theory). Chromatography of the solution vehicle showed no interfering peaks. Benzyl alcohol, present in the sterile solution as a preservative, can be detected (Fig. 4) and quantitated.

Possible impurities or degradation products of clindamycin phosphate are free clindamycin, clindamycin 3-phosphate, clindamycin 4-phosphate, and clindamycin 2-phosphate. Since clindamycin is produced by chemical modification of lincomycin, the lincomyin analogs of the listed compounds are also possible impurities. Figure 4 shows a chromatogram of clindamycin phosphate with clindamycin, clindamycin 2-phosphate, clindamycin 3-phosphate, and clindamycin 4-phosphate present. Clindamycin 3-phosphate and clindamycin 4-phosphate are almost baseline separated from clindamycin 2-phosphate but are not separated from each other under the assay conditions.

The separation and elution order of the 2-, 3-, and 4-phosphate analogs of clindamycin are dependent on the pH and composition of the mobile phase. Mobile phase containing a greater percent of water causes clindamycin 4-phosphate to be eluted after clindamycin 2-phosphate while clindamycin 3-phosphate is eluted at the same time as clindamycin 2-phosphate. This modified mobile phase also shifts the clindamycin elution to a position before the internal standard peak.

Morozowich and Williams (5) chromatographed the clindamycin phosphate analogs on triethylaminoethylcellulose. The order of elution on the cellulose was 4-, 3-, and 2-phosphates. With the mobile phase of this assay, the relative retention times of possible impurities and degradation products are shown in Table I. If the mobile phase was adjusted to pH 6.0 or higher, the clindamycin phosphate peak tailed badly. If the pH was adjusted to 5.6 or less, the clindamycin phosphate peak broadened on the front side. Use of ammonium nitrate or sodium nitrate in the mobile phase did not improve the chromatography. To compensate for column-to-column variation, the amount of water in the mobile phases used for all three antibiotics was modified to give satisfactory chromatography for any particular column.

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pH–Solubility Profiles of Organic Carboxylic Acids and Their Salts

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Abstract □ The solubilities of naproxen, 7-methylsulfinyl-2-xanthonecarboxylic acid, 7-methylthio-2-xanthonecarboxylic acid, and their sodium, potassium, calcium, and magnesium salts were determined as a function of pH. The results on the solubility of naproxen and its salts were in excellent agreement with the theoretical profiles describing the relationship between pH values of the solutions and the dissociation constant of the acid. The solubilities of the two xanthonecarboxylic acids were higher at higher pH values than the values calculated when complete dissociation in solution was assumed. The influence of the salt species on the solubility of organic carboxylic acids, at and above pH values of complete ionization, cannot be predicted even qualitatively from equations used for alkali and alkaline earth metal salts.

Keyphrases □ Solubility—naproxen, two xanthonecarboxylic acids, and various salts, as a function of pH □ Naproxen—and various salts, solubility as a function of pH □ Xanthonecarboxylic acids, substituted—and various salts, solubility as a function of pH □ Anti-inflammatory agents—naproxen and various salts, solubility as a function of pH

Organic carboxylic acids used as medicinal agents generally have poor water solubilities. Since solubility is an important factor in the overall drug absorption process, chemical stability, and formulation of dosage forms, salt-forming agents are used to increase the water solubility. Although salt formation results in an overall increase in solubility, no definitive quantitative methods of predicting the solubility of several salt species of the parent compound are available.

The influence of pH on the solubility of weak electrolytes was reported previously (1–3). The solubility interrelationships of the hydrochloride salt and free base of two amines were investigated extensively (4). Mathematical equations describing the total solubility at an arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to the data with good results.

This paper discusses the solubility of three organic carboxylic acids as a function of pH and the salt species. The data were fitted to mathematical relationships similar to those used for organic hydrochlorides (4). The results of the solubility of naproxen and its salts were in excellent agreement with the theory. However, the solubilities of the xanthonecarboxylic acids and their salts were higher at higher pH values than those calculated when complete dissociation in solution was assumed. The effect of the salt species on the solubility of organic carboxylic acids, at and above pH values of complete ionization, cannot be predicted even qualitatively from equations used for alkali and alkaline earth metal salts.

