CHAPTER 1

Multi-Component Sequential Injection
- A New Challenge?

1.1 Introduction

Since its introduction in 1990 [1, 2], sequential injection analysis' popularity grew tremendously. Several laboratories and research groups [3 - 10] started to exploit the advantages of this third generation of flow injection analysis. Due to its economical sample and reagent consumption and the simplicity and versatility of the sequential injection system, several traditional 'hand' methods and flow injection systems were adapted to SIA systems [11 - 18] and employed as process analysers. Several reasons can be given for the employment of flow and sequential injection systems as process analysers. These reasons include [9]:

- Reduction in cost (personnel, equipment and consumables).
- High and accurate sample throughput.
- Improvement of the work environment (the use of sealed tubing and reactors when working with hazardous and/or toxic reagents).
- Substantial reduction of waste containing toxic reagents or samples [19].

In laboratory applications manual reconfiguration of the flow channel, high reagent consumption due to continuous flow operation, frequent servicing of peristaltic pumps and frequent recalibration of the system are acceptable. In a process environment these are prohibitive in terms of cost and manpower. Early studies in the field of SIA [1] soon showed that a single manifold was sufficient, irrespective of the chemistry to be employed. The manifold, once optimised, could be 'cast in stone'. There is apparently no limit to how many solutions (reagents, samples and standards) or
The biggest advantage of the sequential injection system is that there is no need for physical reconfiguration of the flow path. Any changes (injected sample volume, reaction time, sample dilution and reagent : analyte ratio) are accomplished via flow programming, rather than by physical reconfiguration of the flow path. Indeed, SI is fully computer compatible, as it allows all changes including system calibration, to be controlled from a computer keyboard, a feature that will allow its future optimisation through the use of artificial intelligence [20]. The SI system is further robust, reliable and requires a low frequency of maintenance. In comparison with FIA it is also more flexible for stop-flow and reversed flow operations.

There are presently two drawbacks of SI to be mentioned. First, since aspiration of the wash solution and sequencing of the zones in the holding coil take some time (typically 30 seconds), the sampling frequency of the SI system is presently half that of a conventional FI system, where filling of the injection valve is a matter of a few seconds. Secondly, SI requires specialized software, since the sequencing, injection and data collection are entirely computer driven. This, however, is not an obstacle in using the technique [3].

Automatic methods of analysis have been especially influential in clinic chemistry. Since health is one of the major concerns of our society, it is easy to understand the imperative need for hospitals to obtain a large number of analytical data as quickly and inexpensively as possible.

The situation is much the same in most industries, where quality control laboratories become much more important than they were a few decades ago. Most of the process control systems currently used, are based on physical measurements such as flow rate,
pressure, electrical resistance, etc. While this has resulted in processes which are operated under statistical control, verification of the process performance can only really be achieved by chemical analysis, usually in a remote plant laboratory. This approach is seen as unacceptable in the design of quality management systems for the production process. In such systems, the emphasis is on quality assurance during the process rather than after-the-fact. Process analysis brings the process controller a step closer to ensuring excellent control of the plant and real time quality assurance. At this stage, lengthy development times, the cost of these analysers and their maintenance requirements mean that only a few critical streams are monitored. The demands in this field are dictated by the large number of samples to be analysed, especially in on-line control of automated manufacturing processes and the quality now required in manufactured products. It is therefore necessary to control not only the raw products, but also the intermediate and end products.

Sequential injection systems had been proven to be suitable as on-line process analysers for most single component analysis [8, 9, 21]. Studies revealed that reduced numbers of samples can be used when applying SIA systems as process analysers, provided that the correct regression model is used [21]. The flow systems already adapted to SIA systems involved, however, very simple methods and operations and were mainly restricted to single component determinations. Multi-component analysis using SIA systems seemed to be a bit of a headache, since most of these determinations required extraction and the subsequent separation of the aqueous and organic phases, establishment of pH gradients or effective pH control, long and complex procedures, complex mathematical and statistical calculations and/or multiple detectors. To address these and the many other problems which usually showed up along the way, multi-component analysis might be seen as a new challenge in cost effective analysis.

