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STUDY OF OVERLAPPING OF REAGENT ZONES IN LOV FORMAT AND APPLICATION TO ENZYMATIC ASSAYS

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In flow systems that are based on the use of confluence points to merge sample and reagents solutions, efficient mixing is generally achieved. On the other hand, in sequential injection systems, mixing is based on the degree of overlapping between adjacent zones. Therefore, efficient mixing might be difficult to achieve when various solutions are concerned. That can be the case of enzymatic assays involving sample, enzyme, buffer and frequently cofactor solutions.

One way to overcome this problem is the use of reduced volumes of solutions, which become possible in a repeatable way by using the lab-on-valve format. In this equipment, the reactor/detector is integrated on the selection valve in a robust way and the downscaling of the volumes used provides a good mixture by reversed flow.

In this work a study was carried out to minimize the effect of the deficient overlapping of sample and reagents zones in SIA, by using a LOV system. The study also comprehended the application to the enzymatic assay of ethanol in beverages, based on the conversion of ethanol to acetaldehyde by alcohol dehydrogenase. This reaction occurs between the analyte and the enzyme under specific conditions, in the presence of cofactor (NAD⁺) and at pH 9.5. Therefore the aspiration sequence is: buffer-sample-enzyme-cofactor-buffer plugs.

For the zone overlapping study, a LOV-FIALab-3500 system equipped with a syringe pump (2.5 mL) was used. Using BTB dye as a model solution, efficient overlapping was achieved for volumes corresponding to 50 μ L of buffer (RSD < 1.2%); 15 μ L of sample (RSD < 3.2%); 5 μ L of enzyme (RSD < 2.3%); 5 μ L of cofactor (RSD < 3.1%) and 100 μ L of buffer (RSD < 1.2). This results in a good overall repeatability (RSD < 2.2%) in the enzymatic determination of ethanol using a 15% (v/v) wine reference sample.

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