Regular Article

Ion-macromolecule interactions studied with model polyurethanes

Borja Fernández–d'Arlas [a,b,*], Miguel Ángel Huertos [c,d], Alejandro J. Müller [b,d]

[a] INAMAT (Institute for Advanced Materials) y Departamento de Física, Universidad Pública de Navarra (UPNA), Centro Jerónimo de Ayanz, Campus Arrosadía, 31006 Pamplona, Spain
[b] POLYMAT and Polymer Science and Technology Department, Faculty of Chemistry, University of the Basque Country UPV/EHU, Paseo Manuel de Lardizábal 3, 20018 Donostia-San Sebastián, Spain
[c] Grupo de Investigación Química Inorgánica, Departamento de Química Aplicada, Universidad del País Vasco UPV/EHU, Avda. Manuel de Lardizábal 3, 20018 San Sebastian, Spain
[d] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

GRAPHICAL ABSTRACT
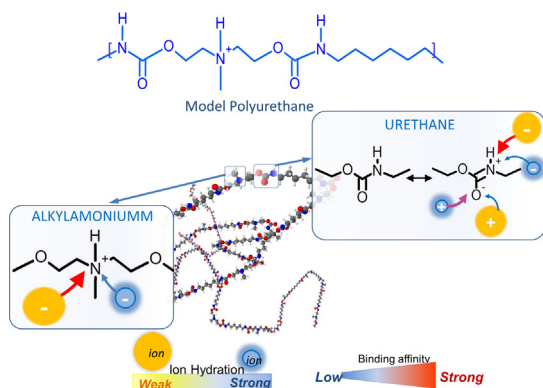
ABSTRACT

Hypothesis: The solubility and self-assembly of macromolecules in solution can be tuned by the presence of different salts. Natural proteins have been long manipulated with the aid of salts, and natural silk is processed in the gland tip across a gradient of different salts which modifies its solubility. Hence, the comprehensive understanding of the role of ion-macromolecule interactions should pave the way towards a biomimetic processing of macromolecules.

Experiments: A model polyurethane catiomer (PU^+) with high density of hydrogen donors and acceptors (similar to proteins) has been designed and synthesized in order to study ion-macromolecule interactions by means of dynamic light scattering (DLS), infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{13}C NMR).

Findings: The PU^+ solubility in the presence of different salts exhibited a reversed anion Hofmeister series (i.e., the anion ability to precipitate the PU^+ was $F^- < Cl^- < Br^- < NO_3^- < CH_3COO^- < H_2PO_4^- < H_2CO_3^- < I^- < ClO_4^- < SCN^-$). The ordering of this series was found to be predicted, for the first time, by the Born-Landé-Ephraim-Fajans-Bjerrum model used here to estimate the degree of macromolecule-ion pairing in water solution. This work also helps understanding the role of cations and anions nature on their interaction with macromolecules backbone.

© 2017 Elsevier Inc. All rights reserved.

* Corresponding author at: INAMAT (Institute for Advanced Materials) y Departamento de Física, Universidad Pública de Navarra (UPNA), Centro Jerónimo de Ayanz, Campus Arrosadía, 31006 Pamplona, Spain.
*E-mail address:* borja.fernandezdarlas@unavarra.es (B. Fernández–d'Arlas).

1. Introduction

Ion-macromolecule interactions are involved in a large number of physico-chemical [1–9] and biological processes [9–12].

Pioneering works reported in the XIX century by Denis [1], Bernard [13] and Matthieu and Urbain [14] led to a deep understanding of the impact of certain salts over some protein solutions [15]. With the aim of understanding the nature of the salt-protein interaction, Lewith and Hofmeister carried out a number of experiments to elucidate the role of the salt nature on either stabilizing or coagulating egg white aqueous solutions [16]. These experiments are considered the first systematic studies of ions specificity on the ion-macromolecule interactions. According to the results of Hofmeister et al., the effectiveness of anions of sodium salts in precipitating egg white solutions followed the series:

$$SO_4^{2-} \approx HPO_4^{2-} > CH_3COO^- > HCO_3^- > CrO_4^{2-} > NO_3^- > ClO_3^-$$
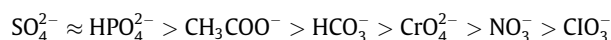
Although not as conclusive as with the anion series, comparing different chloride salts with different cations, Hofmeister et al. also came to the conclusion that the effectiveness of cations on coagulating egg white solutions followed the order:

$$Li^+ > Na^+ > NH_4^+ > Mg^{2+}$$
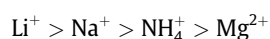
Extension of ion-macromolecule specific effects with other macromolecules and salts has led to the generalization [17] of the anionic series in terms of its precipitating effectiveness as follows:

$$CO_3^{2-} > SO_4^{2-} > H_2PO_4^- > F^- > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^- \\ > ClO_4^- > SCN^-$$
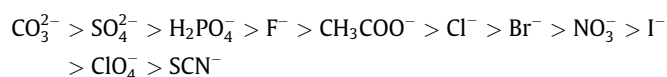
Different experiments carried out with different proteins have revealed that when the protons concentration lead to a net positively charged protein (pH < pI) the general tendency is opposite to the Hofmeister series [18–20]. Experiments performed with positively charged latex have shown that inverse series, in terms of precipitating efficiency, are obtained when different salts were added to their solutions [4,21]. At this point it is necessary to recall that when Hofmeister published their works, the concept of pH was still not introduced [10]. The mayor component of egg white is ovoalbumin (pI = 4.5–5.1) and therefore it is expected that in Hofmeister experiments it was fulfilled that pH > pI.

