Experimental report

28/02/2017

Proposal: 9-13-660 **Council:** 4/2016

Title: SANS study of a pH-sensitive Polymer Interacting with a Model Lipid Membrane

Research area: Physics

This proposal is a new proposal

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Samples: DOPC
PP-50

Instrument	Requested days	Allocated days	From	To
D11	3	3	10/09/2016	13/09/2016

Abstract:

In order to improve the therapeutic effect of a drug, a great deal of research has been dedicated to developing drug delivery systems that are meant to facilitate the transport of a pharmaceutical compound in the human body. To this end, we have been studying a pH-sensitive polymer, PP-50, and how it interacts with model lipid membranes. It has been hypothesized that PP-50 has two modes of action: at pH = 7.4, it circulates in the extracellular medium, protecting its cargo (a drug molecule), but when pH drops to 5.5, a value characteristic for endosomes, PP-50 changes its conformation and becomes a membrane-disruptive agent, which leads to the drug release within a cell. Unfortunately, the detailed understanding of such triggered response, as well as the molecular mechanism of PP50-lipid membrane interactions remains elusive, which hinders further its further development as a drug delivery system. Using SANS, we aim to obtain a comprehensive picture of molecular interactions of PP50 with liposomes, which would enable rational design of novel polymer with enhanced membrane-perturbing capabilities.

INSTITUT MAX VON LAUE - PAUL LANGEVIN (ILL)

EXPERIMENTAL REPORT

EXPERIMENT N° **9-13-660**

INSTRUMENT **D11**

DATES OF EXPERIMENT 10-13/09/2016

TITLE **Neutron Reflectometry of a pH-sensitive Polymer Interacting with a Model Lipid Membrane**

EXPERIMENTAL TEAM
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Date of report: 25/02/2017

Motivation

PP50 is a promising, pH sensitive polymer that changes its physicochemical properties upon external stimuli (e.g. pH change). It has been hypothesized that PP50 has two modes of action – in a typical, physiological pH of 7.4, it circulates in the extracellular medium, protecting its cargo (e.g. a hypothetical drug molecule). When pH drops to 5.5, a value characteristic for endosomes, PP50 changes its conformation and becomes a membrane-disruptive agent, which leads to drug release within a cell.[1,2] Thus, PP50 is an attractive example of a stimuli-responsive drug delivery system, designed to perform its role under strictly defined conditions. Our preliminary results (Isothermal Titration Calorimetry (ITC), Confocal Microscopy and Small-angle X-ray Sattering (SAXS)) suggest that at pH=7.0 PP50 does not bind to the lipid membrane, whereas at pH=5.0 the polymer interacts strongly with the bilayer. SANS technique allows us to understand in detail this process, especially in the pH near the p*Ka* of the polymer.

Measurements

Translocation of PP50 though model lipid bilayer was examined at three different liposomal systems, namely *a)* DOPC in D_2O, *b)* DOPC/Cholesterol (8/2) in D_2O and *c)* POPC-d_{31} in H_2O; at various pH conditions. LUVs were prepared by simple extrusion technique, resulting in the final concentration of 1mg/ml. To test the concentration dependence, two different polymer concentration (1mg/ml and 3mg/ml) were used. All samples were left for 1h incubation prior measurements.
Moreover, we measured pure polymer solution at different pH. All measurements were performed at room temperature. The scattering curves of PP50 and LUVs/PP50 systems are shown in Figure 1.

Results

First, we followed changes in polymer's conformation upon variation of pH (Figure 1*a*). Raw data were fitted with Gaussian model using SasView software.[3] The highest radius of gyration, R_g=121Å , is observed for pH corresponding to p*Ka* (i.e. 6.5) of polymer, while for pH 7.0 R_g shows the lowest value of 56Å.
The scattering curves of LUVs/PP50 mixture are presented in Figure 1*b,d,g.* There is a clear difference in the scattering behaviour depending on the presence of the polymer and the pH value. We started our analysis with a simple evaluation directly from the scattering curve, which gives the membrane thickness and vesicles diameter. The Guinier radius ($R_{guinier}$)[4] was extracted from Guinier plots (Figure 1*c,e,h*) at low *q*-range ($0.000012 < Q^2 < 0.00004$). Comparing calculated $R_{guinier}$ results of LUVs w/ and w/o polymer, we conclude that in all tested systems at low pH (i.e. 5.0), there is decrease in vesicle diameter (not observed at physiological pH).

