# Electrical Conductivity of a Docusate Sodium Coacervate System

**CHRISTOPHER HUGHES** *§ **and BERNARD ECANOW** ‡x

Received February 17, 1983, from * *Stanford University, Dept. of Biological Sciences, Stanford 94305* and the ‡ *College of Pharmacy, University of Illinois, Chicago IL 60612.* Accepted for publication August 16, 1983. § Present address: College of Medicine, University of Illinois, Chicago IL 60612.

**Abstract** □ The conductivity of three xanthines (caffeine, theobromine, and theophylline) and three sugars (glucose, sucrose, and glucose-6-phosphate) was measured as part of a docusate coacervate system. The conductivity of the coacervate phase was always lower than the equilibrium phase. Conductivity of xanthines showed that caffeine had the highest conductivity, followed by theobromine and theophylline. At high concentrations, these values fell and the highest conductivity was seen in samples containing theobromine, caffeine, and theophylline. For sugars at high concentrations, the lowest values of conductivity were seen in sucrose samples. Saturated theophylline samples exhibited an increased volume in the coacervate phase while the saturated sugar systems displayed a decreased coacervate phase volume. These findings are discussed with reference to various clinical parallels and thus supply evidence that coacervates can provide a useful model for human cytoplasm.

**Keyphrases** □ Conductivity—docusate sodium coacervate, caffeine, theobromine, theophylline, glucose, sucrose, concentration effects □ Docusate sodium—coacervate system, partition coefficients, caffeine, theobromine, theophylline, glucose, sucrose, concentration effects

Coacervates have been suggested as a model for human cytoplasm since cytoplasm is also essentially an aqueous phase of water–macromolecule colloid complexes (1, 2). Ecanow (3) has shown that coacervate systems react with more similarity to cytoplasm than do presently accepted organic solvent–water models when subjected to pH change or polar–nonpolar molecule partitioning. This report will deal with the electrical conductivity of a sodium dioctyl sulfosuccinate (docusate sodium) coacervate system as a function of pharmaceutical drug and sugar concentration. The drugs (theophylline, theobromine, and caffeine) were chosen because of their known clinical effects. The sugars (sucrose, glucose, and glucose-6-phosphate) were chosen to investigate the known phenomenon of sugar acting as a depressant at high concentrations (4).

## EXPERIMENTAL SECTION

**Materials and Instrumentation**—All chemicals used for the HPLC analysis were used as supplied[1] and the docusate used for the preparation of the coacervate system was analytical reagent grade[2]. The sugars and xanthines were purchased from commercial sources. The liquid chromatographic apparatus used for these investigations consisted of a solvent delivery system[3], a variable-wavelength detector set at 254 nm[4], and a variable-volume injector[5]. The column used was a reverse-phase $C_{18}$[6] and the mobile phase consisted of acetonitrile–water (6:94, v/v). The conductivities of the samples were determined by the use of a conductivity bridge[7] and a glass dip conductivity cell[8].

**Preparation of Coacervate**—The docusate coacervate system was produced using the technique outlined by Acharya *et al.* (5). Solid docusate (10%, w/v) was added to distilled $H_2O$ and mixed thoroughly. Sodium chloride was then added (2.5%) until oil-like droplets began to separate out of solution. Within a few minutes the biphasic system was obtained.

**Procedure**—Nine docusate sodium coacervate samples were made for each of the three drugs and eight samples were made for each of the three sugars. Each sample contained 5 mL of the coacervate plus 5 mL of the equilibrium solution. Solutions of each additive were made (100 μL) and added to the coacervate system in amounts ranging from 2.44 to 21.96 mg of chemical. Previous studies have been done to determine the partition coefficients of theophylline and theobromine in coacervate systems (6). In this study, the concentration of the drug in each phase was accurately measured by HPLC and the partition coefficients were thereby obtained for this docusate system.

Chromatographic conditions for the HPLC analysis were as follows: the flow rate was set to 3.0 mL/min with a chart speed of 10 cm/h and sensitivity set at 0.02 AUFS for the theophylline and theobromine systems. Due to the wide peaks for caffeine, the chart speed was set at 5 cm/h with a sensitivity setting of 0.005 for the detection of this compound. A 100-μL aliquot from each layer of every sample was diluted with water due to the high concentrations of the drugs in the samples and the high sensitivity of this method of analysis.

Each layer in the control sample was diluted 1:10 and run with the other samples. No concentration of any of the drugs was found in this control sample. The conductivity of each phase of this control sample was measured; these were used as control values. From the dilution of each layer, a 10-μL sample was injected into the column and chromatographed.

**Standards**—Standard solutions were prepared for theophylline, theobromine, and caffeine at concentrations of 10, 20, and 40 μg/mL and also injected into the column. Peak heights on the chromatogram were measured for each specimen and the concentration was determined from the respective standard curve using peak height *versus* concentration. Retention times for each of the drugs were: theophylline, 6.0 min; theobromine, 3.9 min; caffeine, 12 min.

