



PLURONIC COPOLYMER NANOSTRUCTURE FOR SOLUBILIZATION OF HYDROPHOBIC BENZOFURAZAN DERIVATIVES IN WATER. A SPECTROPHOTOMETRIC STUDY

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Polymeric micelles solubilization is a powerful alternative for dissolving hydrophobic compounds in aqueous environments. For this purpose in this work, a micellar solution of triblock copolymer Pluronic L64 in water has been used. An absorption and fluorescence spectrophotometric study on three hydrophobic benzofurazan derivatives has been conducted both in micellar Pluronic L64 and in absolute ethanol solutions. The three chosen compounds were: (N-1-Naphthyl-N'-(7-nitro-2,1,3-benzoxadiazole-4-yl)ethane-1,2-diamine) (1), (7-Nitro-N-(pyridine-2-yl-methyl)-2,1,3-benzoxadiazole) (2) and (2-(Hydroxymethyl)-2-[(7-nitro-2,1,3-benzoxadiazole-4-yl)amino]propane-1,3-diol) (3). Researches undertaken have highlighted the performance of micellar aggregates for the solubilization in water of these hydrophobic compounds, which have numerous applications in various fields.

INTRODUCTION

Pluronic block-copolymers are used in numerous applications such as cosmetics, detergency, textiles, emulsification and drug adjuvants, because of their ability to form various aggregates in water and their low toxicity,¹⁻¹¹ these amphiphilic three block co-polymers PEO (poly(ethylene oxide))-PPO (poly(propylene oxide))-PEO, (PEO being hydrophilic block and PPO hydrophobic block) can form spherical core-shell micelles in aqueous solution. The micellar core consists of the hydrophobic blocks and the shell region consists of the hydrophilic blocks. Pluronic L64 (PEO₁₃PPO₃₀PEO₁₃), in certain conditions can form spherical micelles.^{9,12-14}

The low toxicity and the ability of Pluronic micelles to increase the solubility of sparingly soluble substances in water have particular significance in pharmacy or as drug carriers.^{4-6,10} Within this context, the utilization of micelles as drug carriers presents some advantages when

compared to other alternatives such as soluble polymers and liposomes.^{6,10,11} In addition, the size of the micelles, around 5-100nm, presents an advantage (compared with soluble polymers and liposomes) for pharmaceutical applications.

The ability to solubilise hydrophobic compounds in water using micelles can be monitored with relative ease in two situations: the solubilized compound has an absorption band in visible spectral range and is fluorescent. If the solubilized compound presents solvatochromie, it can be used as polarity probe¹⁵⁻¹⁷ and thus, to study the microstructure of aggregates formed, which in many cases provide important information on the types of interactions between the hydrophobic compound and Pluronic chain.¹⁸

In previous works, 4-amino-7-nitrobenzofurazan hydrophobic derivatives have been synthesized and characterized.¹⁹ They show chromogen and fluorescence properties due to the nitrobenzofurazan (NBD) part.^{20,21} Some of these compounds have biological activity as antileukemic, immunosuppressive, or monoamine oxidase inhibiting activity.²²⁻²⁴

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Because the compounds are fluorescent they could have applications as molecular fluorescence probes for receptors of chemical and/or biomedical interest,²¹ because such compounds, by their structural design, are in the category of multivalent structures, that allow noncovalent interactions.²⁵⁻³⁰

This paper tries to demonstrate the ability of Pluronic L64 micelles to solubilise in water of three hydrophobic compounds having a nitrobenzofurazan (NBD) rest in their structure, and then, to obtain structural information on the aggregates formed in water. The results, obtained from UV-VIS absorption and fluorescence measurements, will be presented and discussed comparatively in absolute ethanol and in L64 micellar-water solutions. The quenching ability of NBD derivatives fluorescence by nitrite and nitrate ions is also investigated both in ethanolic and micellar aqueous solutions.

EXPERIMENTAL

Materials. Triblock copolymer poly(ethylene oxide)-poly(propylene oxide)- poly(ethylene oxide) Pluronic L64 (PEO₁₃PPO₃₀PEO₁₃) (MW about 2900) was obtained from BASF and used without purification. A stock solution of 20%

w/w L64 in water was used, which belong to L₁ phase (direct micelles) at 23°C.³¹ The micellar solution was prepared by weighing appropriate amounts of polymer in water, stirred and stored overnight to ensure homogenization.