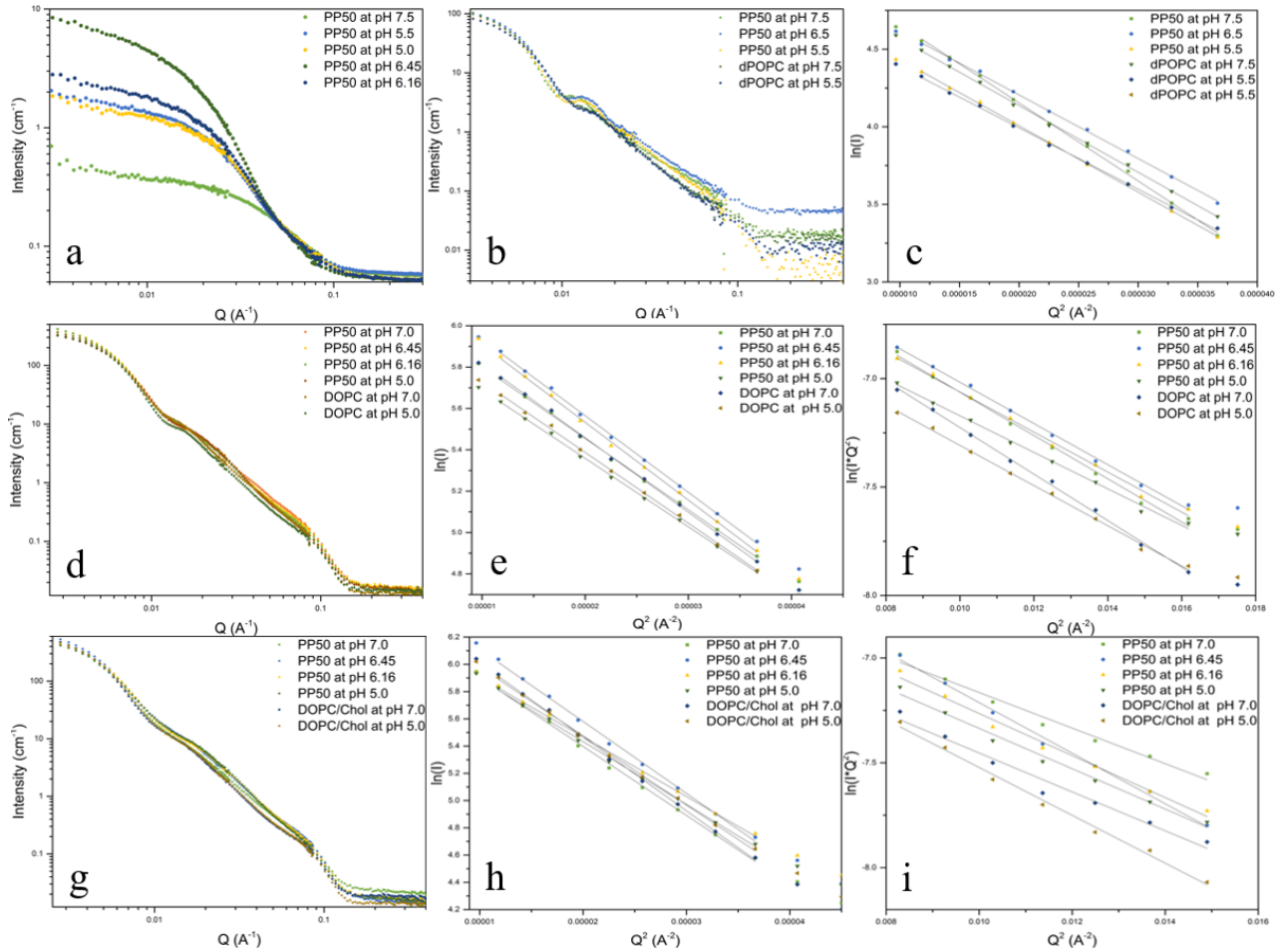

Figure 1. *SANS data of polymer only and PP50/LUVs interaction and at vary pH condition: a) pure PP50 in D_2O; b) raw SANS data of dPOPC/ PP50 mixture (in H_2O) with corresponding c) Guinier plot; d) raw data of DOPC/PP50 mixture (in D_2O) together with e) Guinier plot and f) Kratky-Porod analysis; g) raw data of DOPC/Chol (8/2) mixed with PP50 (in D_2O) h) Guinier plot of DOPC/Chol/PP50 system, together with f) Kratky-Porod plot. To simplify, only 3mg/mL concentration of polymer is presented here.*

We also evaluated the membrane thickness using *Kratky-Porod* approximation[5] (Figure 1*f,i*) in the intermediate q-regime with relation $d^2 = 12R_{kp}^2$. As expected, more pronounced changes in membrane thickness are observed at pH corresponding to p*Ka* of the polymer. Surprisingly, DOPC/Chol system seems to be more affected by polymer's presence, compared to the pure DOPC system.

The experimental setup is quite complicated (unsaturated lipid used and no accumulation of the polymer at the membrane surface), therefore care has to be exercised with choosing the appropriate statistical model. Currently we are working to implement *three strip vesicle* model in order to obtain more details about membrane parameters affected by PP50 (hydration, hydrophobic core of membrane, polymer-induced changes in lipid headgroup), as well as the effect of cholesterol on polymer translocation.

