CHEM 3211

Introduction to Flow Injection Analysis (FIA)

Determination of Chloride Ion Concentration

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Flow Injection Analysis Principles

Flow injection analysis (FIA) is based on the injection of a liquid sample into a moving, nonsegmented continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that continuously records the changes in absorbance, electrode potential, or other physical parameter resulting from the passage of the sample material through the flow cell.

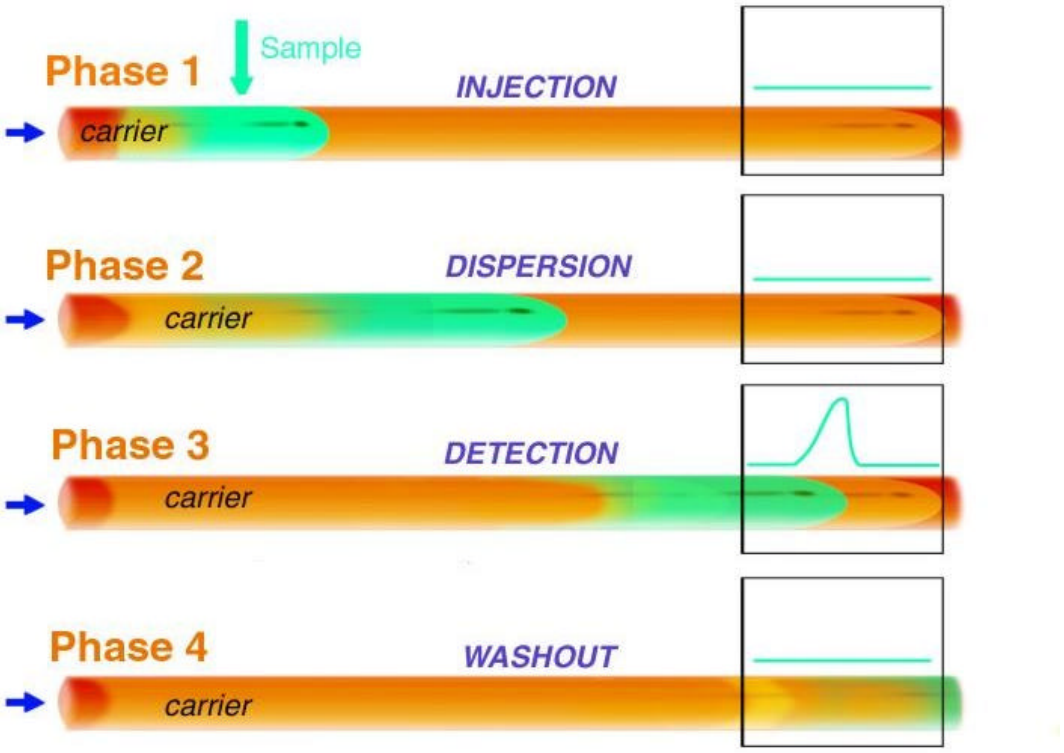

Figure 1. Four phases of FI Analysis

An example of one of the simplest FIA methods, the spectrophotometric determination of chloride, is shown in Figure 2. This is based on the release of thiocyanate ions from mercury(II) thiocyanate and its subsequent reaction with iron (III) and measurement of the resulting red color (for details, see Experiment). The samples, with chloride contents in the range 5 -75 ppm chloride, are injected (S) through a 30 µL valve into the carrier solution containing the mixed reagent, pumped at a rate of 0.8 mL/min. The iron(III) thiocyanate is formed on the way to the detector (D) in a mixing coil (0.5 m long, 0.5 mm i.d.), as the injected sample zone disperses in the carrier stream of reagent. The absorbance A of the carrier stream is continuously monitored at 480 nm in a micro flow-through cell (volume of 10 µL) and recorded (Figure 2b). To demonstrate the reproducibility of the analytical readout each sample in this experiment was injected in quadruplicate, so that 28 samples were analyzed at seven different concentrations of chloride. As this took 14 min, the average sampling rate was 120

samples / h. The fast scan of the 75- and 30-ppm sample peaks (shows on the right in Figure 1b) confirms that there was less than 1% of the solution left in the flow cell at the time when the next sample (injected at S2) would reach it, and that there was no carryover when injecting the samples at 30-s intervals.

