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## Preservation of Solubilized and Emulsified Systems II: Theoretical Development of Capacity and Its Role in Antimicrobial Activity of Chlorocresol in Cetomacrogol-Stabilized Systems

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

Received June 20, 1977, from the *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5*. Accepted for publication January 13, 1978. \*Present address: Faculty of Pharmacy, University of El-Fateh, Tripoli, Libya.

**Abstract** □ The preservation of solubilized and emulsified disperse systems against microbial spoilage depends on the free (unbound) preservative concentration in the aqueous phase and the capacity of the system. The capacity may be defined as the system's ability to resist losses in free preservative concentration. The theory of capacity is developed quantitatively for solubilized and emulsified systems containing the preservative chlorocresol stabilized by the nonionic surfactant cetomacrogol. Equations are derived for solubilized systems that relate capacity to surfactant concentration and the interaction between the surfactant and the preservative. Additional terms are included in the equations to account for the effects of the oil phase on the capacity of oil-in-water emulsions.

**Keyphrases** □ Chlorocresol—in solubilized and emulsified systems, role of capacity in antimicrobial activity □ Capacity—theoretical development, role in antimicrobial activity of chlorocresol in solubilized and emulsified systems □ Antimicrobial activity—chlorocresol, role of capacity in solubilized and emulsified systems

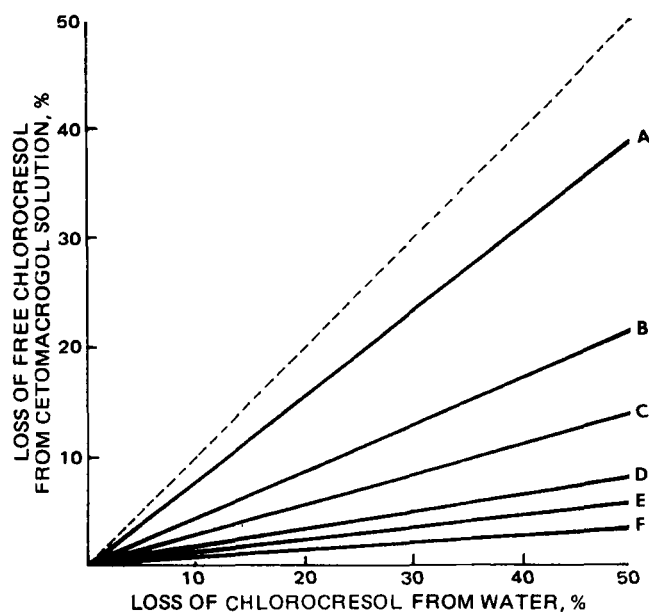
For solubilized systems and oil-in-water emulsions stabilized with the nonionic surfactant cetomacrogol, the kill rate of *Escherichia coli* over 6–8 hr depends on the free chlorocresol concentration (1). However, for long-term protection against microbial contamination, the preservative's effectiveness should depend on the capacity of the system in addition to the free preservative concentration.

The term capacity was first used (2) in discussing the antibacterial activity of iodine solubilized by a nonionic surfactant<sup>1</sup>. The saturation solubility of the iodine was used as a measure of capacity. This definition implies that depletion of preservative due to interaction with microorganisms or foreign materials<sup>2</sup>, volatilization, chemical decomposition (4), or metabolism by microorganisms (5) will be relatively greater from a solution in water than from a surfactant solution where the micelles act as a reservoir of preservative.

In this paper, the theoretical basis of capacity is developed more fully for solubilized systems and the theory is extended to oil–water dispersions and oil-in-water emulsions. The necessary physicochemical parameters were obtained previously (1, 6).

#### THEORETICAL

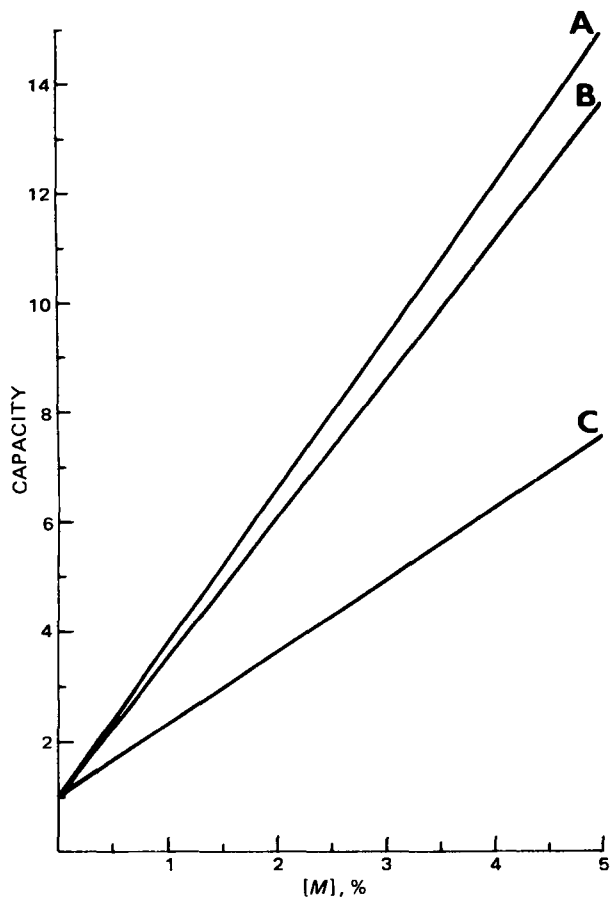
**Capacity of Solubilized Systems**—For a given loss of preservative, the decrease in concentration of free (unbound or nonmicellar) preservative in a solubilized system is less than from a solution in water and decreases with surfactant concentration. The percent decrease in con-



**Figure 1**—Correlation between percent loss of chlorocresol from water and free chlorocresol from cetomacrogol solution when equal amounts of chlorocresol are removed from water and the surfactant solution. Key [cetomacrogol concentration (percent)]: A, 0.1; B, 0.5; C, 1.0; D, 2.0; E, 3.0; and F, 4.0. Curves were calculated using Eq. 1.

