# How flow-injection analysis (FIA) over the past 25 years has changed our way of performing chemical analyses

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**Briefly looking back on the impact of flow-injection analysis (FIA), as reflected in the rapid growth of publications in the scientific literature, and touching upon many of the novel and unique analytical chemical possibilities that FIA and its sequels, sequential injection analysis (SIA) and lab-on-valve (LOV), have offered, we emphasize assays based on kinetic discrimination schemes, where, even subtle differences in the reaction rates of the chemical reactions that occur are judiciously exploited. We give a number of examples, covering homogeneous as well as heterogeneous conversion techniques, determinations of low levels of metals in complex matrices via suitable pre-treatment procedures, and soil-fractionation schemes.**


*Keywords:* Complex matrix; FIA; Flow-injection analysis; Kinetic discrimination; Lab-on-valve; Pre-treatment; Sequential injection analysis; Soil fractionation

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## 1. Introduction

Since the concept of flow-injection analysis (FIA) was first introduced in 1975 [1], it has had a profound impact on how modern analytical procedures are implemented. This is amply reflected in the many scientific publications that it has generated in all corners of the world: thus, by the middle of 2006, there had been more than 16,500 papers, to which should be added well over 20 monographs and numerous theses [2]. The reason for this momentum is clearly that FIA has allowed us to execute novel and unique procedures, which are difficult and, in many cases, not even feasible by the traditional batch procedures, the use of which has for centuries literally dominated the analytical scene; or, maybe more precisely expressed as: generation upon generation of analytical chemists were taught, or rather indoctrinated, to believe that this was the only sensible way to perform chemical assays.

With the advent of FIA, this conceptual restriction was exorcized, and, as a result, we have witnessed a surge in the development of new analytical approaches and applications. Yet, common to all them is that they rely on the exploitation of the three cornerstones of FIA, as expressed in the very first FIA publication, namely:

(i) injection, or insertion, of a discrete, well-defined volume of sample solution into a flowing carrier stream (inert or itself a reagent, additional reagents being added subsequently);
(ii) reproducible and precise timing of the manipulation to which the injected sample zone is subjected in the system, from the point of injection to the point of detection (i.e. the so-called controlled, or rather controllable, dispersion); and,
(iii) the creation of a concentration gradient of the injected sample, providing a transient, but strictly reproducible readout of the recorded signal.

The eventual readout, as monitored by a suitable detection device, is therefore always a result of two kinetic processes that occur simultaneously, namely the *physical* process of zone dispersion and the superimposed *chemical* processes resulting from reaction between analyte and reagent species.

As a result of growing environmental demands for reduced consumption of sample and reagent solutions, the first generation of FIA (or just FI, to emphasize


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that it is a conceptual approach, in addition to a means of performing analysis), which utilizes continuous pumping of carrier and reagent solutions, was in 1990 supplemented by the second generation, termed sequential injection analysis (SIA – equally well often referred to merely as SI) [3,4]. Fully computer controlled and based on the use of a multi-position valve from the ports of which individual, precisely metered zones of sample and reagent(s) are aspirated sequentially by means of a syringe pump and stacked in a holding coil, and then, subsequently, under dispersion within each other, are forwarded to a suitable detector, the SI system implies substantial savings in not only consumables, but also inherently waste generation.

Then, in 2000, we saw the appearance of the third generation of FIA, the so-called lab-on-valve (LOV), with which even further downscaling was achieved and the concept of bead injection (BI), involving bead-renewal approaches, was introduced, also offering new avenues for chemical assays [5–7].

Finally, within the past couple of years, SI chromatography (SIC) has emerged, permitting low-pressure separation procedures and analysis of multi-component samples, especially facilitated via incorporation of commercially-produced Chromolith columns (Merck) formed from a single piece of porous silica gel (monolith) [8,9].

While much of the attention to using FI initially was on the feasibility of achieving high sampling rates, as facilitated by exploiting transient rather than conventional steady-state signals, the focus was soon shifted to exploiting the concentration gradient created, which, as a result of the axial and radial dispersion processes, in reality corresponds to an innumerable number of sequential liquid segments representing all concentrations from zero to the maximum of the FI peak readout, each of which can potentially be used for the analytical readout. This, in turn, gave rise to a number of gradient methods [10], among which should be especially highlighted the stopped-flow method, which has proved to be a very powerful tool in many contexts, not least for enzymatic procedures, for both assay of substrates and determination of enzymatic activities, as the latter has traditionally been very difficult to execute.

Later, there followed the introduction of methods relying on detection by bioluminescence and chemiluminescence, where the alpha and the omega is the inherently accurate timing of the FI manifold, which allows the maximum intensity of the generated transient light emission to be related to the analyte concentration. These very sensitive analytical procedures, which were virtually non-existent prior to the introduction of FI, have blossomed to the extent that more than 1600 publications have emerged in the scientific literature over the years, thus accounting for ca. 10% of all the published FI papers. Essentially, these assays are all based on enzymatic conversion procedures, and these types of kinetic *modus operandi* are some of the most frequently encountered. In the beginning, they relied primarily on the use of solubilized enzymes, but later on immobilized enzymes as affixed on various supports and packed in column reactors, whereby advantage can be taken of the fact that the costly enzymes, even though they participate in the reactions with the substrate, are not consumed, so they can be reused. This again is reflected in the many publications, where the word ''enzym*'' is found in the title of more than 900 papers. There is also evidence in the numerous reviews, among them the biannual reviews on kinetic methods, which until 2002 were regularly published in *Analytical Chemistry*.

Intermediate or metastable constituents with specifically attractive analytical characteristics, in contrast to the ultimately formed products, have also been utilized to provide the analytical readout [11]. However, among the many exciting novel techniques that we would especially like to emphasize are those based on the so-called kinetic discriminating schemes, where even subtle differences in the reaction rates of the chemical reactions that occur are judiciously exploited. This is because these conversion methods more than anything else demonstrate the unique capabilities of FI and its sequels to perform novel and original applications. And, since it is impossible even to attempt to cover all aspects of FI, we have, for the very same reason, opted to devote the present article to a closer survey of these schemes by using some selected examples. Of course, we make no pretence that those we have chosen are exhaustive; rather, we have picked them subjectively to demonstrate different intriguing approaches.

## 2. Exploiting homogeneous conversion reactions

### *2.1. Determination of chlorate*

One of our favorite examples to demonstrate the essence of kinetic discrimination schemes is the determination of chlorate via a homogeneous conversion procedure. We have picked it, firstly because it is simple, yet elegant, and secondly because it is one of the first (if not the first) practical example presented in the literature [12]. Aimed at analyzing chlorate in process liquor, the reaction scheme is as follows:

$$2ClO_3^- + 10Ti^{3+} + 12H^+ \rightarrow 10Ti^{4+} + Cl_2 + 6H_2O \quad \text{(fast)}$$

$$Cl_2 + LMB \rightarrow MB \quad \text{(fast)}$$

$$MB + Ti^{3+} \rightarrow LMB + Ti^{4+} \quad \text{(slow)}$$

The assay is performed by injecting a sample of chlorate into an acidic carrier stream of titanium(III), which is subsequently merged with a second stream of leucomethylene blue (LMB). While the first two of these