

Report of the Summer Student Program DESY 2009

Joanna Kwiatek

Jagiellonian University, Kraków

Supervisor: Dr. Sérgio S. Funari

Abstract

This report describes my work during the Summer Student Program 2009. I worked, at the A2 - Small Angle X-ray Scattering (SAXS) beam line at the HASYLAB in DESY. I measured POPE lipids with molecules (quinone and hydroquinone) in the buffer which had got different pH. The main expectation was to determine the structure of samples.

Experimental setup

The Small angle X-ray scattering - A2 beam line presented on Fig. 1 is located at the DORIS III source at HASYLAB in DESY. The beam line provides high flux of photons and is an ideal instrument for small (**SAXS**) and wide angle X-ray scattering (**WAXS**) experiments dedicated for materials such as polymers, lipids, muscles and colloids analysis. The results of performed experiments at A2 beam line provide information on structure of the analyzed materials.

Capillaries with the sample are placed into a holder, which can be moved in horizontal and vertical direction. The temperature of the sample is controlled by a JUMO IMAGO 500 multi-channel process and program controller connected to a computer. The movement of the sample holder is also controlled by a software available at the beamline. Synchrotron radiation is provided by the DORIS III storage ring. The synchrotron radiation is scattering on the sample and is directed to a CCD camera. The detector gathers the signal which is transformed to the data.



Fig. 1. A2 @ DORIS III [1]

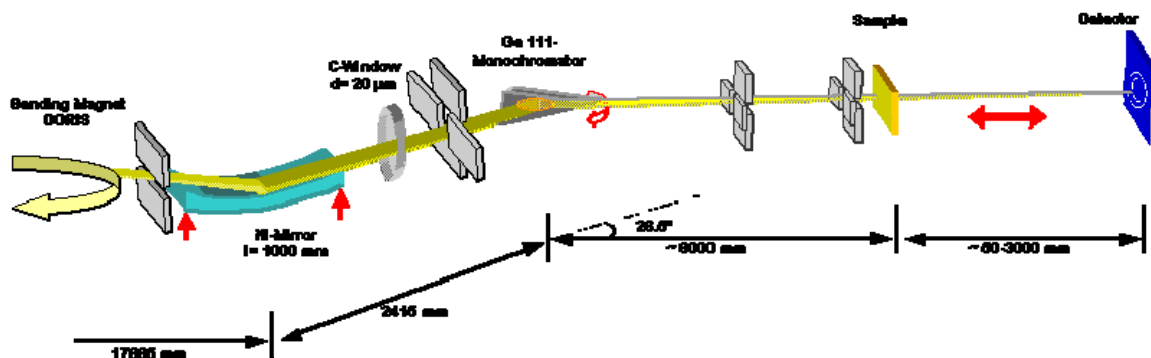


Fig2. Schematic diagram of the A2 beam line [2]

Types of crystals

In nature exists a lot of different types of crystals. They have got very characteristic parameters which can describe distance between planes . There are seven crystal systems:

- **triclinic** - usually not symmetrical from one side to the other,
- **monoclinic** -often forming prisms and double pyramids
- **orthorhombic** - forming rhombic prisms or dipyramids
- **tetragonal** -, forming double pyramids and prisms
- **trigonal** - possess a single 3-fold axis of rotation instead of the 6-fold axis of the hexagonal division
- **hexagonal** - six-sided prisms
- **cubic** - not always cube shaped

In order to find the crystal structure one has to find a ratio between plane distances. The received value has to be compared with the table value and then the right structure can be determined.

