

Ingo Hoffmann  
Institut Laue-Langevin  
71 avenue des Martyrs  
38000 Grenoble, France  
tel: +33(0)4 76 20 72 66  
email: hoffmann@ill.fr

---

Ingo Hoffmann, Institut Laue-Langevin , 71 avenue des Martyrs , 38000 Grenoble, France , tel: +33(0)4 76 20 72 66 , email: hoffmann@ill.fr

Mgr. Valerie Havrdová  
Charles University in Prague  
Přírodovědecká fakulta UK  
Studijní oddělení  
Albertov 6, 128 43 Praha 2, Czech Republic

February 15, 2021

**Review for the Submitted Thesis “Multidimensional characterization of polyelectrolytes and interpolyelectrolyte complexes in aqueous solutions” by Msc. Anastasiia Murmiliuka**

Dear Prof. Obšil,

I received a copy of the doctoral thesis “Multidimensional characterization of polyelectrolytes and interpolyelectrolyte complexes in aqueous solutions” submitted by Msc. Anastasiia Murmiliuk.

It deals with the characterisation and regulation of the charge state of polyelectrolyte copolymers as much as with the self-aggregation of other block-copolymers with at least one charged block. Self-aggregation is triggered either by the addition of an oppositely charged polyelectrolyte or by hydrophobic interactions, if at least one block in the copolymer is hydrophobic. Furthermore, the uptake and release properties of the obtained materials are studied.

A number of different characterisation methods is used, including calorimetry, microscopy, fluorescence spectroscopy, nuclear magnetic resonance as much as different scattering and electrochemical methods.

The results presented in the thesis have already resulted in 6 publications in respected journals, in 3 of which the candidate is the first author. She also is the second author of two more publications which are not part of the thesis.

After a short description of the aim of the study, section 2 of the thesis comprehensively presents the current state of the art in the field. Sections 3 and 4 introduce the characterisation methods and the different polyelectrolytes which were used in the thesis. Section 5 defines the aims of the research presented in the thesis. Sections 6 and 7 list the candidate’s publications and presentations during the time of her thesis.

Finally, sections 8 and 9 give an overview and summarise the obtained results.

The first paper deals with charge regulation in two short ampholytic model peptides, namely Glu<sub>5</sub>-His<sub>5</sub> and Lys<sub>5</sub>-Asp<sub>5</sub> both peptides each contain five acidic and five basic aminoacids. Depending on the difference in  $pK_A$  between the two amino acids in the peptide, either a smooth transition of the overall charge with pH or two distinct inflection points are observed. Coarse grained simulations reproduce the experimental results and such simulations may be used to predict the charge response of peptides.

In the second paper a fluorophore, umbeliferone, with pH dependent fluorescence is attached to a polymethacrylic acid chain and its degree of ionisation as a function of pH is recorded and compared to MD simulations. It is shown that the observed shifts are not exclusively due to changes in local pH but the distribution of counterions around the fluorophore also plays a role and the term “local pH” should be used with caution.

The third paper deals with fluorescent properties of novel poly(3-hexylthiophene) based fluorescent polyelectrolytes with pendant tributylphosphonium and and tributylammonium groups. The fluorescence of these new compounds is sensitive to the solvent and quenching with ferrocyanides. Therefore, they may have applications as sensors.

In the fourth paper, the PMAA polyelectrolyte labeled with umbeliferone that was already used in the second paper is mixed with an oppositely charged double hydrophilic block copolymer poly[3,5-bis(trimethyl ammoniummethyl)-4-hydroxystyrene iodide]-block-poly(ethylene oxide) QNPHOS-PEO which also acts as a quencher. It is shown that core-shell aggregates are formed and interestingly, fluorescence measurements show that the core is in fact dynamic.

In the fifth paper, a hydrophobic-cationic block copolymer poly(lauryl acrylate)-block-poly(trimethylammonioethyl acrylate) (PLA-QPDMAEA) is complexed with hydrophilic-anionic poly(ethylene oxide)-block-poly(methacrylic acid) (PEO-PMAA). Combining hydrophobic and electrostatic self-assembly multilayered materials with a central hydrophobic layer, surrounded on both sides by an IPEC layer and an outermost PEO layer. These multilayers are forming closed structures reminiscent of vesicles.