EXPERIMENTAL

Materials—Naproxen [*d*-2-(6-methoxy-2-naphthyl)propionic acid], 7-methylsulfinyl-2-xanthonecarboxylic acid, 7-methylthio-2-xanthonecarboxylic acid, and their sodium salts were at least 99% pure[1]. Other chemicals were analytical reagent grade unless otherwise indicated.

Preparation of Naproxen Potassium—Potassium bicarbonate, 21.7 g, was added to a 500-ml round-bottom flask and dissolved in 26 ml of distilled water. An equimolar quantity of naproxen was added slowly to the flask, and the contents were refluxed for about 2 hr or until all naproxen dissolved. Then the contents of the flask were poured into a petri dish and allowed to crystallize. The crystals were filtered, rinsed with

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acetone, and dried in a vacuum oven at 50°. The crystals were ground with a pestle and mortar before solubility determinations.

Preparation of Naproxen Calcium—The calcium salt of naproxen was prepared by dissolving 3.5 g of calcium acetate monohydrate in 50 ml of distilled water and adding an equimolar quantity of naproxen sodium dissolved in 25 ml of distilled water. After overnight standing, the precipitate was filtered, washed with one part of water and one part of acetone, and dried in a vacuum oven at 50°.

Preparation of Naproxen Magnesium—The magnesium salt of naproxen was prepared by dissolving 4.2 g of the monohydrated magnesium chloride in 50 ml of distilled water and adding an equimolar quantity of naproxen sodium dissolved in 25 ml of distilled water. After overnight standing, the precipitate was filtered, washed with one part of water and one part of acetone, and dried in a vacuum oven at 50°.

Preparation of Potassium Salts of Xanthonecarboxylic Acids—Potassium bicarbonate, 5 g, was dissolved in 100 ml of distilled water, and 200 ml of 1-propanol was added. An equimolar quantity of the xanthonecarboxylic acid was added and refluxed for 2 hr. Then the reaction mixture was allowed to cool overnight. The crystallized salt was filtered, washed with 1-propanol, and dried in a vacuum oven at 50°.

Preparation of Calcium Salts of Xanthonecarboxylic Acids—The calcium salt of the xanthonecarboxylic acid was prepared by dissolving 2.5 g of the potassium salt in 400 ml of hot distilled water and adding an equimolar quantity of calcium acetate dissolved in 25 ml of distilled water. 1-Propanol, 50 ml, was added to the reaction mixture, and it was then allowed to stand overnight. The crystallized calcium salt was filtered, washed with methanol, and dried in a vacuum oven at 50°.

Preparation of Magnesium Salts of Xanthonecarboxylic Acids—The potassium salt of the xanthonecarboxylic acid, 2.5 g, was dissolved in 400 ml of hot distilled water, and an equimolar quantity of magnesium chloride dissolved in 4 ml of distilled water was added. The crystallized salt was allowed to stand overnight and was then filtered, washed with ethanol, and dried in a vacuum oven at 50°.

Solubility Determinations—The solubility of the carboxylic acids around pH 2.0 was determined by equilibrating the carboxylic acids in dilute hydrochloric acid solutions of different pH values at 25°. Naproxen solubility above pH 4.0 was determined by adding different amounts of dilute sodium hydroxide or dilute potassium hydroxide and equilibrating at 25° to obtain appropriate pH values. The naproxen salts were equilibrated at 25° in water containing small amounts of dilute sodium hydroxide to reach the desired pH values.

The solubility of 7-methylsulfinyl-2-xanthonecarboxylic acid was obtained similarly, except that only potassium hydroxide solution was used to titrate the acid and equilibrated to obtain intermediate pH values.

All four salts of the 7-methylthio-2-xanthonecarboxylic acid were used to obtain the pH profiles. Appropriate amounts of sodium hydroxide solution or dilute hydrochloric acid were used followed by equilibration at 25°. The pH values were determined after equilibrium had been reached.

The samples were prepared in culture tubes, wrapped with aluminum foil, and allowed to equilibrate in a vibromixer[2] at 25°. After equilibration, the samples were filtered through 13-mm diameter and 0.45-μm average pore size filters[3]. The solution pH was determined with a pH meter[4]. The concentrations of the solutions were determined spectrophotometrically. The UV absorbance was determined at 332 nm for naproxen, at 342 nm for 7-methylsulfinyl-2-xanthonecarboxylic acid, and at 262 nm for 7-methylthio-2-xanthonecarboxylic acid.

