] + K_W^O q \right) \right] \right\} \left\{ 1 + ka/[H^+] + \right.$$
$$\left. K_W^O q \right\} \left\{ e^{k't} \right\} \qquad (Eq. 34)$$

Where f1, the binding enhancement factor; f2, oil-water distribution

and ionization enhancement factor; f3, instability enhancement factor;
K1, intrinsic dissociation constant for preservative-surfactant complex;
$k'$, 1st order rate constant for the decomposition of preservative and t,
the time required for decomposition.

(c)   Bean, Konning and Malcolm (1969)

$$[D] = [Df] \left\{ R + K_w^0 q \right\} / (q + 1) \quad (Eq. 35)$$

Basically all these equations are similar except for the binding
parameters used.  In the equation of Kostenbauder (1962), Bean, Konning
and Malcolm (1969) and Patel and Romanowski (1970), the term $1 + n K [M]/$
$(1 + K [Df])$ is replaced by R, where $R = 1 + n K [M]$, i.e. for a given
macromolecule concentration the R value, or binding or solubilization
constant, was assumed to be independent of $[Df]$.  As will be discussed
later, this is true only in special cases.  In Garrett's equation (1966)
the term $1 + n K [M]/(1 + K [Df])$ is replaced by $1 + n [M]/(K' + [Df])$,
where $K'$ is intrinsic dissociation constant and is equal to $1/K$.  Garrett
(1966) also takes into account an instability factor where the preserva-
tive degrades by 1st order kinetics.  This correction is seldom necessary
because most of the commonly used preservatives are stable under the
conditions of use.

E.  Methodology


(a)  Distribution of Preservatives in oil-water systems.

Methods for the determination of oil-water partition coefficient of preservatives and drugs can be devided into two categories, (I) the shake-out methods, resulting in the formation of oil-water emulsions and (II) methods where emulsion formation is avoided.

I - The shake-out methods:  In these methods (Garrett and Woods, 1953; Hibbott and Monks, 1961; Bean and Heman-Ackah, 1964; Anderson and Cho, 1967; Bean, Konning and Malcolm, 1969) the oil-water mixture with preservative or drug is shaken at constant temperature until equilibrium is attained.  The mixture is allowed to stand and the oil-water phases are separated and analyzed for preservative.  An inherent problem associated with this method is that of emulsification or sometimes dispersion of fine droplets of one phase into the other phase.  As a result complete separation of the two phases becomes very difficult.  Allen and McDowell (1960) reported that shake-out methods can also result in anomalous equilibria.  In spite of these drawbacks, these methods are popular because of convenience and compared with non shake-out methods less time is required for equilibrium to be attained.  Where emulsification is a problem, separation of the oil-water phases can be achieved by ultracentrifugation (Garrett, 1962).

II - Other methods:  To avoid emulsification, other methods have been devised for the determination of oil-water partition coefficient of preservatives and drugs.  Patel and Romanowski (1969) determined partition

coefficients using a two-chambered dialysis technique, with oil in one compartment and water in the other. The two compartments were separated by a semipermeable membrane, permeable only to preservative molecules. They found close agreement between partition coefficients determined by the dialysis technique and those determined by shake-out method. Reese, et al. (1964) and Doluisio and Swintosky (1964) developed a simple rocking apparatus for routine determination of distribution coefficients. With this apparatus, up to 36 two-phase samples in cylindrical tubes were equilibrated by rocking the horizontal tubes at one cycle per minute through an arc of $45^{\circ}$. This rocking causes the interface between the two immiscible phases to expand and contract slowly. It also causes the shape of each phase to vary constantly. These two actions facilitate uniform distribution of solute within each phase and facilitate drug transfer from one phase to the other. Emulsion formation is negligible since little turbulance is created. The authors found good agreement between results obtained by this method and results from shake-out methods. Distribution coefficients of some drugs sparingly soluble in aqueous phase has also been determined by paper chromatography (Bowen, James and Roberts, 1970).

(b) Interaction of preservatives with nonionic surfactants.

The various methods used for assessing preservative-surfactant interaction has been reviewed elsewhere (Wedderburn, 1964; Parker and Barnes,

1967; Elworthy, Florence and Macfarlane, 1968). These methods fall into two groups: (I) physico-chemical methods and (II) biological methods. The biological methods have been reviewed by Wedderburn (1964) and Parker and Barnes (1967), and will not be discussed here. The physico-chemical methods can be divided into two categories. The first group depends upon the properties of the interacting molecule; the second, on the behaviour of macromolecule. Methods depending on the properties of the interacting molecule include solubility analysis (Patel and Kostenbauder, 1958; Blaug and Ahsan, 1961; Goodhart and Martin, 1962; Lundi and Held, 1966; Mitchell and Brown, 1966; Humphreys and Rhodes, 1968; and others), equilibrium dialysis (Patel and Kostenbauder, 1958; Patel and Foss, 1964, 1965; Breuninger and Goettsch, 1965; Mitchell and Brown, 1966; Anderson and Morgan, 1966; and many others), turbidimetric titration (a. visual: Higuchi and Lach, 1954; Guttman and Higuchi, 1956. b. photometric: Kabadi and Hammarlund, 1966), potentiometric titration (Donbrow and Rhodes, 1963a, 1964, 1965; Evans, 1964, 1966; Donbrow and Jacobs, 1966), pH measurement (Mitchell and Brown, 1966), molecular sieve technique (Ashworth and Heard, 1966; Donbrow, Azaz and Hamburger, 1970) etc. Methods depending on the behaviour of macromolecule include differential interference refractometry (Choulis, 1970; Choulis and Rhodes, 1970), surface tension (Horin and Arai, 1970), viscometry (Horin and Arai, 1970), density measurements (Harkins, et. al., 1946) x-ray diffraction (Harkin, et. al., 1946) etc. Spectroscopy has also been applied to study preservative-surfactant interaction

by comparing the spectrum of preservative in presence of surfactant and vice versa (Riegelman, et. al., 1958; Anderson and Slade, 1965; Donbrow and Rhodes, 1966; Kabadi and Hammerlund, 1966). Of all the aforementioned methods only solubility and equilibrium dialysis techniques will be discussed in detail.

1. <u>Solubility</u>: This is one of the simplest methods used to study the interaction of preservatives with surfactants. Various techniques have been used to determine the solubility of preservatives in surfactant solutions. One of the techniques involves the addition of an excess amount of preservative to a series of surfactant solutions of different concentrations [M], a blank being included of preservative and water, and the systems agitated on a shaker at constant temperature until equilibrium is attained. The solutions are filtered to remove any undissolved preservative and the filtrate assayed for preservative contents. Analysis of blank gives water solubility of preservative [Df]. Analysis of surfactant solutions give solubility of preservative in various concentrations of surfactant [Dt]. A plot is made of [Dt] versus [M], as shown in Fig. 2a. From the figure it follows that if there is no interaction between preservative and surfactant, there will be no change of preservative concentration at various concentrations of surfactant (i.e. [Dt]=[Df]). If a soluble complex is formed (Dt] will increase as [M] increases within a range of concentrations which is a characteristic of both preservative and surfactant. Increased quantities of preservative in presence of [M]

represent [Db] since [Df] is a constant under specified conditions and is in equilibrium with [Db] throughout the [M] range.

Some preservatives, e.g. chlorocresol, p-hydroxybenzoic acid, etc., give turbid solutions with nonionic surfactants when they are added in amounts higher than their saturation solubility. It is suggested that this is due to the formation of insoluble complexes between preservative and surfactant (Higuchi and Lach, 1954). Other investigators (Evans, 1964) believe, however, that turbidity results from depression of cloud point of these surfactants, rather than complex formation. In these systems it is generally not possible to separate excess of preservative from surfactant solutions. Turbidimetric titrations have been suggested for determination of solubility of these preservatives in solutions of nonionic surfactants (Higuchi and Lach, 1954; Kabadi and Hammarlund, 1966). These titrations generally involve the addition of an excess of known amount of preservative to a given volume of surfactant solution. The solution is shaken and titrated by adding surfactant solution of the same concentration in small increments. The point where turbidity disappears is taken as saturation concentration of preservative [Dt]. From the total amount of preservative added and total volume of surfactant used, [Dt] is calculated for a given concentration of surfactant [M]. The procedure is repeated for [Dt] at various concentration of surfactant [M]. A plot is made of [Dt] versus [M], as shown in Fig. 2b. Fig. 2b is similar to Fig. 2a except the [Df] in the former is less than the aqueous

solubility of preservative. The reason behind the suppression of aqueous solubility of these preservatives in presence of nonionic surfactants is still unknown.

To predict the concentration of free preservative from solubility data certain assumptions must be made. Firstly, the concentration of free preservative in a saturated macromolecule solution is assumed to be equivalent to the water solubility of the preservative at the same temperature (Fig. 2,a). This assumption may not always be valid since in some cases the solubility in the presence of macromolecule is less than the water solubility (Fig. 2,b) e.g. phenobarbital, resorcinol and catechol with polyethylene glycols (Higuchi and Lach, 1954), p-hydroxybenzoic acid with polyoxyethylene 8·5/octyl phenyl ether (Evans, 1964) and for chlorocresol with cetomacrogol (Kazmi and Mitchell, unpublished results). Furthermore if the results of studies of saturated solutions are applied to undersaturated systems, as is frequently the case, a second assumption is made, namely, that the ratio of bound to free preservative at a given macromolecule concentration is a constant for all preservative concentrations up to saturation. There is evidence to demonstrate that this assumption is not always correct (Breuninger and Goettsch, 1965; Mitchell and Brown, 1966; Anderson and Morgan, 1966).

The main weakness of the solubility method is that it is a "one point method". For all surfactant solutions, [Df] is constant, and therefore only one value of 'r' is obtained (see Eq. 24). This limits
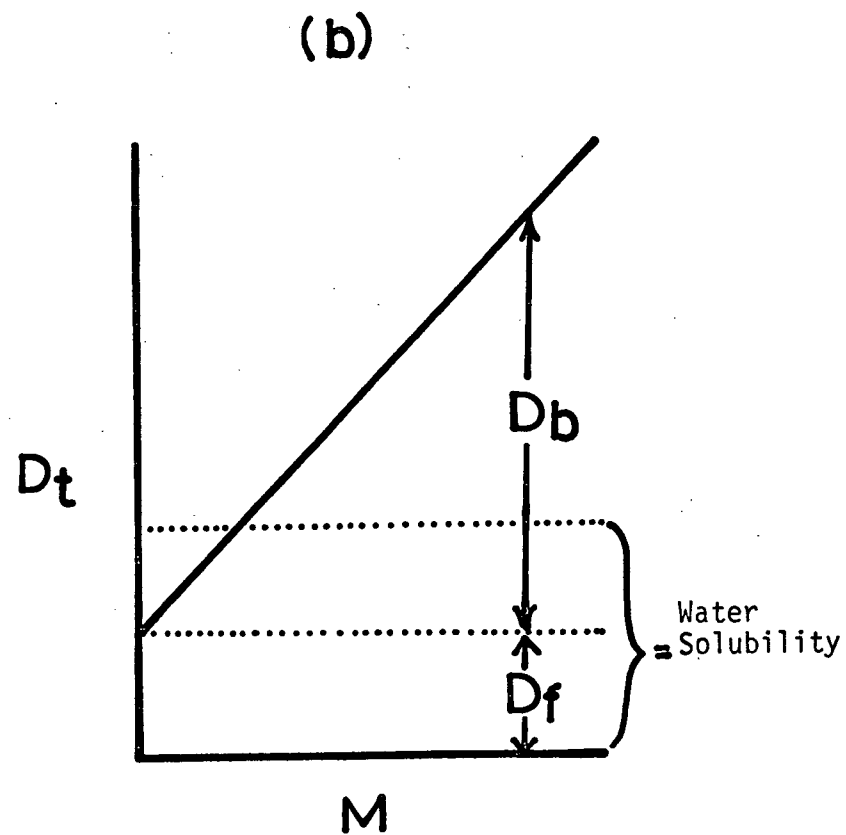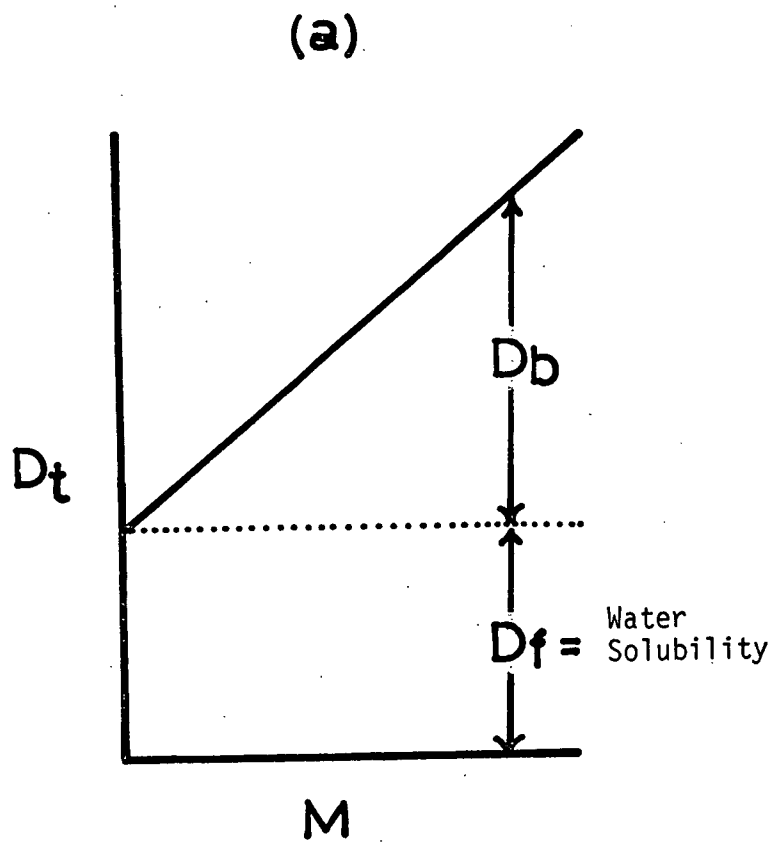
Fig. 2. Solubility of preservative as a function of surfactant concentration.

its application and the amount of information which can be obtained. Certain aspects of the binding process are conveniently studied by this technique e.g. the effects of various solvents, pH (Donbrow and Rhodes, 1964), ionic strength (Eide and Speiser, 1976a) and temperature (Patel and Foss, 1964; Humphreys and Rhodes, 1968) on the extent of binding. The value of 'r' is constant under controlled conditions and therefore a comparison of the 'r' value, under different conditions, gives information about the binding mechanism.

II  Dialysis:  Interactions between preservatives and surfactants may be studied quantitatively using the equilibrium dialysis technique. A container is divided into two compartments by a semipermeable membrane. A surfactant solution with preservative is placed in one compartment and aqueous solution with preservative is placed in the other compartment. The semipermeable membrane is permeable only to preservative molecules but not to surfactant monomer. At equilibrium, the total number of preservative molecules in the surfactant compartment will exceed than in the aqueous compartment. The difference between the concentrations in the two compartments is a measure of [Db]. Two possible sources of error, namely the Donnan effect and membrane binding of preservatives must be considered before applying this technique.

When a charged macromolecule [M], is retained in one of the two compartments, at equilibrium, the concentration of diffusible ions is no longer identical across the membrane. This phenomenon is described as

the Donnan equilibrium (Overbeek, 1956). In dilute solution of macromolecule, the Donnan effect can be neglected only if the concentration of the diffusible ions is reasonably high and the valency of the macromolecule is fairly low. In a solvent system of high ionic strength and pH at which the macromolecule has a small valency charge, the abnormal distribution of small molecules across the membrane due to Donnan equilibrium can be neglected.

The dialysis membrane may act as a binding site for the preservative molecule (Patel and Kostenbauder, 1958) and a correction must be made for this interaction. Corrections are generally made by using a control in which no macromolecule is present in the two chambered dialysis container. It is then possible to measure the 'loss' of small molecule from one compartment to the other across the semipermeable membrane. It has been observed that extent of membrane binding is proportional to the amount of preservative added to the system (Patel and Kostenbauder, 1958; Schoenwald and Belcastro, 1969; Patel and Nagabhushan, 1970).

The main advantage of this method is that an interaction can be studied through a range of free preservative concentration [Df] and in this way it is possible to cover a wide range of 'r' values (see Eq. 24). Thus more information about the interaction can be obtained than with the solubility method.

Techniques employed to carry out equilibrium dialysis have varied from dialysis bag placed in a bottle (Deluca and Kostenbauder, 1960) to

two chambered dialysis cells with a semipermeable membrane separating the two compartments (Patel and Foss, 1964; Eide and Speiser, 1967a). The main advantage of dialysis cells over the former technique is that a better control of membrane binding is attained because the surface area of the membrane remains more or less constant throughout the study.

Various types of dialysis membranes are described by Craig (1965). Kostenbauder, et. al. (1969) discussed the use of nylon membrane in dialysis studies. Nylon membrane react with phenolic compounds (Patel and Kostenbauder, 1958; Patel and Foss, 1964). Hence rubber membranes have been used in the study of the interaction of phenolic preservatives with surfactants (Patel and Foss, 1964; Mitchell and Brown, 1966; Patel, 1967). Several authors have used cellophane membranes in dialysis studies (Matsumoto, et. al., 1966; Patel, 1967). The main disadvantage of cellophane membranes is that they have been shown to be permeable to nonionic surfactants (Patel and Kostenbauder, 1958; Nishida, et. al., 1964). However there is still the question as to whether the surfactant dialyzed consists only of law molecular weight impurities, such as unreacted polyoxyethylene glycol (Matsumoto, et. al., 1966) or impurity plus surfactant. This aspect has been further investigated in the present work. However the main advantage of cellophane membrane is that it is relatively free of fixed charges which would be ion selective (Craig, 1965), and therefore has been used extensively in protein binding work.

(c) Distribution of preservatives in oil-water-surfactant systems.

Very few techniques are available to study quantitatively the distribution of preservatives between the different phases of emulsion systems. Most workers (Baker, 1959; Nowak, 1963; Olson, 1967; Sykes and Smart, 1968; Barnes and Denton, 1969) have employed microbiological techniques to assess the efficiency of preservatives in emulsified systems. However these studies were of a qualitative nature and no observation was obtained with regard to the amount of preservative distributed between the different phases of the emulsion. The concentration of preservative in various phases of an emulsion can be calculated using Eqs. 32 and 35 and previously determined values of $K_w^o$ and R. From these concentrations, the total concentration required in the emulsion to provide a minimum inhibitory concentration in the aqueous phase can be calculated. This theoretical approach to emulsion preservation has been evaluated by Bean, et. al. (1969) and Patel and Romanowski (1970) using microbiological techniques. This approach, however, has definite limitations that will be discussed later. Garrett (1966) suggested an ultracentrifuge technique for the separation of the various phases of an emulsion and subsequent analysis of each phase for preservative content. The main drawback associated with this technique is the destruction of emulsion structure which may disturb the equilibrium concentrations of preservative in the various emulsion phases. Patel and Romanowski (1970) used dialysis technique to determine the concentration of free preservative in the aqueous phase, [Df], of an emulsion. They utilized a two-chambered glass dialysis cell,

with emulsion in one compartment and broth in the other. The two compartments were separated by a semipermeable membrane, permeable only to preservative molecules and impermeable to oil and surfactant phases. At equilibrium the concentration of free preservative in the aqueous phase was assumed to be equal on both sides of the membrane. Analysis of the aqueous compartment gave the concentration of free preservative in the aqueous phase of the emulsion. Subtracting the amount of preservative in the aqueous phase from the total amount of preservative added to the dialysis cell gave the total concentration of preservative in the oil and surfactant phases ($[Do] + [Db]$). Thus with this technique it is not possible to separate the concentrations of preservative in oil $[Do]$ and surfactant $[Db]$ phases. In the present investigation a three-chambered dialysis technique has been developed. With this technique it is possible to measure the amount of preservative in each phase of the emulsion and hence total concentration required to provide desired concentration in the aqueous phase. The technique also differentiates between free surfactant and surfactant adsorbed at oil-water interface or partitioned into the oil phase.

