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STRUCTURAL AND COLLOIDAL-CHEMICAL CHARACTERISTICS OF NANOSIZED DRUG DELIVERY SYSTEMS BASED ON PEGYLATED COMB-LIKE CARRIERS

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Abstract. Novel comb-like polymeric and oligomeric drug carriers combining backbone – copolymers of 5-*tert*-butylperoxy-5-methyl-1-hexene-3-yne (VEP) and glycidyl methacrylate (GMA) – and side PEG chains of various lengths were synthesized. Nanosized delivery systems containing conjugates of water soluble anticancer drug Doxorubicin were developed. The structures of copolymers and their conjugates with drugs were confirmed by IR-spectroscopy. Structural and colloidal-chemical properties of water drug delivery systems were studied using photoluminescent (PL), UV-spectroscopy techniques, surface tension measurements and dynamic light scattering. The scheme of the immobilization of water soluble doxorubicin on developed PEGylated polymeric carriers was assumed.

Keywords: polymeric carriers, drug delivery systems, anticancer preparations.

1. Introduction

The development of novel antineoplastic drugs is a priority task of the pharmaceutical industry. Especially, the creation of novel perspective controlled-release preparations possessing the complex of specific properties (low toxicity, prolonged action, cell targeting), is of great significance. As a rule, novel drug forms based on various natural or synthetic polymer carriers increase their biological accessibility as well as decrease potential side effects in treated organism [1]. Besides, many modern drug delivery systems possess such useful characteristics as addressed drug transportation to the locus of patholo-

gical process while the traditional drug forms do not possess such properties [2]. New carriers and derived effective drug forms were developed for addressed drug transportation to the locus of inflammation or pathological process and for controlling treatment course at cellular level. [3, 4]. Polymeric carriers bearing specific functional fragments facilitating the pathological cell recognition and penetration through cell membrane (phosphatidylcholine, folic, glutaric, lithocholic acids, *etc.*) are of great significance to this aim. Polymers containing tethered polyethylene glycol chains are intensively developed and studied as biologically tolerant carriers for addressed delivery and controlled release of the medicines in the target cells and tissues [5]. Comb-like copolymers containing hydrophilic and hydrophobic parts in their structure have the ability to form micelles in aqueous solutions, due to encapsulating biologically active materials, including insoluble ones [6, 7]. Besides, such carriers can protect these drugs from recognition by multidrug resistance (MDR) transporting systems which block drug action in the target tumor cells [8-10]. Various routes of immobilization of anticancer substances on PEGylated polymer carriers of synthetic, natural or mixed natures can also be useful for developing water-soluble forms of water-insoluble drugs and providing them with prolonged action and addressed delivery to the target organ [11]. Therefore, creating novel drug delivery systems that contain surface active PEGylated polymeric carriers corresponding to these requirements is of great actuality.

The development and study of structural and colloidal-chemical properties of waterborne nanosized

drug delivery systems based on novel comb-like polyethylene glycol-containing polymeric carrier were the aim of this work.

2. Experimental

2.1. Materials

The peroxide monomer 5-*tert*-butylperoxy-5-methyl-1-hexen-3-yne (VEP) was synthesized according to the method described earlier [12]. After purification by vacuum distillation VEP characteristics coincide well with the data: $d_4^{20} = 0.876$, $n_D^{20} = 1.4480$ and active oxygen content 8.79 % (calc. 8.75 %). Poly(ethylene glycol) methyl ether $M_n = 750$ (Aldrich) was used without purification. Azobisisobutyronitrile (AIBN) was purified by re-crystallization from ethanol. Boron trifluoride etherate and doxorubicin hydrochloride (Dox, Fig. 1) were obtained from Aldrich and Arterium (Ukraine), respectively.

