

## Block Copolymers Containing Poly(acrylic acid) for Stabilization of Vitamin E and Silver Nanoparticles in Aqueous Solutions

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We have synthesized asymmetric di- and triblock copolymers containing chemically complementary blocks of poly(acrylic acid) and methoxypoly(ethylene oxide) (PAAc-*b*-MOPEO) or poly(ethylene oxide) (PAAc-*b*-PEO-*b*-PAAc) using a template radical block copolymerization process. At low pH, polyacid blocks of these copolymers were protonated and formed the intramolecular polycomplexes (IntraPCs) with MOPEO or PEO blocks. Due to both the intramolecular complex formation and hydrophobic interactions between non-polar bound segments of the blocks, an intensive micellization in copolymer solutions at low pH took place. Aqueous copolymer solutions were used as nanocontainers for encapsulation of poorly soluble vitamin E and as nanoreactors for the silver nanoclusters/nanoparticles formation.

**Keywords:** Block copolymer, Intramolecular polycomplex, Micellization, Encapsulation, Vitamin E, Silver.

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### 1. INTRODUCTION

Block copolymers with pH-sensitive polyacid blocks have attracted considerable attention due to their capability of reversibly changing its conformation and physicochemical properties in dependence on the solution pH [1,2]. There are only several studies devoted to the micellization process of similar pH-sensitive block copolymers, which ones considered the effect of polymeric structure, the solution pH and ionic strength on micellar parameters [2]. We reported recently that the double hydrophilic block copolymers with the system of cooperative hydrogen bonds between chemically complementary blocks formed stable micelles in aqueous medium [3,4]. These micelles contained hydrophobic “core” with H-bonded segments of interacting blocks and hydrophilic “corona” with free (unbound) segments of longer blocks. The given micellar structures have attracted a considerable attention due to their possible applications as different templates, drug delivery systems, nanoreactors, components of membranes etc [2, 3, 5].

In the present work, we investigated a self-assembly of di- and triblock copolymers based on chemically complementary poly(acrylic acid) and methoxypoly(ethylene oxide) or poly(ethylene oxide) in aqueous medium as a function of the solution pH. The capability of the given copolymers to connect poorly soluble vitamin E and to act as nanoreactor for the silver nanoclusters/nanoparticles formation is studied too.

### 2. EXPERIMENTAL SECTION

#### 2.1 Materials and syntheses

In order to synthesize the PAAc-*b*-MOPEO diblock copolymers (DBC) and PAAc-*b*-PEO-*b*-PAAc triblock

copolymer (TBC), we used methoxypoly(ethylene glycol) (MOPEG) with  $M_n=5.3$  kDa and acrylic acid (AAc) from “Fluka” (USA) and also poly(ethylene glycol) (PEG) with  $M_n=6$  kDa and cerium ammonium nitrate (initiator) from “Aldrich” (USA). Hydroxyl groups of MOPEG/PEG were activated with  $Ce^{IV}$  ions in the block copolymerization process [3]. The reagents were mixed in the deionized water and inert atmosphere at 25 °C for 24 h. The molar ratio  $[Ce^{IV}]/[-OH]=1$  was constant in all the syntheses but the monomer concentration was varied from 0.5 to 2.0 mol<sub>AAc</sub>/base-mol<sub>MOPEG(PEG)</sub>. The sediments of DBCs and TBC in H-form were rewashed by the deionized water after syntheses and transformed to Na-form by dissolution in water with sodium hydroxide.

Chemical structure and molecular parameters of the synthesized block copolymers were characterized by NMR spectroscopy as in the study [6] (Table 1). Thus, a series of DBCs with different length of both the blocks and one TBC sample were obtained.

**Table 1** – Molecular parameters of block copolymers

Sample	$M_{nMOPEO/PEO}$ kDa	$M_{nPANa}$ kDa	$M_{nDBC/TBC^a)}$ kDa	$n^b)$
DBC1	5.3	12.1	17.4	129/120
DBC2	5.3	19.8	25.1	210/120
DBC3	5.3	23.1	28.3	245/120
TBC	6.0	4.2	14.4	52/136

<sup>a)</sup>  $M_{nDBC}=M_{nMOPEO}+M_{nPANa}$ ,  $M_{nTBC}=M_{nPEO}+2\cdot M_{nPANa}$ .

<sup>b)</sup> The ratio between units of PANa and MOPEO/PEO.