III   EXPERIMENTAL

A.   Apparatus

a.   Hitachi Coleman 124, Spectrophotometer.

b.   Fisher Accumet 310, pH Meter.

c.   International Equipment Company HN Centrifuge

d.   Haake Thermoregulator (type FE)

e.   Polarograph with Drop Life Timer and Fisher Calomel Electrode,

No. 13 - 639 - 51 (Polariter, Radiometer PO4, Copenhagen;

Drop Life Timer, type DLT₁, Radiometer, Copenhagen).

f.   Diaflo Ultrafiltration Apparatus.

g.   Hand Powered Homogenizers (Central Scientific Co.).

h.   Water-Bath (50L, ordinary fish tank).

i.   Dialysis - Cells

I.   Two-Chambered Plexiglass Dialysis Cells, as described by

Patel and Foss (1964).

II.   Three-Chambered Plexiglass Dialysis Cells:   two-chambered

dialysis cells, as described above, enlarged by the addi-

tion of an extra spacer in the centre.

j.   Membranes.

I.   Nylon Membrane (0.0005" thick; Capran 77;   Allied Chemi-

cal Corporation, Morristown, New Jersey).

II.   Fisher Cellophane Membrane ($1\frac{47"}{64}$ flat width;   Dialyzer

Tubing; Fisher Scientific Co.).

III. Visking Cellophane Membrane (Union Carbide Ltd.).

IV. Millipore VS Membrane (0.025$\mu$ ± 0,003$\mu$, pore size; Millipore Ltd., 55 Montpellier Blvd., Montreal 379, Canada.).

B. Materials

a. Benzoic acid. Analar grade.

b. p-hydroxybenzoic acid, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, chloroxylenol, as described by Mitchell (1964), Mitchell and Brown (1966) and Brown (1968).

c. Cetomacrogol, B.P.C. (Texofor AIP, Glovers Chemicals Ltd., Leeds, England). Cetomacrogol has the general formula, Me $[CH_2]_m$ $[O \cdot CH_2 \cdot CH_2]_n \cdot OH$. Where m may be 15 or 17 and n may be 19 to 23. The molecular weight was taken as 1300.

d. Polyoxyethylene sorbitan mono-oleate (Tween 80, Atlas Chemical Industries).

e. Sodium lauryl sulfate. U.S.P.

f. Cetylpyridinium chloride. B.P.

g. Peanut oil, commercial grade (Planters Peanut Oil, Standard Brands, Canada).

h. Liquid Paraffin (Primol 355, Imperial Oil Ltd., Canada).

i. Citrate - Phosphate - Buffer

1. Buffer components (citric acid, dibasic sodium phosphate

and sodium chloride).  Reagent grade.

2.   Citrate-phosphate buffer of pH 3.0 was prepared accord-

ing to Cruickshank's buffer tables (Cruickshank, 1965)

and an ionic strength of 0.2 was adjusted by adding

appropriate amount of sodium chloride.  Actual pH value

was determined by using a pH meter.  Unless otherwise

stated the aforementioned buffer was used in all the

studies and for making dilutions in the spectrophoto-

metric analysis of benzoic acid.

C.   Temperature

Unless otherwise stated a temperature of $30^o$ was used in all the

studies.

D.   Analysis of Benzoic Acid in Aqueous and Cetomacrogol Solutions.

Aliquots of solutions were appropriately diluted with buffer and

benzoic acid concentrations were determined spectrophotometrically at

273 m$\mu$.

E.   Analysis of Surface-active Agents.

(a)  Polarographic analysis:  This method is based on the damping of the

polarographic maxima by surface-active agents (Vavruch, 1950; Jehring,

1966).  Potassium chloride solution ($N/500$) gives a very pronounced oxy-

gen maximum which is readily suppressed by surface-active agents.  Thus

by comparing the height, h, of oxygen maximum of potassium chloride solution ($N/500$) in the presence and absence of surface active agents (Fig. 3) it is possible to determine the amount of surface active agent in a given solution.

Hundred mls. each of $N/50$ KCl solution and 0·5% surfactant solution was prepared in glass distilled water. One ml. of 0·5% surfactant solution was transferred to 100 ml. volumetric flask and made up to volume with glassdistilled water, so that the final concentration of surfactant was 0·005%. Five ml. each of $N/50$ KCl solution was pipetted to eleven 50 ml. volumetric flasks and to the first ten volumetric flasks 1-10 mls. of 0·005% surfactant was added respectively. Finally all the volumetric flasks were made up to volume with distilled water. Thus the final concentration of surfactant in first ten volumetric flasks ranged from 1-10 mg.$1^{-1}$ respectively and the final normality of KCl in all the flasks was $N/500$. All the eleven solutions were subjected to polarographic analysis and the height of the oxygen maximum was measured for each solution. The heights of oxygen maxima of $N/500$ KCl solutions containing various concentrations of surfactant (1-10 mg.$1^{-1}$) were subtracted from the height of oxygen maximum of $N/500$ KCl solution respectively. The percentage suppression of oxygen maximum was calculated for each solution.

(b) Method of Crabb and Persinger (1964): The principle behind this method is based on the formation of a blue complex due to an interaction between cobalt thiocyanate and the ethylene-oxide units of polyoxyethy-
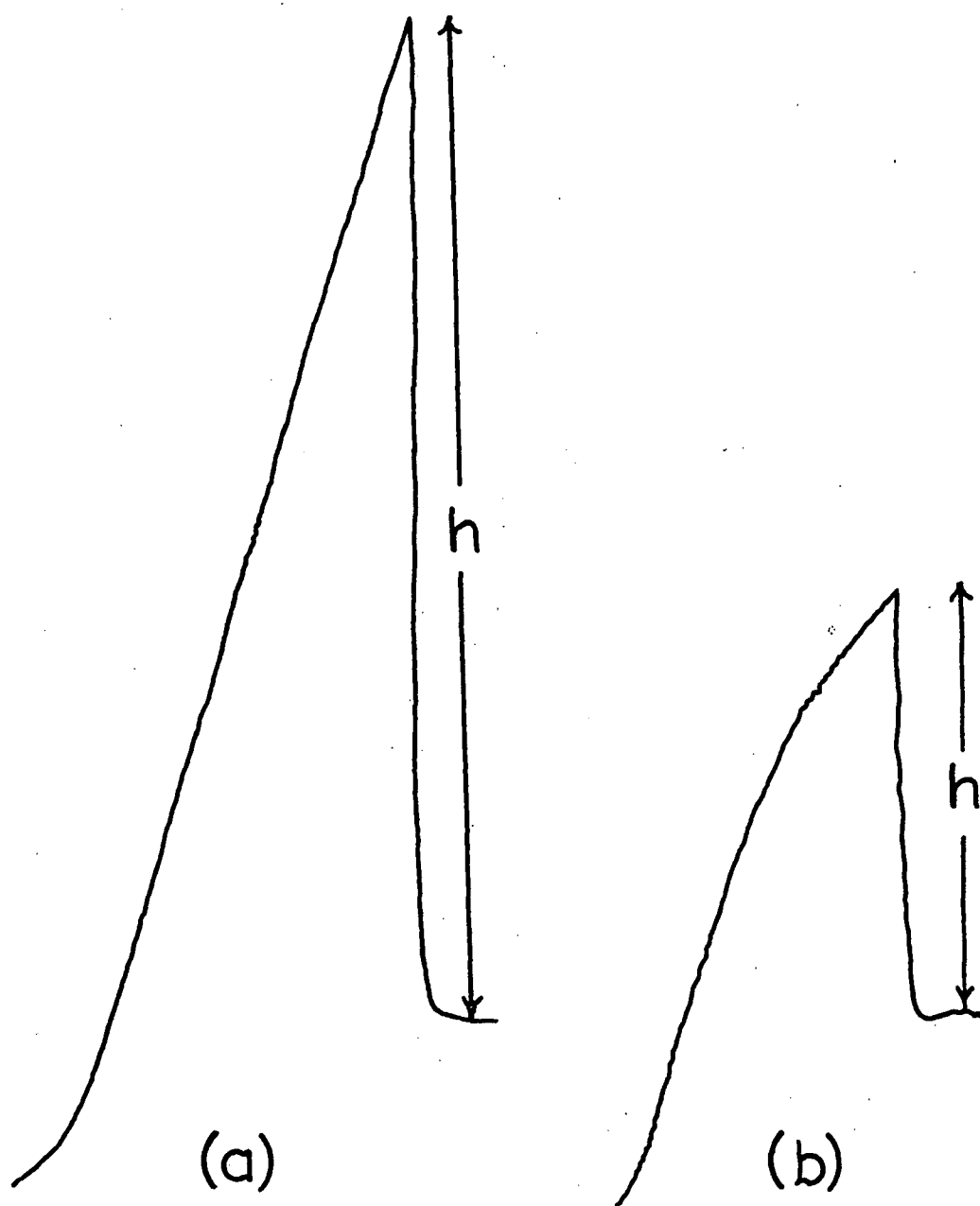
Fig. 3.    Polarographic current voltage curves.

     (a) oxygen maximum of potassium chloride solution
        (N/500).
     (b) suppression of oxygen maximum by surface-
        active agent.   h, height of oxygen maximum.

lene nonionic surfactants. Measuring the absorbance of the blue complex at 620 m$\mu$ at various surfactant concentrations, a calibration curve is made by plotting absorbance versus concentration of surfactant. In the present work this method has been used for the analysis of cetomacrogol.

Various volumes (5, 10, 15, 20, 25 mls.) of cetomacrogol solution were pipetted into 50 ml. volumetric flasks respectively. To each volumetric flask 5 ml. of cobalt-thiocyanate solution (Co (NH$_3$)$_2$, 7·51 g; NH4 SCN, 50·15 g, made to 250 ml with distilled water) was added. All the volumetric flasks were made up to volume with distilled water. A blank was prepared by diluting 5 ml of cobalt-thiocyanate solution to 50 ml with distilled water. The flasks were shaken and the absorbence measured at 620 m$\mu$ . Like other colorimetric methods, the main weakness of this method is fading of the blue colour with time. It was found that the colour faded rapidly in the first fifteen minutes and then became very slow. Therefore the absorbence of the blue complex was always recorded after fifteen minutes.

F.   Permeability of Cellophane Membranes to Cetomacrogol.

The permeability of Fisher cellophane membrane and Visking cellophane membrane to cetomacrogol was studied using the equilibrium dialysis, dynamic dialysis and ultrafiltration techniques.

(a) Preparation of cellophane membranes: The dialyzer tubing was soaked in distilled water, cut flat and then washed with several changes

of distilled water.

(b) Permeability of cellophane membranes to cetomacrogol - equilibrium dialysis: Cellophane membranes were placed between the compartments of the two-chambered dialysis cell. Twenty ml. of cetomacrogol solution was pipetted into one compartment of the cell and 20 ml of distilled water was pipetted into the other compartment. The cells were tumbled in water bath and at 12 hour intervals, equal volumes of solutions were pipetted from both sides of the cell and the cetomacrogol content determined by polarographic analysis.

(c) Permeability of Fisher cellophane membrane to cetomacrogol - dynamic dialysis under sink conditions: A diagram of the apparatus used in this study is shown in Fig. 4. Twenty-five ml. of 10% cetomacrogol was transferred to the cellophane bag and 200 ml. of distilled water was added to the jacketted beaker. The solution in the jacketted beaker was stirred with a magnetic stirrer, while the cetomacrogol solution in the cellophane bag was stirred with a glass stirrer. One hundred ml. of solution was pipetted out from the jacketted beaker at 12 hour intervals and analyzed for cetomacrogol by polarographic technique. The volume of solution in the jacketted beaker was immediately made up to 200 ml. with fresh water, so as to maintain sink condition.

(d) Permeability of Visking cellophane membrane to cetomacrogol - ultrafiltration technique: A Diaflo ultrafiltration cell was used for

Motor

Holes for Introduction
and Removal of Solution

Rubber Stopper

To a Constant-Temperature
Water Bath

BHC-Macromolecule
Stock Solution

Cellophane Bag

Water

Magnetic Stirring Device
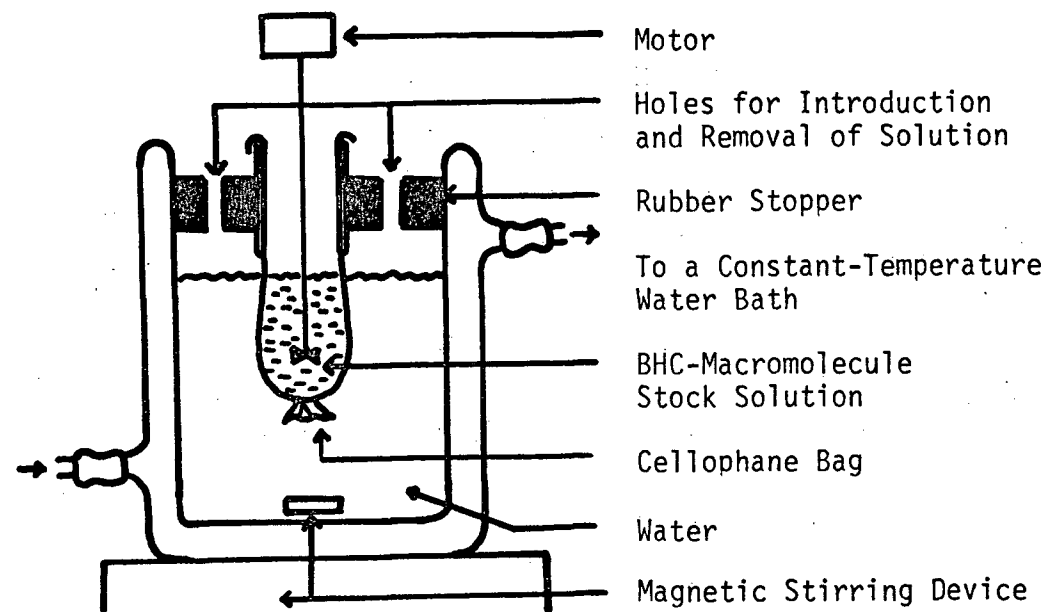
Fig. 4. Dynamic Dialysis Method

this study. The cellophane membrane was cut flat and attached to the filtration chamber. Thirty-five ml. of 1·8% cetomacrogol solution was placed in the filtration chamber and a pressure of 40 lbs./sq. inch was applied till complete filtration of the liquid was attained (about 18 hours). The filtrate was analyzed for cetomacrogol by polarography.

G.    Membrane Binding

(a) Binding of benzoic acid with nylon membrane:  The nylon membrane was washed with several changes of distilled water and placed between the compartments of the two-chambered dialysis cell. Benzoic acid solutions of varying concentrations were made in buffer. Twenty ml. of benzoic acid solution was pipetted into one compartment and 20 ml. of buffer was pipetted into the other compartment. A few glass beads were added to each compartment to ensure continuous stirring during dialysis. The cells were tumbled in the water bath until concentrations of benzoic acid in both compartments were same (about 4 days). Aliquots were taken from each compartment and, after proper dilution with buffer, the benzoic acid concentrations were determined. The percentage recovery was calculated in each case to estimate membrane binding.

(b) Binding of benzoic acid with Millipore VS membrane:  Millipore VS membranes were washed with several changes of distilled water and finally soaked in buffer, pressed between filter paper to remove excess buffer and then placed between the chambers of the two-chambered dialy-

sis cell. The remaining procedure was the same as described under "The binding of benzoic acid with nylon membrane".

(c) <u>Binding of cetomacrogol with Millipore VS membrane</u>: Cetomacrogol solutions of various concentration were made in the buffer. Twenty ml. of the solution was placed in one compartment of a two-chambered dialysis cell and 20 ml. of buffer was placed in the other. The cells were tumbled in the water-bath until the concentration of cetomacrogol was the same in both compartments (about 4 days). Aliquots were withdrawn from each compartment and after proper dilution with distilled water, cetomacrogol concentrations were determined by the method of Crabb and Persinger (1964). The extent of membrane binding was calculated from the per cent recovery.

H.   Distribution of Benzoic Acid in Oil-Water Systems

Equal volumes of benzoic acid solutions in peanut oil or liquid paraffin and buffer were pipetted into glass-stoppered cylinders and agitated using a wrist-action shaker for about one hour at room temperature. The cylinders were then tumbled in a water-bath until equilibrium was reached (about 7 days). The aqueous phase was separated by centrifugation of the oil-water mixture and analyzed for benzoic acid concentration. The concentration of benzoic acid in the oil phase was calculated by substracting the amount of benzoic acid in the aqueous phase from the total amount of benzoic acid added.

I.   Interaction of Preservatives with Cetomacrogol

(a)  Interaction of benzoic acid with cetomacrogol

I.  Solubility of benzoic acid in buffer:  Excess of benzoic acid
was equilibrated with buffer by rotation in a sealed cylinder in a water
bath.  At 2 and 4 days aliquots were withdrawn and their pH measured with
the help of pH meter.  The aliquots were filtered through fine porosity
sintered glass filter stick, diluted appropriately with buffer and anal-
yzed for benzoic acid concentration.

II.  Solubility of benzoic acid in various concentrations of ceto-
macrogol:  Cetomacrogol solutions of varying concentrations were made in
buffer.  The solubility of benzoic acid in varying concentrations of ceto-
macrogol solutions was found using the method given for buffer solubility.

III.  Equilibrium dialysis studies:  Nylon membranes were washed with
several changes of distilled water and placed between the compartments of
the two-chambered dialysis cells.  Twenty ml. of benzoic acid solution in
cetomacrogol was pipetted into one compartment of the dialysis cell and
20 ml. of buffer, or buffer plus benzoic acid was pipetted into the other
compartment of the cell.  A few glass beads were added to each compartment
to ensure continuous mixing.  The cells were tumbled in the water-bath un-
til equilibrium was reached (about 4 days).  Aliquots were removed from
both compartments and after proper dilution with buffer analyzed for ben-
zoic acid concentration.

(b)  Solubility and equilibrium dialysis data for the interaction of p-hydroxybenzoic acid, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate and chloroxylenol with cetomacrogol in unbuffered aqueous solution were derived from the literature (Mitchell, 1964; Mitchell and Brown, 1966; Brown, 1968).

J.    Distribution of Benzoic Acid in Oil-Water-Surfactant Systems

(a)  Preparation of emulsions:  Peanut oil or liquid paraffin and various concentrations of cetomacrogol were mixed in various ratios and passed through hand powered homogenizers, at least five times, to ensure the formation of stable emulsions.

(b)  Two-chambered dialysis technique:  A nylon membrane was placed between the compartments of a two-chambered dialysis cell (Fig. 5).  The nylon membrane was permeable to benzoic acid, but not to oil and cetomacrogol.  Twenty ml. of emulsion plus benzoic acid was placed in one compartment (E) and 20 ml. of buffer or buffer plus benzoic acid was placed in the other compartment (W).  A few glass beads were added to each compartment to ensure continuous mixing.  The cells were tumbled in water bath, until equilibrium was reached (about 7 days).  Aliquots were removed from the buffer compartment and analyzed for benzoic acid.

(c)  Three-chambered dialysis technique:  A Millipore VS membrane was placed between compartments E and S and a nylon membrane between

compartments S and W of a three-chambered dialysis cell (Fig. 6). The Millipore VS membrane was permeable to surfactant and benzoic acid but not to oil, while nylon membrane was permeable to benzoic acid only. Twenty ml. of peanut oil or mineral oil emulsion was pipetted into compartment E, 20 ml. of cetomacrogol solution was pipetted into compartment S and 20 ml. of aqueous buffer solution was pipetted into compartment W. Known amounts of benzoic acid were 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 cells were tumbled in a water-bath 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.
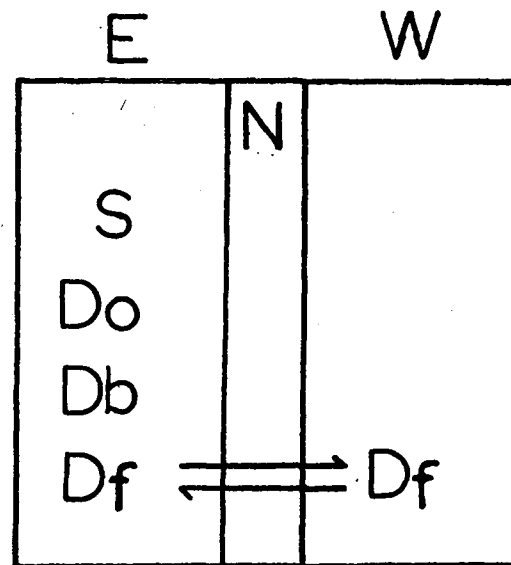
Fig. 5.　Two-chambered dialysis cell:　E, emulsion;　W, water;
S, surfactant;　N, nylon membrane;　Do, preservative
in oil phase;　Db, preservative bound to surfactant;
Df, free preservative in aqueous phase.

