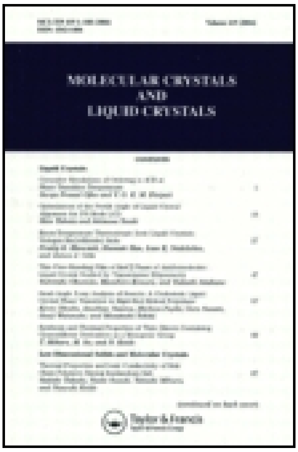

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# The Self-Assembly of Diblock Copolymers MePEG-b-PAAm into Micellar Structures and Their Interaction with Doxorubicin

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*The micellization of asymmetric diblock copolymers (DBC) MePEG-b-PAAm formed by monomethoxy-poly(ethylene)oxide (MePEG) and polyacrylamide (PAAm) has been studied in water, a water/alcohol mixture, and a NaCl solution. The effect of added NaCl on the critical micellization concentration of DBC water solutions was examined. The interaction between DBC and antitumor drug doxorubicin is discussed. The analysis of the UV spectrum of doxorubicin is performed.*

**Keywords** Acrylamide; block copolymers; doxorubicin; micellization; polyethylene glycol

## Introduction

The development of drug delivery systems using synthetic polymers as drug carriers was started about 30 years ago [1,2]. At the present time, the general concept of polymer drug carrier systems has accepted [3–6]. Among such systems, self-assembled polymer micelles based on amphiphilic block copolymers have attracted considerable attention as potential delivery vehicles for anticancer drugs.

The block copolymers of polyethylene glycol (PEG) and polyacrylamide (PAAm) form intramolecular polycomplexes that have hydrophobic areas of binding between PAA and PEO blocks and hydrophilic units of quite long PAA chains. Their self-assembly in water or mixed solvents forms the spherical micelles that have ability to bind most of organic molecules [7,8]. It is to be noted that PEO and PAAm are commercially available, water soluble, non-toxic, and widely used for biomedical

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application. There have enough reasons to be perspective objects for the generation of novel-type drug carrier systems. The aim of the present investigation is to study a self-assembly of diblock copolymers MePEG-b-PAAm to micelles and, for the first time, to examine their interaction with anticancer agent doxorubicin.

## Experimental

The diblock copolymers (DBC) MePEG-b-PAAm were prepared by the radical polymerization of AAm using monomethoxy-poly(ethylene)oxide (MePEG) as a macroinitiator according to the technique described in our previous paper [9]. Four samples of MePEG with $M_v$ from $7.5 \cdot 10^2$ to $5 \cdot 10^3$ produced by "Fluka" (Germany) were used. The molecular weights and block compositions of DBC were determined from $^1H$ NMR spectra which were recorded on a Varian Mercury-400 spectrometer operating at 400 MHz. All samples were examined at room temperature and a concentration of $0.001\,g \cdot cm^{-3}$. The molecular parameters (Table 1) were estimated by comparing the peak integration of methylene protons in the PAAm block ($\delta = 1.6$–$1.7$ ppm) to that of the (Me)PEG block ($\delta = 3.70$ ppm) in the $^1H$ NMR-spectrum.

The optical density of copolymer solutions was detected on a UV/VIS Spectrometer Perkin Elmer Lambda 20. The scattering intensity of vertically polarized light was measured at $\lambda = 520$ nm, scattering angle $\theta = 90°$, and $T = 293°$ K in the range of concentrations $C = 0.01$–$1.00\,kg \cdot m^{-3}$ on FPC-3 (Russia). The optimization of the geometry of a DOX molecule was performed with the semiempirical RM1 [10] built into the software package MOPAC 2002 [11]. The calculation of the localization and the energy of spectral-active molecular orbitals and the subsequent evaluation of the parameters of electronic transitions in the spectra of molecules were done with semiempirical CNDO/S/CI [12]. To calculate the spectra, we used a matrix of configuration interaction incorporating 100 configurations. The determination of the nature of electronic transitions was carried out by analyzing the matrix of configuration interaction, as well as according to special indices of the excitation localization [13,14].