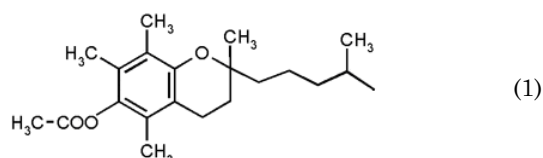
Vitamin E is a major lipid-soluble non-enzymatic antioxidant. We have synthesized and applied  $\alpha$ -Tocopherol acetate with the molecular structure (1) as a stable vitamin E derivative.

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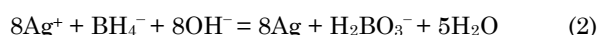
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Syntheses of silver nanoparticles were carried out in DBC3 aqueous solutions by the addition of sodium borohydride (from China) as a reducer to  $\text{AgNO}_3/\text{DBC3}$  blends ( $C_{\text{DBC}}=1 \text{ kg}\cdot\text{m}^{-3}$ ) at a molar ratio of  $[\text{Ag}^+]/[\text{COO}^-]=0.1$ . The excess of  $\text{NaBH}_4$  was necessary to favor the formation of monodispersed Ag-nanoparticles through a fast nucleation process [7]. This Red/Ox process could be described by the (2) stoichiometric equation:



In our experiments, the excess of  $\text{NaBH}_4$  with respect to  $\text{Ag}^+$  ions was 8-80 times.

## 2.2 Characterization

We studied the copolymer micellization using Vis spectroscopy, photography and static light scattering (SLS). The optical density (turbidity) of DBC and TBC solutions at different pH was measured by a Cary 50 Scan UV-Visible Spectrophotometer from "Varian" (USA) at  $\lambda = 500 \text{ nm}$ . The critical micellization concentration (CMC) at  $\text{pH} = 2.5$  for the block copolymers with different relative length of the blocks was determined by SLS method. For this purpose, a modernized instrument FPS-3 (Russia) contained a light-emitting diode ( $\lambda = 520 \text{ nm}$ ) from "Kingbright", an ADC-CPU™ controller from "Insoftus" (Ukraine) and the computer program WINRECORDER was used. The values of pH in copolymer solutions and the dissociation degrees ( $\alpha$ ) of carboxylic groups of polyacid blocks were found from the data of potentiometric titration. The titration was performed with 0.2 N HCl in a thermostatted cell in argon current at  $T=25 \text{ }^\circ\text{C}$  using a 1-160 M pH-meter (Belarus) calibrated with standard buffer solutions.

We obtained the blends of VE with DBCs or TBC by the addition of a small volume of VE ethanol solution to a large volume of the copolymer aqueous solution with a certain pH. The blends were studied by photography, potentiometric titration, UV-Vis spectroscopy. The encapsulation of VE by the copolymer micelles was quantitized by UV-Vis spectroscopy.

UV-Vis spectra of the mixtures  $\text{AgNO}_3/\text{DBC3}$  after  $\text{NaBH}_4$  addition were recorded in the 200-1000 nm region using a Cary 50 Scan UV-Visible Spectrophotometer from "Varian" (USA).

## 3. RESULTS AND DISCUSSION

It is well known that amphiphilic block copolymers form different micellar or vesicular structures in dilute solutions of the solvents, which are selective for one of the blocks [5]. Micelles of the block copolymers with chemically complementary components, which form the intramolecular polycomplexes (IntraPCs), would be essentially differing from those characteristic for ordinary amphiphilic copolymers of a block type.

### 3.1 Micellization of DBCs and TBC in water

The template character of the block copolymerization of PAAc with MOPEG or PEG, which was established earlier [6], confirmed the formation of intramolecular polycomplexes (IntraPCs) in DBC and TBC macromolecules in H-form. Micellization of these copolymers in acidic aqueous solutions ( $\text{pH} \leq 3$ ) developed because of: i) hydrogen bonding of MOPEO/PEO and protomated PAAc blocks and ii) hydrophobic segregation of non-polar bound parts of the blocks in water medium. It is accompanied by the appearance of a significant turbidity in the copolymer solutions (Fig. 1). At the same time, the IntraPC formation and micellization were impossible in the case of fully deprotonated DBCs and TBC in Na-form. Thus, at  $\text{pH}>3$  the micelle destruction, which was initiated by the H-bond ruining in IntraPCs, took place.