RESULTS AND DISCUSSION

The pH–solubility profiles for naproxen and its sodium, potassium, calcium, and magnesium salts are given in Fig. 1. The solid data points were obtained by adding appropriate amounts of dilute hydrochloric acid, dilute sodium hydroxide, or dilute potassium hydroxide and equilibrating to reach the final pH values. The open data points were obtained by adding the salts, adjusting the pH values with a small quantity of dilute hydrochloric acid or dilute sodium hydroxide, and then equilibrating.

The lines drawn through the data points were calculated from the simple equilibrium expression based on the dissociation of an organic acid. At any pH, the total concentration of a compound is the sum of the individual concentrations of its respective species. For example, at an intermediate pH, the total concentration of an organic acid is equal to

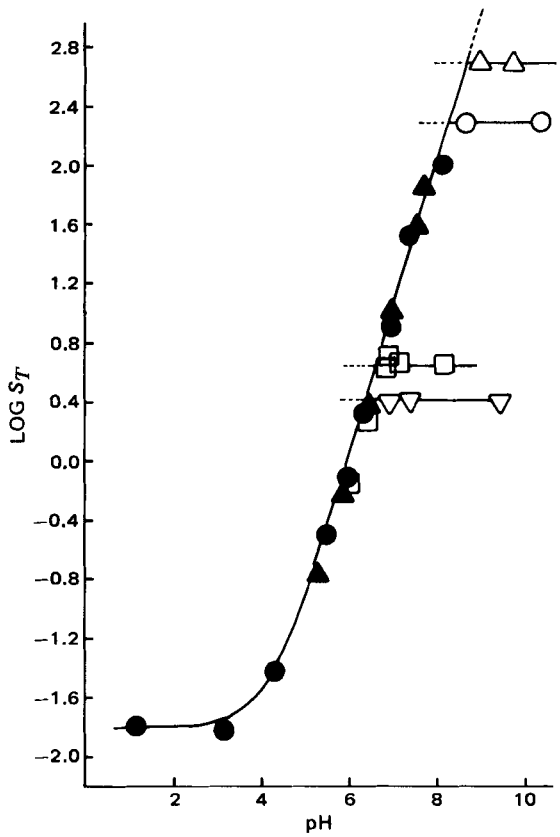

Figure 1—*The pH–solubility profiles for naproxen and its salts at 25°. Symbols represent experimental data: triangles, potassium salt; circles, sodium salt; squares, magnesium salt; and inverted triangles, calcium salt. The lines drawn through the data are theoretical and were calculated using 0.0159 mg/ml as the solubility of the free acid, 507.7 mg/ml as the solubility of the potassium salt, 196.7 mg/ml as the solubility of the sodium salt, 4.4 mg/ml as the solubility of the magnesium salt, 2.55 mg/ml as the solubility of the calcium salt, and 4.15 as the pKa′. Solid symbols indicate that the starting material was the acid, and open symbols indicate that the starting material was the salt.*

[XH] + [X$^-$], where [XH] and [X$^-$] are the concentrations of the unionized and ionized species, respectively. Equations similar to those derived for organic hydrochlorides (4) were used for the calculations at low pH and high pH values. At low pH, the following equations are applicable:

$$S_{T,\mathrm{pH}<\mathrm{pH}_{max}} = [\mathrm{XH}]_s + [\mathrm{X}^-] \qquad \text{(Eq. 1}a\text{)}$$

$$S_{T,\mathrm{pH}<\mathrm{pH}_{max}} = [\mathrm{XH}]_s \left(1 + \frac{K_a'}{[\mathrm{H_3O^+}]}\right) \qquad \text{(Eq. 1}b\text{)}$$

where S_T indicates total solubility, and subscript pH < pH_{max} indicates both that there is a pH of maximum solubility and that this equation is applicable for pH values less than this maximum. The subscript s indicates a saturated species, and K_a' is the apparent dissociation constant.

At high pH, where the solubility of the ionized species is limiting, the following equations hold:

$$S_{T,\mathrm{pH}>\mathrm{pH}_{max}} = [\mathrm{X}^-]_s + [\mathrm{XH}] \qquad \text{(Eq. 2}a\text{)}$$

$$S_{T,\mathrm{pH}>\mathrm{pH}_{max}} = [\mathrm{X}^-]_s \left(1 + \frac{[\mathrm{H_3O^+}]}{K_a'}\right) \qquad \text{(Eq. 2}b\text{)}$$

Equations 1*b* and 2*b* describe an independent curve limited by the solubility of one of the two species. Superposition of these curves produces a pH–solubility profile. The constraint that the solubility of either species cannot be exceeded determines which curve is applicable at a given pH.

