Solvatochromic Behaviour of Rifampicin in Diluted Solutions

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The solvent influence on the maxima of UV-Vis electronic absorption spectra of rifampicin diluted solutions was studied by Bakshiev's solvatochromic theory. The experimental data were analyzed and discussed comparatively for the visible and respectively the near ultraviolet bands - on one side, and for the polar and non-polar solvents – on the other side. The dependence of the frequency in the electronic absorption maxima on the function $(n^2-1)/(n^2+2)$ of the non-polar solvent refractive index, n, evidenced the dispersive nature of the solute-solvent interactions for both studied bands. In polar solvents, the additional effect of orientation-induction forces was emphasized by means of the dependence of the studied band maxima frequency on n *as well as on* ε *(solvent dielectric constant) through the function $[(\varepsilon-1)/(\varepsilon+2)]-[(n^2-1)/(n^2+2)]$. The experimental results were generally consistent with the ones provided by semi-empirical molecular modeling applied to rifampicin optimized geometry. This study may be useful in rifampicin selective extraction from liquid reaction medium as well as in liquid chromatographic technique for final product purity test and also in yielding of polymeric micelles loaded with rifampicin for tuberculosis treatment.*

Keywords: rifampicin, solvatochromism theory, semi-empirical molecular modeling

Antibacterial agents included in the tuberculosis treatment programs can be divided in broad spectrum compounds and narrow ones. Rifampicin is included in the broad spectrum antibiotics according to the above classification; is a semi-synthetic compound derived from the rifamycin B family, produced by strains of *Nocardia (Streptomyces) mediterranei* which focused not only the microbiologist attention, but also the interest of several multidisciplinary research groups. Rifampicin solubility and stability varies according to *p*H due to its amphoteric nature [2]; it exists as a polymorph with two principal forms, I and II, and also in amorphous form [3], the form I, being the most stable, was used in the present study.

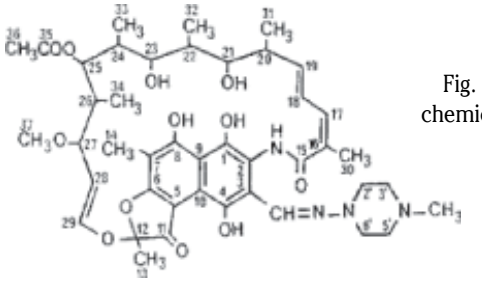

Fig. 1. Rifampicin chemical structure [1]

Spectral studies on the rifampicin and rifampicin solutions were reported by Favila et al (2007) [4], who investigated the IR and UV-Vis absorption spectra of this molecule in comparison to those of other antibacterial compounds. Raman vibrational studies, in parallel with UV-Vis ones, were carried out by Howes et al (2007) [5] in order to discuss rifampicin behaviour in different liquid media. Molecular modeling was also used in various reports to reveal structural and energetic parameters of rifampicin in comparison to other molecules with biological activity (J.K. Rugutt & K.J. Rugutt, 2001 [6]); semi-empirical formalism was applied by Mishra et al (2001) [7] to give a similar molecular approach of several rifampicin different conformers. We also previously reported some data regarding the rifampicin conformers structural and energetic parameters [8].

Aiming to provide a mathematical insight in the spectral behaviour in the UV-Vis range of solvated compounds in diluted solution, by taking into account the solvent influence on the absorption transition energies, various theoretical models of real liquids were proposed [9-13].

The mathematical approach developed by Bakhshiev [12] expressed the solvent influence on the electronic absorption band (EAB) maximum, when passing from vapour to solution, by graphical approach of the dependence on the solvent macroscopic parameters of the difference of the wave numbers ($\Delta\tilde{\nu}$) in solution ($\tilde{\nu}$) and in vapour state ($\tilde{\nu}$), so that for the solute-solvent universal interactions (dispersive, orientation, inductive) the relation (1) results:

$$hc\Delta\tilde{\nu} = hc\left(\Delta\tilde{\nu}_{dispersion} + \Delta\tilde{\nu}_{orientation} + \Delta\tilde{\nu}_{induction}\right) \quad (1)$$

or, briefly, according to [14]:

$$hc\,\Delta\tilde{\nu} = C_1\left(\frac{2n^2+1}{n^2+2}\right)\left(\frac{\varepsilon-1}{\varepsilon+2}-\frac{n^2-1}{n^2+2}\right)+C_2\frac{n^2-1}{n^2+2} \quad (2)$$

where ε and n are the solvent dielectric constant and refractive index.

