

Flow Injection Analysis: An Investigation of N-Phenylbenzohydroxamic Acid

LISA ANN BLYSHAK
AND
A.J.C.L. HOGARTH*
Department of Chemistry
DePauw University
Greencastle, Indiana 46135

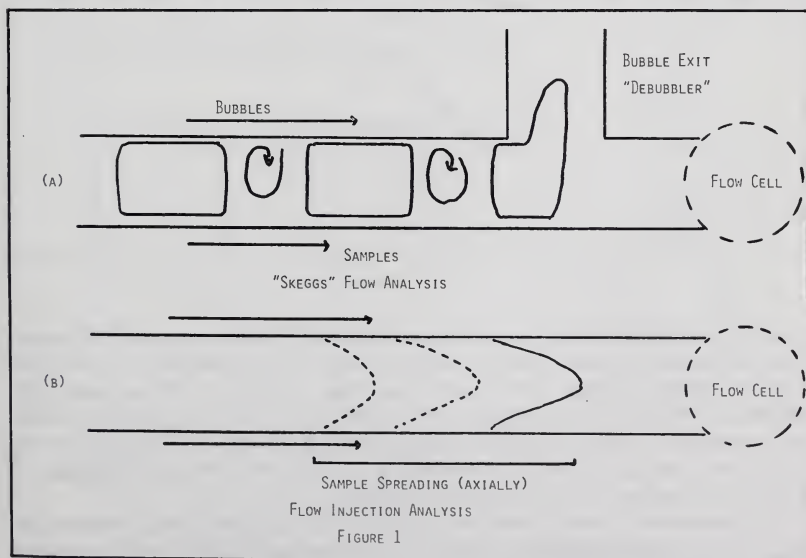
Introduction

Flow Injection Analysis (FIA) is a recent and innovative addition to the analyst's repertoire. It is a very flexible and versatile system, originally designed for undergraduate teaching^(3,4), which has found its way rapidly to the forefront of research.

Generally, FIA may be regarded as a method wherein a sample is injected into a continuous stream of reagent, the concentration of analyte being measured virtually uninterrupted by bubbles or other reactions. Samples pass through narrow bore tubing and are mixed with incoming reagents at various points, and then proceed through a cuvette, potentiometric cell etc., where the appropriate signal is monitored continuously. Recordings of these signals are made in the usual way.

One major difficulty with continuous monitoring is the prevention of two consecutive samples from mixing whilst they pass through the system; one ingenious, and frequently used, solution to the problem was developed by Skeggs⁽⁶⁾. This involved segmentation of the liquid stream using bubbles, and such an artifice is used with the Technicon AutoAnalyzer. This device is one type of instrumentation which may handle many individual tests concurrently.

Although a well-tried method for separating consecutive samples in a flow system, the bubble segmentation concept has a number of disadvantages. One of these is the necessity for removing the bubbles before the sample enters the system (Figure 1):

