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Complex impedance spectroscopy to investigate degradable chondroitin–poly(amino-serinate) complexes

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ABSTRACT

Sodium chondroitin-4-sulfate and poly(amino-serinate) bromide can interact to form a degradable polyelectrolyte complex. The structure of each polymer and of their complex before and after degradation is investigated by complex impedance spectroscopy. Poly(amino-serinate) bromide and sodium chondroitin-4-sulfate exhibit dc conductivity and dielectric relaxation phenomena in the 10^2–10^6 Hz range. On the contrary, no dc conductivity and dielectric relaxation are observed in the polyelectrolyte complex before degradation. After degradation, the released chondroitin-4-sulfate is re-complexed with additional poly(amino-serinate) bromide. Contrary to the original parent complex the restored complex exhibits both dc conductivity and dielectric relaxation phenomena. This difference is assigned to structural defects due to the presence of residual poly(amino-serinate) oligomers which compete with the newly added poly(amino-serinate) to complex the released chondroitin-4-sulfate. This outcome is interpreted assuming the displacement of the low molecular by higher molecular weight chains in contrast to the behavior usually reported for this type of polymeric systems.

1. Introduction

Polyelectrolyte complexes (PECs) exhibit properties potentially or effectively exploitable in various domains of applications such as separation and purification of charged molecules [1,2], molecular chaperoning [3,4] combination with macromolecular prodrugs [5], and drug encapsulation [6]. PECs are also involved in gene transport and transfection to cells [7–10].

PECs based on commercially available polyelectrolytes like poly(acrylic acid), poly(styrene sulfonic acid), poly(ethylene imine), polylysine, etc. have been proposed as model systems in order to get insights into the fate of polyelectrolytes or of their complex when they are introduced in the blood stream where molecules and cells bearing ionic functions are present. Electrostatic interactions with these entities or destabilization of preformed PECs can occur depending on their chemical–physic properties and those of blood [11,12]. On the other hand, high molar mass macromolecules are retained between skin and mucosa i.e. in parental compartments. Ideally, any foreign macromolecular system introduced in blood must be degradable if it has to be eliminated from the body once its mission is completed. In practice, the present trend imposes biocompatibility and bioresorbability i.e. elimination via natural pathways or bioassimilation as biomass at the top of bio-functionality when effective applications of polyelectrolytic systems for time-limited therapy in human are targeted.

It is rather difficult to study the factors that affect the formation and the stability of polyelectrolyte complexes because the energy of interchain cooperative interactions is high and one has to separate the oppositely charged polyelectrolytes using drastic pH or salt conditions prior to analysis. During the last decades, experimental techniques have been developed to obtain information on the structure of PECs [13] as well as molecular simulations [14,15] with limits due to the methodology.

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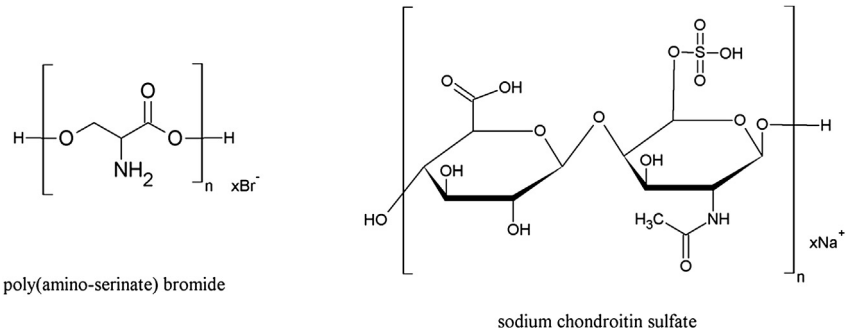

Scheme 1. Chemical structure of PAS-Br and ChS-Na.

Complex impedance spectroscopy (CIS) is generally used to measure the ac electrical response, i.e. ac conductivity or permittivity, of liquids or solids. The principle is well-known and can be found in textbooks [16]. It is a powerful tool to investigate polarization and conductivity phenomena in condensed matter where the conduction is insured by mobile ions [17–19]. It was exploited for studying solid state crystalline or amorphous solids [20], and also charged polymers and polyelectrolytes [21,22]. Recently, investigations on cation mobility and on the effect of adsorbed water in a PEC have been reported by Cramer and co-workers [23–25].