The role of each interaction type could be emphasized only based on experimental measurements and correlations established between the position of EAB maxima ($\tilde{\nu}_s$) and the solvent parameters, as one can see further below. The main purpose of this study was the application of spectral method for revealing the rifampicin interaction ability with various organic solvents.

Experimental part

Pure crystalline powders of rifampicin (SIGMA) was solved in an array of non-polar and polar solvents (table 1) resulting in highly diluted (about 10^{-5} M) solutions.

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Table 1
SOLVENT MACROSCOPIC PARAMETERS

No.	Solvent	n	ε
1	*n*-Hexane	1.3749	1.89
2	Petroleum ether	1.3650	2.20
3	Carbon tetrachloride	1.4602	2.24
4	Toluene	1.4969	2.38
5	Diethyl ether	1.3524	4.33
6	Chloroform	1.4459	4.81
7	Ethyl Acetate	1.3724	6.02
8	Acetic acid	1.3716	6.15
9	*n*-Pentanol	1.4100	13.90
10	Isopentanol	1.4072	15.19
11	*n*-Butanol	1.3993	17.51
12	Isobutanol	1.3959	17.93
13	Isopropanol	1.3772	18.23
14	*n*-Propanol	1.3856	20.45
15	Acetone	1.3587	20.70
16	Formaldehyde	1.3746	23.00
17	Ethanol	1.3614	24.55
18	Methanol	1.3284	32.66
19	N,N-Dimethylformamide	1.4305	36.71
20	1,2-Ethanediol	1.4318	37.70
21	Water	1.3330	80.00

The electronic absorption spectra (EAS) of the rifampicin diluted solutions were recorded by a Shimadzu UV-1700 double beam UV/Visible spectrophotometer (quartz cells of 1 cm width versus the reference corresponding solvent) with data acquisition software.

Computational simulations of rifampicin EAB were carried out by using PM3 semi-empirical method implemented in HyperChem 8.0.10 molecular modeling package [15], for the mathematical reconstruction of rifampicin electronic absorption spectrum.

Results and discussions

In order to evidence the solute interactions with non-polar and polar solvents based on the theory of solvent influence on the electronic transitions energy two bands of rifampicin EAS, from ultraviolet and respectively visible domains, were analyzed.

The nature of the studied electronic transitions was discussed according to the literature data [16-18], so that the band at about 340 nm was assigned to the π-π^* transition of the naphthohydroquinone chromophore, while the band at about 474 nm was assigned to a n-π^* transition with electron charge transfer (CT transition) from the electron donating group (CO) to the (OH) of the naphthohydroquinone chromophore [5]. In the next figures (fig. 2 a-b), the two EAB taken into the study are presented, aiming to evidence the solvatochromic shift of their maxima in various solvents.

The spectral shift of the EAB maxima were discussed based on Bakhshiev's theory [12], as follows: starting from relation (2) and using the notations:

$$f(\varepsilon,n)=\frac{\varepsilon-1}{\varepsilon+2}-\frac{n^2-1}{n^2+2} \text{ and } f(n)=\frac{n^2-1}{n^2+2} \qquad (3)$$