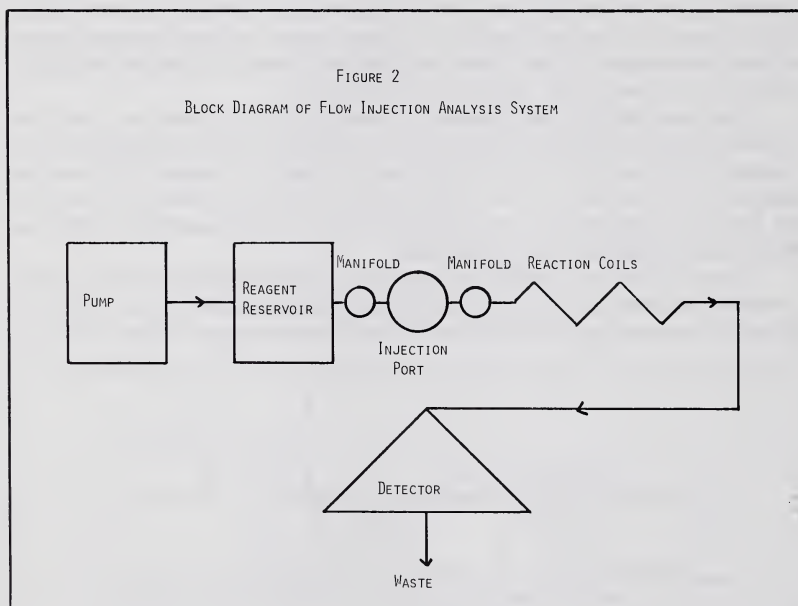


*Current address: Ross Laboratories, 625 Cleveland Avenue, Columbus, Ohio 43216

a "debubbling" device has to be added; also another pump has to be inserted into the system specifically to introduce bubbles. Debubbling has to be accomplished efficiently otherwise the signal obtained would be intermittent and unsuitable for recording.

Another disadvantage of the segmented system is that air is compressible, *i.e.*: when a sample or another air bubble is introduced, the other air bubbles tend to compress slightly. This leads again to a slight oscillation of the measured signal. This disadvantage is coupled also with that of bubbles in plastic tubes being electrical insulators, thus supporting the growth of an electrostatic charge on the tube. This has the effect of disturbing the signals developed by sensors, particularly potentiometric ones which are commonly used with FIA.

FIA, however, has certain distinct advantages over the air segmentation system. In the first place the apparatus is considerably simpler often requiring only one pump for what could be quite an involved analysis (Figure 2). As the analyses become more



complex it is a simple procedure to add more pumps and inlets as required. If reagents and analytes are required to flow at different rates, it is still possible to use the same pump in many instances merely by altering other parameters such as pump tube diameter⁽⁷⁾. Another clear advantage over the Skeggs system is that since the samples are injected into a continuous flowing stream of reagent, and no air bubbles are used to separate consecutive samples, the stream may flow directly through a detector cell without first having to pass through a debubbler.

Consider now a major point with regard to both methods: sample-reagent mixing. In the air-segmented stream each individual segment of fluid may be regarded as a vessel separated from contiguous ones by an air bubble. Within each of these vessels the liquid is mixed by friction against the tubing walls (Figure 1a). Each of

these containers, when debubbling is complete, contributes to an increase in the recorded signal until a stable one is produced. This method thus relies on complete mixing.

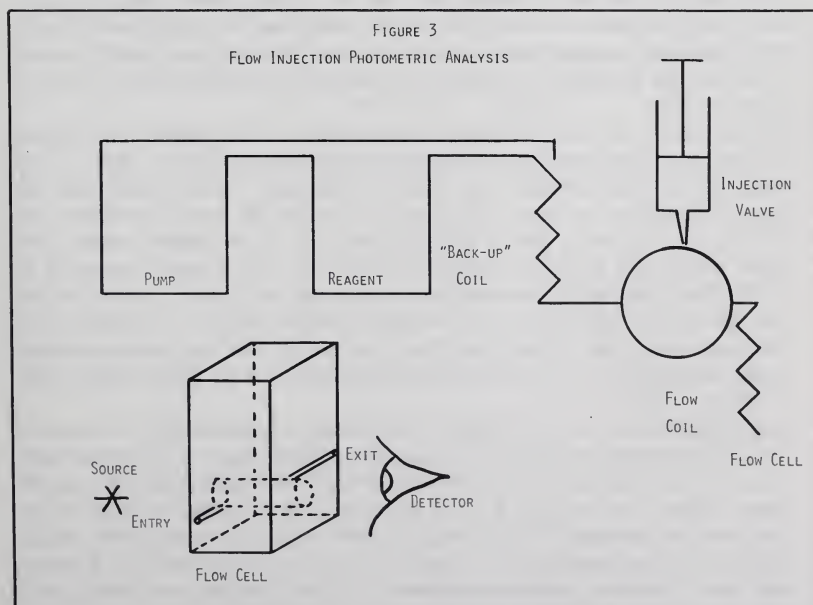
In the case of FIA this is not so. The sample is injected into a continuously flowing stream, progression in which produces some mixing and a parabolic shape for the reaction zone (Figure 1b). Here the mixing is incomplete, but because the mixing pattern for a given experimental arrangement does not change, the shape of the reaction zone is invariant. Often this method leads to rapid results for a determination, whereas time-delay coils are frequently used in the Skeggs system in order to facilitate equilibration before measurement. Merely by using microvolumes of samples and not waiting for complete equilibration increases the frequency with which the analysis may be made.