Lastly, in the sixth paper the self aggregation of a triblock copolymer poly[quaternized 2-(dimethylamino)ethyl methacrylate-b-lauryl methacrylate-b-(oligo ethylene glycol)methacrylate] (QPDMAEMA-b-PLMA-b-POEGMA) consisting of a cationic (PDM-AEMA), a hydrophobic (PLMA) and a neutral hydrophilic (POEGMA) block, is studied and the formation of core shell aggregates with a mixed PDMAEME-POEGMA corona is observed. Adding insulin, dynamic clusters are formed, which renders these complexes potential delivery systems for insulin.

The last part summarises the results from this large variety of different systems.

The thesis contains unique and important results. I recommend acceptance of the thesis.

Nevertheless, I will have a few questions to the doctorand.

1. p. 20, eq. (1): Could the candidate comment on the origin of this equation and why specific values of the packing parameter mark the transition from one shape to another?

2. p. 29, eq. 24: This is not directly a matter of the energy of the light. Could the candidate comment on the origin of the equation?
3. In fig. 5 of the second paper, the bulk value of the counterion concentration seems to be twice the salt concentration. Why is that?
4. p. 63 l. 15ff: It is stated that quenching in these systems is mostly due to electrostatic interaction with multivalent species but iodide is charged, as well. Could the candidate comment on that?
5. In the fourth paper it is found by fluorescence quenching that the IPEC core is dynamic. Could the candidate comment on the time scale on which these measurements imply that the complexes are dynamic?
6. In the fifth paper, based on TEM measurements, it is concluded that a mixture of spherical and rodlike particles is present. How can a spherical particle be differentiated from a rodlike particle with its long axis normal to the plane?
7. In the fifth paper, some SANS curves with fits to rather complex models (fig. 8 of the paper) are presented. How were these fits parametrised and constrained? In the supporting information to the same paper, there is a table (tbl. 2) with scattering length densities (the units of which are not provided). Assuming they are in  $\text{\AA}^{-2}$  they are all essentially the value of  $\text{D}_2\text{O}$ . While this may make sense to some point for the PEO and IPEC layer, the hydrophobic layer should have a significantly different value. Could the candidate please comment on this.
8. In the sixth paper, in scheme 1, the chemical structure of QDPAEMA-b-PLMA-b-POEGMA is shown with a terminating  $\text{C}_{12}$ . Could the candidate comment on the potential impact of this group on the observed structures?

The subsection on scattering methods contains a number of mistakes. This is forgivable given that the candidate's work is more focused on electrochemistry and fluorescence but I would suggest nevertheless to correct these mistakes in an appendix:

1. If eq. 7 is supposed to hold for light scattering, this should read  $\frac{4\pi n}{\lambda} \sin(\theta/2)$
2. Eq. 8 does not hold for neutron scattering.
3. The applicability of eq. 9 does not depend on a homogeneous SLD. However, it is strictly valid only for monodisperse, spherical particles.
4.  $\tau$  in eq. 10 is not some relaxation time but the correlation time.
5. p. 37 l. 20ff: The Porod region is the high-q end of the scattering of a particle. If you insist on a name, the mid-q region could be called fractal region.
6. p. 38 l. 1f: This is specific to polymers.

7. p. 38 eq. 23: This should be  $N(V\Delta\rho)^2P(q)$ . I would also suggest to discuss the intensity prefactor  $K$  right in the beginning of the scattering section as it is of central importance.

In addition to that, there are a few minor typesetting mistakes:

- p. 17 l. 8: “prospective” should probably read “perspective”
- p. 21 fig. 2: chemical structure of PSS is wrong
- p. 27 eq. 3:  $E$  in the equation but  $V$  in the text
- p. 28 l. 12: “nuclei” should probably read “nucleus”
- p. 29 eq. 5: last term should read  $\exp\left(-\frac{h\nu}{k_B T}\right)$
- p. 29 l. 12: “shields” should read “shield”
- p. 39 eq. 24 should read  $d = \frac{\lambda}{n \sin \alpha}$
- p. 44 l. 6: “adsorb” should read “absorb”
- p. 46 l. 12: unitm
- p. 50 fig. 12: The chemical structure is wrong. The C6-spacers are missing.
- p. 65 l. 4: “that, that”

I will join the jury to challenge the doctorand regarding her work.

Sincerely yours,

Dr. Ingo Hoffmann