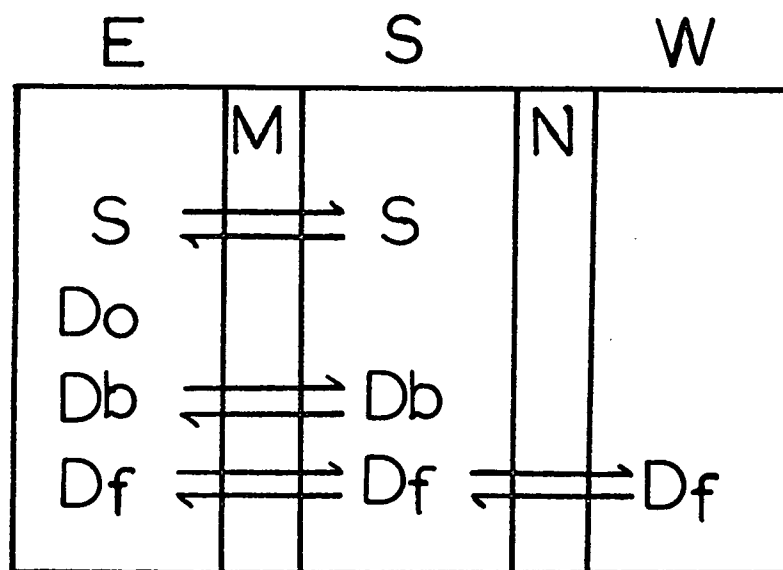Fig. 6.   Three-chambered dialysis cell:  E, emulsion;
          S, surfactant;  W, water;  M, millipore VS membrane;
          N, nylon membrane;  Do, preservative in oil phase;
          Db, preservative bound to surfactant;  Df, free pre-
          servative in aqueous phase.

## IV   RESULTS AND DISCUSSION

### A.   Analysis of Surface-Active Agents

(a)   <u>Polarographic analysis</u>:   The per cent suppression of the oxygen maximum versus concentration of cetomacrogol is shown in Fig. 7.   From a calibration curve such as this, the concentration of cetomacrogol in a given unknown solution can be calculated provided the suppression of the oxygen maximum is determined under the same experimental conditions.   The method was found to be applicable not only to the analysis of nonionic surfactants, but also to anionic and cationic surfactants.   Fig. 8 shows the calibration curves for sodium lauryl sulfate, cetylpyridinium chlorid and Tween 80.

The height of polarographic maximum, h, (Fig. 3) depends very much on the conditions of analysis, such as atmospheric pressure, temperature, electrolyte concentration, diameter of capillary, drop time etc.   Unfortunately it proved to be very difficult to maintain the same experimental conditions.   Since it is impossible to control atmospheric pressure, it was necessary to plot a new calibration curve each time.   Hence in spite of the high sensitivity (0·01 - 1 mg l$^{-1}$), this method was not used further.

(b)   <u>Method of Crabb and Persinger (1964)</u>:   A plot of absorbence versus concentration of cetomacrogol is shown in Fig. 9.   From a calibration curve such as this, the concentration of cetomacrogol in a given unknown solution can be calculated provided the absorbance of blue complex
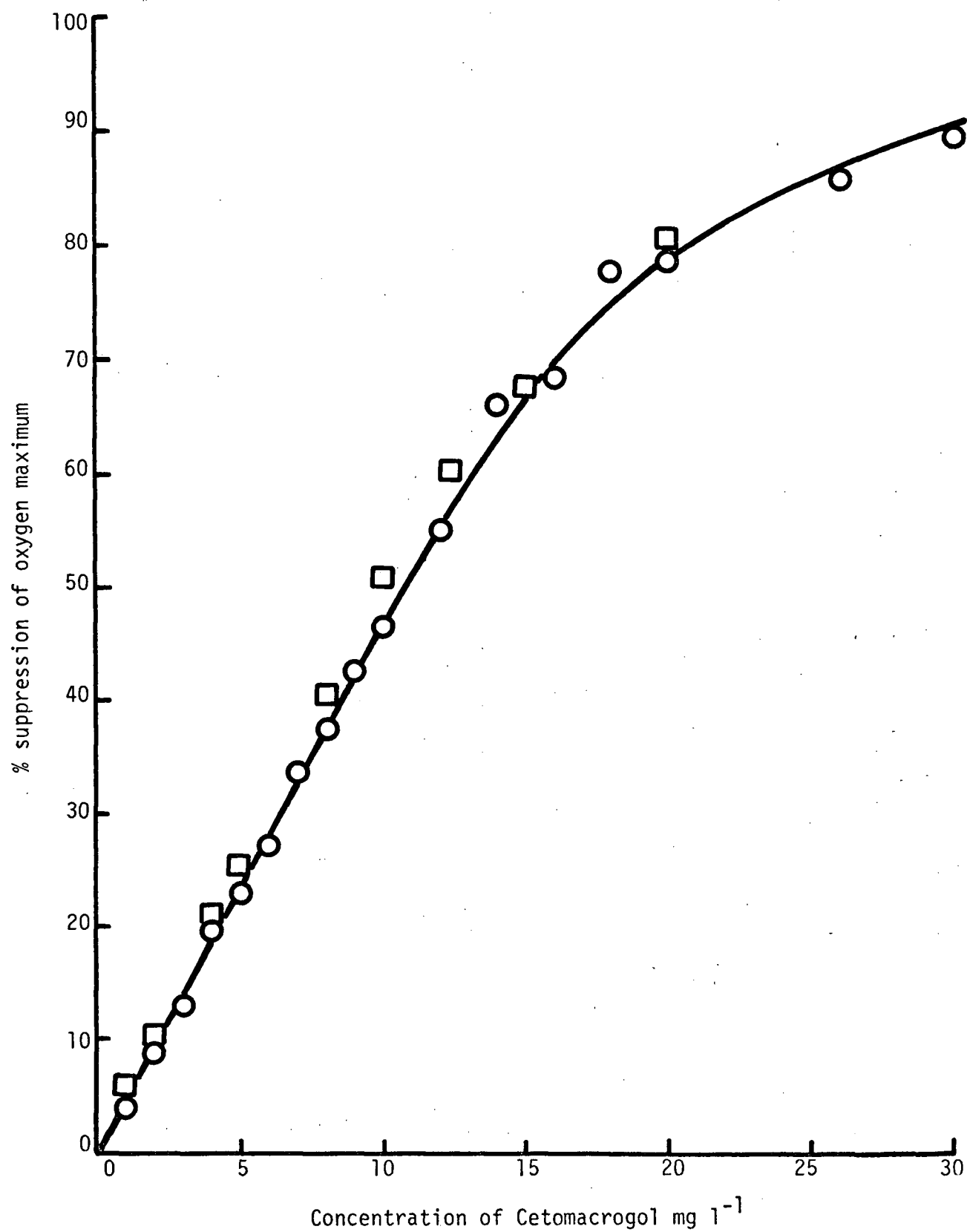
Fig. 7.   Polarographic determination of cetomacrogol:

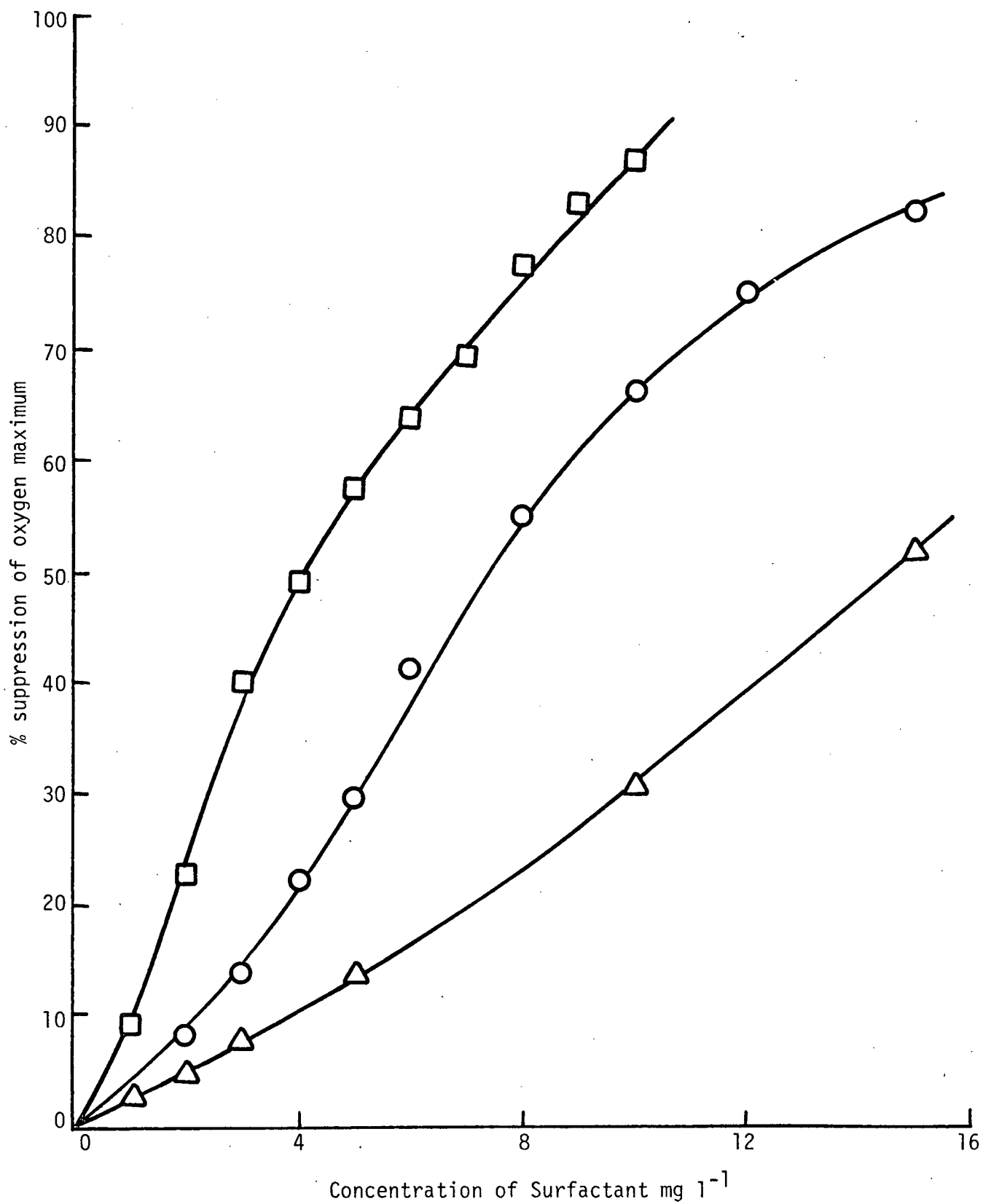O, and ☐, represent separate experiments.

Fig. 8.    Polarographic determination of surface-active agents:

□, cetyl pyridinium chloride;  ○, sodium lauryl sulfate;
△, tween 80.
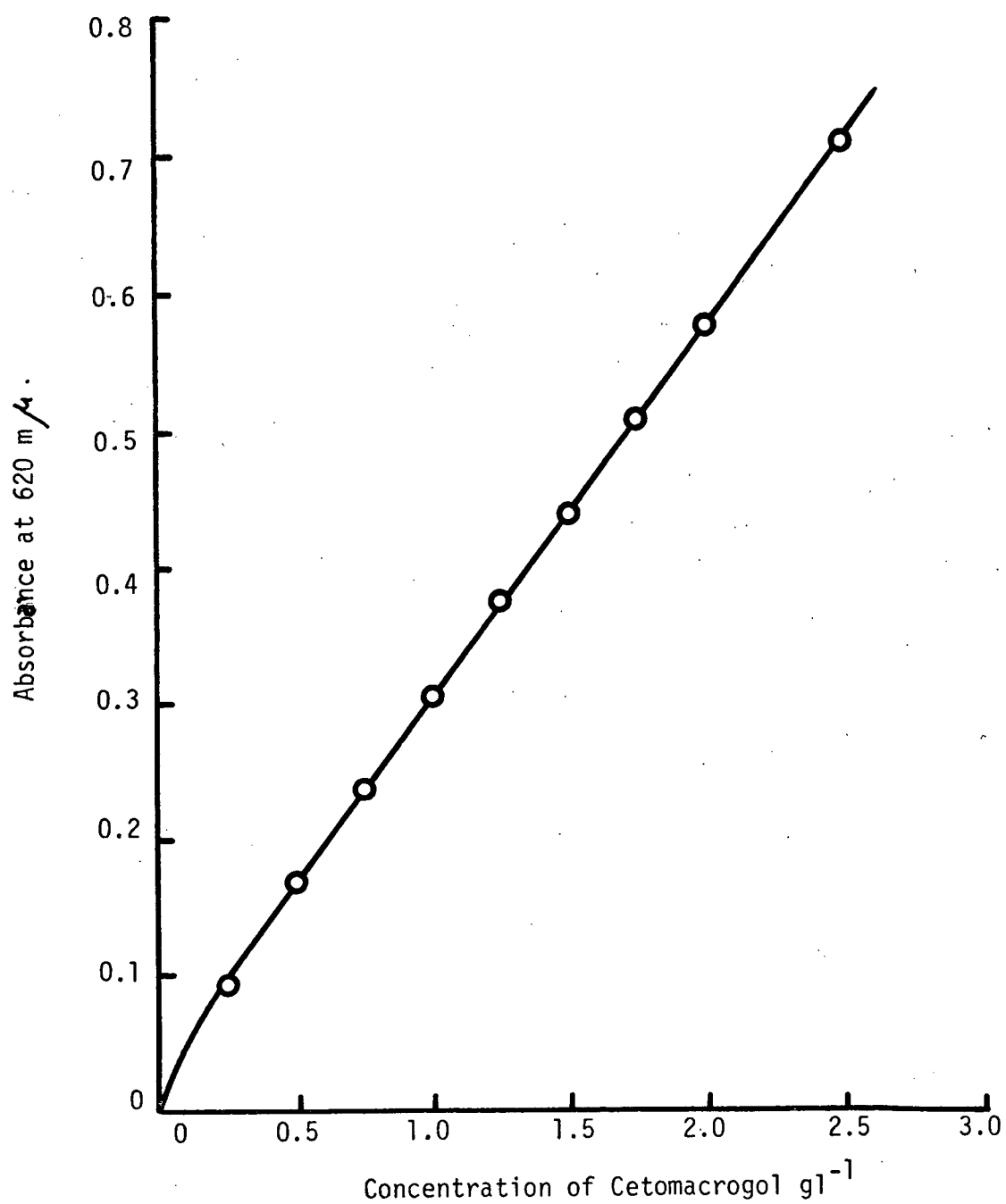
Fig. 9.   Colorimetric determination of cetomacrogol.

is measured under the same experimental conditions.

B.    Permeability of Cellophane Membranes to the Nonionic Surfactant
      Cetomacrogol

Fig. 10 shows per cent cetomacrogol dialyzed through Fisher cello-
phane membrane as a function of time in dynamic dialysis under sink con-
ditions.  The permeability of the membrane to cetomacrogol is rapid in
the beginning but slows down after 320 hours.  It is possible that lower
molecular weight fractions of cetomacrogol dialyze rapidly, while the
high molecular weight fractions dialyze more slowly.  Similar studies
by Matsumoto, et. al. (1966) showed that the permeation of Tween 80 and
Nikkol BL - 25 (Brij 35 type) through Visking cellophane membrane was
initially rapid but reached a plateau after 25 hours.  The authors con-
cluded that the membrane was readily permeable to impurities, especially
low molecular weight polyethylene glycols, but not to the nonionic sur-
factant molecules themselves.  However from Fig. 10 it is apparent that
even after 320 hours the permeation of cetomacrogol through Fisher cello-
phane membrane never reached a plateau i.e. the membrane is permeable
both to lower molecular weight fractions of cetomacrogol and to high
molecular weight fractions assuming that these exist.  Fig. 11 shows the
permeability of Fisher and Visking cellophane membranes to cetomacrogol
in equilibrium dialysis.  These studies show that both membranes are per-
meable to cetomacrogol.  The Visking membrane is slightly more permeable

