Molecular Weight of the Antifungal Antibiotic Saramycetin

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The molecular weight of the antibiotic saramycetin was determined as 2100 by two ultracentrifugal methods: (*a*) approach to sedimentation equilibrium and (*b*) combination of the zero concentration diffusion constant ($D_{20} = 34 \times 10^{-7}$ cm.^{2} sec.$^{-1}$) with the zero concentration sedimentation constant ($S_{20,w} = 1.2 \times 10^{-13}$ cm. sec.$^{-1}$). At concentrations above 5 mg./ml. saramycetin associates, forming aggregates with a molecular weight of 55,000.

THE MOLECULAR weight of the antifungal agent saramycetin had been previously reported by Baudet and Cherbuliez (1) to be 14,000 daltons on the basis of sedimentation and diffusion constant measurements. Evidence will be presented in this paper that saramycetin is capable of molecular association and that the apparent molecular weight is dependent on polarity of solvent and solute concentration.

EXPERIMENTAL

Molecular weights were measured by the Archibald (2) approach to sedimentation equilibrium method. One to 0.1% solutions, in 12-mm. interference cells, were centrifuged in an An-D rotor at 22°. Concentration gradients were formed at speeds ranging from 8766 to 42,040 r.p.m. using the schlieren optical system with a phase bar angle of 75°. The concentration gradient was evaluated using Engelberg's modification (3), at the meniscus and cell bottom from photographs recorded on Kodak metallographic plates magnified tenfold on a Nikon magnifier. Total solute concentration was measured with a synthetic boundary centerpiece in an interference cell centrifuged at 29,500 r.p.m.

Sedimentation coefficients for saramycetin solutions with concentrations from 10 mg./ml. to 1.25 mg./ml. were measured in a 4°, 12-mm. cell centrifuged in an An-D rotor at 42,040 r.p.m. For solutions with concentrations of 1.25 mg./ml. to 0.313 mg./ml., the sedimentation coefficients were evaluated from schlieren peaks produced with the aid of a 12-mm. synthetic boundary centerpiece in an interference cell, rotated at speeds to 42,040 r.p.m.

Diffusion constants were evaluated in the analytical ultracentrifuge (4), at 22°, using a synthetic boundary centerpiece and the schlieren optical system. Solute concentrations were from 10 mg./ml. to 0.313 mg./ml. The first image was recorded at two-thirds of the final speed of 42,040 r.p.m. or 29,500 r.p.m. This first image was considered to be recorded at zero time for the diffusion constant calculations.

Partial specific volume was determined at 20° in a 1-ml. pycnometer.

RESULTS AND DISCUSSION

As the concentration of saramycetin is diminished from a concentration of 10 mg./ml. to 0.313 mg./ml. in 0.2 *M* NaCl–0.02 *M* phosphate buffer, pH 6.85, the sedimentation coefficient (*s*) first increases and then decreases (Fig. 1). Two *S* values at infinite dilution can be calculated by extrapolating to zero solute concentration both the positively sloping and negatively sloping regions of the curve. At the higher concentrations of saramycetin (10 mg./ml. to 5 mg./ml.) $S_{20,w} = 5.28 \times 10^{-13}$ cm. sec.$^{-1}$ and at concentrations of saramycetin below 2.5 mg./ml., $S_{20,w} = 1.2 \times 10^{-13}$ cm. sec.$^{-1}$. The *S* value found for saramycetin depends then on the solute concentration in 0.2 *M* NaCl–0.02 *M* phosphate buffer.

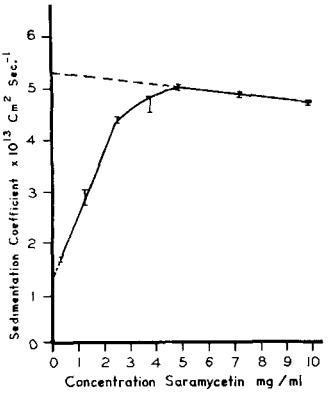

Fig. 1—Dependence of sedimentation coefficient of saramycetin on the concentration of saramycetin in 0.2 M NaCl–0.02 M phosphate buffer, pH 6.85.

Measurements of the diffusion constant also give two values dependent on saramycetin concentration. At the higher saramycetin concentrations, $D_{20} = 6.0 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ and at the lower concentrations, $D_{20} = 34 \times 10^{-7}$ cm.^{2} sec.$^{-1}$, extrapolating to zero concentration.

The partial specific volume, $\bar{V}$, was found to be 0.58, and agrees with the value of 0.60 ± 0.05 published by Baudet and Cherbuliez.