<u>Additionally</u>

To fully exploit the awarded beam time, samples containing DPPC membranes with Polystyrene (PS) nanoparticle were measured, alongside the PP50 samples preparation.

Motivation: Recently, serious concerns have been raised due to the growing plastic waste (mainly polystyrene) caused by the development of nanotechnologies. Although previous studies have shown that nanoscopic scale polymers can accumulate in cells, particularly in cellular membranes,[6] the effects on cell metabolism is far from being understood, and the fundamental mechanisms of their interaction with the different cell components are yet to be addressed.

Previously, we used Laser Scanning Confocal Microscopy together with Calorimetry measurements and Fluorimeter to reveal that presence of polystyrene disrupts the lipid bilayer phase transition by ordering the lipid tails and preventing full melting above transition temperature. Small angle neutron scattering (SANS) is an ideal experimental technique to complement our biophysical investigations and quantify the changes of the internal structure of a DPPC bilayer in the presence of PS.

Measurements: Accumulation of PS within lipid membrane was examined in DPPC-vesicles above and below transition temperature (25°C and 50°C). Samples were prepared by lipid film hydration followed by extrusion. We tested two different molar fraction of PS i.e 10% and 30%.

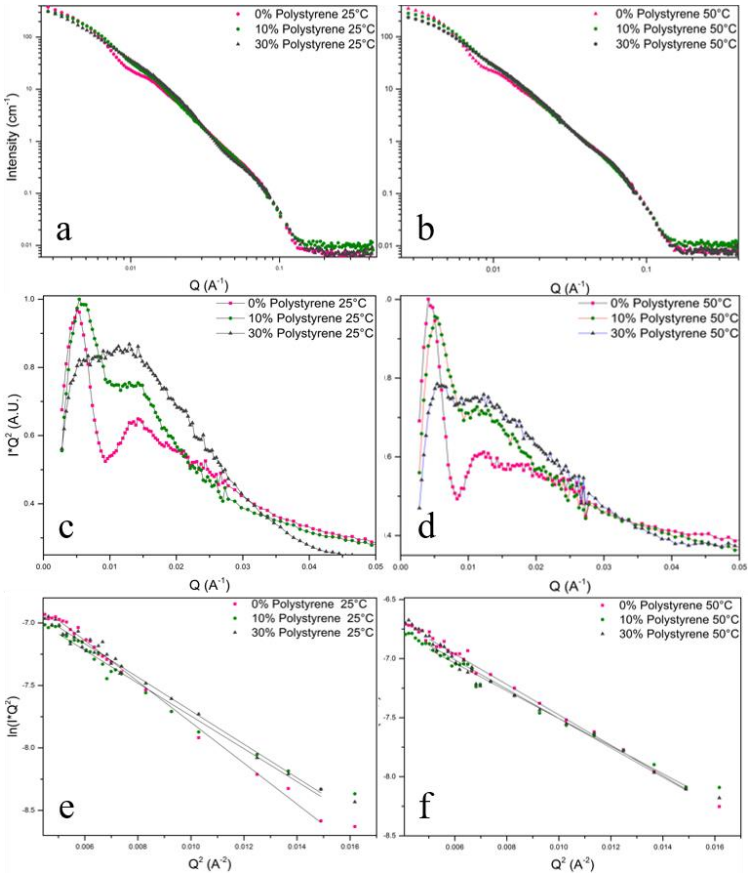

Figure 2. *SANS experiments of DPPC with incorporated polystyrene (10% and 30%): a) DPPC/PS in D_2O at 25°C together with c) Kratky plot and e) Kratky-Porod plot; b) DPPC/PS in D_2O at 50°C with d) Kratky analysis and f) Kratky-Porod plot.*

Results: Raw SANS data are presented in Figure 2*a,b.* The vesicle size i.e. Guinier radius was obtained from scattering vector q_{max} at which Kratky plot (Figure 2*c,d*) shows a maximum ($R_g = (3^{1}/_{2})/q_{max}$).[7] With increasing PS content the vesicle radius is significantly reduced.
Subsequently, SANS intensity I(q) were analyzed in range $0.005<Q^2<0.016$ using Kratky-Porod plot (Figure 2*e,f*) in order to obtain the bilayer thickness. Our analysis shows that increasing polystyrene content influences the bilayer thickness, from 49 Å for pure DPPC up to 64 Å for 30% PS DPPC in fluid phase. In case of gel phase only 30% PS induce changes in bilayer radius.
Using *strip*-function model we explored which part of membrane is occupied by PS and how its presence change hydration of membrane interface. These results are in good agreement with our data form Confocal Microscopy and in great deal helped to understand our Calorimetry results which show partial inhibition of lipid phase transition.

Results of this part of SANS experiment are currently being incorporated into a manuscript.

References

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