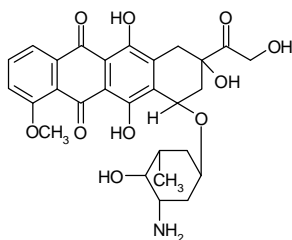


Fig. 1. Chemical structure of Dox

2.2. Methods

2.2.1. Synthesis of poly (VEP-GMA)-graft-mPEG copolymers

Poly (VEP-GMA) copolymer was prepared by radical polymerization of 5-*tert*-butylperoxy-5-methyl-1-hexene-3-yne (VEP) and glycidyl methacrylate (GMA) mixture initiated by AIBN at 343 K under argon atmosphere in ethyl acetate (EA) until 65 % conversion. Monomer conversion was measured using dilatometric and gravimetric techniques. After being cooled to room temperature, the mixture was concentrated, dissolved in acetone and several times purified by precipitation into hexane. The polymer was dried under vacuum at 323 K to a constant weight.

The synthesis of PEG-containing comb-like copolymers was carried out *via* treatment of poly(VEP-GMA) by mPEG in dry dioxane. The mole ratio mPEG:GMA links of initial copolymer was 1:2. The boron trifluoride diethyl etherate $\text{BF}_3 \cdot \text{OEt}_2$ (0.0008 % with respect to the weight of reaction mixture) was used as a catalyst. In a

typical reaction, 1 g of poly(VEP-GMA) was dissolved in 20 ml of dry dioxane and the BTDE (0.027 ml, 0.0318 mol) solution in dioxane (1 ml) was added dropwise to the flask containing 2.5 gm of PEG dissolved in 14 ml of dioxane. The resulting mixture was stirred at 313 K for 5 h. Then residual epoxy groups were deactivated by adding the methanol (0.5 ml) and stirred for 2 h. The resulting solution was concentrated, precipitated into hexane and washed by toluene. Then the washed product was dried at room temperature in vacuum to a constant weight.

2.2.2. Preparation of drug-loaded micelle-like structures of PC

100 mg of poly(VEP-GMA)-graft-mPEG were dissolved in 1 ml of DMSO. 3 mg of Dox were dissolved in 0.5 ml of DMSO. Drug solution was added to the polymer solution and stirred. Then 8.5 ml of 0.9 % water solution of NaCl was added dropwise under stirring. The resulting solution was sonicated for 20 s.

2.2.3. Characterization

Surface tension of the polymeric carrier solution and derived Dox conjugate on its basis were measured by the instrument PPNL-1 (Ukraine) using the maximum bubble pressure method (MBPM) [13]. The size of the polymers and their conjugates was measured by dynamic light scattering on Zetasizer Nano ZS (Malvern) photon correlation spectrometer using NIBS (Non-Invasive Back-Scatter) technology at 298 K. The concentration of the samples solutions was 0.4 mg/ml. UV spectrometry was performed at Nanodrop ND-1000 spectrophotometer (Thermo Scientific, USA). Photoluminescence spectra were recorded using an automated spectrometer. PL was excited by light with the wavelength of 260 nm, which is allocated by the primary monochromator MDR-2 (LOMO St Petersburg, Russia). Fluorescent light from the sample fell on MDR monochromator. Light record was carried out by photo multiplier tube FEU-100 ("MELZ FEU", Ltd., Moscow, Russia). Photo multiplier signal was recorded by the computer. Conductivity was measured by conductometer CON 2700 (USA).

3. Results and Discussion

The assumed general structure of PCs used for preparing waterborne drug delivery systems is presented in Fig. 2.

In contrast to initial copolymers of VEP – GMA PEGylated PCs are highly soluble in water and polar organic solvents. It is obvious (Fig. 3) that they possess surface activity and form micelle-like structures at the achievement of definite concentration in water.

