Novel Approaches to Flow Injection Measurements: Sinusoidal Flow, Sequential Injection, and Sensor Injection

Gary D. Christian
Department of Chemistry, BG-10
University of Washington
Seattle, Washington 98195

Quimica Nova
Proceedings of VI En Centro Nacional de Química Analítica
Araraquara, SP, Brasil, September 3—6, 1991

Following the inception of flow injection analysis (FIA) by Ruzicka and Hansen (1), much of the early pioneering work on the development of this important new analytical technique was performed in Brazil (2). This work provided the foundation for the rapid growth of FIA, with over 3,000 publications now in the scientific literature. The versatility of the technique is illustrated by examples of merging zone methods (3), stopped flow analysis (4), the interfacing to a variety of instruments such as atomic spectrometers (5, 6), and multicomponent analyses. Numerous advances have continued in recent years in the sophistication of operations that can be performed by using FIA. Yet, the increased flexibility and capabilities that have resulted are often at the expense of increased complexity, which can lead to decreased long term reliability in routine operations (7). The Flow Injection Analysis laboratory at the University of Washington has been investigating approaches to simplify the mechanics of operation in FIA and thereby increase the reliability of operations. The long term goal is to develop systems that can be run automatically and unattended for extended periods of time, e.g., in field operations.

Summarized here is some recent work on a new approach to pumping solutions, based on a piston pump that delivers sinusoidal flow, and the new concept of sequential injection analysis, both in single line modes. These combined technologies are utilized in various applications, including sensor injection and a new stopped flow coulometric titration system that eliminates reagent preparation, calibration and storage.

Sinusoidal Flow Pump

The most common way of propelling solutions in various FIA applications is by means of peristaltic pumps. While these work well for many applications, are relatively inexpensive and are readily available, they are subject to pulsations; and the pump tubing wears and stretches with use. Since the flow rate is proportional to the square of the tube radius, relatively frequent recalibration may be required in continuous operation. And frequent tube replacement adds to the cost and the attention to maintenance. In addition, the pumping of nonaqueous solvents requires solvent-resistant pump tubing, and even then the tubing tends to be attacked.

In order to overcome some of these limitations, a syringe pump based on a cam driven piston was developed (8). The principle of the pump is shown in Figure 1. The cam driven syringe piston moves just as the piston in an automobile engine, i.e., it moves in one direction during one-half of the cam cycle (to aspirate solution) and then in the opposite direction during the other half of the cycle (to deliver solution). Dual syringes are used in conjunction with an automatic electrically activated injection valve, first to aspirate sample into the sample loop and reagent solution into the carrier line (reverse direction flow), and then to inject the sample into the carrier (forward direction flow). Solution flow is maximum at the 90° and 270° positions of the cam and zero at the 0° and 180° positions. The result is a sinusoidal flow pattern. The flow rate at any given position of the cam is given by:

$$Q = 2R\pi^2 r^2 v \sin \phi \quad (1)$$

where R is the cam radius, r the syringe radius, ϕ the angle of the piston arm relative to the beginning (extreme piston) cam position, and v is the frequency of cam rotation in herz. The maximum flow rate is

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

The flow rate is most readily varied by changing the speed setting (frequency of cam rotation), although the syringe may be changed. Also, the cam is designed to allow positioning of the piston drive arm at different radius positions on the cam. A high gear ratio, 1:625, is used, so very slow flow rates can be precisely delivered.

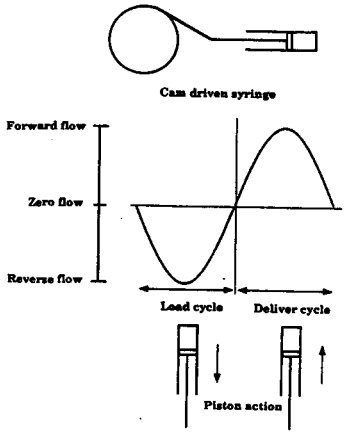

Figure 1. Conceptual representation of a sinusoidal flow syringe pump.

The operation of the pump in the flow injection mode is shown in Figure 2. A microswitch is activated by a stop on the cam at the 0° and 180° positions, which triggers switching an electrically activated injection valve at those positions (when the flow is zero—avoiding pressure pulsations); the valve is thereby switched between the load and inject positions. Two syringes are driven simultaneously by the cam. In the reverse flow direction, sample is loaded into the sample loop while carrier (reagent) is loaded into the second syringe. At the 180° position, the valve is switched to the inject position, the flow is reversed to the forward direction, and the sample is injected into the carrier stream and carried toward the detector, as in conventional FIA. The sequence is repeated for each rotation of the cam. Each cycle can be manually initiated by means of a start switch, for injection and measurement of a series of samples (with pumping of wash solution between samples).

