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Review

Enzyme based assays in a sequential injection format: A review

Cristina I.C. Silvestre, Paula C.A.G. Pinto*, Marcela A. Segundo, M. Lúcia M.F.S. Saraiva, José L.F.C. Lima

REQUIMTE, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal

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ABSTRACT

Sequential injection analysis systems have been extensively exploited in the last decades for the implementation of enzyme based assays aiming the evaluation of enzyme activity or the determination of specific analytes. The most prominent aspects of the automation of enzymatic assays in these systems are discussed in this review. Special attention is devoted to the mode of enzyme manipulation in homogeneous or heterogeneous media and to the comparison with batch and flow injection enzyme methodologies. The possibility of implementing strategies for the enhancement of selectivity in specific determinations is also addressed. The more recent trends in this field are discussed focusing mainly on the miniaturization resorting to the lab-on valve platform as well as on the bead injection concept.

Cristina Silvestre is a PhD candidate in Faculty of Pharmacy of Oporto University. She obtained her Master Degree in Quality Control in 2006 and her Degree in Biochemistry in 2004. Her research interests focus on automation, flow analysis, quality control and green chemistry. She has published 7 papers in international journals.

Paula C.A.G. Pinto is an Assistant Researcher at the associate laboratory REQUIMTE, Faculty of Pharmacy of Oporto University. She obtained her PhD degree, in Analytical Chemistry (2006) from Faculty of Pharmacy of Oporto University, under the supervision of Professors Lúcia Saraiva and José Costa Lima. Her research interests focus generally on sequential injection analysis and specifically on biocatalysis in aqueous and ionic liquid media. She has published 18 papers in international journals with the SCI over 75 times cited.

Marcela A. Segundo received her BSc degree in Microbiology (1997) and PhD degree in Biotechnology, speciality in Chemistry (2002), from College of Biotechnology, Porto. She is currently Assistant Professor at the Faculty of Pharmacy, University of Porto and researcher at REQUIMTE Associate Laboratory. Her research is focused on bio- and analytical chemistry applied to food and environmental issues, with emphasis on automation of sample preparation. She has published 42 papers in international journals with the SCI over 330 times cited.

M. Lúcia M.F.S. Saraiva received her PhD in Analytical Chemistry (2000) in the Faculty of Pharmacy, University of Porto where she is currently Professor. She is also research scientist at REQUIMTE Associate Laboratory. Her research fields are related with automated flow methodologies mainly based on the sequential injection concept. She is now focusing on studying and applying biocatalysis in non-conventional media (ionic liquids, lipid aggregates) and nanomaterials (biosilica, magnetic and metallic). She has published 47 papers in international journals with the SCI over 214 times cited.

* Corresponding author. Tel.: +351222078939; fax: +351 222078961.
E-mail address: ppinto@ff.up.pt (P.C.A.G. Pinto).

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José Luís F. Costa Lima received his BSc in Chemistry (1970) and PhD in Chemistry (1985) from Faculty of Sciences, University of Porto. He is currently Director of Faculty of Pharmacy, University of Porto, where he leads a research group with more than 30 PhD researchers and more than 25 PhD and MSc students, comprehending research lines in Analytical Chemistry and Biochemistry fields. He has published about 400 papers in international journals with the SCI over 4400 times cited.

1. Introduction

Analytical methodologies resorting to enzymes have increased exponentially and gained popularity and significance over the last decades. This is mainly related with the high selectivity and specificity of enzymes that enable the determination of single species in complex samples by means of nonspecific detection techniques [1]. On a distinct perspective, the utilization of enzymes in analytical methodologies, in replacement of hazardous chemical substances, results in cleaner processes that are in good agreement with the present concerns of Green Chemistry, due to their inherent biodegradability. Notwithstanding, some shortcomings such as high sensitivity to environmental factors (pH, ionic strength and temperature), dependence on cofactors, limited life-time and inhibition by sample compounds hinder the utilization of enzymes in some specific situations [1].

The implementation of biocatalytic procedures in flow systems as an alternative to conventional batch assays, can minimize some of these drawbacks by guaranteeing an effective control of the reaction conditions, allowing the maximization of enzyme activity [2]. These features are a consequence of the well defined transport conditions inherent to flow systems that result in high reproducibility during sampling and detection steps. At the same time, since measurements can be performed in non-equilibrium conditions, the analysis time can be dramatically reduced conducting to faster and more economic methodologies.

