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Novel flow injection analysis systems for drug analysis*

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Abstract: Flow injection analysis (FIA) has become a versatile tool for rapid and automated analyses. As its capabilities have increased, so have the complexity and operation of the apparatus. We have been investigating ways to simplify both the apparatus and the application of injection techniques. A novel cam-driven syringe pump and the development of sequential injection analysis (SIA) are reviewed, and some applications presented. Flow injection coulometric titrations are presented as a means to further alleviate reagent stability and calibration problems. These systems have potential for automatically carrying out many drug assays.

Introduction

The technique of flow injection analysis (FIA) was conceived in 1975 [1], and has become a useful tool for rapid and automated analysis [2, 3]. There are now over 3000 publications in the scientific literature on the development and applications of FIA. As it has matured, the capabilities have increased to the point that nearly any analysis involving solution chemistry can be performed. Hence, it has great potential for improving the efficiency of many drug analyses performed today. Multiple reagents can be used and even multicomponent analyses are possible. Physical operations such as solvent extraction can be performed [4], as well as dialysis and gas diffusion. Small volumes of samples and reagents are used, minimizing waste disposal problems.

Since its early days, flow injection analysis has been recognized as a valuable tool for drug analysis. An obviously valuable application is solvent extraction [5, 6]. Various detection techniques have been utilized, including amperometry [7] voltammetric adsorptive preconcentration [8], chemiluminescence [9, 10], turbidimetry [11], and dual wavelength monitoring [12]. Several authors have developed automated FIA systems for drug analysis [13–17] and the technique has found use in drug–protein binding studies [18–22].

As the capabilities of FIA have increased, the apparatus has tended to become less simple [23]. One goal of research in the Flow Injection Analysis Laboratory at the University of Washington has been to simplify the instrumentation while maintaining maximum flexibility and automation, and increasing reliability. Specifically, we have been investigating ways to make pumping more reliable and to utilize single channel systems for most analyses, while maintaining versatility. To this end, a cam-driven syringe pump with sinusoidal flow [24] and the technique of sequential injection analysis (SIA) were developed [25, 26] and are being applied for various types of analyses. These have the potential for application to many pharmaceutical analyses and tests. A summary of the basis for the techniques and some applications will serve to illustrate this potential.

Cam-Driven Syringe Pump

Peristaltic pumps are the most common means of propelling solutions in various flow injection systems. These are relatively inexpensive and widely available, and work fine for many applications. The restrictions are that they are subject to pulsations and the pump tubing stretches and wears with use, changing calibrations and necessitating intermittent changing, and adding to operational costs and maintenance requirements. The pumping of nonaqueous liquids is restricted.

In order to alleviate some of the limitations of peristaltic pumps, a cam-driven syringe pump was designed and constructed [24].

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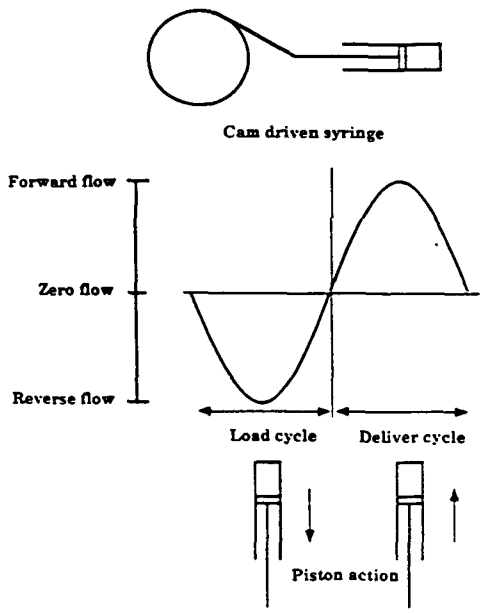

Figure 1
Principal of cam-driven sinusoidal flow syringe pump.