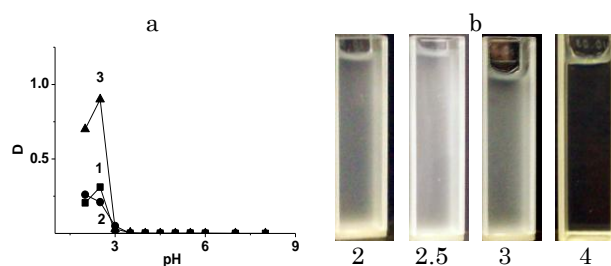


Fig. 1 – (a) The turbidity of the copolymer solutions at different pH for DBC1 -1, DBC2 -2 and TBC -3; (b) photos of DBC1 at different pH;  $C=1 \text{ kg}\cdot\text{m}^{-3}$ ,  $\lambda = 500 \text{ nm}$

The CMC values and the Gibbs free micellization energies calculated by the relation:  $\Delta G^\circ = RT \cdot \ln \text{CMC}$  [8] are represented in Table 2. The raise in CMCs and reduction in  $-\Delta G^\circ$  values indicated the enhance in a micellar stability with growth of DBC asymmetry. One could be assumed that DBC1 macromolecules formed spherical "crew-cut" micelles [8] comprised relatively large hydrophobic "core" with H-bonded segments of both the blocks and a short "corona" with free segments of PAAc blocks (Fig. 2 a).

Table 2 – Parameters of the micellization process

Sample	CMC $\cdot 10^6$ , $\text{mol}\cdot\text{dm}^{-3}$	$-\Delta G^\circ$ , $\text{kJ}\cdot\text{mol}^{-1}$	n
DBC1	4.24	30.65	129/120
DBC2	6.33	29.66	210/120
TBC	2.78	31.7	52/136

An alternative micellar structure would be attributed to DBC2, which contained longer PAAc blocks (Fig. 2 b). In this case, the formation of "hairy-type" micelles with relatively small hydrophobic "core" and developed "corona" would be expected [5].

The smallest CMC and largest  $-\Delta G^\circ$  values were characteristic for TBC sample (Table 2). In this case, the most intense micellization developed due to insolubility of two "tail" short PAAc blocks, which were H-bonded with longer central PEO block. Therefore, the appearance of the "flower-like" micelles (Fig. 2 c) would be waiting [9]. Insoluble "tails" of the bound PAAc and PEO segments would form a large micellar "core", while soluble "loops" of free PEO segments would be concentrating in a "corona".

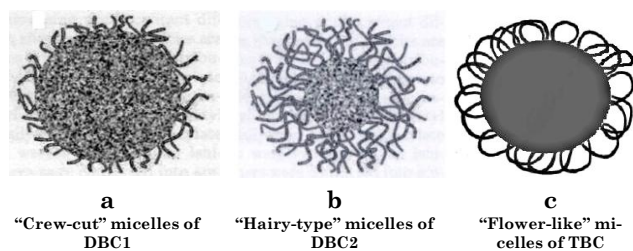


Fig. 2 – Micellar structures of the copolymers at  $\text{pH} \leq 3$

### 3.2 Encapsulation of vitamin E

Taking into account a poor solubility of VE in water, we dissolved it in a small volume of ethanol and blended with aqueous copolymer solutions. The interaction of VE with DBCs and TBC in the mixed ( $\text{H}_2\text{O}/\text{EtOH} = 95/5$  v/v) solvent at the ratio  $\varphi = 0.1$  mol<sub>VE</sub>/base-mol<sub>PAAc/PANa</sub> and the contact time  $\sim 24$  h resulted in the appearance of a solution turbidity in the region of  $\text{pH} = 3\div 9$  and a phase separation at  $\text{pH} < 3$ . Thus, the development of the micellization process in the first pH region and its intensification in the second one were observed.

We characterized VE encapsulation by DBC and TBC micelles at  $\text{pH} = 2.5$  by UV spectroscopy using a phase separation in the system. Thus, the precipitates with micellar phase and encapsulated VE were separated by a centrifugation ( $\omega = 6000$  rot/min) and supernatants included unbound VE were obtained and studied. UV spectra of the supernatants and initial VE solutions are shown in Fig. 3. The intense absorption bands near 260 and 300 nm in the spectra of a pure VE (Fig. 3 a, b, curves 1) corresponded to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  electronic transitions [10]. Significantly less intensity of these bands was observed in the spectra of supernatants (Fig. 3 a, b, curves 2).

The degree of VE encapsulation was calculated as the ratio between the integral intensities of the given absorption bands in the spectra of supernatant and initial pure VE. The results are represented in Table 3.