Fig. 10. Permeability of Fisher cellophane membrane to cetomacrogol in dynamic dialysis under sink conditions. Initial cetomacrogol concentration, 10%.
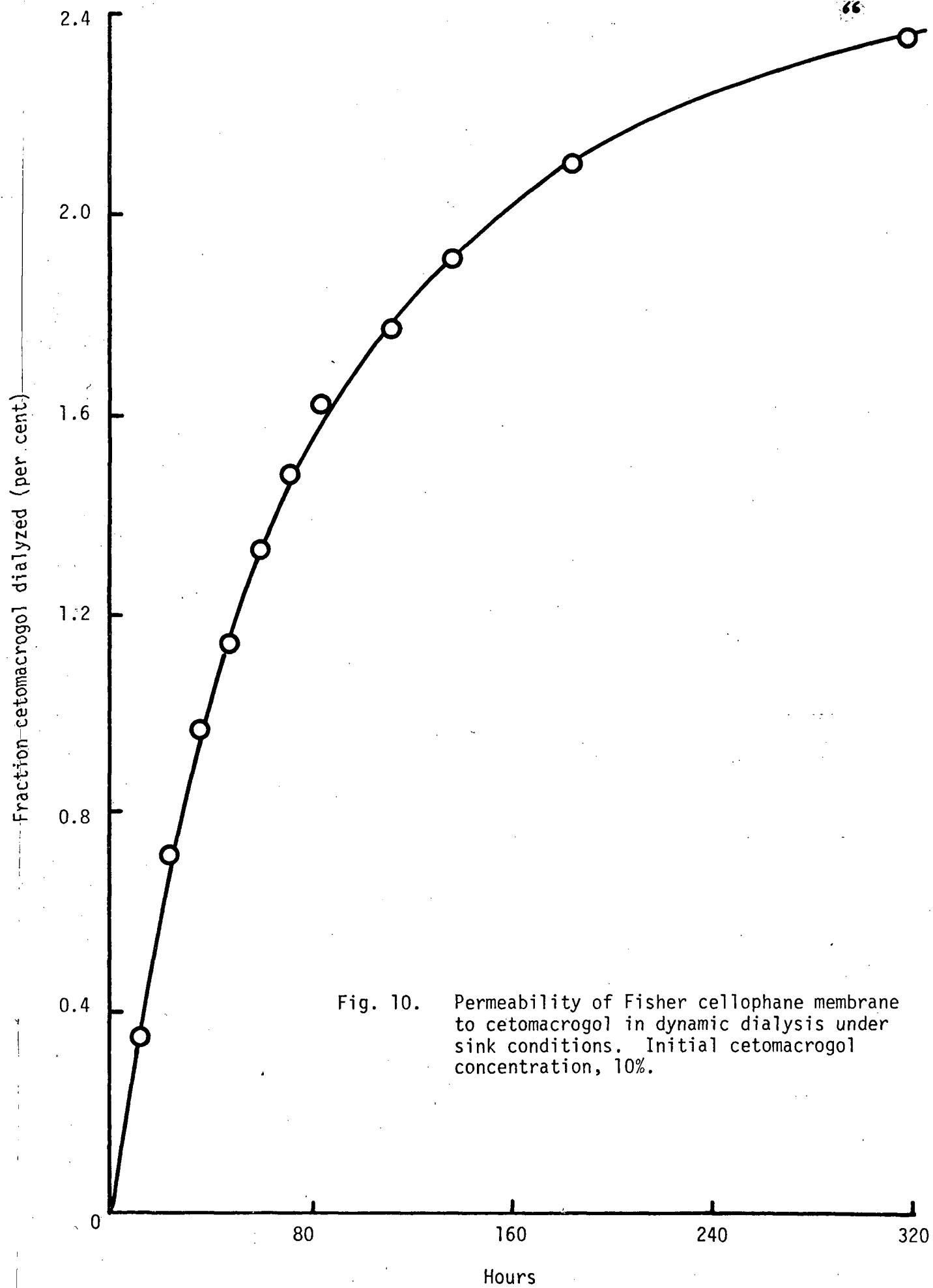
Fig. 11.    Permeability of cellophane membranes to cetomacrogol
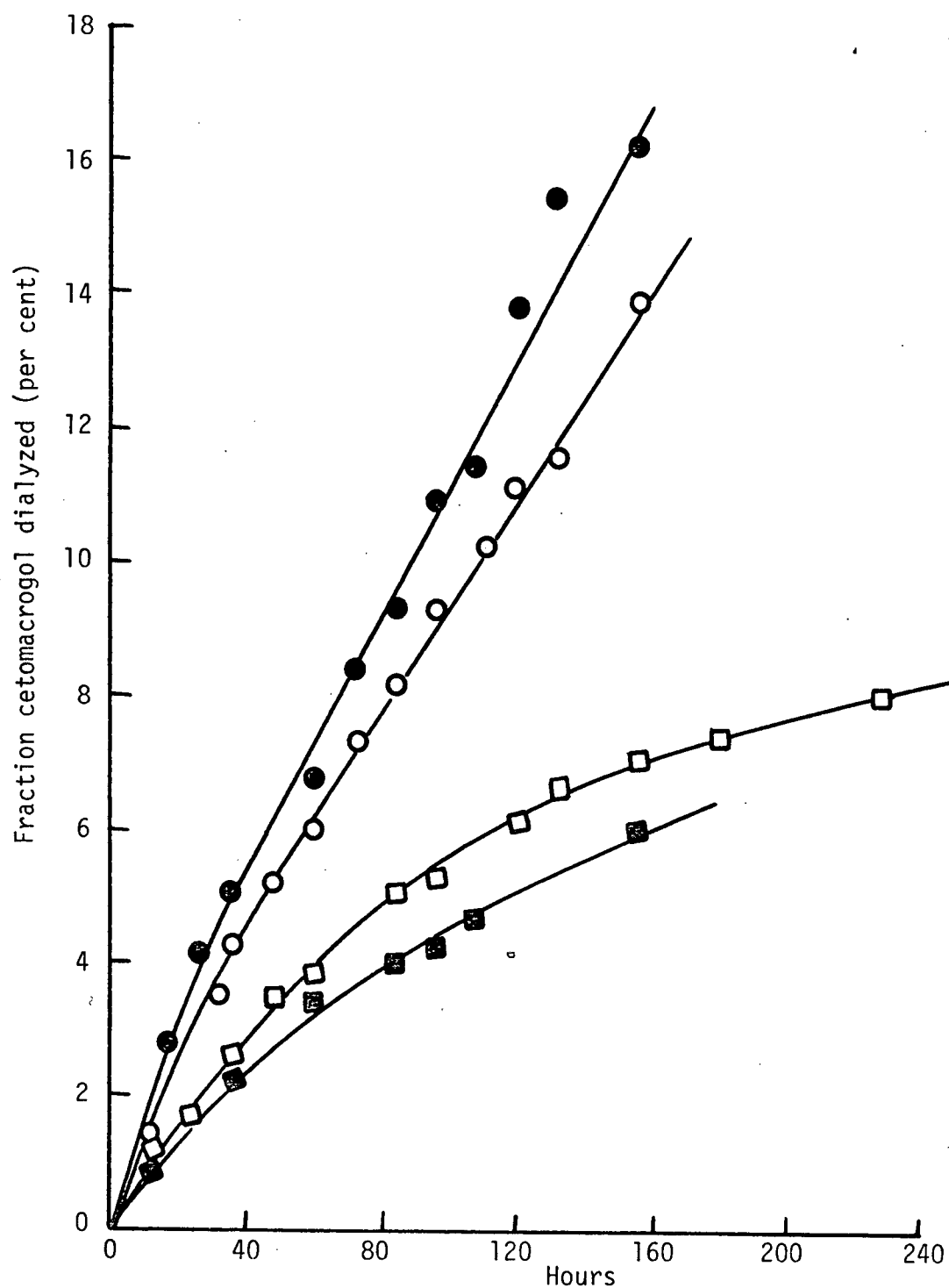            in equilibrium dialysis.  Initial cetomacrogol con-
            centrations:  □, 5%;   O, 10%.  Closed and open sym-
            bols represent Visking and Fisher membranes respec-
            tively.

to cetomacrogol than the Fisher membrane at high cetomacrogol concentrations, while the reverse is true at lower concentrations of cetomacrogol. Ultrafiltration of cetomacrogol solution (1·8%) through Visking cellophane also showed that an appreciable amount (2·7% of total amount) of cetomacrogol passed through the membrane in 18 hours. It is apparent that cellophane membranes are permeable to nonionic surfactants, such as cetomacrogol and therefore are not suitable as a semipermeable membranes in dialysis studies involving the interaction of preservatives and drugs with nonionic surfactants.

C.    Membrane Binding

The binding effect of solute with dialysis membranes is one of the main sources of error in dialysis studies. It was, therefore, necessary to correct for this interaction in all binding studies. However, it was found that benzoic acid did not bind with the nylon membrane. Corrections for the binding of benzoic acid and cetomacrogol with Millipore VS membrane were made using Figs. 12 and 13 respectively.

D.    Distribution of Benzoic Acid in Oil-Water Systems

Fig. 10 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 (1930) the equation of the line

Fig. 12.    Binding of benzoic acid with
Millipore VS membrane.
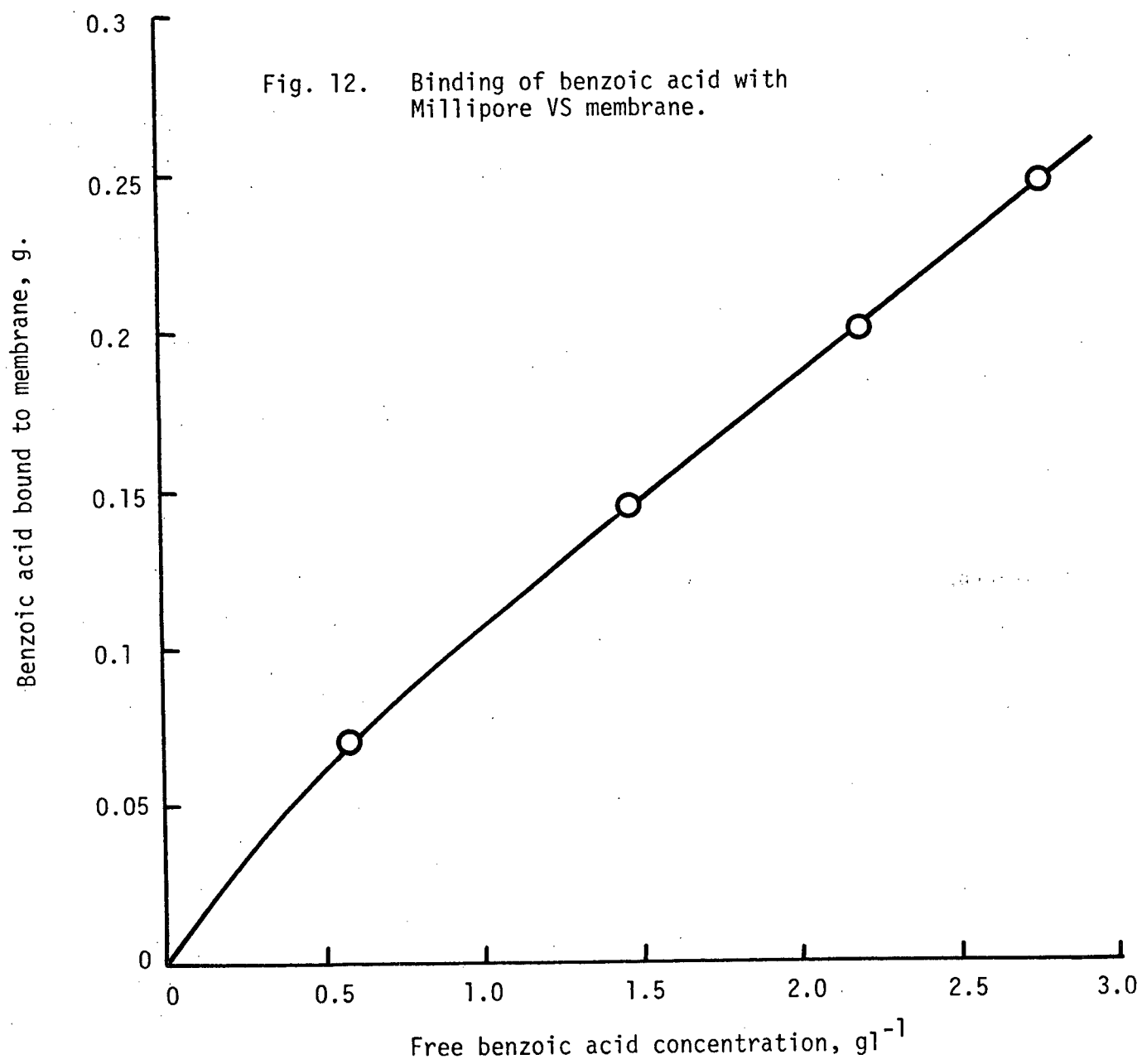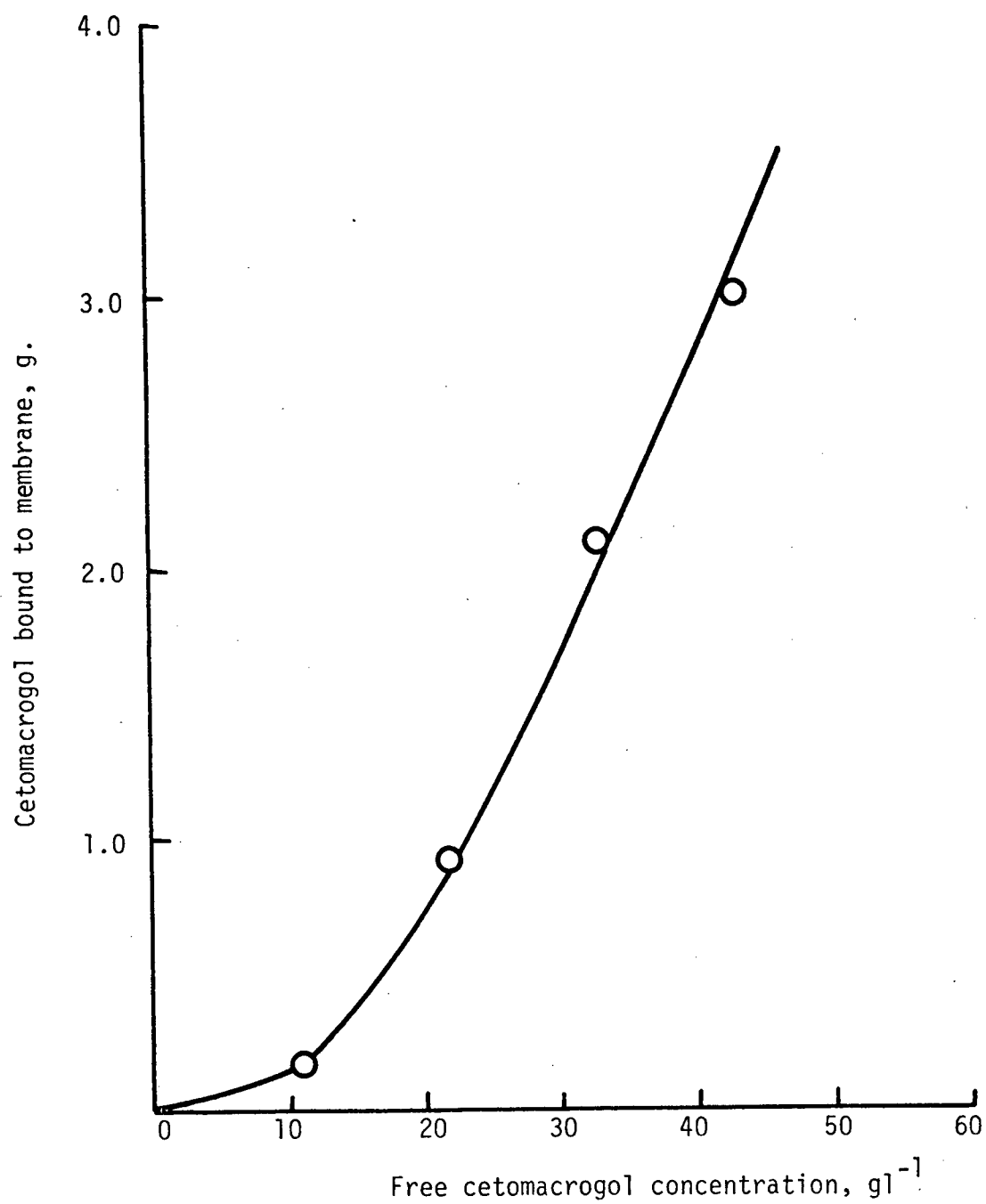
Fig. 13.   Binding of cetomacrogol with Millipore VS membrane.

is:

$$K_W^{'O} = K_{dw}^O + m\,k'(K_{dw}^O)^m\,[D_f']^{m-1} \qquad \text{(Eq. 17)}$$

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. 14, indicates dimerization of benzoic acid, where m = 2. The curve for peanut oil indicates m merization in the oil phase. Whereas Anderson and Cho (1967) showed $K_W^{'O}$ to be a constant independent of $[D_f']$. However they determined $K_W^{'O}$ values over a very narrow range of benzoic acid concentration ($1 \cdot 22 - 2 \cdot 44$ g $1^{-1}$), as compared with the range used in this study ($1 \cdot 89 - 30 \cdot 2$ g $1^{-1}$). The intercept on $K_W^{'O}$ axis at $[D_f'] = o$, gives $K_{dw}^O$ values of $5 \cdot 84$ and $0 \cdot 23$ for peanut oil and mineral oil respectively. For substitution in Eq. 30, the pH dependent observed partition coefficient, $K_W^O$, Fig. 15, is more convenient than $K_W^{'O}$ or $K_{dw}^O$.

E.   Interaction of Benzoic Acid with Cetomacrogol

(a) Solubility:  Analysis of benzoic acid in saturated buffer solution and saturated buffered cetomacrogol solutions gives [Df] and ([Df] + [Db]) respectively and hence [Db].

(b) Equilibrium dialysis studies:  Analysis of benzoic acid in aqueous and cetomacrogol compartments, at equilibrium, gives [Df] and ([Df] + [Db]) respectively and hence [Db]. Preliminary membrane binding studies revealed that benzoic acid did not bind with the nylon membrane. Moreover correction for membrane binding is not necessary in this case because both compartments were analyzed for benzoic acid. Nylon membranes were also

Fig. 14.  pH-independent partition coefficient of benzoic acid versus the aqueous concentration of unionized benzoic acid: ◯, peanut oil; ☐, mineral oil.

Fig. 15. pH dependent partition coefficient of benzoic acid versus the concentration of benzoic acid in aqueous phase: ◯, peanut oil; ▢, mineral oil.
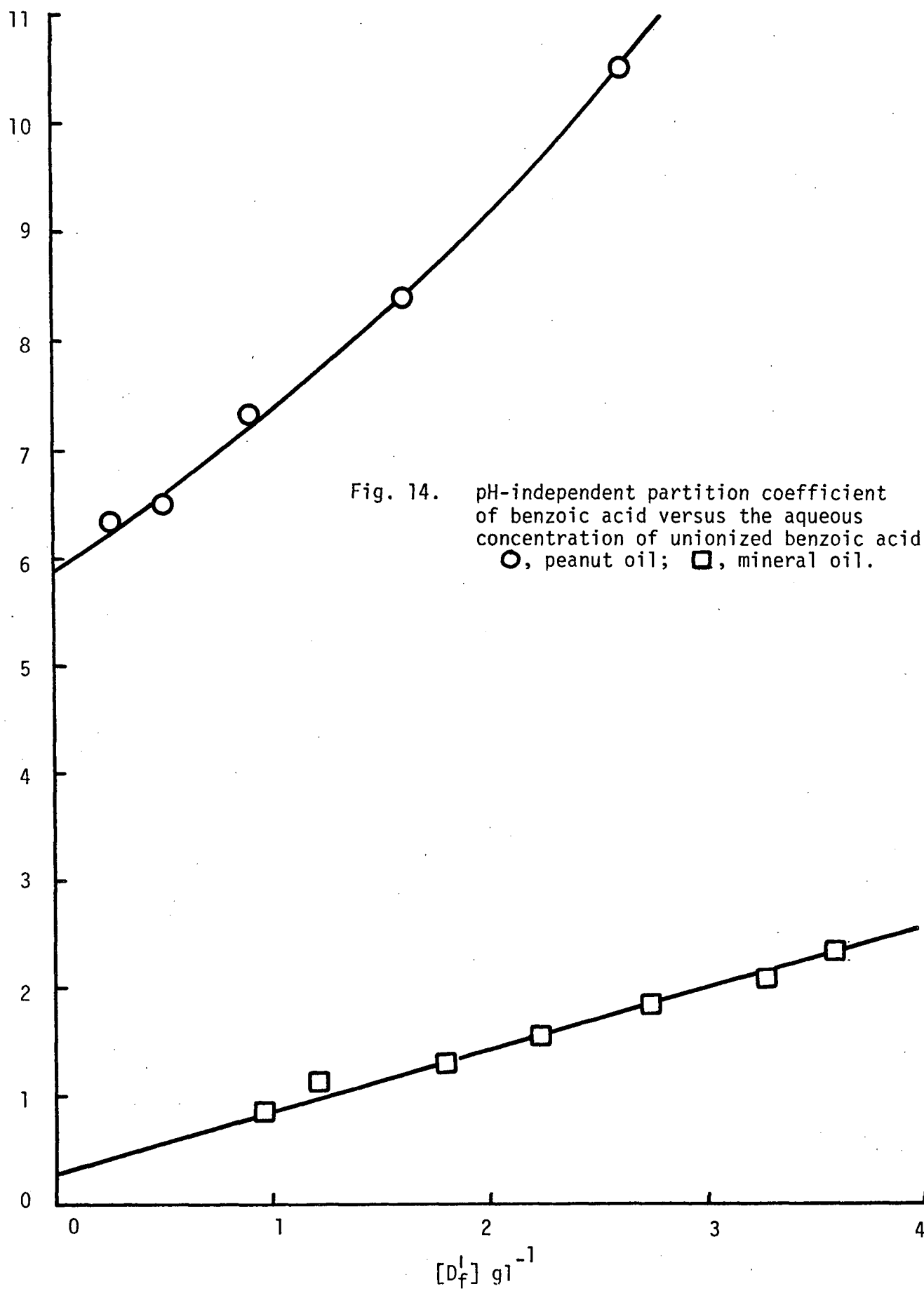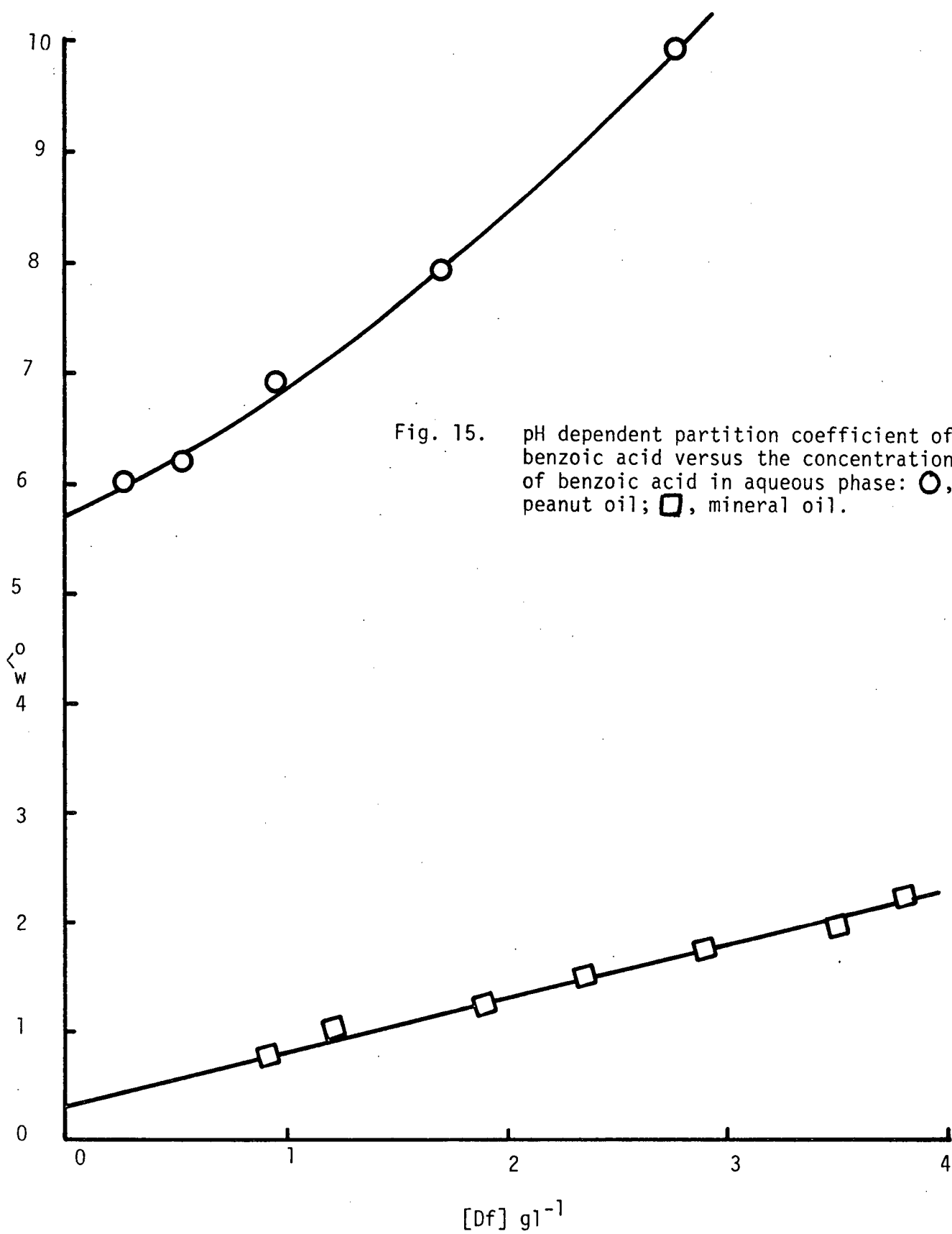
found to be impermeable to cetomacrogol. Error due to Donnan equilibrium was neglected because a high ionic strength was maintained in all the dialysis studies.