<sup>1</sup> AntaroX A-400.

<sup>2</sup> See Tables III and IV of Ref. 3.



**Figure 2**—Capacity as a function of surfactant concentration,  $[M]$ , for the interaction of chlorocresol with: I, where  $n_1 = 0.538$ ,  $n_2 = 142$ ,  $K_1 = 2.12 \times 10^3$  liters/mole, and  $K_2 = 1.65$  liters/mole (A); cetomacrogol, where  $n_1 = 0.697$ ,  $n_2 = 136$ ,  $K_1 = 1.87 \times 10^3$  liters/mole, and  $K_2 = 2.09$  liters/mole (B); and II, where  $n_1 = 0.432$ ,  $n_2 = 148$ ,  $K_1 = 4.97 \times 10^3$  liters/mole, and  $K_2 = 3.16$  liters/mole (C). Curves were calculated using Eq. 8.

centration of free preservative from a solubilized system is given by:

$$\text{percent decrease} = \frac{([D_f] - [D_f'])}{[D_f]} \times 100 \quad (\text{Eq. 1})$$

where  $[D_f]$  is the initial free preservative concentration in a solution that contains an initial total preservative concentration,  $[D_t]$ ;  $[D_f']$  is the free preservative concentration remaining after a loss of preservative from solution,  $[D_{t1}]$ ; and  $[D_t] - [D_{t1}]$  is the remaining total preservative concentration,  $[D_t']$ .

The total preservative concentration,  $[D_t]$ , present initially in the chlorocresol-cetomacrogol system described previously (1) is given by:

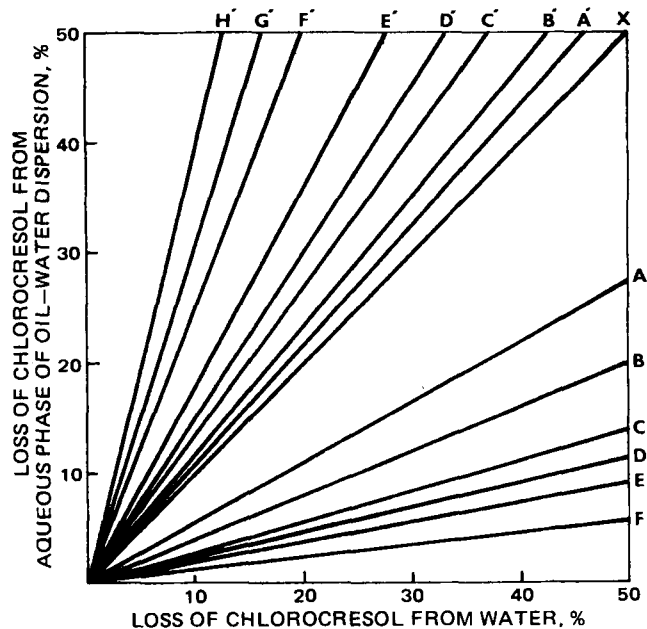
$$[D_t] = [D_f] \left[ 1 + \frac{n_1 K_1 [M]}{1 + K_1 [D_f]} + \frac{n_2 K_2 [M]}{1 + K_2 [D_f]} \right] \quad (\text{Eq. 2})$$

where  $n_1$  is the number of independent binding sites on the surfactant molecule of Set 1 and  $K_1$  is the corresponding association constant,  $n_2$  is the number of independent binding sites on the surfactant molecule of Set 2 and  $K_2$  is the corresponding association constant, and  $[M]$  is the surfactant concentration. Rearrangement of Eq. 2 gives Eq. 3, from which  $[D_f']$  can be obtained:

$$K_1 K_2 [D_f']^3 + (K_1 + K_2 + n_1 K_1 K_2 [M] + n_2 K_1 K_2 [M] - K_1 K_2 [D_t'] [D_f']^2 + 1 n_1 K_1 [M] + n_2 K_2 [M] - K_1 [D_t'] + K_2 [D_t']) [D_f'] - [D_t'] = 0 \quad (\text{Eq. 3})$$

The roots of Eq. 3 were calculated using the Bairstows method<sup>3</sup>.

When equal amounts of chlorocresol are removed from water and a cetomacrogol solution, there is a direct relationship between the loss of preservative from water and the loss of free preservative from the solubilized system (Fig. 1). The slope of the curve increases as the surfactant



**Figure 3**—Comparison between percent loss of chlorocresol from water and the aqueous phase of a hypothetical oil-water dispersion when equal amounts of preservative are removed from water and the dispersion. When  $K_w^0 = 0.1$ ,  $q = 0.1$  (A'),  $0.2$  (B'),  $0.4$  (C'),  $0.6$  (D'),  $1.0$  (E'),  $2.0$  (F'),  $3.0$  (G'), and  $5.0$  (H'). When  $K_w^0 = 1.0$ ,  $q = 0.0-\infty$  (X). When  $K_w^0 = 10.0$ ,  $q = 0.1$  (A),  $0.2$  (B),  $0.4$  (C),  $0.6$  (D),  $1.0$  (E), and  $5.0$  (F). Curves were calculated using Eq. 1.

concentration decreases and approaches that of water, which, with a value of unity, offers no resistance to losses of preservative.