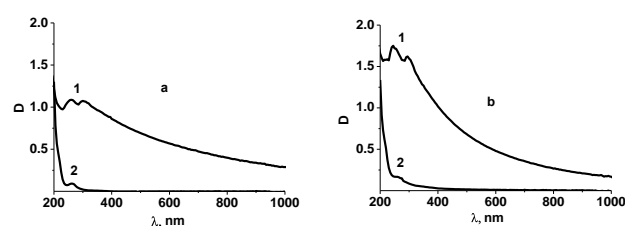


Fig. 3 – UV spectra of pure VE –1 and supernatants –2 for the blends: (a) DBC1 + VE and (b) TBC + VE.  $C_{\text{DBC}/\text{TBC}} = 1$  kg·m<sup>-3</sup>;  $\varphi = 0.1$  mol<sub>VE</sub>/base-mol<sub>PAAc</sub>;  $C_{\text{VE}} = 0.08$  kg·m<sup>-3</sup> (a) and  $0.09$  kg·m<sup>-3</sup> (b)

Table 3 – VE encapsulation by copolymer micelles

System	$C_{\text{VE}}$ initial, kg·m <sup>-3</sup>	$C_{\text{VE}}$ supernatant, kg·m <sup>-3</sup>	$X_{\text{VE}}$ a), wt %
DBC1+VE	0.250	0.003	99.0
TBC+VE	0.280	0.002	99.4

a) The degree of VE encapsulation.

It is seen that the micelles of both the copolymers encapsulated practically all VE at the selected its concentration and  $\varphi$  ratio.

### 3.3 Formation of silver nanoparticles

A color of the reaction mixtures after chemical reduction of silver ions in DBC solution with different solution pH by  $\text{NaBH}_4$  (at  $[\text{NaBH}_4]/[\text{Ag}^+] = 80$ ) gradually changed. So,  $\text{Ag}^+/\text{DBC}$  mixture with  $\text{pH} = 9.6$  showed a yellow color, while analogous mixture with  $\text{pH} = 2.5$  demonstrated a blue one. Nano-scaled metal particles exhibit an intense band of the surface plasmon resonance, which is conditioned by the movement of conducting electrons at the particle surface [7]. In our case the absorption band with  $\lambda_{\text{max}}$  near 400 nm characterizes the surface plasmon resonance of Ag-nanoparticles [7,11]. Typical plasmon resonance bands, which were recorded during the process of Ag-nanoparticle synthesis in DBC3 solutions, are shown in Figure 4 a. We observed a steady increase in the intensity of the absorption band with  $\lambda_{\text{max}} = 381$  nm at earlier reaction stages and further decrease in the band intensity and its red shift in 27-29 nm (up to  $\lambda_{\text{max}} = 408\text{-}410$  nm) at longer time. According to the Mie's theory, [7] the observed plasmon resonance band would correspond to the particles, whose size is less than  $\sim 30$  nm.

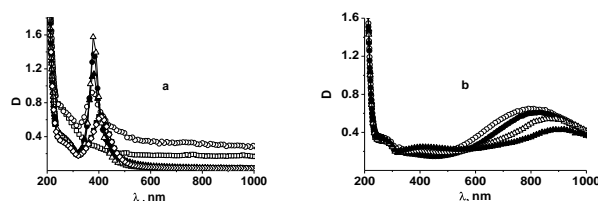
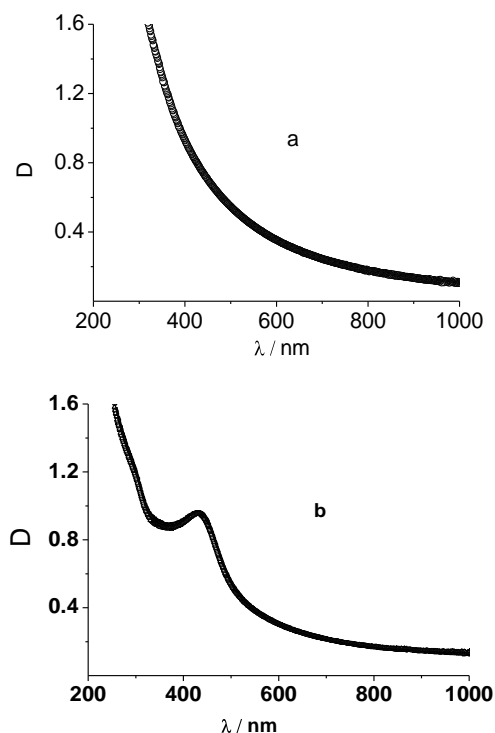


Fig. 4 – Absorption spectra of (a) Ag-nanoparticles obtained in DBC3 solution ( $\text{pH} = 9.6$ ) in different time after reduction beginning:  $\square = 5$ ,  $\circ = 15$ ,  $\blacktriangle = 35$ ,  $\bullet = 120$ ,  $\Delta = 270$ ,  $\blacksquare = 5760$ ,  $\diamond = 14400$  min; (b) Ag-nanoclusters obtained in DBC3 solution ( $\text{pH} = 6$ ) in different time after reduction beginning:  $\circ = 5$ ,  $\bullet = 141$ ,  $\Delta = 1440$ ,  $\blacktriangle = 5760$  min;  $C_{\text{DBC}} = 1$  kg·m<sup>-3</sup>,  $C_{\text{AgNO}_3} = 1.4 \cdot 10^{-2}$  kg·m<sup>-3</sup>,  $[\text{NaBH}_4]/[\text{Ag}^+] = 80$ .

