EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Interaction of various forms of DNA with phospholipids monolayers	**Experiment number**: SC 1872
Beamline: ID10-B	**Date of experiment**: from: 10th February 06 to: 14th February 06	**Date of report**: 1st March 06
Shifts: 16	**Local contact(s)**: Leide Cavalcanti	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Giovanna Fragneto (ILL)*

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Report:

Aims of the experiment

The main aims of the present work were to gain an understanding of the interaction of phospholipids, in the form of a monolayer, with different forms of DNA as well as the effect of divalent cations, such as calcium, on this interaction. These interactions are of particular interest in the preparation of non-toxic gene delivery vectors (ie vectors prepared from phospholipid, DNA and $CaCl_2$). The aims of the study were achieved using X-ray specular reflection in combination with monolayer film techniques. These experiments complemented neutron reflectivity experiments previously performed at ISIS on the same systems. In the same study, a number of experiments were performed in which deuterated materials were substituted for protonated materials in an attempt to determine whether isotope effects exist that may affect the interpretation of the neutron reflectivity data.

Experiments performed

In our study DSPC was used as phospholipid; DSPC was the chosen lipid for the study because it forms stable vesicles. The monolayers formed by DSPC were first characterised at 5 surface pressures, namely 10, 20, 30, 40 and 50mN/m. The effect of the presence in the sub-phase of 20mM $CaCl_2$ (chosen as it is a physiologically relevant amount and also successfully mediates the interaction of DSPC with DNA) was studied at 4 of these surface pressures (namely 10, 20, 30, 40mN/m). The effect of (calf-thymus) DNA in the sub-phase present at 0.67mg/mL was similarly studied. Finally the effect of the presence of both calf-thymus DNA and 20mM $CaCl_2$ on the DSPC monolayer was determined.

The effect of isotopic substitution was investigated in the following manner. D_2O replaced H_2O in an experiment investigating the DSPC monolayer at 4 surface pressures. Then protonated and deuterated forms of chromosomal DNA (kindly provided in a collaboration with the biological deuteration laboratory of the ILL/EMBL) was investigated in the system containing both Ca^{++} and DNA in the sub-phase. This experiment also allowed us to see if the molecular weight of the DNA had any influence on the behavior of the monolayer.

Results

The following results have yet to be fully analysed as the experiments were only very recently performed. Figure 1 shows the reflectivity profiles obtained for DSPC at the various surface pressures examined in the presence of the various additives tested. It is clear from the Figure 1 that while there is little difference in the profiles obtained from DSPC alone to when either Ca^{++} or DNA was present there is a significant interaction of DNA with DSPC in the presence of Ca^{++} with a second, thicker layer being seen (no second layer is seen in any of the other systems tested).

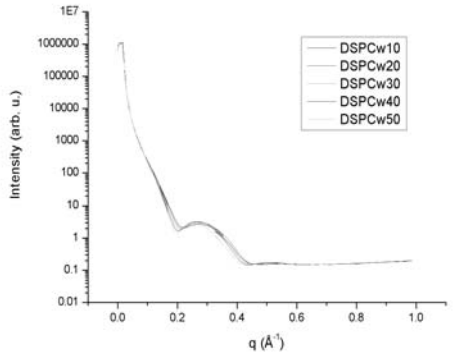

Figure 1a DSPC alone

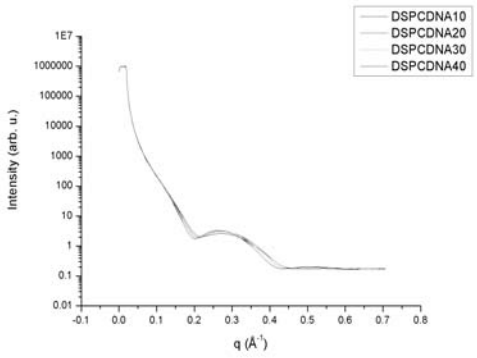

Figure 1b DSPC and DNA

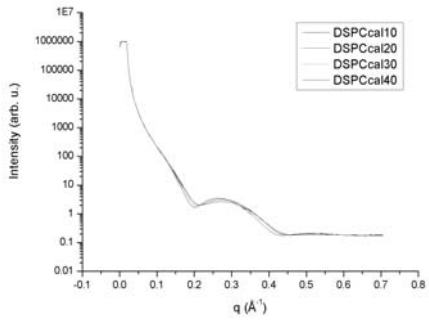

Figure 1c DSPC and Ca^{++}

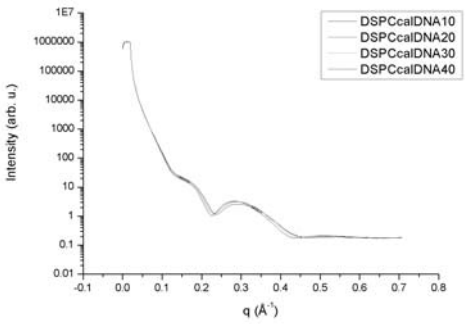

Figure 1d DSPC, DNA and Ca^{++}