If computer control of the pump and valve is employed, then the flow can be operated over a narrow range of the cam position, in mid-cycle. In this manner, the flow variation is small and reagent and sample consumption is minimized.

This pump has been demonstrated to provide precise FIA measurements (8). Thus, while it is generally assumed constant flow rate is necessary to perform flow injection measurements, a non-constant flow such as the sinusoidal flow employed here is perfectly satisfactory, provided timing is reproducible. In addition to avoiding the use of pump tubing and its inherent problems, this pump provides pulseless flow.

Sequential Injection Analysis

The sinusoidal flow syringe pump is very useful for a new injection technique called sequential injection analysis (SIA) (9), particularly since it is readily computer controllable at very slow flow rates, a requirement for SIA. The principle of the SIA technique is shown in Figure 3. The injection valve is substituted by an electrically activated computer-controlled directional valve, which is operated in synchronization with the pump. A single line system is again used. But rather than injecting the sample into a carrier or reagent in the usual manner, the sample plug (a few microliters, as in FIA) is aspirated into the line by operating the syringe pump in the reverse flow direction for a fixed period. (This step is preceded by aspirating wash solution into the syringe and a holding coil, to prevent cross contamination between samples.) The flow is stopped and the valve is switched to the reagent inlet and then a few microliters of reagent are aspirated, next to the sample; so the sample and reagent are sequentially stacked next to each other. The valve is then switched to the port leading to the detector, and the flow is changed to the forward direction, causing the two zones to disperse and merge in the reaction coil as they are propelled to the detector. The point along the merged zones at which the sample and reagent are equally dispersed is called the isodispersion point.

The detector may be placed between the pump and the valve, with a reaction coil preceding the detector. In this case, a sharp peak is recorded as the sample passes through the detector in the reverse flow direction, followed by a broader, more dispersed peak when the flow is changed to the forward direction to flush the sample from the system. If no chemical reaction is needed, i.e., the carrier stream is simply used to carry the sample to a selective detector (e.g., electrode), then the system is operated in a sensor injection mode. Again, a double peak is recorded as the sample is washed from the system. The sensor injection technique minimizes exposure of the sensor to the sample matrix, as in FIA.

The SIA technique may be operated in a variety of configurations (10). A spacer (e.g., water) may be aspirated between the sample and the reagent, to

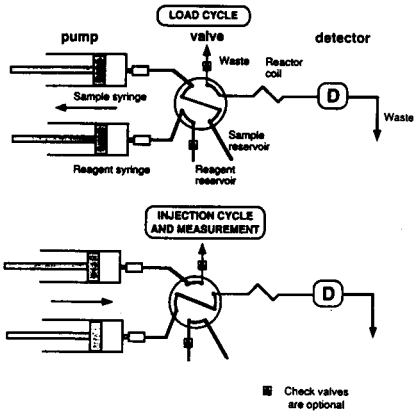

Figure 2. Flow injection manifold employed with the sinusoidal flow pump and an eight port injection valve. The role of the check valves is to prevent movement of the liquid in the lines. If the line is hydrodynamically balanced, the check valve is not needed. This mode of operation can be carried out without computer control, since the valve is activated by two microswitches and

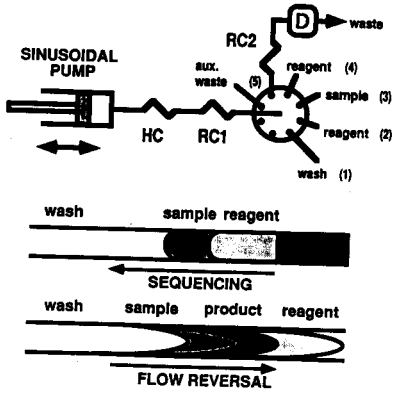

Figure 3. Sequential injection system using directional valve (top) and the structure of sequenced and interdispersed zones (below). HC is the holding coil, R1 and R2 are reactor coils, and D is the flow through detector. The pump and valve are under computer control.

decrease the degree of overlap and provide gradient dilution, for operating over a wide range of concentrations. Or, the sample may be sandwiched between two different reagent zones, for multireagent chemistries; hence, a simple single line system may be used for measurements that would require multiple lines in the usual FIA mode. If the two reagent concentrations are large compared to the analyte concentration in the sample, then the peak maximum will occur at or near the isodispersion point.