If capacity is defined as the ability of a system to resist losses in  $[D_f]$ , then the inverse of the slope of a given curve in Fig. 1 becomes a numerical expression of capacity:

capacity = 1/slope

$$= \frac{\text{percent loss of preservative from water}}{\text{percent loss of free preservative from solubilized system}} \quad (\text{Eq. 4})$$

$$\text{capacity} = \frac{[D_{t1}]/[D_f]}{([D_f] - [D_f'])/[D_f]} \quad (\text{Eq. 5})$$

where:

$$[D_{t1}] = [D_t] - [D_t'] \quad (\text{Eq. 6})$$

Capacity increases with an increase in the surfactant concentration according to Eq. 4 and Allawala and Riegelman's (2) definition. The relationship between capacity as defined by Eq. 4 and surfactant concentration can be derived from Eqs. 6 and 2:

$$[D_{t1}] = [D_f] \left[ 1 + \frac{n_1 K_1 [M]}{1 + K_1 [D_f]} + \frac{n_2 K_2 [M]}{1 + K_2 [D_f]} \right] - [D_f'] \left[ 1 + \frac{n_1 K_1 [M]}{1 + K_1 [D_f']} + \frac{n_2 K_2 [M]}{1 + K_2 [D_f']} \right] \quad (\text{Eq. 7})$$

Substituting  $[D_{t1}]$  from Eq. 7 into Eq. 5 and rearranging give:

$$\text{capacity} = 1 + \left[ \frac{n_1 K_1}{1 + K_1 [D_f] + K_1 [D_f'] + K_1^2 [D_f] [D_f']} + \frac{n_2 K_2}{1 + K_2 [D_f] + K_2 [D_f'] + K_2^2 [D_f] [D_f']} \right] [M] \quad (\text{Eq. 8a})$$

$$\text{capacity} = 1 + \left[ \frac{n_1 K_1}{x_1} + \frac{n_2 K_2}{x_2} \right] [M] \quad (\text{Eq. 8b})$$

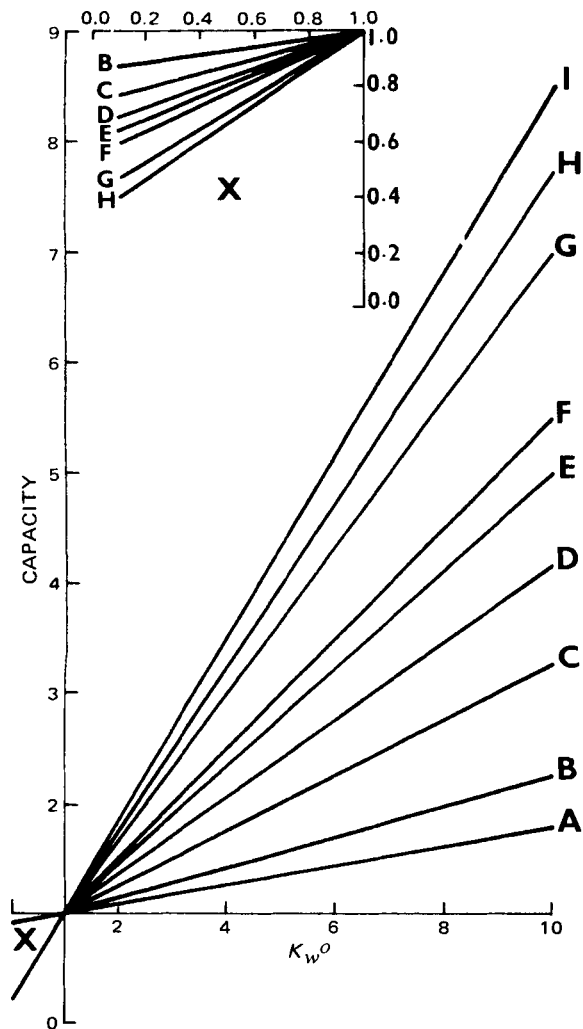
where:

$$x_1 = 1 + K_1 [D_f] + K_1 [D_f'] + K_1^2 [D_f] [D_f'] \quad (\text{Eq. 9a})$$

$$x_2 = 1 + K_2 [D_f] + K_2 [D_f'] + K_2^2 [D_f] [D_f'] \quad (\text{Eq. 9b})$$

Investigations of the effect of surfactant concentration on the antimicrobial activity of preservatives frequently include solutions of the preservative in water for comparison (1, 2, 7). Because of the difference

<sup>3</sup> University of British Columbia computer program Z POLY.



**Figure 4**—Capacity as a function of  $K_w^o$  for the distribution of chlorocresol in hypothetical oil-water dispersions. Key (oil-water ratio): A, 0.1; B, 0.2; C, 0.4; D, 0.6; E, 0.8; F, 1.0; G, 2.0; H, 3.0; and I, 5.0. Curves were plotted according to Eq. 12b.