Figure 1 illustrates its principle of operation. The piston moves back and forth in the syringe as the cam rotates. The volumetric flow is maximum at mid-cycle and zero at each end of the cycle. Hence, a sinusoidal flow pattern results during a complete revolution (cycle) of the cam. Solution is aspirated into the syringe in the reverse direction and is delivered in the forward direction. The flow is pulse free, and while the flow rate varies with time, the flow pattern is reproducible, making it well suited for flow injection applications.

The volumetric flow rate, Q, is governed by a number of parameters, as expressed by:

$$Q = 2R\pi^2r^2v\sin\alpha, \qquad (1)$$

where R is the cam radius, r the syringe radius, α the angle of the piston arm relative to the beginning (extreme piston) cam position, and v is the cam rotation speed in hertz. Hence, the flow rate can be controlled by the speed setting of the cam motor, by changing the syringe, or by adjusting the radius of the cam. The maximum flow occurs at the 90° and 270° cam positions and is given by:

$$Q_{max} = 2R\pi^2r^2v. \qquad (2)$$

Zero flow occurs at the 0° and 180° positions. By employing a high gear ratio (1:625), flow rates can be precisely controlled.

The sinusoidal nature of the flow pattern allows rapid filling or flushing of the system at mid-cycle positions, while providing very precise delivery near the 0° and 180° positions, a feature of critical importance in the sequential analysis technique described below.

The manner in which the cam drives the syringe is shown in Fig. 2. A peristaltic pump (Alitea USA, Medina, WA 98039) is modified by incorporating a 1:625 ratio gear box to provide the slow drive ratio necessary for precise control. On the cam, there is a wheel which can be positioned in either of four holes at different radii, marked 2, 3, 4 and 5 in Fig. 2. During operation, the wheel runs in a

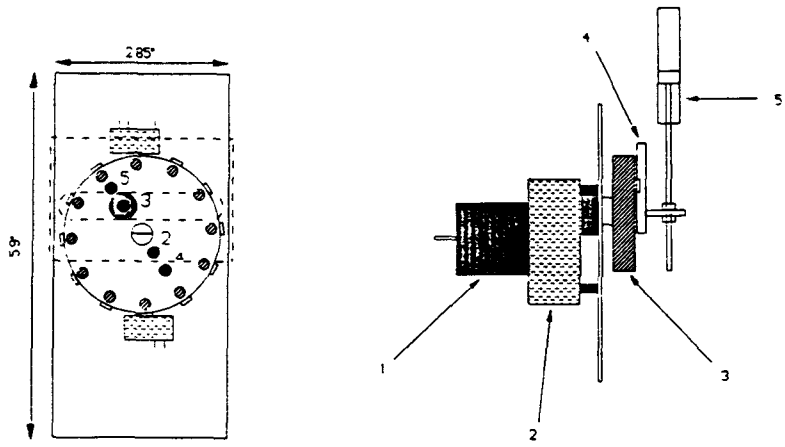

Figure 2
Design of cam drive. (Left) Cam and microswitches. 1, Rotating cam; 2, wheel in track of plate (dashed line) in which the wheel moves as the cam rotates (the numbers on the cam identify different holes for mounting the wheel); 3, cylinder stops (the set screws are on the rim of the cam) (only one stop is set to contact the switches); 4, microswitches; 5, plate that moves up and down during each cycle, to move the syringe pistons (dashed rectangle). (Right) Side view of pertinent components. 1, DC motor; 2, gear box (1:625); 3, cam; 4, moving plate; 5, syringe.

track that is milled in a rectangular plate. The plate is connected to two rails, on each side of the plate, which are part of the chassis. The plate thus moves up and down as the cam rotates. The pistons for two syringes are connected to this plate and consequently they also move with the cam. The cam contains cylinder stops (set screws are located on the perimeter of the cam) that are positioned 30° apart and can be set to activate microswitches at 0° and 180°. The switches can be used to (1) substantially trigger switching of an electronically actuated two-position valve in the stand-alone operation of the pump (complete displacement mode) or (2) in computer-controlled operation. Under computer control, any flow scheme can be implemented.

