

Dynamic Measurements of Small Molecule Partitioning into C18 Membrane Mimics using Dual Polarisation Interferometry

Introduction

Dual Polarisation Interferometry (DPI) is an important and highly sensitive technique for determining interfacial properties. DPI can be used to provide a quantitative and sensitive measure of a range of processes such as thin film permeability, solute partitioning and changing of solvation state.

Surface immobilised C18 is a common hydrophobic surface used for a range of applications, often functioning as a biological *membrane mimic*. The behaviour of C18 under differing solvation conditions has a clearly understood impact on the system being investigated. Hydrophilic solvents collapse a typical C18 layer to a thickness of around 10Å whilst hydrophobic solvents solvate C18 up to a thickness of 40Å.

The hydrophobicity of solute molecules can also have a dramatic impact on the solvated structure and hence the performance of C18 surfaces. Better understanding of these effects will lead to design of higher performance organic surfaces and to better quantitation when such surfaces are used to measure retention in applications such as logP determination.

This application note describes the use of DPI to demonstrate the dynamic effects of solute molecules partitioning into thin C18 films through real time, quantitative measurement of the behaviour of the C18 surface.

Experimental

The DPI experiments were performed on a Farfield **AnaLight**[®] instrument. The surface used was an unmodified silicon oxynitride **AnaChip**[™] (**Farfield Part No. 2007-110c**) covalently functionalised *ex-situ* with C18. Surface coverage post-functionalisation was confirmed by contact angle measurement. The temperature of the samples was controlled to 20°C throughout. Reagents were analytical grade or higher, water was high purity and all solutions were degassed prior to use.

The C18 functionalised **AnaChip**[™] was inserted into the **AnaLight**[®] instrument and re-solvated by flowing running solvent (high purity (UHQ) water, 5% acetonitrile, 0.1% phosphoric acid) over the **AnaChip**[™] surface at 200µl/min until a stable baseline was obtained. A series of solute molecules of varying hydrophobicity (**Figure 1**) were introduced as 300µl injections of stock solutions (1mg/ml in running solvent) into the flow across the **AnaChip**[™] surface at 400-second intervals. The thickness and density of the C18 surface in the presence of the different solute molecules was measured by DPI.

Results and Discussion

Figure 2 shows the dimensional and structural response of the C18 surface in the presence of the solute series of increasing hydrophobicity. Compared to the thickness of the C18 surface in running buffer (approximately 2nm), the structure of the C18 surface increases in thickness and decreases in density in the presence of hydrophilic molecules (caffeine-phenol series) and decreases in thickness and increases in density in the presence of hydrophobic molecules (nitrophenol-anisole series). Therefore, partitioning of high logP (hydrophobic) compounds into the C18 layer causes it to densify whilst low logP (hydrophilic) compounds cause the C18 layer to swell. The transition between C18 densification and C18 swelling occurs sharply at a logP value of approximately 1.5. Furthermore, the degree of collapse and compaction of the C18 layer structure continues with increasing solute hydrophobicity.

Solute	logP Value
Caffeine	- 0.07
Benzamide	0.64
Acetanilide	1.18
Phenol	1.47
Nitrophenol	1.43
Benzonitrile	1.56
Anisole	2.11

Figure 1: The range of solute molecules studied and their logP values

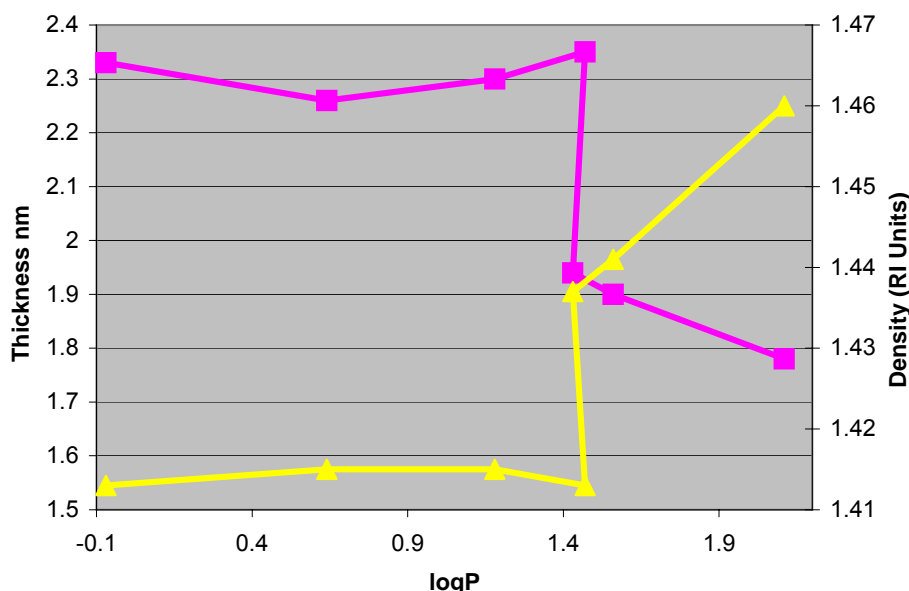


Figure 2: Thickness (pink) and density (yellow) of C18 surface as a function of solute logP

Initially, this seems to be a counterintuitive result, since a hydrophobic solvent would increase rather than decrease C18 solvation and therefore C18 layer thickness. However, the results can be explained by considering the effect a hydrophobic molecule would exert on the C18 as it *partitions into* the layer. The resulting equilibrium system will have a higher concentration of solute in the C18 than in the bulk solution above it, causing the C18 to collapse away from the bulk and densify around the partitioned solute. The opposite effect will be seen with a hydrophilic solute molecule as the partitioned solute displaces the C18, causing an increase in molecular length and an increase in the thickness of the C18 layer.

One obvious conclusion of this effect is that there is a larger volume of C18 in the presence of hydrophilic molecules for the solute to partition into, and therefore more partitions occur than would be expected. In the presence of hydrophobic molecules the C18 collapses and less partitions occur than expected. Therefore, both the volume and the density need to be determined to accurately measure the degree of partition in applications where quantitation is necessary, such as logP value studies.

Conclusions and Benefits

These experiments show DPI can be applied to demonstrate the *dynamic* effects of molecules partitioning into C18 thin films. Importantly, equilibrium conditions are reached very rapidly as the partitioning process takes only a few seconds. This is a significant time saving over classical octanol-water partitioning methods. The quantitative nature of the measurements also provide great insights into the *structural* behaviour of the C18 layer and how it might be tailored for highly specific applications.

The **AnaLight**[®] instrument range and associated experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index (density) and surface coverage in an easy to use, bench-top technique. The **AnaLight**[®] is an important enabling tool for surface and interfacial scientists giving them the ability to:

- Measure thin film structure and dimensions at high resolution and follow and understand dynamic thin film processes and the role played by supporting solvent systems
- Sensitively quantify surface behaviour in real-time, providing rapid information on permeability, solute partitioning and changing solvation state in a range of thin films, including cellular membrane mimics

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