The relative volumes of sample and reagent are important in establishing the degree of sample/reagent overlap (10). Increased equal volumes of sample and reagent result in less complete overlap, but increased signal (decreased dispersion) at the isodispersion point, where $D_{sample} = D_{reagent}$. The result is also increased analysis time since larger volumes must be transported through the detector and washed from the system. On the other hand, increased volumes and the resulting broader peak allow for a greater range of gradients from which to make measurements.

If the sample volume is increased relative to the reagent volume, the completeness of overlap (zone penetration) is decreased, but the signal is increased due to less dispersion at the isodispersion point. When the sample volume is small compared to that of the reagent, the zone penetration is nearly complete, and mimics conventional FIA. For optimum conditions for SIA, the reagent volume should be at least twice the sample volume, which in turn should be no larger than the $S_{1/2}$ volume (the volume required to reach a steady state signal).

Zone penetration, especially for viscous samples, may be enhanced by flow reversal (11). Band spreading, σ^2, is proportional to the number of reversals, N, and the square of the length traveled, l^2, and so the distance traveled during flow reversal is more important than the number of reversals.

The limitation of the sequential injection technique is that computer control of the pump and valve is required, and the measurement process is longer than in conventional FIA, but the rugged and versatile nature of the single line technique makes is potentially useful for a number of routine applications.

Stopped Flow Coulometric Titrations

In the technique known as "flow injection titration" (12), a sample (e.g., acid) is injected into a carrier titrant (e.g., base) that contains an appropriate indicator (e.g., acido-basic). The analyte and titrant will be equivalent at each edge of the dispersed sample plug, at which point the indicator changes color. The width recorded signal (from measuring one form of the indicator) is proportional to the logarithm of the analyte concentration. This allows for a wide dynamic range, but at some expense of precision. In addition, the carrier (titrant) must be calibrated, and frequently checked for stability.

The new technique of "stopped flow injection coulometric titration" overcomes some of these limitations (13). The principle of the measurement technique is shown in Figure 4. The injected sample is carried into a mixing chamber (*ca.* 0.7 ml) containing a magnetic stir bar, and the flow is stopped at a fixed time, arresting a certain fraction of the sample in the chamber. An exponential gradient dilution results, in which the ratio of injected amount, C_o, to determined amount, C_{det}, of analyte is given by:

$$\frac{C_o}{C_{det}} = e^{t/T_i} \qquad (3)$$

where t is the stop time and T_i is equal to V_m/Q (the ratio of the mixing chamber volume to the volumetric flow rate). This gradient dilution is similar to the split

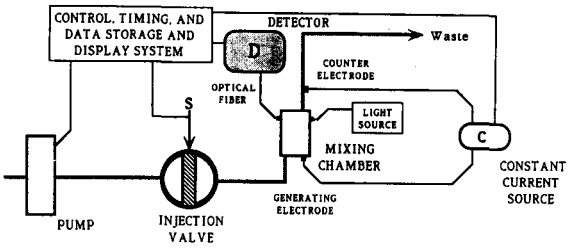

Figure 4. Stopped flow coulometric titration system. Light is transmitted from the light source, through the gradient (mixing) chamber, and to the detector via optical fibers. The injection valve, pump, and constant current source are controlled by computer, and the titration signal is collected by the computer.

zone dilution technique (14). When the sinusoidal flow pump is used, the dilution is a convolution of the sinusoidal pattern and the exponential gradient. A wide range of concentrations may be measured without prior dilution by stopping the flow at different t-values. The amount stopped in the flow cell is determined by coulometric titration (15), by electrolytically generating the titrant at an appropriate electrode in the chamber (e.g., by electrolysis of water at a platinum cathode to generate base—the carrier is an electrolyte suitable for generating the appropriate titrant). The end point is detected by photometric measurement of an acido-basic indicator, pH electrode, etc. The amount of analyte titrated is calculated from Faraday's law, i.e:

$$N = Q/nF = it/nF$$

where N is the number of moles titrated, n is the electron change, F is the Faraday constant, and Q is the number of coulombs required to generate the titrant and is given by the product of the generating current (amperes) and the time to reach the end point (seconds).

The entire system is under computer control. The flow and stop time of the sinusoidal flow pump is preset, an electrically activated injection valve automatically injects the sample, the generating current is automatically started after the flow is stopped, and the computer collects the data from the end point sensor.

Using this system, injected acid over a range of 0.1 M to 15 M was accurately determined (13). While the dilution is an exponential function, the end point is a linear function of the amount of analyte at a given stop time, and the titration range may be varied also by changing the generating current. Hence, the stopped flow coulometric titration technique is useful for analyzing samples over a wide dynamic range through the use of flow programming and current control, and it provides "reagentless titration" in which the titrating agent

is generated in situ, thus eliminating the need for calibration and storage of reagents. A large number of reagents may be generated coulometrically, i.e., acids and bases, redox agents, complexometric agents and precipitating agents (15, 16). So this simple automated technique should find use in a wide range of applications, especially for concentrated samples that require dilution.