The benzofurazan derivatives: **1** (N-1-Naphthyl-N'-(7-nitro-2,1,3-benzoxadiazole-4-yl)ethane-1,2-diamine), **2** (7-Nitro-N-(pyridine-2-yl-methyl)-2,1,3-benzoxadiazole) and **3** (2-(Hydroxymethyl)-2-[(7-nitro-2,1,3-benzoxadiazole-4-yl)amino]propane-1,3-diol) have been synthesized as in literature.¹⁹ Their chemical structures are presented in Table 1.

Absolute ethanol was from Merck, and water was Millipore purity.

Spectrophotometric measurements. Absorption spectra were recorded with a Perkin –Elmer Lambda 35, UV-VIS spectrophotometer, with a 0.5nm increment. 0.5 cm quartz cells have been used for measurements.

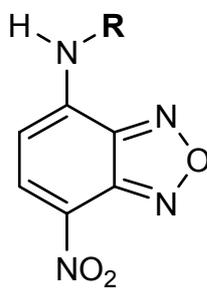
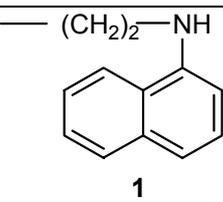
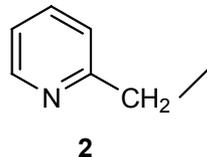
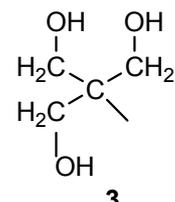
The steady-state fluorescence spectra (emission and excitation) were recorded with JASCO FP-6500 spectrofluorimeter.

RESULTS AND DISCUSSION

Table 1 presents the chemical structures of NBD derivatives **1-3** and their molecular hydrophobicity, R_{M0} .^{19,32,33} The hydrophobic/hydrophilic balance of compounds **1-3** was studied by reverse phase TLC (RP-TLC) method.

Table 1

Chemical structure and molecular hydrophobicity (R_{M0}) of NBD derivative compounds **1-3**

Compounds	R	R_{M0}
	 <p>1</p>	2.708
	 <p>2</p>	1.543
	 <p>3</p>	0.497

Compounds **1-3** (Table 1) were chosen in this research due to the following characteristics: i) high absorption in the visible spectrum; ii) to have fairly high the hydrophobic/hydrophilic balance, appointed by the experimental parameter, R_{M0} , function of structural design and iii) a high fluorescence emission intensity Fl , and high quantum yield, Φ .

Regarding the hydrophobic/hydrophilic property, the compounds **1-3** are hydrophobic (preferentially soluble in organic solvents) in the order $R_{M0}1 > R_{M0}2 > R_{M0}3$.

Only **3**, which have an amphiphilic structure, shows a low solubility in water.¹⁹ The compounds **1-3** are intense fluorescent in ethanol, in the order $Fl\ 2 > Fl\ 3 > Fl\ 1$.

It is thus expected that, in the presence of hydrophobic environment provided by micelles in water, the compounds can be transferred relatively easy in micellar core or shell, function of the hydrophobic/hydrophilic balance. If the solubilization takes place, this can be easily observed and studied by spectrophotometric methods, thanks to chromogenic and fluorescence properties of the compounds. The spectral parameters determined from absorption and fluorescence spectra of the compound in micellar

solutions are compared with the corresponding values obtained in absolute ethanol.

In the following will be presented and discussed experimental results from absorption and fluorescence spectra of compounds **1-3** after solubilization in L64 micelles/water.

UV-VIS absorption spectra

The compounds **1-3** were introduced, in certain quantities, in the stock 20% L64 /water solution, obtaining different concentrations of compounds. The intense yellow solutions were stirred and were stored overnight to ensure homogenization. In parallel, solutions in absolute ethanol were prepared with about the same concentrations of the compounds. The absorption spectrum presents three bands, two in UV and one in visible range. The bands in UV are $\pi \rightarrow \pi^*$ transitions. The long-wavelength band (450-470nm) is an intramolecular charge transfer (ICT) band.³⁴ Table 2 presents the experimental results from the absorption spectra obtained in the two situations. In the table 2, the UV band (at 260-270nm) has been omitted to avoid errors due to the absorption band of the polymer.