This article is aimed at reporting on the results of a structural investigation of solid state polyelectrolytes and degradable PECs using CIS. The selected PECs derived from degradable polyelectrolytes of therapeutic interest. The first one is poly(amino-serinate) under the bromide form referred to as PAS-Br. It is an artificial soluble polycation hydrolytically degradable in aqueous solution at physiologic pH (7.4) and temperature (37 °C). The second one is sodium chondroitin-4-sulfate (ChS-Na) (Scheme 1). Contrary to PAS-Br, ChS-Na is a natural polyanion constituent of human cartilage. It is chemically stable in water and degradable *in vivo*. PAS-Br and ChS-Na are soluble in aqueous media whereas their complex, referred to as PAS–ChS PEC, is insoluble. However PAS is known to be hydrolyzable even though it is complexed by ChS [26]. Therefore, because of hydrolyzation of PAS, PAS–ChS PEC is degraded and, hence, becomes constituted of ChS partially complexed only with the remaining non-hydrolyzed PAS. The addition of extra PAS-Br to the solution containing the degraded PEC can thus lead to the re-complexation of the degraded domains and the formation of a renew PEC.

In this work, we investigated the ageing of PAS–ChS PEC in aqueous solution. Ac conductivity and ac permittivity were first measured on PAS-Br and ChS-NA and then on their PEC at different degradation states: before and after degradation, and after re-complexation. Data are discussed with respect to the ChS-Na/PAS-Br complexation phenomenon. The results obtained are compared with a PEC formed with ChS complexed with poly(L-lysine bromide) (PLL-Br) that is known to be very stable in the absence of protease.

2. Experimental

2.1. Reagents

N-Carbobenzoxy serine (N-Z), acetone, trifluoroacetic acid (TFA), sodium chondroitin sulfate (ChS-Na, reference 27043), poly(L-lysine bromide) (PLL-Br, 10,000 and 30,000 g/mol) were purchased from Sigma Aldrich (France) and used as received.

2.2. Methods

2.2.1. Size exclusion chromatography (SEC)

Protected polymer molecular weight was evaluated by THF SEC using a column PLgel 5 μm Mixed-C (length 60 cm). The elution rate was 1 ml min^{-1}. A differential diffractometer (Water 40) has used for detection. Polystyrene standards were used for calibration.

2.2.2. High performance liquid chromatography (HPLC)

Aqueous HPLC analyses were performed using an Alltech Macrosphere WAX 7 μm column (L. 250 mm, diam. 4.6 mm) fitted to a Waters 570 pump and a UV/Visible Waters 484 spectrometer operated at $\lambda = 514$ nm. The mobile phase was phosphate buffer 0.05 M pH 6.8 flowed at 0.8 ml min^{-1}. Data were collected using Millennium softwares from Waters Polymers and PEC preparation.

2.2.3. Polymers

PAS-Br was synthesized from N-Z to yield protected poly(amino serinate) (PASZ) according to a procedure previously described [26]. Briefly, 2 g of Z-serine was dissolved in 80 ml of anhydrous acetone. 4 g of anhydrous sodium carbonate was added. After 2 h, 400 μl of mesyle chloride was introduced and the mixture was allowed to stir for 12 h. After filtration and evaporation, the collected pasty PASZ presented a bimodal molecular mass distribution. It was fractionated by selective precipitation from a solution in acetone using diethyl ether as a non-solvent. The fraction with $M_w = 25{,}000$ g mol^{-1} and $I = 1.8$ according to SEC in THF fraction was selected. PAS-Br was obtained by quantitative deprotection of PASZ using a mixture of TFA–HBr– acetic acid.

2.2.4. PEC formation

Typically, 0.5 ml of 0.2 N PAS-Br and ChS-Na in buffer 0.15 NaCl and PBS (pH 7) solutions were mixed. Supernatant and precipitate phases were separated by centrifugation and freeze-dried. A similar protocol was used to prepare a PLL–ChS complex.

2.2.5. PEC degradation

A 30 mg sample of PAS–ChS PEC dispersed in deionized water with 0.15 NaCl and PBS (pH 7.4) was allowed to age for 3 weeks at 37 °C. The degradation studies of PAS–ChS PEC are performed by HPLC (Fig. 1). After 6 h, HPLC reveals the presence of serine monomers in solution (peak 4 min) and in the same time the quantity of Br^- increases (peak 11.3 min). After 12–15 day, the solid PEC totally disappears of solution. In addition, ChS is known to be stable under these conditions. Thus the degradation process of PEC is only due to PAS hydrolysis [12].