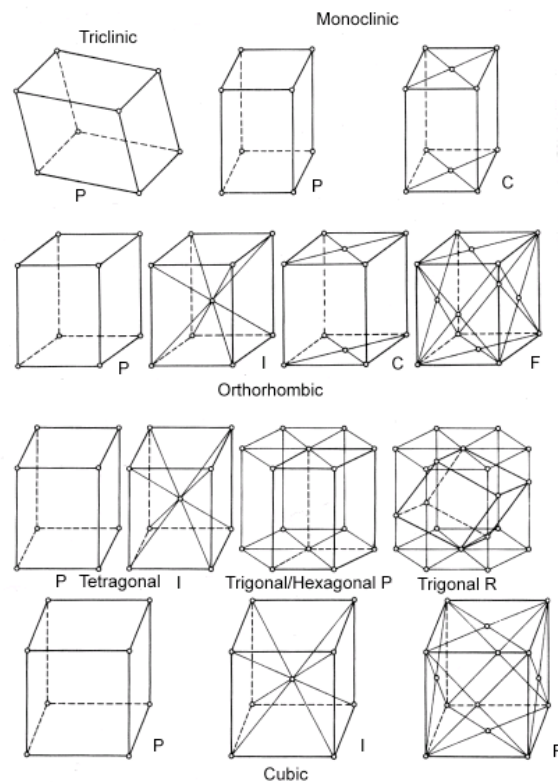


Fig3. Types of crystals

Sample preparation

During experiments two types of samples were measured: POPE / 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine/ & BATH /hydroquinone/ and POPE & BATQ /quinone/ which were have been mixed with buffer. The pH of solvents were: 3.5, 6.0, and 7.0.

The samples were prepared of the POPE which average mass was 6-8 mg and guest molecules /hydroquinone or quinon/. Afterwards ratio between components was calculated (100:1) and the components were dissolved. In the end samples were mixed with buffer.

Tab. 1 Presents sample numbers of different samples in dependence on pH concentration.

Type of the sample	pH 3.5	pH 6.0	pH 7.0
POPE+buffer+2.6BATH	1	2	3
POPE+buffer+2.6BATQ	4	5	6
POPE+buffer	8	9	10,11

Biological description of the samples

Lipids are a group of natural molecules which includes sterols, waxes, fats and others. They are amphiphilic, which means that lipids has got hydrophobic heads and hydrophilic carbon chain.

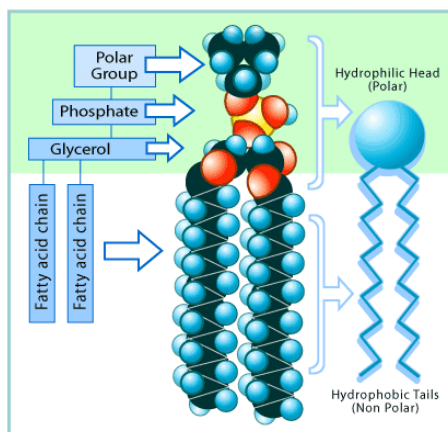


Fig4. Schema of the lipids [3]

The lipids of physiological importance for humans have several major functions:

1. They serve as structural components of biological membranes.
2. They provide energy reserves, predominantly in the form of triacylglycerols.

The experimental work was focused on the POPE / **1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine**/ which is unsaturated lipid. It has got two carbon chains and double bond between Carbons in the one of them.

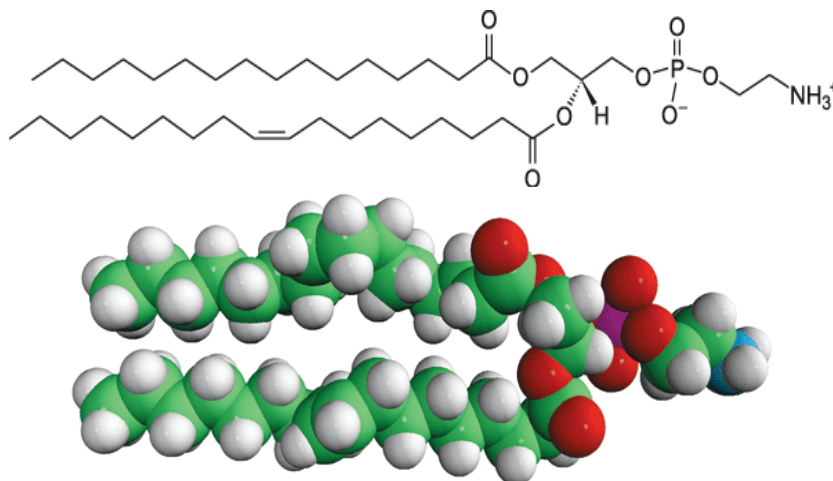


Fig5. Structure and model of POPE [4]

BATH is the hydroquinone molecule formed by the reduction of quinone. BATQ is the quinone molecule. Quinones are prepared by oxidation of the corresponding aromatic ring systems containing amino (NH_2) or hydroxyl (OH) groups on one or both of the carbon atoms being converted to the carbonyl group¹.

