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Synthesis and characterization of novel drug delivery nanoparticles based on polyzwitterionic copolymers

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ABSTRACT

Copolymer (vinyl acetate (VA)-co-3-dimethyl(methacryloyloxyethyl)ammonium propane sulfonate (DMAPS)) nanoparticles have been synthesized by radical copolymerization in water. It was established that the variation of the initial monomer feed (DMAPS concentration was 10 mol% (copolymer 1) and 90 mol% (copolymer 2)) changes the copolymerization type, nanoparticles morphology, self-organization and size distribution. The shape, average diameter, size distribution and zeta potential of the copolymer nanoparticles are determined by atomic force microscopy (AFM), dynamic light scattering and zeta potential data, respectively. While the copolymer 1 nanoparticles are solid with spherical shape, average diameter 276 nm and zeta potential −25.2 mV, the copolymer 2 nanoparticles have bean-like shapes with an average diameter 49.3 nm and zeta potential −4.4 mV and contain many domains with different density. For the first time the AFM images of the copolymer 2 nanoparticles presented the unique self-organization of the dipole–dipole clusters of DMAPS units. The results indicated that the obtained copolymer nanoparticles with specific structure could be used as drug delivery systems.

1. Introduction

In recent years the nanotechnology has created one of the fast growing research fields in the pharmaceutical science [1,2]. Many scientific groups have focused their research interests in synthesis and characterization of drug delivery formulations with shapes and sizes spanning within the nanometer scale range [3,4]. The investigators' efforts involve elaboration of new synthetic methods, improvement of the stability of drug carriers, and their drug release properties for better targeting to the recipient cells and tissues [5–7]. In this sense, one promising approach is the synthesis of new polymer carriers with increased delivery efficiency and improved drug absorption as well as with reduced toxic effects [8]. Taking into account the requirements imposed to the polymer materials for drug formulation preparation, the study of homo- and copolymer zwitterions (PZIs) characterized by their specific self-association ability in aqueous solutions is of considerable interest [9,10].

In our previous study [11] we reported the preparation of new zwitterionic copolymer latexes, based on vinyl acetate (VA) and 3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate (DMAPS) (p(VA-co-DMAPS)). Using emulsifier-free emulsion copolymerization, latexes with different compositions were obtained. These latexes were used as matrix carriers for Metoprolol tartrate and Verapamil hydrochloride sustained delivery [12,13]. Using SEM, the changes in the structure of p(VA-co-DMAPS) latexes containing Metoprolol tartrate and Verapamil hydrochloride, during pressing and swelling have been studied. It has been established that the grainy (microspherical)

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structure of p(VA-co-DMAPS) is retained during matrix preparation and swelling, which shows its potential usage as a nanocarrier [14].

In the present study nanoparticles based on p(VA-co-DMAPS) with highest and lowest concentration of zwitterionic monomer DMAPS in the initial monomer feed (M_{DMAPS}) were synthesized. For the nanoparticle characterization and size distribution we employed atomic force microscopy (AFM) and compared those measurements with dynamic light scattering (DLS) and zeta potential data. AFM is relatively new technique which has found a variety of applications in many areas of research including materials and nanoparticles characterization [15]. It is well established that for metallic nanoparticles, transmission electron microscopy (TEM) is often considered as a preferable technique, because metallic particles have high contrast in TEM and do not require additional coating. But sample preparation for TEM measurements for polymer materials i.e. nanoparticles require additional treatment which is avoided when AFM is used. One of the inconveniences of the AFM utilization is the existing imaging artifact known as "tip convolution effect". Nevertheless, for structures with defined geometrical shapes (i.e. spheres) the AFM application is preferable because only the sizes measured in z-direction could be considered.

The objectives of this study were to determine the influence of the DMAPS content in the initial monomer feed (M_{DMAPS}) on: (i) the size and size distribution, morphology and structural organization of the nanoparticles obtained by radical copolymerization of VA and DMAPS in water; (ii) characterization of the obtained nanoparticles and determination of their cytotoxicity using the MTT-dye reduction assay [16,17].

2. Experimental section

2.1. Materials

Vinyl acetate (VA) (Merck, Darmstadt, Germany) was further purified by vacuum distillation. 3-Dimethyl(methacryloyloxyethyl)ammonium propane sulfonate (DMAPS) was obtained from Merck (Darmstadt, Germany). The initiator potassium peroxide disulfate (KPS, Fluka, Switzerland) was purified by recrystallization from water. Distilled water was used in copolymerization. RPMI 1640 medium (DMEM) (Sigma–Aldrich, Munich, Germany), fetal calf serum (FCS) (Sigma–Aldrich, Munich, Germany), trypsin (Merck, Darmstadt, Germany), ethylenediaminetetraacetic acid (EDTA) (Sigma–Aldrich, Munich, Germany), 96 well flat-bottomed micro plates (Nunc, Roskilde, Denmark), 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Merck, Darmstadt, Germany), PBS tablets (phosphate buffered saline (pH 7.4) as tablets) (Sigma–Aldrich, Munich, Germany), formic acid (Merck, Darmstadt, Germany), 2-propanol (Merck, Darmstadt, Germany). The cell lines DOHH-2 (non-Hodgkin lymphoma), KE-37 (T-cell leukemia) and K-562 (chronic myeloid leukemia), were supplied from DSMZ GmbH, Germany.

2.2. Radical copolymerization of VA and DMAPS in water

The chemical structures of the two monomers VA and DMAPS, respectively are presented in Fig. 1. Radical copolymerization of VA and DMAPS was performed in purified distilled water in a glass flask with a ground-glass stopper [11]. The VA/DMAPS mol% ratios in the initial monomer feed were 90/10 (copolymer 1) (described previously [11]) and 10/90 (copolymer 2). The total monomer concentration was 7.5×10^{-2} mol/L. The KPS concentration was 1 wt.%. The reaction mixtures were purged with nitrogen to remove oxygen from the system. The glass flask was thermostatted at 49 ± 1 °C. At the VA to DMAPS 90/10 mol% ratios in the initial monomer feed, the copolymerization occurs as emulsifier-free emulsion copolymerization (EFC). At the beginning the EFC was homogeneous, but latex phase was formed on the 5th hour from the beginning of the process then the system becomes heterogeneous. The radical copolymerization at a molar part of zwitterionic monomer DMAPS in the initial monomer feed (M_{DMAPS}) 90 mol% was homogeneous and there was no such phase separation. The copolymerization occurs as radical copolymerization in water solution. The conversion (q) was monitored gravimetrically. Purification of the synthesized copolymer 1 latexes and copolymer 2 solution was performed by dialysis in purified water (dialyze membranes Cut off 8000) for 7 days, thus removing the residual unreacted monomers. The purified copolymer 1 latexes and transparent solution of copolymer 2 were lyophilized. The copolymer 1 and copolymer 2 were white porous powders, insoluble and slightly soluble in water, respectively.

2.3. Elemental analysis

The copolymer compositions were determined by nitrogen analysis using Vario EL III elemental analyzer (Elementar Analysen systeme GmbH, Hanau, Germany).

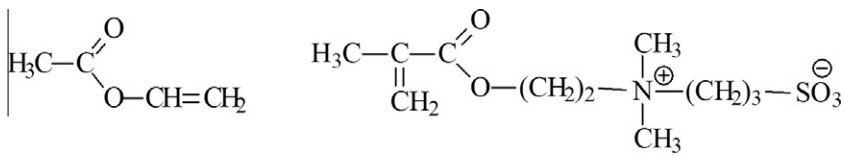

Fig. 1. Chemical structures of monomers VA and DMAPS.