Block Copolymers of Polyacrilamide and Poly(ethylene oxide) as Nanocarriers for Drug Delivery: Micellization and Bulk Structure

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Summary: The self-assembly of diblock copolymers series (DBC) of polyacrylamide and methoxypoly(ethylen oxide) (MOPEO-*b*-PAAm) with a variable length of both the blocks were studied in water and water-ethanol solutions. The tendency of DBCs to micellization in water-ethanol mixture grew with increased molecular weights of the blocks. The addition of NaCl resulted in increase of micellar stability, while the introduction of dimethylformamide (DMF) destructed DBC micelles. In DBC bulk structure, MOPEO blocks either lost or considerably reduced their ability of crystallization due to interaction with PAAm blocks. DBC micelles encapsulated anticancer drug doxorubicin (DOX) that led to lowering the crytical micellization concentration.

Keywords: diblock copolymers; differential scanning calorimetry (DSC); intramolecular polycomplex; micelles; self-assembly

Introduction

For the past few decades, growing interest has been devoted to selection of polymer compounds that could be used in pharmacology as drug carriers.[1,2] At the present time, the general concept of polymer drug delivery systems has been accepted.[3,4] Among such systems, amphiphilic block copolymers composed of hydrophobic and hydrophilic components were in the focus of considerable attention. Micelles formed by self-assembly of amphiphilic block copolymers offer an excellent platform for hydrophobic drugs delivery since the hydrophobic ''core'' can act as a drug vessel and also improve the drug solubility in aqueous medium, while the hydrophilic ''corona'' can mediate the compatibility of the nano-scale carrier with the biological surrounding.[5–7]

From this viewpoint the block copolymers of poly(ethylene oxide) (PEO) and PAAm can have good perspectives in numerous therapeutic strategies. PAAm and PEO are water soluble, non-toxic, non-immunogenic and so suitable for biomedical applications. Furthermore, the block copolymers of PEO and PAAm form the intramolecular polycomplexes (IntraPCs) that have hydrophobic areas of binding PAAm and PEO blocks. These block copolymers (especially of asymmetric character) have tendency to self-assembly in aqueous solution and form micellar structures.[8–9] There are enough reasons to regard them to be perspective objects for the generation of novel-type drug carriers. The aim of the present investigation is to study a self-assembly and a bulk structure of a lot of DBCs with different length of MOPEO and PAAm blocks.

Materials and Methods

All DBCs were prepared by the radical block copolymerization of PAAm with methoxypoly(ethylene glycol) (MOPEG)

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using ammonium cerium nitrate as initiator.[10] Four samples of MOPEG with $M_v = 7.5 \cdot 10^2$–$5 \cdot 10^3$ from "Fluka" (Germany) were used for this purpose. The weight compositions and the number average molecular weights of MOPEG (M_{nMOPEG}) and DBCs (M_{nDBC}) were determined from ^{1}H NMR spectra, which were recorded in D_2O at $C = 1\,kg \cdot m^{-3}$ and a room temperature on a Varian Mercury-400 spectrometer operating at 400 MHz (Table 1). The values of M_{nMOPEG} were calculated from MOPEG NMR using the following relation:

$$M_{nMOPEG} = (3 \cdot M_{oMOPEO} \cdot A_a)/4 \cdot A_b, \quad (1)$$

where M_{oMOPEO} is the molecular weight of MOPEO (or MOPEG) units, A_a and A_b are the integral intensities of the proton signals of methylene and methoxy groups of MEPEG with $\delta = 3.70$ and 3.36 ppm, respectively.

The number average molecular weights of PAAm blocks (M_{nPAAm}) were calculated using the following equation:

$$M_{nPAAm} = M_{oPAAm} \cdot M_{nMOPEG} \cdot A_c / M_{oMOPEO} \cdot A_d, \quad (2)$$

where M_{oPAAm} and M_{oMOPEO} are the molecular weights of PAAm and MOPEO links, A_c and A_d are the integral intensities of the proton signals of methylene groups of MOPEO and PAAm blocks from DBC spectra, correspondingly. The values of M_{nDBC} were found by the equation:

$$M_{nDBC} = M_{nMOPEG} + M_{nPAAm} \quad (3)$$

The critical micellization concentration (*CMC*) of DBCs was determined by two methods. The *CMC* measurements in water-ethanol solutions were carried out on a UV/Vis spectrometer Perkin Elmer Lambda 20 (Sweden) at $\lambda = 490$ nm. Determination of analogous values in a pure water and water/salt solutions was performed by static light scattering (SLS) using a modernized light scattering instrument FPS-3 (Russia), which contained a WP7113VGC/A light-emitting diode ($\lambda = 520$ nm) from "Kingbright", a ADC-CPU^{TM} controller from "Insoftus" (Ukraine) and the computer program WINRECORDER. In order to define CMCs, the scattering intensities of the vertically polarized light were measured at the $\theta = 90^\circ$ scattering angle and $T = 20\,^\circ C$ in a certain region of DBC concentrations.

