



Polyelectrolyte Nanocomplexes with pH-controlled Solubility

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(Received 10 June 2013; published online 01 September 2013)

The prepared polyelectrolyte complexes of anionic copolymer of divinyl ester and maleic anhydride and cationic water soluble chitosan proved to be able to undergo reversible phase transition, in particular at physiological pH and ionic strength. Formation of either soluble or insoluble complexes occurred depending on pH value, charge ratio and degree of polymerization of the polymers. The stability of positively or negatively charged complexes against destruction action of the added salt drastically increased in acidic media presumably owing to H-bonds formation. According to light-scattering data, the size of the soluble complexes varied from 100 to 300 nm and remained virtually unchanged under storage after lyophilizing drying and resuspending in the same medium.

Keywords: Nanoparticles, Polyelectrolyte complexes, Water-soluble chitosan, Copolymer DIVEMA, Drug delivery.

PACS numbers: 61.25H 82.35 jK 82.35 Np 82.35 Rs

1. INTRODUCTION

In the past decade a huge amount of drug delivery systems based on nanoparticles have been investigated and some of them were accepted for clinical applications [1]. These systems improves existing treatments though their altered pharmacokinetics and biodistribution profiles. The next urgent task for further enhancement therapeutic efficacy is the creation of environmentally responsive systems that could respond to environmentally changes or enable triggered on-demand responses by application of a suitable stimulus such as temperature, pH, and salt concentration. Among these environmental stimuli pH gradient seems challenging whereas different organs, tissues and sub-cellular components, as well as their pathophysiological states can be characterized by their pH value.

Polyelectrolyte complexes (PECs) are the products of coupling reaction between oppositely charged polyions and represent the example of so-called “smart systems”, i.e. the systems that could change their properties under the external impulse; what is more PECs mainly have nanoscale sizes. These features make PECs particularly attractive for drug delivery. PECs formed by natural polymers or synthetic polymers that possess biological activity are the most interesting for these application.

The objective of the present study was to develop polyelectrolyte nanocomplexes based on synthetic copolymer of divinyl ester and maleic anhydride and modified water soluble chitosan which are able to undergo reversible phase transition, in particular at physiological pH and ionic strength.

2. MATERIALS AND METHODS

2.1 Materials

Samples of modified chitosan (mCht) were synthesized by alkylation of primary amino groups of the corresponding parent chitosan with glycidyltrimethylammonium chloride as described elsewhere [2]. The

prepared mCht samples with degree of polymerization 320, 640, and 860 (measured via static light scattering in 0.33 M acetic acid containing 0.2 mol/l sodium acetate) were denoted as mCht₃₂₀, mCht₆₄₀, mCht₈₆₀. The DIVEMA samples of different degrees polymerization 600, 1350, 2850 (GPC data) were prepared and characterized according to [3]. Hereafter, the samples referred to as DIVEMA_{DP}.

Polymer weighed portions were dissolved in 0,02M solutions of the buffers. Polymer concentrations were expressed in terms of molar concentration [Nq] of primary amino groups for the mCht solutions and total concentration charged and uncharged carboxylic groups [COOH+COO⁻] for the DIVEMA-anion solutions.

Polyelectrolyte complexes were prepared by mixing of corresponding volumes of polyelectrolytes solutions in a selected buffer. Compositions of mixtures $Z = [Nq] / [COOH+COO^-]$ were expressed in terms of the ratio of molar concentrations of mCht and DIVEMA. The concentration of polyelectrolyte in excess in the mixture was $8,7 \times 10^{-4}$ M.

2.2 Method

Turbidimetric titration was carried out via successive addition of the concentrated solution of a titrant (polysaccharide or KCl) in a standard quartz cuvette containing the solution of the DIVEMA anion or the transparent mixture of polyelectrolytes of a selected composition. Measurements were performed on a Specord M40 spectrophotometer (Carl Zeiss, Jena, Germany) under constant stirring at a temperature of 25 °C. Turbidity was estimated from the values of optical density D_{450} measured at a wavelength of 450 nm, at which none of the polymer components absorbed light. Mixtures meeting the condition $D_{450} < 0.02$ were regarded as transparent.

