



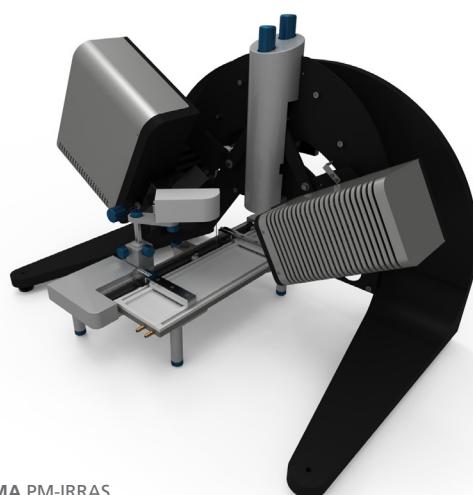
Characterizing molecular orientation and hydrogen bonding at the air-water interface using PM-IRRAS

This application note illustrates how the analytical instruments from KSV NIMA can be used to create cell membrane models and study biomolecular interactions in these models.

Introduction

Floating monolayers (Langmuir films) are two-dimensional ordered systems formed by insoluble molecules floating on a subphase. One of the most important applications of the Langmuir films is to function as a model for cell membranes and other ordered systems. Molecular orientation often has an important role on molecular interactions, and it can affect catalytic properties and intermolecular binding of biologically active molecules.

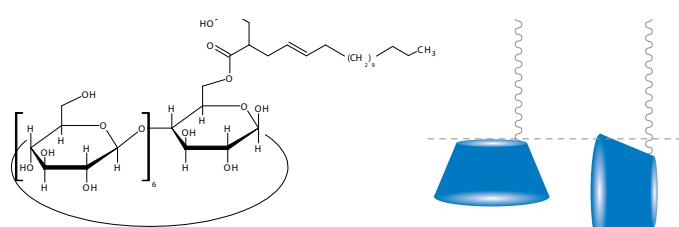
The KSV NIMA PM-IRRAS is specifically designed for polarization modulated infrared reflection absorption spectroscopy. The PM-IRRAS method reduces greatly unwanted signals from environmental factors such as CO₂ and H₂O-vapor compared with conventional IR. It allows the instrument to have a very open design and enables IR measurements of liquid surfaces. The PM-IRRAS method can distinguish between dipoles parallel and perpendicular to water surface, making it a powerful tool when measuring molecular orientation. Polarized light interacts only with the corresponding transition moment of the dipole in the molecule. Reflection of p- and s-polarized light from liquid interface differs therefore significantly from each other. In a PM-IRRAS spectra, dipoles parallel to the surface show as negative peaks while perpendicular dipoles appear as positive peaks compared to the baseline.



KSV NIMA PM-IRRAS

Cyclodextrins are cyclic oligosaccharides that are capable of forming reversible non covalent complexes inside a cavity formed by the ring structure. The ring can also be selectively functionalized, making it possible to modify the function of the molecules. Both native and modified cyclodextrins have been shown to have biological functions, and self assembly properties of the molecule are interesting for nanofabrication and self assembling structures. Fundamental understanding is crucial in order to provide a basis for their use in nanoconstruction.

Behaviour and properties of monoacylated β -cyclodextrin Langmuir films were recently studied by Vico et al. [1] where the molecular orientation of the cyclodextrin in the monolayer could be assessed using the KSV NIMA PM-IRRAS. We present here a summary of the study.



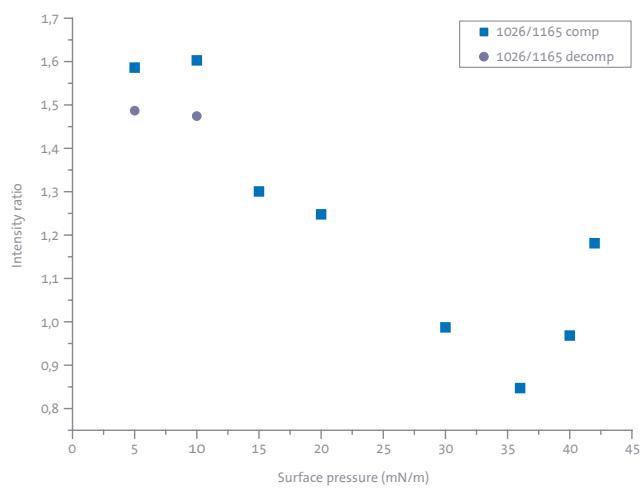
Structure of monoacylated β -cyclodextrin C16-B CD (1) and picture of the molecule when the β CD is oriented parallel (2) and perpendicular (2) to the interface.

Materials and methods

Synthesized and purified monoacylated cyclodextrin (16- β CD) [2, 3] was spread on a Langmuir Trough from 1:1:1 chloroform:methanol:dimethylsulfoxide solution, and the compression- and expansion experiments were done with a 10 mm/min compression rate. The PM-IRRAS spectra were recorded with incident angle of 80°, modulator retardation set to 1500 cm⁻¹ in order to take advantage of the polarization modulation effect on reflectance of p- and s- polarized light. Background corrected spectra were normalized so that the highest peak in the 890 – 1700 cm⁻¹ region was set to 1 and the lowest to 0. The PM-IRRAS spectra presented and used in the consecutive data analysis were produced by averaging at least three different spectra from different experiments.