F.    Representation of Preservative-Surfactant Interaction Data

The interaction of preservatives, drugs and other solutes with various macromolecules such as surfactants, polymers and proteins has been studied extensively. Methods used to express the interaction with proteins are well established (Goldstein, 1949; Klotz, 1953; Meyer and Guttman, 1970) and have been applied successfully to polymers such as methyl cellulose and polyvinyl pyrolidone (Eide and Speiser, 1967a; 1967b; Cho, Mitchell and Pernarowski, 1971). Results for the interaction between solute and surfactant, however have been presented in a variety of ways depending essentially on the particular theory adopted to explain the mechanism of interaction. In the following discussion some of these methods are compared using results obtained in studies of the interaction between several commonly used preservatives and the nonionic surfactant cetomacrogol.

(a)  Interaction as a partition phenomenon:

One of the earliest attempts to express solubilization quantitatively was due to McBain and Hutchinson (1955). They suggested that the formation of micelles and in particular the occurance of a hydrocarbon region in the centre of micelles justifies the treatment of micelle formation as

a phase separation. Solubilization may be regarded therefore as the distribution of solute between water and a micellar phase. McBain and Hutchinson expressed this:

$$Km = \frac{\text{moles micellar solute / mole micellar surfactant}}{\text{moles free solute / mole water}}$$

(Eq. 36)

Where Km is the apparent partition coefficient for the distribution of solute between the micelles and aqueous phase. This approach has been used by Evans (1964) and Mitchell and Brown (1966). However Eq. 36 does not include the volumes of the aqueous or micellar phases and the values of Km cannot therefore be compared with classical oil-water partition coefficients. An estimate of micellar volume can be made from the partial molar volume of the surfactant and Km expressed according to Eq. 37 (Donbrow and Rhodes, 1963; Mitchell and Broadhead, 1967)

$$Km = \frac{Db/v}{Df/(1-v)}$$

(Eq. 37)

When Db is the amount of solute in the micellar phase, Df is the amount of solute in the aqueous phase, v is the volume of the micellar phase and (1-v) is the volume fraction of the aqueous phase. Apparent partition coefficients calculated according to Eq. 37 for various preservatives in cetomacrogol solutions are shown in Fig. 16. The Km values are not constant but depend on the free drug concentration.

A major problem associated with the application of Eq. 37 is that the value assigned to the volume of the micelles is somewhat arbitrary

since the volume could be (a) the hydrocarbon core of the micelles, (b) the entire micelle or (c) the entire micelle including bound and trapped water. Humphrey and Rhodes (1968) attempted to overcome this problem in a study of solubilization of benzoic acid in a series of nonionic surfactants, but extrapolating the solubility curve to 100% w/w surfactant. This value was taken to represent the solubility of the solute in the micellar phase, Sm and

$$Km = Sm/Sw \qquad (Eq. 38)$$

where Sw is the solubility in aqueous phase.

This technique will normally entail a very large extrapolation to 100% w/w surfactant and like all methods based on solubility measurements is, in effect, a one-point method. It cannot be assumed that the value of Km obtained from Eq. 38 will be applicable to undersaturated systems. Moreover it has been shown that the solubilization of benzoic acid is not governed by the distribution law (Donbrow and Rhodes, 1964; Donbrow, Molyneux and Rhodes, 1967; Donbrow, Azaz and Hamburger, 1970).

(b) Interaction as a "binding" phenomenon

An alternative and widely used method is to express interaction data according to Eq. 39 (Patel and Kostenbauder, 1958; Blaug and Ahsan, 1961a; 1961b; Bahal and Kostenbauder, 1964; Patel and Foss, 1965; Ashwarth and Heard, 1966; Patel, 1967; Bean, Konning and Malcolm, 1969).

$$[Dt]/[Df] = 1 + k [M] \qquad (Eq. 39)$$

Fig. 16.  Variation of apparent partition coefficient with free preservative concentration for the partition of preservative between micelles and aqueous phase of cetomacrogol.
Solutions A. Benzoic acid at 30°; cetomacrogol concentrations (moles/litre): 0.0077;0.015; 0.031.
B. Parahydroxybenzoic acid.  C. Methyl p-hydroxybenzoate.  D. Propyl p-hydroxybenzoate, all at 25° (Brown, 1968); cetomacrogol concentrations (moles/litre); 0.01; 0.04; 0.06; 0.1.  E. Chloroxylenol, at 20° (Mitchell & Brown, 1966);  cetomacrogol concentrations (moles/litre): 0.005;  0.01;  0.049;  0.096.

A, B, C, [Df] x $10^2$; D, E, [Df] x $10^3$ (moles litre$^{-1}$)

Where $[Dt]/[Df]$, represented by R, is the ratio of total solute concentration to the free solute concentration and $[M]$ is the surfactant concentration. Plots of R as a function of surfactant concentration are normally presented as a single curve, the slope of which, k, is taken as a measure of the binding capacity of the surfactant. The total preservative concentration is calculated by multiplying the concentration of free preservative required for antimicrobial action by the R value at the appropriate surfactant concentration. However, as will be shown later (see Figs. 17 and 18) the R value at any given surfactant concentration is constant only under limited conditions.

Since the "partition" and binding approaches to solubilization are so widely used it is of interest to compare Eqs. 37 and 39. Over a limited concentration range, the volume of micellar phase, v is directly proportional to the surfactant concentration, $[M]$, i.e. $v = k'[M]$. Hence $Db/v$ in Eq. 37 can be written $[Db]/k'[M]$ where $[Db]$ is the concentration of solute in moles per litre. Similarly, for relatively dilute solutions $Df/(1-v)$ is proportional to concentration of free solute in moles/litre, $[Df]$, and Eq. 37 can be written

$$Km = \frac{[Db]/k'[M]}{[Df]} = \frac{[Db]}{k'[M][Df]} \quad \text{(Eq. 40a)}$$

or

$$[Db] = Km \, k'[M][Df] \quad \text{(Eq. 40b)}$$

$$\therefore \frac{[Db]}{[Df][M]} = k' \, Km = k \quad \text{(Eq. 41)}$$
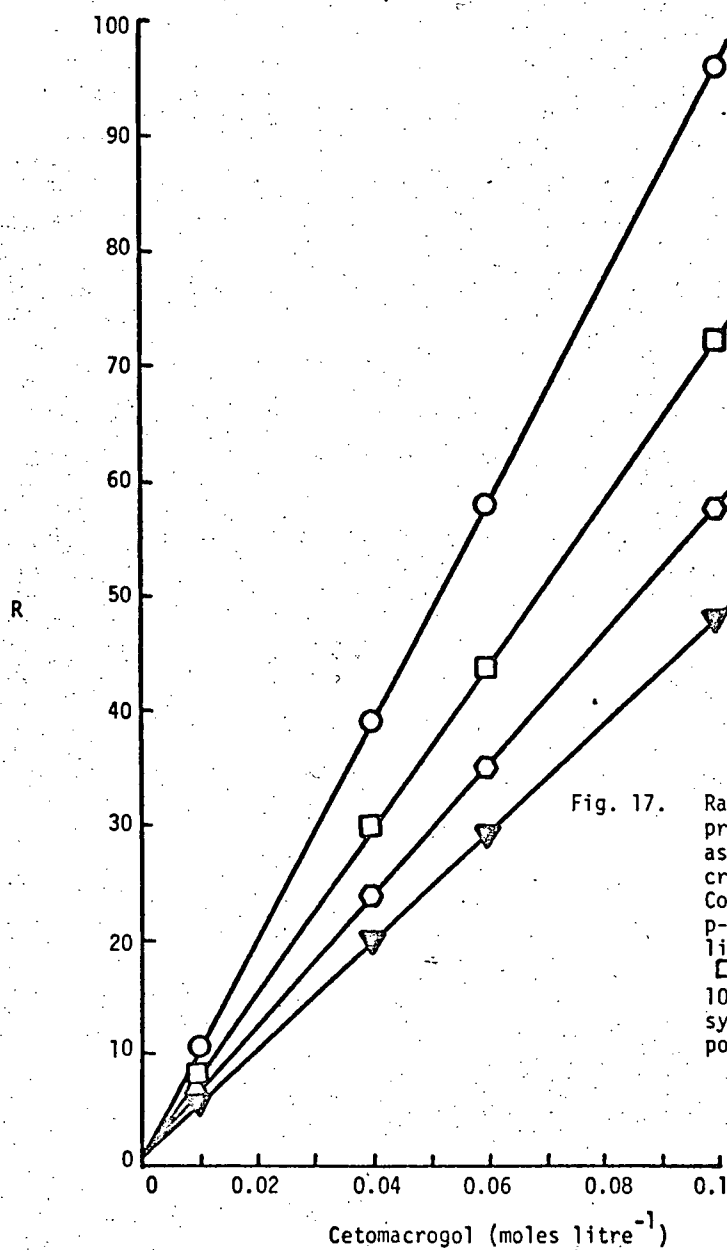
Fig. 17. Ratio of Total/Free propyl p-hydroxybenzoate as a function of cetomacrogol concentration at $25^{\circ}$. Concentration of free propyl p-hydroxybenzoate (moles/litre): $\bigcirc$, $0.21 \times 10^{-3}$; $\square$, $0.5 \times 10^{-3}$; $\bigcirc$, $1.23 \times 10^{-3}$; $\triangledown$, $2.0 \times 10^{-3}$. Closed symbols represent solubility points.

Fig. 18. Ratio of Total/Free benzoic acid as a function of cetomacrogol concentration at 30°. Concentration of free benzoic acid (moles/litre): O, $5 \times 10^3$; △, $10 \times 10^{-3}$; □, $20 \times 10^3$; ⬡, $31.27 \times 10^{-3}$.
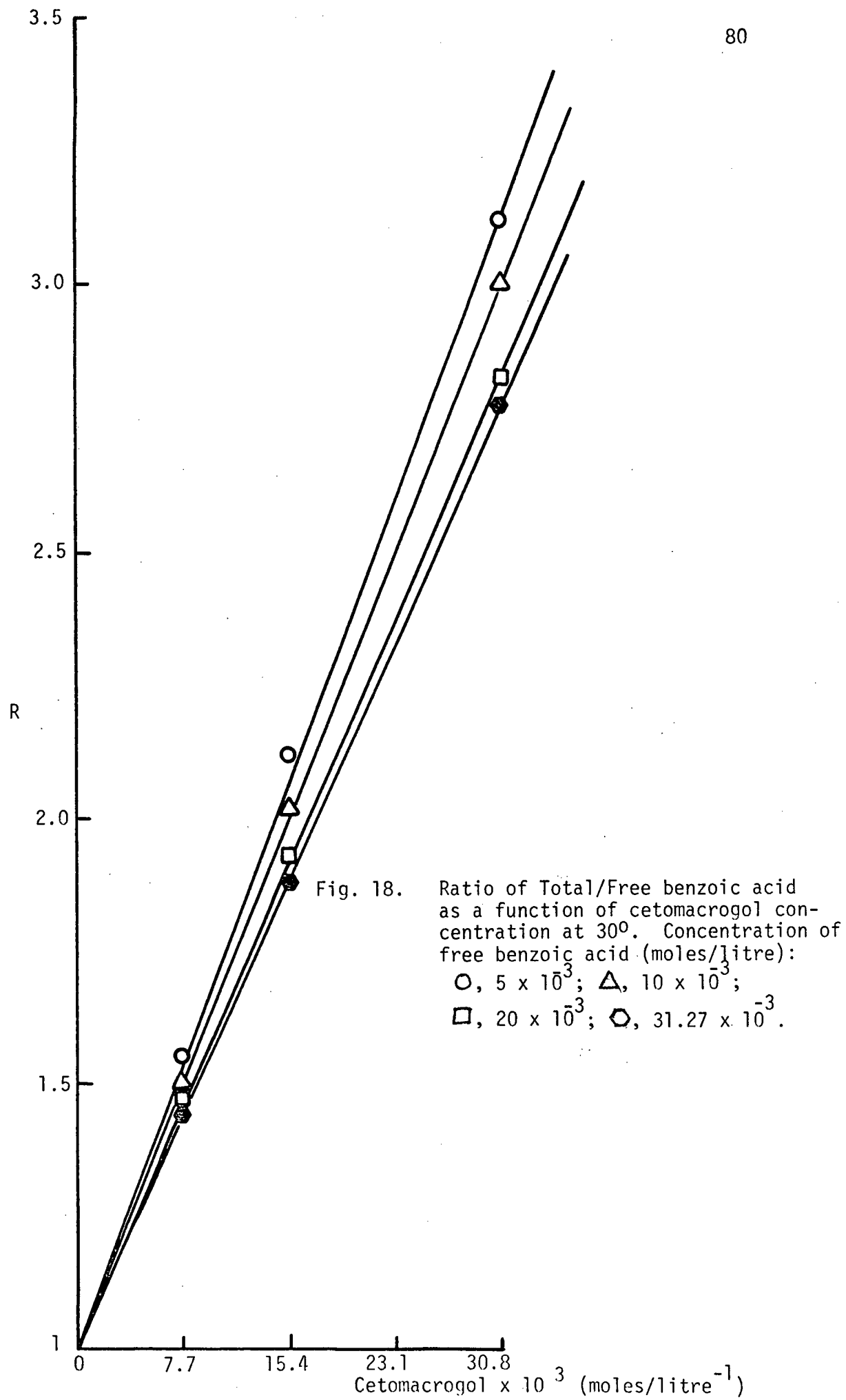
Since [Dt] = [Db] + [Df], Eq. 39 can be rearranged into the same form as Eq. 41 as follows:

$$\frac{[Db] + [Df]}{[Df]} = 1 + k \ [M] \qquad \text{(Eq. 42)}$$

or

$$\frac{[Db]}{[Df]} + \frac{[Df]}{[Df]} = 1 + k \ [M] \qquad \text{(Eq. 43)}$$

Therefore

$$\frac{[Db]}{[Df]} = k \ [M] \qquad \text{(Eq. 44)}$$

or

$$\frac{[Db]}{[Df] \ [M]} = k \qquad \text{(Eq. 41)}$$

Hence both the 'partition' and simple 'binding' approaches to solubilization depend on the same relation and a fit of data to either equation does not permit any assumptions to be made about the mechanism of the interaction. Although many authors have expressed solubilization in terms of a partition coefficient or as a binding constant, neither of these constants fully characterizes the interaction.

In contrast to the controversy surrounding methods used to describe the interaction of solute with surfactant, the fundamental concepts dealing with the interaction of solute with proteins are well established. The interaction can be expressed by Eq. 45 which is derived from the law of mass action:

$$r = \frac{n \ K \ [Df]}{1 + K \ [Df]} \qquad \text{(Eq. 45)}$$

Where r is the molar ratio of bound solute to total protein [Db]/[M], n

is the maximum number of independent binding sites on the protein and K

is the association constant. Garrett (1966) suggested that the binding

of preservatives to macromolecules, other than proteins, may be treated

in a similar way i.e. r = [Db]/[M], where [M] is the concentration of any

macromolecule including surfactant. An important difference between sur-

factants and other macromolecules is that interaction occurs between sol-

ute and surfactant micelles rather than monomer surfactant molecules.

Theoretically [M] in Eq. 45 should be the concentration of micelles, n

the number of binding sites per micelle and K the association constant

for reaction with the micelles. From a practical viewpoint however, it

is more convenient to express [M] in terms of surfactant concentration.

The critical micelle concentration of commonly used nonionic surfactants

is sufficiently low for the monomer concentration to be neglected.

Eq. 45 has the same form as the Langmuir equation which has led some

authors to suggest that the mechanism of interaction between solute and

surfactant is one of adsorption onto the surface of the micelle or some

other site within the micelle (Donbrow and Rhodes, 1964; Donbrow, Moly-

neux and Rhodes, 1967). However, as pointed out by Goldstein (1949) and

Klotz (1953) for solute-protein interaction, although the equations are

similar it is not necessarily correct to assume that binding and adsorp-

tion are identical processes.

Figures 17 and 18 show the results plotted as the ratio of total

preservative to free preservative, R, as a function of surfactant concentration according to Eq. 39. Contrary to the manner in which data is normally presented for this type of plot, the results cannot be represented by a single surve. Eq. 39 is in fact a special case of Eq. 45 and a single curve will be obtained only under two conditions: (a) when $[Df] \xrightarrow{\lim} o$ then $nk/(1 + K [Df]) = n K$ and $R = 1 + k [M]$ where $k = n K$, but the practical application of this assumption is not always valid because the minimum inhibitory or minimum lethal concentration of most of the commonly used preservatives, such as p-hydroxybenzoates, phenolics, benzoic acid etc., lies very close to their saturation concentration; (b) when $[Df]$ is constant, as in the solubility method, then $n K/(1 + K [Df]) = $ a constant, $k''$, and $R = 1 + k''[M]$. Hence $k$ (or $k''$) does not fully characterize the interaction. A macromolecule or micelle has a limited binding capacity for solute molecules and a single value of $k$ (or $k''$) will be obtained only over a limited range of free solute concentration. It is impossible to maintain $[Df]$ constant using the equilibrium dialysis technique and Fig. 17 and 18 were constructed using calculated values of $[Df]$. The slope decreases with increasing values of $[Df]$ and the lowest limiting slope, corresponding to a solubility curve, represents the saturation-point in the Langmuir type plots, Figs. 19 and 20.

The simplest way to express the binding data is a Langmuir-type plot of r versus $[Df]$. Eq. 45 is a segment of a ractangular hyperpola passing through the origin. If $[Df]$ becomes infinite, the r value approaches n as a limit

Fig. 19. Langmuir-type plot for the interaction of preservatives with cetomacrogol solutions: A. Benzoic Acid, B. Parahydroxybenzoic acid. Cetomacrogol concentrations as in Fig 16. Closed symbols represent solubility points.

[Df] x 10$^2$ (moles litre$^{-1}$)

Fig. 20. Langmuir-type plot for the interaction of preservatives with cetomacrogol solutions: C. Methyl para-hydroxybenzoate; D. Propyl para-hydroxybenzoate; E. Chloroxylenol. Cetomacrogol concentrations as in Fig 16. Closed symbols represent solubility points.