## Results and Discussion

The micellization of block copolymers containing hydrophilic and hydrophobic areas has been studied mainly in water solutions. Meanwhile, an investigation of non-aqueous or mixed soutions can give opportunity to form new regular and

**Table 1.** Molecular characteristics of DBC

| Copolymer | $M_{vMePEG} \cdot 10^{-3}$ | $M_{nPAAm} \cdot 10^{-4}$ | $M_{DBC} \cdot 10^{-4}$ | $w_{MePEG}$, %[1] | $M_w/M_n$ | $n^2$ |
|---|---|---|---|---|---|---|
| DBC1 | 0.75 | 0.94 | 1.01 | 7.4 | 1.36 | 7 |
| DBC2 | 1.10 | 1.40 | 1.51 | 7.3 | 2.03 | 8 |
| DBC3 | 2.00 | 2.98 | 3.18 | 6.3 | 1.06 | 9 |
| DBC4 | 5.00 | 22.25 | 22.75 | 2.3 | 1.19 | 27 |

[1] Weight fraction of MePEG block.

[2] Ratio of PAA blocks and PEO blocks.

reverse micelles. Alcohol dissolves a lot of drug molecules. At the same time, it is a selective solvent for a PEG block, so the DBC micellization process in a water/alcohol mixture has been studied on the first stage of this work. A change of the optical density was determined at $\lambda = 490\,\text{nm}$ and $C_{DBC} = 2\,\text{kg}\cdot\text{m}^{-3}$. The weak opalescence for all samples of DBC has been observed starting from 30 vol.% of EtOH and becomes more intense with increase in the alcohol content up to 50–60 vol.%. Thereafter, a sharp reduction of the optical density has been noticed. This can best be explained by forming the bulk micelles and the fast phase separation in the measuring cell. Since the alcohol is a "bad" solvent for PAAm, it is reasonable to suggest that the "core" of such micelles is formed by PAAm blocks, whereas the "corona" consists of hydrophobic PEO-PAAm H-bonded segments. Such reversible "crew-cut" micelles are well-known in the literature and typical of amphiphilic block copolymers.

Micellization occurs in dilute solutions of block copolymers at a fixed temperature above a concentration called the critical micellization concentration (CMC) [15]. The CMC of DBC was determined by spectrophotometry at the ratio $H_2O$/EtOH = 50/50 vol.%. The Gibbs free micellization energy was calculated as $\Delta G^\circ \approx RT \cdot \ln CMC$ on the base of thermodynamic theory of micellization and under the assumption of a large number of DBC molecules in a micelle [15].

As shown in Table 2, the CMC goes down, and the absolute value of Gibbs free micellization energy increases from DBC1 to DBC4, i.e., the micellization process is more intensive for DBC that has longer MePEG blocks. Probably, it is induced by the creation of a more extended H-bond system between MePEG and PAAm blocks that intensifies the hydrophobic interactions.

It was interesting to study the association of DBC macromolecules in a water/saline solution containing 0.85 wt.% NaCl which has been considered as a physiological solution simulator. The DBC4 sample with the longest MePEG block has been chosen and examined in the range of concentrations $0.01\text{–}1.00\,\text{kg}\cdot\text{m}^{-3}$ by the method of static light scattering.

The macromolecules of PEG are able to link the ions of metals, including $Na^+$ in non-aqueous and mixed solvents [16]. However, in water-salt environments, the interaction of separate oxygen atoms of PEG with a hydrated ion $Na^+$ is poorly expressed and affects slightly the polymer conformation. A stronger influence is rendered by the ejecting effect of the environment which is a result of the interaction of NaCl with molecules of a solvent and is shown up under a strong compression of water, increasing its intrinsic pressure and the energy necessary for the formation of a cavity which will be occupied by a molecule of the dissolved substance

**Table 2.** Thermodynamic parameters of DBC

| Copolymer | The solvent composition $H_2O$/EtOH, vol.% | $CMC \cdot 10^{-4a}$ $\text{mol}\cdot\text{dm}^{-3}$ | $-\Delta G_0{}^b$ $\text{kJ}\cdot\text{mol}^{-1}$ |
|---|---|---|---|
| DBC1 | 50/50 | 9.24 | 17.02 |
| DBC2 | | 5.95 | 23.70 |
| DBC3 | | 1.44 | 27.16 |
| DBC4 | | 0.38 | 30.42 |

[a] Critical micellization concentration.
[b] Gibbs free micellization energy.