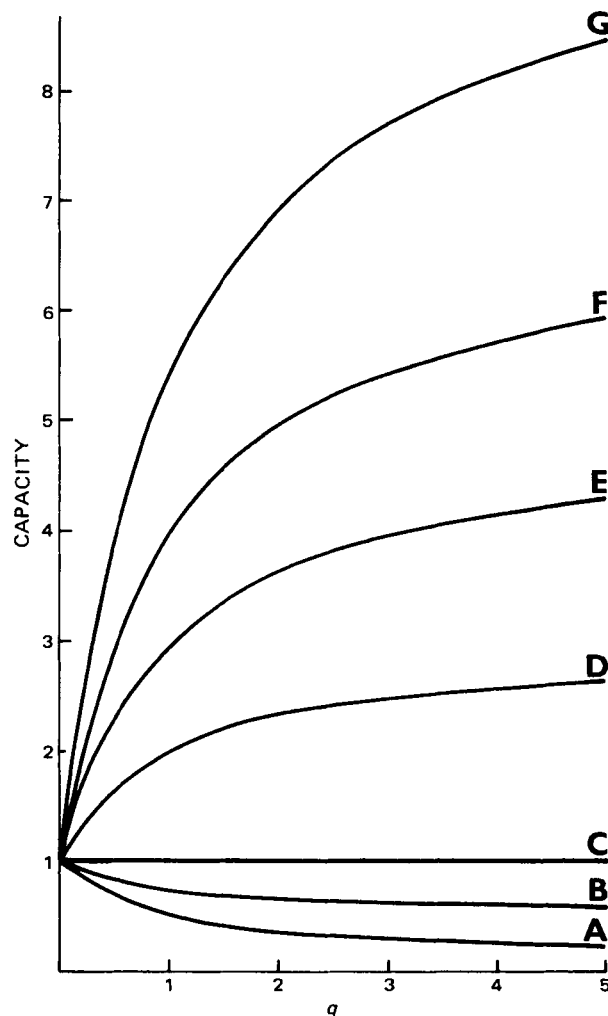
in capacity between surfactant solutions and solutions in water, such comparisons may be misleading unless either  $[D_f]$  remains comparable throughout the experiment or the duration of the experiment is too short for the capacity of the system to exert an effect. It is better to compare the antimicrobial activity of various solubilized systems having the same initial value of  $[D_f]$ . During an experiment, changes in  $[D_f]$  due to interaction of preservative or surfactant with the microorganisms or other factors will be relatively small, so any enhancement or diminution of activity can be attributed to the differences in surfactant concentration.

The interaction of chlorocresol with cetomacrogol and two other *n*-alkylpolyoxyethylene surfactants, designated<sup>4</sup> I and II, when plotted according to Eq. 8 (Fig. 2) shows that the capacity of the solubilized systems becomes smaller as the ethylene oxide chain length decreases. The efficiency of solubilization decreases with an increase in the number of ethylene oxide units (6).

**Capacity of Oil-Water Dispersions**—The percent loss of preservative from the aqueous phase of an oil-water dispersion for a given loss of preservative from the total system can be calculated in a similar manner to surfactant solutions, except  $[D_f']$  for a given  $[D_f]$  is calculated using (8):

$$[D_f'] = \frac{[D_f](q+1)}{K_w^o q + 1} \quad (\text{Eq. 10})$$

<sup>4</sup> Texofor A16 and Texofor A60, respectively, Glovers Chemicals Ltd., Leeds, England. The general formula of *n*-alkylpolyoxyethylene surfactants is  $\text{CH}_3(\text{CH}_2)_m(\text{OCH}_2\text{CH}_2)_n\text{OH}$ , where  $m = 15$  and  $n = 16$  for A16,  $m = 15$  or 17 and  $n = 20$ –24 for cetomacrogol, and  $m = 15$  and  $n = 77$  for A60 (6).



**Figure 5**—Capacity as a function of the oil-water ratio,  $q$ , for the distribution of chlorocresol in hypothetical oil-water dispersions. Key ( $K_w^o$ ): A, 0.1; B, 0.5; C, 1.0; D, 3.0; E, 5.0; F, 7.0; and G, 10.0. Curves were plotted according to Eq. 13.

where  $K_w^o$  is the experimentally determined oil-water partition coefficient and  $q$  is the oil-water ratio.

Figure 3 shows a direct relationship between the percent loss of chlorocresol from water and the aqueous phase of hypothetical oil-water dispersions when an equal amount of preservative is removed from water and the oil-water dispersion. The percent loss from the aqueous phase of oil-water dispersions increases with an increase in  $q$  when  $K_w^o < 1.0$ ; it is independent of  $q$  when  $K_w^o = 1.0$  and decreases with an increase in  $q$  when  $K_w^o > 1.0$ . As before, the capacity of a system to resist changes in  $[D_f]$  is inversely proportional to the slope of a given line. A slope equal to or greater than one means the system offers no resistance to changes in  $[D_f]$ .

The relationship between capacity and  $K_w^o q$  can be defined as follows. From Eqs. 6 and 10:

$$[D_{t1}] = [D_f] \left[ \frac{K_w^o q + 1}{q + 1} \right] - [D_f'] \left[ \frac{K_w^o q + 1}{q + 1} \right] \quad (\text{Eq. 11})$$

