Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1291–1300, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Flow injection analysis of pharmaceuticals*

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Abstract: An overview of the most representative problems solved by flow injection analysis (FIA) in drug analysis is presented. Different aspects of this technique which can be manipulated with specific purposes are discussed and special emphasis is placed on the possibilities of FIA in dissolution test control.

Keywords: *Flow injection analysis; pharmaceuticals; review.*

Introduction

Flow injection analysis (FIA) [1, 2] is a technique characterized by its simplicity, economy, fast sampling rate and extreme versatility. It is an excellent tool for solving problems in various areas including clinical [3–5], environmental [6, 7], and drug analysis [8, 9]. This paper describes some examples in which FIA has solved various problems in drug analysis and is arranged according to the particular aspect of FIA which has been exploited for solving the problem, namely:

(i) The use of a conventional chemical reaction and the exploitation of the intrinsic kinetic character of FIA to boost performance using the usual FIA measurements (peak height) or peak-width measurements at a preselected peak height [10–12]. Adequate reaction development may call for the presence of a solid interface to improve sample–reagent mixing [13] or to facilitate a reaction [14].

(ii) The use of FIA alternatives such as the merging zones of the closed-loop mode, or those allowing typical kinetic measurements [15].

(iii) The on-line coupling of a separation technique [16–19] can eliminate or minimize interferences resulting from the sample matrix.

(iv) The detection system used markedly influences the sensitivity and selectivity achieved (FIA can be implemented with virtually any type of detector).

(v) The degree to automation afforded by an FIA analyser is indicative of its versatility and flexibility of adaptation to different situations and needs.

(vi) Continuous and discontinuous on-line control are of great significance to the drug industry, to which FIA can make major contributions.

All of these aspects, which are discussed below, show the suitability of FIA for solving typical problems encountered in drug analysis.

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
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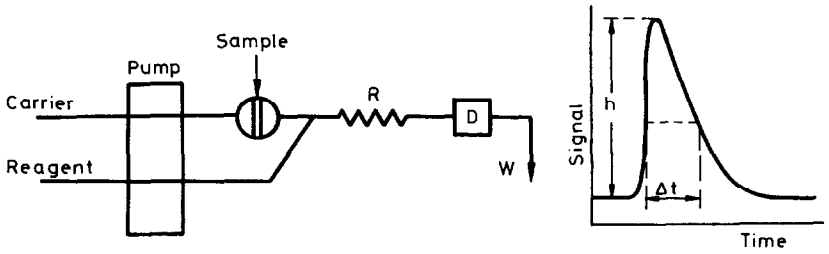

Figure 1
Conventional two-channel FIA manifold and recording obtained from peak-height and peak-width measurements.

(i) Conventional FIA and FIA Titrations

Figure 1 shows a simple two-channel FIA configuration in which the sample is first injected into a carrier solution and later merged with the reagent solution. The reaction developed along reactor R is monitored as the plug passes through the detector flow-cell. The non-steady state of the measurements (fixed-time kinetic measurements [15]) allows one to eliminate or minimize the potential interferences of other species present in the sample. Thus, the determination of zinc with Zincon [20] or that of hydrogen peroxide with the sulphite-5,5′-dithiobis(nitrobenzoic acid) system [21] in insulin preparations and dopamine, respectively, are excellent examples of the improved selectivity resulting from the kinetic character of FIA, in addition to its high sampling frequency (up to 80 h^{-1}) and reproducibility (relative standard deviation, RSD <1%).

The determination of water in organic compounds by the Karl Fischer method is another example of conventional methods noticeably improved by using an FIA system which minimizes interferences with respect to the standard batch titration method and affords a determination range of 0.01–5% (v/v) of water, with RSD <0.5% (v/v). Detection can be photometric [22, 23] or potentiometric [23] and the usefulness of the method has been demonstrated with iodine-consuming samples [23].

Peak-width measurements made at a preselected peak height, in which the time interval between both signal values and the logarithm of the analyte concentration are proportional, are the basis of FIA titrations in their normal (with a mini-mixing chamber) and high-speed modes [10–12]. Conventional acid–base [24, 25], redox [26], catalytic [26] and dye formation [27] reactions have been used for drug analysis in FIA titrations, which have also been employed in studies of drug–protein binding interactions with fluorimetric detection [28]. In all cases, the results obtained clearly improve those provided by conventional methods. The determination of parameters inaccessible to conventional methods [28] is also possible.

