J. Korean Dairy Technol. Sci. 21(2) : 77 ~ 80, 2003

FLOW-INJECTION ANALYSIS

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I. Introduction

After the paper entitled "Flow Injection Analysis, Part 1. A New Concept of Fast Continuous Flow Analysis" by Ruzicka and Hansen (7), the flow-injection analysis (FIA) has gained wide acceptance within the scientific community. FIA is the type of continuous flow analysis that utilizes an analytical stream, unsegmented by air bubbles, into which highly reproducible volumes of sample are injected. Further downstream a flow-through detector monitors the reaction products. The reaction products are measured before steady-state conditions are established, and in many analyses the readout is fast and thus high sample throughput is possible. Application of this principle to automated analysis yields a fast, precise, accurate, and extremely versatile system that is simple to operate.

Gas bubbles, usually air, are introduced into the streams in conventional continuous flow analysis (10, refer Fig. 1). In the past, the presence of the air bubbles in the analytical stream of a continuous flow system had been deemed necessary to effect three primary functions: (1) to limit sample dispersion; (2) to promote mixing of the sample with reagents by generating turbulent flow; and (3) to scrub the walls of the analytical conduits. Further study has shown that, not only are all of these functions possible in the unsegmented stream, but also that the absence of the air bubbles actually expands the capabilities of the analytical system (6). The apparatus used for FIA is less complicated than that used in gas-segmented flow analysis (SFA) systems (1, 5, 6, refer Fig. 2).

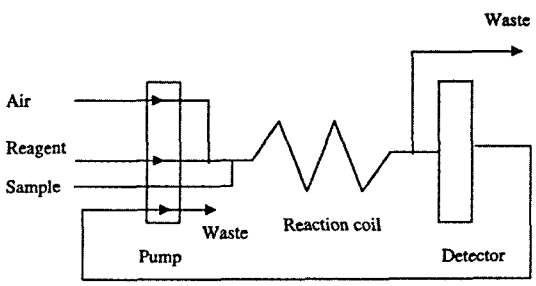

Fig. 1. Configuration of a simple SFA system.

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While it was originally thought that turbulent flow is present (7, 11), it was later determined that FIA operates only in the laminar flow region (1, 8, 9). The flow conditions under which most FIA systems operate generate dispersion through both diffusion and convection (6). The Vanderslice group (13) confirmed that it is radial rather than axial dispersion that contributes most significantly to sample dispersion in FIA systems. This type of dispersion operates to move

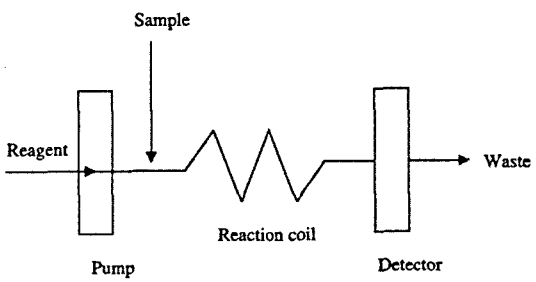

Fig. 2. Configuration of a simple FIA system.

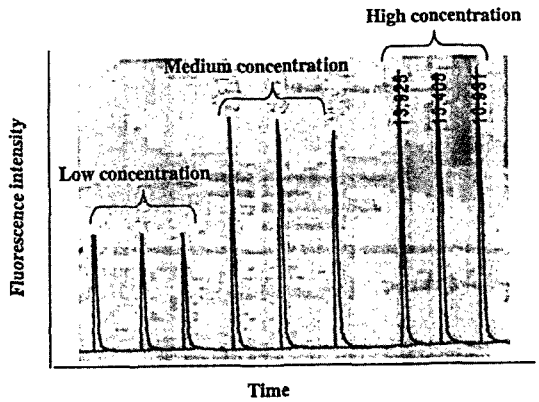

Fig. 3. An example of FIA curves, showing reproducible fluorescence intensities at various concentrations of sulforhodamine B solution.
(Low: 6 μM SRB, medium: 10 μM SRB, high: 12 μM SRB)

the fluid both toward and away from the tubing walls and thus serves as an efficient scrubbing mechanism, leading the low carryover and cross-contamination exhibited between samples processed in an unsegmented stream. This flow also serves to limit band spreading. Asymmetrical peak shapes are typically found in FIA skewed to the positive slope. Thus some peak tailing is normally observed. Fig. 3 shows an example of FIA curves.