The need to measure several parameters rapidly in the same sample in areas such as clinical chemistry, environmental pollution and industrial control has urged the development of automated methods of analysis, which in both the continuous and discrete modes offer the possibility of carrying out simultaneous determinations. In the
discrete mode, the same sample must be present in as many locations as there are parameters that are going to be determined. In the continuous mode or segmented flow analysis, the sample is split up into as many channels as there are parameters to be determined. Reaction and detection units exist for each of them.

1.2 Single-component sequential injection analysis

Determination of single components can range from simple analysis involving only one zone (the analyte zone) to complex systems using up to six different zones [22]. Single zone determinations involve mainly pH determinations, ion-selective electrodes, amperometric determinations or the use of chemical sensors. The properties of the unknown substance usually dictate the type of sensor needed for the detection. No mixing of analyte and reagent is needed and the analyte is analysed directly without chemical modification. The most important parameter needed to be controlled is the physical dispersion the analyte zone undergoes. In these type of applications it is advisable to work under low dispersion conditions (D < 3) in order to minimise the extent of sample dilution by mixing with the carrier, thereby ensuring a minimum decrease in sensitivity.

These methods offer substantial advantages which are noted below [23]:

- Simplicity. No sample pretreatment is necessary. Satisfactory sample dilution is achieved by controlling the dispersion (volume and flow rate) of the aspired zone.
- Rapidity. High sample frequencies are achieved this way.
- Sensitivity. Since it is a micromethod it is of great usefulness for the analysis of samples collected in capillary tubes.
- Reproducibility. The precision is comparable to that of conventional methods.
Determinations that involve chemical modification of the analyte, result in SIA systems where two or more zones must be sufficiently mixed to yield a product with measurable properties. In general, the dispersion is larger than in the case of single zone analysis ($D = 2 - 10$) and the occurrence of the chemical reaction is one of the major factors contributing to dispersion. Most of these systems employ colorimetric determination, fluorescence or chemiluminescence, since these methods of detection are cost effective and the apparatus are generally available. In photometric determinations parameters governing physical dispersion usually play a lesser role compared to the optimisation of the chemical parameters.

A typical SIA system is represented in Fig. 1.1. It is usually constructed (assembled) from a liquid driver (this can be a peristaltic, piston or electroosmotic pump or a burette [8]), an electrically actuated selection valve, Tygon or Teflon tubing to form holding and reaction coils and a single detector. Additional components can be added to perform specific duties. These components include dialysers (for separation of the analyte from the matrix) [24], hydride generators [25] and mixing chambers (for effective mixing or dilution) [22]. Since these applications are more advanced, it proves to be necessary that when describing it, the following aspects must always be highlighted: (i) the components it was constructed of, (ii) the specific manifold dimensions, as well as (iii) the device sequence or method construction (see addendum 1 for an example).

![Fig. 1.1](image-url) A typical sequential injection system used to determine single analytes (components). HC - holding coil, SV - selection valve, RC - reaction coil and D - detector.
1.3 Multi-component sequential injection analysis

Since most of the SIA systems originate from conventional flow injection methods, it is wise to first highlight the different options possible. There are mainly two groups of FIA methods used: One group of methods in which a detector for each species to be analysed exists and another group in which the number of detectors is lower than the number of species to be determined [26]. The application of pH gradients [27] and differential kinetic determinations [28, 29] dominate the simultaneous determination of two or more closely related species, like cobalt and nickel, in mixtures without prior separation. Using the same reagent, PAR in most cases [27, 28], the different analytes can be determined spectrophotometrically at the same wavelength. In some applications of flow injection analysis to kinetic analysis the, FIA system is used as a sample inlet system whereby the reactants are transported to the detector cell. On arrival the flow is stopped and the rate of the reaction is monitored by change in absorbance. FIA has received growing attention for making catalytic methods of analysis simpler and more reliable and has been used to implement differential kinetic methods for binary mixtures of metals.