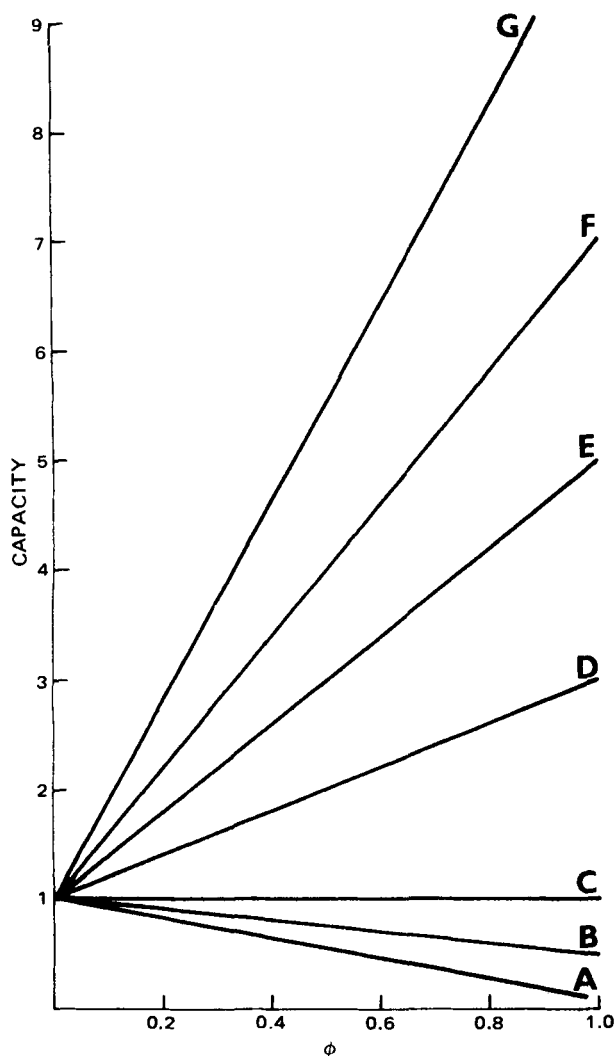
Substituting  $[D_{t1}]$  from Eq. 11 into Eq. 5 and rearranging give:

$$\text{capacity} = \frac{K_w^o q + 1}{q + 1} \quad (\text{Eq. 12a})$$

or:

$$\text{capacity} = \left( \frac{1}{1+q} \right) + \left( \frac{q}{q+1} \right) K_w^o \quad (\text{Eq. 12b})$$

The capacity of various hypothetical oil-water dispersions plotted according to Eq. 12b is shown in Fig. 4. For a given value of  $q$ , the capacity increases when  $K_w^o > 1.0$  and decreases when  $K_w^o < 1.0$ . When  $K_w^o = 1.0$ , the capacity is independent of  $q$  (see also Fig. 5) and its value is unity.



**Figure 6**—Capacity as a function of the volume fraction of the oil phase,  $\phi$ , for the distribution of chlorocresol in hypothetical oil-water dispersions. Partition coefficients of curves A–G are as in Fig. 5. Curves were plotted according to Eq. 14.

Rearrangement of Eq. 12b shows that the relationship between capacity and  $q$  is nonlinear:

$$\text{capacity} = 1 + \left( \frac{K_w^\circ - 1}{1 + q} \right) q \quad (\text{Eq. 13})$$

Figure 5 shows the curvilinear relationship between capacity and  $q$  for various values of  $K_w^\circ$ . The intercept of 1.0 is the capacity of water in the absence of an oil phase. The capacity decreases with an increase in  $q$  when  $K_w^\circ < 1.0$ , increases with an increase in  $q$  when  $K_w^\circ > 1.0$ , and is independent of  $q$  when  $K_w^\circ = 1.0$ .

When  $q$  is expressed in terms of the volume fraction of the oil phase,  $\phi$ , Eq. 12b becomes:

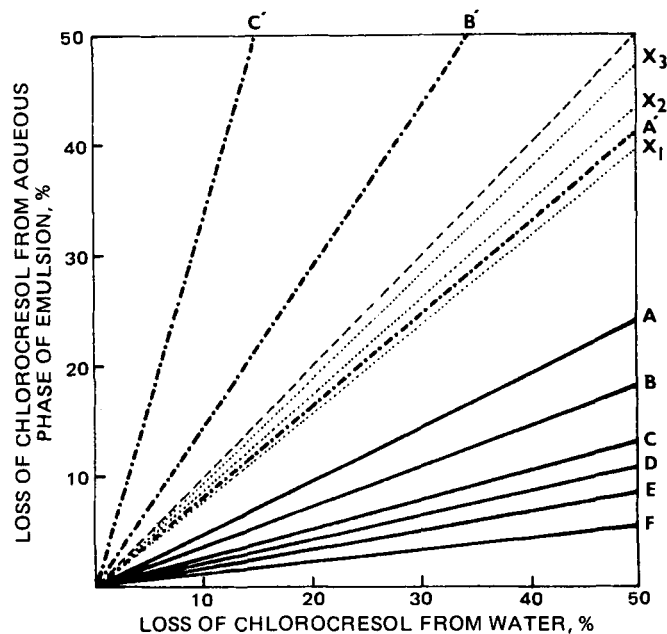
$$\text{capacity} = 1 + (K_w^\circ - 1)\phi \quad (\text{Eq. 14})$$

where  $\phi = q/(q + 1)$ .

Equation 14 gives a linear relationship between capacity and  $\phi$  for a given  $K_w^\circ$  (Fig. 6). When  $\phi$  approaches a value of 1.0, the capacity becomes equal to  $K_w^\circ$ . Hence,  $K_w^\circ$  is a measure of the capacity of the oil phase in the absence of an aqueous phase.