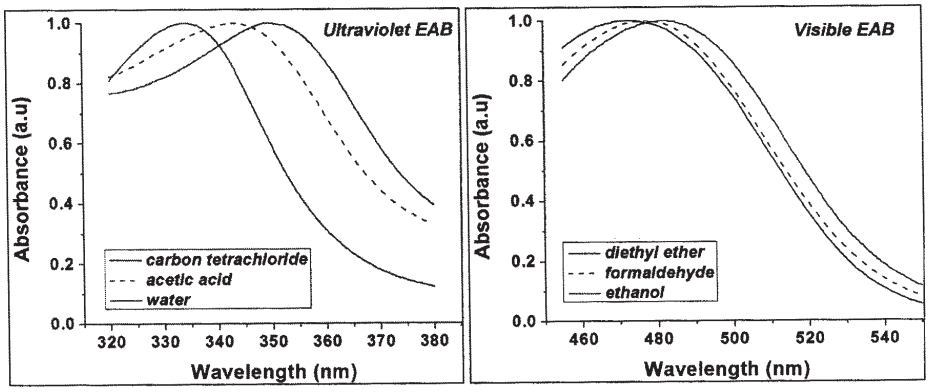
with the observation that for the used solvents the term $(2n^2+1)/(n^2+2)$ is close to the unit, one can write:

$$\Delta\tilde{\nu}=C_1 f(n,\varepsilon)+C_2 f(n) \qquad (4)$$

Following this approach, one can consider that, when dispersive interactions dominate into the solute-solvent systems, C_1 becomes non-significant (as in the case on non-polar solvents, fig. 3 a-b), where linear regression line was fitting the dependence of experimental wavenumbers on the function $f(n)$. The statistical significance of the correlation coefficient was found to be $p < 0.0005$.

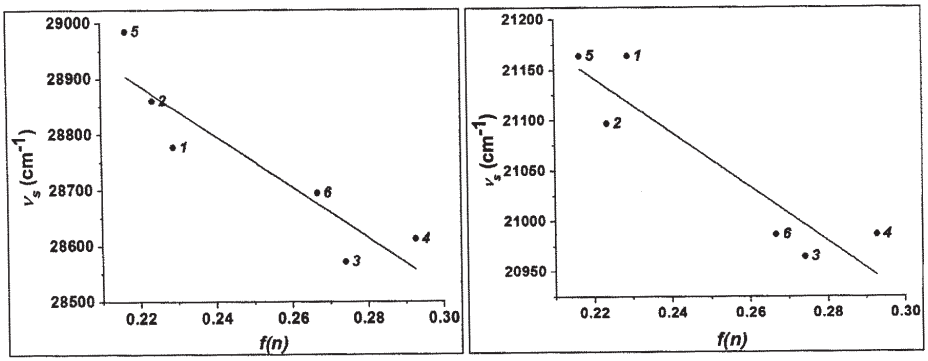
In the case of polar solvent solutions, we evidenced that the wavenumbers of rifampicin EAB maxima depend linearly of the function $f(\varepsilon,n)$ (fig. 4 a-b), which can be assigned to the dominancy of orientation induction universal interactions (equivalent with the C_1 much higher weight compared to C_2); good statistic significance of the correlation coefficient was also found for these regression lines ($p < 0.0005$).

According to the regression equations (table 2) in figure 3 a-b, bathochromic shift was revealed when passing from vapour to non-polar solvents (i.e. $\tilde{\nu}_s < \tilde{\nu}_v$); accordingly, negative slope values were obtained (-4547.2 cm^{-1} for the UV band and -2698 cm^{-1} for the visible band, table 2) from the linear dependences of $\tilde{\nu}$, on $f(n)$.