Table 2

Absorption spectra parameters (λ_{max} and absorbance, A) for compounds **1-3** in absolute ethanol and in 20% L64/water solutions with different concentrations

Compound	λ_{max} , nm (Absorbance, A)	Concentration (M)
in absolute EtOH solutions		
1	466(2.825), 333(2.297)	1.72×10^{-4}
	466(1.002), 333(0.776)	5.73×10^{-5}
	466(0.596), 333(0.464)	3.05×10^{-5}
2	455.5(3.199), 326(2.051)	2.21×10^{-4}
	455.5(2.039), 326(0.909)	1.47×10^{-4}
	455.5(1.018), 326(0.448)	8.57×10^{-5}
3	462(3.109), 329(1.760)	2.82×10^{-4}
	462(1.453), 329(0.649)	1.88×10^{-4}
	462(0.741), 329(0.334)	8.77×10^{-5}
in 20% L64 /water solutions		
1	470(1.300), 334.5(1.062)	1.72×10^{-4}
	470.5(0.794), 335.5(0.644)	6.88×10^{-5}
2	463(3.088), 331(1.459)	2.21×10^{-4}
	468(1.258), 334.5(0.528)	1.47×10^{-4}
	469(0.793), 335(0.351)	5.88×10^{-5}
3	469(3.302), 336.5(2.063)	2.82×10^{-4}
	471(1.313), 337(0.505)	5.64×10^{-5}
	470.5(0.283), 334.5(0.122)	1.86×10^{-5}

The values in Table 2 show that: i) in absolute ethanol, λ_{max} is not influenced by concentrations of the compounds **1-3**; ii) regardless of the type of **1-3** compounds, the absorption bands of compounds in

L64/water are bathochromic shifted and iii) the bathochromic shifts of long-wavelength band in L64 compared with ethanolic solutions are greater at lower **1-3** compounds concentrations. The shifts

are largest in the case of low-energy band (ICT transition), which is strongly influenced by the solvents. It is well known for these compounds (NBD-NH-R derivatives) that by dissolving the compounds, the environment (*e.g.* solvent, possible additions) may change λ_{\max} values, by the effects

of polarity, hydrophobic interactions, hydrogen bridges etc.³⁵ Figure 1 illustrates the absorption spectra of compound **2** ($c = 1.47 \times 10^{-4} \text{M}$) in absolute ethanol and in 20% L64/water, which proves the solubilization of the compound in micellar solution.

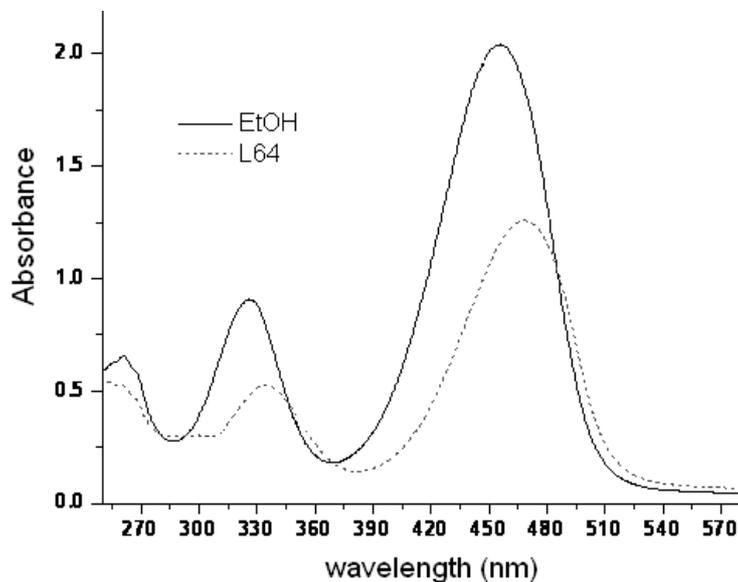


Fig. 1 – Absorption spectra of compound **2** ($1.47 \times 10^{-4} \text{M}$) in ethanol and L64 micelles.

Using a set of molecular probes, it was shown that there is a non uniform distribution of water in L64 direct micelles, *i.e.* there is a hydration gradient,¹⁴ in other words, the water concentration decreases from the surface towards the core of the micelle, with a completely hydrophobic core. The solubilization site of the compound depends on its hydrophobic/hydrophilic balance, the hydrophobic molecules will be solubilized in the micellar core, and the molecules with intermediate polarity will be distributed on the poly (ethylene oxide) chain, near the interface or in the external part of the shell (function on the compound polarity). The observed bathochromic shifts of the absorption bands in micellar solutions compared with solutions in ethanol demonstrate that the molecules are solubilized in a slightly more polar area than ethanol. Previously, positive solvatochromie of the

compounds **1-3** was demonstrated in different solvents.¹⁹ It is possible that at higher concentrations, a part of molecules are aggregated and are dissolved in an outside area (the molecules “feel” a higher hydration).