**Capacity of Emulsions**—The method used to calculate the percent loss of chlorocresol from the aqueous phase of an emulsion stabilized with cetomacrogol for a given loss of preservative from the total system is similar to that already given except that  $[D_f']$  in Eq. 1 is calculated using:

$$\begin{aligned} & (K_1K_2 + K_w^\circ q K_1K_2)[D_f']^3 + (K_1 + K_2 + n_1K_1K_2[M] + n_2K_1K_2[M] \\ & + K_w^\circ q K_1 + K_w^\circ q K_2 - K_1K_2[D_f']q - K_1K_2[D_f']^2) \\ & + (1 + n_1K_1[M] + n_2K_2[M] + K_w^\circ q - K_1[D_f']q - K_1[D_f'] \\ & - K_2[D_f']q - K_2[D_f']^2)[D_f'] - [D_f'](q + 1) = 0 \quad (\text{Eq. 15}) \end{aligned}$$



**Figure 7**—Comparison between the percent loss of chlorocresol from water and the aqueous phase of a hypothetical oil-in-water emulsion stabilized with 0.1% cetomacrogol when equal amounts of preservative are removed from water and the emulsion. When  $K_w^\circ = 0.1$ ,  $q = 0.1$  (A'), 1.0 (B'), and 5.0 (C'). When  $K_w^\circ = 1.0$ ,  $q = 0.1$  (X<sub>1</sub>), 1.0 (X<sub>2</sub>), and 5.0 (X<sub>3</sub>). When  $K_w^\circ = 10.0$ ,  $q = 0.1$  (A), 0.2 (B), 0.4 (C), 0.6 (D), 1.0 (E), and 5.0 (F). Curves were calculated using Eq. 1.

which is a rearrangement of (1):

$$[D_t] = [D_f] \left[ 1 + \frac{n_1K_1[M]}{1 + K_1[D_f]} + \frac{n_2K_2[M]}{1 + K_2[D_f]} \right] / q + 1 \quad (\text{Eq. 16})$$

The roots of Eq. 15 were calculated using the Bairstows method<sup>3</sup>.

Figure 7 shows a direct relationship between the percent loss of chlorocresol from water and the aqueous phase of hypothetical oil-in-water emulsions stabilized with 0.1% (w/v) cetomacrogol when an equal amount of preservative is removed from water and the emulsion. For a given percent loss of preservative from water, the percent loss from the aqueous phase of the emulsion is a function of  $q$ ,  $K_w^\circ$ , and  $[M]$ . As before, the capacity of a system to resist changes in  $[D_f]$  is inversely proportional to the slope of a given line. A slope equal to or greater than one means that the system offers no resistance to changes in  $[D_f]$ .

For a given oil-water ratio, the value of the slope is a function of  $[M]$  and  $K_w^\circ$ . Figure 7 shows that for a given  $[M]$ , an increase in  $q$  results in a decrease in the value of the slope when  $K_w^\circ = 10.0$ . For the same  $[M]$ , increasing  $q$  results in an increase in the value of the slope when  $K_w^\circ = 0.1$  and  $K_w^\circ = 1.0$ . Thus, unlike surfactant and oil-water systems where capacity is determined only by  $[M]$  and  $K_w^\circ q$ , respectively, in emulsified systems the capacity is a result of the interaction among the parameters  $[M]$ ,  $K_w^\circ$ , and  $q$ .

The relationship between the capacity of oil-in-water emulsions and  $[M]$ ,  $K_w^\circ$ , and  $q$  can be derived from Eqs. 6 and 16:

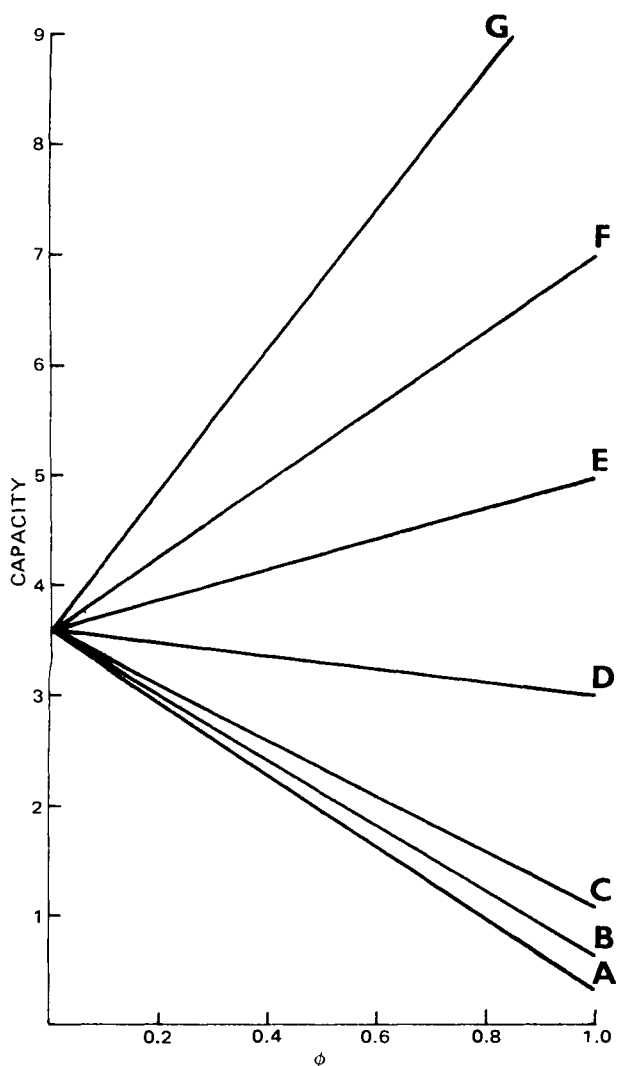
$$\begin{aligned} [D_{t1}] = & \left\{ \left[ [D_f] \left( 1 + \frac{n_1K_1[M]}{1 + K_1[D_f]} + \frac{n_2K_2[M]}{1 + K_2[D_f]} + K_w^\circ q \right) \right] / (q + 1) \right\} \\ & - \left\{ \left[ [D_f'] \left( 1 + \frac{n_1K_1[M]}{1 + K_1[D_f']} + \frac{n_2K_2[M]}{1 + K_2[D_f']} + K_w^\circ q \right) \right] / (q + 1) \right\} \quad (\text{Eq. 17}) \end{aligned}$$

