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Controlled phase separations in solutions of soluble polyelectrolyte complex of DIVEMA (copolymer of divinyl ether and maleic anhydride)

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Abstract

Copolymer of divinyl ether and maleic anhydride (DIVEMA) is known to possess some anti-tumor and immune-stimulating activity and use as a drug carrier in anti-tumor drug delivery systems. Samples of DIVEMA of different degrees of polymerization were synthesized and characterized. Interaction of the hydrolyzed water-soluble DIVEMA polyanions with poly(*N*-ethyl-4-vinylpyridinium) cations (PEVP) has been studied. According to the potentiometry data, almost all carboxylic groups of the polyanions were able to form ion pairs with PEVP. In aqueous and water–salt solutions, formation of either soluble or insoluble polyelectrolyte complexes occurred depending on pH, ratio of the oppositely charged groups, and degree of polymerization of PEVP and/or DIVEMA. The phase separations followed general rules revealed by studying mixtures of PEVP and polycarboxylic acids. However in the case of DIVEMA, a significant broadening of the region for insoluble complexes at the expense of the region of soluble complexes was established. The data obtained demonstrate plausible advantages of the complex formation as the non-covalent modification of the polymeric carrier that endow DIVEMA with the ability for reversible soluble–insoluble transformation, in particular at physiological pH and ionic strength.

Keywords: DIVEMA; Phase separations; Polyelectrolyte complex; Pyran copolymer

1. Introduction

Polyelectrolyte complexes (PECs) are the products of coupling reaction between oppositely charged polyions of high charge density [1,2]. The cooperative electrostatic interaction of the partners imparts PECs a specific set of new properties, in particular the ability to undergo reversible phase transitions in a narrow and enzyme-friendly range of pH and/or ionic strength [3]. This feature enables one to classify these new polymer compounds as so-called "smart" polymers that have great potential for applications in medicine and biotechnology. Thus, the data obtained on studying "smart" PECs formed the basis for development of soluble–insoluble self-adjusted enzymatic systems with controlled activity, effective isolation and purification of proteins and nucleic acids, enhanced assay of endonucleases activity, vehicles for gene delivery, polyelectrolyte multilayer

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films and capsules with controlled stability, etc. Information on possible applications of PECs as clearly defined "stimuli response" polymers is summarized in reviews [4–6]. Inasmuch as the components of PECs might be charged polymers of different nature and structure, in particular nucleic acids [5] and globular proteins [6], the complex formation can be considered as a powerful tool to impart some technologically useful properties to charged (bio)polymers.

Copolymer of divinyl ether and maleic anhydride (DIVEMA) which is also known as pyran copolymer, has aroused considerable interest in medicine as a polymer modifier because of its anti-tumor and immune-stimulating activities [7,8]. To reduce side effects and enhance anti-tumor activity, the anti-tumor reagents are conjugated with polymeric carriers. The conjugates with DIVEMA demonstrated a dramatic anti-tumor effect that could be approximately 100 times greater than the non-modified drugs being much less toxic. Application of DIVEMA conjugates seems a promising new approach to attenuate intrahepatic inflammatory processes [9]. DIVEMA-modified drugs are candidates for development of cancer vaccines and organ protection in the transplantation medicine.

DIVEMA is readily transformed to water-soluble polyanion of high charge density by alkaline hydrolysis of the anhydrous groups (Scheme 1). To the best of our knowledge, no evidence was published on preparing and properties of polyelectrolyte complexes of this polyanion with polycations. This is the surprising thing when taken into account the plausible advantages of the complex formation as the non-covalent modification of the polymeric carrier that could endow DIVEMA or DIVEMA conjugates with the properties required by pharmacologists. Despite the practical importance, the results obtained on studying above interpolyelectrolyte interactions could be of scientific importance as well. Complexing of DIVEMA with polycation could permit one to gain a better understanding of a mechanism of PECs formation since the numerous carboxylic groups positioned in close proximity along DIVEMA chain might stabilize (or destabilize) the polyelectrolyte complex.

In the current paper, the complex formation between DIVEMA polyanion and poly(*N*-ethyl-4-vinylpyridinium bromide) (PEVP) has been investigated. To verify whether the specific structure of DIVEMA could influence the complexing, we contrasted PEVP/DIVEMA complex and complex of PEVP with poly(methacrylic) acid that was already studied in some detail [3,4]. We aimed to elucidate factors controlling phase diagrams of PEVP/DIVEMA mixtures in aqueous and water–salt solutions. Of special interest was to impart DIVEMA the capability for reversible soluble–insoluble transformation, in particular at physiological pH and ionic strength. The latter could be an important stage in approach the problem of the targeted delivery of anti-cancer drugs.

2. Experimental

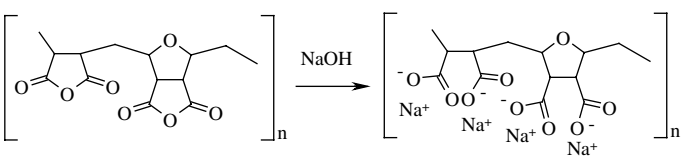
2.1. Materials

HCl, NaOH, NaCl, TRIS and MES buffers were purchased from Sigma (USA). In all experiments twice distilled and additionally purified by Milli-Q (Millipore, USA) water was used.

Samples of DIVEMA of different molecular mass and molecular mass distribution were prepared and characterized according to the developed approach [10]. In brief, DIVEMA copolymer was synthesized from the corresponding monomers in glass sealed tube by the reaction of radical cyclopolymerization in acetone, then purified by extraction in ether, and finally, dried to a constant mass in vacuum. DIVEMA samples with $M_w = 34\,800$ ($M_w/M_n = 2.5$), $M_w = 170\,000$ ($M_w/M_n = 3.5$), and $M_w = 345\,000$ ($M_w/M_n = 3.2$). The molecular weights were determined by GPC with the use of PEG-PEO standards (Waters, USA).

Samples of exhaustively alkylated poly(*N*-ethyl-4-vinylpyridinium bromide) (degree of alkylation > 92%) of different molecular masses were prepared by alkylation of fractions of poly(4-vinylpyridine) with ethyl bromide and characterized as described elsewhere [11]. PEVP samples with $M_w = 53\,500$ ($M_w/M_n = 1.3$), $M_w = 73\,000$ ($M_w/M_n = 1.4$), $M_w = 120\,000$ ($M_w/M_n = 1.4$), and $M_w = 350\,000$ ($M_w/M_n = 1.5$) were used. The molecular weights were measured by GPC with light-scattering detector.

Poly(methacrylic acid) (PMAA) was prepared by radical polymerization of the monomer and fractionally precipitated in methanol/ethyl acetate mixture. The sample with $M_w = 220\,000$ was used.



Scheme 1. Structure of DIVEMA and sodium salt of DIVEMA.