Ph.D. Thesis Review Report

Thesis by: Raju Lunkad
Department of Physical and Macromolecular Chemistry (Faculty of Science, Charles University, Prague)
Title: “The Role of Charge Regulation in Weak Polyampholytes”

This thesis represents a significant contribution to the understanding of the ionization processes taking place in polyelectrolytes and, in particular, in polyampholytes and polyzwitterions. The work is very comprehensive since the problem is jointly addressed from the theoretical, experimental and computational standpoints.

Although much research has been devoted to the study of ionization properties of polycations or polyanions, rigorous studies of flexible polyampholytes are scarce. The followed approach is based on a coarse graining approach in which several atoms are grouped into beads linked by spring-like bonds. This allows for much shorter computational effort than that demanded by full-atom simulations. Despite the simplicity of the model, the agreement between simulations and experiment is generally remarkable.

The work is structured in seven chapters. In chapter one the motivation, methodology and main objectives of the work are presented. Chapter two represents a rigorous summary of the main concepts concerning protonation equilibria taking place in polyacids, polybases and polyampholytes. This chapter could be useful as an introductory text for researchers non familiar to the field. In general, the references throughout the work are very up-to-date, which is acknowledged by the reader.

In chapter three the simulation methodology and the dynamic equations are presented. The potentials used are of the type Weeks-Chandler-Andersen (WCA) and Lennar Jones (LJ) potentials. The following questions arise:

Q1) In page 27, the values used for the parameters in the potentials are reported. It is said “All simulations were carried out by setting $\varepsilon_{LJ} = 1k_BT$ and $\sigma = 0.355$ nm.” Which is the physical justification for these values? Have other possible values been tested? In the case of the LJ potential, is there an attractive interaction at short distances? If this is the case, could it produce crossed-linking effects during the simulation?

Charter four is devoted to charge regulation of single polyampholytes containing two ionisable groups, one of them acid and the other one basic. The effect of two key parameters is investigated: the difference between pKa between acid and basic group (ΔpKa) and the distribution of the aminoacids along the chain: bi-block or alternating. The investigation of this point has produced one publication (MSDE 6, no. 2 (2021): 122-131). The methods used are able to predict the experimental ionization curves obtained by potentiometry and NMR:

Q2) In the analysis of NMR data it is assumed that the chemical shift of a group only depends on the ionization degree of the group itself, not of the ionization degree of its neighbours. In this case, the chemical shift of a group would linearly depend on the degree of ionization of the other groups too. Has this possibility been considered? Is there some argument to omit the inter-group interaction in the NMR analysis? Is this assumption common in the peptide literature?

Chapter four continues with a discussion about the consequences of charge regulation on the peptides conformational structure, which has rendered a second publication (*Polymers* 13, no. 2 (2021): 214). These interesting results are included in the publication but they are not too much discussed in the thesis itself. In particular, Fig. 7 of the paper shows that the conformational properties of diblock and alternating polymers are rather different, despite the similarity of their ionization properties (Figs. 5 and 6). Near the IP the end-to-end distance of the diblock structure is significantly smaller than the one of the alternating peptide, especially for the case ΔpK_A=6.89.

Q3) Which is the mechanism for the observed differences in the polymer end-to-end distances between the diblock and the alternating structure?

Chapter five is a particularly innovative part of the thesis. It addresses the adsorption of polypeptides (PP) (of the same type of the previous chapters) onto a poly-cation (PC), paying special attention to the adsorption in the "wrong side" of the isoelectric point (WSIP), i.e., when both PP and PC have the same nominal charge. This study has produced a publication (*JACS*, 144, no. 4 (2022): 1813-1825), and it focus on the two possible mechanism for WSIP: charge patchiness (asymmetric distribution of chares) and charge regulation. The two key parameters are ΔpK_A and the dipolar moment. A small ΔpK_A value indicates large CR and therefore the capability of the PP to invert the charge near the PC. Large dipolar moments are signature of patchiness and therefore the PP is able to link the PC through the negative side, without charge inversion. The main result is summarized in the figure in page 53. All the concepts presented in the previous chapters (one to four) are used, which gives to the work a clear argumentative unity.

Q4) In Fig. 5.5 the density profiles of peptides around the PC are shown. Which procedure has been used to calculate them: probability of the centre of mass position, monomer concentration?

If I am not mistaken, there are two writing errors, which by no means affect the quality of the results. In page 41, point i) bellow the table 4.5 it is said $\Delta pK_A >> 0$ for the case ΔpK_A=1.75 and in point ii) $\Delta pK_A \approx 0$ for ΔpK_A=6.89. A similar confusion seems to be in page 67 (chapter 5) for the case $\Delta pK_A \approx 0$ where it is written: "This confirms that the adsorption of the diblock peptide to the star on the wrong side of the isoelectric point is *not attributed to charge inversion* but rather to the presence of charge patches…" which is in contradiction with the conclusive paragraph in page 68: "Therefore, we conclude that the adsorption of the peptide to the star on the wrong side of the isoelectric point is *due to only charge regulation when ΔpKA is small*, while it is due to only charge patchiness when Δp*K*A is large." If I am correct in my appreciations, an *erratum* is recommended.

Finally, in chapter six the same simulation methodology is applied to poly-zwitterions. Up to my knowledge, this is a rather novel investigation. Three systems are analysed (PDAGA, with two acidic and one basic ionisable groups per monomer) and PDha and PImAA (both with one acidic and one basic ionisable groups per monomer). Simulations are compared to experiments coming from two different techniques (potentiometry and zeta-potential). For PDAGA and PDha the agreement of simulations and experiments are reasonable. However, this is not the case of PImAA. A question arises about the potentiometric experiments:

Q5) In Fig. 6.5a, the charges obtained by potentiometry for PDAGA at large pH-values (pH>12) are smaller than -2. But the most negative charge which a PDAGA monomer can achieve is -2. Which is the reason for this apparent inconstancy? The same concern is in the Figure 6.5c for PImAA, where points with charges less than -1 are shown (in this case, the minimum possible charge is -1)

In summary, this is an interesting contribution which provides new guides and insights to the field of charge regulation in soft matter systems. Furthermore, four manuscripts, published in very high quality peer-reviewed journals, are included at the end. Undoubtedly, the results and the innovative solutions presented will be acknowledged by other research laboratories working in this field. In conclusion, this work by far fulfils the requirements of a doctoral degree.

Josep Lluís Garcés González
Assistant professor
Department of Chemistry, Physics and Environmental and Soil Sciences
Lleida University

20-6-2023