Substituting  $[D_{t1}]$  from Eq. 17 into Eq. 5 and rearranging give:

$$\begin{aligned} \text{capacity} = & \left( 1 + \frac{n_1K_1[M]}{1 + K_1[D_f] + K_1[D_f'] + K_1^2[D_f][D_f']} \right. \\ & \left. + \frac{n_2K_2[M]}{1 + K_2[D_f] + K_2[D_f'] + K_2^2[D_f][D_f'] + K_w^\circ q} \right) / (q + 1) \quad (\text{Eq. 18a}) \end{aligned}$$

$$\text{capacity} = \left( 1 + \frac{n_1K_1[M]}{x_1} + \frac{n_2K_2[M]}{x_2} + K_w^\circ q \right) / (q + 1) \quad (\text{Eq. 18b})$$

where  $x_1$  and  $x_2$  are as already defined.



**Figure 8**—Capacity as a function of the volume fraction of the oil phase,  $\phi$ , for the distribution of chlorocresol in hypothetical oil-in-water emulsions stabilized with 1.0% (w/v) cetomacrogol. Key ( $K_w^o$ ): A, 0.1, B, 0.5; C, 1.0; D, 3.0; E, 5.0; F, 7.0; and G, 10.0. Curves were plotted according to Eq. 19.

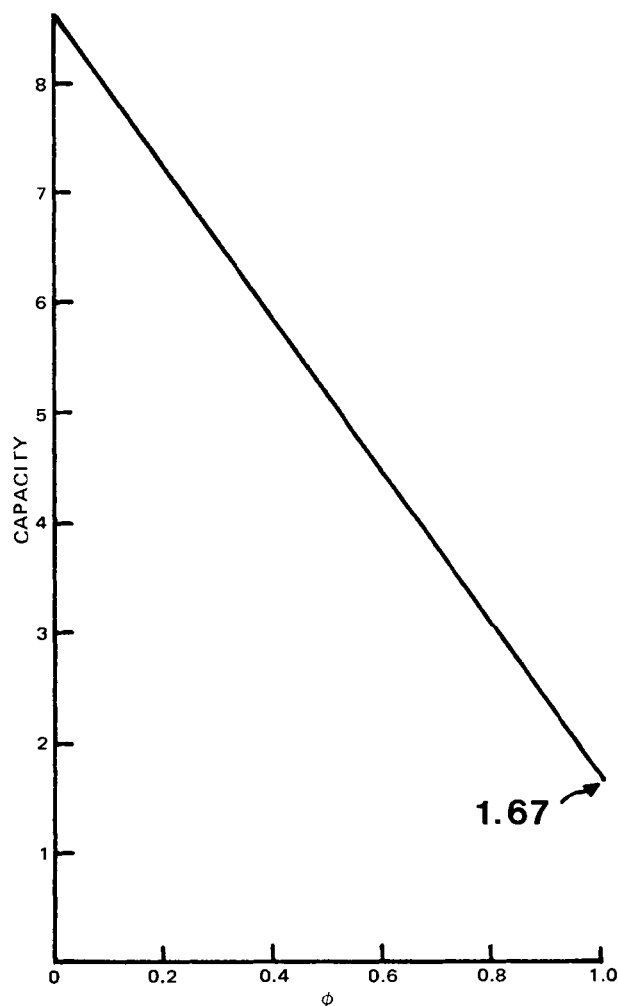
When  $q$  is expressed in terms of the volume fraction of the oil phase,  $\phi$ , the relation between capacity and  $[M]$ ,  $K_w^o$ , and  $\phi$  is given by:

$$\text{capacity} = \left(1 + \frac{n_1 K_1 [M]}{x_1} + \frac{n_2 K_2 [M]}{x_2}\right) - \left(1 + \frac{n_1 K_1 [M]}{x_1} + \frac{n_2 K_2 [M]}{x_2} - K_w^o\right) \phi \quad (\text{Eq. 19})$$

Figure 8 shows the results for the distribution of chlorocresol in hypothetical emulsions stabilized with 1.0% (w/v) cetomacrogol plotted according to Eq. 19. The intercept gives the capacity of the aqueous phase (containing surfactant) in the absence of an oil phase; *i.e.*, when  $\phi = 0$ , the capacity is given by Eq. 8b.

At fixed  $[M]$ , an increase in  $\phi$  decreases the capacity up to a certain value of  $K_w^o$  (curves A–D, Fig. 8); beyond that value, an increase in  $\phi$  results in an increase in capacity (curves E–G). For all curves, when  $\phi^{\text{lim}} = 1$ , then capacity =  $K_w^o$ , which is the capacity of the oil phase in the absence of an aqueous phase. According to Eq. 19, the overall capacity of the emulsion is determined by two factors: the capacity of the oil phase and the capacity of the surfactant-containing aqueous phase. The capacity of the oil phase is governed by  $K_w^o$ ; the capacity of the aqueous phase is controlled by the surfactant concentration,  $[M]$ , and the binding affinity of the surfactant for the preservative ( $n$ 's and  $K$ 's).