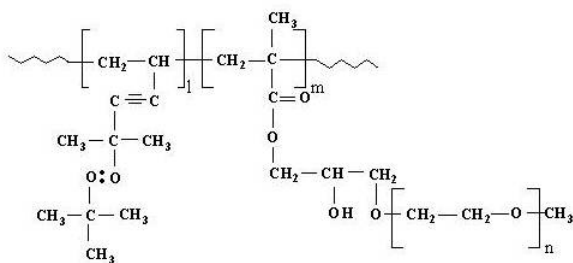


Fig. 2. Chemical structure of poly(VEP-GMA)-graft-mPEG

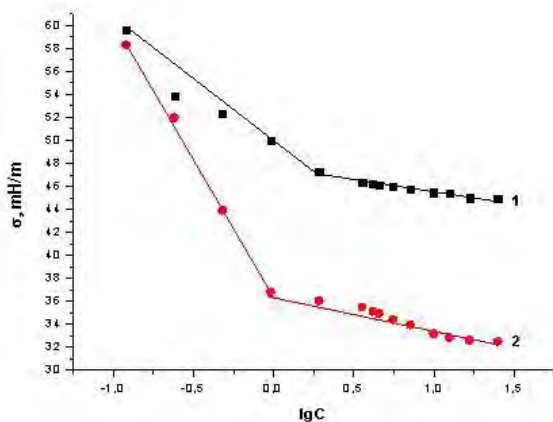


Fig. 3. The surface tension isotherms of water solutions of poly(VEP-GMA)-graft-mPEG (1) and Dox-PC (2)

Moreover, one can see the enhancement of surface activity and the decrease of point inflection value attributed to the formation of micelle-like structures as a result of Dox binding by PEGylated PC molecule. This shows, in our opinion, not only the binding Dox by PC molecules but also a high stability of the Dox-PC conjugates formed. The amount of Dox bound by PC can be determined from the dependence of conductivity of the PC solution on the amount of added Dox (Fig. 4).

The extremum of the conductivity dependence on the amount of Dox in water solution is specific characteristic of the plot presented in Fig. 4. First, the decrease of the conductivity is explained, probably, by the binding Dox, which is electrolyte unlike nonionic PC, and conjugation of Dox with side PEG branches of PC due to the formation of hydrogen bonds of Dox amino group with oxygen atoms of PEG branches. The minimal value on the curve of the dependence corresponds to the amount of the Dox bound by the PC molecules at the weight ratio Dox: PC = 1:30. The further increase of the conductivity is caused by the presence of free Dox in the solution.

A significant shift of the Dox absorption band at 190 nm in bathochromic region in UV-spectrum also confirms binding Dox with PC molecule as a result of their interaction (Fig. 5).

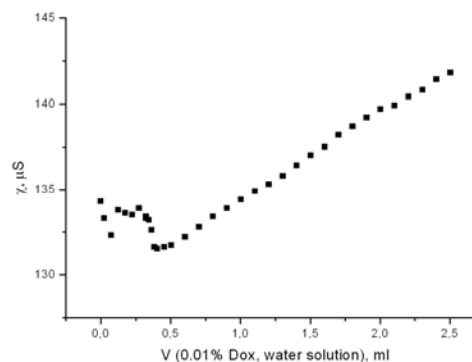


Fig. 4. Dependence of the added Dox amount on the conductivity of PC water solution

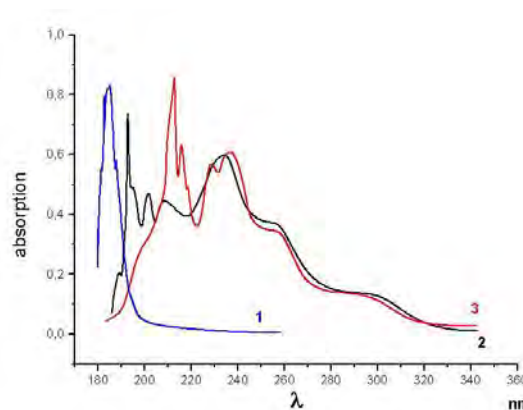


Fig. 5. UV spectra of PC (1), Dox (2), and Dox-PC water solutions

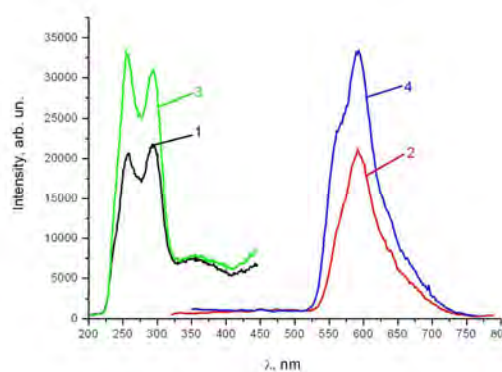


Fig. 6. Spectrum of excitation (1, 3) and photoluminescence (2, 4) of free Dox (1 and 2) and immobilized Dox (3, 4)

