## Flow Injection Analysis

Almost all reagent based assays can be downscaled and automated by Flow Injection Analysis. FIA was first described in a patent filed in Denmark by Ruzicka and Hansen in 1974. Since then the technique has grown into a discipline covered by 22 monographs and over 20,000 research papers. The scope of the method grew from a tool for automation of serial assays to a method for enhancing the performance of spectroscopic and electrochemical instruments. The conventional FIA methodology, the Flow Injection (FI) is based on continuous flow, while its most recent variant, the Programmable Flow Injection uses different flow rates during individual steps of the assay protocol with aim to optimize the analytical readout. The second generation of FI techniques, the Sequential Injection (SI) also utilizes flow programming to optimize the assay protocol, but in contrast to FI it uses discontinuous flow generated by a syringe pump, and operates at microliter scale.

The Conventional Flow Injection Method
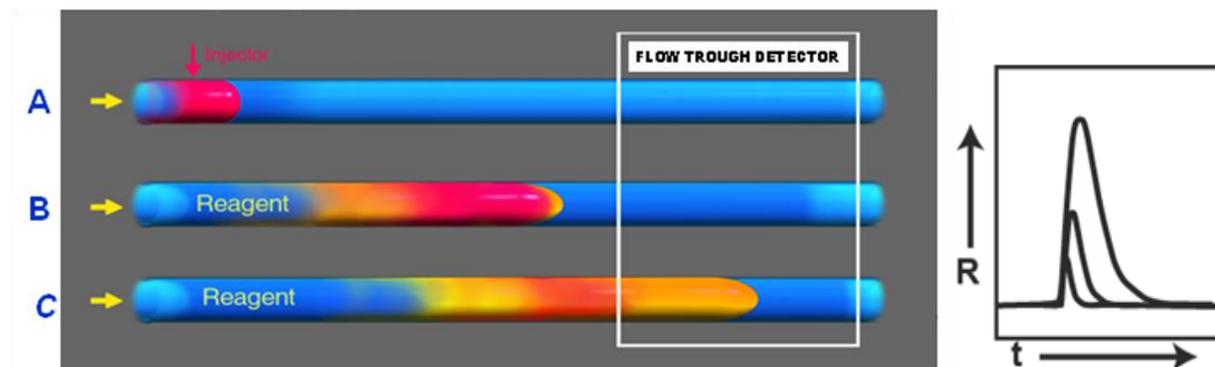
The conventional FI technique is based on injection of sample solution into a carrier solution that is continuously moving at a constant flow rate. The carrier stream transports the analyzed species through the reactor and into the detector. The assay protocol comprises the following steps:

A) Sample injection is designed to meter an exact volume of analyte solution into a flowing stream of reagent.

B) As the sample zone (red) moves downstream, the dispersion process mixes sample with reagent forming a reaction product (yellow). The extent of mixing and the length of reaction time is controlled by the flow rate, by channel volume, and by channel geometry.

C) The reaction mixture flows through the detector yielding an analytical readout.   Since all standards and samples to be analyzed are individually processed in exactly the same way, the calibration curve is valid for unknown samples to be assayed

The peak height recorded by the detector is proportional to the analyte concentration.

Single Line System: Chloride Assay
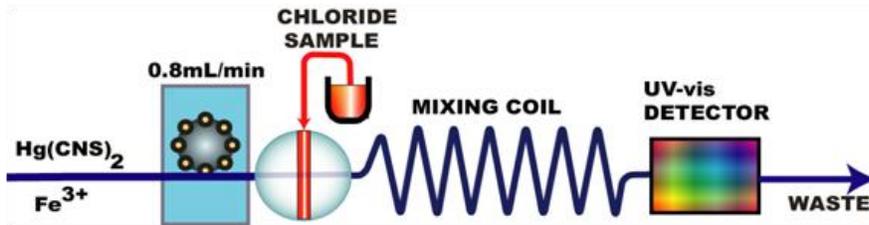
Flow diagram for colorimetric assay of chloride
.
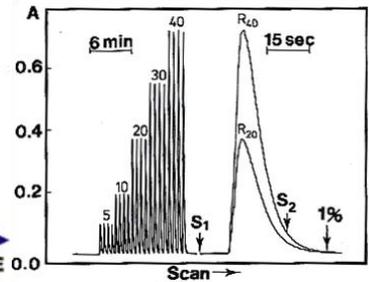
Colorimetric Assay of Chloride.
Each sample injected four times.

