Micellar Solubilization of Cetaben Sodium in Surfactant and Lipid Solutions

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Abstract □ Cetaben sodium solubilities were evaluated by micellar solubilization in various surfactants and lipid solvents. At pH 8, the relationship between cetaben sodium solubility and surfactant concentration delineated apparent saturable kinetics; at pH 4.9, the relationship between the two parameters was linear. In the presence of 0.5% sodium taurocholate and polysorbate 80, cetaben sodium solubility increased as the medium pH was increased; however, in the presence of 0.5% poloxamer 188, cetaben sodium solubility revealed a hyperbola when the pH was changed from 4.9 to 8.0. Cetaben sodium solubility was enhanced greatly by mixed physiological surfactants, full-strength caprylic–capric monodiglycerides or monodiglycerides, when compared to a single surfactant system. Cetaben sodium solubility is influenced by pH, surfactant type, surfactant concentration, lipid solvent type, and the simultaneous presence of surfactants or phospholipids.

Keyphrases □ Cetaben sodium—micellar solubilization in surfactant and lipid solutions □ Solubility—micellization of cetaben sodium in surfactant and lipid solutions □ Surfactants—micellar solubilization of cetaben sodium □ Micellization—cetaben sodium solubilization in surfactant and lipid solutions □ Antiatherosclerotic agents—micellar solubilization of cetaben sodium in surfactant and lipid solutions

Cetaben sodium, sodium 4-(hexadecylamino)benzoate, is a new synthetic drug (1) highly effective in preventing and ameliorating the condition of atherosclerosis (2, 3). Cetaben sodium is an odorless, tasteless, white, soapy-textured powder; it is chemically stable under normal conditions and is not expected to present compatibility problems with the usual formulation excipients. However, cetaben sodium is practically insoluble in water at all pH values. As a consequence, analysis, bioavailability, and formulation problems have been encountered.

This paper reports findings that characterize some solubilities for cetaben sodium and describes the solubilization mechanism using surfactants and lipid solvents.

EXPERIMENTAL

Materials—Cetaben sodium, >99% pure, was used as obtained[1]. Purified grade sodium taurocholate[2] was found to have <1% impurities by TLC (4). In some experiments, polysorbate 80[3] and poloxamer 188[4] were used as obtained as nonionic surfactants for solubilizing cetaben sodium. L-α-Lecithin[5] (99% pure) and monoolein[6] and oleic acid[6] (>99% pure) were obtained commercially.

Caprylic–capric monodiglycerides[7], monodiglycerides[8], polyoxypropylene 15 stearyl ether[9], caprylic–capric triglycerides[10], triacetin[11], ethyl citrate[12], and medium-chain triglycerides oil[13] were used as lipid solvents as obtained. Analytical reagent grade monobasic and dibasic sodium phosphates[14] were used as buffer components. The total concentration of buffer solution was 65 m*M*. Micellar solutions of the surfactants in phosphate buffer were prepared by ultrasound irradiation for 5 min with a sonifier[15].

Solubility Determinations—An excess of cetaben sodium was added to 20 ml of micellar solutions contained in 50-ml amber screw-capped bottles. The bottles were shaken with a wrist-action shaker[16] for 48 hr in a shaker bath[17] (24 ± 0.1°). The time required for equilibration was established by a repetitive sampling technique. After equilibration, samples were filtered through 0.45-μm filters[18] held in adapters[19] and diluted appropriately if required. The samples were assayed for cetaben content by high-performance liquid chromatography (HPLC).

Analysis—Analytical determinations were carried out on an HPLC system consisting of a solvent-delivery unit[20], an injector[21], a 3.9-mm × 30-cm reversed-phase analytical column with 10-μm particles[22], a variable-wavelength UV detector[23], and a strip-chart recorder[24]. The mobile phase contained 90% methanol[25] in 2.5 m*M* ammonium acetate[25]. The system was operated at 280 nm and ambient temperature, and the mobile phase flow rate was 3 ml/min. Cetaben content was quantitated by comparing peak heights obtained from the chromatograms of experimental samples with those of chromatographed standards. Standard calibration plots of peak height *versus* concentration were linear in the concentration ranges studied.