Sequential injection multi-detection systems used, include the use of two detectors placed in series [30 - 32], a sandwich technique employing large sample volumes [33], ICP [34], a single detector using two chemiluminescence reactions involving luminol [35, 36], HPLC (SIA systems were used for sample preparation) [37, 38], diode array spectrophotometry [39, 40], redox reactions (nitrate and nitrite) [41], photometry (using the same reagent for Mg(II) and Ca(II)) [42], hydride generation DCP-AES [25], f-AAS [43], potentiometry [44] and other chromatographic detection [45].

Most of the above systems were merely used for sample preparation and detection was done by more expensive apparatus such as ICP, AAS, AES and chromatography. The systems employing the chemiluminescence reactions also seemed to be too expensive, since the instrumental setup included three pumps (one peristaltic pump and two piston pumps) and three valves (one injection valve and two multi-position valves) [35].
The best solution to produce cost effective multi-component SIA systems appeared to be the use of inexpensive detectors in series (if detection is non-destructive) or detectors in parallel (if detection is destructive). Non-destructive detectors include photometric detection (LED) and ion-selective electrodes. Single detector systems will be even cheaper. These type of systems involve reaction of the analytes with the same reagent and the subsequent detection of the formed products. The selectivity of these determinations are controlled by either controlling the pH of the different reactions or by employing the difference in reaction rates of the analytes with the chromophore. Extraction of the analytes into the aqueous or organic phase prior to the reaction will also increase selectivity.

The manifolds used for multi-component systems or systems employing complex chemistries can usually be accommodated with minimal changes to the manifold. The SIA system used for the simultaneous determination of Co(II) and Ni(II) is shown in Fig. 1.2. Programming of the methods used for multi-component detection is more complex as for single component analysis and the mathematical and statistical manipulations of the data also require more skill.
Since these applications are even more complicated, it proves (like in the case of more advanced single component SIA systems) to be necessary that in its description, the following aspects need special attention: (i) the components it was constructed of, (ii) the specific manifold dimensions, (iii) the device sequence (see addendum 1 for an example), (iv) a sketch of the device sequence (if applicable) as well as (v) the operational conditions.

Optimisation of the multi-component SIA systems is done in the same way as for single component analysis. It is, however, sometimes necessary to optimise more than one response peak or value. Chemical parameters need more attention than physical parameters since most of the reactions employed is dependant on pH. The sample frequency is also lower for multi-component systems, because longer and more complex flow paths are followed. The fact that two or more analytes are determined in one cycle compromise for the longer analysis time.

1.4 Aim of this study

Process monitoring in the chemical and pharmaceutical industries has moved towards more automated and computer controlled systems. The need for information about processes, process components and the changes in component concentrations has increased in pace. Unfortunately, most SIA methods are designed to deliver analytical results for just one analyte at a time. Literature surveys showed that 72% of all articles published describe single component analysis, while only 28% contain information about multi-component analysis. Multiple determinations are feasible, not only because it is more cost effective, but also because it reduces or eliminates interferences of species which are closely related to the analyte. With multi-component analysis it is not necessary to separate the interfering specie(s) from the analyte, since they can be determinated at the same time. This results in less sample preparation and eliminates loss of analyte due to long and sometimes ineffective separation methods.

1 Literature used to calculate the statistical data are the same as the references cited in Chapter 2. Speciation studies were included as multi-component analysis.
Cobalt is invariably associated with nickel, and often also with copper and lead, and it is usually obtained as a byproduct or coproduct of the recovery of these metals [46]. Since cobalt and nickel both have dual importance (biological as the metal complexes from certain enzymes and as toxic components in higher concentrations), sequential injection analysis was used to determine cobalt and nickel in natural waters as well as in soil extracts, using a differential kinetic method (Chapter 7). SIA is ideally suited for kinetic determinations due to its discontinuous nature. Longer stop-flow periods can be included into the method to allow longer reaction time for slower reactions.