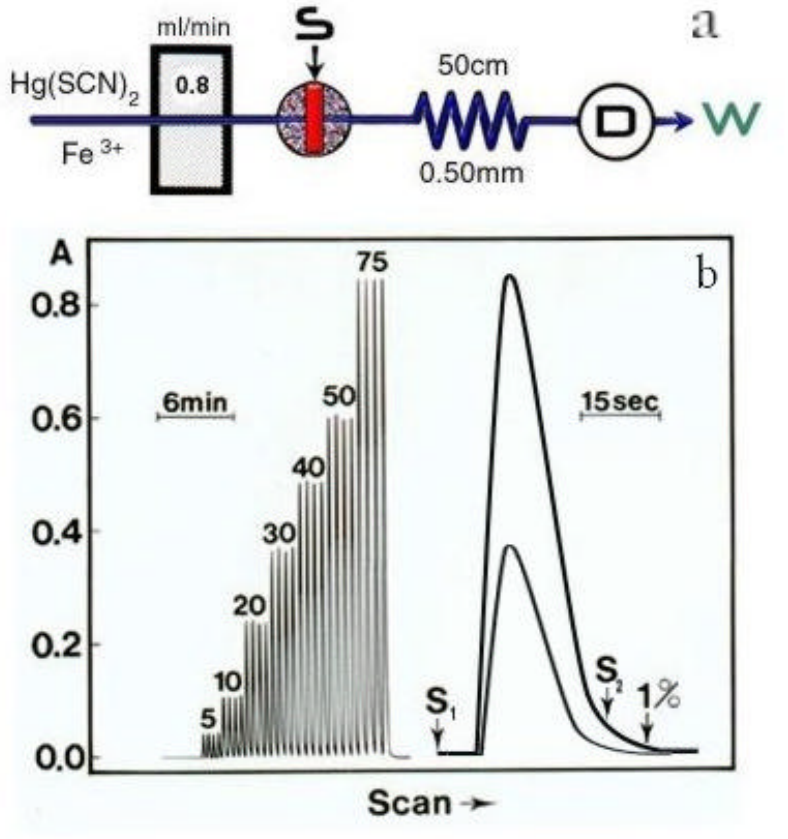

Figure 2. (a) Flow diagram for the specrophotometric determination of chloride: S is the point of sample injection, D is detector, and W is waste. (b) Analog output showing chloride analysis in the range of 5 - 75 ppm Cl^- with the system depicted in (a).

A key feature of FIA is that since all conditions are reproduced, dispersion is very controlled and reproducible. That is, all samples are sequentially processed in exactly the same way during passage through the analytical channel, or in other words, what happens to one sample happens in exactly the same way to any other sample. FIA is a microchemical technique which consumes minute quantities of sample and reagents and therefore generates less than 1 mL of waste per assay (Figure 2).

The FIA System

The simplest flow injection analyzer (Figure 3a) consists of a pump, which is used to propel the carrier stream through a narrow tube; an injection port, through which a well-defined volume of a sample solution S is injected into the carrier stream in a reproducible manner; and a microreactor in which the sample zone disperses and reacts

with the components of the carrier stream, forming a species which is sensed by a flow-through detector and recorded. A bypass loop allows passage of carrier when the injection valve is in the load position. A typical recorder output has the form of a peak (Figure 3b), the height H, width W, or area A of which is related to the concentration of the analyte. The time span between the sample injection S and the peak maximum, which yields the analytical readout as peak height H, is the residence time t during which the chemical reaction takes place. A well-designed FIA system has an extremely rapid response, because T is in the range 5 - 20 s. Therefore, a sample cycle is less than 30 s (roughly T + t_b) and thus, typically, two samples can be analyzed per minute. The injected sample volumes may be between 1 and 200 μL (typically 25 - 50 μL), which in turn requires no more than 0.5 mL of reagent per sampling cycle. This makes FIA a simple, automated microchemical technique, capable of a high sampling rate and minimum sample and reagent consumption.