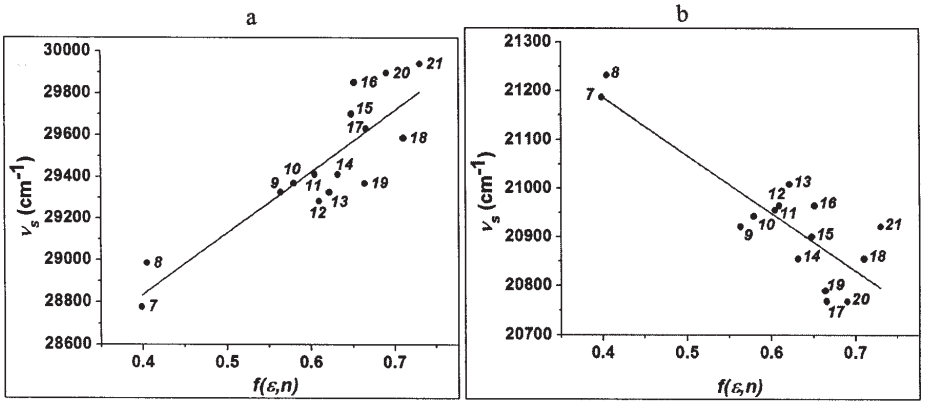
a b

Fig. 2 a-b. The rifampicin ultraviolet and visible EAB recorded in various solvents emphasizing the solvatochromic shift



a b

Fig. 3 a-b. The linear dependence on the function *f(n)* of the experimental wavenumbers from a) UV and b) Vis range, in the case of non-polar solvents



a b

Fig. 4 a-b. The linear fitting on the function f(ε,n) of the experimental wavenumbers from a) UV and b) Vis range in rifampicin solutions with polar solvents

This red shift is in concordance with aromatic chromophores behaviour due to the dispersive solute-(non-polar) solvent interactions, the shift being usually larger, for larger refractive index of the solvent [19]. The interpretation of the bathochromic shift may be understood based on the stabilization of the excited state more than that of the ground state.

In polar solvents, the presence of orientation-induction interactions was evidenced by fitting the experimental $\tilde{\nu}_s$ on *f(ε, n)*. For the UV band, the dominancy of this type of interactions is obvious from the hypsochromic shift ($\nu_s > \tilde{\nu}_{so}$), as well as from the positive slope (2873.3 cm^{-1}, table 2) of the linear dependence ν_s on *f(ε,n)* (fig. 4a). One can presume that in polar solvents, the excited state energy of the solute molecule was enhanced relatively to the ground state for the UV transition. In the case of the visible EAB (fig. 4b), the same as for the non-polar solvents (fig. 3b), a bathochromic shift was evidenced, but the absolute value of the line slope (-1185.3 cm^{-1}, table 2) was more than twice smaller in comparison to that for the non-polar solvents (-2698 cm^{-1}, table 2); this could be due to the significant but non-dominant role of the orientation-induction forces comparatively to the dispersive ones.

The experimental rifampicin EAS (fig. 5) were compared with the simulated electronic spectrum obtained following semi-empirical quantum-chemical modeling (PM3 method) for rifampicin isolated state (after previous molecule geometry optimization) (fig. 6).

From the simulated spectrum, the wavelengths (λ'_o) corresponding to the UV and visible range of the vapor state rifampicin EAS were identified, then converted to wavenumbers ($\tilde{\nu}_o$) and compared with the $\tilde{\nu}_o$ EAB recorded maxima, statistically deduced from regression equations (table 2). The concordance between the results of the application of theoretical and experimental methods to the study of rifampicin EAS could be discussed as follows.

The differences noticed between the values of EAB maxima provided by quantum-chemical approach calculation and respectively the statistically deduced regression equations could be related to the hypotheses of the theoretical solvatochromic shift model as well as to the semi-empirical basis of the molecular modeling simulation. Especially the limited number of solvents could affect the statistical significance of the linear fitting (as could be seen from the figures above, where some correlation coefficients are under 0.9). More, the specific interactions that are expected in some polar solvents are actually neglected in the frame of the solvatochromic shift theory (Bakhshiev's mathematical approach); they still may occur in the polar solvent solutions since some rifampicin conformers [8] were found to exhibit

