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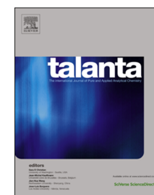

From continuous flow analysis to programmable Flow Injection techniques. A history and tutorial of emerging methodologies

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ABSTRACT

Automation of reagent based assays, also known as Flow Analysis, is based on sample processing, in which a sample flows towards and through a detector for monitoring of its components. The Achilles heel of this methodology is that the majority of FA techniques use *constant continuous forward flow* to transport the sample – an approach which continually consumes reagents and generates chemical waste. Therefore the purpose of this report is to highlight recent developments of flow programming that not only save reagents, but also lead by means of advanced sample processing to selective and sensitive assays based on stop flow measurement. Flow programming combined with a novel approach to data harvesting yields a novel approach to single standard calibration, and avoids interference caused by refractive index. Finally, flow programming is useful for sample preparation, such as rapid, extensive sample dilution. The principles are illustrated by selected references to an available online tutorial http://www.flowinjectiontutorial,com/.

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1. Introduction

The individual steps of an assay protocol, i.e., sample and reagent metering, mixing, incubation, monitoring and efficient washout are carried most efficiently at different time frames and flow rates. Therefore processing samples by means of constant continuous flow compromises the efficiency of an assay, as much as it would impair driving of a car, equipped with only one forward gear. Yet, the majority of flow based assays are still performed on continuous flow basis, the legacy of Skeggs' [1,2] Auto Analyzer®

In contrast to all flow based methods, including Flow Injection [3] (Fig. 1A), Sequential Injection [4], (Fig. 1B) uses bidirectional stop flow to perform the essential steps of assay protocol, within

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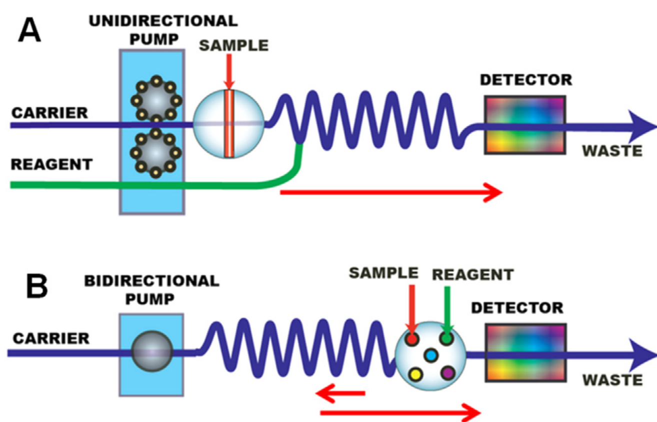

Fig. 1. A) Flow Injection manifold. B) Sequential Injection manifold (from Ref. [T17]).

the holding coil situated *upstream* from a mutliposition valve.

In the first step of SIA protocol (Fig. 2A) sample (red) is injected into a carrier stream of water by flow reversal, followed by reagent (Fig. 2B, blue). As the reaction product (yellow) (Fig. 2C) starts to form at the interface of sequentially stacked zones, a flow reversal (Fig. 2D and E) is applied, to transport the reaction mixture into the detector for monitoring. [T1]. Note: references [T..] are to hyperlinks to Flow Injection Tutorial where the additional information on the discussed topic is available.

2. Stop flow techniques

Incubation of sample with reagents by stopping the flow can take place either in the *holding coil* (SHC mode Fig. 2D) or in the *flow cell* (SFC mode Fig. 2E) The readout in the SHC mode resembles a Flow Injection peak, the height of which is proportional to the concentration of analyte, since the reaction product flows interruptedly through a flow cell. The readout in SFC mode is a reaction rate curve, the slope, or end point of which is proportional to the concentration of analyte, since the reaction mixture is monitored while the chemical reactions proceed. The SHC mode offer higher sampling frequency, and it is therefore suitable for serial assays, while the SFC method is more sensitive and selective. Arresting the reaction mixture in the holding coil or in the flow cell offers a convenient opportunity to control the temperature of incubation, and in this manner the reaction rate and sensitivity of the assay. As Fig. 2 indicates, temperatures of the holding coil and of the flow cell should be independently controlled, because in the SHC mode there is no need to heat the flow cell. In the SFC mode on the other hand, it is advantageous to heat the reaction mixture only in the flow cell (Fig. 2E) since this approach yields, after initial warming up lag phase, a response which is larger than it would have been if the reaction mixture was preheated within the holding coil [T2].

