Micelle Size and Stability of Aqueous Amphotericin B (Polyene) Systems

By JOEL KIRSCHBAUM and SAMUEL G. KAHN

Concentrations of components were varied in aqueous amphotericin B systems containing cetyl dimethyl benzyl ammonium chloride, dimethylsulfoxide, amphotericin B, and water. Using the analytical ultracentrifuge and correcting for density and viscosity, the systems containing the smallest micelles were found by determination of sedimentation coefficients. This procedure proved valuable in subsequent formulations, containing amphotericin B for veterinary purposes.

AMPHOTERICIN B is a polyene antifungal antibiotic which has a molecular weight of approximately 960. It is relatively insoluble in water and other common solvents, but exceedingly soluble in dimethylsulfoxide (DMSO) (1). The preparation of an aqueous suspension, colloid, or solution of amphotericin B for use as a medicament would be very desirable. This study was undertaken to investigate how changes in concentration of each component in an aqueous DMSO–amphotericin B–cetyl dimethyl benzyl ammonium chloride system will affect the micelle size and stability of amphotericin in such a system.

EXPERIMENTAL

Solutions were prepared by dissolving the appropriate amounts of amphotericin B and cetyl dimethyl benzyl ammonium chloride in DMSO and diluting with deionized water to the desired concentration. The concentrations of amphotericin B (900 mcg./mg.) were varied from 12.5 to 400 mcg./ml. The alkyl quaternary ammonium salt, cetyl dimethyl benzyl ammonium chloride[1] was used in concentrations from 12.5 to 200 mcg./ml. DMSO (pharmaceutical grade) was varied in concentration from 0.25% to 8% of solution.

Sedimentation coefficients (S) were measured using 20-mm. centerpiece, 4° cells, and centrifuged at speeds of 12,590; 29,500; or 50,740 r.p.m. in the An-E rotor, the temperature being maintained at 20°C. The Schlieren phaseplate angles were from 50° to 65°. Images were recorded on Kodak metallographic plates and tenfold magnifications were traced using a Nikon shadowgraph. The areas under the Schlieren peaks or peak areas, were measured with a planimeter. These areas are proportional to the concentration of sedimenting substances.

Viscosities were measured at 20° C. employing capillary and Zimm viscometers (2). Densities were measured at 20° C. using a 10-ml. pycnometer. For molecular weight calculations, the partial specific volume of amphotericin B was determined in a 1-ml. pycnometer and found to be 0.64 ± 0.02.

Micelle weights were calculated using the Archibald approach to sedimentation equilibrium method (3). Concentration gradients were measured in 12 mm. interference cells at speeds of either 8,766 or 42,040 r.p.m. and evaluated using Engelberg's modification (4). Total concentration was measured with a synthetic boundary centerpiece in an interference cell. The Schlieren phaseplate angle was 75°. Concentration gradients at the meniscus and cell bottom were evaluated from enlarged images as previously described for the determination of S values.

RESULTS AND DISCUSSION

Table I presents data of peak areas and S values, corrected for viscosity and density. When the level of amphotericin B was maintained at 100 mcg./ml. and the concentrations of cetyl dimethyl benzyl ammonium chloride and DMSO varied, S values and peak areas were at a minimum when DMSO levels equalled 1% to 2% and cetyl dimethyl benzyl ammonium chloride concentrations were 25 to 100 mcg./ml. Interestingly, the S values for amphotericin B at these component concentrations did not change after 24 hr., thus indicating that at these concentrations cetyl dimethyl benzyl ammonium chloride and DMSO stabilized amphotericin B against increased micelle growth and precipitation. Increasing cetyl dimethyl benzyl ammonium chloride and/or decreasing DMSO concentrations increased the S values and permitted precipitation of the preparation. Very high concentrations of cetyl dimethyl benzyl ammonium chloride (200 mcg./ml.) and DMSO (8%) promoted large increases in S values and peak areas, indicating increased micelle size.

When DMSO was kept at 1% concentration and the levels of both cetyl dimethyl benzyl ammonium chloride and amphotericin B varied from 12.5 to 400 mcg., the S coefficients were minimum in the region where amphotericin B concentration was 100 mcg./ml. and the cetyl dimethyl benzyl ammonium chloride level was approximately 100 mcg./ml. (Table II). Similar results were obtained when amphotericin B and DMSO levels were varied and cetyl dimethyl benzyl ammonium chloride concentration was kept at a constant 100 mcg./ml. Preparations of greatest stability (lowest S value) were observed when the amphotericin level was 100 mcg./ml., the cetyl dimethyl benzyl ammonium chloride level was 50 to 100 mcg./ml., and the DMSO concentration equaled 1% to 2%. Micelle weight determinations gave parallel results and correlated positively with S values. In an aqueous amphotericin B system (amphotericin B, 100 mcg./ml.; cetyl dimethyl benzyl ammonium chloride, 50 mcg./ml.; DMSO, 1%), the minimal micelle weight of an amphotericin micelle was calculated to be 400,000 daltons. The micelle weight increased into the millions when the concentration of any component of the system was increased or decreased.

The molar ratio between cetyl dimethyl benzyl ammonium chloride (mol. wt. 395), amphotericin B (mol. wt. 960), and DMSO (mol. wt. 78) at minimal S value concentrations (or micelle size) is approximately 1:1:1000.