Potentiometric titration of aqueous solutions was conducted using a Beckman F-70 (USA) potentiometer equipped with a combined electrode. The measurements were performed in water-thermostatic stirred cell holder under argon atmosphere at 25 °C.

The average particle size and polydispersity index were measured by photon correlation spectroscopy using a zeta-seizer NanoZS (Malvern Instruments, Malvern, UK) at 25 °C and a backscatter detector. The zeta-potential was determined by micro-electrophoresis in a dip cell.

3. RESULTS AND DISCUSSION

Both components of studied system are water soluble and biological active. Copolymer DIVEMA possesses its own antivirus, antitumor and immune stimulating activities and is used in design of delivery systems for drug and imaging agents. In water solution copolymer DIVEMA is a polyanion with high density of charges due to hydrolysis of the anhydrous cycles. The mCht - modified N-(2-hydroxy3-trimethylammonium)propyl chitosan used in present study as a polycation is combining such advantages of chitosan as its low toxicity, biocompatibility and biodegradability with solubility in water medium though the whole pH range.

The complexes formation in present system is a complicated process controlled mainly by pH value since DIVEMA's macromolecules bears a large amount of carboxylic groups and modified chitosan contains in each modified unit besides of positively charged at any pH quaternized amino groups secondary pH-dependent amino groups [2].

3.1 Effect of pH on DIVEMA-mCht Complexes

The charges balance and its dependence on pH value were studied by ζ -potential measurement and potentiometry. In the alkaline medium at pH 9.0 zero value of ζ -potential formed particles was achieved at $[+]/[-] \approx 0.85$ (fig. 1). At the same time the only about 30% mCht in the mixture was enough to achieve the zero value of $[+]/[-]$ at pH (pH 5.5). This data is in full agreement with potentiometry data (not shown) according that at pH = 9 the degree of DIVEMA dissociation equal 0,85 while the secondary groups of mCht is uncharged. Its mean that in basic medium the charge ratio equal 0,85 corresponds to stoichiometric PEC.

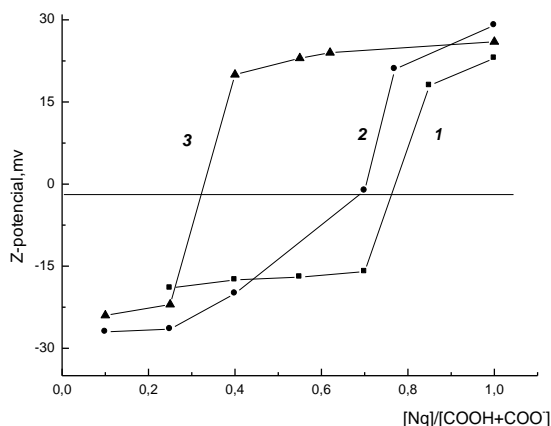


Fig. 1 – ζ -potential of mCht/DIVEMA complexes as a function of $[+]/[-]$ ratio measured at different pH: 9,0 (1); 7,2 (2); 5,5 (3). Concentration of carboxylic groups 1×10^{-3} M, 25 °C.

3.2 Turbidimetric Titration

The formation of either soluble or insoluble polyelectrolyte complexes occurred depending on ratio of the oppositely charged groups and degree of polymerization of mCht and/or DIVEMA, also on pH value. The regions of existence of positive or negative charged soluble complexes were determined by the turbidimetry data. At pH=9 under titration the solution of DIVEMA by mCht solution up to certain compositions $Z = [Nq] : [COOH + COO^-]$ system remained transparent that indicated the formation of soluble nonstoichiometric polyelectrolyte complexes (fig. 2). When a critical value Z_{cr} was achieved, stable opalescence appeared and did not vanish during mixing. The addition of polysaccharide at $Z > Z_{cr}$ resulted in the appearance and further accumulation of the insoluble complex.