The forthcoming of sequential injection analysis (SIA) in 1990 [3] broadened the scope of chemical analysis by overcoming some of the drawbacks that hindered FIA routine utilization such as the continuous circulation of reagents and the need of physical reconfiguration to perform different determinations. SIA soon showed to exhibit operational characteristics amenable to the implementation of enzymatic procedures, namely those common to flow injection analysis as mentioned above. Moreover, solutions do not flow continuously in SIA as happens in FIA. This is an important aspect for automation of enzyme based assays, as waste of enzyme solutions/suspensions is minimized because well defined portions are applied in SIA, in opposition to the waste that occurs during tubing washing between injections verified in FIA.

The research in the field of enzyme based SIA assays has conducted to the publication of about 70 scientific papers, in which enzymes are used either as catalyst or analyte. A careful analysis of the literature reveals an exponential growth of the publications with application in several areas, mainly in the biotechnological, pharmaceutical and food fields until mid-90s, with a relative slow down in the past few years (Fig. 1). Hence, this review intends to summarize and discuss the conjugation enzymes–SIA with the aim of highlighting the main features, particularities as well as shortcomings of this association, opening new possibilities for further developments in this field.

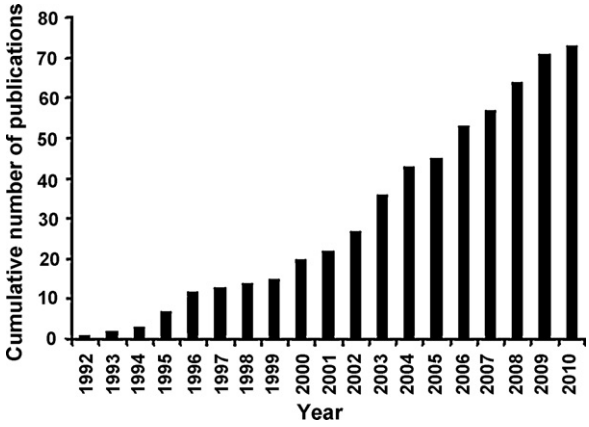

Fig. 1. Cumulative distribution by year of papers reporting SIA systems incorporating enzymatic reactions.

2. Enzymatic applications based on the SIA concept

The most significant features of enzymatic methodologies based on SIA are defined, to a great extent, by SIA mode of operation as well as by the particularities of enzyme catalyzed chemical reactions. A schematic representation of a basic SIA system is depicted in Fig. 2. The core of the system is the selection valve that supports all the solutions and devices involved in the analysis. The functioning of a basic SIA system is characterized by the sequential aspiration of aliquots of solutions to a holding coil followed by the propulsion of the composite reaction zone to the detector by flow reversal.

The sequential aspiration of solutions stacked to each other influences the implementation of analytical methodologies since it defines the effective overlap of the different aliquots. Focusing on the automation of enzyme based reactions, it is mandatory to assure that the implemented sequence enables the contact between enzyme, substrate(s) and cofactor(s) (whenever needed) guaranteeing a sufficient mixing in order to maximize the yield of the chemical reactions involved. On the other hand, the sequence of aspiration must also assure optimum conditions for the immediate beginning of the reaction and a reduced dispersion of the enzyme zone which is usually the last aliquot to be inserted. As discussed in the following section, the optimization of the sequence of insertion and mixing efficiency of the solutions is one of the biggest challenges of the automation of enzymatic assays resorting to SIA.

Compared to batch enzymatic analysis, the strict control of reaction time, offered by the computer control inherent to SIA, is the key feature of SIA as an enhanced automatic approach. In batch enzymatic procedures, detection generally takes place at end-point conditions, after substrate exhaustion, requiring reaction times between 5 and 30 min. In SIA and related techniques, as strict

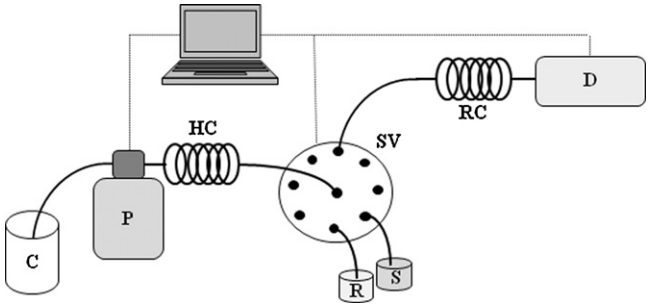

Fig. 2. Schematic representation of a basic SIA system. C: carrier, P: pump, SV: selection valve, HC: holding coil, RC: reaction coil, D: detector, S: sample, R: reagent.