Structural investigations of MOPEO-*b*-PAAm and individual polymer components were carried out by differential scanning calorimetry (DSC) using a DSC-910 microcalorimeter and 1090 "Du Pont" thermoanalyzer (USA). DBC and MOPEG samples and also the sample of a pure PAAm with $M_v = 630$ kDa were dried in a vacuum case at $\sim 50\,^\circ C$ for 2 days and a vacuum-desiccator for a week. Then 5–10 mg of each sample were placed in open pans, cooled with liquid nitrogen and heated with a rate of $16^\circ \cdot min^{-1}$. To define the thermodynamic parameters of structural transitions, the instrument was calibrated with indium and zinc. Moreover, a sapphire crystal was heated with each polymer sample in order to transform the heat flow curves to the temperature dependences of the specific heat capacity (C_p) according to the following equation:

$$C_p(T) = C_p(T) \cdot l/l^o \cdot m_o/m, \quad (4)$$

Table 1.
Molecular parameters of the diblock copolymers

Copolymer	M_{vMOPEG} [a] kDa	M_{nMOPEG} kDa	M_{nPAAm} kDa	M_{nDBC} kDa	w_{MOPEO} [b] %	n [c]
DBC1	0.75	0.72	10.37	11.09	6.49	8.9
DBC2	1.10	1.13	14.38	15.51	7.29	7.9
DBC3	2.00	2.50	37.25	39.75	6.29	9.2
DBC4	5.00	5.29	235.41	240.70	2.20	27.6

[a]The viscosity-average molecular weight of MOPEG from "Fluka". [b]The weight fraction of MOPEO block in DBC. [c]The ratio between the units in PAAm and PEO blocks.

where C_p° is the heat capacity of a sapphire at a current temperature, l^o and l are the distances of both the sapphire and polymeric thermograms at a current temperature from the base line, m^o and m are the weights of a sapphire crystal (61.66 mg) and a sample, correspondingly.

The studies of viscosity of DBC sample in water and water/DMF solutions were carried out using the Osvald-type viscosimeter with $\tau_0 = 99.9$ s at 25 °C.

The UV spectra of DBC, DOX and their mixture were recorded at $C_{DBC4} = 0.12\,\mathrm{kg \cdot m^{-3}}$, $C_{DOX} = 2.5 \cdot 10^{-5}\,\mathrm{mol \cdot dm^{-3}}$ and room temperature on a UV/Vis spectrometer Perkin Elmer Lambda 20 (Sweden). The concentrations of DBC and DOX were constant both for the individual solutions and for DBC-DOX mixture. The molar ratio DOX/DBC = 0.0122 in their mixture was constant for water and water-ethanol solutions.

Micellization

Four samples of DBCs with the different length of MOPEO block have been synthesized and their bulk structure and behavior in a solution have been examined. All the samples had strongly asymmetric character because the molecular weight of PAAm block was essentially higher than that of MOPEO block. In addition, they contained chemically complementary components so they were expected to form steady micellar structures in aqueous solutions. In this case, the process of micellization is developed in dilute aqueous solution and a hydrophobic ''core'' of micelles is formed due to interaction of MOPEO and PAAm blocks by the system of hydrogen bonds followed by segregation of hydrophobic bound parts in aqueous medium, analogously to a similar micellization process in solutions of asymmetric PAAm-*b*-PEO-*b*-PAAm triblock copolymers.[8] Hydrophilic ''corona'' in these micelles consists of the surplus (unbound with MOPEO) segments of PAAm blocks. To prove this fact, the viscosity researches of DBC water and water/DMF solutions have been carried out.