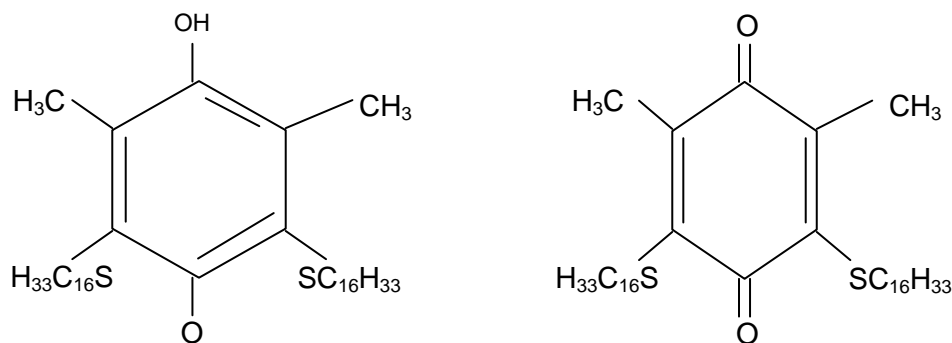


Fig6. Structure of BATH /left/ and BATQ /right/

During the Summer Students Program 2009 the interaction between lipids and hydroquinone/quinone has been measured.

Way of carry on measurements

Firstly, the experimental setup was calibrated by Standard RTT. With the knowledge of the reflection order, the channel number and the repeat distances (d) [nm], the channels could be converted into s -values ($s=1/d$) [nm^{-1}].

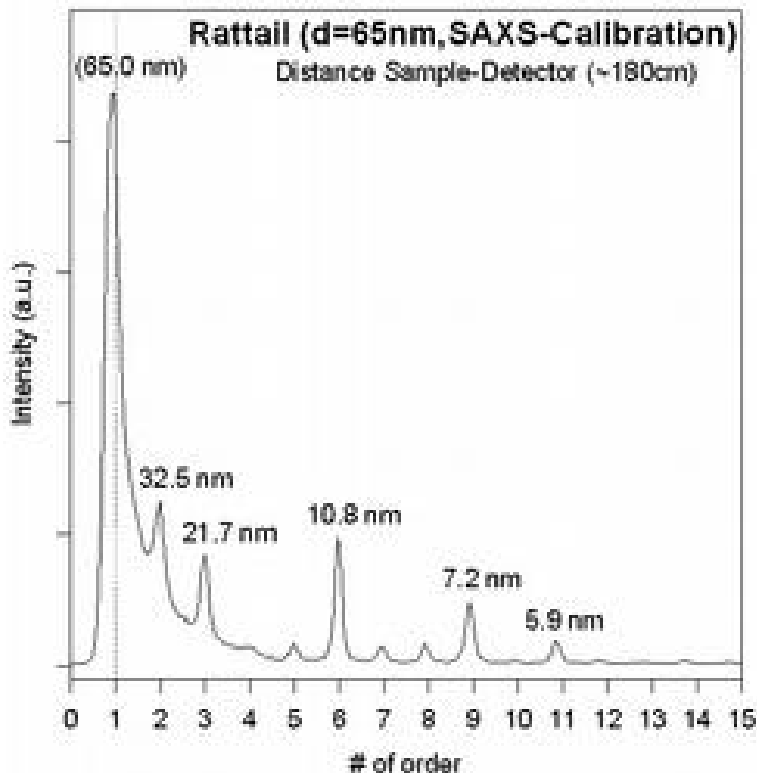


Fig7. Spectrum of RTT [5]

Each sample was measured twice or even more times. Firstly samples were heated from 25°C to 83°C, afterwards they were cooled down to 25°C. Some of them had changed their structure: on the bottom was orange or white layer, called orange or white rings. Those layers were heating and cooling.

