Interaction of Amphotericin B with Ricinoleic Acid and 12-(cholesteryloxicarbonyloxi)-9-Octadecenoic Acid

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The interaction between amphotericin B and ricinoleic acid, respectively 12-(cholesteryloxicarbonyloxi)-9-octadecenoic acid has been studied by UV-Vis absorption spectroscopy, in order to determine of the binding parameters: the number of binding sites (n) and the binding constant (K). The results were rationalized in terms of several literature models: Benesi-Hildebrand, Scott and Scatchard, taking into account both 1:1 drug – sterol system and cooperativity effects.

Keywords: amphotericin B, ricinoleic acid, 12-(cholesteryloxicarbonyloxi)-9-octadecenoic acid, UV-Vis absorption spectroscopy

Cholesterol (Ch), the principal sterol of mammalian cell, is known to play a fundamental role in maintaining their proper structure and function [1]. A large amount of experimental and theoretical work showed that cholesterol, increases the membrane mechanical strength, and decreases the permeability of the bilayer to small molecules [2,3]. At the molecular level, cholesterol is known to effectively order the hydrocarbon chains of adjacent lipid molecules, but at the same time, it preserves the relatively high degree of their lateral mobility [4].

Amphotericin B (AmB, fig. 1) is one of the main polyene macrolide antibiotics widely used to treat deep-seated fungal infections [5]. The mechanism of biological action of AmB is most probably directly related to the ability of the drug to form hydrophilic pores in the hydrophobic membrane core, where it increases the permeability of the cells to ions and small molecules [6,7]. It has been proposed in the 1970s that the interaction between membrane sterols and AmB is responsible for the selectivity of the drug.

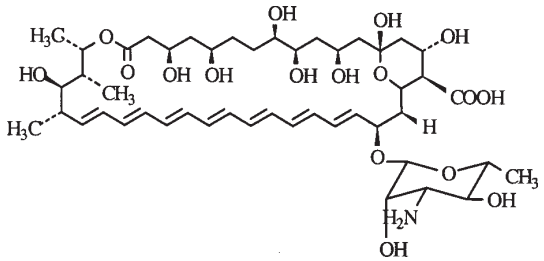


Fig. 1. Structure of AmB

The spectral properties of the free amphotericin B and their interactions with cholesteryl linoleate and cholesteryl trifluoromethylphenyl-carbamate, were previously [8-10] investigated by absorption spectroscopy. A lot of absorption maxima and the gradually decreasing of the absorption with the concentration of drug were noticed. Considering the dimerization process of the AmB, a dimerization constant of K_d=5000M^{-1} has been determined [8].

In this paper, the results of the interaction between AmB and ricinoleic acid (RicAc, fig. 2a), respectively 12-(cholesteryloxicarbonyloxi)-9-octadecenoic acid (cholesteryl carbonate from ricinoleic acid, ChOCORic, fig. 2b), obtained by UV-Vis absorption spectroscopy, are presented. The study of the interaction between AmB and RicAc is a principle study because this acid can only be found in vegetable lipids.

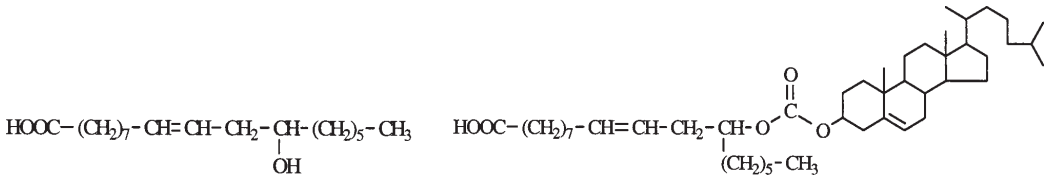
Experimental part

Amphotericin B from *Streptomyces sp.* was Sigma-Aldrich product. The stock solutions of AmB were prepared in ethanol and their concentration was determined using the molar absorption coefficient value: ε_{408nm} = 160000 $M^{-1}cm^{-1}$. Ricinoleic acid was Merck product and cholesterylchloroformate was Aldrich product. 12-(cholesteryloxicarbonyloxi)-9-octadecenoic acid was obtained by reaction of cholesteryl chloroformate with ricinoleic acid, a hydroxyl unsaturated fatty acid, in the presence of pyridine as acid acceptor [11].

