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Design and development of a bovine erythrocyte acetylcholinesterase (BEA) activity-based biosensor.

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Many biological phenomena can be characterized through the use of biosensors. The use of enzymes as a biological component allows to achieve specificity and speed in measurements.

In our laboratory we have been working on the design of a biosensor based on the activity of bovine erythrocyte acetylcholinesterase BEA, testing the effect of different compounds on the catalytic activity of the enzyme. To do this, Langmuir films (LF) build up from bovine erythrocyte membrane (BEM) are transferred to an alkylated glass substrate through the Langmuir Schaefer method (LS films). To improve the reproducibility of results and enhance the biosensor efficiency, different techniques were assayed. for LF preparation. Membrane spreading was done applying drop by drop deposition over the air-water interface or drop slipping up to the interface along a glass rod. Moreover, the film transference from the air-water interface to the glass substrate was done with or without the elimination, by aspiration, of the monolayer not adhered to the glass. The catalytic activity of BEA and the protein surface density were determined at 96 discrete points covering the entire surface of the glass substrate. These data were plotted as a 3D mesh and submitted to a statistical analysis using the Moran's index to evaluate the film homogeneity. In some cases, the LS film was also prepared with a trace amount of the dye DiI-C18 and the fluorescence intensity was scanned along the whole plate using an Odyssey scanner.

At the macroscopic level, fluorescence images exhibited a homogeneous film. The biochemical data exhibited a varied degree of heterogeneity distribution in the 3D mesh. However, statistical analysis using Moran's index showed that the values consisted of almost randomly distributed spatial data, with slight variations depending on the technique applied to the LF and LS preparation. Moreover, the least coefficient of variations were obtained with rod spreading without interface aspiration.

It can be useful to take into account possible disturbances in the monolayer organization due to the dynamics followed by the catenoidal shaped capillary bridge formed between the solid substrate and the liquid interface during the elevation of the LS substrate. To get deeply into this problem, modifications in the substrate design will be assayed.

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