

Characterization of the Interaction of Amphotericin B with Cholesteryl Trifluoromethylphenyl-Carbamate by UV-Visible Spectroscopy

LOREDANA ELENA VIJAN*, CARMEN MIHAELA TOPALA

University of Pitesti, Targu din Vale No. 1, Pitesti, 110040, Romania

Amphotericin B, a polyene antibiotic, self-associates in ethanol. The self-association of antibiotic has characterized by UV-visible spectroscopy. Starting from a simple dimerization model and using different methods described in literature, one determined the molar absorption coefficient of monomer, the molar absorption coefficient of dimer and the dimerization constant. The binding of amphotericin B to cholesteryl trifluoromethylphenyl-carbamate has investigated using absorbance measurements and the results have rationalized in terms of literature models, taking into account both 1:1 drug-sterol system and cooperativity effects. The binding constant of amphotericin B to cholesteryl trifluoromethylphenyl-carbamate has determined using Benesi-Hildebrand, Scott and Scatchard methods.

Keywords: amphotericin B, self-association, cholesteryl trifluoromethylphenyl-carbamate, binding, UV-visible spectroscopy

Optical probes have provided very useful information about protein structure and function relationship. These probes are generally polyunsaturated molecules, which either acquire fluorescence emission or modify their absorption spectrum when they are bound to a protein. Previous reports have shown that polyunsaturated linear fatty acids such as linoleic acid and retinoic acid can be used as optical probes to detect conformational changes in proteins, biological membranes, and liposomes [1]. The polyene antibiotic amphotericin B is unsaturated macrolides of amphipatic nature used in the therapy of systemic fungal infections [2].

The effect of amphotericin B on organization of lipid membranes and the effect of the lipid phase on molecular organization of the drug has been the subject of the numerous studies, carried out applying different instrumental techniques including spectroscopic ones, mainly electronic absorption [3-5]. Amphotericin B has been widely applied as a probe for sterol location in biological membranes since an alteration of its adsorption spectrum is induced by formation of sterol-polyene antibiotic complex [6].

In this work we study the interaction between amphotericin B (AmB), a polyene antibiotic, and the cholesteryl trifluoromethylphenyl-carbamate (Ch-CF₃), a mesogen lipid derivative. Ch-CF₃ is a lipid, steroid derivative, which possess a hydrophobic moiety (rigid skeleton and lateral chain at C-17), a charged head group (CF₃ at C-3' from aromatic substituent at C-3 sterolic) and a linker functional group such as carbamate at C-3 sterolic to bind these two moieties together covalently. Our little work has focused on the design of the optimum CF₃ head group to interact with the AmB.

The molecular structures of the compounds used in the experimental study are shown in figure 1. AmB (fig. 1a) has quite a special structure, with one hydrophobic side containing seven conjugated double bonds and the other

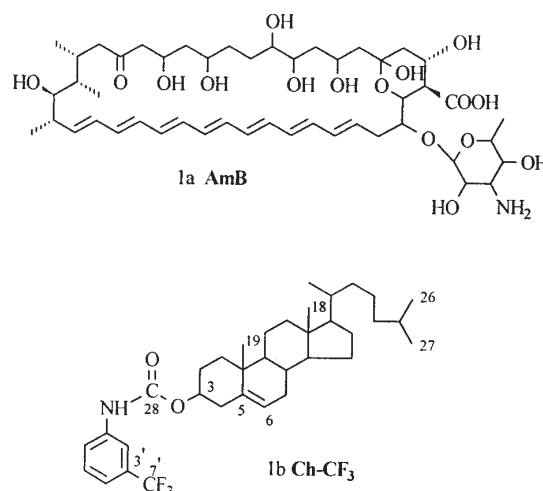


Fig. 1. The structure of AmB and Ch-CF₃

side, hydrophilic, containing several polar substituents. Ch-CF₃ (fig. 1b) presents a bulky polarizable substituent on *meta* position of aromatic substituent at C-3 of steroidal ring.

Experimental part

Cholesteryl trifluoromethylphenyl-carbamate was obtained from cholesterol and 2,4,6-trifluoro-*m*-tolyl isocyanate and characterized chromatographically and spectroscopically as previously described [7]. Amphotericin B from *Streptomyces sp.* was obtained from Sigma-Aldrich, Germany. The AmB and Ch-CF₃ solutions were prepared in ethanol. The concentrations of the stock solutions of AmB were determined using the molar absorption coefficient value: $\epsilon_{408\text{nm}} = 160000 \text{ M}^{-1} \text{ cm}^{-1}$. The absorption spectra were recorded at the range of 460-320nm, in a Lambda 25 PerkinElmer spectrometer, with quartz cells, at room temperature.