In this, the simplest configuration, a chloride sample is injected, by means of a two position valve, into a continuously flowing carrier stream of reagent. As the injected zone moves downstream, the sample solution disperses within the reagent, forming a reaction product. A flow through detector placed downstream records absorbance, yielding readout within 15 seconds after sample (S1) injection.

Three Line Systems: Nitrate Assay
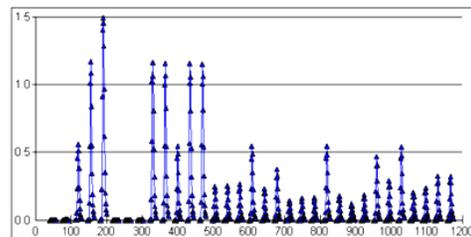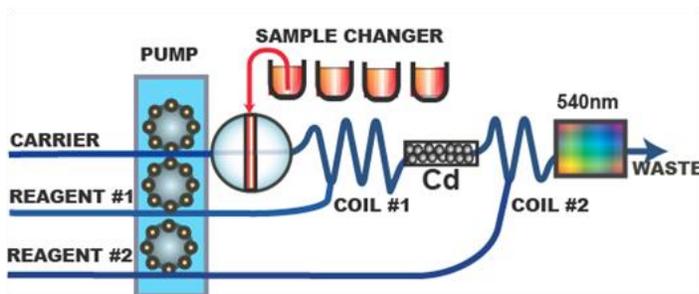
FI manifold for automated assay of nitrate with integrated cadmium column using sulfanilamide reagent.. The calibration record of 0, 2, 5 and 8 ppm nitrate is followed by a routine run of nitrate assay extracted from soil samples.

Hydride Generation for Atomic Spectroscopies (AA and ICP)
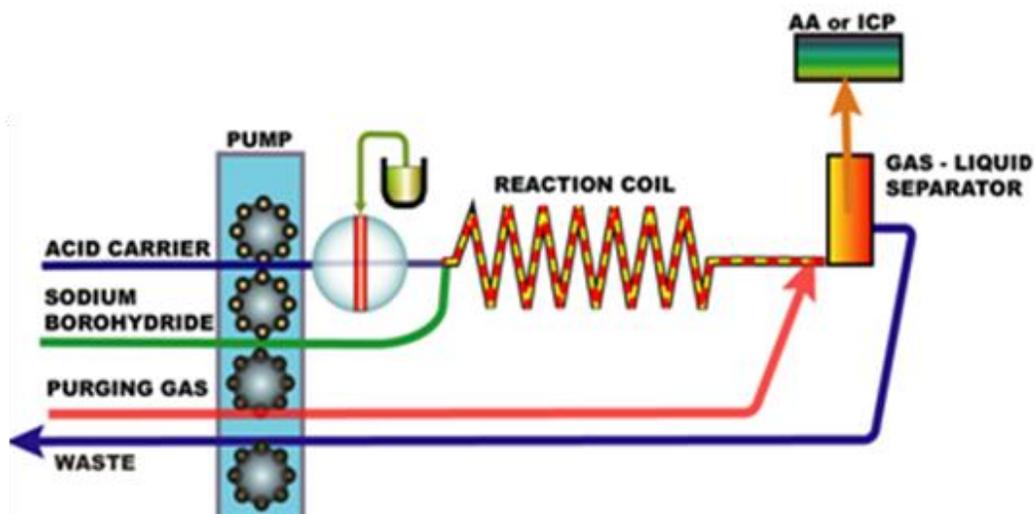
This method is routinely used for trace analysis of As, Bi, Ge, Hg, Pb, Se, Sn and Te. In addition, assay of volatile compounds of Ag, Co, Cu, Ni, and Zn has been reported. Separation of the trace metals from complex matrices, analyte enrichment, fast reaction speed, and ease of automation were first demonstrated by Astrom (1982) in his pioneering work on the FI-based hydride AA assay of bismuth. By combining an acidified sample stream with a strong reducing agent (sodium borohydride), hydrogen and metal hydride are rapidly released, and the gaseous phase is separated with the aid of purging gas (air or argon) and swept into AA or ICP for detection.