Fluorescence spectra

The compound **1** is weak fluorescent, its fluorescence being higher in acidic environments,¹⁹ this is why this compound was not involved in the investigation by fluorescence method of its solubilization in L64 direct micelles. Table 3 presents the fluorescence parameters of the compounds **2** and **3**. It is noted that the fluorescence excitation was performed at 450nm, *i.e.* at intramolecular charge transfer band.

Table 3

Fluorescence parameters: maximum emission wavelength (λ_{mem}), maximum fluorescence intensity (I_f) at mentioned molar concentration, C, and fluorescence quantum yield Φ , for compounds **2** and **3** in ethanol and 20 %L64/water solutions

Compound	absolute ethanol solutions				L64/water solutions			
	C(M)	λ_{mem} , nm	I_f a.u.	Φ	C (M)	λ_{mem} , nm	I_f a.u.	Φ
2	4.4×10^{-5}	530	125.6	0.058	4.4×10^{-5}	531	40	0.025
3	5.5×10^{-5}	531	123.9	0.039	5.5×10^{-5}	532	44	0.013

The hydrophobicity of compounds **2** and **3**, evidenced by R_{M0} parameter (table 1), show a decrease in the order $R_{M0}2 > R_{M0}3$. The compounds **2** and **3** are practically insoluble in water and are non-fluorescent. In a mixture 80% water-ethanol, the fluorescence intensity is about 7 times lower than in absolute ethanol, at the same concentration of compound **2** (fig.2-a) and the emission wavelength (λ_{mem}) bathochromic shifted at 536nm. Using a micellar aqueous solution, these

hydrophobic compounds can be dissolved. The fluorescence intensity in L64/water is lower than in ethanol, but is higher than in water-ethanol solution. From Table 2 and figure 2 one can observe a very small bathochromic shift of emission band in L64-water solutions compared with ethanolic solutions. Fluorescence quantum yield is lower in micellar solution, perhaps because of the quenching effect of hydration water.

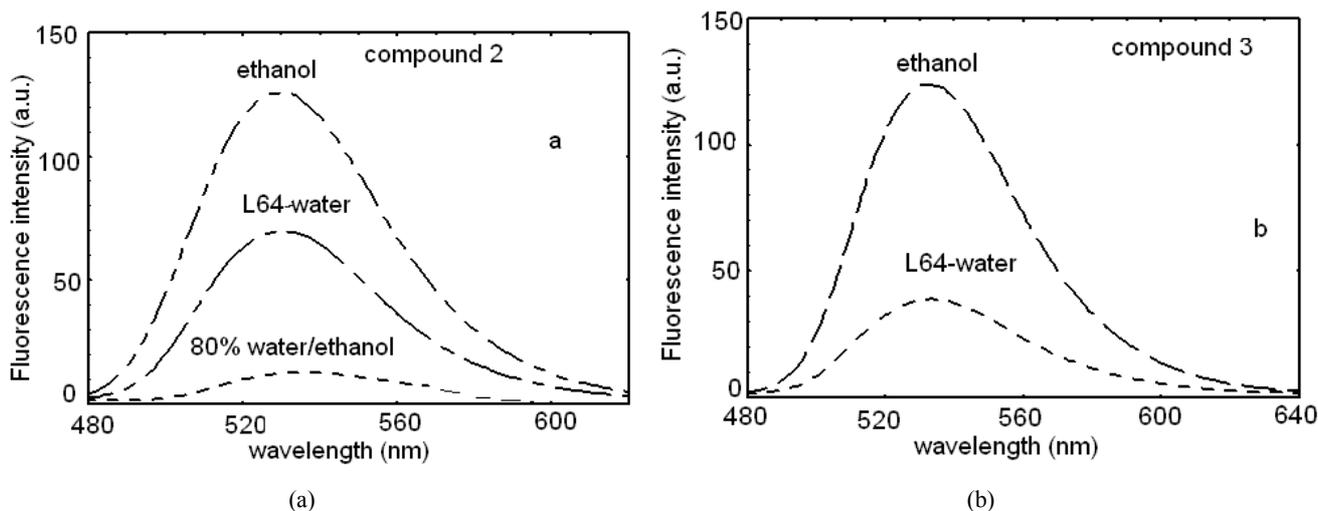


Fig. 2 – The fluorescence emission spectra of compounds **2** (Fig2-a) and **3** (Fig2-b) in L64/water, ethanolic and ethanol-water solutions: $C = 4.4 \times 10^{-5} M$; $\lambda_{ex} = 450 nm$.