Results and discussions

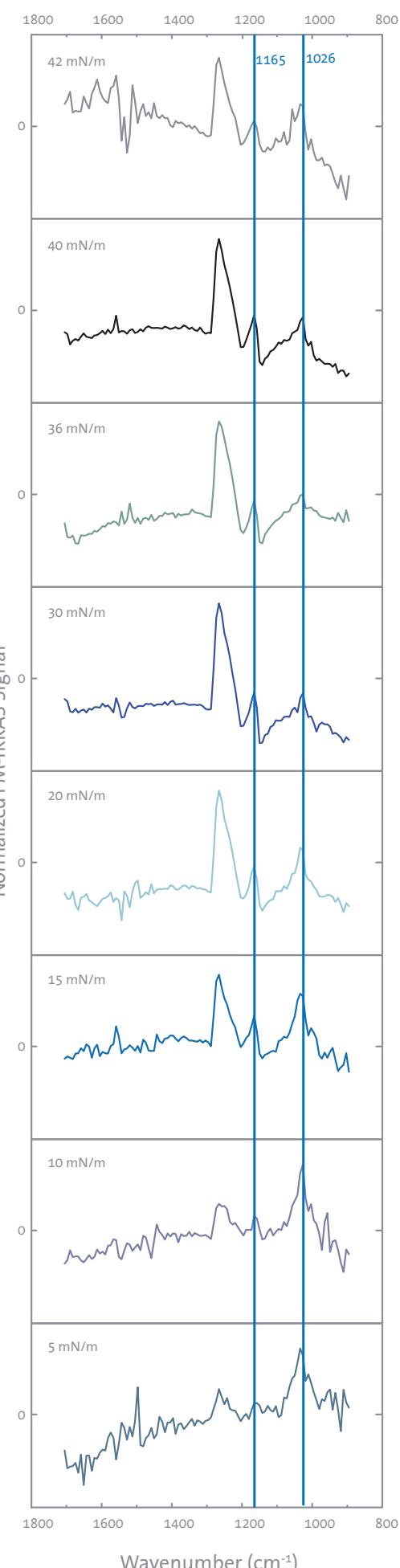
The C16- β CD had two PM-IRRAS bands with the contribution from the ring structure (BCD): 1026 cm⁻¹ when in x-y plane (cyclodextrin ring cavity parallel to the air-water interface) and 1165 cm⁻¹ when the cyclodextrin ring cavity is perpendicular to the air-water interface. By comparing the ratios of these bands as a function of surface pressure, the change in the cyclodextrin orientation can be seen (Figure 1 and Figure 2).



[Figure 2] The PM-IRRAS peak intensity ratios of 1026/1165 (β CD parallel/ β CD perpendicular) as a function of surface pressure obtained during the first compression and first decompression.

The PM-IRRAS spectra of C16- β CD had bands at 1265 cm⁻¹ in compressed monolayers which are not present in a reference absorbance IR spectra of C16- β CD. This band was attributed to ring-ring hydrogen bonding that was present in the monolayer after the ring structures were packed close enough to form such bonding.

The Figure 1 shows how the peak intensity of the C16- β CD monolayer changed during the first compression. The band intensity ratio of 1026/1165 (Figure 2) shows that the molecules start to tilt (as the intensity of 1165 cm⁻¹ increases) when the surface pressure was increased over 10 mN/m. Similarly hydrogen bonding started to increase significantly after the 10 mN/m surface pressure, as seen from the ratio of 1265/1026 bands (Figure 3). The C16- β CD molecules also remained slightly tilted in the decompression, and some of the hydrogen bonding is retained.

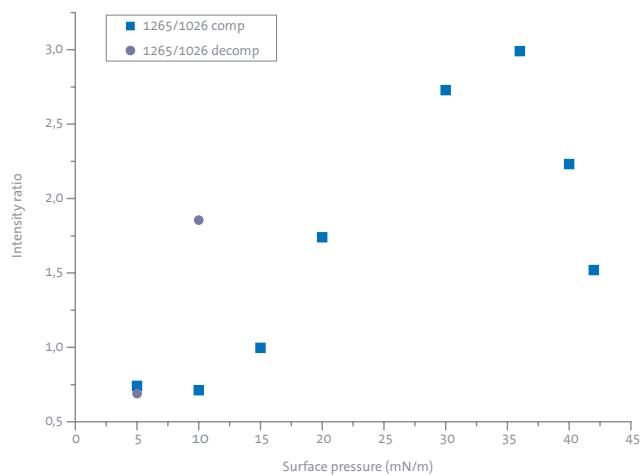


[Figure 1] PM-IRRAS spectra of the C16- β CD fingerprint region at different surface pressures. The background corrected spectra was normalized according to lowest and highest band intensities.

Conclusions

It was possible to study the molecular orientation and hydrogen bonding of monoacylated cyclodextrin at the air-water interface by comparing peak ratios of PM-IRRAS spectra. Both the orientation and hydrogen bonding of the molecules in the monolayer had a large dependency on the packing density in the monolayer, and the effect was shown to be partially irreversible as during the decompression the intermolecular hydrogen bonds created during compression did not break. The results can be used further to determine optimal deposition parameters when such molecules are used in nanofabrication of sensors or catalytic devices.

The same method can also be applied to study other cyclodextrin derivatives or other molecules for which the molecular orientation and intermolecular interactions are important for their function. PM-IRRAS utilization is of considerable interest in nanoscience and nanotechnology focused on the development of functional materials and self-organized surfaces for applications in electric and optical devices and nanoreactors.



[Figure 3] The PM-IRRAS peak intensity ratios of 1026/1165 (hydrogen bonding/BCD parallel) as a function of surface pressure obtained during the first compression and first decompression.

Acknowledgement

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References

- [1] Vico, R. V.; de Rossi, R. H.; Maggio, B. Langmuir 2010, 26, 8407-8413
- [2] Vico, R. V.; Silva, O. F.; de Rossi, R. H.; Maggio, B. Langmuir 2008, 24, 7867-7874.
- [3] Silva, O. F.; Fernandez, M. A.; Pennie, S. L.; Gil, R. R.; de Rossi, R. H. Langmuir 2008, 24, 3718-3726