* Tel.: 0724289007

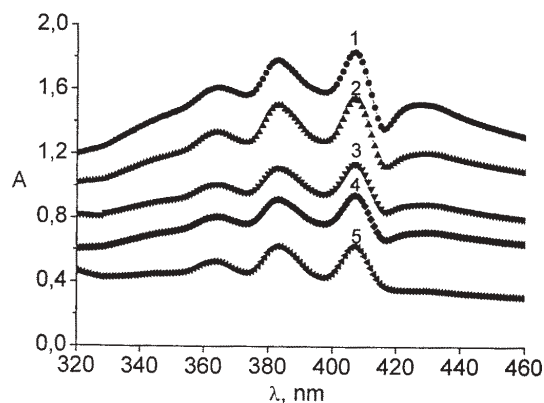


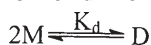
Fig. 2. Absorption spectra of amphotericin B solutions

Results and Discussion

The self-association of amphotericin B has been followed by measurement of the electronic absorption in the UV-VIS region as a function of drug concentration. Figure 2 presents the absorption spectra of the amphotericin B solutions. In the range of 320-460nm, one observes four distinct maxima centered at 428, 408, 383 and 364nm. It may be noted that the spectra change gradually with increasing antibiotic concentration.

On this basis and taking into account literature data on the aggregation of dyes or drugs [8, 9], the band at 408nm was assigned to the monomer, the band at 428nm was assigned to the monomer, the bands at 364 and 383nm were assigned to the higher aggregates.

The literature describes several computation methods [10, 11] for estimating the self-association constant. Starting from the equilibrium monomer-dimer:



and from the following equations:

$$\sqrt{\frac{C_D^0}{\epsilon_M - \epsilon_{app}}} = \frac{1}{\epsilon_M - \epsilon_D} \sqrt{C_D^0(\epsilon_M - \epsilon_{app})} + \sqrt{\frac{1}{2K_d(\epsilon_M - \epsilon_D)}}$$

$$\sqrt{\frac{\epsilon_M - \epsilon_{app}}{C_D^0}} = \sqrt{\frac{2K_d}{\Delta\epsilon}} [\Delta\epsilon - (\epsilon_M - \epsilon_{app})]$$

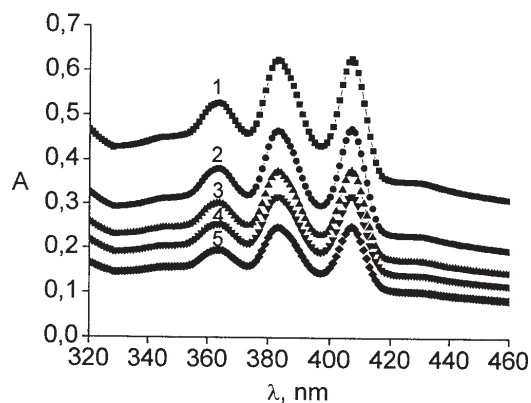


Fig. 3. Absorption spectra of AmB in the presence of varying amounts of Ch-CF₃. The various *p* ratios are: (1) 0; (2) 4,46; (3) 10,84; (4) 16,58; (5) 29,34

described in Tipping [10], respectively Schwarz [11] theory, we have calculated the dimerization constant (K_d). In these equations, ϵ_M is the molar absorption coefficient of the monomer, ϵ_D - the molar absorption coefficient of the dimer, $\Delta\epsilon$ - the difference in the molar absorption coefficients of the monomer and dimer, C_D^0 - the total concentration of bleomycin in terms of monomer. Both methods lead to a dimerization constant of $K_d = 5000 \pm 30 M^{-1}$.

A family of curves obtained at the titration of AmB solutions of concentrations in the range 10^{-6} - $10^{-5} M$ with Ch-CF₃ is presented in figure 3.

It may be observed that the AmB - Ch-CF₃ complex is characterized by the decrease of the major bands at 408, 383 and 364nm at small and medium $\frac{Ch-CF_3}{AmB}$ (noted *p*) ratios.

In the case of 1:1 AmB - Ch-CF₃ interaction, the binding constants may be evaluated from the methods proposed by Benesi-Hildebrand [12], Scott [13] and Scatchard [14]. The equations utilized and the results obtained for AmB - Ch-CF₃ system are summarized in table 1. In figures 4, 5 and 6 are presented Benesi-Hildebrand, respectively Scott and Scatchard plots.