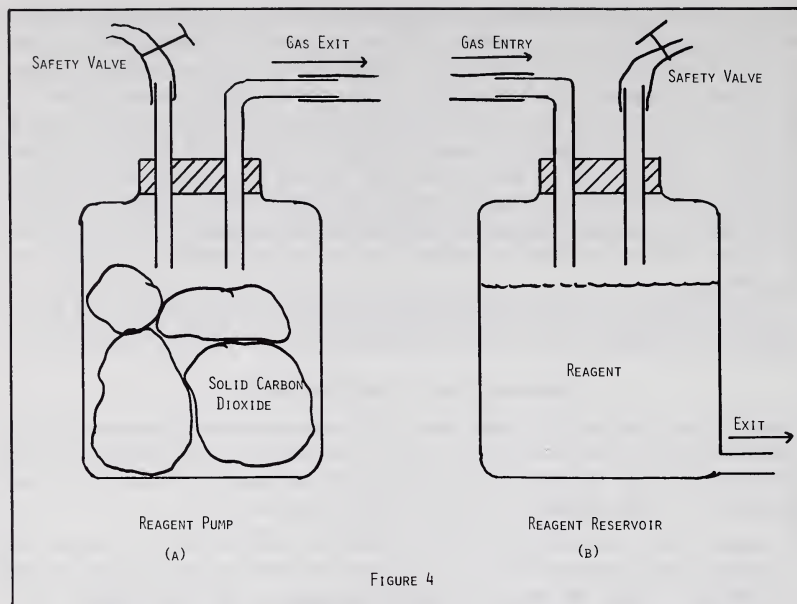
In conclusion, this paper deals with the construction, testing and use of a very simple and inexpensive flow injection analysis system for a student research project.

Equipment Design and Construction

General: Figure 3 shows the arrangements of the various sections of the complete FIA system. Basically it may be broken up into a) a reagent pump; b) a reagent reservoir; c) an injection port; d) a spectrophotometric flow cell. Most items used in the construction are readily available in most chemistry departments.

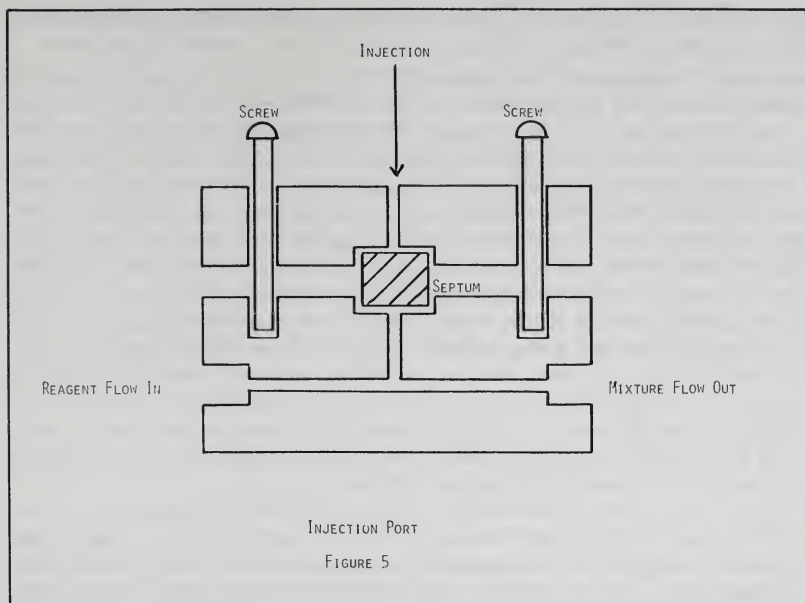
a) *Reagent Pump:* Several devices are available for this purpose, for example gas pumps⁽⁴⁾ and peristaltic pumps⁽¹⁾. The former was selected for its simplicity and availability. Figure 4a shows its detailed construction. The gas generator was a wide mouth plastic bottle (1 lb. capacity) containing dry ice and fitted with an appropriately bored rubber bung. One of the two holes in the latter was fitted with a safety rapid pressure release valve consisting of a piece of glass tubing attached to a piece of rubber tubing fitted with a Mohr clip. The whole bung assembly,