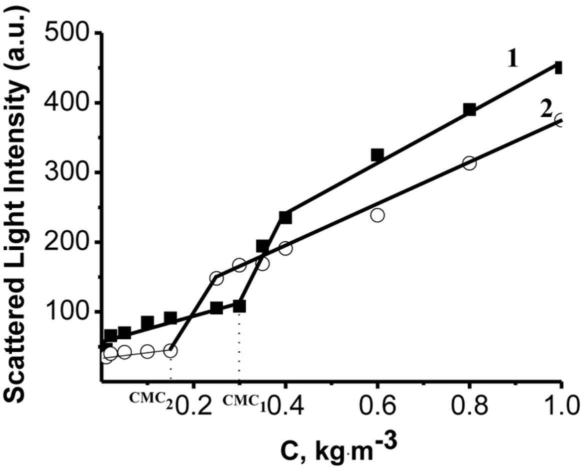


**Figure 1.** Plots of the scattered light intensity at an angle of 90° versus the concentration of DBC4 in water (1) and 0.85 wt.% NaCl.

[17,18]. The addition of NaCl causes a reduction of the sizes of polymer coils and the fall of the lower critical temperature of PEG dissolution [18]. Thus, the thermodynamic quality of a water-NaCl mixture is getting worse for PEG as compared to water. Therefore, it is possible to suppose that the quality of the solvent with added NaCl will also get worse in relation to DBC4. As follows from the experiment and Figure 1, the CMC of DBC4 decreases from $0.3\,\mathrm{kg \cdot m^{-3}}$ in a water solution to $0.15\,\mathrm{kg \cdot m^{-3}}$ in a saline solution, indeed. The calculation of $\Delta G$ gives $-32.98\,\mathrm{kJ \cdot mol^{-1}}$ and $-34.67\,\mathrm{kJ \cdot mol^{-1}}$. respectively. This means that the micellization of DBC4 becomes easier in a water/NaCl solution.

In order to estimate the ability of DBC micelles to bind the hydrophobic drugs, the interaction between DBC4 and doxorubicin (DOX), one of the most effective anticancer agent, has been examined. The analysis of the DOX structure (Fig. 2a) shows that the absorption of a molecule in the UV and visible ranges is due to the anthraquinone segment of a molecule having the system of $\pi$- and n-electrons. There are five highest occupied and five lowest unoccupied molecular orbitals of the anthraquinone fragment of DOX involved in the formation of electronic transitions in the range 200–700 nm. Filled orbitals are alternately localized on fragments A and B (Fig. 2), while the vacant orbital is delocalized throughout the anthraquinone segment.

In the calculated spectrum of DOX, there are 14 electronic transitions of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ types in the range 200–700 nm. Most of them are associated with low strength oscillators ($f < 0.01$), so the appropriate bands in the experimental absorption spectra are absent. The most intense transitions ($f > 0.01$) are in the short-wave (200–300 nm) and long-wave (400–550 nm) spectral regions. Their parameters are given in Table 3.

The analysis of configurations of these transitions shows that a redistribution of the electron density during the excitation of molecules affects the whole anthraquinone fragment of DOX, and, in particular, the localization of occupied orbitals A or B. Figure 2 shows the comparison of experimental and calculated spectra of molecules. Since the influence of the solvent was not taken into account in the

