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Functional micelles formed by branched polymeric surfactants: Synthesis, characteristics, and application as nanoreactors and carriers

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ABSTRACT

Branched polymeric surfactants composed of grafted non-ionic polyethylene glycol (PEG) and anionic polyelectrolyte chains were synthesized via radical polymerization initiated by the comb-like PEG-containing polyperoxide. Above definite concentration in solution, these surfactants form micelle-like structures (MLS). The MLS formed by branched polymeric molecules are of larger size in comparison with size of MLS formed by the initial PEG-containing polyperoxide that is caused mainly by different mechanism of their self-organization and morphology of formed MLS. The availability of grafted polyelectrolyte chains in the MLS provides a possibility of their use as the containers for immobilization of bio-active substances and nucleation of the inorganic nanoparticles, as well as formation of their stable colloidal systems in water in a wide pH range. Noticeable compaction and narrowed size distribution of the MLS were revealed after immobilization of doxorubicin (Dox) molecules or Fe_2O_3 nanocrystals. MLS-based systems were used for delivery of Dox and maghemite particles at treatment of tumor cells. Both MLS-based formulations of Dox and Fe_2O_3 were efficiently engulfed by rat glioma C6 cells. A significant (10 times) decrease in the effective therapeutic dose of Dox was found when this drug was delivered by a MLS-based formulation of Dox. That effect might be explained by a specific structure and functionality of the novel carrier used for immobilization of drug that should be delivered to target cells.

1. Introduction

In spite of successful synthesis of a variety of functional polymers, the creation of new polymeric surfactants and derived magnetic nanoparticles is still a topical task for the development of novel non-toxic organ targeted drug carriers and delivery systems for application in medicine and biotechnology. Thus, tailored syntheses of micelle-forming polymeric surfactants, as well as their use as carriers for drug delivery and for the nucleation of functionalized nanoparticles, are of high interest for

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researchers engaged in the creation and study of the formulations for chemo- and hyperthermia therapies used for tumor treatment.

During last years, numerous functional polymeric surfactants of linear, block or branched type and other desired architecture aimed at creation of micelle-like drug carriers and synthesis and functionalization of the nanoparticles of biomedical applications were synthesized and studied [1–3]. Particularly, the comb-like and branched water-soluble surface-active copolymers and derived micelles possessing high solubilization ability were developed [4].

Recently, several publications devoted to tailored synthesis of polymeric surfactants, as well as their application as carriers of bioactive substances [5–10] and soft templates for nucleation and functionalization of micro- and nanoparticles, appeared. These are amphiphilic polymers of linear, block, comb-like, star-like and dendritic structures that are capable of immobilizing drugs, particularly water insoluble ones [11–19], and can be used in biology and medicine.

Three main approaches for synthesis of comb-like copolymers were used [20–23]: (1) direct grafting side polymeric branches to a backbone via polymer analogous transformation; (2) copolymerization of the macromers with conventional functional monomers; (3) "grafting from" via radical polymerization initiated by the multi-site polymeric initiators containing peroxide or azo- side groups disposed along the backbone.

To obtain the comb-like copolymers via the method of «grafting from», backbone polymers containing reactive side sites along a chain are synthesized first [23–25]. At present, the ATRP technique based on using the polymeric macroinitiators for graft-copolymerization is more often applied [26,27].

The methods of obtaining amphiphilic comb-like copolymers based on water-soluble backbone and grafted side hydrophobic polymeric chains and vice versa the synthesis of similar polymeric surfactants consisting of water insoluble backbone and grafted hydrophilic chains were developed [17,28,29]. Grafted chains can be constructed from the identical [30] or distinct [31] monomer links and they can also contain grafted polysaccharide molecules [32]. To increase water solubility of the comb-like polymeric surfactants, hydrophilic, for instance, quaternary ammonium salt [33] or polyethylene glycol (PEG) [34] fragments are included into the grafted side chains. Such amphiphilic polymers form in water solution micelle-like structures (MLS) [28] or vesicles [35]. The architecture and functionality of grafted side branches, as well as the grafting degree cause a direct influence on the parameters and morphology of self-organized colloidal structures formed by the molecules of functional comb-like polymeric surfactants in solution [17,28,32,36,37].

New surface-active functional polyelectrolytes containing side peroxide fragments were successfully used for synthesis of the polymeric surfactants of branched structure. Many functional polymeric and inorganic nano- and microparticles were synthesized and tested for their potential biomedical application [25,38–42]. Previously, we have developed synthesis of comb-like PEG-containing polymers with side peroxide groups and tested them *in vitro* and *in vivo* for tumor chemotherapy [43,44]. Branched polymeric surfactants with the anionic polyelectrolyte chains and PEG were studied as thermo- and pH-sensitive drug carriers that can deliver drugs to the target organ and release them under the influence of the microenvironment conditions.

The main goal of this study was developing tailored synthesis of branched polymeric surfactants combining the non-ionic PEGylated and the anionic polyelectrolyte branches, and colloidal-chemical characterization of the obtained novel micelle-like structures. Big advantage of these structures, comparing with other similar created structures, is based on their universal capability to serve both as carriers for the organic molecules (ex. drugs) and containers for the nanoscale particles (ex. Fe_2O_3 nanocrystals). Other potential applications of these bifunctional micelle-like structures are discussed. An existence of stable colloidal systems and their low toxicity for the mammalian cells are additional features that are of great value at the biomedical use of the created materials.

2. Experimental

2.1. Materials

The peroxide monomer 5-*tert*butylperoxy-5-methyl-1-hexen-3-yne (PM) [CH_2═CH—C≡C—$C(CH_3)_2$—O—O—$C(CH_3)_3$ was synthesized from the dimethylvinylethynyl carbinol (2-methylhex-5-en-3-yn-2-ol) [CH_2═CH—C≡C—$C(CH_3)_2$—OH] (0.23 mol, 96 g) (Nairit Plant CJSC, Armenia) and *tert*-butyl hydroperoxide [HO—O—$C(CH_3)_3$] (0.24 mol, 118 g) (70% in H_2O, AkzoNobel, Netherlands) was purified by vacuum distillation and characterized by: [O] = 8.7%, d_4^{20} = 0.867 g/ml, n_d^{20} = 1.4482 [45]. N-vinyl-2-pyrrolidone (NVP) (Merck) and acrylic acid (AA) (Merck) were purified by distillation under vacuum. Iron (II) chloride ($FeCl_2 \times 4H_2O$) (99%, ABCR, Germany) and iron (III) chloride ($FeCl_3 \times 6H2O$) (99%, ABCR, Germany) were used as received. Doxorubicin (Dox) was purchased from Arterium (Ukraine). The solvents were used after additional purification [46]. Aqueous ammonia solution (25%) (Aldrich), hydrogen peroxide (35 wt.% solution in water, stabilized) (Fluka) were obtained from ACROS Organics.

2.2. Synthesis

2.2.1. Synthesis of poly(PM-co-GMA)-graft-PEG macroinitiator

Synthesis of poly(PM-co-GMA)-*graft*-PEG macroinitiator was carried out via subsequent stages as Refs. [43,44,47].

1) <u>*Preparation of the PM-GMA Copolymer*</u> was carried out as Ref. [48]. *5-tert* butylperoxy-5-methyl-l-hexene-3-yne (PM, 0.41 g, 0.5 mol) and glycidyl methacrylate (GMA, 7.72 g, 12.2 mol) (Aldrich) were dissolved in ethyl acetate (7.9 ml)