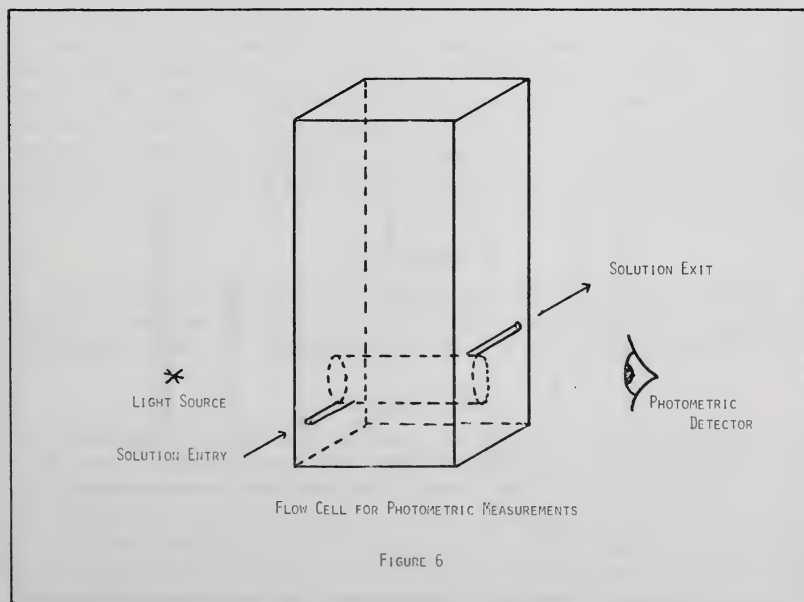
after charging the bottle with dry ice, was wired in place. The supply lasted for at least fifteen hours of continuous use.

- b) *Reagent Reservoir*: Figure 4b shows the very simple construction of this item. The heavy-walled, glass two-liter bottle was fitted with a bored rubber bung containing an angled glass tube to receive gas from the pump, and a straight piece of glass tubing attached to a short length of rubber tubing. The latter was closed by means of a Mohr clip and acted as a safety valve and pressure control device. It too was wired in place.
- c) *Injection Port*: This was constructed of two pieces of clear plastic block (Figure 5). The rough dimensions of the prototype blocks were: top $\frac{3}{4}$ in. wide, $1\frac{1}{2}$ in. long, $\frac{3}{8}$ in. thick; bottom $\frac{3}{4}$ in. wide, $1\frac{1}{2}$ in. long, $1\frac{1}{8}$ in. thick. The top piece of plastic had two screw holes flanking a $\frac{3}{64}$ in. dia. hole flush countersunk $\frac{1}{32}$ in. on one side to form a receptacle for a $\frac{1}{2}$ in. dia. rubber septum. The lower portion was drilled vertically with a $\frac{3}{64}$ in. hole to about two-thirds of its thickness, and then the top was flush countersunk by $\frac{1}{16}$ in. to hold the rubber septum. This hole was drilled completely through the block to intersect with the lower end of the vertical hole. Each end of this hole was countersunk and tapped appropriately to hold polypropylene connectors for the Teflon tubing (0.047 in. dia.).
- d) *Spectrophotometric Flow Cell*: Figure 6 shows the spectrophotometric flow cell which was constructed from a rod of 1 cm square section clear plastic. The rod was bored with a $\frac{1}{8}$ in. dia. hole, and $\frac{3}{64}$ in. holes were bored tangentially to carry the plastic tubing. Grooves $\frac{1}{16}$ in. deep and wide were cut along the length of the rod from the entrances of the $\frac{1}{16}$ in. diameter holes to hold the plastic tubing. The rod was long enough to just fit neatly into a square cuvette holder for a Bausch and Lomb Spectronic 20 spectrophotometer. The hole/light path was closed at each end by attaching a window of thin clear plastic sheeting.