As in the case of cobalt and nickel, iron, zinc and copper also have important biological as well as industrial roles to play. Contrary to their importance they also become very toxic to humans, animals and plants in high concentrations. Lead and mercury are the only elements in this study which do not have any essential biochemical role to play. Lead is generally considered to be a non-essential toxic element which accumulates in the organism, while all forms of mercury are considered to be poisonous. Due to (i) the toxicity of mercury, (ii) the fact that organo-mercury compounds can be formed in nature (methyl-mercury and probably dimethyl-mercury), and (iii) the bio-accumulation of methyl-mercury [47], there is a great need for the accurate determination of mercury. These determinations are not only needed to locate polluted areas, but definitely to prevent mercury pollutants to enter the environmental chain by controlling industrial and research effluents. Since the determination of organo-mercury is complicated by the different species that can be formed, this study concentrate more on prevention than cure. Aqueous samples as well as extracts of inorganic mercury from soil and urine spikes were analysed.

Mercury was determined together with cobalt using a simplified sequential injection extraction method (Chapter 8) and together with cadmium using a sequential injection extraction which involved a pH gradient (Chapter 9). Mercury was also determined along with six other elements using sequential injection extraction and a diode array spectrophotometer as detection system (Chapter 10). The other elements were: Lead, copper, cobalt, cadmium, zinc and iron. The extractions all involved Dithiozone as extractant and chromophore. Different solvents were used. The solvents were chosen
for their ability to form thin organic layers on the walls of the tubing, into which the analytes could be extracted. Film thickness depends on the ratio between the viscosity and surface tension of the organic solvent used as well as the affinity of the solvent for the tubing material (Teflon).

The kinetic determination as well as the different extractions involved complex programming and mathematical calculations to get the systems running and to evaluate the data obtained in these systems. Compared to these complicated systems, a very simple sequential injection system was also evaluated. A cation, Fe(III), and an anion, SO₄²⁻, were determined using a tandem SIA system (Chapter 6). The reaction between Fe(III) and tiron (4,5-dihydroxy-1,3-benzene-disulfonic acid) was monitored spectrophotometrically at 667 nm, while the sulphate was determined at the same wavelength using a turbidimetric determination. This system was able to do either sequential or simultaneous determinations of the two analytes in the same or different samples. The same analytes are used to evaluate the sandwich technique proposed by Estela et al. [33].
1.5 References

CHAPTER 2

Sequential Injection Analysis

2.1 Introduction

The use of process control strategies represent a significant shift in the thinking of many process control engineers. Increasing pressure on the chemical manufacturing industry to produce higher quality products in an economically viable and environmentally acceptable manner, increases the requirements to maintain strict control of plant conditions throughout the production process.

The sophisticated instrumentation of laboratory facilities are unlikely to be suitable for manufacturing environments, and hence, dedicated systems offering long-term dependability must be developed. The demand for mechanically simple and robust flow-Injection methodology has been the driving force behind the development of the sequential injection technique. The simplicity of the sequential injection (SI) manifold and its low need for maintenance makes it an ideal tool in process analysis. As miniaturization and reduction of reagent consumption are also ultimate goals in chemical sensing, it is useful to review the use of combined injection and programmed flow as a central issue in designing chemical sensors and structurally simplified chemical analysers.

Extraction, separation, pre-concentration, dialysis (matrix removal), titrations and dilution methods were adapted for use in sequential injection manifolds. Colorimetric, electrochemical and other detectors, equipped with suitable flow-through cells, were also incorporated into these manifolds. A new scope of SIA manifolds were developed for use in both industrial applications as well as in the laboratory.
2.2 Historical background

Sequential injection analysis (SIA), introduced in 1990 [1, 2], is a simple and convenient concept of flow analysis. Although this technique is a mere nine years old today, its roots can be traced back as far as 1974 [3]. To understand where sequential injection fits in and why Růžička refers to it as "a new look at a familiar landscape" [3], it is best to start at the very beginning.