The absorption bands near 400 nm were practically absent in spectra of reaction mixtures  $\text{Ag}^+/\text{DBC}$  at the  $\text{pH} \sim 6$  (Fig.4 b). Instead them, the absorption bands in the region of  $\lambda > 800$  nm appeared. In fact, the formation of so-called “blue silver” took place in these cases. According to the literature data, [11] this result could be interpreted by the formation of very long sequences of the bound silver nanoclusters (and  $\text{Ag}^+$  ions) along polyacid blocks of DBC. From such point of view the “blue silver” is a product of the incomplete reduction of silver ions by sodium borohydride. Therefore, the chemical reduction of  $\text{Ag}^+$  ions in DBC solutions at  $\text{pH} \sim 6$  (unlike to  $\text{pH} \sim 9$ ) led to formation of silver nanoclusters and their complexes with polyacid blocks. The absence of any sediment in the reaction mixtures denoted the presence of fully stable silver nanoparticle/nanocluster dispersions. The main task of the last experimental series was to carry out the silver ion reduction in micellar structures of the copolymers. In order to solve this problem, we have decreased the molar ratio of  $[\text{NaBH}_4]/[\text{Ag}^+]$  in the reaction mixture in 10 times (up to 8). In this case the solution  $\text{pH} = 3$  was not changed. Results of these tests are represented in Figure 5 b.



**Fig. 5** – Absorption spectra of (a)  $\text{Ag}^+/\text{DBC3}$  micellar solution (pH $\sim$ 3) without  $\text{NaBH}_4$  and (b) in 5-60 min after  $\text{NaBH}_4$  addition;  $C_{\text{PAAc}}=1 \text{ kg}\cdot\text{m}^{-3}$ ,  $C_{\text{AgNO}_3}=1.4\cdot 10^{-2} \text{ kg}\cdot\text{m}^{-3}$ ,  $[\text{NaBH}_4]/[\text{Ag}^+]=8$

At once after  $\text{NaBH}_4$  addition, relatively broad absorption band with  $\lambda_{\text{max}} = 431\text{-}433 \text{ nm}$  arose in the  $\text{Ag}^+/\text{DBC3}$  micellar solutions due to formation of silver nanoparticles. The intensity of this band was practically unchanged during some times ( $\sim 24 \text{ h}$ ) and then dis-

appeared because of the microphase separation in the system. Evidently, that in DBC micellar solutions at pH $\sim$ 3 a very quick development of the reduction process and formation of Ag-nanoparticles with larger size (than in DBC solutions at pH $\sim$ 9) took place.

#### 4. CONCLUSION

The given block copolymers with interacting PAAc and MOPEO (PEO) blocks were stimuli responsive copolymers. It was shown that their behavior in aqueous solutions strongly depended on the ionization degree of PAAc blocks. At low pH PAAc blocks were protonated and connected with chemically complementary MOPEO (PEO) blocks. Due to this, block copolymer macromolecules self-assembled into the micelles of different construction (with different nature of stabilizing “corona”) in dependence on relative length of nonionic and polyacid blocks.

The ability of DBC and TBC micelles to connect poorly soluble vitamin E was established. DBC and TBC matrixes demonstrated higher VE encapsulation efficiencies up to 100%.

Highly stable Ag nanoparticle dispersions were obtained with an excess of sodium borohydride ( $[\text{NaBH}_4]/[\text{Ag}^+] = 8\text{-}80$ ) in aqueous solutions of fully deprotonated DBC (pH = 9). Ag nanoparticle formation in DBC micelles (pH = 3) under  $[\text{NaBH}_4]/[\text{Ag}^+] = 8$  was established. It was revealed the partial reduction of  $\text{Ag}^+$  to the nanoclusters and their further stabilization by partially protonated DBC (pH = 6).

Thus, the capabilities of given block copolymer matrixes to act as the templates for encapsulation of fat-soluble VE and for silver nanoclusters/nanoparticles formation were shown.

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