Using these data (Figure 1), the intrinsic viscosities were calculated by the extrapolation of linear parts to $C = 0$. It is known that electron-donor capacity of carbonyl groups of DMF is higher as compared to ether groups of PEO.[11,12] Due to this, DMF is a competitor to form hydrogen bonds with -NH_2 fragments of PAAm amide groups and capable of destroying hydrogen bonds between PAAm and MOPEO blocks.

It was found that the intrinsic viscosity of DBC aqueous solutions (0.38) turns out to be essentially lower than that of solutions containing 5 v % of DMF (0.29). This fact could be attributed to the destruction of H-bonds responsible for the appearance of DBC micelles, thus implies their existing. Further increase in DMF content up to 10 v % did not influence the viscosity of DBC solutions. It should be supposed that even a small quantity of DMF could be able of ruining DBC micellar structure.

It was interesting to compare the micellization of DBC macromolecules in water and water/salt solution containing 0.85 wt % NaCl, which could be considered as a physiological solution model. The DBC4 sample with the longest MOPEO block has been chosen and examined in the range of concentrations 0.01–1.00 $\mathrm{kg \cdot m^{-3}}$ by SLS method.

Micellization takes place in dilute solutions of amphiphilic block copolymers since some concentration called *CMC*. [13] As it follows from Figure 2, the *CMC* for DBC4

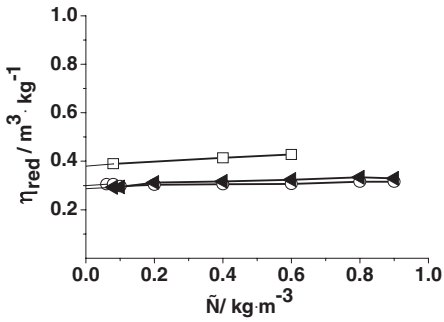

Figure 1.
The reduced viscosity vs concentration of DBC4 in H_2O and H_2O/DMF (50/50 v/v) mixed solvent: □ - H_2O, ◀ - 5 v % DMF, ○- 10 v % DMF.

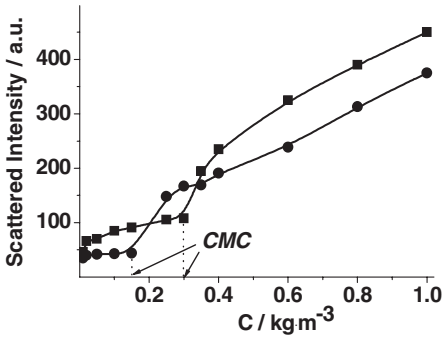

Figure 2.
The scattered light intensity *vs* concentration in DBC4 solutions; ■ = a pure H_2O, ● = 0.85 wt % NaCl.

decreases from $0.3\,kg \cdot m^{-3}$ in a water to $0.15\,kg \cdot m^{-3}$ in a saline solution. The Gibbs free micellization energies calculated as: $\Delta G^{\circ} = RT \cdot \ln CMC$ were equal to $-32.98\,kJ \cdot mol^{-1}$ and $-34.67\,kJ \cdot mol^{-1}$ for DBC4 in water and a saline solution, respectively. It was suggested that DBC4 micellization increased at the presence of sodium chloride and the micellar stability kept at less DBC concentrations that important in the context of application of micelles as drug carriers.

The described phenomenon is not unexpected if we take into account the well known effect worsening thermodynamic quality of water as a solvent with respect to non-polar and nonionic polar parts of polymers at the addition of sodium chloride.[14] In fact, the presence of NaCl strengthens hydrophobic interactions in a micellar ''core'' and assists in the growth of micelles.

The most of drugs are poorly soluble in water but they have good solubility in ethanol. Therefore, it was reasonable to study the behavior of DBCs in water-ethanol mixture. Micelle formation was examined by the measuring optical density at different ratio water-ethanol. Slight opalescence for all DBC samples was observed from 30 v % of ethanol and became more intensive with increasing in ethanol content up to 50–60 v %. After that sharp reduction in the optical density has been noticed. This effect could be explained by the formation of the micellar aggregates that initiated a phase separation. The *CMC* values were determined by UV-Vis spectroscopy at the ratio water/ethanol = 50/50 v/v. As it is shown in Table 2, the *CMC* decreased while the Gibbs free micellization energy increased, when the length of PEO and PAAm blocks grew (Table 1).

