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Editorial

Automated Enzymatic Methods Using Flow and Sequential Injection Analysis

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EDITORIAL

The use of enzymes in analytical chemistry has gained tremendous attention during the last 2-3 decades. The obvious and straightforward reason has to do with the specificity of enzymatic reactions. In other words, by incorporating an enzyme-related step in the analytical cycle, someone can achieve high selectivity by using non-selective detection techniques (e.g. spectrophotometry, fluorescence, chemiluminescence, potentiometry etc).

Another important advantage of enzymatic methods of analysis is their versatility. The analytical chemist has several options when developing a method, including:

i) Determination of the enzyme itself (e.g. its activity in biological material)

ii) Determination of the substrate as an analyte (e.g. determination of fluorophosphates after hydrolysis by alkaline phosphatase [1]).

iii) Determination of inhibitors (e.g. heavy metals, toxins)

iv) Determination of activators (e.g. zinc in the case of alkaline phosphatase)

When developing analytical methods based on enzymatic reactions special attention has to be paid on the kinetic nature of the latter. Strict control of experimental conditions such as for example the pH, temperature and most important the reaction time is, in many cases, extremely critical and requires experienced users.

Flow injection analysis (FI) is a well-established automated technique with numerous and widespread applications in quantitative chemical analysis. In contrast to conventional continuous flow procedures (and all batch methods), FI does not rely on complete mixing of sample and reagent(s) (physical homogenization). Combined with the inherent exact timing of all events it is neither necessary to wait until all chemical reactions have proceeded to equilibrium (chemical homogenization). These features, that allow transient signals to be used as the readout, do not only permit the procedures to be accomplished within a very short time (typically in less than 30 s), but have open new and novel avenues to perform an array of chemical analytical assays that are mainly of kinetic nature (e.g. enzymatic methods) [2].

Sequential-injection analysis (SI) is considered to be the second generation of flow injection techniques and was initially developed as an alternative sample-handling technique to FI. The heart of a SI manifold is a multiposition selection valve (Figure 1). Fluids are manipulated within the manifold by means of a bi-directional pump. A holding coil is placed between the pump and the common port of the multiposition selection valve. The selection ports of the valve are reservoirs, detectors, pumps, reactors, separators, special cells, other manifolds etc. After aspiration of a discrete volume (zone) of sample into the holding coil via the sample line, the sample can be subjected to very complex physical and chemical pre-treatment in different ways within the SI manifold. SI offers great potential for sample handling because it is a bidirectional, stopped-flow sample-handling technique enabling the sample to be serially processed in the different modules connected to the selection valve by means of repetitive aspiration and delivery steps [3].

The most straightforward approach to implement an enzymatic method under flow injection conditions is to use the enzyme in solution, flowing as a reagent. A representative example is in fact the first report of a FI enzymatic method by

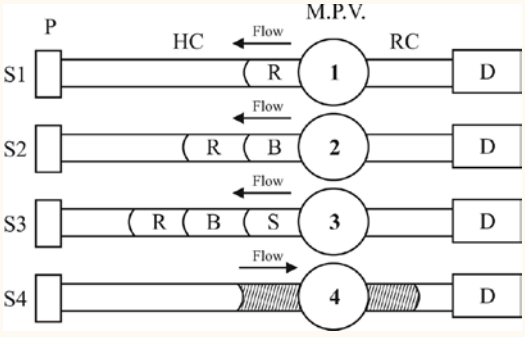

Figure 1 Typical setup and steps of a SI system utilizing three-zone chemistry. S1-S4: SI steps; P: pump; HC: holding coil; M.P.V.: multi-position valve (1-4 are the valve ports); RC: reaction coil; D: flow through detector; R: reagent; B: buffer; S: sample.

the inventors of the technique Ruzicka and Hansen, for the determination of glucose in blood [4]. As can be seen in the flow diagram of Figure 2, the sample is injected in an inert carrier (C) and is merged downstream with the mixed-enzymes reagent stream (R). The main concern when using flowing streams of enzymes is the consumption. Some ingenious strategies in order to overcome this are based on the concepts of "merging" or "chasing" zones (Figure 3) [5,6].

However the most viable solution towards the minimization of the consumption of the enzymes is their immobilization on solid supports, leading to the formation of enzymatic reactors. One of the first and classical immobilization protocols was reported by the group of Townshend and was based on the covalent binding of the enzyme on activated controlled pore glass beads [7]. Alternative solid supports may include membranes, monolithic materials, sepharose beads etc. Depending on the immobilization procedure (e.g. physical adsorption vs covalent binding), enzymatic reactors have shown impressive stability for several months. As can be seen in Figure 4, enzymatic reactors are typically incorporated in the flow configure ration either before the injection valve, or between the injector and the flow-through detector. One of my favorite and most characteristic examples on the potentials of using immobilized enzymes in FI has been reported by the group of Pacey for the determination of urea in biological samples [8]. In brief, the enzyme urease was immobilized on a PTFE membrane housed in a typical gas-diffusion unit (Figure 5). Upon contact with the membrane, the analyte was converted to volatile ammonia that simultaneously diffused through the porous membrane to a suitable acceptor stream. The latter contained an indicator and ammonia could be determined photometrically.

The second generation of flow injection techniques, that is SI, has also been a useful platform for the automation of enzymatic

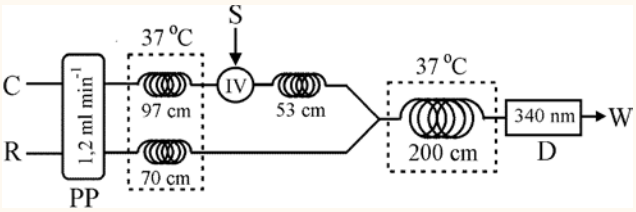

Figure 2 Flow Injection setup for the determination of glucose in blood [4]; C = carrier, R = mixed enzymes reagent, PP = peristaltic pump, S = sample, IV = injection valve, D = detector, W = waste.