One can see from spectrum of excitation and luminescence of water solutions of doxorubicin and doxorubicin-PC conjugate (Fig. 6) that the main peaks characteristic of Dox coincide. However, it is obvious that the intensity of Dox luminescence significantly increases due to the immobilization of Dox on PC. This is caused, evidently, by localization of Dox

molecules inside of micelle-like structures formed by conjugates in water and protection of Dox from the contact with H₂O molecules, which are scotophors. This conforms well to the results of study of colloidal-chemical properties of conjugates presented in Figs. 3, 7 and 8.

One can see from the results of DLS study (Figs. 7 and 8) that free Dox molecules form in water solution colloidal structures sized in the range of 1 μm. The Dox size distribution is generally narrowed and monodispersed. The curves of the size distribution of free PC molecules in water are more complicated. It is evident that three main fractions of colloidal particles of different size formed by PC molecules are present in water solution. As a result of Dox-PC conjugation the narrowing and decrease of the size of the particles depending on Dox: PC ratios are observed. The increase of doxorubicin in the system leads to a

substantial decrease of the conjugate size, probably, due to the hydrophobization and compactness of the core of micelle-like structures containing Dox bound by PC molecules in accordance with the scheme (Fig. 9). Therefore, it can be assumed that displacement of water molecules and binding Dox molecules via hydrogen bonds with oxygen atoms of PEG fragments $-\text{CH}_2\text{CH}_2-\text{O}-\text{CH}_2\text{CH}_2-$ leads to the formation of micelle-like structures with hydrophobic core and free exterior hydrophilic PEG chains providing stabilization of the nanoparticles. The presence of other fractions of particles on the curves in PC solution and systems containing Dox conjugates can be explained, in our opinion, by polydispersity of initial PC (polydispersity coefficient is 3.5) and the presence of secondary structures formed by the molecules of carrier or its conjugates with Dox, as a result of aggregation.

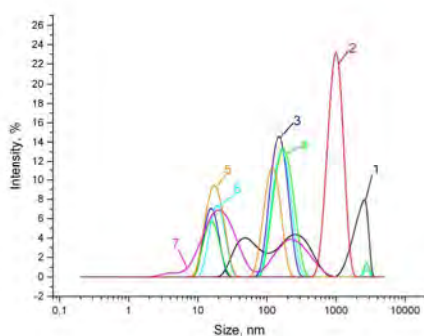


Fig. 7. Size distribution by the intensity of the micelles Dox:PC=0:100(1); 100:0 (2); 1:100 (3); 1:50 (4); 1:40 (5); 1:20 (6) and 1:10 (7)

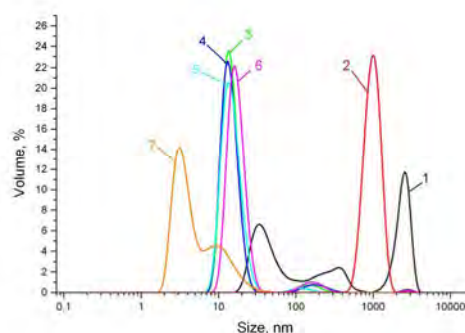


Fig. 8. Size distribution by the volume of the micelles Dox:PC=0:100(1); 100:0 (2); 1:100 (3); 1:50(4); 1:40 (5); 1:20 (6) and 1:10 (7)