At high content of ethanol unlike to water medium, the self-assembly of DBC macromolecules proceeds because of insolubility of PAAm blocks analogously to the behavior of PAAm-*b*-PEO-*b*-PAAm triblock copolymers at high ethanol content.[15] In this case the micelles of a classical type (due to insolubility of only one block) are formed. It is seen (Table 2), that such micellization process is more intensive in the case of DBCs with longer MOPEO and PAAm blocks, which ones define the size of micellar ''corona'' and ''core'', respectively. Thus, in the given DBC series, the stability of micelles regularly increases.

Bulk Structure

The structure of block copolymers with cooperatively interacting polymer components in the bulk state has attracted less attention than their behavior in solutions, but such investigations are very important. They help to prove the fact of IntraPC formation and establish a correlation between structural features and molecular architecture of block copolymers. On the other hand, these studies could demonstrate some differences between bulk structures of the IntraPC-forming block copolymers and corresponding polymer blends belonging to the intermolecular

Table 2.
Thermodynamic parameters of DBC micellization

Copolymer	H_2O/EtOH composition v/v	$CMC \cdot 10^{-4}$ a) $mol \cdot dm^{-3}$	$-\Delta G^{\circ}$ b) $kJ \cdot mol^{-1}$
DBC1	50/50	9.24	17.02
DBC2		5.95	23.70
DBC3		1.44	27.16
DBC4		0.38	30.42

polycomplexes (InterPCs). Finally, the studies of morphology are very important for a practical application of IntraPCs as the drug-delivery systems.

A bulk structure of MOPEO-*b*-PAAm diblock copolymers (DBC1, DBC3 and DBC4) was studied by DSC and compared with that of individual PAAm and MOPEGs. Some DSC thermograms are shown in Figure 3.

The main parameters of thermal transitions in DBC structure such as the glass and melting transition temperatures (T_g and T_m), the temperature regions for corresponding transitions (ΔT_g and ΔT_m), the specific heat capacity jump (ΔC_p) for glass transitions, the enthalpy of melting process (ΔH_m) and the crystallinity degree (X_{cr}) of MOPEO (or MOPEG) chains were determined and indicated in Table 3.

DSC thermogram of amorphous PAAm contained an endothermic peak of water evaporation at $T \sim 100\,^{\circ}\mathrm{C}$ and a single capacity jump that reflects the glass transition.

Thermograms of DBCs comprised a similar single glass transition, which T_g values were lower than for individual PAAm. The reduction in T_g values compared to that in pure PAAm indicated the compatibility of both the blocks in DBC structure.[17] We attribute this fact to the presence of the interactions between PAAm and MOPEO blocks both intra- and intermolecular type. It should be noted that the first type of the interactions between the blocks was realized only in the diluted solutions of DBCs.