The shaded area defined the region of insoluble PEC and the region 1 and 3 corresponded to negatively and positively charged soluble complexes. The region of existence of soluble complexes is the most extended in basic medium. Under acidification of solution, this region becomes narrower up to $Z_{cr} = 0.2$ and 0.1 at pH 7,2 and 5,5 corresponding for negatively charged complexes.

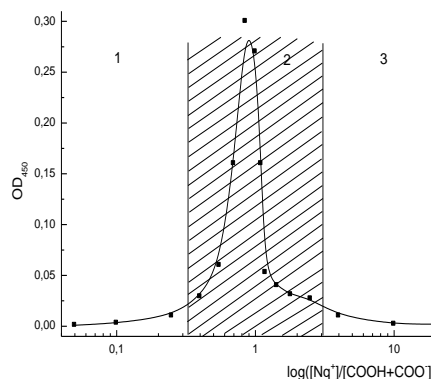


Fig. 2 – Turbidity vs ratio of $[Nq]$ to $[COOH + COO^-]$ at pH 9,0. $[DIVEMA] = [Nq] = 8,7 \times 10^{-4}$ M, $[TRIS] = 0,02$ M.

3.3 Effect of Ionic Strength on Complexes Stability

The systems for drug delivery should be stable under physiological condition. The critical concentration of the salt, $[KCl]_{cr}$, which corresponds to full dissolution of the suspension of the mCht–DIVEMA mixture with the composition $[Nq]:[COOH + COO^-] = 1.0$, depends on the solution pH (Fig. 3) as was shown by the turbidimetric titration data. With a decrease in pH from 9.0 to 7.2, an insignificant stabilization of the complex occurs, whereas further reduction in the pH to 5.5 is accompanied by a marked increase in $[KCl]_{cr}$. Such behavior was detected previously for modified chitosan mixtures with other polyanions⁴⁻⁵ and may reflect both contribution of ionization of secondary amino groups and H-bond formation at the acidic medium.

The increase in DIVEMA's degree of polymerization has led to improvement of complexes stability. The most important is that prepared PEC kept them stability under physiological condition.

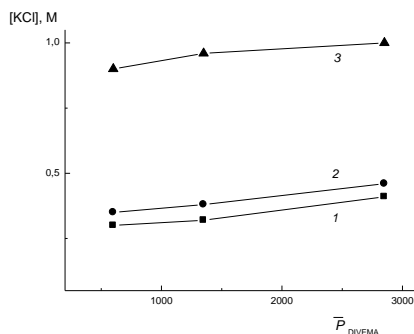


Fig. 3 – Critical salt concentration $[KCl]_{cr}$ corresponding to the full dissolution of the mixture of the mCht and DIVEMA of the composition $Z = 1.0$ vs. degree of polymerization of DIVEMA at pH (1) 9.0, (2) 7.2, and (3) 5.5.

3.4 PEC Sizes

The other critical property for delivery systems that alters them the biological distribution profile as well as

possible rout of administration is their sizes. As it follows from the light scattering data the sizes of the complexes prepared in neutral medium at pH = 7.2 depending on mixture composition were about 140-260 nm depending on mixture composition. Only one narrow mode was observed for different $[Nq]:[COOH + COO^-]$ ratio with the exception of the narrow region closed to point of system overcharging. In addition, complexes were stable under storage and didn't change their sizes after drying and resuspending in the same medium.

CONCLUSION

The PECs based on synthetic copolymer of divinyl ester and maleic anhydride and modified water soluble chitosan, were able to undergo reversible phase transition in particular at physiological pH and ionic strength value. The data obtained demonstrated the potential of the prepared PECs as a promising platform for development of biocompatible and pH-tunable drug delivery systems.

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