3. Programming of flow rates

While duration of the stop flow period, applied to incubation, determines sensitivity of the assay, programming of flow rates applied to individual assay steps allows optimization of sampling frequency. As an example the serial assay of phosphate [T3], carried out in the SHC mode at 50 °C, (Fig. 3) comprised the following steps: 1/ initial washout for 1 s at 1000 μL/s, 2/aspiration of molybdate reagent for 3 s at 75 μL/s, 3/sample aspiration for 3 s at 25 μL/s, 4/aspiration of ascorbic acid for 2 s at 50 μL/s, 5/monitoring period of 6 s at 20 μL/s and final washout for 2 s at 1000 μL/s. In this way sample and reagents were metered at low flow rates, incubation of 2 s in SHC at zero flow, monitoring was performed at moderate flow rate, while washout at a very high flow rate, (1000 μL/s corresponds to linear velocity of 2 m/s), ensured vigorous sweep of flow channel and flow cell, as documented by the perfectly straight and stable baseline (Fig. 3.).

4. Exploiting concentration gradients

It is well known that the concentration profile of any FIA/SIA signal, as based on the injection of a sample of concentration C^0, essentially represents innumerable concentrations below C^0 that are correlated with the associated signal. Instead of trying to model the dispersion profile – which was attempted by many in the past – in this report we take a direct experimental approach of exploiting the dispersion profile by *selecting a suitable section of the concentration gradient* in order to obtain the desired analytical readout. This principle is applied for various protocols: reaction rate measurement, single standard gradient dilution and single standard reaction rate, as well as for extensive sequential dilution. The concept and implementation of these techniques became

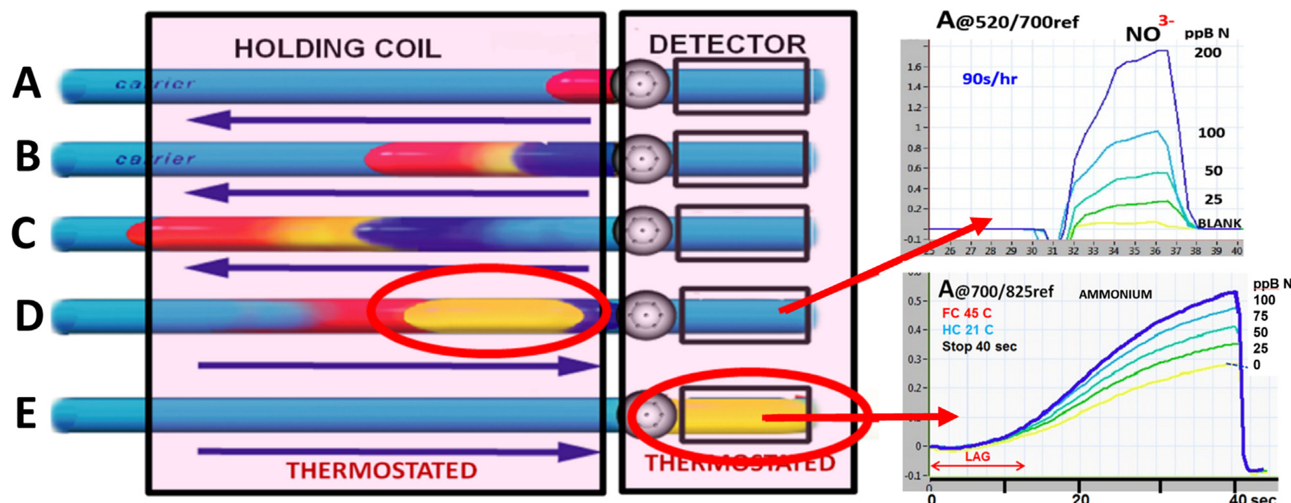

Fig. 2. Steps (A to E) of Sequential Injection assay protocol (from Ref. [T17]). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

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