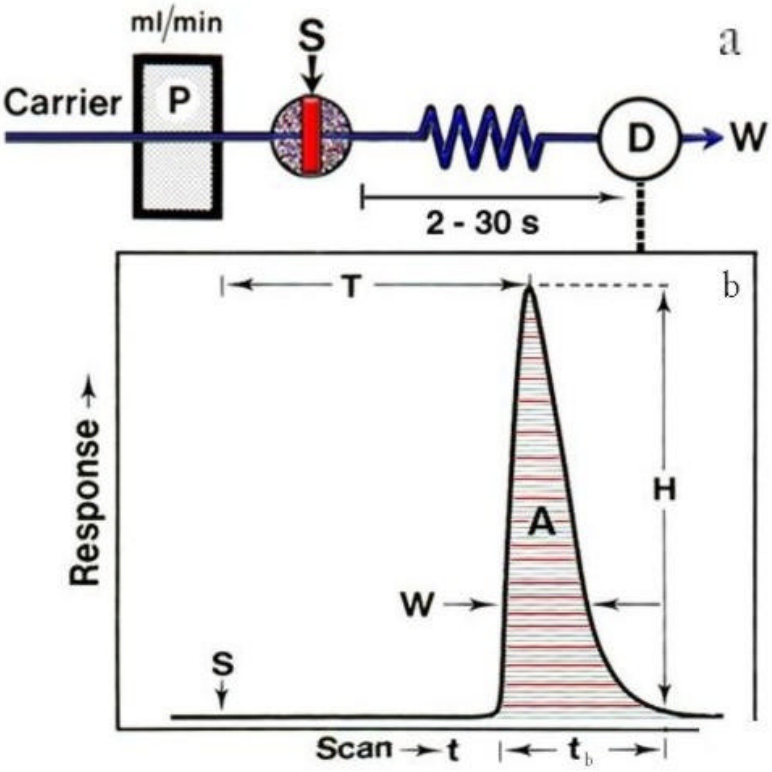

Figure 3. (a) The simplest single line FIA manifold utilizing a carrier stream of reagent; S is the injection port, D is the flow cell, and W is the waste. (b) The analog output has the form of a peak, the recording starting at S (time of injection t_o). H is the peak height, W is the peak width at a selected level, and A is the peak area. T is the residence time corresponding to the peak height measurement, and t_b is the peak width at the baseline.

FIA, then, is based on a combination of three principles: sample injection, controlled dispersion of the injected sample zone and reproducible timing of its

movement from the injection point to the detector. Thus, in contrast to all other methods of instrumental analysis, the chemical reactions are taking place while the sample material is dispersing with the reagent, that is, while the concentration gradient of the sample zone is being formed by the dispersion process.

FIA is a general solution-handling technique, applicable to a variety of tasks ranging from pH or conductivity measurement to colorimetry, titrations, and enzymatic assays. To design any FIA system properly, one must consider the desired function to be performed. For pH measurement, or in conductometry, or for simple atomic absorption, when the original sample composition is to be measured, the sample must be transported through the FIA channel and into the flow cell in an undiluted form in a highly reproducible manner. For other types of determinations, such as spectrophotometry, the analyte has to be converted to a compound measurable by a given detector. The prerequisite for performing such an assay is that during the transport through the FIA channel, the sample zone is mixed with reagents and sufficient time is allowed for production of a desired compound in a detectable amount.

Besides the single line system, a variety of manifold configurations may be used to allow application to nearly any chemical system. The two-line system (B) is the most commonly used, in which the sample is injected into an inert carrier, and then merges with the reagent. In this manner, the reagent is diluted by a constant amount throughout, even when the sample is injected, in contrast to the single line system. Reagent dilution by the sample in a single-line system is feasible so long as there is excess reagent (and $D > 1$) and the reagent does not exhibit a background response that would shift upon dilution. If two reagents are unstable when mixed, they may be mixed on-line (C or D), or they may merge with the sample following injection (E). Mixing coils may be interspersed between confluence points to allow dispersion before merging.