The absorption measurements were performed on a Perkin-Elmer Lambda 25 UV-Vis spectrophotometer using the 1cm optical path length quartz cell, at room temperature.

Results and discussion

The influence of RicAc, respectively ChOCORic on AmB is presented in figure 3 by two families of curves obtained



(a) , (b)

Fig. 2. Structure of RicAc (a) and ChOCORic (b)

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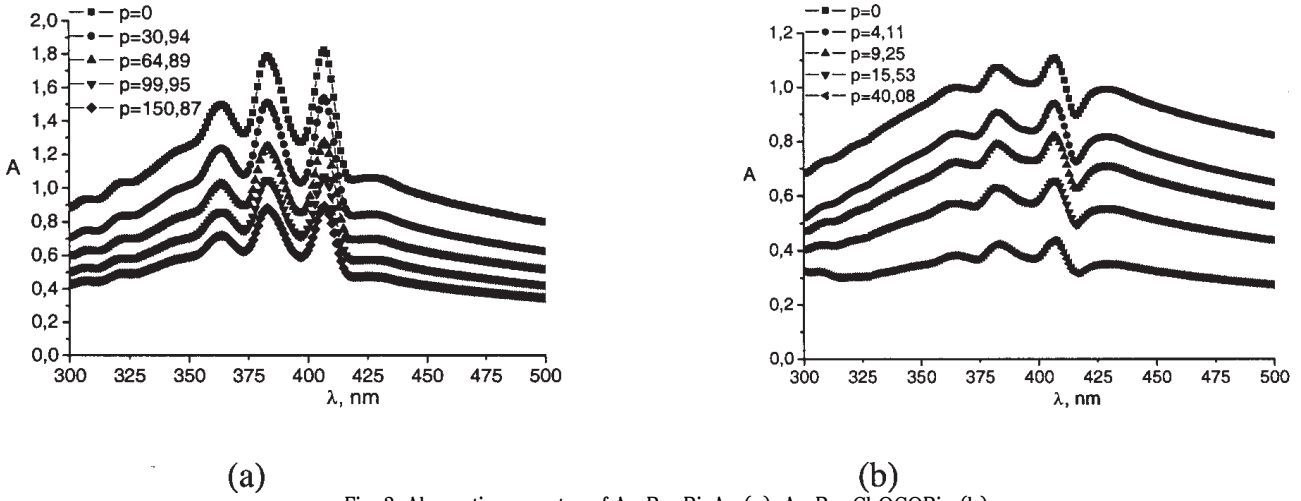


Fig. 3. Absorption spectra of AmB – RicAc (a), AmB – ChOCORic (b)

