Spectral Study of the Amphotericin B - cholesteryl Linoleate Interaction

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The interaction of amphotericin B with cholesteryl linoleate has investigated by UV-VIS spectroscopy. The binding results have rationalized in terms of methods Benesi-Hildebrand, Scott and Scatchard, taking into account 1:1 amphotericin B - cholesteryl linoleate system.

Keywords: amphotericin B, cholesteryl linoleate, UV-VIS spectroscopy

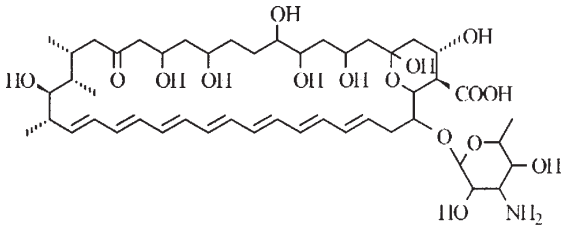
Cholesteryl linoleate (Ch-Lin) is one of the important component of the low density lipoproteins (LDL). Spectral and optical studies about its mesomorphism reveal that in mixtures with other mesogen compounds forms an interphase state at Ch-Sm transition boundary [1]. Ch-Lin together with cholesteryl arachidonate, another polyunsaturated cholesteryl esters, are smectic liquid crystals and in fibrous plaques (one of the human atherosclerotic lesions) are isotropic at 23°C (nesaturation decreases the melting point of cholesteryl ester). The physical state of cholesteryl esters mixture was obtained by polarizing microscopy and proved by XRD [2]. Another studies about mesogenous behaviour of cholesteryl derivative, such as optical and thermo-optical effects [3, 4], method of thermally stimulated depolarization currents [5] were recently realized.

In this work we study the interaction between amphotericin B (AmB), a polyene antibiotic, and cholesteryl linoleate (Ch-Lin), a mesogen lipid derivative. Ch-Lin is a simple lipid, steroid derivative, which possess a hydrophobic moiety: rigid skeleton and lateral chains at C-17 and C-3 sterolic (alkyl chain with two C=C double bonds at C-3) and a linker functional group such as carboxilate at C-3 sterolic to bind these two lateral chains. 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 side, hydrophilic, containing several polar substituents. In Ch-Lin structure (fig. 1b), the double nesaturation (C-9′ and C-12′) at C-3 sterolic presents the special importance for binding of steroid derivative with amphotericin B.

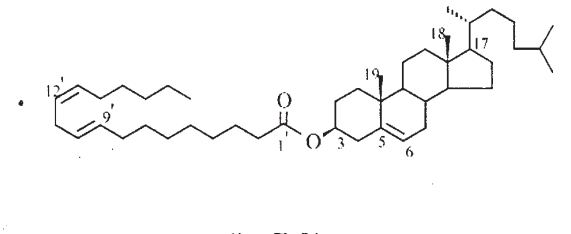
The present work has focused on the interaction of amphotericin B (AmB) with cholesteryl linoleate (Ch-Lin). Our study is based on the results obtained from UV-Vis absorption spectroscopy and follows the determination of the binding parameters from Benesi-Hildebrand, Scott and Scatchard methods, on the assumption that the binding is in 1:1 system.

Experimental part

Cholesteryl linoleate was obtained by esterification cholesterol with linoleic acid in the presence of *p*-toluensulfonic acid, in anhydrous benzene by method described in literature [6]. Amphotericin B from *Streptomyces sp.* was obtained from Sigma-Aldrich, Germany. The AmB and Ch-Lin solutions were prepared



1a **AmB**



1b **Ch-Lin**

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

in ethanol. The concentrations of the stock solutions of AmB were determined using the molar absorption coefficient value [7]:$\varepsilon_{408nm}=160000M^{-1}cm^{-1}$.

The absorption spectra were recorded at the range of 460-320nm, in a Lambda 25 PerkinElmer spectrometer, with quartz cells, at room temperature.

Results and discussion

Figure 2 presents the absorption spectra of the amphotericin B solutions. In the range of 300-500nm, it can be observed five distinct maxima centered at 428, 408, 383, 364 and 310nm. It may be noted that the spectra change gradually with increasing the antibiotic concentration.