The presence of a solid interface in the FIA manifold can boost its performance in different ways, depending on its nature:

(a) The use of a reactor packed with an inert solid [SBSR (13)] can dramatically increase reaction development and hence sample–reagent mixing with small dispersion. This is a means of increasing sensitivity and sampling frequency, as shown by van Veen *et al.* for paracetamol [29].

(b) The little beads of SBSR can be treated chemically (silylation, glutaraldehyde coupling and enzyme immobilization) to facilitate the development of a reaction and introduce the physical effects described in (a). The determination of penicillins by

immobilizing penicillinase on this type of support provides a sampling frequency of 150 versus 60 h^{-1} achieved by using controlled pore glass (CPG) or reactor glass wall as support [30]. The use of CPG as a support, though, has the advantage of its large surface area, which allows the immobilization of larger amounts of catalyst per reactor length unit [14]; however, its compactness and overpressure surpass those of SBSR and open-glass reactors.

(c) Packed reactors containing group-specific receptors can be used to effectively reduce the background fluorescence of biological fluids such as serum, as shown by Miller *et al.* [32] in FIA studies of interacting biochemical systems.

(ii) Other FIA Modes

More sophisticated configurations than those commented on above have been used from the point of view of the chemical system employed.

Whenever reagent consumption is a limiting factor, a merging zones configuration (Fig. 2a) reduces the amount of reagent required for mixing with the sample; such an amount can be further reduced by the asymmetric merging zones mode [33]. In addition, suitable hydrodynamic working conditions allow one to minimize the sample and reagent volumes simultaneously injected [34].

Another way to reduce reagent consumption lies in the use of closed-loop systems, proposed by Mottola *et al.* [35–37]. In these configurations (Fig. 2b), the reagent from a reservoir is circulated in a closed regime through a circuit in which the derivatizing reaction takes place, the reagent being regenerated and returned to the reservoir. The determination of enzymes and other species acting as catalysts [38] and the calculation of kinetic parameters on phenothiazines [39] have been successfully carried out on closed-loop configurations.

The different rate of reaction of two analytes with the same or different reagents can be exploited for their simultaneous determination (differential kinetic methods). In addition, this involves increased selectivity inherent in these methods: the elimination of the blank or sample matrix contribution and the interferences of other species present in the sample reacting faster or more slowly than the analytes. Two approaches (Fig. 3a) have been designed with this purpose for the determination of magnesium (or calcium) and strontium ions based on the differences between the dissociation rates of the cryptand (2.2.2) complexes of the metal ions in the presence of potassium ions as

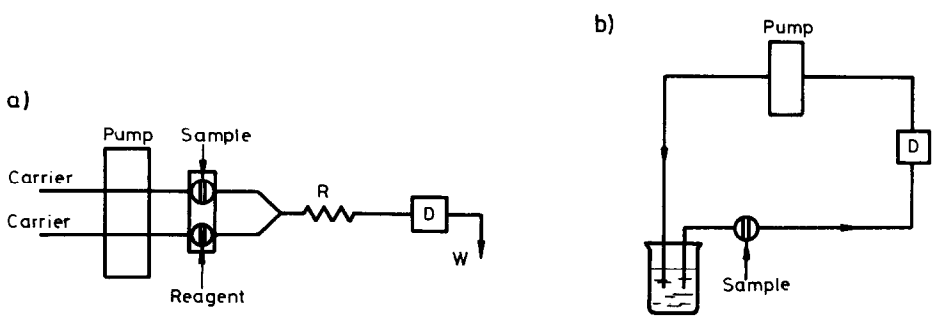

Figure 2
Two reagent-saving modes: (a) Merging-zones, and (b) closed-loop configurations.