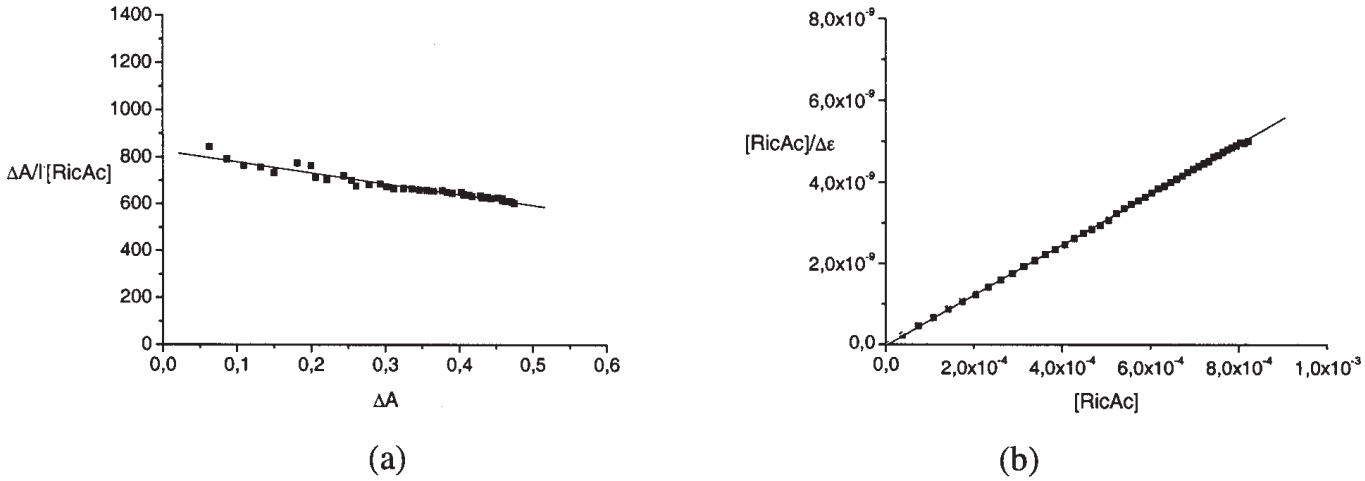


Fig. 4. Scatchard (a) and Wolfe (b) plots of AmB – RicAc system

at the titration of AmB solutions (10^{-6}-10^{-5}M) with RicAc, respectively ChOCORic. It was noticed the gradually decreasing of the absorption with the RicAc or ChOCORic to AmB ratios, this variation being identical to those observed on decreasing the concentration of drug.

Supposing that the interaction of AmB with RicAc, respectively ChOCORic is in system 1:1, the total absorbance represents the sum of the absorbance of the free and bound species, weighted by their respective concentrations:

$$A = f_0 \cdot (C_D^0 - C_B) + f_B \cdot C_B \quad (1)$$

$$A_0 = f_F \cdot C_D^0 \quad (2)$$

where:

A_0 – the absorbance of free drug;

A – the absorbance of drug measured at each RicAc or ChOCORic concentration,

C^o – the total drug concentration,

C_B – the bound drug concentration.

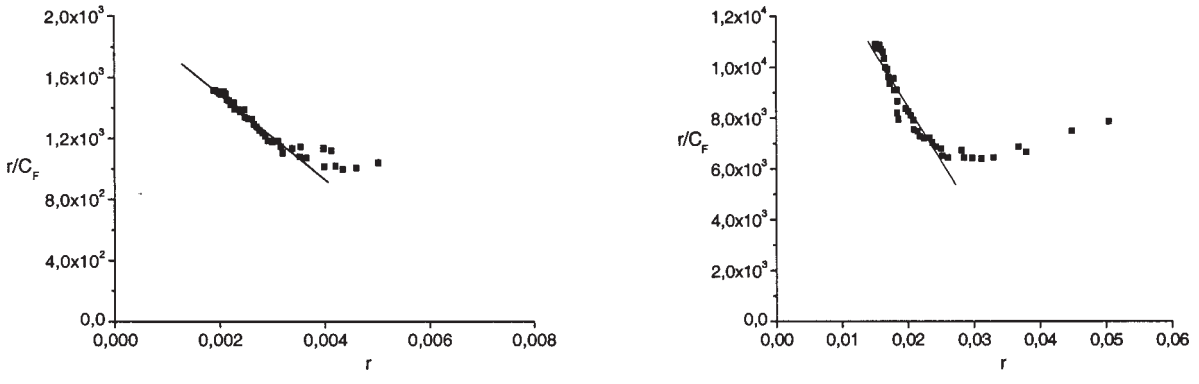
On the assumption of the absorption is due only to the free form of drug (f_B=0), the concentrations of free and bound drug are given by:

(3)

(4)