Experimental

General: All chemicals used were of analytical grade; all solutions were made up with distilled water as the major solvent. The *N*-phenylbenzohydroxamic acid (PHB) was



obtained from Aldrich Chemical Company, Inc. and was recrystallized twice from dilute acetic acid, and stored in a desiccator. Triton X-100 was used as the surfactant.

- a) *Testing the Apparatus:* The apparatus was tested by injecting small volumes of bromothymol blue solution (0.440g in 25ml 96% ethanol + 75ml 10^{-2} M borax) into a flowing stream of 10^{-2} M borax. The wavelength was set at 620nm in order to monitor the dye. Flaws in the system such as air bubbles, leaks and blockages were rapidly detected and corrected. Reproducibility of the system was also tested at this stage (Figure 7b), and this will be discussed in the Results Section.
- b) *Iron(III) Chloride as the Reagent:* Iron(III) chloride and PBH were made up to be 0.01M in 0.01M hydrochloric acid. It was found that more concentrated solutions would induce precipitation. Test injections were made of the hydroxamic acid into iron(III) chloride as the reagent solution, after appropriate setting of the wavelength. Results are shown in Figures 7a and 7b. The wavelength used was 520nm.
- c) *N-Phenylbenzohydroxamic Acid as the Reagent:* Again, individual solutions of iron(III) chloride and PBH were made up to be about 0.01M, and the hydroxamic acid was used this time as the flowing reagent. Test injections were made of varying quantities of the iron(III) solution. The results are identical to those in Figures 7a and 7b. The wavelength used was 520nm.
- d) *Interferences:* Using approximately 0.01M N-phenylbenzohydroxamic acid as the flowing reagent and constant volume injections of iron(III) solution "spiked" with varying concentrations of copper(II), cobalt(II), nickel(II), chromium(III) and manganese(II), the interference of these metals, commonly found in steel alloys, was ascertained. The wavelength used was 520nm.

Results and Discussion

Visually the overall results obtained were similar to those of other FIA analyses using spectrophotometric detection (Figure 7a)^(1,2). The initial experiments with

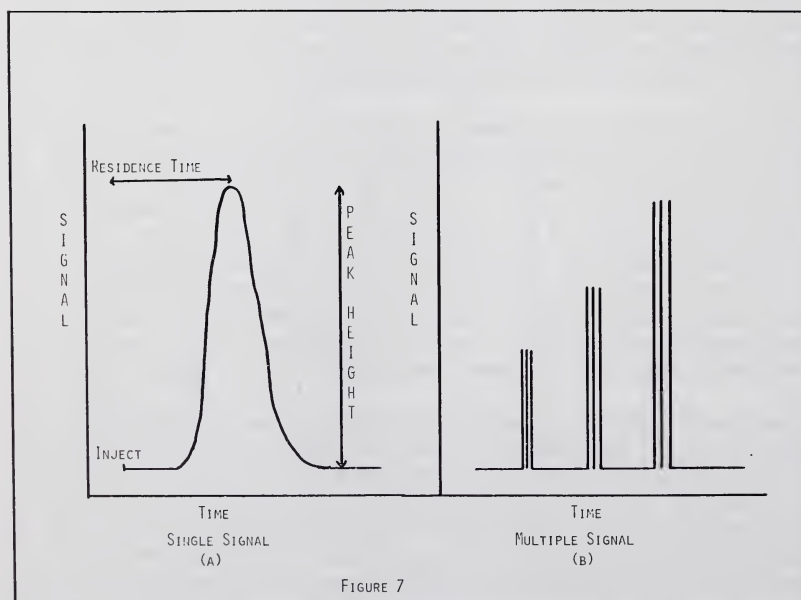
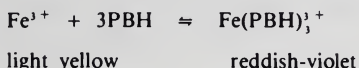