D, E. $[Df] \times 10^3$; C. $[Df] \times 10^2$ (moles litre$^{-1}$)

85
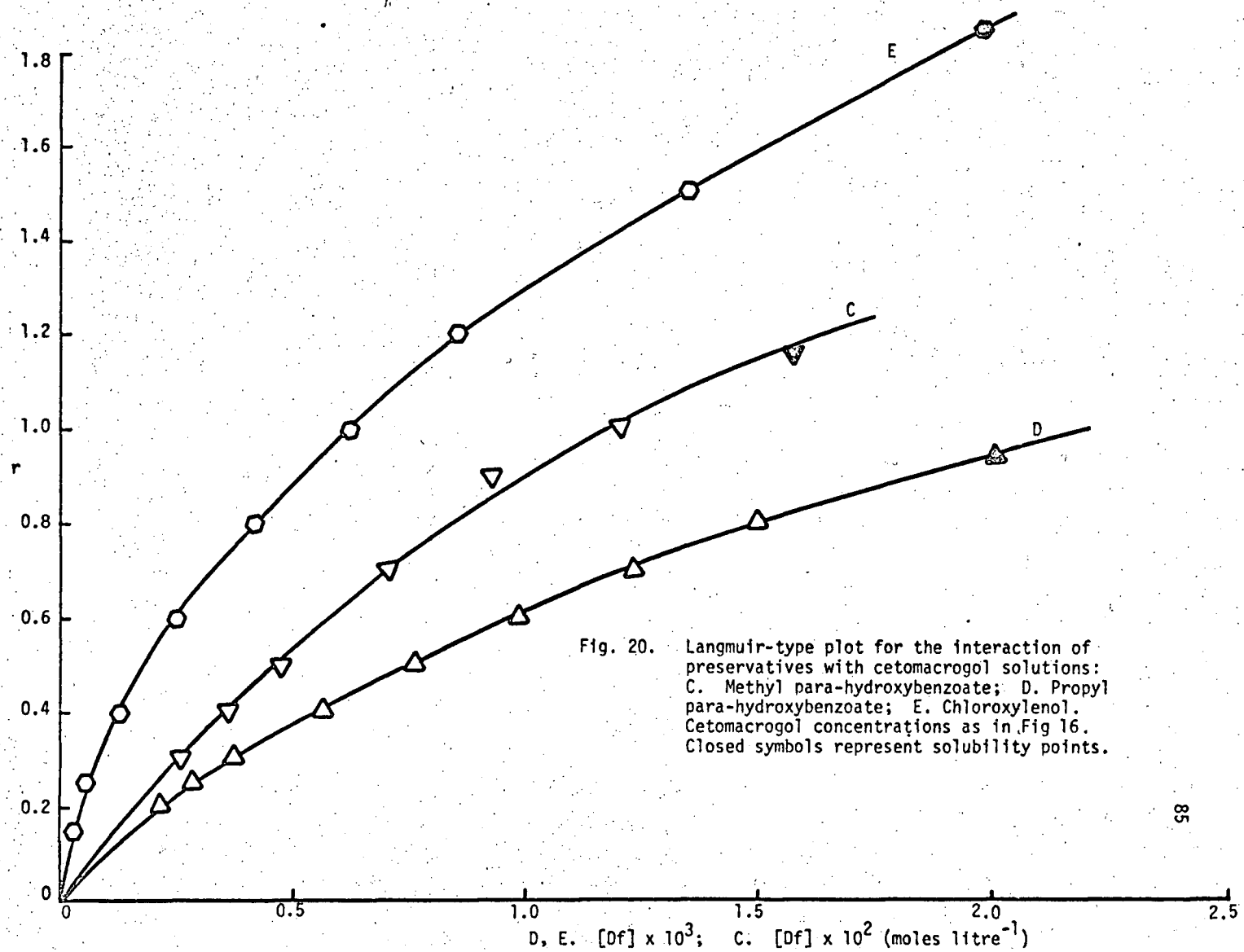
$$[Df] \xrightarrow{\quad \lim \quad} \infty \quad r = n \qquad \text{(Eq. 46)}$$

and at $r = n/2$

$$[Df] = 1/K \qquad \text{(Eq. 47)}$$

Eqs. 46 and 47 indicate the importance of a wide concentration range of free solute in any binding study. Fig. 19 and 20 show that at low concentrations the preservatives are more easily bound to cetomacrogol than at high concentrations. Only results obtained from solubility experiments show saturation of binding sites. Hence binding parameters were not derived from these plots.

Eq. 45 is normally rearranged into forms more convenient for graphical presentation of the results. Figs. 21 and 22 show results for the interaction of some preservatives with cetomacrogol plotted according to the reciprocal form of the equation,

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{nK\,[Df]} \qquad \text{(Eq. 48)}$$

A line passing through the origin rather than an intercept corresponding to a limiting binding capacity has been taken as evidence that the mechanism of interaction is partitioning into the micelles rather than adsorption on a micellar surface or to specific sites on the macromolecule (Bahal and Kostenbauder, 1964). This plot, however, heavily weights those experimental points obtained at low concentrations of free drug and may lead to large errors on extrapolation to infinitely high preservative concentrations. An alternative arrangement of Eq. 45 is known as the Scatchard equation (Scatchard, 1949):
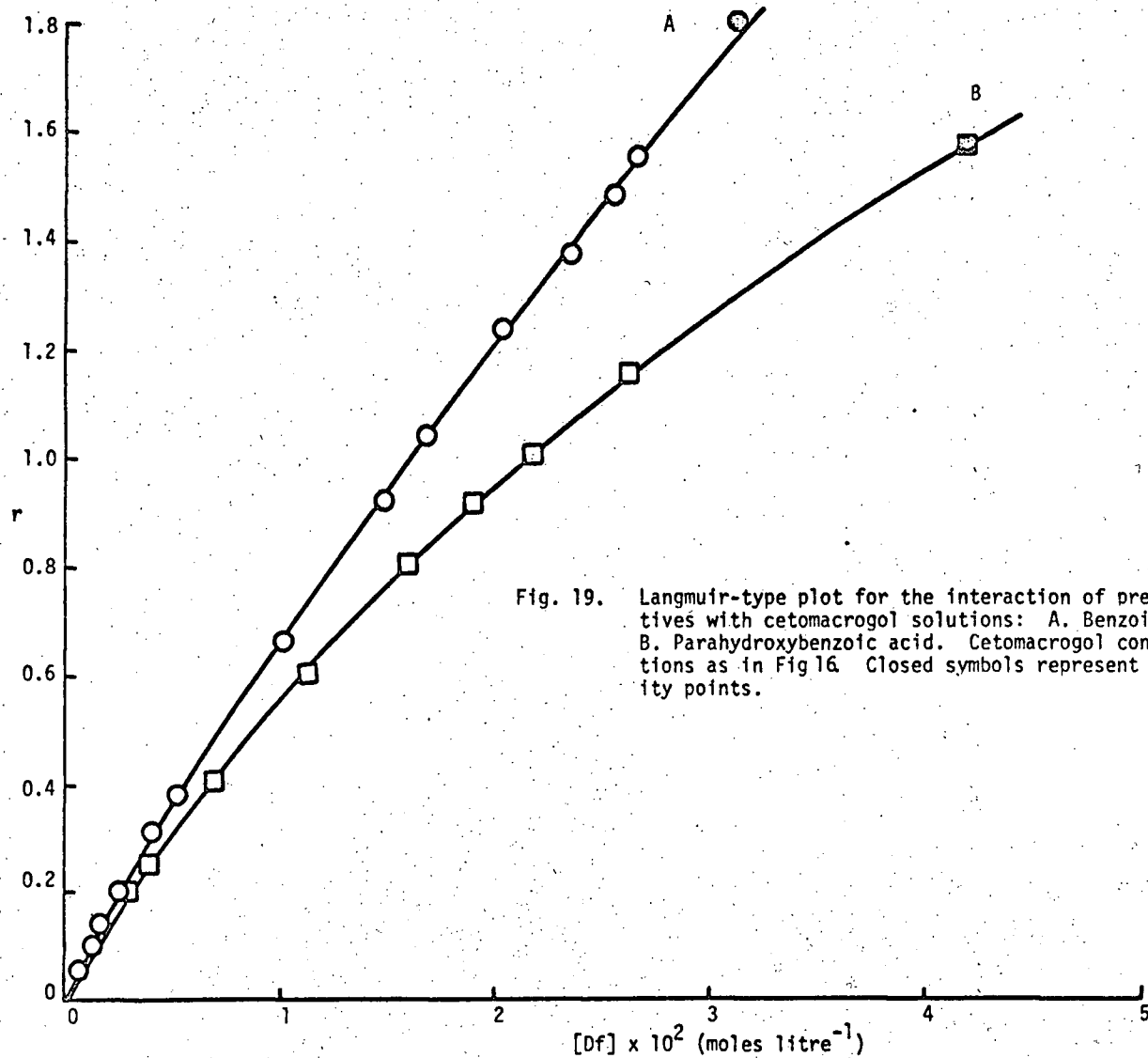
Fig. 21. Double-reciprocal plot for the interaction of preservatives with cetomacrogol solutions: A. Benzoic acid, B. Parahydroxybenzoic acid. Cetomacrogol concentrations as in Fig. 16. Closed symbols represent solubility points.
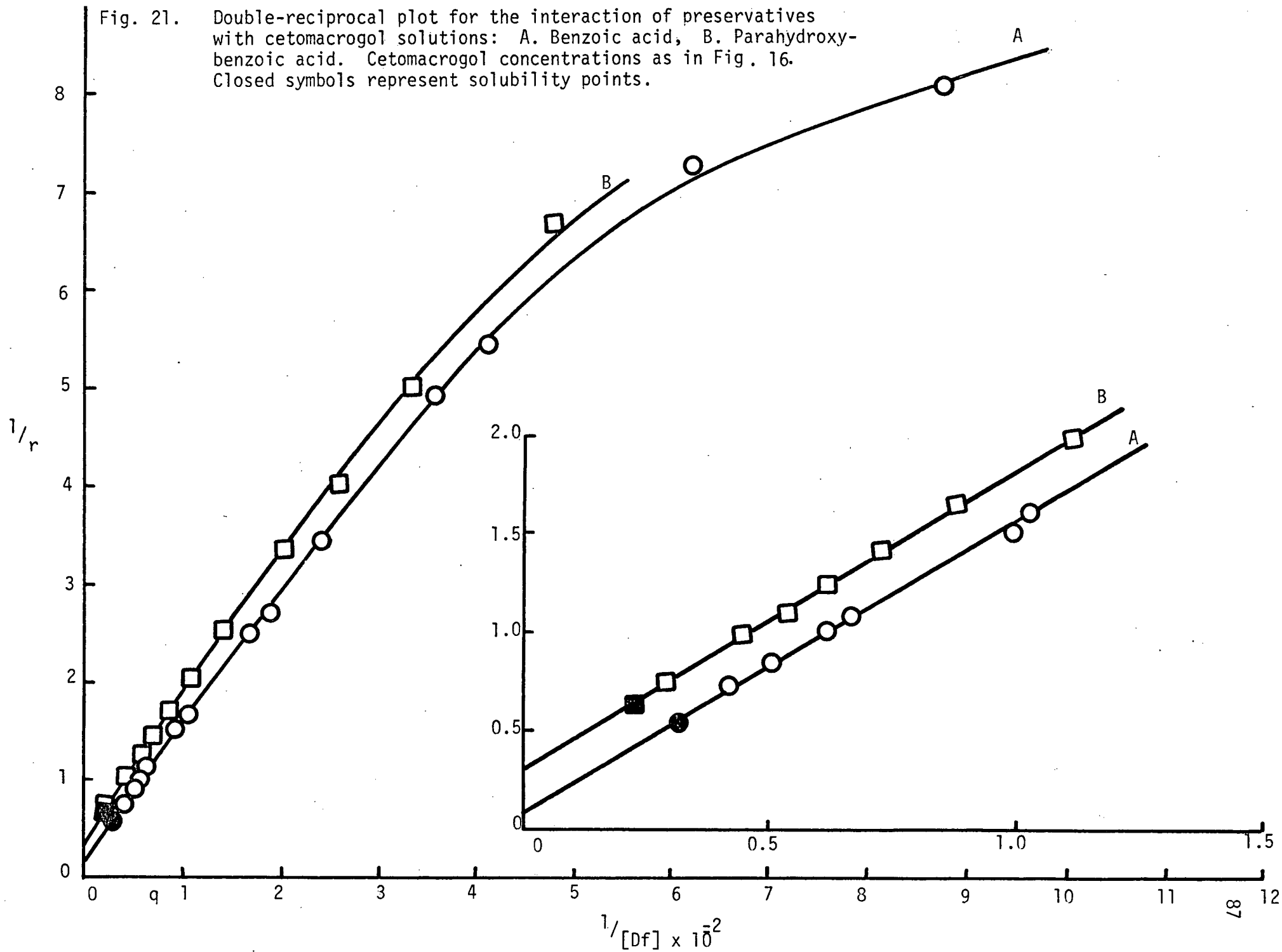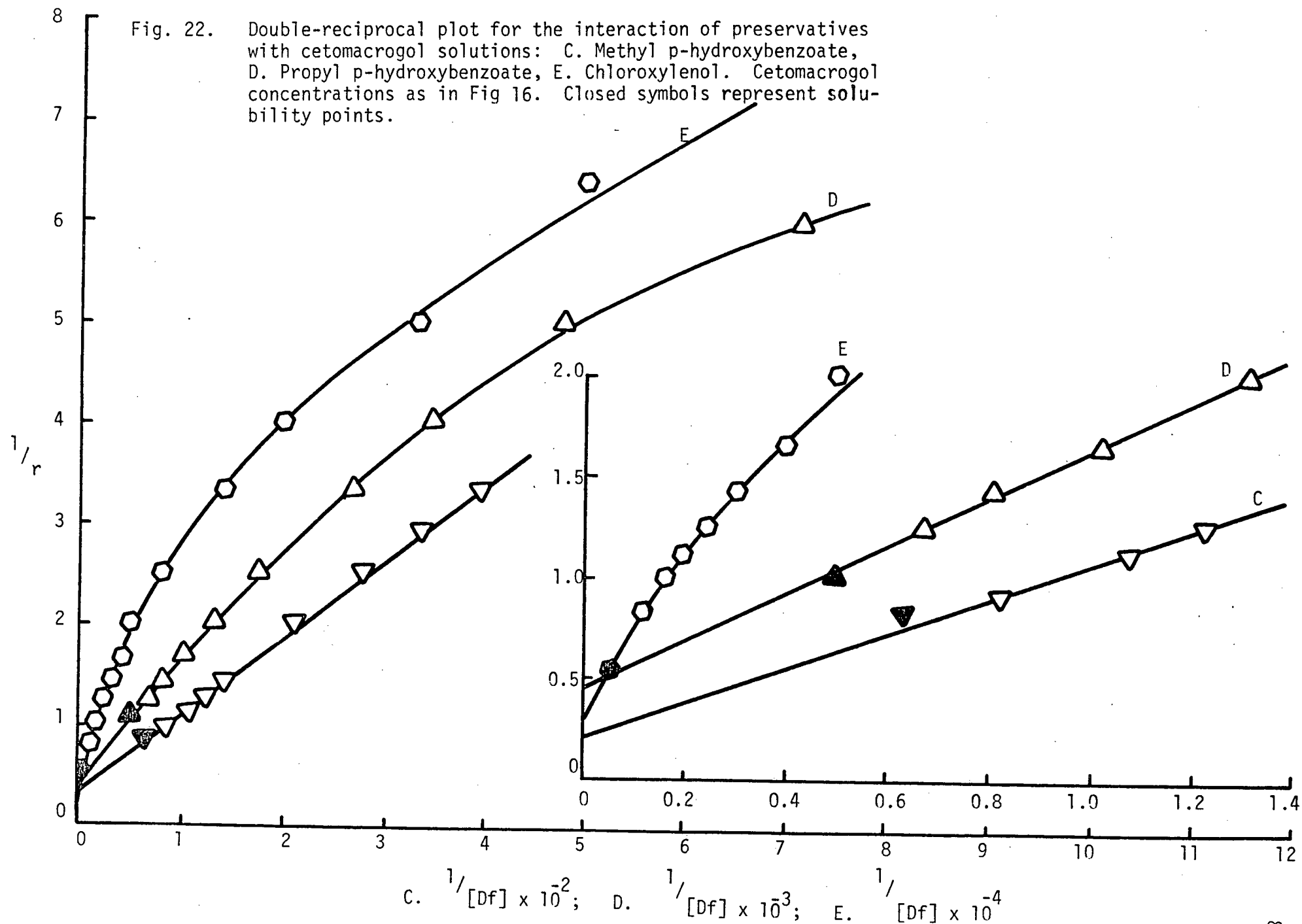
Fig. 22. Double-reciprocal plot for the interaction of preservatives with cetomacrogol solutions: C. Methyl p-hydroxybenzoate, D. Propyl p-hydroxybenzoate, E. Chloroxylenol. Cetomacrogol concentrations as in Fig 16. Closed symbols represent solubility points.

C. $\dfrac{1}{[Df]}$ x $10^{-2}$;  D. $\dfrac{1}{[Df]}$ x $10^{-3}$;  E. $\dfrac{1}{[Df]}$ x $10^{-4}$

$$\frac{r}{[Df]} = nK - rK \qquad \text{(Eq. 49)}$$

Which on plotting gives a more even weighting to the different points on the curve. The plot for each preservative shown in Fig. 23, has a definite curvature. In protein binding studies, this is taken as evidence for the existence of more than one type of binding site. In the case of solute-surfactant interaction the binding sites within the micelles probably do not behave independently of one another as required by Eq. 45. It is possible that uptake of solute into the micelle progressively alters the interaction between the binding sites and solute leading to a change in both the number of sites available and the association constant. Hence to describe the interaction it is necessary to plot the curve over a wide range of [Df] and determine n and K values from the slope in the region of interest. In case of preservatives, this is the concentration of free preservatives required for antimicrobial activity e.g. a concentration equal to or greater than the minimum inhibitory concentration. Table 3 gives the minimum inhibitory concentrations for a number of preservatives and values of n and K for the interaction with cetomacrogol. Substitution of n, K, [Df] and [M] into Eq. 51 enables the required preservative concentration to be calculated.

$$[Dt] = \left[ \frac{n \, K \, [Df] \, [M]}{1 + K \, [Df]} \right] + Df \qquad \text{(Eq. 50)}$$

or

$$[Dt] = [Df] \left[ \frac{n \, K \, [M]}{1 + K \, [Df]} + 1 \right] \qquad \text{(Eq. 51)}$$