EXPERIMENTAL SECTION

Single-line FIA: Spectrophotometric Determination of Chloride

Principle

The analytical procedure is based on the following reactions:

$$Hg(SCN)_2 + 2Cl^- \Rightarrow HgCl_2 + 2SCN^-$$

$$2SCN^- + Fe^{3+} \Rightarrow Fe(SCN)_2^+$$

The carrier stream contains $Hg(SCN)_2$ and Fe(III). The chloride of the injected sample reacts with $Hg(SCN)_2$, liberating SCN^-, which in turn forms with Fe(III) the red-colored complex ion $Fe(SCN)_2^+$, which is measured spectrophotometrically at 480 nm. The height of the recorded absorbance peak is then proportional to the concentration of chloride in the sample. Besides $Fe(SCN)_2^+$, other (higher) complex ions between Fe(III) and SCN^- might be formed; thus, the calibration curve cannot be expected to be linear over a wide range of concentrations.

NOTE: The reagent used is toxic, since it contains mercury. Collect the waste for safe disposal. Since FIA generates much smaller amounts of waste than manual procedures, the volumes of waste produced will be easily manageable. Further reduction of waste can be accomplished by neutralizing the waste with an excess of sodium hydroxide and subsequent coprecipitation of Fe_2S_3 and HgS with Na_2S.

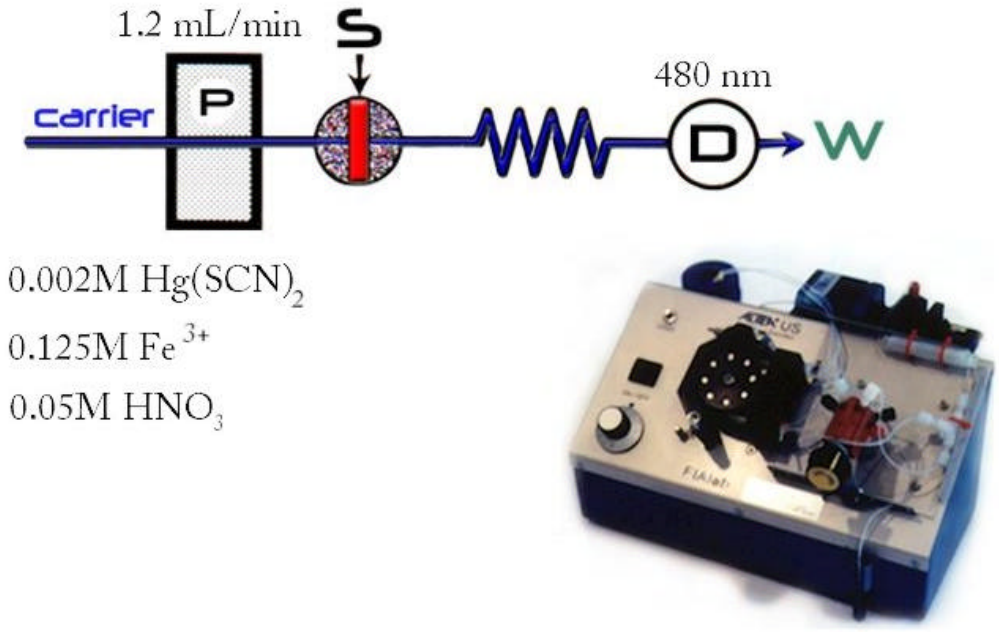

Figure 4. Experimental setup.

Solutions and Chemicals Required

Reagent. The carrier stream is prepared by dissolving 0.626 g of mercury(II) thiocyanate, 30.3 g of iron(III) nitrate, 3.3 mL of concentrated nitric acid, and 150 mL of methanol in water, making the final volume up to 1 L.

Standard Solutions. Standard solutions in the range 5 - 75 ppm Cl^- are made by suitable dilution of a stock solution containing 1000 ppm Cl^- (1.648 g of sodium chloride per liter).