Method	Equations	K, M^{-1}	
		AmB – RicAc	AmB – ChOCORic
Benesi-Hildebrand	$\frac{1}{\Delta A} = \frac{1}{C_D^0 \cdot K \cdot \Delta\varepsilon} \cdot \frac{1}{C_{RicAc}} + \frac{1}{C_D^0 \cdot \Delta\varepsilon}$	$0,29 \cdot 10^3 M^{-1}$	$2,94 \cdot 10^3 M^{-1}$
Scott	$\frac{l \cdot C_{RicAc}}{\Delta A} = \frac{1}{C_D^0 \cdot \Delta\varepsilon} \cdot C_{RicAc} + \frac{1}{C_D^0 \cdot K \cdot \Delta\varepsilon}$	$0,23 \cdot 10^3 M^{-1}$	$1,89 \cdot 10^3 M^{-1}$
Wolfe	$\frac{C_{RicAc}}{\Delta\varepsilon_{app}} = \frac{C_{RicAc}}{\Delta\varepsilon} + \frac{1}{K \cdot \Delta\varepsilon}$	$1,67 \cdot 10^5 M^{-1}$	$1,62 \cdot 10^5 M^{-1}$
Scatchard	$\frac{r}{C_F} = (n - r) \cdot K$	$2,72 \cdot 10^5 M^{-1}$	$3,09 \cdot 10^5 M^{-1}$
	$r = \frac{n \cdot K \cdot C_F}{1 + K \cdot C_F}$	$2,66 \cdot 10^5 M^{-1}$	$2,92 \cdot 10^5 M^{-1}$

Table 1
RESULTS OF SPECTRAL STUDY



(a) (b)

Fig. 5. Scatchard plot of AmB – RicAc (a), AmB – ChOCORic (b)

$$C_B = C_D^0 \cdot \frac{A - A_0}{A_0}$$

$$C_F = C_D^0 - C_B$$

On the basis of assumptions above mentioned, the binding constants were evaluated from the methods proposed by Benesi-Hildebrand [12], Scott [13], Scatchard [14] and Wolfe [15]. The experimental data lead to the linear Benesi-Hildebrand, respectively Scott, Scatchard and Wolfe plots, two examples being presented in figure 4 for AmB – RicAc system. The equations utilized and the results obtained for the two systems are summarized in table 1.

In addition, the experimental data fitted either to the linear Scatchard plot [14]:

$$\frac{r}{C_F} = (n - r) \cdot K \quad (5)$$

or to a non-linear regression:

$$r = \frac{n \cdot K \cdot C_F}{1 + K \cdot C_F} \quad (6)$$

corresponding to a single class of non-interacting binding sites that do not exhibit cooperative behaviour. In these relationships, C_F is the free drug concentration, n – the number of binding sites, r – the binding ratio: $r = C_B / C_{RicAc}$ or $r = C_B / C_{ChOCORic}$.

For both systems, the Scatchard plots (an example is presented in fig. 5) attest the presence of two binding processes. The solid line represent the best fit of the linear portion of the plot and it is characteristic for non-cooperative binding to one class with n equivalent sites. Considering this solid line, the binding constant and the number of binding sites were obtained.

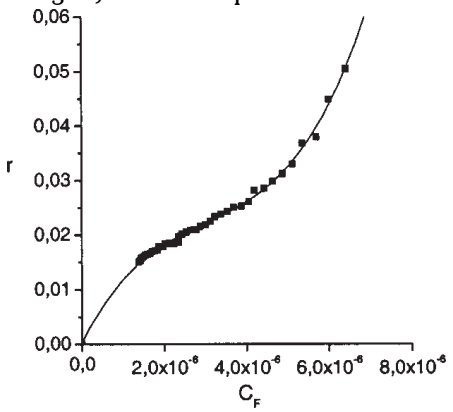


Fig. 6. Non-linear fitting of binding processes of AmB-RicAc

The non-linear fitting of both processes (fig. 6) yield similar binding parameters for the process (II), the results being presented in table 1.

Conclusions

The analysis of the AmB interaction with RicAc, respectively ChOCORic, using several methods points out two binding types. The first binding process was analysed by Benesi-Hildebrand, Scott and Scatchard models, when one assume of 1:1 binding ratio and do not account explicitly for either the dimerization of the drug or cooperativity effects on the binding. The second binding process was analysed by Wolfe and Scatchard methods, supposing the cooperative interaction between AmB and RicAc, respectively AmB and ChOCORic.

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