EAB	Linear regression equation	*R	$\tilde{\nu}_o$ (cm^{-1})	λ'_o (nm)	$\tilde{\nu}_o'$ (cm^{-1})
UV – non-polar solvents	$\tilde{\nu}_s = -4547.2\, f(n) + \tilde{\nu}_0$	0.914	29888	338.90	29507
UV – polar solvents	$\tilde{\nu}_s = 2873.3\, f(\varepsilon,n) + \tilde{\nu}_0$	0.870	27699		
Vis – non-polar solvents	$\tilde{\nu}_s = -2698.0\, f(n) + \tilde{\nu}_0$	0.914	21736	416.90	23987
Vis – polar solvents	$\tilde{\nu}_s = -1185.3\, f(\varepsilon,n) + \tilde{\nu}_0$	0.855	21661		

*R – correlation coefficient

Table 2
THE COMPARISON OF EXPERIMENTAL AND SIMULATED RIFAMPICIN EAB MAXIMA

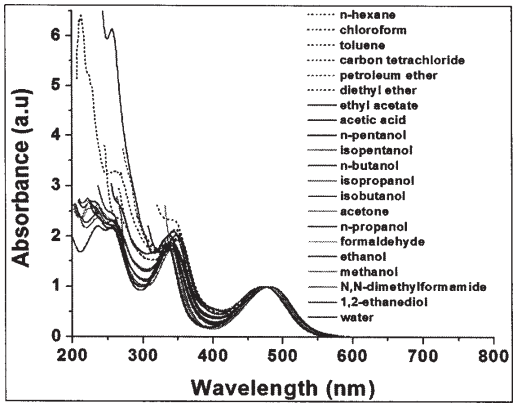


Fig. 5. Experimentally recorded EAS of rifampicin (UV-Vis range)

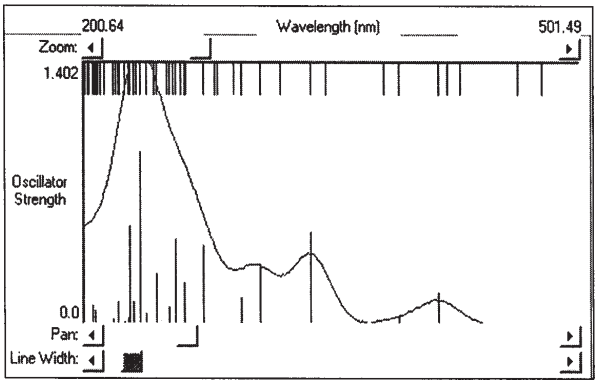


Fig. 6. Simulated EAS of rifampicin in vapour state

intramolecular hydrogen bonds that could be destabilized – for instance in alcohols, so that specific local interactions in the form of intermolecular hydrogen bonds could be developed. The study of such interaction could be further developed aiming to study the interactions ensuring polymeric micelles formation for rifampicin delivery in pulmonary disease treatment; such polymeric systems incorporating rifampicin were already reported in [20].

Conclusions

Linear dependences between the frequencies in the absorption band maxima and distinct functions on the solvent macroscopic parameters (*n* - the refractive index of non-polar solvents, and both *n* and ε - the dielectric constant - in the case of polar solvents) were found. From the accomplished study it was found that to the increase of the solvent refractive index the red shift also increased, as proof of the dominancy of dispersive interactions in the rifampicin diluted solutions. Higher sensitivity of the UV band was revealed (regression line slope of - 4547.2 cm^{-1}) compared to the visible range band (-2698 cm^{-1} slope). The development of orientation-induction forces in polar solvents diminished the regression line slope (-1185.3 cm^{-1}) for the visible range band, while for the UV band higher solvatochromic sensitivity was emphasized by the reversal of the regression line slope (2873.3 cm^{-1}) as well as by the blue shift to the increase of solvent polarity. One may conclude that the dispersive interaction dominancy in non-polar solvents stabilized the excited state electronic level relatively to the ground state one for both analyzed bands, while the orientation-induction interactions, developed in polar solvents, lowers the excited state energy in the case of the ultraviolet range transition of rifampicin. In the next research project, extended computational studies are planed on the rifampicin structural and energetic parameters in parallel with solvent effect investigations on the vibration bands by means of Raman spectroscopy.

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