References

1. J. Ruzicka and E. H. Hansen, Anal. Chim. Acta, 78, 145(1975)
2. J. Ruzicka and E. H. Hansen, "Flow Injection Analysis," 2nd ed., Wiley Interscience, New York, 1988
3. H. Bergamin F°, E.A.G. Zagatto, F.J. Krug and B.F. Reis, Anal. Chim. Acta, 101, 17(1978)
4. G. D. Christian and J. Ruzicka, Anal. Chim. Acta, submitted
5. A.O. Jancintho, E.A.G. Zagatto, H. Bergamin F°, F.J. Krug, B.F. Reis and B.R. Kowalski, Anal. Chim. Acta, 130, 243(1981)
6. G. D. Christian and J. Ruzicka, Spectrochim. Acta, 43B, 157(1987)
7. G. D. Christian, J. Flow Injection Anal., 7, 86(1990)
8. J. Ruzicka, G.D. Marshall, and G. D. Christian, Anal. Chem., 62, 186(1990)
9. J. Ruzicka and G.D. Marshall, Anal. Chim. Acta, 237, 329(1990)
10. T. Gübeli, G. D. Christian and J. Ruzicka, Anal. Chem., submitted
11. G.D. Clark, J. Zable, G. D. Christian and J. Ruzicka, Talanta, 38, 119(1991)
12. J. Ruzicka, E. H. Hansen, and H. Mosbaek, Anal. Chim. Acta, 92, 235(1977)
13. R.H. Taylor, J. Ruzicka and G.D. Christian , Talanta, in press
14. G.D. Clark, J. Ruzicka and G.D. Christian, Anal. Chem., 61, 1773(1989)
15. G.D. Christian, "Analytical Chemistry," 4th ed., John Wiley & Sons, New York, 1986, Chapt. 12
16. G.D. Christian and J.E. O'Reilly, "Instrumental Analysis," 2nd ed., Allyn and Bacon, Boston, 1986, Chapt. 4

BIOSENSORS FOR EXPERIMENTAL MEDICINE

M. Mascini
Dipartimento di Sanita' Pubblica e Chimica Analitica Ambientale
Universita' di Firenze
Via Gino Capponi 9, 50121 Firenze (Italy).

Abstract

The application of biosensors in medicine fall in three major areas: analysis of clinical samples, ex vivo monitoring and in vivo monitoring. Clinical chemistry is the most advanced area and biosensors for glucose are commercially available; in the next future we expect the availability of new biosensors. Continuous monitoring has been achieved with large instruments and only recently with portable instruments. Two major approaches are described; the fabrication and use of needle electrodes and the coupling between microdialysis and biosensors. By using the last technique a prototype has been assembled for glucose continuous measurement of reduced dimensions for 24 hours monitoring (Glucoday).

Introduction

Electrochemical biosensors found wide interest in clinical chemistry and medicine. Physiologists, cardiologists, diabetologists dream for years about the possibility to monitor continuously chemical parameters to feed back appropriate action to restore the values to normal levels. In the last 15 years a large number of publications, reviews, books, workshops have been devoted to this topic. No operating completely implantable biosensor is presently commercially available, but many approaches have been reported and great advances have been achieved (1-4).

I will report the major advancements in three areas of interest in medicine, namely clinical chemistry, ex vivo monitoring, in vivo monitoring.

Clinical chemistry

The most successful approach in this area has been the "glucose pen" commercialized in USA and in Europe (5).

The "glucose pen" has been accepted by thousands of patients for glucose home testing for two main features: high reproducibility which eliminate calibration; reduced size (and cost) for the transducer and recording apparatus. Now several other researches are in progress for coupling enzymes with mediators to obtain similar features for other metabolites; therefore in the next future an alcohol pen, lactate pen, cholesterol pen, will be available in the future. However the main disadvantages of the glucose pen are: 1) it is suitable only for a single measurement i.e. it cannot be used twice, and 2) it cannot be applied in continuous monitoring.

Two approaches can be discussed for continuous monitoring, the ex vivo and the in vivo monitoring.

Ex vivo monitoring

Continuous measurement for glucose has been introduced several years ago with complex instruments called "artificial pancreas". These instruments have also an insulin feedback delivery system regulated by an algorithm function of the blood glucose concentration (6-7). These instruments represent a great improvement in the treatment of diabetes, but they still do not completely normalize altered concentrations of intermediary metabolites such as lactate, pyruvate, alanine and ketone bodies. Information on the concentration of these metabolites might be