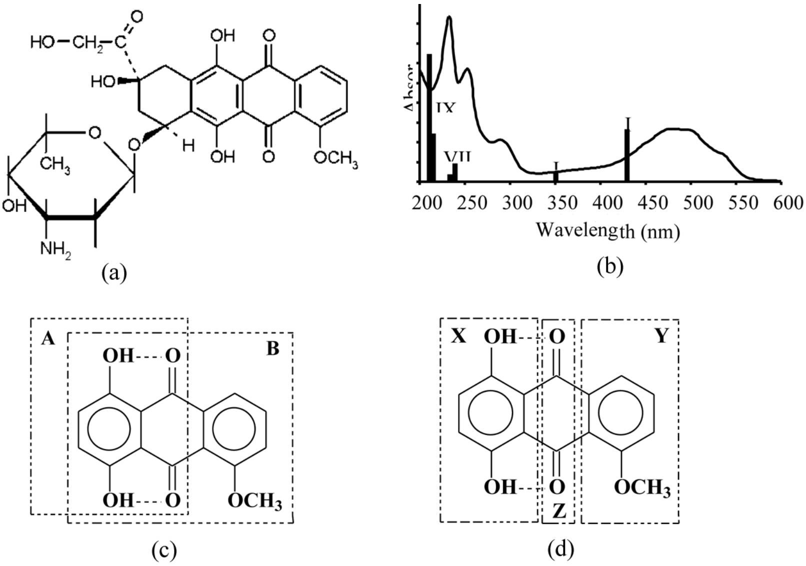


**Figure 2.** Molecular structure of DOX (a), its experimental and calculated spectra (b), fragment with a localized filled MO (c), and fragment with calculated indices of the excitation localization (d).

**Table 3.** Characteristics of intense electronic transitions in the calculated spectrum of DOX

| Transition number | $\lambda_{abs}$ (nm) | $f^1$ | Configuration of the transition[2] | Indices of the excitation localization (%)[3] | | | | | Localization of the transition[4] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $L_X$ | $L_Y$ | $L_Z$ | $L_X+L_Z$ | $L_Y+L_Z$ | |
| I | 430 | 0.408 | $0.83\cdot\chi^2_{1\to1'}$ | 72.6 | 10.2 | 17.2 | 89.8 | 27.4 | A |
| II | 352 | 0.074 | $0.67\cdot\chi^2_{2\to1'}$ | 11.5 | 73.2 | 15.3 | 26.8 | 88.5 | B |
| VII | 238 | 0.141 | $0.18\cdot\chi^2_{2\to1'}+$<br>$0.28\cdot\chi^2_{2\to2'}$ | 18.1 | 64.6 | 17.3 | 35.4 | 81.9 | B |
| VIII | 232 | 0.054 | $0.33\cdot\chi^2_{1\to3'}+$<br>$0.20\cdot\chi^2_{5\to1'}$ | 59.7 | 18.1 | 22.2 | 81.9 | 40.3 | A |
| IX | 215 | 0.367 | $0.20\cdot\chi^2_{5\to1'}$ | 41.5 | 33.9 | 24.6 | 66.1 | 58.5 | General mol. |
| X | 210 | ~1.0 | $0.22\cdot\chi^2_{1\to4'}+$<br>$0.32\cdot\chi^2_{3\to1'}$ | 54.4 | 28.9 | 16.7 | 71.1 | – | A |

[1] $\lambda_{abs}$ – transition wavelength, $f$ – transition oscillator strength.

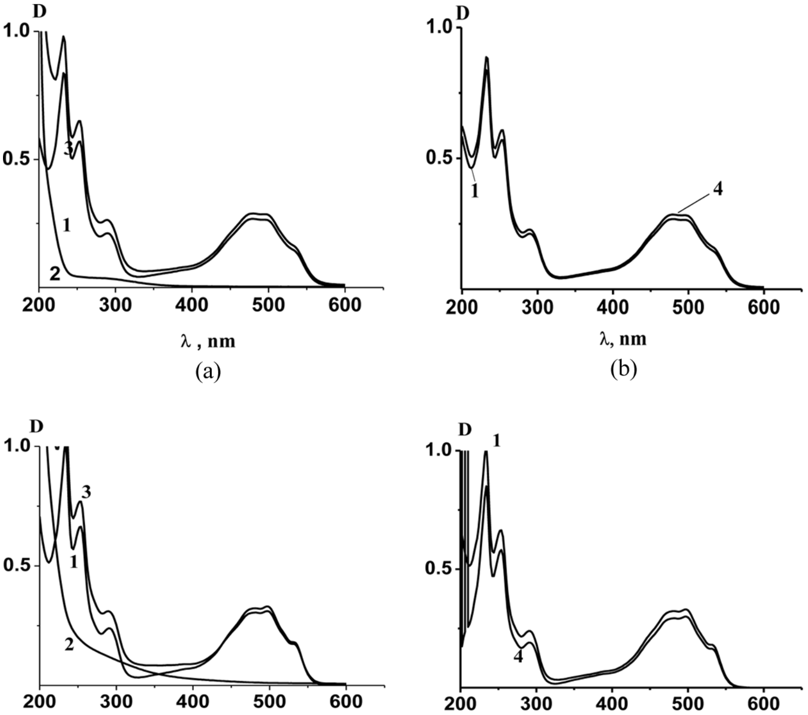
[2] Configuration of the transition $\Psi_k$ is expressed by the relation $\Psi_k^2 = d_{ij}^2 \sum \chi_{i\to j}^2$, $k$ – transition number, $i$ – number of filled MO, $j$ – number of vacant MO. In the table, the configurations with $d^2 > 0.1$ are shown.