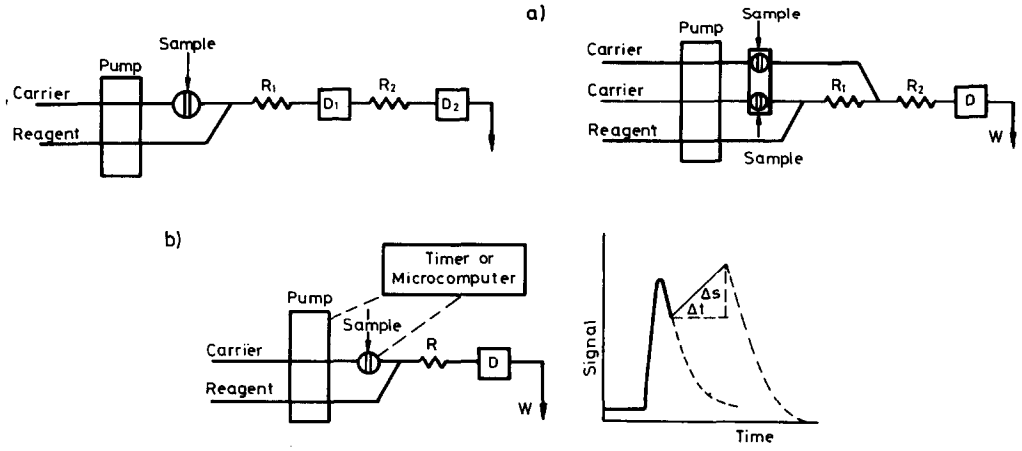

Figure 3
Configurations used to perform kinetic measurements: (a) by differential kinetic methods, and (b) by stopped-flow methods.

scavengers and phthalein complexone as the chromogenic reagent for the released metal ions [40].

Another way to perform kinetic measurements is the halting of the reacting plug at the flow-cell during the interval required to monitor the reaction evolution. In addition to the usual components of the conventional configuration, the FIA/stopped-flow mode requires a timer or an active interface connected to a computer or microprocessor to synchronize the halting of the pump with the injection (Fig. 3b). The advantages of this mode have been shown by Tougas and Curran in the determination of dopamine [41].

(iii) Use of On-line Separation Techniques

Although FIA has been used with virtually all types of separation techniques [16–19] only some of them (e.g. liquid–liquid extraction and HPLC) have been employed for drug analysis.

Liquid–liquid extraction was one of the first separation techniques coupled to FIA. Karlberg *et al.* proposed a number of methods for drugs [42–45], methods for calculation of constants and parameters characteristic of the process [42, 44] and the design of the separation system components [44, 45]. Improved phase segmentors [46] and separators [46–48] have been reported by different authors, who demonstrated the value of their designs by the determination of drugs such as enalapril [46], caffeine [47] and procyclidine [48]. In all cases, the determinations were carried out in the organic phase.

Possibly, the most important achievement in the FIA/liquid–liquid extraction coupling was due to Fossey and Cantwell, who achieved perfect separation between both phases by using a dual membrane (porous Teflon and paper) and determined one analyte in each. They accomplished in this way the quantitative extraction of diphenhydramine into a cyclohexane phase at pH 10, while 8-chlorotheophylline remained in the aqueous buffer phase. The assays were performed at the rate of two per minute and with precision and accuracy of 1% [49]. One approach which improves the efficiency of liquid–liquid

extraction uses no phase separation nor the conventional units, but the iterative change of the flow direction [50]. The results clearly improve those of conventional configurations; in addition, the system being simpler and more convenient. In some cases, liquid–liquid extraction can be avoided by using turbidimetric detection. The determination of an anthelmintic drug (lavamisole) with HgI_4 is an example of the use of ion-association compounds in drug analysis [51].

Recently, our research team used precipitation–filtration–washing–dissolution continuous systems coupled on-line with an atomic absorption spectrometer to perform the indirect determination (inorganic and organic anions) and preconcentration of traces and subtraces of metal ions [52]. Several sulphonamides in pharmaceutical formulations and in urine have been indirectly determined by continuous precipitation with Ag(I) or Cu(II) by measuring the signal decrease caused by injection of the sample into a stream of cation reagent. The determination range is between 2.5–35.0 $\mu g\ ml^{-1}$ with a RSD of 1.5–3.0% and a sampling frequency of 100 h^{-1}. It is interesting to note the absence of interferents from the sample matrix [53]. Local anaesthetics (ledocaine, tetracaine and procaine) have been determined in pharmaceutical formulations by using a Co(II) solution as carrier–reagent [54].

FIA has also been used as a derivatization/detection post-column system in HPLC to decrease the workload associated with repetitive control determination. The coupling allows for minimal set-up and changeover time between samples and also provides the high accuracy and precision required in a testing laboratory [55]. In addition, the HPLC–FIA coupling contributed other advantages to the routine analysis of analytes with similar properties which require a long time for separation by HPLC, and whose allowable or permissible level is surpassed only in some samples. In such cases, the basic FIA system is used for the fast overall quantitation of the total analyte contents. Only those samples surpassing the established level are injected into the chromatograph in order to determine which analyte(s) cause(s) the out-of-range situation. In these instances, the FIA manifold acts as a derivatization/detection post-column system [56, 57].