By combining the *S* and *D* data (5) from each concentration range, two molecular weights have been calculated: 55,000 for the 10 to 5 mg./ml. solute concentration and 2100 for the 2.5 to 0.313 mg./ml. concentration range. Acid titration yields an equivalent weight of 2200. This evidence suggests that 2100 represents the true molecular weight. In this solvent system, if it can be assumed that the mass action law is valid, the monomer concentration term in an equilibrium gradient expression would have an exponent of about 25.

Further evidence for aggregate formation is summarized in Table I, which gives the molecular weight of saramycetin in various solvents.

Approach to equilibrium sedimentation (6) yielded a molecular weight of 54,000 ± 2000 daltons at the meniscus and cell bottom for a 1% solution of saramycetin dissolved in any of the following buffers: 1 *M* acetate buffer, pH 4.05; 0.2 *M* NaCl–0.02 *M* phosphate buffer, pH 6.85; 2 *M* NH_4OH–2 *M* NH_4Cl, pH 9.4; or an isotonic solvent containing the inorganic cations and anions found in human serum.

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TABLE I—MOLECULAR WEIGHT OF 1% SARAMYCETIN SOLUTIONS IN VARIOUS SOLVENTS AS DETERMINED BY APPROACH TO EQUILIBRIUM SEDIMENTATION

Solvent	Mol. Wt.
1 *M* acetate buffer, pH 4.05	55,000 ± 2,000
0.2 *M* NaCl–0.02 *M* phosphate buffer, pH 6.85	56,000 ± 2,000
2 *M* NH_4OH–2 *M* NH_4Cl buffer, pH 9.4	55,000 ± 2,000
Isotonic phosphate buffer containing K^+, Na^+, Ca^{++}, Mg^{++}, Cl^-, and $SO_4^=$	54,000 ± 2,000
0.68 *M* borate buffer, pH 8.6	6,000–20,000
8 *M* urea in NaCl–phosphate buffer	7,000 ± 1,000
90% ethanol (95%)–10% NaCl–phosphate buffer	2,100 ± 100

Sedimentation of 1% saramycetin in 8 *M* urea–NaCl–phosphate buffer diminished the apparent molecular weight to 7000 ± 1000 daltons, indicating a disruption of the aggregate.

Borate buffer, pH 8.6, 0.68 *M*, also affected the aggregate molecular weight with several components found with uncorrected molecular weights of from 6000 to 20,000 daltons.

By diminishing the concentration of the phosphate–NaCl buffer to concentrations of $^1/_{100}$ of the original, a molecular weight of 5500 ± 1000 daltons was found ($S = 1.37 \times 10^{-13}$ cm. sec.$^{-1}$). The physical effect of diluting the buffer is to diminish the apparent molecular weight of the saramycetin aggregate. The *in vitro* microbiological activity of 0.1% saramycetin solutions varied inversely with the ionic strength of the buffer (7). This indicates that the biological effect of using a more dilute buffer is to increase the *in vitro* antifungal activity of saramycetin solutions.

In summary, saramycetin, molecular weight 2100, may form aggregates in solution. The molecular weight of these aggregates may range upward to 55,000 depending on the solvent and on electrolyte ionic strength.

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Communications

Mathematics of the Three-Phase *In Vitro* Absorption Models

Sir:

Several reports on three-phase models for drug absorption have recently appeared in the literature (1–3). However, it appears that volume terms should be included in the rate equations as well as in the equilibrium equations to obtain more meaningful values for the transfer rates. In these models, the drug (usually weakly basic or weakly acidic) is transferred from an aqueous buffer (*A*) of a pH found in the alimentary tract, through a water immiscible organic phase (*B*) acting as the "membrane," to a buffer of blood pH (*C*). Assuming that only the unionized drug is soluble in the organic phase, then the equilibrium concentrations (C_A, C_B, C_C) can be expressed in the equations derived below in terms of the initial concentration of the drug (a_0), the true distribution coefficient of the drug between the organic layer and water (D), the pH's of the buffers (pH_A and pH_C), the pKa of the drug, and the volumes of the phases (V_A, V_B, and V_C).

For a monobasic acid

$$C_B = \frac{a_0 D}{1 + 10^{pH_A - pK_A} + \frac{V_C}{V_A}(1 + 10^{pH_C - pK_A}) + \frac{V_B D}{V_A}}$$

$$C_A = \frac{C_B}{D}(1 + 10^{pH_A - pK_A})$$

$$C_C = \frac{C_B}{D}(1 + 10^{pH_C - pK_A})$$

For a mono-acidic base

$$C_B = \frac{a_0 D}{1 + 10^{pK_A - pH_A} + \frac{V_C}{V_A}(1 + 10^{pK_A - pH_C}) + \frac{V_B D}{V_A}}$$

$$C_A = \frac{C_B}{D}(1 + 10^{pK_A - pH_A})$$

$$C_C = \frac{C_B}{D}(1 + 10^{pK_A - pH_C})$$

In the kinetics of the three-phase distribution