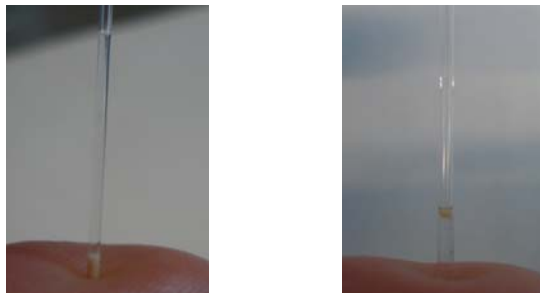


Fig8. Capillaries with the samples which had got white /left/ and orange /right/ rings

Subsequently samples were heated “degree to degree” – it means that temperature was raising one degree per minute with five minutes of break

The data which has been collected were analyzed with the use of the A2 Tool Software.

The data from Rat Tendon Tail was transformed by linear regression, for the dependence between S [1/nm] and pixels. Using those data the final version of the plot has been created: intensity as a function of S . All peaks which have been observed were analyzed: all peaks position were found to calculate the ratio between them. That ratio was compared with tables value in order to assign a type of the structure.

Data analysis

Sample 4 /POPE+BATQ+buffer pH3,5/

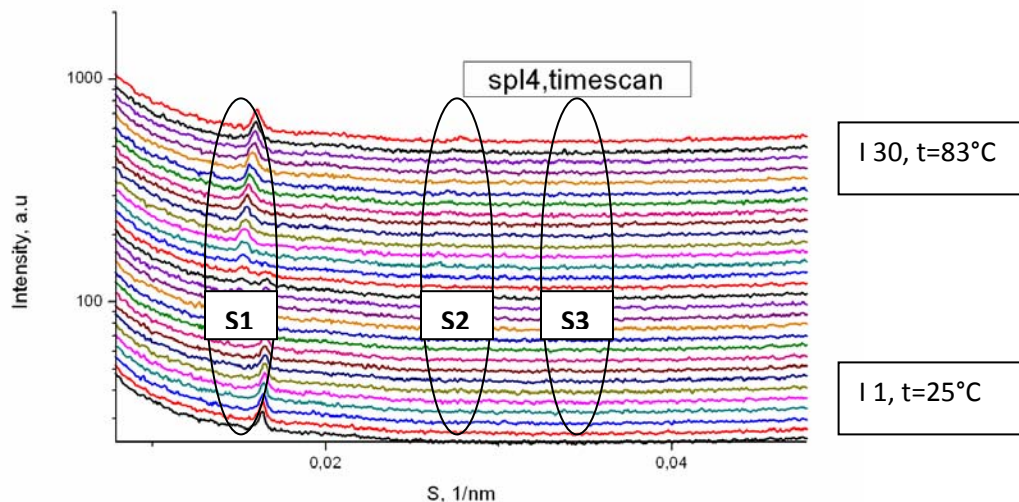
In the volume “Timescan” were observed three groups of peaks. Their position were:

$$S1:0.0163; S2:0.0282; S3:0.0313$$

Ratio between those group of peaks was :

$$S_1/S_1 = 1; S_2/S_1 = 1.73; S_3/S_1 = 1.93$$

which suite to the hexagonal structure.



Plot 1. Sample 4, time scan

Results of the measurements of the “orange ring” have been more difficult for interpretation.

Plot was divided into three parts. In the first one /P1/ three groups of peaks, describe hexagonal structure because, ratio between position was similar to that structure.