(iv) Improvements in Detection Systems

Although in most cases the use of a flow-cell in a conventional detector has sufficed to monitor the measured parameter, some situations require less common techniques.

Among optical techniques, photometry has been by far the most frequently used, whether with direct measurements of the analyte [58] or its product after a redox [59–62], complex formation [63–65] or dye formation [66] reaction. The enormous sensitivity characteristic of chemiluminescent reactions has been exploited by Townshend *et al.* for the determination of narcotics in the fmol range [67, 68]. The features of this technique call for special cells [69–71]. In any case, the advantages of this detection technique are boosted by the continuous flow system.

As stated above, the most important advantage of turbidimetric detection is the possibility of avoiding the use of on-line separation techniques [51, 72].

The coupling of a fast-scan detector and FIA for multicomponent determination has also been exploited in drug analysis. A photodiode array detector coupled to a single-channel manifold allowed the accurate resolution of mixtures of two, three and four components with completely overlapped spectra by the normal and derivative modes [73].

The large variety of electroanalytical techniques offer a wide range of possibilities for continuous detection, further broadened by the design of new cells and operation modes [74]. Conventional direct current amperometry with a glassy carbon working electrode has so far been the technique most frequently used in the FIA determination of various types of drugs used as antihypertensive agents [75, 76], anticoagulants [77], analgesics [78] and others [79–82]. The Kel-F-graphite electrode has provided excellent results in the determination of oxytocic drugs [83]. In spite of the difficulties inherent in the use of dropping mercury electrodes in flowing systems, they have provided interesting methods in FIA/drug analysis [84–87]. Distinct advantages in this detection technique have been provided by a carbon fibre array electrode, as that proposed by Belan and Anderson for the determination of phenotiazines [88]. Other amperometric modes as those of pulse [89, 90], biamperometry [91], linear-sweep [41] and subtractive stripping [92] voltammetry have demonstrated their excellent performance in these fields. Potentiometry is the electroanalytical technique least used for this type of analysis [23, 93, 94].

An unusual detection technique in FIA that again demonstrates the versatility of FIA, is enthalpimetry. Decristoforo and Danielsson have developed a method for the fast (40–45 samples h^{-1}), reproducible (RSD 0.8%) and specific determination of β-lactams based on the measurement of the enthalpy of enzyme reactions by means of thermistors. Enzyme reactions were carried out on small solid reactor columns, whereby the selectivity for various analytes was exclusively dependent on the specificity of the chosen enzyme system. Accuracy of enzyme thermistor–FIA coupling was evaluated by comparison with HPLC. Statistically no significant difference was found between the two methods [95].

(v) Degrees of Automation

The degree of automation of an FIA analyser ranges from completely manual operation [96] (manual injection and data collection through a recorder or display) to the analytical black box [97]. A passive interface is the most elementary and common means of automating this technique [20, 25]. Dispensable active interfaces controlling the impulsion and injection systems have been used in FIA titrations, in addition to the indispensable passive interface to perform accurate peak-width measurements at preselected peak height(s) [24, 25]. Nevertheless, there are other FIA modes such as stopped-flow [41], iterative change of the flow direction [98] and open–closed [99] configurations, in which the presence of an active interface is essential to their performance.

(vi) On-line Control of Evolving Systems

Flow analysis is the easiest and most convenient way to perform near-real time measurements. When the evolution of the system under control is very fast, a completely continuous analyser [100] is required to monitor in a continuous fashion the state of the system. For evolutions requiring sampling frequencies $\leqslant 100\ h^{-1}$, FIA affords great reagent and sample economy. Dissolution tests, which permit one to elucidate the kinetics of drug dissolution, pose problems still unsolved owing to the wide variety of such kinetics and to the impossibility of obtaining results in real time. The earliest attempt in this context was made by Koupparis *et al.*, who performed dissolution studies according to a program loaded in a microcomputer's memory. A calibration graph was