At the juncture where two solubility curves (Eqs. 1*b* and 2*b*) intersect, both ionized and unionized species coexist at saturation. At the pH of maximum solubility, Eqs. 1*b* and 2*b* are simultaneously satisfied and can be set equal to one another. However, the resulting equation is of limited practical value because it involves small differences in large numbers (4).

[2] Chempac Inc., Hoboken, NJ 07030.
[3] Metricel GA-6, Gelman Instrument Co., Ann Arbor, MI 48103.
[4] Model 10, Corning Scientific Instruments.

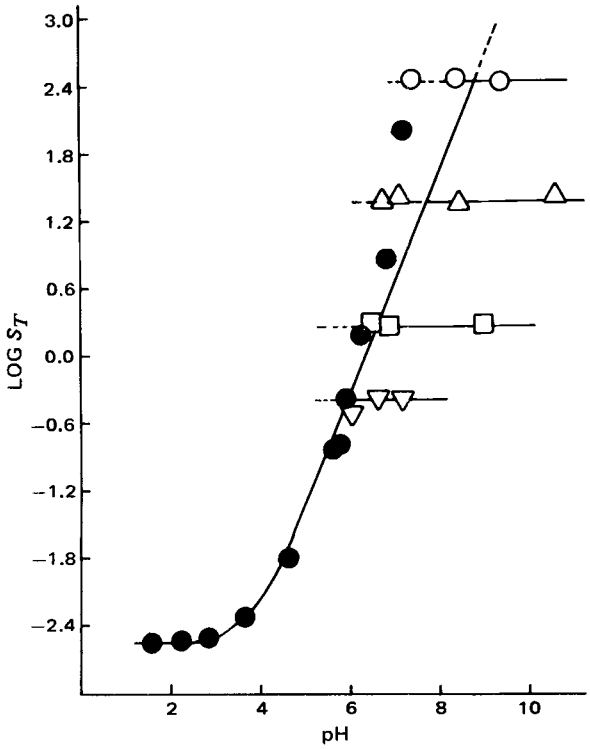

Figure 2—*The pH–solubility profiles for 7-methylsulfinyl-2-xanthonecarboxylic acid and its salts at 25°. Symbols represent experimental data: circles, potassium salt; triangles, sodium salt; squares, calcium salt; and inverted triangles, magnesium salt. The lines drawn through the data are theoretical and were calculated using 0.00274 mg/ml as the solubility of the free acid, 270.0 mg/ml as the solubility of the potassium salt, 23.84 mg/ml as the solubility of the sodium salt, 1.855 mg/ml as the solubility of the calcium salt, 0.35 mg/ml as the solubility of the magnesium salt, and 3.8 as the pKa′. Solid symbols indicate that the starting material was the acid, and open symbols indicate that the starting material was the salt.*