**Conductivity**—The conductivity of each phase of every sample was measured 10 times and an average value for each was obtained. To ensure no contamination, the conductivity cell was rinsed completely between each measurement.

## RESULTS

**Partition Coefficient**—The partition coefficient for each drug was obtained by plotting the concentration of the drug in the coacervate phase *versus* the concentration in the equilibrium phase (Fig. 1). The slopes of the resulting curves (in these cases, straight lines) are equal to the partition coefficients (P). For this coacervate system, the P values for these drugs are: theophylline = 1.600; caffeine = 1.522; theobromine = 1.260.

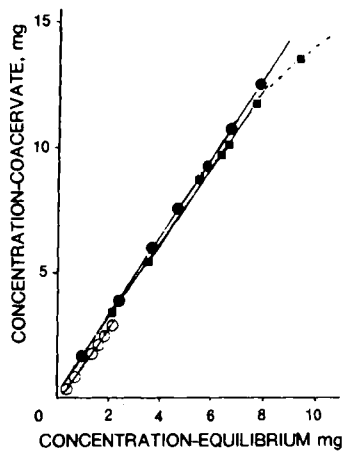


**Figure 1**—*Drug concentration in coacervate phases* versus *equilibrium phase. Key: (●) theophylline; (■) caffeine; (○) theobromine.*

[1] Spectrograde, A & C American Chemicals.
[2] Aldrich Chemical Co.
[3] Model 6000A; Waters Associates.
[4] Model 440; Waters Associates.
[5] Model U6K; Waters Associates.
[6] μ-Bondapak; Waters Associates.
[7] Model RC-20; Beckman Co.
[8] Model CEL-A001; Beckman Co.

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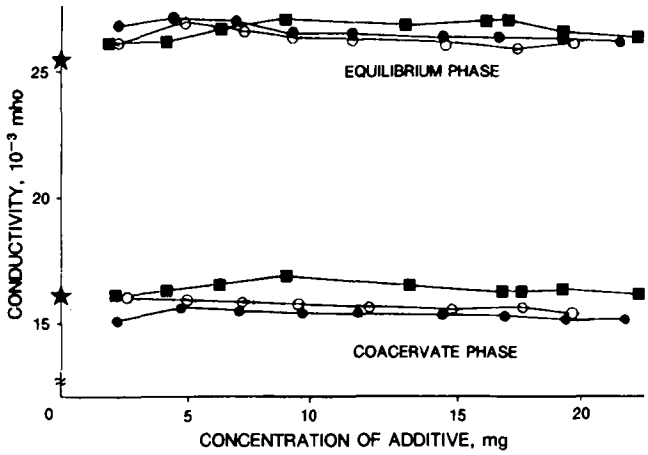


**Figure 2**—*Drug conductivity as a function of total concentration. Key: (●) theophylline; (■) caffeine; (○) theobromine; (★) control.*

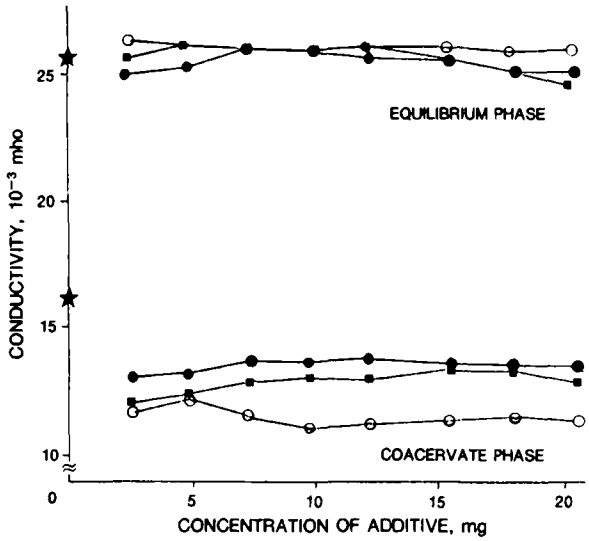


**Figure 3**—*Sugar conductivity as a function of total concentration. Key: (●) glucose-6-phosphate; (■) sucrose; (○) glucose; (★) control.*

Theobromine, having a low solubility constant in water, reached its saturation level in the sample containing 4.88 mg theobromine; therefore, precipitation was noted in the samples with a higher concentration of this drug. This saturation and precipitation account for the abbreviated partition coefficient graph for this drug.