Procedure

Assemble the apparatus in the single-line mode. Use 1.02 mm i.d. pump tubing for the carrier and the sampling tubes. This should provide a flow rate of about 1.2 mL/min when using 15 rpm.

Turn on the detector and allow to warm up for several minutes to stabilize. If a monochromator is used, set to 480 nm. (Since the color produced is specific for the analyte, a simple visible light source-detector system may be used without a monochromator.)

Reagent carrier solution is pumped through the system and the individual chloride standards are injected successively in triplicate, yielding a series of peaks for each. Determine the precision of the procedure by injecting a single standard (in the middle concentration range) ten times. Report the percent relative standard deviation.

Obtain an unknown from your instructor and inject at least three times to obtain an average peak height.

Prepare a calibration curve from the peaks recorded for the standard solutions, and from this calculate the concentration of chloride in your unknown solution.

At the end of the experiment, wash the system thoroughly by pumping distilled water for a few minutes. Include flushing the sampling tube and valve loops. Then release the pump cassettes and pump tubes. If the system is not to be used for an extended time, your instructor will instruct you to empty it.

Flow System Characterization

Determination of flow rates. Fill a 10 mL graduate cylinder with distilled water, record the volume and insert the carrier tube in the cylinder. Turn on the pump and simultaneously start a stop watch (or begin timing with a clock). Pump for five minutes, remove the tube, and turn off the pump. Record the volume of water remaining in the cylinder. Calculate the flow rate in milliliters per minute and record on the form at the end of the experiment.

Perform a similar experiment for the sample flow (injector in the load position).

Finally measure the flow rate of the waste stream from the volume collected for five minutes.

If pump tubing of equal internal diameter is used for all channels, the flow rates should be similar. Also, the flow rate of the waste stream should equal the flow rate of the carrier stream.

Note that the flow rate is directly proportional to the square of the internal radius of a pump tube (i.e., flow is proportional to cross section area = πr^2).

Estimation of sample loop volume. With the pump turned on, place the valve in the load position and remove the sampling tube from the sample. This will allow the loop to fill with air. Then insert the tube in the sample solution and with a stopwatch measure the time from when the sample just enters the loop to when it leaves the loop. Perform the measurement several times and take the average. From a knowledge of the sample flow rate determined above and the measured time to fill the loop, calculate the sample loop volume in microliters. NOTE: This is an estimate and will not include the dead volume of the injector.

Estimation of the flow system volume. Fill the sample loop chloride solution and inject into the carrier. Measure the time to reach the detector. From a knowledge of the carrier flow rate determined above and the measured time, calculate the volume from the injection valve to the detector, in microliters.

Determination of total dead time. With the recorder on, simultaneously inject a sample and start a stopwatch. Measure the time for the sample to reach the detector, i.e., the time for the recorder to begin deflection. This represents the dead time from injection to initial measurement.

Time to flush sampling tube and sample loop. When injecting different samples, it will be necessary to flush the previous sample completely from the sampling tube and the injection loop. Determine the time required to just flush the previous sample by introducing air into the tube and then reinserting the tube in the sample solution with the pump running. Measure the time for the sample solution to reach and fill the sample

loop. Use at least twice this time for introduction of each sample in all future experiments, to allow adequate washing of the loops by the new sample.

From a knowledge of the sample flow rate, also calculate the volume of solution required to flush the line.

Determination of dispersion: Single-line system. Make several injections of the sample and determine the average peak height. This represents H^{max}. Turn the pump off and insert the carrier tube in the solution of mixed sample and carrier (0.5mL/5.0mL respectively). Turn the pump on and record the signal until a steady state signal is obtained. This represents $H°$. Calculate the dispersion at the peak height, D^{max}.

Determination of maximum sample frequency. Inject a sample and record the rise and fall of the peak. Measure the distance from baseline-to-baseline on the peak and convert this to seconds. This will represent the minimum time between injections. Report the maximum sampling frequency in samples per hour.