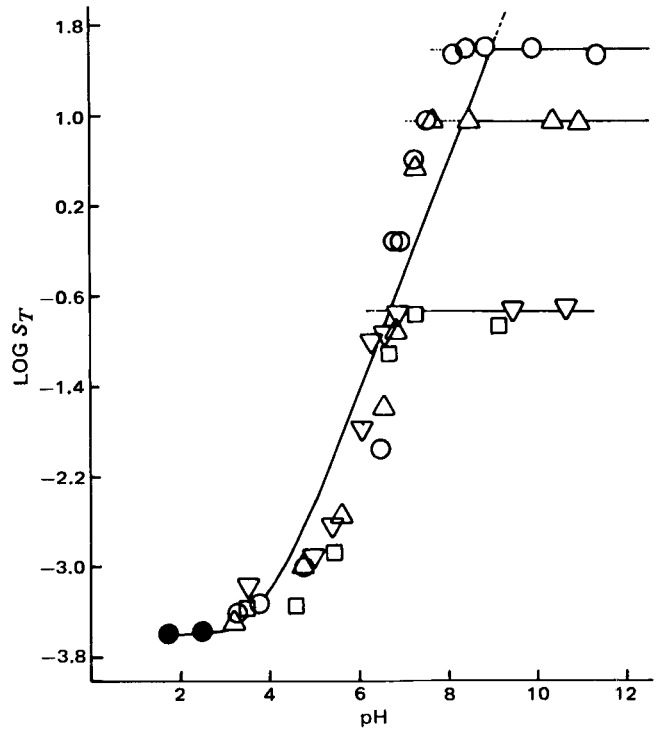

Figure 3—*The pH–solubility profiles for 7-methylthio-2-carboxylic acid and its salts at 25°. Symbols represent experimental data: circles, sodium salt; triangles, potassium salt; squares, magnesium salt; and inverted triangles, calcium salt. The lines drawn through the data are theoretical and were calculated using 0.00026 mg/ml as the solubility of the free acid, 37.2 mg/ml as the solubility of the sodium salt, 8.87 mg/ml as the solubility of the potassium salt, 0.185 mg/ml as the solubility of magnesium and calcium salts, and 3.8 as the pKa′. Solid symbols indicate that the starting material was the acid, and open symbols indicate that the starting material was the salt.*

The pH–solubility profiles for 7-methylsulfinyl-2-xanthonecarboxylic acid and its sodium, potassium, calcium, and magnesium salts are given in Fig. 2. Equations 1*b* and 2*b* were used to calculate the lines. The theoretical lines and the data points show a good fit for lower pH values and for the calcium and magnesium salts. However, the solubilities of the sodium and potassium salts at higher pH values are considerably higher compared to the calculated values.

The solubilities of 7-methylthio-2-xanthonecarboxylic acid and its sodium, potassium, calcium, and magnesium salts are given in Fig. 3. The lines drawn through the data points were calculated as described. Comparisons between theory and experiment indicate some deviations, both at low and high pH values.

Excellent agreement between theory and experiment was obtained for naproxen, although concentrations rather than activities were used in the calculations and pKa′ is not the thermodynamic value. This finding suggests that the higher solubilities of xanthonecarboxylic acids at higher pH values could be due to the complex formation or hydration of the salts. The presence of charged complexes having a different proportion of ions or the presence of undissociated species in solution results in a greater salt solubility than that calculated when complete dissociation in solution is assumed. If this is so, the solubilities predicted by Eq. 1*b* would be lower than the experimental values. Similar deviations were reported (3) for sulfadiazine.

A general prediction of the effect of salt species on the solubility of organic carboxylic acids is not possible (Figs. 1–3). The order of decreasing solubility of naproxen salts is $K^+ > Na^+ > Mg^{2+} > Ca^{2+}$; the order for 7-methylsulfinyl-2-xanthonecarboxylic acid salts is $K^+ > Na^+ > Ca^{2+} > Mg^{2+}$; and the order for 7-methylthio-2-xanthonecarboxylic acid is $Na^+ > K^+ > Ca^{2+} = Mg^{2+}$.

The solubility is determined by the molar free energy change on dissolving a solid in solution. For a monovalent organic salt, this molar free energy change is equal to the molar free energy change of hydration of the cation plus the anion, minus the molar free energy change for the formation of crystal lattice. Mathematically, this change is expressed by:

$$\Delta F_{\text{solution}} = \Delta F_{\text{hx}^-} + \Delta F_{\text{hc}^+} - \Delta F_{\text{lattice}} \qquad \text{(Eq. 3)}$$

where $\Delta F_{\text{solution}}$ is the molar free energy change on dissolving a solid in solution, ΔF_{hx^-} is the molar free energy change of hydration of the anion, ΔF_{hc^+} is the molar free energy change of hydration of the cation, and $\Delta F_{\text{lattice}}$ is the molar free energy change for the formation of the crystal lattice.

Equations describing the change in solubility in a series of similar salts in which the anion is varied and the cation is kept constant, or the cation is varied and the anion is kept constant, were derived (5). These equations represent the differences between two quantities: the change in the hydration energy and the change in the lattice energy. On increasing the value of the radius of the cation at a constant anion radius, both the hydration and the lattice energies decrease. The change in solubility depends on the amount by which these two quantities decrease. If the lattice energy decreases by a larger amount than the hydration energy, a solubility increase occurs. If the lattice energy decreases by a smaller amount than the hydration energy, a solubility decrease occurs. Similar behavior occurs on increasing the radius of the anion at a constant cation radius. For those salts in which the lattice energies are much greater than the hydration energies (and which, as a result, have slight solubility), changes in the ionic radii influence solubility mainly through changes in the lattice energies. Conversely, changes in solubility reflect changes primarily in the hydration energies when the hydration energies are much larger than the lattice energies (highly soluble salts).