All MOPEG samples demonstrated one endothermic melting peak that is typical for

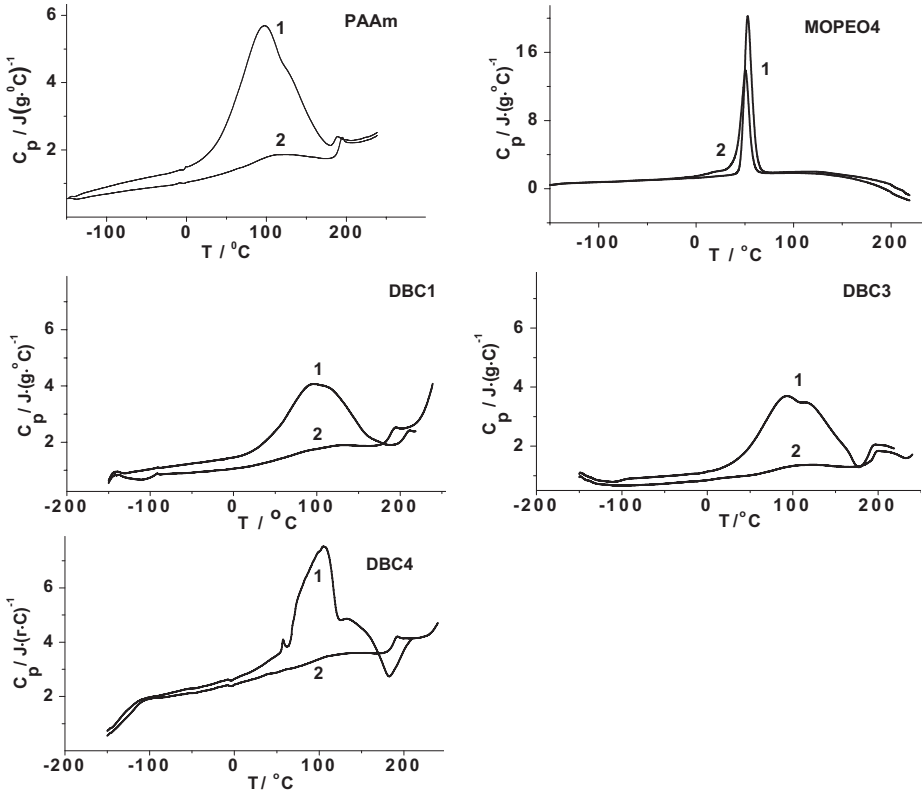

Figure 3.
The DSC thermograms of PAAm, MOPEO4, DBC1, DBC3, DBC4; the 1-st scan —*1* and the 2-nd scan —*2*.

Table 3.
Parameters of structural transitions in MOPEO-*b*-PAAm, PAAm and MOPEG

Copolymer	Scan	T_g °C	ΔT_g °C	ΔC_p J · (g · K)$^{-1}$	T_m °C	ΔT_m °C	ΔH_m J · g^{-1}	X_{cr} [a] %
PAAm	2	190.9	8.0	0.55	–	–	–	–
MOPEG1	1	–	–	–	21.3	64.1	132.4	67.3
MOPEG3	2	–	–	–	23.5	64.1	117.8	59.9
MOPEG4	1	–	–	–	53.0	39.0	194.8	99.0
	2	–	–	–	50.6	85.0	167.2	85.0
	1	–	–	–	61.5	48.9	196.8	100.0
	2	–	–	–	59.5	56.7	175.9	89.4
DBC1	2	186.9	12.8	0.67	–	–	–	–
DBC3	2	190.0	10.9	0.56	–	–	–	–
DBC4	1	–	–	–	57.9	16.0	1.3	31.5
	2	186.1	9.9	0.66	41.0	20.0	0.4	9.7

[a] For MOPEG $X_{cr} = \Delta H_m/\Delta H^\circ_m$, where ΔH°_m is the melting enthalpy of the 100% crystalline polymer (196.8 J · g^{-1}); for DBC $X_{cr} = \Delta H_m/(\Delta H^\circ_m \cdot w_{MOPEO})$.[16]

crystallizable polymers. At the 2-nd scans the parameters T_m and ΔH_m, which reflected MOPEG melting, and the calculated values of X_{cr} were lower to some extent (Table 2) that is connected with a very quick crystallization of MOPEG chains during sharp cooling of samples after the 1-st scan. We did not see such peaks at DBC1 and DBC3 thermograms; it implied the absence of any crystalline regions in the block copolymer structure. Unlike this, DBC4 thermogram (the 1-st scan) showed a weak melting peak, which belonged to the MOPEO small crystalline domains. This peak disappeared at the 2-nd scan after the transition through a melting state. This fact confirmed a high compatibility of MOPEO and PAAm blocks in DBC bulk structure because of their interaction. Really, it is known that the thermodynamic immiscibility of some blocks in block copolymers is essentially strengthened at the transition through a melt.[17] In this case DSC thermograms show two glass transitions (instead a single one), which T_g values correspond to those for individual polymer components. The other situation was observed at the 1-st scan of DBC4 thermogram that was discussed above.

All the samples of initial MOPEG were characterized by a high value of X_{cr}. But in DBCs corresponding MOPEO blocks either lost (as in DBC1 and DBC3) or essentially reduced (as in DBC4) their ability of crystallization that led to the reduction in T_m, ΔH_m and X_{cr} values as compared to those of initial MOPEG. These facts are produced by the interaction of chemically complementary blocks in DBCs.

Interaction with Doxorubicin

In order to estimate the ability of DBC micelles to bind toxic hydrophobic drugs, the interaction between DBC4 which forms the most stable micelles, and one of the most effective anticancer agent doxorubicin (DOX) has been examined. The DOX molecule (Figure 4) has sufficiently developed hydrophobic part and also active hydroxyl-, ether-, carbonyl- and amine groups.