By Tipping [10] and Schwarz [11] methods, the values for the molar absorption coefficient of the monomer $\varepsilon_{408nm}=160000M^{-1}cm^{-1}$ and the auto-association constant $K_d=5000M^{-1}$ have been determined [7].

A family of curves obtained at the titration of AmB solutions with Ch-Lin is presented in fIgure 3.

It may be observed that the AmB – Ch-Lin complex is characterized by the decrease of the major bands at small and medium Ch-Lin to AmB concentration ratios ($\frac{Ch-Lin}{AmB}$, noted *p*).

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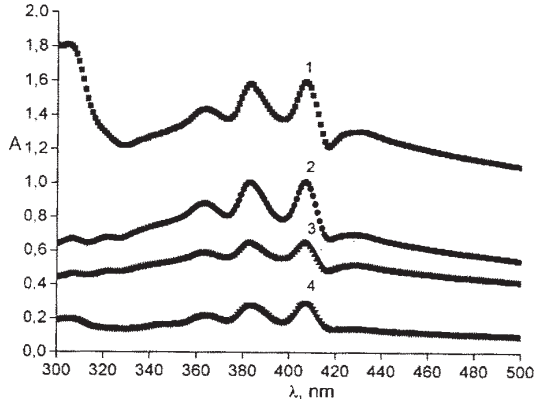

Fig. 2. Absorption spectra of amphotericin B solutions, at four drug' concentrations: (1) 10 . 10^{-6}M, (2) 6.31 . 10^{-6}M, (3) 4.06 . 10^{-6}M, (4) 1.85 . 10^{-6}M

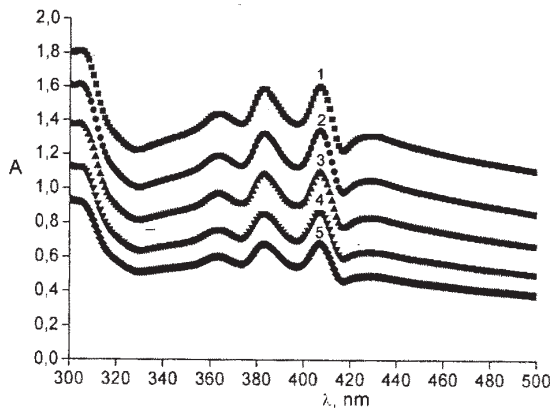

Fig. 3. Absorption spectra of AmB in the presence of varying amounts of Ch-Lin. The concentration Ch-Lin to AmB ratios (p) are: (1) 0; (2) 3.5; (3) 11.6; (4) 40.6; (5) 92.8

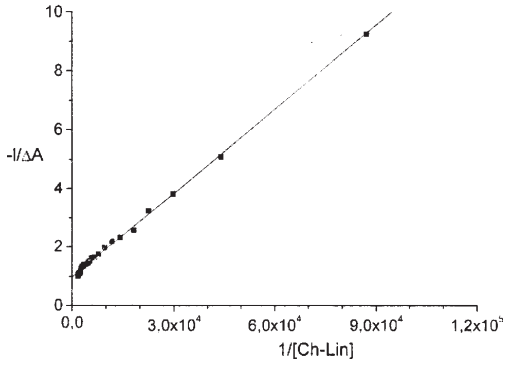

Fig. 4. Benesi-Hildebrand plot for AmB - Ch-Lin system

In the case of 1:1 AmB – Ch-Lin interaction, the binding constants may be determined from the methods proposed by Benesi-Hildebrand [8], Scott [9], Scatchard [10] and Wolfe [11]. In figures 4 - 7 are presented Benesi-Hildebrand, respectively Scott, Scatchard and Wolfe plots. The equations used and the results obtained for AmB – Lin system are summarized in table 1, where $\Delta\varepsilon_{app}=\varepsilon_{app}-\varepsilon_F$, $\Delta\varepsilon=\varepsilon_B-\varepsilon_F$, ε_{app}, ε_F and ε_B are the apparent, free and bound drug absorption coefficients, l is path length, ΔA is the

