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Miniaturization of enzymatic assay based on the LOV-sequential injection system: Application to the determination of ethanol in alcoholic beverages

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In this work a miniaturized sequential injection-lab-on-valve (SI-LOV) system was developed for an enzymatic assay based on two different measurement modes: peak height and initial-rate measurement. The initial reaction rate measurement can be an efficient way to overcome the difficulties when samples present either an intrinsic absorption or potential interferences with reaction kinetics different from the analyte. The LOV format is particularly suitable for kinetic based measurements due to the easy manipulation of time sequences and the low volumes involved.

As a case study, we selected the enzymatic determination of ethanol in alcoholic beverages, an important parameter to define the quality and the stability of the product. The reference methods proposed for this determination are complex and laborious, while an enzymatic assay requires only a sample dilution step before analysis. The present study exploits the reaction between alcohol dehydrogenase and ethanol in the presence of NAD^+ under controlled pH conditions.

A linear range up to 0.040% (v/v) was achieved for both initial rate and peak height measurement, with good repeatability ($\text{RSD} < 5.0\%$ and $< 1.0\%$, respectively). Enzyme; NAD^+ ; buffer and sample consumption per assay were 0.12 U; 0.066 mg; 150 μL and 15 μL , respectively. The results obtained for several alcoholic beverages, including a certified sample, were not statistically different from those obtained by the reference procedures.

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