RESULTS AND DISCUSSION

Influence of Surfactants on Cetaben Sodium Solubility—Surfactants are one of the most important groups of formulation excipients used for pharmaceutical preparations. Their unique physicochemical properties have prompted considerable interest in their possible effects on drug absorption. However, these effects seem to be specific and not unique (5). Since dissolution usually is the rate-determining step for hydrophobic drug absorption, the influence of surfactants on cetaben solubility was investigated.

The solubilities of cetaben sodium in 0.5% sodium taurocholate, polysorbate 80, and poloxamer 188 at pH 4.9 were 0.028, 0.021, and 0.016 mg/ml, respectively; at pH 8, the solubilities were 0.468, 0.129, and 0.004 mg/ml, respectively. With the same surfactant concentration, cetaben sodium was solubilized in the following order: sodium taurocholate > polysorbate 80 > poloxamer 188. This cetaben solubilization is ascribed to the micellization of the drug by the surfactant systems. In such aqueous systems, micellization is due primarily to hydrophobic interactions between lipophilic moieties of the amphiphilic molecules (6).

A striking difference in solubility with respect to pH change also can be noted from these results, as will be discussed.

Influence of pH on Cetaben Sodium Solubility—The intralumenal intestinal pH varies under normal absorptive conditions and depends on the proximity of the absorbing segment to the pylorus. Therefore, the influence of pH on cetaben sodium solubility was evaluated. The pH of the aqueous media was varied from 4.9 to 8 by changing the relative amounts of the monobasic and dibasic salts of phosphate in the media.

With sodium taurocholate and polysorbate 80, a significant increase in the cetaben sodium solubility was observed as the medium pH was

[1] Metabolic Disease Research Section, Medical Research Division, American Cyanamid Co.
[2] Calbiochem Co., San Diego, Calif.
[3] Tween 80, Fisher Scientific Co., Fair Lawn, N.J.
[4] Pluronic F68, BASF Wyandotte Corp., Wyandotte, Mich.
[5] Type III-D from egg yolk, Sigma Chemical Co., St. Louis, Mo.
[6] Sigma Chemical Co., St. Louis, Mo.
[7] Capmul 8210, Capital City Products, Cleveland, Ohio.
[8] Arlacel 186, ICI, Wilmington, Del.
[9] Arlamol E, ICI, Wilmington, Del.
[10] Neobee M5, PVO, Boonton, N.J.
[11] Triacetin, Union Carbide, New York, N.Y.
[12] Pfizer Laboratories, New York, N.Y.
[13] MCT, Mead Johnson Laboratories, Evansville, Ind.
[14] J. T. Baker Chemical Co., Phillipsburg, N.J.
[15] Branson Ultrasonic Corp., Stamford, Conn.
[16] Model 75, Burrell Corp., Pittsburgh, Pa.
[17] Eberbach Corp., Ann Arbor, Mich.
[18] Millipore Corp., Bedford, Mass.
[19] Swinnex-25, Millipore Corp., Bedford, Mass.
[20] Model M6000A pump, Waters Associates, Milford, Mass.
[21] Model U6K, Waters Associates, Milford, Mass.
[22] μBondapak C_{18}, Waters Associates, Milford, Mass.
[23] Model 450, Waters Associates, Milford, Mass.
[24] Omniscribe B-5000, Houston Instrument Division, Austin, Tex.
[25] Fisher Scientific Co., Fair Lawn, N.J.