The Programmable Flow Injection Method

Advances in hardware and computer control, recently allowed a further refinement of FI technique, by applying the principle of flow programming, originally designed for Sequential Injection. Each step of an assay protocol, sample injection, mixing, incubation, product monitoring and instrument washout is best performed at an different flow rate. Thus instrument washout is most efficiently performed at a maximum flow rate, while for a slow chemical reaction a low flow rate is preferable, and data collection is favored by moderate flow rates. Conventional assay protocols, based on constant flow rate are therefore necessarily compromised, as they operate at averaged flow rates, or use excessively long reaction coils to gain reaction time for slower chemical reactions. In contrast, flow programming saves reagents, by applying very low

flow rates for sample incubation and fast flow rates of short duration for washout of the system. The unprecedented advantage of flow programming is the increased sensitivity and detection limit of most reagent based assays, achieved by adjusting the flow rate and incubation time from the computer keyboard, without need for system reconfiguration.

High Sensitivity Assay of o- Phosphate

This assay is based on a fast reaction of o-phosphate with ammonium molybdate forming molybdenum yellow, which is in turn reduced at a much slower rate to molybdenum blue, which is monitored at 880nm.
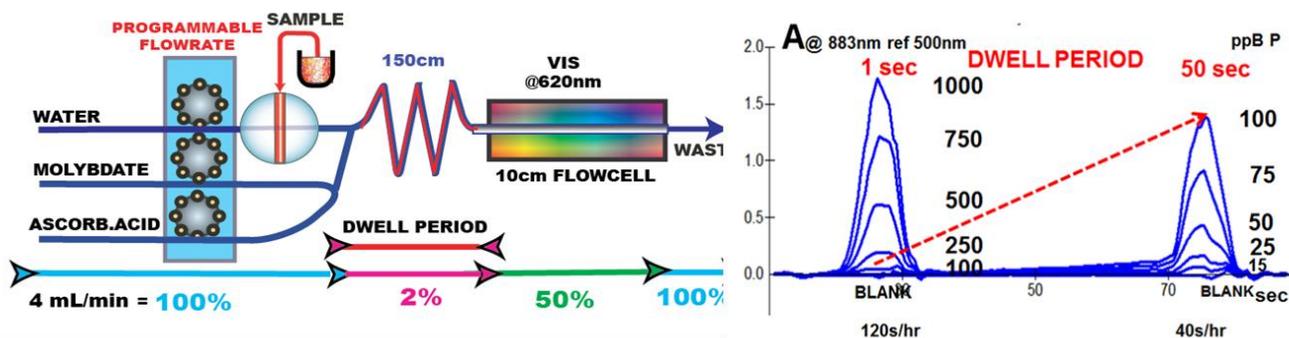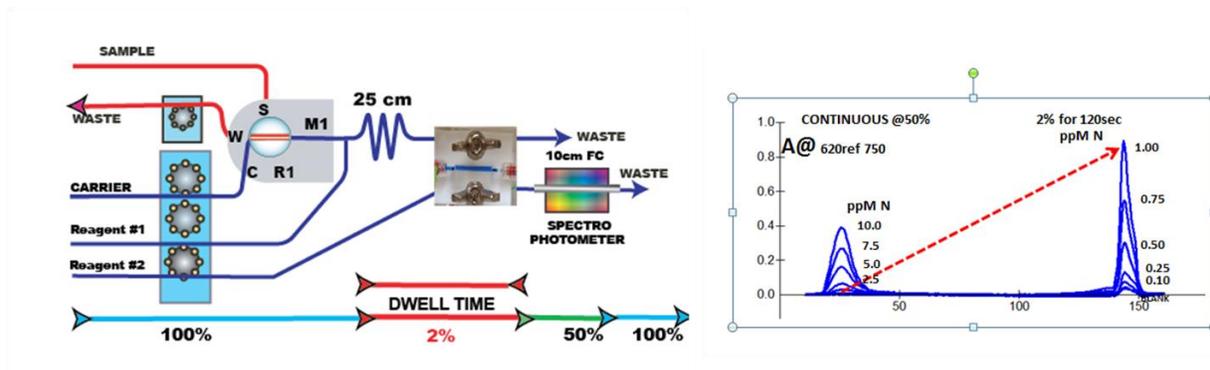