If the capacity of the oil phase is less than the capacity of the aqueous surfactant phase, then an increase in  $\phi$  results in a decrease in the overall capacity of the system (curves A–D, Fig. 8). This aspect is further illustrated in Fig. 9, which shows the decrease in capacity with  $\phi$  for mineral



**Figure 9**—Capacity as a function of the volume fraction of the oil phase,  $\phi$ , for the distribution of chlorocresol in mineral oil emulsions stabilized with 3.0% (w/v) cetomacrogol, plotted according to Eq. 19, with  $K_w^o$  (mineral oil) = 1.67.

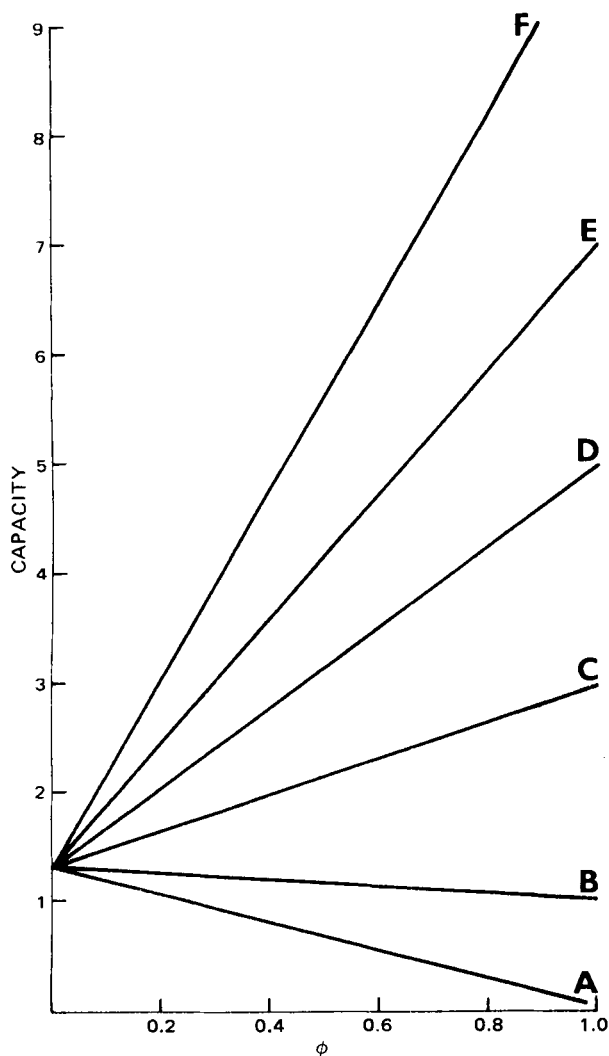
oil emulsions (1) stabilized with 3.0% (w/v) cetomacrogol. Conversely, if the capacity of the aqueous phase is less than the capacity of the oil phase, then an increase in  $\phi$  results in an increase in the capacity of the emulsion (curves E–G, Fig. 8). As the capacity of the aqueous phase approaches the capacity of water, *e.g.*, with dilute surfactant solutions [0.1% (w/v) cetomacrogol in Fig. 10], an increase in  $\phi$  results in an increase in the capacity for all values of  $K_w^o > 1.0$ .

## CONCLUSIONS

The concept of capacity, defined as the ability of solubilized and emulsified systems to resist losses in the concentration of free preservative, was developed theoretically from equations used previously to quantitate the distribution of chlorocresol in solubilized and emulsified systems stabilized with the nonionic surfactant cetomacrogol. The treatment should apply to other preservative–nonionic surfactant combinations for which the interaction can be described by the same binding expression (6).

The capacity of a system should affect its ability to withstand microbial contamination. High capacity systems should be able to resist losses in preservative concentration due to such factors as: (a) adsorption onto, or complexation with, the container and closure surfaces; (b) adsorption, absorption, or chemical reaction with contaminants including microorganisms; and (c) chemical decomposition and volatilization.

The effectiveness of a preservative in a complex disperse system should depend on the capacity of the system and the concentration of the free preservative. However, the magnitude of the capacity factor's contribution to the overall antimicrobial activity of a preservative will vary according to the testing procedure adopted. Thus, the effect of capacity is likely to become apparent in procedures that involve sampling for



**Figure 10**—Capacity as a function of the volume fraction of the oil phase,  $\phi$ , for the distribution of chlorocresol in hypothetical oil-in-water emulsions stabilized with 0.1% (w/v) cetomacrogol. Key ( $K_w^0$ ): A, 0.1; B, 1.0; C, 3.0; D, 5.0; E, 7.0; ad F, 10.0. Curves were plotted according to Eq. 19.

microorganisms over prolonged periods, e.g., days, weeks, or months [compared with hours as used in Part I (1)], or challenge tests (9, 10) in which the preparation is reinoculated with microorganisms over a long storage period. Such procedures allow time for the preservative to re-equilibrate between the various phases following any depletion and for this reequilibration to affect the antimicrobial action. Incomplete understanding of the capacity factor and its significance is a probable reason for some controversy regarding the evaluation of preservative effectiveness in solubilized and emulsified systems.

Capacity equations should be useful in the formulation of high capacity systems containing the least amount of preservative necessary for adequate preservation. In view of the number of variables involved in the preservation of solubilized and emulsified systems, however, a choice of preservative concentration based solely on physicochemical measurements cannot replace a final microbiological evaluation of the product for its ability to withstand microbial contamination. Nevertheless, determination of the appropriate physicochemical parameters provides a logical first step in estimating the total amount of preservative required and should help to avoid much "trial-and-error" formulation when purely microbiological techniques are employed.

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