Received September 2, 1966, from the Squibb Institute for Medical Research, New Brunswick, N. J. 08903.
Accepted for publication October 21, 1966.

[1] Marketed as Cetol by Fine Organics, Inc., Lodi, N. J.

TABLE I.—SEDIMENTATION COEFFICIENTS (S) AND PEAK AREAS (cm.^{2}) FOR AMPHOTERICIN B MICELLES WHEN AMPHOTERICIN CONCENTRATION IS KEPT CONSTANT (100 mcg./ml.) AND CETYL DIMETHYL BENZYL AMMONIUM CHLORIDE AND DMSO LEVELS ARE VARIED

Cetyl Dimethyl Benzyl Ammonium Chloride, mcg./ml.	DMSO, %[a]					
	0.25	0.50	1	2	4	8
12.5	88[b] (0.008)[c]	38 (0.012)	23 (0.017)	31 (0.016)	60 (0.009)	662 (0.079)
25	43 (0.011)	21 (0.007)	13 (0.007)	9 (0.007)	28 (0.011)	437 (0.149)
50	23 (0.016)	12 (0.003)	23 (0.008)	20 (0.005)	24 (0.057)	58 (0.011)
100	34 (0.011)	31 (0.012)	24 (0.017)	46 (0.024)	76 (0.036)	42 (0.014)
200	47 (0.012)	37 (0.010)	41 (0.008)	67 (0.038)	139 (0.060)	140 (0.145)

[a] DMSO, dimethylsulfoxide. [b] S, sedimentation coefficient. [c] Peak area.

TABLE II.—SEDIMENTATION COEFFICIENTS (S) AND PEAK AREAS (cm.^{2}) FOR AMPHOTERICIN B MICELLES WHEN DMSO[a] CONCENTRATION IS KEPT CONSTANT (1%) AND CETYL DIMETHYL BENZYL AMMONIUM CHLORIDE AND AMPHOTERICIN B LEVELS ARE VARIED

Cetyl Dimethyl Benzyl Ammonium Chloride, mcg./ml.	Amphotericin B, mcg./ml.					
	12.5	25	50	100	200	400
12.5	160[b] (0.04)[c]					207 (0.020)
25		269 (0.007)			54 (0.014)	
50	91 (0.08)		58 (0.006)	24 (0.004)		49 (0.048)
100			53 (0.006)	25 (0.004)		
200		196 (0.011)			77 (0.008)	
400	485 (0.026)					331 (0.070)

[a] DMSO, dimethylsulfoxide. [b] S, sedimentation coefficient. [c] Peak area.

These results show that in formulating an aqueous system for a water-insoluble substance the concentrations of the substance and other components of the system are critical in determining the physical characteristics of a molecular species. The results suggest that physical chemical analysis, with an analytical ultracentrifuge, as used in these studies, can help select the more stable aqueous system for a relatively water-insoluble substance. This method proved practical when a stable water-miscible amphotericin B formulation was prepared as medication in the drinking water of poultry (5). Using this procedure, other water-insoluble substances, such as the polyene antifungal antibiotic nystatin, can be formulated into water-miscible systems.

REFERENCES

(1) Donovick, R., Steinberg, B. A., Dutcher, J. D., and Vandeputte, J., *Del Giornale Microbiologia*, **2**, 147(1956).
(2) Zimm, B. H., and Crothers, D. M., *Proc. Natl. Acad. Sci.*, **48**, 905(1962).
(3) Archibald, W. J., *J. Phys. Colloid Chem.*, **51**, 1204 (1947).
(4) Engelberg, J., *Anal. Biochem.*, **6**, 530(1963).
(5) Kahn, S. G., and Slocum, A., *Poultry Sci.*, **45**, 761 (1966).

Isolation and Identification of Three Alkaloids from the Bark of *Zanthoxylum elephantiasis*

By ALBERT T. AWAD, JACK L. BEAL, SUNIL K. TALAPATRA*, and MICHAEL P. CAVA†

The bark of *Zanthoxylum elephantiasis* (*Rutaceae*) was found to contain three major alkaloids which were identified as 6-canthinone, 5-methoxy-6-canthinone, and laurifoline.

THE GENUS *Zanthoxylum* (*Rutaceae*) contains some 200 species dispersed over the world, many of which have been studied because of their alkaloid content. Price (1) in an excellent review of the distribution of alkaloids in the *Rutaceae* alludes to the nomenclature confusion between the two genera *Zanthoxylum* and *Fagara* when he points out that a number of *Fagara* species are alternatively named as *Zanthoxylum* and vice versa. Indeed, the particular *Zanthoxylum* species which forms the subject of the present investigation was at one time placed in the genus *Fagara* (2). Fosberg (3) recently clarified the situation regarding the relationship of *Fagara* and *Zanthoxylum* in such a way that today less confusion exists.

Zanthoxylum elephantiasis Macf. (*Z. aromaticum* DC., *Fagara elephantiasis* Kr. and Urb.) is indigenous to the Caribbean region having been found

Received April 26, 1966, from the College of Pharmacy, Ohio State University, Columbus 43210.
Accepted for publication September 27, 1966.
This investigation was supported by research grant GM-05640 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
* Present address: Department of Chemistry, University College of Science, Calcutta, India.
† Present address: Department of Chemistry, Wayne State University, Detroit, Mich.