The Hofmeister series were first attempted to be explained by the effect of the ions on the three dimensional order of water, classifying them into *chaotropes* or *kosmotropes*, depending on their ability to disrupt or to reorganize water molecules, respectively [22,23], what would modulate water hydrating capacity. The stability of macromolecules in solutions would be therefore linked to measurable properties of ion-water interactions in solution, such as the viscosity coefficient [3], *B*. Nevertheless, different experiments suggest that the presence of ions has a minimal effect on the bulk structure of water far beyond its first shells of hydration [24,25]. In addition, different experiments have demonstrated the relevance of ion-macromolecule interactions for understanding the Hofmeister phenomena, minimizing the importance of water structure and emphasizing the importance of the nature of the solutes and the ions [26–28].

The complicated nature of proteins, composed of multiple amino acids, makes difficult the assignment of the nature of the interactions between the ions and the macromolecules that determines their behavior in solution. It is for this reason that studies with synthetic molecules, with a defined structure, are important in order to discriminate the nature of the ion-macromolecule predominating interactions. Studies of López-León et al. [4,21] with latexes positively functionalized with amidinium groups, R_1C $(=NHR)NR_2^+$, or negatively with sulphate (RSO_4^-) charges, have pointed out the importance of macromolecules charge on determining the ordering of ions on influencing their stability in solution. Stability of positively charged latexes followed an inverse

Hofmeister series, while negatively charged latexes followed a direct Hofmeister series in terms of latexes stability in solution. In addition, López-León et al. have also shown the relevance of the solute hydrophobicity on determining ion-specific effects [29,30]. Zhang et al. have studied the ion specific effects onto the solubility of poly(N-isopropylacrilamide), PNIPAN [31], chosen as a non-charged polymer model to study the specificity of amide-ion interactions, presumably present in proteins. They have found that the solubility of PNIPAN with the addition of salts follows a direct Hofmeister series (i.e., $ClO_4^- > SCN^- > Br^- > NO_3^- > Cl^-$) and interpreted their observation in terms of different types of interactions between the anions and the PNIPAN amide (—NH—CO—) groups. Similar results have been obtained more recently by López-León et al. with thermally sensitive charged PNIPAM microgels [31].

Naturally occurring proteins are complex polymers composed of different amino acids which can be characterized by having different types of side groups. Some amino acids like Lys, Hys, Arg, gain positive charges, while some others as Asp and Glu, get a negative net charge after ionization. Therefore, in proteins both electrostatic and dipole-dipole type of interactions with ions might be responsible of their stability in solutions. Fox et al. [32] have rationalized that two mechanism might be responsible of the uncertainty concerning the interaction between proteins and ions in aqueous solution: (i) what attributes of ions and proteins determine the binding sites and the degree of interactions; and (ii) how do ions alter the structure of solvating water molecules.

Here, with the aim of elucidating ions binding sites on a defined synthetic macromolecular structure, a synthetic catiomeric polyurethane (PU$^+$) has been design, synthetized and studied as a model macromolecule. The polyurethane has been designed to have a high density of urethane groups (—NH—CO—O—) in parallel to amide groups in proteins. In addition, the urethane groups are intercalated with one alkyl-ammonium group, which has a positive charge in solution, as certain proteins do at pH values below their isoelectric point. The nature of the interactions between the PU and different salts has been studied by Fourier transformed infrared spectroscopy (FTIR) and nuclear magnetic resonance (^{13}C NMR).

2. Materials and methods

2.1. Materials

The following chemicals were used in this investigation: Hexamethylene diisocyanate, HDI (Sigma-Aldrich), N-methyldiethanolamine, MDEA (Riedel de Haën), Ethylene glycol, EG (Panreac) LiBr (Panreac), LiCl (Panreac), CH$_3$COONa (Fluka), H$_2$CO$_3$Na (Probus), NaH$_2$PO$_4$ (Probus), NaF (Panreac), NaCl (Panreac), NaBr (Panreac), NaI (Probus), NaSCN (Pantreac), NaClO$_4$ (Panreac), NaNO$_3$ (Probus). The HDI was dried at 110 °C under vacuum for 14 h before being used. MDEA and EG were dried (before use) into a rotary vapor for 2 h at 80 °C, with reduced pressure and constant air flux. Salts were used as received, except LiCl and LiBr, which were dried under vacuum at 100 °C, before being used for the preparation of solutions.

2.2. Polyurethane synthesis

Here two polyurethanes were synthesized. One consisted in a homopolymer (**PU$_0$**) synthesized by the stoichometrical reaction between 1,6-hexamethylene diisocyanate (HDI) and N-methyl diethanol amine (MDEA). For this 3.516 g of HDI were introduced into a vial and 2.463 g of MDEA were added dropwise while stirring vigorously at room temperature. The second polyurethane (**PU$_{3/2}$**) was a copolymer based on HDI, MDEA and ethylene glycol