Peaks positions:

$$S_1: 0.0155; S_2: 0.027; S_3: 0.0316$$

Ratio:

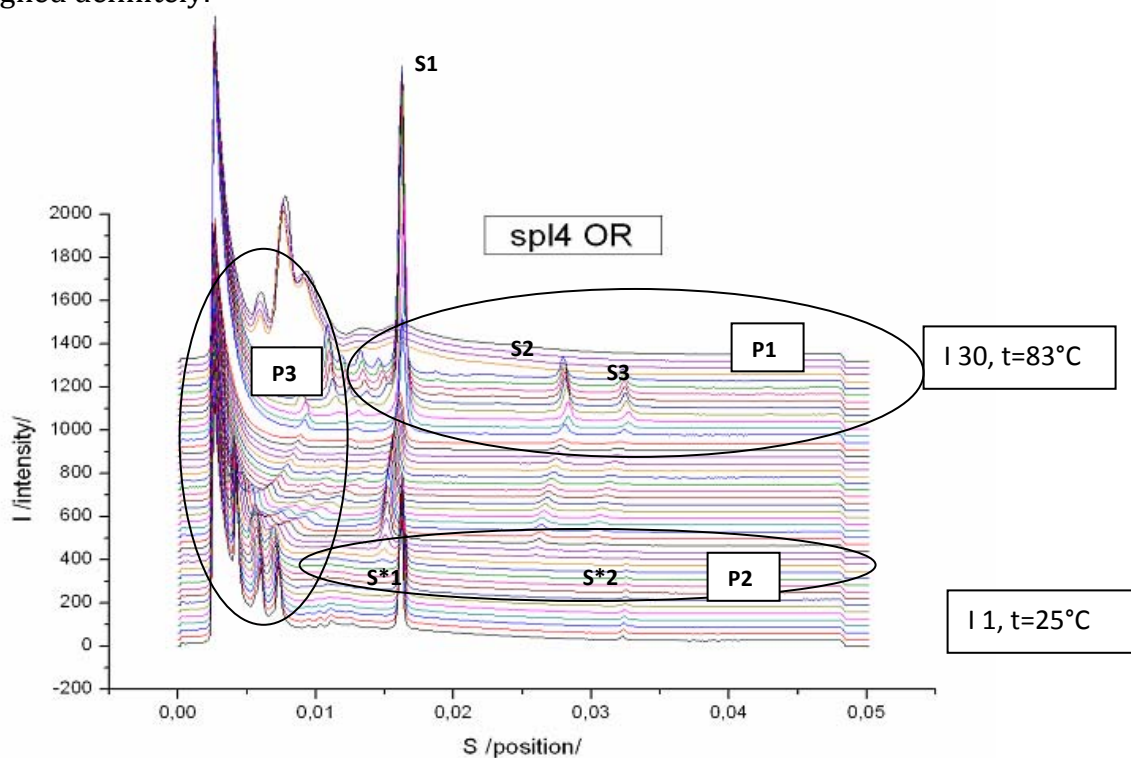
$$S_1/S_1 = 1; S_2/S_1 = 1.73; S_3/S_1 = 2.03$$

In the second part /P2/ there is lamellar structure, because there were two groups of very strong peaks.

Ratio between them is:

$$S^*_1/S^*_1 = 1; S^*_2/S^*_1 = 2$$

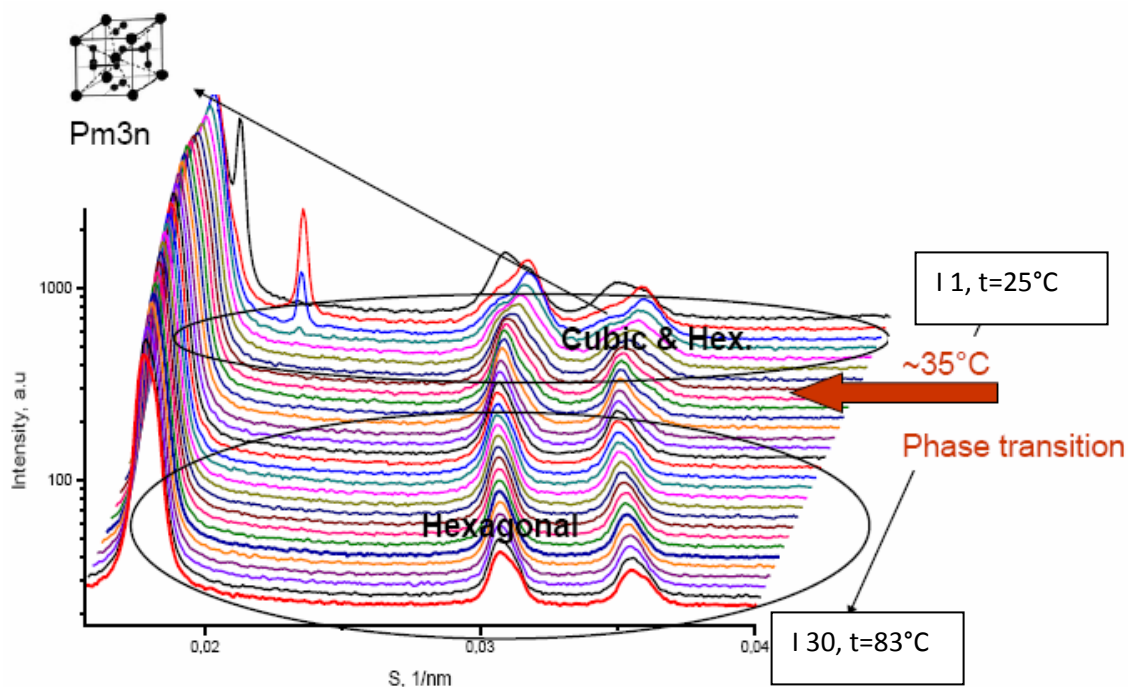
Third part /P3/ of the plot is the most difficult, because there were cubic structure but it was not assigned definitely.