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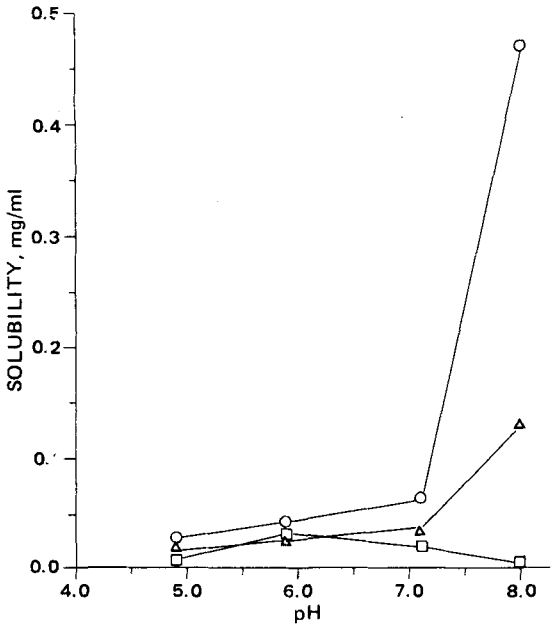

Figure 1—*Cetaben sodium solubilities in the presence of 0.5% (w/v) sodium taurocholate (○), polysorbate 80 (△), and poloxamer 188 (□) at pH 4.9, 5.9, 7.1, and 8.0.*

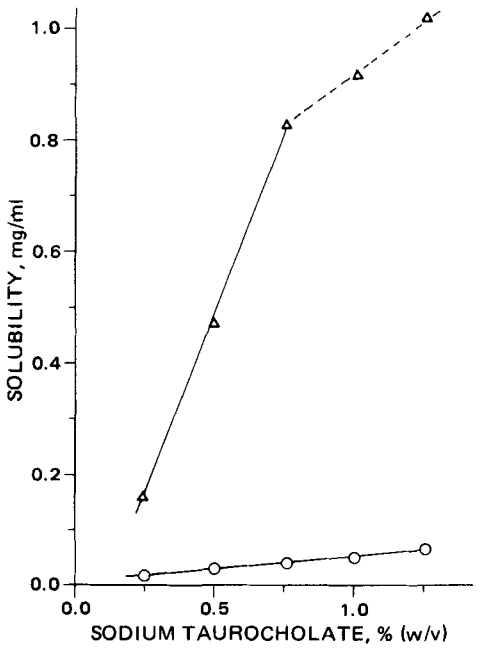

Figure 2—*Cetaben sodium solubilities in the presence of various strengths of sodium taurocholate at pH 4.9 (○) and 8.0 (△).*

increased (Fig. 1). Two separate mechanisms may account for this observation. The first mechanism is related to the ionization of cetaben itself. The apparent basic pKa_1 and acidic pKa_2 of cetaben were 2.6 and 5.3, respectively, in acetic acid when titrated with standardized perchloric acid that also was in acetic acid (7). Therefore, within pH 4.9–7.3, the proportion of cetaben in its anionic form increases as the hydrogen-ion concentration decreases, resulting in an increase in the aqueous solubility of cetaben as the pH is increased. The free acid form of cetaben had a much lower solubility than its sodium salt, indicating a consistency with the pH-partition hypothesis.

Changes in the critical micelle concentration (CMC) and micellar structure may offer another explanation for the changes in cetaben solubility under different pH conditions. Structurally, cetaben itself appears to have micellar properties. The alkaline pH conditions may enhance the mixed micellar formation, which results in a greater increase in cetaben solubility beyond pH 7.1–8. Bloor *et al.* (8) showed that the CMC for polysorbate 40 decreased with the pH increase as a result of the higher entropy change, which, in turn, was due to the greater degree of water structure at higher pH.

No evidence has proved or disproved the general applicability of these findings for surfactants other than polysorbate 40. Nevertheless, these postulations provide a possible explanation for the present experimental observations. It is likely that cetaben would partition more into the micellar phase resulting from a lower CMC at higher pH as compared to the lower pH conditions. The overall magnitude of the changes in cetaben solubility would depend entirely on the relative contribution of each mechanism. At pH values greater than 7.1, cetaben exists >98% as the anionic species. The sharp increase in cetaben solubility from pH 7.1 to 8 (Fig. 1) then is most likely due to changes in the CMC and micellar structure that are predominant under alkaline pH conditions.