The flow rate for this assay is programmed to 100% for startup and sample injection, to 2% for incubation time, to 50% for monitoring and 100% for system washout. During the selected dwell period the combined flow rate of 2% was 600 microiters per minute. By adjusting the duration of the dwell period the sensitivity and the detection limit can be selected to cover the range of 0.5 ppB P to 1000 ppB P (10 cm light path flow cell, flow rate at 100% 4mL/min per channel).

For details see Programmable Flow Injection for o-phosphate assay

Ammonia Assay by Gas Diffusion

This assay is based on conversion of ammonium ion into ammonia gas that diffuses through a gas permeable membrane into a receptor stream of an acid/base indicator. The indicator, bromothymol blue is converted from acid (yellow) form into a basic (blue) form that is monitored at 620nm. Since acid /base reaction in both donor and acceptor

stream are very fast, the rate limiting step is ammonia diffusion across the membrane. Therefore the flow rate of the donor stream is decreased when the sample zone is in contact with the diffusion membrane during the dwell time.

The flow rate for this assay is programmed to 100% for startup and sample injection, and mixing, to 2% for incubation time, 50% for monitoring and 100% for system washout. During the selected dwell period the combined flow rate was only 600 microiters per minute. By adjusting the duration of the dwell flow period from 0 seconds (continuous flow) to 120 seconds the sensitivity and the detection limit improved 10 times. The method   covers the range 0.1 to 1 ppM N. 10 cm light path flow cell, 2 cm long diffusion path, flow rate at 100% 4mL/min per channel).

For details see Gas Diffusion Methods and Protocols (Ammonia)

Flow Injection or Air Segmented (Auto Analyzer) Technique?

Both techniques automate a wide range of wet chemical assays, by replacing manual solution handling with flow based methodology. While Air Segmentation (originally marketed as AutoAnalyzer®)
has served well for over 60 years, it has become a legacy technique, and is being replaced by Flow Injection in both routine as well as research laboratories. This, is because FIA techniques offer:

Higher sampling frequency: 2 samples/minute - or even up to 6 samples/minute.
Short residence time: readout within less than 30 seconds after sample injection.
Low sample consumption: typically 50 to 200 microliters
Low reagent consumption: typically 1ml per assay
Small instrument footprint: about 1/5 of a typical Auto Analyzer bench space.
Transparency: the entire flow path and cell can be visually inspected during the assay
Enclosed system: no air segmentation. Processed solutions isolated from ambient air.

Low cost replaceable components: No glass coils, no de bubblers, only tubing.
Short startup and close down Times: internal volume of system is less that 1/10th that of a typical Auto Analyzer system.