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Use of DPI to Study Interactions of Anionic and Cationic Polymers with Liposomes

Introduction

Dual Polarisation Interferometry (DPI) is a major enabling tool for polymer chemists. Farfield's ***Ana*****Light**® DPI instrument embodies a truly quantitative analytical technique, rather than a simple 'mass sensor' response, providing absolute mass, thickness and refractive index (RI) measurements of soft surfaces [1]. Changes in these physical parameters can be directly related to the structure and behaviour of molecules at the measurement surface. Here we describe how DPI was used to examine the effects of two different polymers, dextran and polyethyleneimine (PEI), on negatively charged liposomes.

PEI is an organic polymer with a high density of amino groups which can be protonated. At physiological pH, this polycation has been shown to be very effective in binding DNA. Dextran is a complex, branched polysaccharide comprising numerous glucose subunits joined into chains of varying lengths. It is anionic at physiological pH. These experiments were performed in order to:

- Establish the amounts of cationic and anionic polymers binding to the negatively charged liposomes at pH7
- Measure structural changes caused in the liposomes on binding of polymers

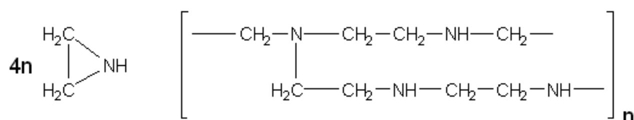

Figure 1: Molecular Structures of Ethyleneimine and Polyethyleneimine (PEI)

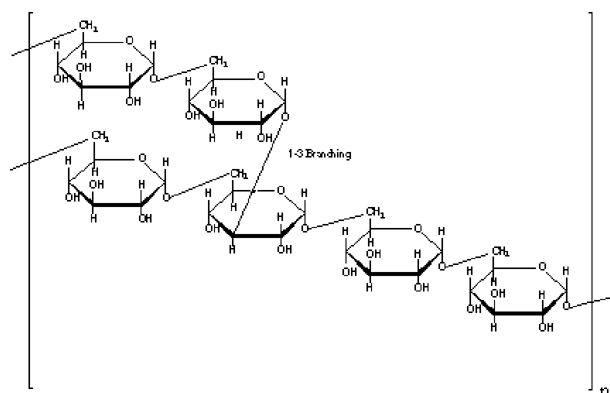

Figure 2: Molecular Structure of Dextran

Results and Discussion

Liposome Layer

The liposomes used in this study were formed from a mixture of phosphatidylserine, phosphatidylcholine and phosphatidylethanolamine and had an overall negative charge. Liposomes were adsorbed onto an unmodified ***Ana*****Chip™** surface to give high surface coverage (15ngmm^{-2}). The thickness of the adsorbed liposome layer was measured at 29nm.

Polymer Additions at pH7

Additions of dextran and PEI at pH7 were made to the liposome surface. **Figure 3** shows that at pH7, PEI bound very strongly to liposomes (3.4ngmm^{-2}), and remained bound on return to buffer. This is in contrast to dextran which showed negligible binding at the same pH. The structural information provided by DPI revealed that there was a large increase in thickness following addition of the PEI polymer, probably a result of:

- The cationic polymer binding to the outside of the liposomes, giving the liposome an extra layer
- Alteration of the liposome interaction with the ***Ana*****Chip™** surface

The negligible change in RI indicates that there was neither binding of the polymer to the ***Ana*****Chip™** surface, nor insertion into the liposome structure.

	Mass Change (ngmm^{-2})	Thickness Change (nm)	RI Change
Dextran	0.005	0.032	-0.0004
PEI	3.4	9.7	0.0017

Figure 3: Effects of Adding Polymers pH7 Values to an Immobilised Liposome Layer

Conclusions and Benefits

These experiments show how DPI can be applied to the study of lipid-polymer interactions. The ***Ana*****Light**® instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index and mass in a benchtop instrument.

The ***Ana*Light**® is an important enabling tool for polymer chemists, giving them the ability to:

- Clearly understand the molecular mechanisms involved in liposome-polymer interactions
- Study the effect of liposome composition in order to optimise applications benefits
- Avoid the limitations and ambiguities that are inherent in other techniques
- Obtain final results and analysis quickly and efficiently

Farfield gratefully acknowledges that these experiments were carried out in collaboration with Dr Alison Paul, School of Chemistry, Cardiff University, Cardiff, UK

For further applications information contact: applications@farfield-group.com

(1) Cross et al., ***Biosens. Bioelectron*** , **19** (2003) 383-390.