The use of the pump for flow injection requires two syringes, as illustrated in Fig. 3, operated in conjunction with an automatic electronically actuated valve [24]. The two syringes are driven by the same cam. In the load cycle (reverse flow), sample is aspirated into the sample loop of the injection valve by means of one syringe, and reagent is aspirated into the other syringe. In the inject cycle (forward flow), the valve is turned to inject the sample in the sample loop into the reagent stream, which is then propelled to the detector in the usual way. This operation is performed automatically by placing microswitches at the 0° and 180° positions of the cam. The microswitches activate the valve, causing it to turn to either the load or the inject position. This occurs at zero flow positions, avoiding pressure build-up. The sequence is repeated for each rotation of the cam, and each cycle can be manually initiated by means of a start switch on the motor controller. Pumping of wash solution is done between samples.

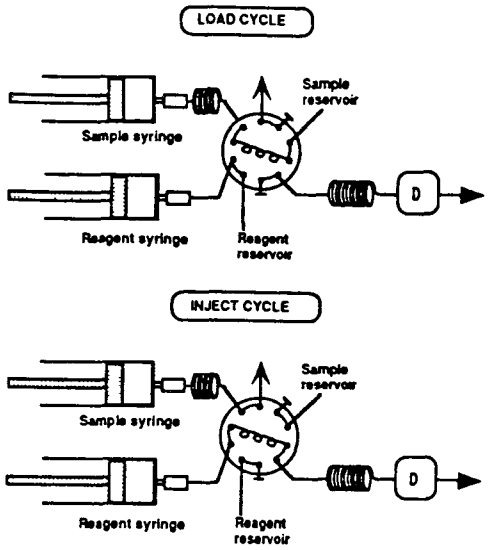

Figure 3
Operation of syringe pump in flow injection analysis mode.

If the pump and valve are controlled by a computer, then the syringe can be operated over a narrow flow range, e.g. in mid-cycle. This minimizes reagent and sample consumption. Computer control is mandatory in the SIA applications below.

The reproducible timing of the pump results in precise flow injection measurements [24], even though the flow is not constant. The use of pump tubing is avoided, the pump provides pulse free flow, and nonaqueous solvents can be readily handled.

Sequential Injection Analysis

While complex manipulations and the use of multiple reagents can be accomplished using conventional flow injection analysis, the design of the apparatus and flow manifolds becomes complicated. The technique of sequential injection analysis [25, 26] is a modified flow method that simplifies the manifold design and allows most analyses to be performed using a single line. The sinusoidal flow syringe pump is well suited for this new injection technique, particularly since it is readily computer controllable at very slow flow rates. But a high quality peristaltic pump that can be precisely stopped will work as well, e.g. the Alitea USA C4-V pump that was modified for the syringe pump.

The principle of the SIA technique is shown in Fig. 4. Since it employs a single line, only one syringe is used. Rather than an injection valve, a computer-controlled selection valve is used (e.g. Valco, Houston, TX) in synchronization with the pump. In operation, the pump is

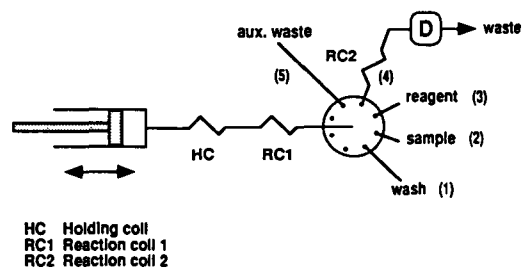

Figure 4
Operation of the syringe pump in sequential injection analysis mode.

first operated in the reverse direction to sequentially aspirate different solutions through different parts in the valve into a holding coil. The holding coil and syringe are first filled with wash solution to prevent solutions from getting into the syringe and to wash the system between samples. Then a few microliters of sample is aspirated, the pump is stopped, the valve turned to a reagent port, and a few microlitres of reagent is aspirated next to the sample. Hence, the sample and reagent are sequentially stacked next to each other. Then the valve is switched to the port leading to the detector, and the zones are propelled through a reaction coil and through the detector cell; the zones merge, causing reaction to occur as in conventional FIA, and a transient signal is recorded. The system is flushed with wash solution from the syringe, preparing it for the next cycle and sample.