Table 1
THE RESULTS OF THE BINDING CONSTANTS FOR AmB - Ch-CF₃ INTERACTION

| Methods | Equations | K, M^{-1} |
|-------------------|--|-------------------|
| Benesi-Hildebrand | $\frac{1}{\Delta A} = \frac{1}{C^0 \cdot K \cdot \Delta\epsilon} \cdot \frac{1}{[Ch-CF_3]} + \frac{1}{C^0 \Delta\epsilon}$ | $1,32 \cdot 10^4$ |
| Scott | $\frac{1 \cdot [Ch-CF_3]}{\Delta A} = \frac{1}{C^0 \cdot \Delta\epsilon} \cdot [Ch-CF_3] + \frac{1}{C^0 \cdot K \cdot \Delta\epsilon}$ | $1,21 \cdot 10^4$ |
| Scatchard | $\frac{\Delta A}{1 \cdot [Ch-CF_3]} = -\frac{K}{1} \cdot \Delta A + C^0 \cdot K \cdot \Delta\epsilon$ | $1,13 \cdot 10^4$ |

where $\times \square_{app} = \square_{app} - \square_F$, $\times \square = \square_B - \square_F$, \square_{app} , \square_F and \square_B are the apparent, free and bound drug absorption coefficients, *l* is path length, $\times A$ is the observed absorbance change, C^0 is

the total concentration of drug and $[Ch-CF_3]$ is sterol concentration (concentration in moles per unit volume).

In conclusion, the binding process of amphotericin B to cholesteryl trifluoromethylphenyl-carbamate, analysed by

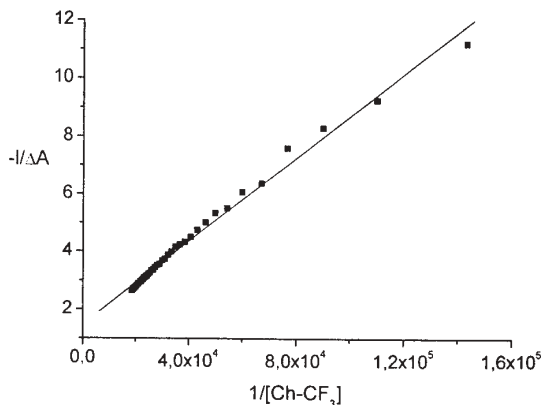


Fig. 4. Benesi-Hildebrand plot

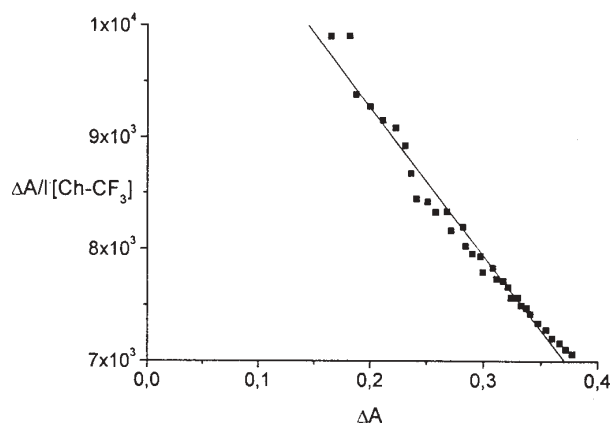


Fig. 6. Scatchard plot

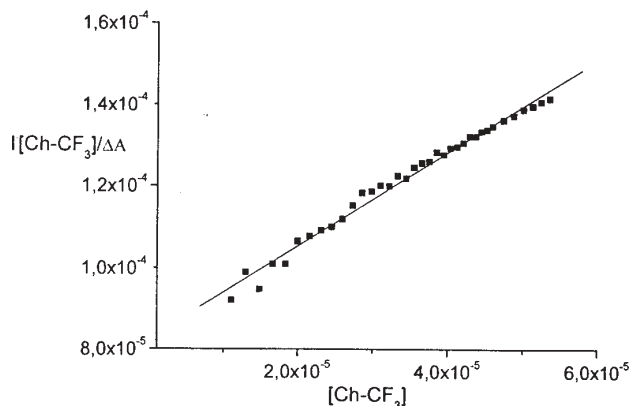


Fig. 5. Scott plot

Benesi-Hildebrand, Scott and Scatchard models suppose a 1:1 binding ratio and do not account explicitly for either the dimerization of the drug or cooperativity effects on the binding.

Further, different models that accounts for dimerization of the drug and cooperativity effects will be further employed.

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