In contrast to the sodium taurocholate and polysorbate 80 systems, the pH–solubility profile for poloxamer 188 delineated a hyperbola (Fig. 1). The described mechanisms still are applicable, except to the lowering of cetaben solubilities under alkaline pH values. This exception indicated that poloxamer 188 was not stable at alkaline conditions as compared to acidic conditions, a rationalization that was justified by a control experiment under the same experimental conditions using a UV spectral shift technique. As the medium pH becomes more alkaline, poloxamer 188 may degrade extensively, lose its surfactant properties, and, thus, cause the gradual dropping in cetaben solubility.

The complexity of the mixed micellar system makes analysis of the mechanisms contributing to the observed pH–solubility profiles difficult. The proposed mechanisms should be viewed as reasonable hypotheses explaining the present experimental observations. Further investigation should clarify the actual contribution of each mechanism to the overall pH–solubility profiles.

Influence of Surfactant Concentration on Cetaben Sodium Solubility—The low cetaben solubility requires surfactants for solubilization in an aqueous phase. To examine the effect of surfactant concentration on cetaben solubility, the surfactant concentration was increased in a stepwise fashion.

In the first series of experiments, the sodium taurocholate concentration was varied from 0.25 to 1.25% (w/v) at a constant pH of 4.9. The relationship between cetaben sodium solubility and sodium taurocholate concentration was linear (Fig. 2). This linearity suggests a typical solubilization plot for a micellar system where the solubility increases linearly with surfactant concentration beyond the CMC (9). As the surfactant concentration is increased, more drug is dissolved into the micellar phase (10). As a result, at higher sodium taurocholate concentrations, the amount of cetaben in the micellar phase would increase. Similar findings also were observed for nonionic surfactants, polysorbate 80, and poloxamer 188 (Figs. 3 and 4).

In the second series of experiments, all experimental conditions were kept the same but the medium pH was 8. The solubility–concentration profile delineated apparent saturable kinetics, revealing two linear segments with different slopes (Figs. 2–4). This change into two linear segments was statistically significant for sodium taurocholate and polysorbate 80 but not for poloxamer 188. The sudden change in slope could be due to size or shape changes in the micellar structure (11, 12). At

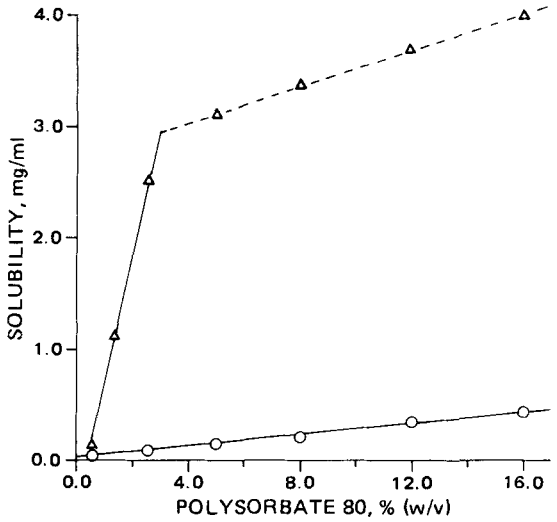

Figure 3—*Cetaben sodium solubilities in the presence of various strengths of polysorbate 80 at pH 4.9 (○) and 8.0 (△).*