It is reasonable to suppose that DBC macromolecule can interact with the quinine portion of DOX as well as the

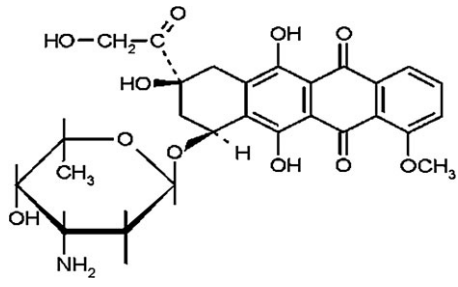

Figure 4.
Molecular structure of doxorubicin.

hydrophobic effect between them. In addition, the –OH and $–NH_2$ groups of DOX can form a strong hydrogen-bonding interaction with ether- and amide groups in DBC. UV visible spectra of DBC in water and water-ethanol mixture shows a slight peak at 240–300 nm that belongs apparently to $n\pi^*$-electron transition of carbonyl group in $–CO-NH_2$ fragment. Free DOX solutions absorbs strongly at 233, 253, 289, 480, and 495 nm (Figure 5). We expected to find out more evident changes in the spectra of DBC, DOX and DBC-DOX solution.

However, even so small differences indicate the redistribution of electron density in DOX molecule under the influence of DBC micelles.

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. In water-ethanol mixture the value of optical density in X spectrum has more deferences in short-wave region as compared to DOX spectrum. Obviously, this fact can be explained by DOX-DBC interaction. The value of optical density of DBC-DOX mixture in water-ethanol = 1:1 solvent is redouble in compare with water. It means that DOX improves the DBC micelle formation. At the presence of DOX the CMC of DBC4 water-ethanol solution decreases from $3.8 \cdot 10^{-5}\,mol \cdot dm^{-3}$ to $0.53 \cdot 10^{-5}\,mol \cdot dm^{-3}$ indeed.

Conclusion

In this paper, the bulk structure of diblock copolymers MOPEO-*b*-PAAm and their behaviour in water and water-alcohol solutions were investigated. The total compatibility of the polymer components and the homogeneous bulk structure of DBCs were found. This fact could be attributed to the presence of additional cooperative interactions between covalently bonded PAAm and MePEO blocks that is the existence of IntraPC in MePEO-*b*-PAAm copolymers.

It was shown that diblock copolymers MOPEO-*b*-PAAm form the H-bonded IntraPCs in aqueous solutions. The lowering of DBC solution viscosity at the presence of DMF means the destruction of H-bonds between PAAm and MOPEO blocks thereby indirectly confirms their existing. It is seen that even small quantity of DMF could be able to ruin DBC micelles. At the same time, the addition of NaCl to the DBCs water solution improves their micellization due to stabilizing micellar structure.

The tendency of DBCs to micellization in water-ethanol solvent grows with increase in the molecular weights of MOPEO and PAAm blocks. It is induced by increasing the length of both the ''core'' and ''corona'' blocks that is known to enhance a stability of the micellar structure. Since the ethanol is a selective solvent for PEO, it is reasonable to assume that the

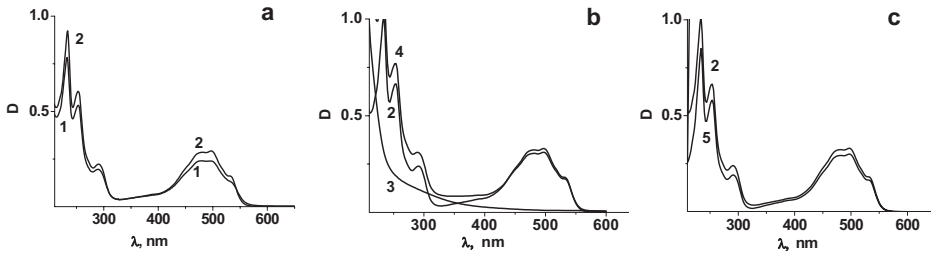

Figure 5.
UV spectra of: (**a**) pure DOX –*1,2*, (**b**) DBC4–*3*, (DBC4 + DOX) –*4* and (**c**) the difference (DBC4 + DOX)-DBC4–*5*, which were recorded in water –*1*(**a**) and water- ethanol (50/50 v/v) solutions –*2-5*(**a-c**). $C_{DOX} = 2.5 \cdot 10^{-5}\,mol \cdot dm^{-3}$, $C_{DBC4} = 0.12\,kg \cdot m^{-3}$.

''core'' of such micelles is formed by PAAm blocks, while ''corona'' consists of the soluble MOPEO blocks.

The slight changes noticed in UV spectra of DOX-DBC mixture can be attributed to interaction that most probably have hydrophobic nature. However the final conclusion should be carefully considerate.

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