II. Components of FIA

The FIA system consists of a carrier stream of reagent propelled by a pump injection system; a flow-through detector; and a recorder or other data handling device (5). Fig. 2 shows the diagram of a simple FIA system. Sample and reagents move into the reaction manifold where the analytical processing occurs. In the reaction manifold, the sample may be reacted with a variable number of reagents, incubated, dialyzed, distilled, or extracted. The reaction products flow to the flow-through cell of an appropriate detector to generate a signal, which is directed to a recorder and microprocessor for data reduction.

The technique of FIA is a combination of the following three principles: sample injection, controllable sample dispersion, and reproducible timing. The purpose of sample injection is to insert a discrete plug of sample into a continuously moving carrier stream in such a way that the movement of the stream is not disturbed (1). It must be introduced into the carrier stream precisely, so that the volume and length of the plug can be reproduced exactly from sample to sample. When the sample is first injected, it forms a well-defined sample plug in the stream. In the absence of gas segmentation, the plug disperses into and, thus, mixes with the carrier stream under laminar flow conditions to form a gradient, as the sample is swept downstream through the analytical conduits of narrow-bore tubing. This type of diffusion-induced dispersion has a moderating effect on the longitudinal dispersion caused by carrier flow, which explains the low carryover and high sample throughput possible with FIA. Changes in mean flow velocity, tube diameter, monitoring distance, diffusion coefficient of analyte, or any combination of these will obviously alter the dispersion of the sample in the carrier stream. Varying the values of these parameters confers a significant degree of control over the dispersion characteristics and facilitates optimization of a flow injection system. Reproducible timing is of utmost importance in FIA. The time from introduction of the sample into the carrier stream until it is detected is dependent on the pumping speed.

III. FIA vs. HPLC

FIA bears resemblance to HPLC, which is itself a type of continuous flow procedure, in their small sample volume and signal profile. The major difference is that while the sample is always separated on a column in HPLC, this is not necessarily the case with FIA. And also, FIA uses low pressure, while HPLC needs high pressure to push the sample through the tightly packed

Table 1. Comparison of characteristics of FIA, HPLC, and SFA.

Parameter	FIA	HPLC	SFA
Sample introduction	Injection	Injection	Aspiration
Sample volume	Small (μL)	Small (μL)	Large (mL)
Analytical stream	Unsegmented	Unsegmented	Segmented
Pump speed	Variable	Variable	Fixed
Column	Possible	Yes	Possible
Dada reduction Integration	Integration	Peak height	peak height

analytical column (5, refer Table 1).

IV. Applictions of FIA

The FIA technique offers many opportunities for automation. The ever-increasing demand for analyses in clinical, agricultural, pharmaceutical, industrial, and other types of analytical control has led to the development of a large number of different instruments for automated analysis. Immunoassays can also be combined with various techniques. Hybrid systems, such as gas chromatography with electron capture and liquid chromatography with ultraviolet absorption, are well accepted in analytical chemistry laboratories. Antibody affinity columns and immunomagnet separations can be used in sample preparation to selectively concentrate and purify chemicals. Individual compounds then can be quantified by separating them by HPLC following immunoconcentration. The development of the flow-injection immunoassay, beginning with one of the first publications in 1980 by Lim and Miller (2), has resulted in a growing field which combines the precise and reproducible timing of FIA with immunoassays to yield assays which are carried out in a non-equilibrium time frame (4). These often faster assay methods require small volumes of sample and reduced sample handling. Traditional batch type immunoassays are time-consuming, partially because of the equilibrium-based measurements carried out. FIA-based techniques are capable of non-equilibrium-based measurements (3). Therefore, the sample throughput in an FIA system is substantially increased.

V. References

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