**Conductivity**—Figures 2 (drug series) and 3 (sugar series) were obtained by plotting the electrical conductivity, measured in $10^{-3}$ mho, of each phase of the samples as a function of the total concentration of the additive in the system. Note that control values are indicated at the zero concentration points. In the concentration range of the drug series, the conductivity of theophylline was lowest, followed by theobromine and caffeine; for the sugar series, the lowest was glucose, then sucrose and glucose-6-phosphate. However, the conductivity did not vary significantly as a function of the concentration of the additive in the system. Therefore, samples containing additives at 2–4 times the original concentration were prepared to investigate the behavior of the coacervate system under increased concentrations.

Figures 4 and 5 are plots of conductivity *versus* total concentration of additive, but these graphs have a larger concentration scale to include the additional data mentioned above. These figures show that the high concentrations of the additives in some cases greatly affected the conductivity of the coacervate system. In the drug series, the conductivity decreased in both the coacervate and equilibrium phases and were usually lowest in theophylline and highest in theobromine, especially in the coacervate phase. A similar decrease was noted in the sugar series, with a marked change in the sucrose values. Glucose demonstrated a reversal or increase in conductivity before a later decrease with highest concentrations. These high concentrations also affected the coacervate system in other ways, as seen in the sugar samples containing 30–80 mg of additive; in these samples a clear, viscous, gel-like layer formed at the bottom of the tube. Because of the formation of this third layer, the volume of the coacervate layer decreased ~2 mL in these samples.

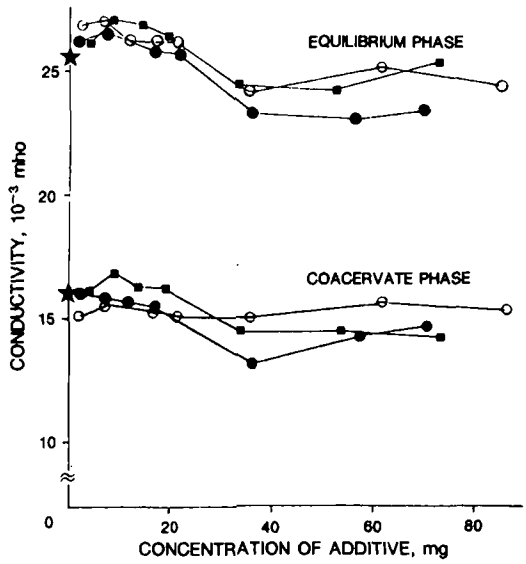


**Figure 4**—*Drug conductivity at increased concentrations. Key: (●) theophylline; (■) caffeine; (○) theobromine; (★) control.*

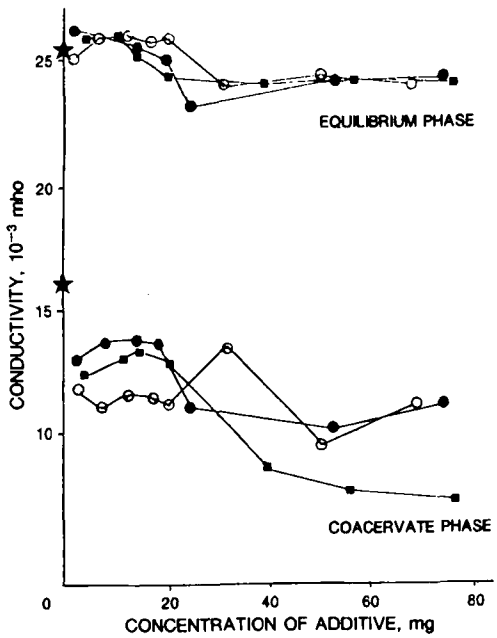


**Figure 5**—*Sugar conductivity at increased concentrations. Key: (●) glucose-6-phosphate; (■) sucrose; (○) glucose; (★) control.*

**Table I—Coacervate Layer Volume Increases in Theophylline Samples**

| Sample | Theophylline Conc., mg | Coacervate Volume, mL | |
|---|---|---|---|
| | | Total | Increase |
| 1 | 36.41 | 5.6 | 0.6 |
| 2 | 57.64 | 6.5 | 1.5 |
| 3 | 71.03 | 6.8 | 1.8 |

The high-concentration theophylline series (30–80 mg) exhibited an increase in the coacervate layer volume as shown in Table I. Furthermore, the sample containing the highest concentration (71.03 mg) also had a white precipitate, indicating that the sample had reached the saturation point.

## DISCUSSION

At high concentrations beyond the solubility point, sugars, like most compounds, will precipitate out of solution. However, because of the high solubility of the sugar and a strong physicochemical attraction to water, the sugar and bound water that separates as an additional phase from the rest of the system

is not a white, chalk-like powder, but rather a clear, viscous, sugar-water gel. This characteristic explains the formation of the third layer in the high-concentration sugar samples.

The increase in volume of the coacervate phase in the high-concentration theophylline samples (Table I) indicates a general increase in the structuring of this phase. In this case, the additives also act as general structure makers causing an increased nonpolarity of the equilibrium phase. This hypothesis is consistent with the decrease in electrical conductivity of these samples (Fig. 4), indicating a more structured system.