initially obtained by using standards of the compound (phenothiazine) examined. Then, a dosage form was placed into a screen basket immersed and rotating in a double-wall beaker. The dissolution medium was thermostatted at 37 ± 0.5°C. The filtered dissolution medium was recirculated continuously through the sample loop and was injected into the carrier stream at pre-set time intervals. At the end of the experiment, the entire dissolution profile was presented on the chart recorder as a series of absorbance peaks versus time, and also on the computer's printer as a table of time, absorbance, drug concentration and percentage dissolution values [101]. Recently, these authors reported new automated dissolution studies [27] involving sulphonamide formulations in which an automated FIA analyser was coupled with the USP rotating basket apparatus as shown in Fig. 4a, similarly as in their earlier experiments. When measuring sample solutions with undissolved material from the excipients, the solution was centrifuged or a disposable filter (filter for pipette tips) was attached to the end of the sample probe so as to provide on-line filtration [27]. An automated system corresponding to the block diagram in Fig. 4b has been patented by Valcárcel *et al.* [102]. It is a highly versatile modular design adaptable to a variety of needs, which consists of four modules: (1) Dissolution module, comprised of three or six externally thermostatted glass reservoirs with individual stirring (paddle or basket) systems which work simultaneously. This module responds to the USP's specifications. (2) Storage–solvent addition–waste–washing module, consisting of one or several solvent reservoir(s)

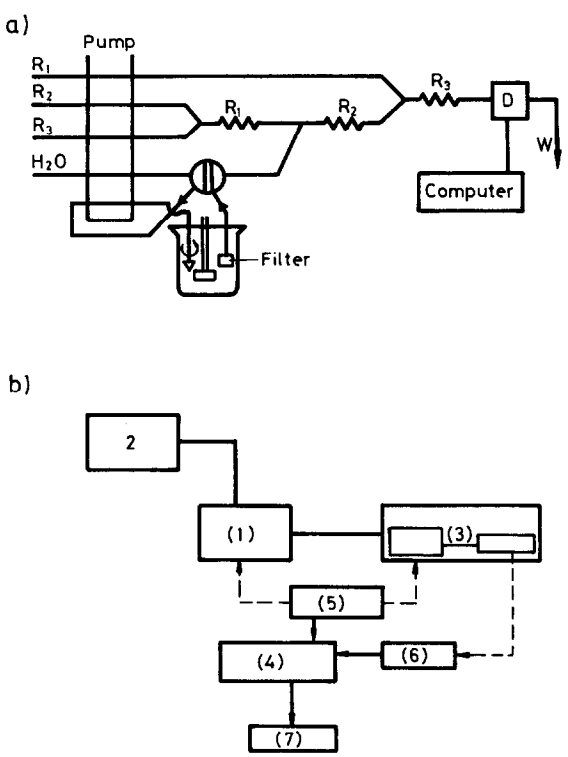

Figure 4
FIA-dissolution test approaches. (a) Manifold used by Koupparis *et al.* [26], (b) block diagram of the analyser patented by Valcárcel *et al.* [101] (for details, see text).

connected through a distributing valve to each of the dissolution glasses in the module (1). This module allows the contents of each glass to be discarded and the glass to be washed and re-filled for automatic re-utilization. (3) An FIA module of variable design suited to the particular requirements. It consists of one or several peristaltic pumps, injection and selecting valves, reactor(s), separation system(s) and an optical or electroanalytical detector. (4) A microcomputer furnished with an active interface (5) to control modules (1), (2) and (3), and a passive interface (6) to collect the signals from the detection system and display them directly via the printer (7) — kinetic curve — and/or treat them as required. The aims of this analyser are reducing the cost of automatic systems; reducing human intervention in these processes; controlling systems affording very fast dissolution kinetics; allowing the control of systems with fast evolution of the active compounds; allowing the control of systems with extremely slow dissolution kinetics involving the appearance of very small concentrations of the active compounds or their degradation products; meeting the needs imposed by the evaluation of the dissolution of active compounds occurring at small concentrations; allowing the use of very small sample volumes whenever the solution volume is a critical variable; improving the sensitivity and selectivity of the detection system through the continuous development of chemical derivatization reactions; and creating new detection modes in addition to those used to date with dissolution tests in order to increase the potential of single and multi-determinations.

Conclusions

The large variety of problems it solves in drug analysis shows the usefulness of FIA in this field, where, despite its great potential, it has not been fully exploited, probably because of its scarce commercial diffusion in some countries and because chemists are not yet fully aware of its great possibilities as an efficient tool for routine analysis.

Acknowledgement — The CICYT is thanked for financial support (Grant No. PA86-0146).

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[Received for review 16 May 1989]