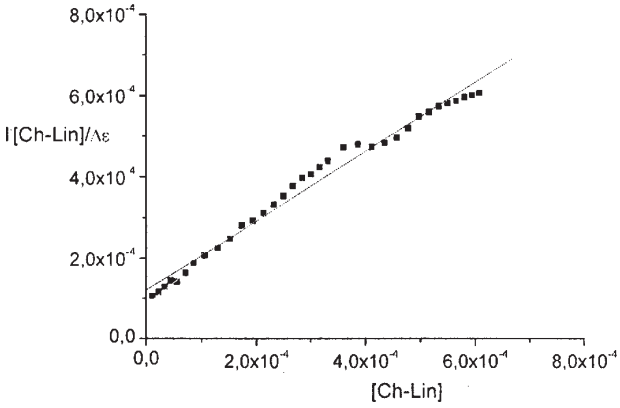

Fig. 5. Scott plot for AmB - Ch-Lin system

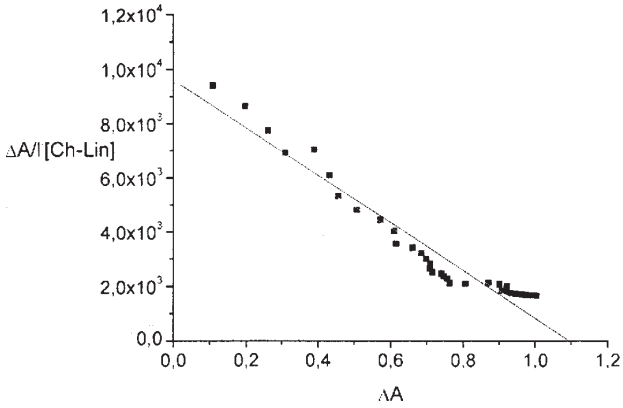

Fig. 6. Scatchard plot for AmB-Ch-Lin system

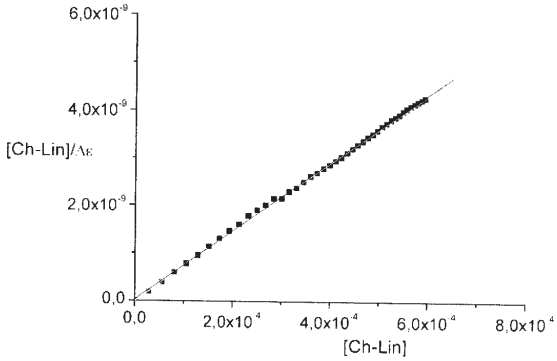

Fig. 7. Wolfe plot for AmB - Ch-Lin system

Methods	Equations	K, M^{-1}
Benesi-Hildebrand	$\frac{l}{\Delta A}=\frac{1}{C^0\cdot K\cdot\Delta\varepsilon}\cdot\frac{1}{[Ch-Lin]}+\frac{1}{C^0\Delta\varepsilon}$	$1{,}01\cdot10^4$
Scott	$\frac{l\cdot[Ch-Lin]}{\Delta A}=\frac{1}{C^0\cdot\Delta\varepsilon}\cdot[Ch-Lin]+\frac{1}{C^0\cdot K\cdot\Delta\varepsilon}$	$0{,}93\cdot10^4$
Scatchard	$\frac{\Delta A}{l\cdot[Ch-Lin]}=-\frac{K}{l}\cdot\Delta A+C^0\cdot K\cdot\Delta\varepsilon$	$0{,}68\cdot10^4$
Wolfe	$\frac{[Ch-Lin]}{\Delta\varepsilon_{app}}=\frac{[Ch-Lin]}{\Delta\varepsilon}+\frac{1}{K\cdot\Delta\varepsilon}$	$1{,}41\cdot10^4$

Table 1
THE RESULTS OF THE BINDING CONSTANTS FOR AmB – LIN INTERACTION

observed absorbance change, C^0 is the total concentration of drug and *[Ch-Lin]* is cholesteryl linoleate concentration (concentration in moles per unit volume).

In conclusion, the interaction of amphotericin B to cholesteryl linoleate, analysed by four methods: Benesi-Hildebrand, Scott, Scatchard and Wolfe, suppose a 1:1 binding ratio and does not account explicitly for either the dimerization of the drug or conjugated effects on the binding.

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