[3] Localization of three fragments X, Y, Z is shown in Figure 2. Localization of the transition is A if $(L_X + L_Z) >> (L_Y + L_Z)$; localization of the transition is B if $(L_X + L_Z) << (L_Y + L_Z)$; and the transition is general molecular if $L_X \approx L_Y$.

[4] Localization of the fragments A and B is shown in Figure 2.

calculations, the energy of electronic transitions in the calculated spectrum has higher values (about 0.4 eV more) than that in the experimental spectrum. However, the calculated spectrum fairly describes the location and the relative intensity of the experimental absorption bands. The above data gives a reason to assign the most intense absorption bands in the long- and short-wave parts of the spectrum to the excitation at chromophore A (Fig. 2c). DBC4 has no chromophore segments with intensive absorption. Nevertheless, its spectrum contains a low-intensity band at 240–300 nm which may be assigned to the band of $n\pi^*$ transition in a carbonyl group of the fragment -CO-$NH_2$.

The polymer produces a medium with certain parameters, including some local polarity. If the polarity is high, the electric field produced by a micelle should polarize other molecules. At the excitation of a molecule, the states with different dipole moments are produced, so the polarity of the medium should affect differently various excited states. So, if DBC interacts with a molecule of DOX, the shifts of bands in the UV spectra, as well as the changes in their intensities (optical density) would be different. The analysis of the electronic spectra of DBC4, DOX, and their mixtures was performed as follows. The spectrum of DBC was subtracted from the total spectrum (DOX + DBC), and the obtained difference (X = (DOX + DBC) – DBC)) was compared with the spectrum of "pure" DOX (Fig. 3).



(a) (b)

(c) (d)

**Figure 3.** Spectra of DOX – *1*, DBC4 – *2*, mixture DOX + DBC4 – *3* and (DOX + DBC4)-DBC4 – *4* in water (a, b) and a mixture of water/alcohol = 1:1 (c, d). $C_{DOX} = 2.5 \cdot 10^{-2}\,\mathrm{mol} \cdot \mathrm{м}^{-3}$, $C_{DBC4} = 0.12\,\mathrm{kg} \cdot \mathrm{м}^{-3}$.

The analysis of UV spectra is not able to confirm exactly the formation of any bonds between the molecules of the copolymer and doxorubicin. At the same time, we can not state with certainty that the interaction between molecules is absent. As is known, the weak interactions of components may not always produce the considerable UV spectral changes. It is quite probable that the observable changes in the spectrum of a DBC4/DOX mixture is caused by hydrophobic interactions, but the final decision requires a careful consideration.

## Conclusions

We have found the self-assembly of diblock copolymers MEPEO-b-PAAm to micelles in a water/alcohol mixture. The critical micellization concentration and the Gibbs free micellization energy go down with increase in the length of a MePEG block. The addition of NaCl to a water solution improves the micellization. The determination of the nature of electronic transitions in a DOX molecule and the assignment of intense bands in its absorption spectra to the corresponding chromophoric fragments give a reason to assign the most intense absorption bands in the long- and the short-wave parts of the spectrum to the excitation of the anthraquinone segment chromophore.

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