If the coacervate state is a model for human cytoplasm, then clinical parallels should be found in man. The decrease in the volume of the coacervate layer in the high-concentration sugar samples may be related to the known delayed healing of wounds in diabetics with low insulin and therefore high blood sugar levels. Ecanow *et al.* (7) have hypothesized that this delay in healing is partially caused by a rupturing of the cells in the wounded area from the high sugar concentrations acting as a structure breaker of these cells. This hypothesis is consistent with the decrease in the volume of the coacervate in the high-concentration sugar samples (acting as a structure breaker in the highly structured coacervate phase) without noticeable effect on the loosely bound equilibrium phase.

For each additive, the conductivity of the coacervate phase is reduced compared with the equilibrium phase (Figs. 2–5). This decreased conductivity of coacervates is consistent with both the hypothesis of Ecanow *et al.* (7) that conditions like malignancy occur in a nonpolar aqueous matrix (coacervate) and the findings of Lowenstein and Kanno (8) that malignant cells have a reduced action potential. Also, a low conductivity of the sugars is shown which may be related to the known depressant effect of sugars on the central nervous system, culminating in diabetic coma with high glucose concentrations (4). The effect of high concentrations of sucrose on membrane interface disorientation (structure breaking) can account for the known low absorption rate of sucrose in the intestine, compared to glucose (9).

The conductivity of caffeine was highest, followed by theobromine and theophylline (Fig. 2). These data may have a relationship to the decurarizing effects of these three drugs with caffeine showing the greatest effect, followed by theobromine and theophylline (10). With higher concentrations (Fig. 4) the conductivity was generally highest in theobromine, then caffeine and theophylline. These data may then be related to the effects of these drugs on urine production with theobromine showing the greatest effect followed by caffeine and theophylline, as reported by Scott *et al.* (11).

The increase over control values of the caffeine (Figs. 2, 4) followed by a reversal of a decrease with high concentrations (Fig. 4) finds a clinical parallel in the known increased cardiac contraction effect of caffeine at concentrations of 0.25–1.50 mM and a decreased contraction effect with concentrations >2.0 mM as reported by Guboreff and Sleator (12).

This discussion has shown a number of clinical parallels in the conductivity changes from the drug and sugar series and provides evidence in favor of the usefulness of a coacervate system as a model for human cytoplasm.

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# Urinary Metabolites of the Antiprotozoal Agent *cis*-3a,4,5,6,7,7a-Hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole in the Rat


**W. J. A. VANDENHEUVEL ˣ, B. H. ARISON, H. FLYNN, G. J. GATTO, H. E. MERTEL, and P. G. WISLOCKI**



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**Abstract** □ $^1$H-NMR and MS were employed to identify 13 rat urinary metabolites of $^{14}$C-labeled *cis*-3a,4,5,6,7,7a-hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole (MK-0436). The major free (unconjugated) metabolite was *cis*-3a,4,5,6,7,7a-hexahydro-3-carboxamido-1,2-benzisoxazole; it was also the second most abundant metabolite released during hydrolysis of the conjugated fraction. All other identified metabolites were hydroxylated analogues substituted at C(4)—C(7a) of the cyclohexane ring. The 4-equatorial,5-axial,7a-triol was the second most abundant metabolite excreted in an unconjugated form. Four monohydroxy (5-axial, 6-axial, 6-equatorial, 7-equatorial) metabolites of the drug were identified; they were found in the conjugated fraction only and were released by hydrolysis. The 5-axial hydroxy compound is the major conjugated metabolite and is overall the most abundant of all the metabolites. Six dihydroxy metabolites were identified: one was found exclusively in the free state, three as conjugates only (including the 7-axial,7a-diol, which is the major dihydroxy species), and two both free and conjugated. A second triol was found both free and conjugated.



**Keyphrases** □ *cis*-3a,4,5,6,7,7a-Hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole—urinary metabolites, rat, NMR, MS □ Hydroxylation—conjugation, *cis*-3a,4,5,6,7,7a-hexahydro-3-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,2-benzisoxazole, NMR, MS


Elliott and co-workers (1) reported that in the rabbit, the urinary metabolites of cyclohexane were cyclohexanol and cyclohexane-*trans*-1,2-diol (as conjugates); Renwick and Williams (2) showed that cyclohexylamine was converted to mono- and dihydroxy metabolites (free and conjugates). Testa and Jenner (3) also discussed the metabolic hydroxylation of cyclohexyl ring systems. Substituted nitroimidazoles are known to undergo hydroxylation on their hydrocarbon moieties: metronidazole is hydroxylated on the 2-methyl group (4), ipronidazole on the 2-isopropyl group (5). The antipro-


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