At the end of the experiment, wash the system thoroughly by pumping distilled water for a few minutes. Also flush the sampling tube and valve loops. Then release the pump cassettes and pump tubes. If the system is not to be used for an extended time, your instructor will instruct you to empty it. In your report, list all instrument parameters and solutions employed.

REFERENCES

1. J. Ruzicka, E. H. Hansen; Flow Injection Analysis, 2nd Ed, 1988,
2. FIAlab Operation Manual
3. Skoog, Holler, Nieman *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing, Fort Worth, TX 1997, Ch. 10.

QUESTIONS

1. Name two processes responsible for FIA response.
2. Name main components of the FIA system.
3. What is the reason behind using stopped flow FIA?
4. What makes dispersion controlled in FIA?
5. What are the parameters responsible for dispersion?
6. What is the role of the radial mixing?
7. Name advantages and drawbacks of multiline systems.
8. Why do double peaks form? Name at least two reasons.
9. What is the main difference between FIA and SIA systems?
10. Name main advantages of Bead Injection Analysis.

Use CD ROM "FIA_BIBLE" to answer those questions.

Operating software WinFIA 3.0 for Windows

1. Open WinFIA
2. Select “Manual Control”
3. In dialog box ”Data readings” click “OFF” button
4. Select “Settings”
5. Click “Spectrometer Config” button
6. Switch off the light source and click “Dark Scan” button
7. Switch on the light source and click “Reference Scan” button
8. Choose absorbance mode (if not on) by pressing “A” button
9. Close “Spectrometer Configuration”
10. Click “Logon” button (make sure that logon was successful)
11. Select “Manual Control”
12. In dialog box ”Data readings” click “OFF” button
13. Select “Plots”
14. After injecting the sample click “Start” (on the right-hand side) and “Recording Off” buttons
15. Click button “Time Series” to view data collection *in situ*
16. When peak starts turn injection valve to the “load” position
17. When signal stabilizes at baseline make a new injection
18. After running all samples click “Reset” and “Recording On” buttons
19. Select “Analysis “
20. Click “Raw Data” button
21. Save data in folder “DATA” and exit

Analyse data with Microsoft Excel.

Things required in the lab report and grade brakdown

1. **Introduction (20 points)**
   - ❖ Theoretical principle of FIA
   - ❖ Apparatus and its components
   - ❖ Description of the principle of the experiment

2. **Experimental part (30points)**
   - ❖ Description of the work done
   - ❖ Results and calculations
   - ❖ Analysis of results
   - ❖ Error analysis

3. **Discussion (25 points)**
   - ❖ Conclusions
   - ❖ Summary

4. **References (5 points)**

Answers to the questions (20 points)

RESULTS OF FLOWSYSTEM CHARACTERIZATION

Determination Of Flow Rates

CARRIER FLOW RATE

Volume at beginning _____mL
Volume after 5 minutes _____mL
Volume pumped _____mL
Flow rate _____mL/min = _____µL/s carrier flow

SAMPLE FLOW RATE

Volume at beginning _____mL
Volume after 5 minutes _____mL
Volume pumped _____mL
Flow rate _____mL/min = _____µL/s sample flow

Estimation Of Sample Loop Volume

Time to fill loop: _____s Average = _____s
_____µL/s ×_____ s = _____µL loop volume

Estimation Of Flow System Volume

INJECTOR TO DETECTOR VOLUME

Time to reach coil: _____s Average = _____s
_____µL/s ×_____ s = _____µL system volume

Determination Of Total Dead Time

Time to reach detector: _____s Average = _____s dead time

VOLUME REQUIRED TO FLUSH SAMPLING TUBE AND SAMPLE LOOP

_____ s × _____ µL/s = _____µL

Determination Of Dispersion, D^{max}

SINGLE-LINE SYSTEM

Steady state signal, $H°$: _____ cm
Peak height, H^{max}: _____ cm Average = _____ cm
$D^{max} = H° / H^{max}$ = _____ cm / _____ cm = _____

Determination Of Maximum Sampling Frequency

Baseline-to-baseline: _____ cm = _____ s
Maximum sampling frequency = _____ samples/h