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Electrical biosensors for triglycerides based on lipase immobilization in layer-by-layer films

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Abstract – The preserved activity of immobilized biomolecules in layer-by-layer (LbL) films can be exploited in various applications, including biosensing. In this study, lipase layers were alternated with layers of poly(allylamine hydrochloride) (PAH) in LbL film. The adsorption time and growth of each layer deposited was monitored with UV-vis. spectroscopy. The concept based on the use of enzyme to increase sensitivity and selectivity was extended to biosensing with impedance spectroscopy measurements. The PAH/Lipase sensor was able to detect triglycerides in aqueous solutions, with the sensitivity and selectivity being attributed to the molecular-recognition interaction.

The detection of triglycerides is important because high blood triglyceride levels are indication of further heart disease. Triglycerides are fatty acid esters of glycerol, which yield glycerol and fatty acids when hydrolysed by a hydrolase enzyme, e.g. lipase. The estimation of triglyceride content in food, in particular, is crucial not only due to the increased health awareness in our society, but also owing to the stringent regulatory laws in the food industry. Here we report an alternative method for estimating triglycerides, where the sensing units were made with layer-by-Layer (LbL) films with immobilized enzymes onto interdigitated electrodes. This builds upon previous work in our groups, in which enzymes such as cholesterol oxidase and phytase [1,2] were used. This process of enzymes immobilization retains their activity because the film-fabrication is carried out under mild conditions, and entrained water remains in the film structure [3]. The enzyme immobilized was a lipase, which interacts specifically with triglycerides, whose layers were alternated with poly(allylamine hydrochloride) (PAH). The adsorption kinetics and growth of PAH/lipase onto PAH/PVS LbL film were monitored with UV-vis. spectroscopy. The time for a complete layer is achieved within 10 minutes and the linear increase at the maximum absorption with the number of layers indicated that the same amount of material was adsorbed in each deposition step, as shown in Figure 1. The concept of a sensor array to increase sensitivity, widely employed in electronic tongues, was extended to biosensing with impedance spectroscopy measurements. Using three sensing units, made of LbL films of PAH/Lipase, PAH/Glucose oxidase (GOX) and PAH/PVS (polyvinyl sulfonic acid), we were able to detect triglycerides in aqueous solutions down to the 10^{-6} M level with PAH/Lipase sensor. The sensing units containing PVS and GOX were used as reference.

The PAH/lipase biosensor showed high sensitivity and specificity that can be attributed to the molecular-recognition interaction between lipase and triglycerides. This system opens the way for clinical tests and quality control in the food industry to be made with low-cost, fast experimental procedures.

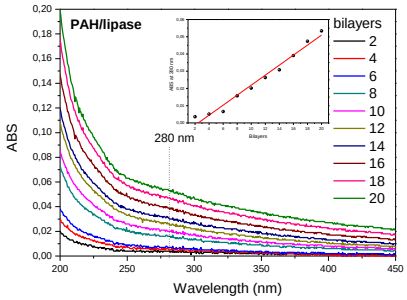

Figure 1: UV-VIS. spectra for LbL films with various numbers of bilayers of PAH/lipase. Inset: increase in the intensity at 280 nm for a PAH/lipase film as a function of the number of deposited bilayers.

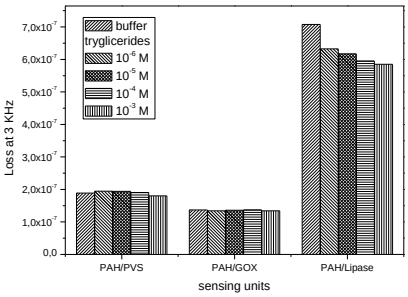

Figure 2: Loss at 3 kHz for three sensor units: bare electrode, PAH/PVS and PAH/Lipase films, for different triglycerides concentrations.

References

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