Fig. 23. Scatchard plot for the interaction of preservatives with cetomacrogol solutions: A. Benzoic acid. B. Parahydroxybenzoic acid, C. Methyl p-hydroxybenzoate, D. Propyl p-hydroxybenzoate, E. Chloroxylenol. Cetomacrogol concentrations as in Fig 16.
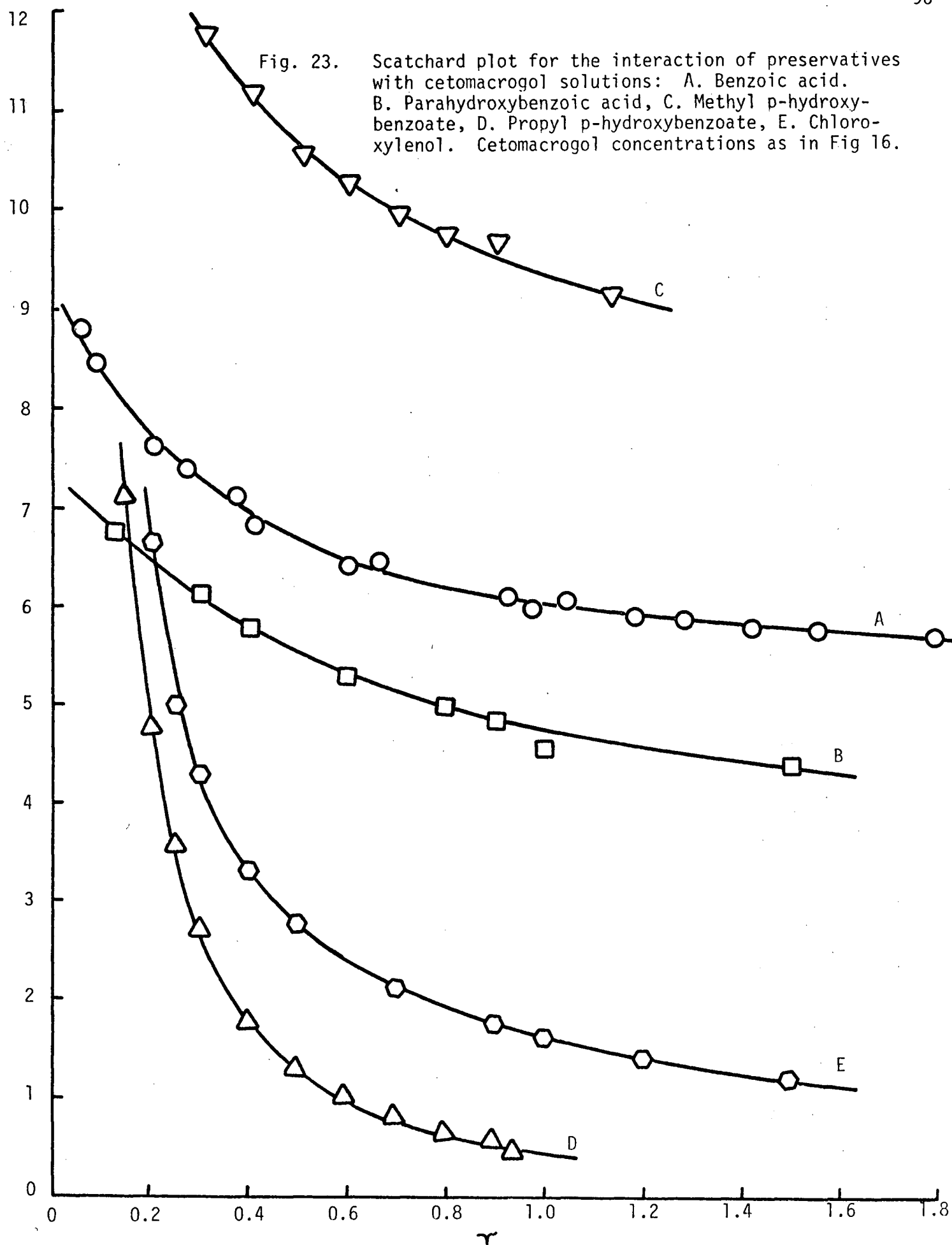
TABLE 3

Minimum Inhibitory Concentrations and Binding Parameters for

The Interaction of Preservatives with Cetomacrogol

| Preservative | Minimum inhibitory Concentration (per cent)(a) | n | $K$ $(L \ mole^{-1})$ | n | $K$ $(L \ mole^{-1})$ |
|---|---|---|---|---|---|
| | | Calculated using monomer molecular weight (e) | | Calculated using micellar molecular weight (f) | |
| Benzoic acid | 0. 1 (b) | 4.6 | 16 | 371 | 16 |
| Methyl p-hydroxybenzoate | 0.15 (c) | 5.2 | 22 | 445 | 22 |
| Propyl p-hydroxybenzoate | 0.06 (c) | 2.2 | 343 | 176 | 341 |
| Chloroxylenol | 0.02 (d) | 2.8 | 942 | 216 | 949 |

(a)   Highest Concentration quoted in each reference (b) Bandelin (1958 (c) Nowak (1963)

(d)   Aist Gucklhorn (1969) (e) taken as 1300 (f) from Altwood, Elworthy and Kayne (1969).

G.. Distribution of Benzoic Acid in Oil-Water-Surfactant Systems

(a) Two-chambered dialysis technique: Analysis of the concentration
of preservative in the aqueous compartment, W, at equilibrium enables the
free preservative concentration, [Df], to be determined. From [Df] and
the total amount of preservative added, ([Do] + [Db]) can be determined,
although it is not possible to separate these quantities.

(b) Three-chambered dialysis technique: At equilibrium analysis of
the concentration of preservative in compartments W and S gives [Df] and
([Df] + [Db]) respectively and hence [Db]. From these terms and the
amount of preservative added initially, [Do] can be obtained.

Analysis of surfactant in compartment S gives the free surfactant
concentration. From the free surfactant concentration and the total
amount of surfactant added, the amount of surfactant adsorbed at the oil-
water interface or partitioned into the oil phase can be calculated.

(c) Validity of two and three-chambered dialysis techniques: The
amount of benzoic acid added to dialysis cells was calculated using Eq.
30

$$[D] = \left\{ [Df] \left[ 1 + nK[M]/(1 + K[Df]) + K_W^O q \right] \right\} / (q + 1)$$

$$(Eq. 30)$$

For given [Df], the parameters n and k were calculated from the Scatchard
plot (Fig. 23) and $K_W^O$ from Fig. 15. The final concentrations were cor-
rected for the binding of benzoic acid with the Millipore VS membrane (Fig.

13).

Fig. 24 is a plot of [D] versus [Df] for the distribution of benzoic acid in an emulsion of peanut oil, cetomacrogol and aqueous buffer using the two-chambered dialysis technique. Data from three-chambered dialysis technique was plotted in a variety of ways. Figs. 25 and 26 are ternary plots of [Do],[Db] and [Df] for the distribution of benzoic acid in peanut oil-cetomacrogol-aqueous buffer systems and mineral oil-cetomacrogol-aqueous buffer systerms respectively. Figs. 27 and 28 are three dimensional plots of [Do], [Db] and [Df] for the distribution of benzoic acid in peanut oil-cetomacrogol-aqueous buffer systems and mineral oil-cetomacrogol-aqueous buffer systems respectively. Figs. 29 and 30 are three dimensional plots of [Do], [Db]/[M] and [Df]. In each figure there is close agreement between the experimentally determined values and the curves predicted using Eq. 30. Hence both the two - as well as the three - chambered dialysis techniques are valid and give correct value of [Df] for a given [D].

(d) <u>Applications of three-chambered dialysis technique</u>: 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 an emulsion to provide a minimum inhibitory concentration. Determination of the various physico-chemical parameters for substitution into Eq. 30 is a time-consuming process particularly if, as is usual in most pharmaceutical and cosmetic emulsions, there is more than one type of macromolecules present. Moreover even a slight change

Fig. 24. Variation of free benzoic acid concentration [Df], 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% w/v cetomacrogol: O, observed [Df]; curve calculated from Eq. 30 .

Fig. 25. Ternary graph of the distribution of benzoic acid in an o/w peanut oil emulsion stabilized with cetomacrogol: ○, experimental values; curve calculated using Eq. 30.

Fig. 26. Ternary graph of the distribution of benzoic acid in an o/w mineral oil emulsion stabilized with cetomacrogol: O, experimental values; curve calculated using Eq. 30.

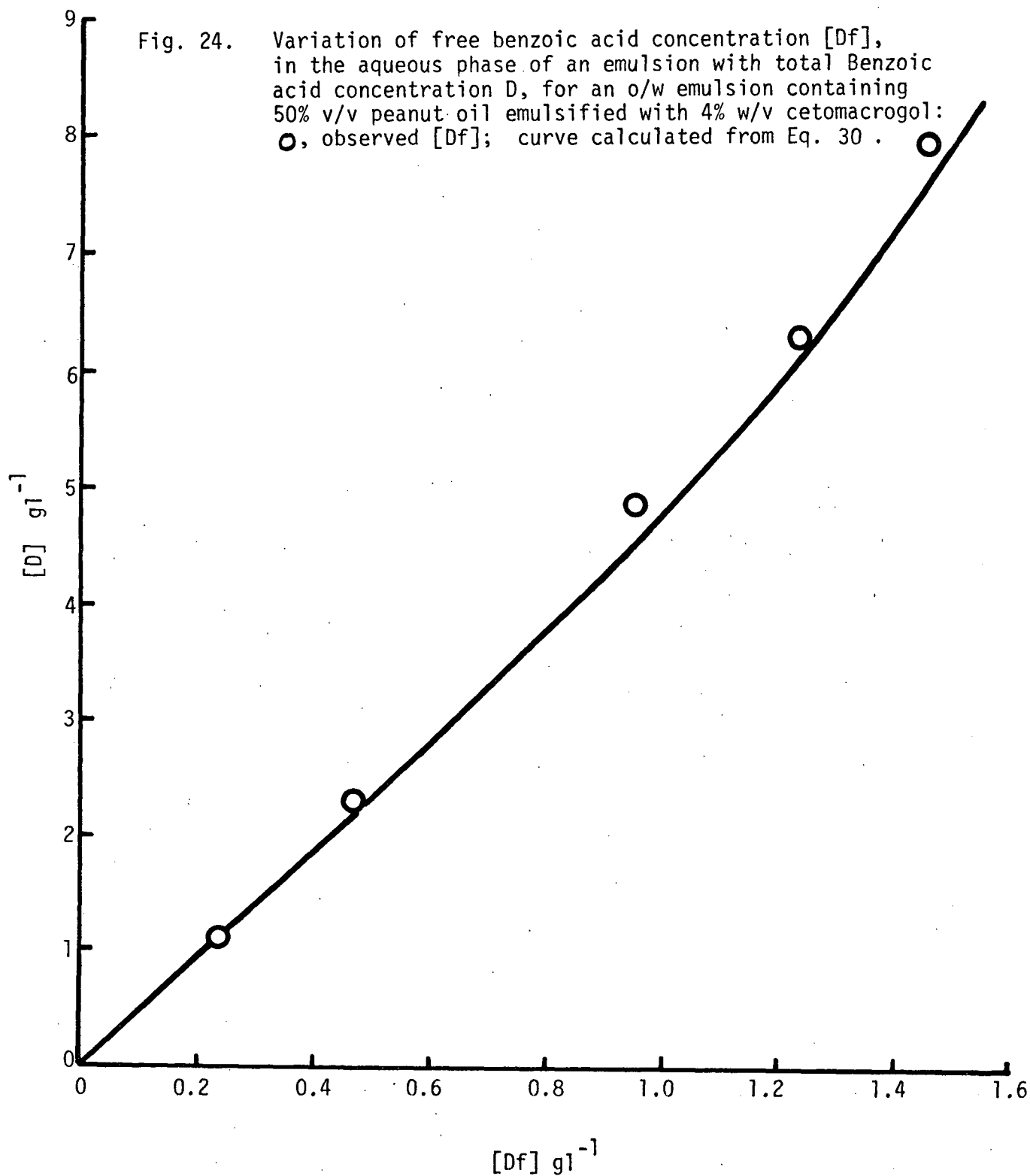Fig. 27. Three-dimensional graph of the distribution of benzoic acid in an o/w peanut oil emulsion stabilized with cetomacrogol: O, experimental values; curves calculated using Eq. 30.

Fig. 28.  Three-dimensional graph of the distribution of benzoic acid in an o/w mineral oil emulsion stabilized with cetomacrogol: O, experimental values;  curves calculated using Eq.  30.

Fig. 29.  Modified three-dimensional graph of the distribution of benzoic acid in an o/w peanut oil emulsion stabilized with cetomacrogol: O, experimental values; curves calculated using Eq. 30.