FIGURE 7

bromothymol blue indicator gave a certain amount of practice to the operator in terms of "what to look for"; they also gave some data with regard to reproducibility *i.e.*: about $\pm 2\%$. This compares well with those data obtained with more sophisticated systems *i.e.*: about $\pm 1-2\%$. Naturally, however, this will vary from system to system and should be determined experimentally for each.

When working with the iron(III)/N-phenylbenzohydroxamic acid system, it was shown that relatively low levels of either iron(III) or PBH could be determined depending upon which was used as the reagent. The results for PBH as the reagent are quite interesting. Up to a concentration of 150ppm of iron(III), if peak height is plotted against concentration, an excellent straight line is produced (correlation coefficient >0.985). Increasing the concentration range to about 1750ppm and plotting this against peak height as the analytical parameter, produces a graph of considerable curvature. This effect is due to the hydroxamic acid system becoming flooded with iron(III) according to the reaction:



A plot of peak width at half-height against concentration at this stage is a useful exercise because the injected iron(III) does diffuse with respect to a concentration gradient, and although the optical system may still be saturated, how long it remains so becomes important. Plotting peak width at half-height versus concentration produces a graph in which a steady and quite linear increase is evident. There is an initial curvature to the graph, but this is due to the fact that the height is still increasing but the width is remaining essentially constant. Thus the linear portion of this graph occurs between about 150ppm and 1750ppm of iron(III), and at concentrations below 150ppm it is more appropriate to use peak height as the analytical parameter.

With regard to interferences, studies on solutions of iron(III) containing between zero and 1000ppm of copper(II), cobalt(II), nickel(II), chromium(III) and manganese(II) were made. Although in a few cases a certain overall decrease in signal was observed, this was seen to be a proportional decrease, *i.e.*: the signal decreased in the same proportion all along the curve also causing a decrease in the intercept. The latter effect was observed also when peak width at half-height was plotted against iron (III) concentration. The slope of the graph did not change and so sensitivity was not affected, although it was noted that the results showed a little more scatter than just with iron(III).

Acknowledgments

The authors wish to thank the Chemistry Department at DePauw University for the use of chemicals and equipment; the Johnson's Wax Fund, Inc. for equipment funding; and Wilbert C. McCluer for the gift of a research scholarship (LAB).

Literature Cited

1. Betteridge, D., Cheng, W.C., Dagless, E.L., David, P., Goad, T.B., Deans, D.R., Newton, D.A., and Pierce, T.B. 1983. An Automated Viscometer Based on High-precision Flow Injection Analysis. *Analyst* 108:1
2. Faizullah, A.T. and Townshend, A. 1985. Application of a Reducing Column for Metal Speciation by Flow Injection Analysis. *Analytica Chimica Acta* 167:225
3. Hansen, E.H. and Ruzicka, J. 1979. The Principles of Flow Injection Analysis as Demonstrated by Three Lab Exercises, *J. Chem. Ed.* 56:677

4. Lown, J.A., Koile, R. and Johnson, D.C. 1980. Amperometric Flow-through Wire Detector: A Practical Design with High Sensitivity. *Analytica Chimica Acta* 116:33
5. Ruzicka, J. and Hansen, E.H. *Flow Injection Analysis*. Wiley Interscience, New York, 1981
6. Skeggs, L.T. 1966. New Dimensions in Medical Diagnoses. *Anal. Chem.* 38(6):31A
7. Vanderslice, J.T., Stewart, K.K., Rosenfeld, A.G., and Higgs, D.J. 1981. Laminar Dispersion in Flow Injection Analysis. *Talanta* 28:11