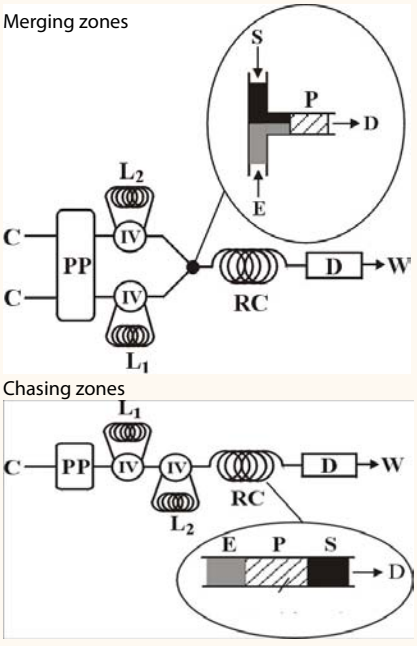

Figure 3 Merging/chasing zones flow configurations; C = carrier, PP = peristaltic pump, L1/L2 = sample loops, IV = injection valve, RC = reaction coil, D = detector, E = enzyme, P = product, S = substrate.

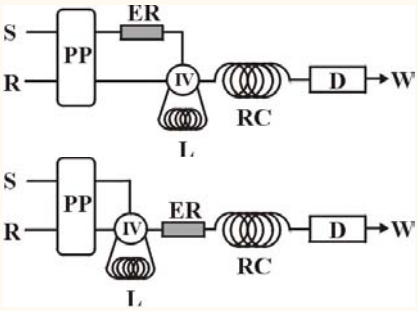

Figure 4 Incorporation of enzymatic reactors in flow injection configurations; S = sample, R = reagent, PP = peristaltic pump, IV = injection valve; L = sample loop; ER = enzymatic reactor; RC = reaction coil, D = detector, W = waste.

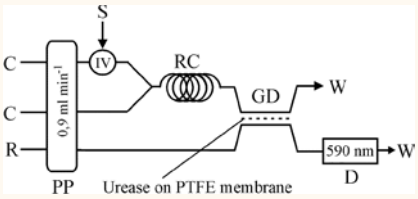

Figure 5 Flow manifold for the determination of urea in biological samples [8]; C = carrier, R = reagent for the determination of ammonia (indicator), PP = peristaltic pump, S = sample, IV = injection valve, RC = reaction coil, GD = gas-diffusion unit, D = detector, W = waste.

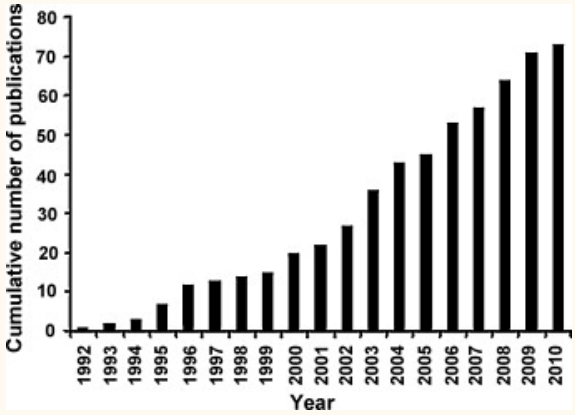

Figure 6 Evolution of SI methods using enzymatic reactions (from [9]).

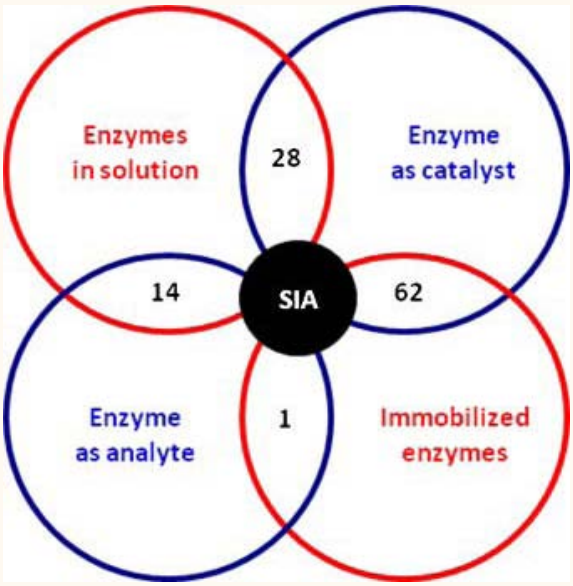

Figure 6 Evolution of SI methods using enzymatic reactions (from [9]).

methods. A graphical depiction on the evolution of enzymatic SI methods is depicted in Figure 6 [9]. The zone-fluidics principle of SI is ideal for applications of enzyme in solutions, since each analysis cycle utilizes a specific volume of the enzyme at the micro-liter level, minimizing the consumption and reducing the cost of the method (Figure 7). Of course immobilized enzymes in the form of a suitable enzymatic reactor can be used in a similar manner as in FI.

To conclude this short editorial piece, automated flow injection methods have proven to be excellent platforms for the implementation of enzymatic methods of analysis, as has been demonstrated by hundreds of scientific reports. These techniques are perfectly compatible with the kinetic nature of enzymatic reactions offering enhanced precision and accuracy. Stopped-flow experiments can be applied for kinetic measurements (reaction rate), while the possibilities of using both soluble and immobilized enzymes increase the versatility of the developed procedures. Additional specific topics on flow enzymatic methods that were not discussed in this editorial and are equally important are flow immunoassays, on-line biosensors and bead injection systems.

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