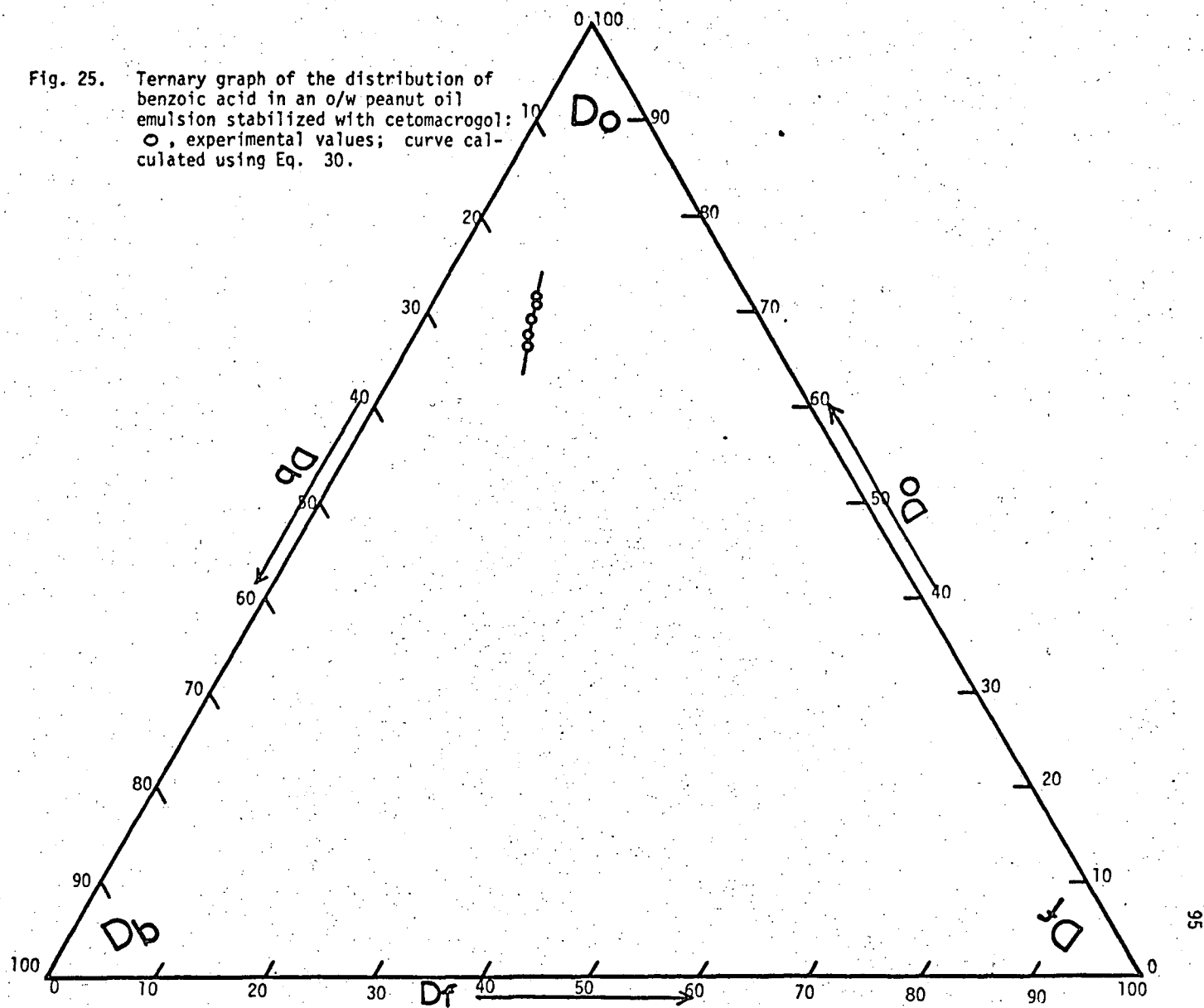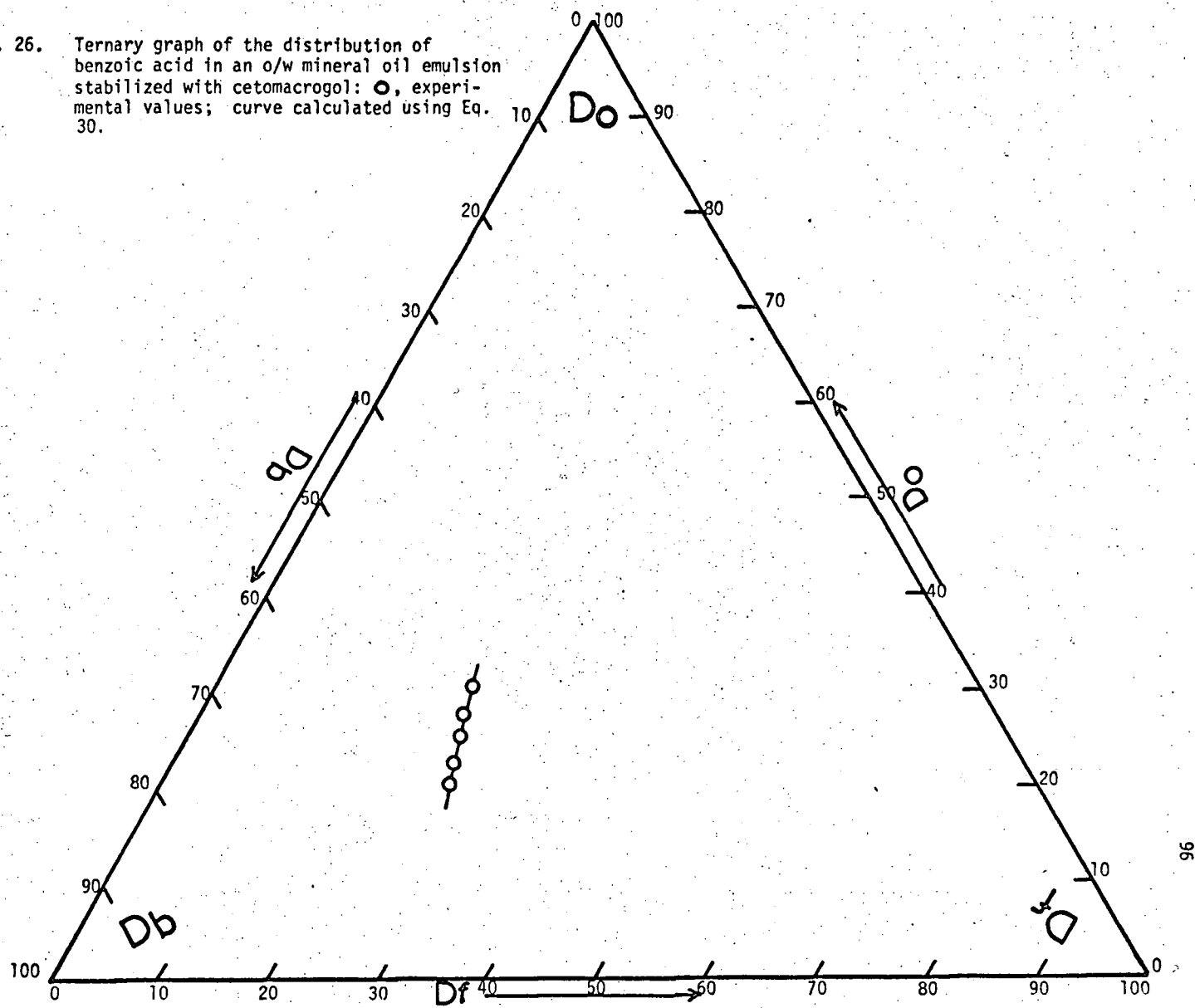Fig. 30.  Modified-three dimensional graph of the distribution
of benzoic acid in an o/w mineral oil emulsion stabil-
ized with cetomacrogol:  O, experimental values;
curves calculated using Eq.  30 .
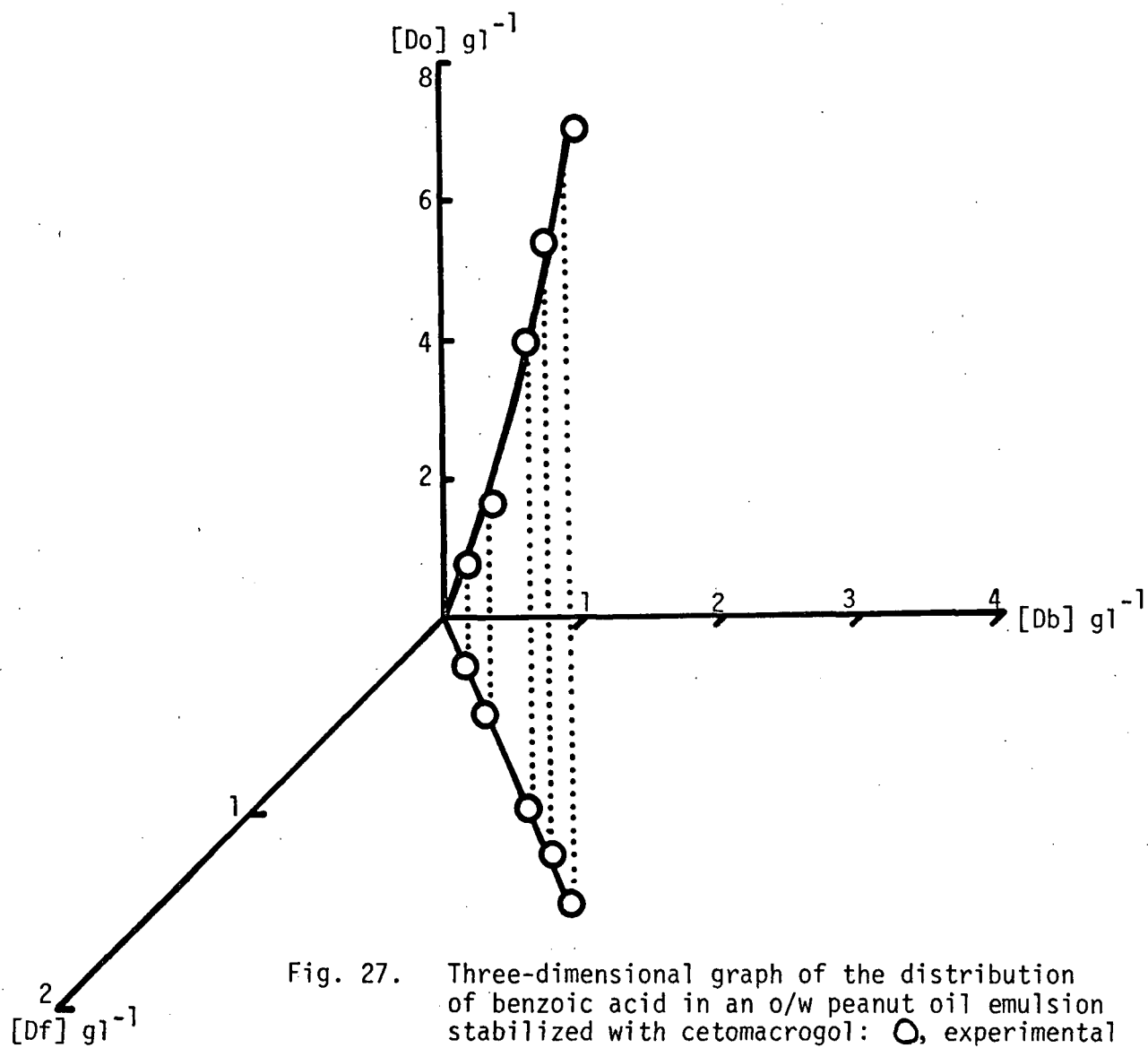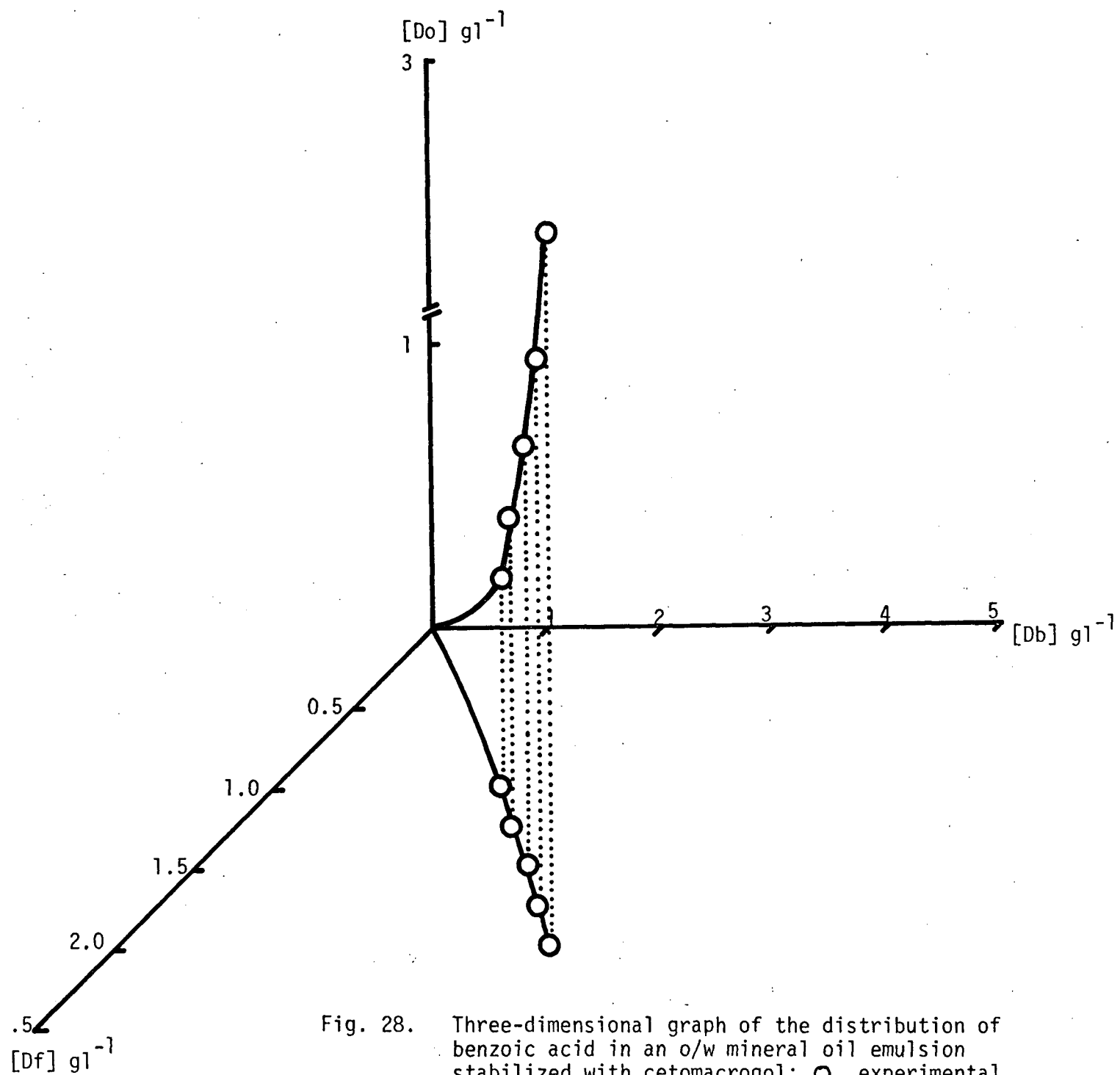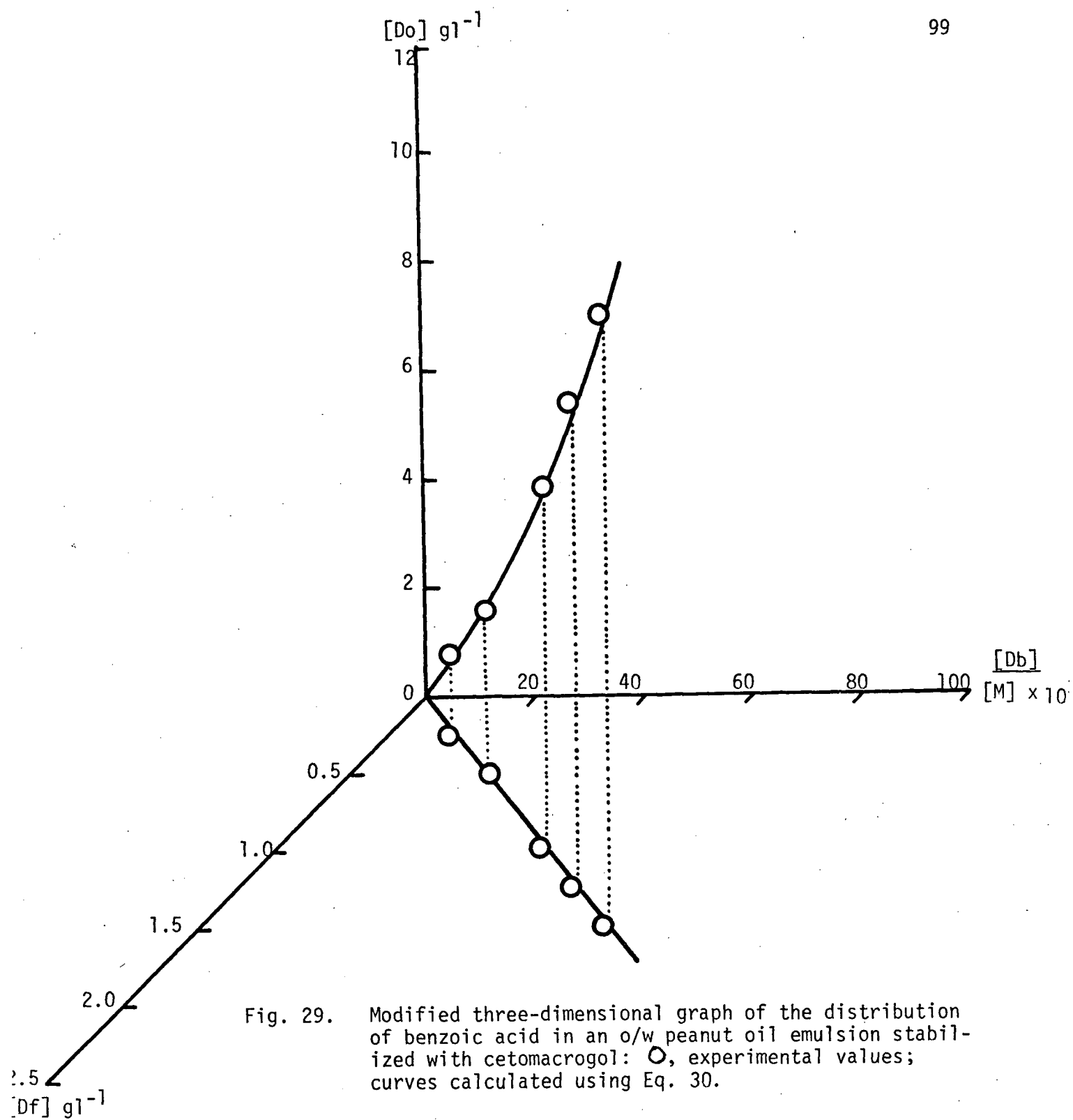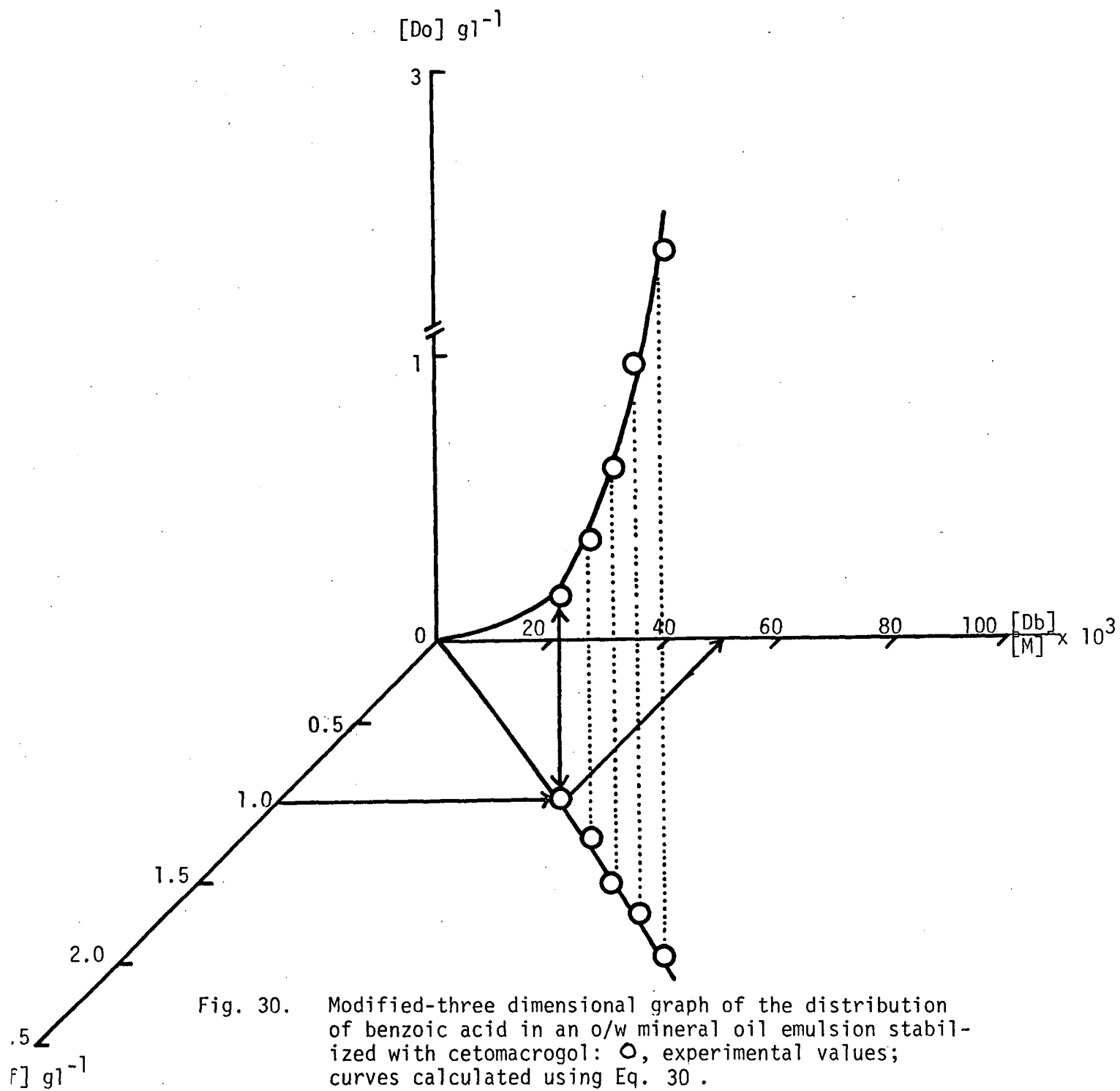
in the formulation of the emulsion necessitates a re-evaluation of the various terms. However agreement between predicted and observed values shows that the three-chambered dialysis technique provides a relatively simple method by which the total concentration of preservative necessary can be estimated. The first step is to construct a calibration curve using values of [Do], [Db] and [Df] obtained by dialysing the emulsion containing varying known concentrations of preservative in the three-chambered cell. A variety of calibration curves can be obtained from the values of [Do], [Db] and [Df]. Figs. 25 and 26 show data plotted on triangular coordinate graph paper. The main weakness of this type of plot is that [Do], [Db] and [Df] are expressed as a per cent of the total preservative concentration, [D], in the emulsion. It is not possible therefore to obtain values of [Do], [Db] for a given [Df] directly from the plot. Secondly, scales for Do, Db and Df are fixed and if the values of one variable are very close, there is no possibility of expanding that particular scale. In this way some accuracy in the data derived from this plot is lost. These weaknesses were overcome by plotting the data in three-dimensional figures. Figs. 27 and 28 are three-dimensional plots of [Do], [Db] and [Df], the lower line gives the relation between [Db] and [Df] and the upper line gives the relation between [Do], [Db] and [Df]. For a given value of [Df], [Do] and [Db] can be obtained directly from the graph. Scales for [Do], [Db] and [Df] can be expanded as much as desired. These plots are independent of the oil-water ratio, but are dependent on the surfactant concentration. Hence a

change in surfactant concentration would require a new calibration curve to be made. These plots can be made independent of surfactant concentration if [Db] in Figs. 27 and 28 is replaced by [Db]/[M], where [M] is the surfactant concentration (Figs. 29 and 30). This was confirmed experimentally for oil-water ratios from 0.18 to 1.0, Table 4; and for cetomacrogol concentrations from 1% to 4%, Table 5. To calculate the total concentration of preservative which is adequate to preserve an oil in water emulsion the graph is entered at a [Df] value corresponding to the minimum inhibitory concentration. The corresponding values of [Do] and [Db]/[M] are determined, as shown by the arrows in Fig. 30. 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, shown in Fig. 30:

Let the minimum inhibitory concentration $= 1.0$ gl$^{-1} = $ [Df]

The corresponding value of [Db]/[M] $= 0.05$

The corresponding value of [Do] $= 0.75$ gl$^{-1}$

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

$$[D] = \left[ ([Df] + [Db])\, Vw + [Do]\, Vo \right] / 1000 \text{ gl}^{-1}$$

$$(\text{Eq. } 52)$$

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

$$[D] = \left[ (1.0 + 0.05 \times 40)\, 500 + 0.75 \times 500 \right] / 1000 = 1.88 \text{ gl}^{-1}$$

In addition to the simple emulsions used in this work the method should be applicable to more complex emulsions in which existence of reversed

TABLE  4


Validity of Three Dimensional Calibration Curve For

The Distribution of Benzoic Acid in

Peanut Oil-Water-Cetomacrogol Systems at Various Oil-Water Ratios


| Oil : Water ratio | [D] Calculated from Fig. 29 $gl^{-l}$ | Predicted [Df] $gl^{-l}$ | Observed [Df] $gl^{-l}$ |
|---|---|---|---|
| 0.176 | 3.5 | 1.0 | 0.91 |
| 0.333 | 3.9 | 1.0 | 0.94 |
| 1.0 | 4.9 | 1.0 | 0.94 |

Cetomacrogol Concentration = 4%

TABLE 5

Validity of Three Dimensional Calibration Curve For

The Distribution of Benzoic Acid Between

Peanut Oil-Water-Cetomacrogol Systems at Various Concentrations

| Cetomacrogol Concentration % | [D] Calculated from Fig. 29 $gl^-$ | Predicted [Df] $gl^-$ | Observed [Df] $gl^-$ |
|---|---|---|---|
| 1.0 | 2.48 | 0.71 | 0.69 |
| 2.0 | 2.48 | 0.66 | 0.63 |
| 4.0 | 2.48 | 0.58 | 0.57 |

Oil : Water ratio = 0·6

liquid crystalline phases and the presence of reversed micelles in the oil phase (Friberg and Mandell, 1970) would make the use of mathematical model difficult or impossible.

The total preservative concentration can also be determined from a calibration curve of [D] versus [Df], Fig. 24 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 in that it differentiates between preservative in the oil phase and preservative associated with the surfactant. The effect of modifications 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 of the surfactant is adsorbed at the oil-water interface and, depending on its oil-solubility, some will partition into the oil phase. Both factors will reduce the amount of surfactant available for interaction with the preservative, and will effect the oil-water partition coefficient. Analysis of compartment S of the three-chambered dialysis cell for surfactant, enables the distribution of surfactant between 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 [Db].

Where appreciable amounts of surfactant are adsorbed or partitioned into the oil (Greenwald, et. al., 1961; Lin and Lambrechts, 1969), determination of the parameters necessary for substitution into Eq. 30 would become 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.

# V. SUMMARY AND CONCLUSION

## A. The Permeability of Cellophane Membranes to Cetomacrogol

The permeability of two brands of cellophane membrane to the nonionic surfactant, cetomacrogol was tested using equilibrium dialysis, dynamic dialysis and ultrafiltration techniques. The results show that the cellophane membranes are permeable to cetomacrogol and therefore cannot be used as dialysis membranes.

## B. Distribution of Benzoic Acid in Oil-Water Systems

The distribution of benzoic acid between peanut oil-water and mineral oil-water systems was studied. The distribution did not obey the simple partition law. This indicates that benzoic acid associates in both oils.

## C. Interaction of Preservatives with Cetomacrogol

The interaction of a number of commonly used preservatives with cetomacrogol was examined and a comparison made of various methods of expressing this interaction. It is suggested that the Scatchard equation is the most satisfactory equation for describing the binding data. Binding parameters determined from a Scatchard plot in the concentration range of free preservative appropriate for antimicrobial activity can be used to calculate the total concentration of preservative required in the surfactant system.

D.  Distribution of Benzoic Acid in Oil-Water-Surfactant Systems

A three-chambered dialysis cell was used to estimate the distribution of benzoic acid between the oil phase and the aqueous phase of an emulsion. The method also differentiates between the preservative bound, or solubilized by the surfactant and free in the aqueous phase. The distribution data was plotted on a three-dimensional graph from which the total concentration of preservative required to provide a given free concentration in the aqueous phase can be determined. Results from the dialysis method were compared with those calculated using mathematical models for preservative distribution. It is suggested that this method is more practical and time saving than methods based on predictions using mathematical models. This is particularly true in the case of complex emulsions where the use of more than one type of oil or macromolecule in the formulation, or the existence of liquid crystalline phases or reversed micelles in the oil phase, or the adsorption of an appreciable amount of surfactant at the oil-water interface or partition into the oil phase would make the use of mathematical models difficult or even impossible.

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