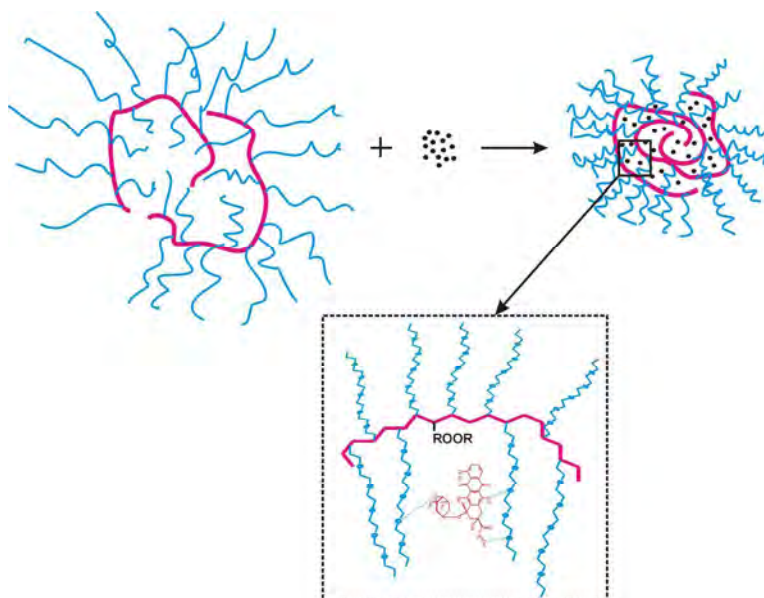


Fig. 9. Assumed scheme of nanosized Dox...PEGylated carrier conjugate

Such structure and size of Dox conjugates in waterborne delivery systems based on novel PEGylated polymer carrier provides their stability and low cytotoxicity. Moreover, due to these specific structural and colloidal-chemical properties they showed high therapeutic efficiency *in vitro* and *in vivo*, and strong acceleration of the action on cancer cells. So, such anticancer preparations can be of great significance for Dox cancer therapy providing avoidance of negative side effects.

4. Conclusions

Study of structural and colloidal-chemical characteristics of novel PEGylated polymeric carriers confirmed their ability to bind water soluble anticancer medicine doxorubicin forming highly stable nanosized waterborne systems. Using spectral techniques testifies to the formation of conjugates and their stability in water nanosized systems as well as the DLS method displayed the dependence of conjugate size on Dox-PC ratio. The scheme of the Dox conjugation via formation of hydrogen bonds with oxygen atoms of PEG branches was assumed and confirmed by independent methods. Testing developed drug delivery systems based on novel PEGylated carrier *in vitro* and *in vivo* showed that specific structure and controlled size of Dox bearing conjugates provides their low toxicity, the acceleration of cytostatic action on cancer cells and high therapeutic effect in comparison with using free doxorubicin.

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СТРУКТУРНІ ТА КОЛОЇДНО-ХІМІЧНІ ХАРАКТЕРИСТИКИ НАНОРОЗМІРНИХ СИСТЕМ ДОСТАВКИ ЛІКІВ НА ОСНОВІ ПЕГЕЛЬОВАНИХ ГРЕБЕНЕПОДІБНИХ НОСІЇВ

Анотація. Синтезовані нові гребенеподібні полімерні та олігомерні носії лікарських засобів, що поєднують основний ланцюг – кополімер 2-трет-бутилперокси-2-метил-5-гексен-3-іну (ВЕР) та гліцидил метакрилату (ГМА) – та бічні ланцюги поліетиленгліколю різної довжини. Розроблені нанорозмірні системи доставки, що містять кон'югати водорозчинного протиракового препарату Доксорубіцину. Структури кополімерів та їх кон'югатів з лікарською речовиною були підтверджені за допомогою ІЧ-спектроскопії. Структурні та колоїдно-хімічні властивості водних систем доставки ліків вивчені за допомогою фотолумінісцентної (ФЛ)-, УФ-спектроскопії, вимірювання поверхневого натягу та динамічного світлорозсіювання. Запропонована схема іммобілізації доксорубіцину на синтезованих пегельованих полімерних носіях.

Ключові слова: полімерні носії, системи доставки ліків, протиракові препарати.