The effect of $NaNO_2$ and $NaNO_3$ on the fluorescence of **2** and **3** in L64/water and absolute ethanol has been investigated. It was observed a slight decreases of fluorescence intensity only in the case of $NaNO_2$ in ethanolic solution. Figure 3 show this fluorescence quenching in the case of **2** in ethanol. Instead, in micellar solutions fluorescence

intensity remains unchanged. This is another proof of the solubilization of compounds in L64 micelles, nitrite and nitrate ions, very soluble in water, do not reach fluorescent molecules (shielded by polymeric chains). It also was found that L64 can protect the compounds by the action of ions, which in some cases are very toxic.

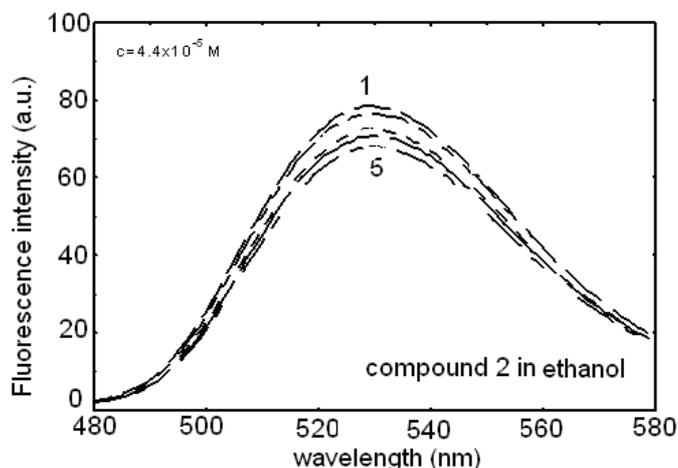


Fig. 3 – The effect of $NaNO_2$ addition on the fluorescence of compound **2** ($4.4 \times 10^{-5} M$) in ethanol. The $NaNO_2$ concentrations are: 0 (1); 1.5 mM (2); 2.0 mM (3); 2.9 mM (4); 3.8 mM (5).

Figure 4 shows the fluorescence excitation spectra for compounds **2** and **3** in ethanol and L64/water solutions. Spectra have been obtained at 530nm emission wavelength. It is interesting that one can recognize the band at 330nm, but the second absorption band is splitted, both in ethanolic

and in micellar solutions. It is possible that the relaxation of the excited molecules from ICT (S_1^*) energetic level to take place through twisted intramolecular charge transfer (TICT) state, as was noted in the literature for similar compounds.²¹

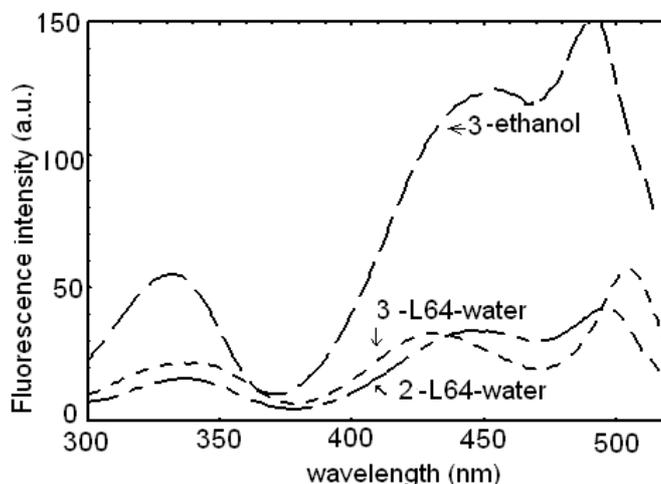


Fig. 4 – Fluorescence excitation spectra of compounds **2** and **3** in absolute ethanol and in 20% L64/water micellar solutions.

CONCLUSIONS

Research undertaken has shown that triblock copolymer Pluronic L64 micelles in water facilitate solubilisation of three hydrophobic compounds (**1-3**), type 4-amino-7-nitrobenzofurazan, by noncovalent interactions of the compounds with poly(ethylene oxide) chain.

The spectral characteristics determined from absorption and fluorescence spectra of the compounds indicate modifications which lead to the conclusion that the molecules are dissolved in micelles on the poly(ethylene oxide) chain, in sites slightly more polar than ethanol, and also arguing on the molecular interactions which cause these processes. It also was found that L64 can protect the compounds by the action of nitrite ions (do not observe the fluorescence quenching).

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