A variety of operational configurations may be employed to extend the range of samples that can be analysed [26]. For example, a water spacer may be aspirated between the sample and reagent to decrease the degree of overlap, so that higher concentrations can be measured. The sample may be aspirated between two or more different reagents, for multiple reagent chemistries. The relative volumes of reagents and samples may be adjusted to provide a wide working range. If complex chemistries are involved, then a small mixing chamber may be placed at one of the ports [27]. Multiple solutions can be aspirated from the holding coil into the chamber for reaction, followed by aspiration to the detector.

The technique of SIA is in its infancy, but has the potential of automatically performing most assays done with conventional FIA. The apparatus is relatively inexpensive, is readily automated, and easy to adapt to a particular chemical method.

Flow Injection Coulometric Tritrations

The pioneering work of the late Prof. Gaston Patriarche and his students in the application of coulometric titrations to pharmaceutical analyses established this technique as a valuable one for performing many analyses without the necessity of stable reagents or standards [28]. Nearly every common titrant can be generated electrochemically, including acid–base, redox, precipitation and complexometric titrants [29, 30]. In addition, unstable titrants can be readily generated, such as Cu(I), Br_2, Cl_2 and Cr(II).

The new technique of stopped flow injection coulometric tritrations combines the advantages of FIA and coulometric titrations [31]. Figure 5 illustrates this measurement system. An injected sample is carried into a 0.7 ml mixing chamber containing a magnetic stirring bar. The flow is stopped at a predetermined time, arresting a fixed portion of the sample in the chamber, creating a gradient dilution. The sample is then titrated by electrochemical generation of the titrant as a coiled platinum electrode at the bottom of the cell, until an end point is recorded, in this case a photometric end point. Other methods of detection may be employed, such as potentiometric, amperometric or conductometric. Faraday's law is used to calculate the amount of analyte titrated. The system must be calibrated for a given flow stop time. The range of sample concentrations that can be titrated is a function of the stop time and the generating current selected. Thus, 0.1–15 M nitric acid has been titrated without prior dilution [31]. The tech-

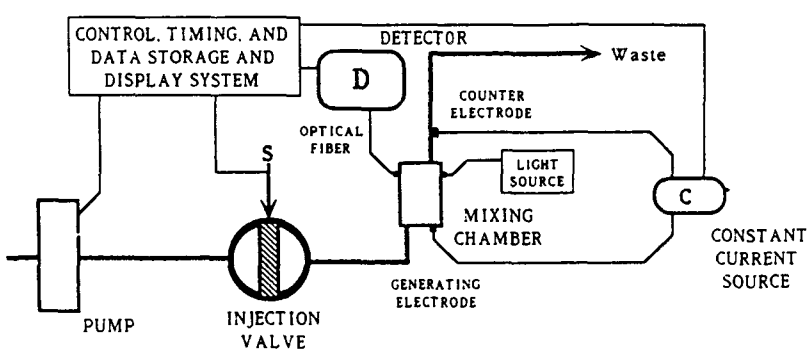

Figure 5
Flow injection coulometric titration system.

nique has been used for bromine number determinations [32] and for iodometic titration of starch and metabisulphite in corn syrup [R.H. Taylor, G.D. Christian and J. Ruzicka, unpublished].

The entire system is under computer control, including injection of the sample, stopping the flow, activating the generating current, detecting the end point and reading out the signal. It should be a valuable tool for the automatic assay of many pharmaceuticals using only a few microlitres of sample solution.

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