Plot 2. Sample 4, orange ring

Sample 11/POPE+buffer pH7/

In the "orange ring" there were three different structures. Difference between them was in the peaks positions. In the temperature 25°C to 35°C two structure were observed :cubic and hexagonal. Afterwards phase transition were observed and structure changed to the hexagonal.



Plot 4. Sample 11, orange ring

Conclusions

Samples have changed their structure in the different pH. It depends on molecule which was used for the sample preparation. Hydroquinone change structure in the higher pH such as: 6.0, and 7.0. Quinones changed structure in the acid environment as pH = 3.5. In the sample 8, 9, 10, 11 where were only POPE and buffer structure were changed in the all pH.

Tab2. Results of measurements

Type of sample	pH 3.5	pH 6	pH 7
POPE+buffer+2.6BATH	1 No structure	2 or Q+LAM	3 or 1 ring
POPE+buffer+2.6BATQ	4 volume LAM->Hex 4 or Q+LAM->Q+Hex	5 No structure	6 No structure
POPE+buffer	8 or LAM+Q-> Hex	9 or LAM -> Hex	10,11 or Hex+Q->Hex.

References

- [1] http://hasylab.desy.de/facilities/doris_iii/beamlines/e5748/index_eng.html
 - [2] http://hasylab.desy.de/facilities/doris_iii/beamlines/a2/beamline/index_eng.html
 - [3] http://images.google.de/imgres?imgurl=http://www.bioteach.ubc.ca/Bio-industry/Inex/graphics/TypesOfLipidStructures.gif&imgrefurl=http://www.bioteach.ubc.ca/Bio-industry/Inex/&usg=__4NqlBteHqkN0v35sB1zmb2-nZBc=&h=250&w=449&sz=36&hl=de&start=100&um=1&tbnid=5QaAbP7e76l1HM:&tbnh=71&tbnw=127&prev=/images%3Fq%3Dlipid%2Bstructure%26ndsp%3D18%26hl%3Dde%26sa%3DN%26start%3D90%26um%3D1
 - [4] http://www.avantilipids.com/index.php?option=com_content&view=article&id=1542&Itemid=335&catnumber=110637
 - [5] http://hasylab.desy.de/facilities/doris_iii/beamlines/a2/calibration/saxs/index_eng.html
- ¹ <http://www.answers.com/topic/quinone>

Personal remarks

During Summer Student Program 2009 I have got a lot of experience. So if I had a chance once more to come to DESY for such a program I would do it. Unfortunately one could apply only once...

I would like to thank my supervisor Sérgio S. Funari for his care, help and patience. He gave me the opportunity to learn and practice in a very comfy and cordially atmosphere. I wish to thank him for all advices, gags during the beam time /specially for “diamonds” 😊/ and frankness. Thank to Maria Zhukova for her sense of humor 😊 .