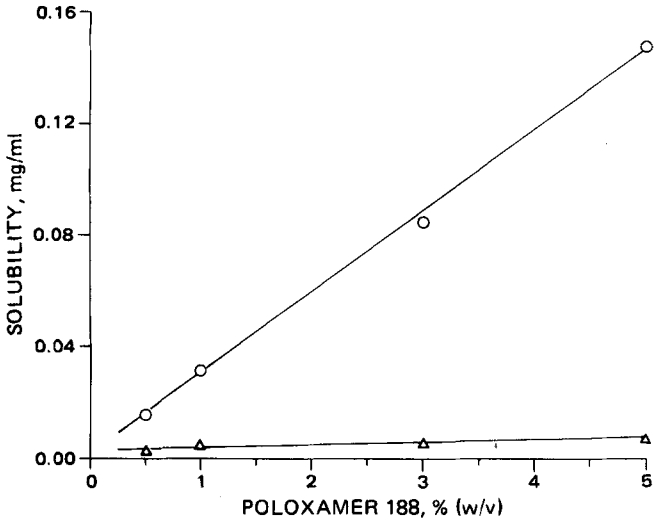

Figure 4—*Cetaben sodium solubilities in the presence of various strengths of poloxamer 188 at pH 4.9 (○) and 8.0 (△).*

concentrations higher than the CMC, micelles tend to coalesce into long rod-shaped micelles and cylindrical aggregates; finally, at very high concentrations, a phase transformation into lamellar structures takes place. Each transformation point varies and depends on the type of surfactant, the nature of surfactant, and the medium conditions.

Influence of Mixed Physiological Surfactants on Cetaben Solubility—Bile salts and phospholipids have been recognized as physiological surfactants important to lipid absorption (13, 14). The present study examined the effect of some physiological surfactants and mixed surfactant systems on cetaben sodium solubility.

Sodium taurocholate, lecithin, oleic acid, and monoolein were prepared in different proportions (Table I). Cetaben solubility was increased ninefold by taurocholate–monoolein, 23-fold by taurocholate–lecithin, 368-fold by taurocholate–oleic acid, 376-fold by taurocholate–oleic acid–lecithin, and 631-fold by taurocholate–oleic acid–monoolein when compared to taurocholate alone. Solubility enhancement can be attributed to an increased effective surface area when mixed disk micelles are formed (15). It also is possible that the inclusion of a second solubilizate may cause rearrangement of the mixed micelle structure, thereby altering the distribution coefficient favorable to solubilization (16).

Influence of Lipid Solvents on Cetaben Solubility—Lipid solvents

Table I—Cetaben Sodium Solubilities in Solutions Containing Physiological Surfactants Prepared in Different Proportions

Surfactant	Molar Ratio[a], m*M*	Solubility, mg/ml	Solubility Ratio[b]
Taurocholate	10	0.029	1
Taurocholate–monoolein	10:5	0.243	9
Taurocholate–lecithin	10:5	0.647	23
Taurocholate–oleic acid	62.5:3540	10.313	368
Taurocholate–oleic acid–lecithin	62.5:3540:0.78	10.547	376
Taurocholate–oleic acid–monoolein	62.5:3540:1.75	17.658	631

[a] The 10 m*M* was chosen because of its similarity to physiological concentrations of bile salts and its frequent use in previous studies. The ratios were chosen for convenience in preparation. [b] Solubility ratio = solubility in mixed surfactants/solubility in sodium taurocholate.

greatly enhance the bioavailability of hydrophobic compounds (10). Therefore, the effect of lipid solvents on cetaben solubility was studied to provide additional formulation information.

The selected lipid solvents were used at 100% strength. The solubilization capacity of lipid solvents for cetaben was in the following order: caprylic–capric monodiglycerides (23.191 mg/ml) > monodiglycerides (22.821 mg/ml) > triethyl citrate (0.61 mg/ml) > polyoxypropylene 15 stearyl ether (0.305 mg/ml) > medium-chain triglycerides (0.224 mg/ml) > caprylic–capric triglycerides (0.108 mg/ml) > triacetin (0.043 mg/ml).

Unlike solutions in aqueous media, micelles are formed by a stepwise aggregation rather than by a simple monomer–micelle equilibrium characteristic of aqueous systems (6, 17). The driving force behind aggregation and micellization in these nonaqueous media likely is due to dipole–dipole interactions between polar heads of the amphiphilic molecules (6).

Cetaben sodium is a promising antiatherosclerotic agent. Knowledge regarding the mechanism of its solubilization by surfactants and lipid solvents, as well as factors modifying its solubility, is essential for development of optimal formulation conditions.

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