The radii of the anions of organic carboxylic acids are large and, based on the calculated changes in solubility of alkali metal salts, the solubility of the potassium salt should be lower than the sodium salt. The data indicate that the solubility of the potassium salts of one of the three carboxylic acids is lower than that of the sodium salt. Similarly, based on the calculated changes in the solubility of alkaline earth metal ions, the solubility of the magnesium salt should be larger than that of the calcium salt. The solubility of the magnesium salt of one of the carboxylic acids is larger than that of the calcium salt. Lower or same solubility of the

magnesium salt of the other two carboxylic acids compared to the calcium salt again suggests that qualitative trends reported for alkali halides or alkaline earth metal salts are not seen in organic carboxylic acid salts.

In view of these results, the equations (5) predicting only qualitatively the solubilities of a series of inorganic salts cannot be used for organic salts. The solubility of several salts of the organic compound of interest should be determined experimentally, and this parameter should be considered in the selection of the most suitable drug entity for further development.

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Preservation of Solubilized and Emulsified Systems I: Correlation of Mathematically Predicted Preservative Availability with Antimicrobial Activity

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Abstract □ Mathematical models were investigated for the distribution and antimicrobial activity of chlorocresol in solubilized and emulsified systems stabilized with a nonionic surfactant. The concentration of free preservative in the solubilized systems was described adequately by an equation widely used to describe the binding of small molecules to macromolecules. For the emulsions, this equation was combined with an expression for the partitioning of the preservative between the oil and water phases. It was confirmed that short-term antimicrobial activity can be related to the free (unbound) preservative concentration in the aqueous phase and that preservative solubilized within the surfactant micelles or partitioned into the oil phase does not contribute to short-term preservation.

Keyphrases □ Chlorocresol—in solubilized and emulsified systems, mathematical model relating phase distribution to antimicrobial activity □ Models, mathematical—relating phase distribution to antimicrobial activity, chlorocresol in solubilized and emulsified systems □ Antimicrobial activity—chlorocresol in solubilized and emulsified systems, related to phase distribution with mathematical model □ Distribution, phase—chlorocresol in solubilized and emulsified systems, related to antimicrobial activity with mathematical model

The ability of preservatives to prevent microbial spoilage of solubilized and emulsified products is normally assessed by empirical tests involving inoculation of the finished product and examination during prolonged storage. Such methods are laborious, time consuming, and usually qualitative.

Mathematical models have been developed that predict the preservative concentration required in a surfactant solution (1–3) or an emulsion (2–9). These models were based on the assumption that antimicrobial activity is a function of the free (unbound or nonmicellar) preservative concentration in the aqueous phase.

Attempts to correlate the predictions made by mathematical equations with the observed antimicrobial activity of preservatives in solubilized systems generally confirmed that the antimicrobial action is largely a function of the free preservative concentration and that preservative associated with the surfactant micelles has little or no activity (10–14).

Although much work has been done on the distribution and antimicrobial activity of preservatives in oil–water systems (15–21), few studies have been made in oil-in-water emulsions stabilized with nonionic surfactants (5, 6). In simple oil-in-water dispersions, the antimicrobial activity appears to depend mainly on the preservative concentration in the aqueous phase while preservative partitioned into the oil phase is biologically inactive. However, Bean *et al.* (18, 20) suggested that preservative adsorbed at the oil–water interface also contributes to the overall activity; an increase in the oil–water ratio results in an increase in interfacial area and, therefore, should enhance the antimicrobial activity. Since the interfacial area in oil-in-water emulsions is much larger than in simple oil-in-water dispersions, antimicrobial activity might increase enormously with an increase in the oil–water ratio.

In this study, a physicochemical and microbiological evaluation of two mathematical models, one for surfactant solutions and the other for emulsions, was made to test their utility. The influence on antimicrobial activity of factors such as surfactant concentration, oil–water ratio, and interfacial area was given particular attention.

THEORETICAL

Garrett (3) suggested that the binding of preservatives with surfactant macromolecules can be quantified using expressions similar to those established for the protein binding of drugs.

Kazmi and Mitchell (1) compared various methods of expressing the interaction of a number of commonly used preservatives with the nonionic surfactant cetomacrogol and found that protein binding equations such

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