Flow injection analysis (FIA) is an analytical technique that is based on the injection of a known volume of sample, with a geometrically well-defined shape, into a moving, unidirectional carrier or reagent stream. In this moving stream, the sample undergoes physical and chemical transformation until a detectable specie causes a detector response downstream of the injection point. If all critical parameters (reproducible injection, controlled reaction time and controlled dispersion) are held within certain tolerance levels, the result will be reproducible [4]. The instrumentation needed for an FIA system are a multichannel pump, an injection valve, a flow-through detector and a recorder. Except for the last component, which was replaced when computers invaded laboratories some ten years later, this basic flow scheme remained essentially unchanged [5].

When FIA research became orientated towards the exploitation of concentration gradients formed by the dispersion process [6], new techniques using stopped-flow, reversed flow, sinusoidal flow, reagent injection, sequential injection and single solution calibration were developed. Transformation of FIA into SIA signifies recognition of the tremendous versatility of this method, originally designed as a mere tool for automation of serial assays [7]. Much work has been done to assess the basic parameters of flow injection systems [8]. With the introduction of sequential injection analysis the basic parameters for flow injection were assumed to be applicable, because the same basic components (with minor changes) were used. Sequential injection (SI) is mechanically simpler than flow injection for it uses only a single pump, a single valve and a single channel. SI uses a selection (rather than an injection) valve, through which precisely
measured volumes of sample and reagent solutions are aspirated into a holding coil by means of a pump that is capable of a precise, controlled stop-go-forward-reverse movement [9 - 12]. A basic sequential injection analysis manifold is shown in Fig. 2.1.

Mainly four different liquid drivers were used in sequential injection analysis. Papers dealing with its applications used a sinusoidal flow piston pump specially designed for SIA [11, 13 - 16]. With this pump, the flow rate is dependent on the rotation angle, the radius of the pump, the cam, and the frequency of the pump. Repeatability and reproducibility are good, but it is difficult to maintain a constant flow rate during an analysis. The performance of peristaltic pumps to be used in SIA has been investigated by Ivaska and Růžička [17]. These pumps are suitable for SIA applications, especially when used with Neoprene tubing. Automatic burettes that are computer programmable and have variable speed are mainly used by the Spanish group [18 - 20]. A drawback of these liquid drivers is that it is impossible to use flow rates lower than 2 ml/min. A field-decoupled electro-osmotic pump is described by Liu and Dasgupta [21, 22] as an ideal pumping system for SIA, because the flow direction is readily and reproducibly reversed and the flow rate can be maintained with a high degree of reproducibility.

Full exploitation of these flow techniques in automated modes of operation necessitates computer control. Specialized software packages were designed to
control both the movement of the apparatus (pumps and valves) and to handle data acquisition and storage. The FlowTEK package (obtainable from MINTEK, Randburg, South Africa) was designed by Marshall and coworkers [9, 23] and is widely used. The Spanish group uses the program DARRAY, obtainable from SCIWARE, Palma de Mallorca, Spain [20]. Růžička's group [24] uses a program called FIALab for control and data acquisition.

The advantages of SIA have been discussed in detail by Růžička and Gübeli [13] and in comprehensive reviews and congresses [3, 9, 10, 12, 15, 25 - 28]. A valuable contribution by the group at the University of Washington was the exploitation of new sensor systems which broadened the scope of sequential injection analysis tremendously and opened new horizons in the field of flow analysis. First, Scudder et al. [29] developed a fountain cell for use in fluorescence microscopy. A chemiluminescence system that combines the simplicity and reproducibility of SIA with the unique radial flow properties of the fountain cell was then successfully employed for the chemiluminescence determination of hydrogen peroxide and glucose [30]. The fountain cell design was further used as basis for a perfusion chamber to perform the characterization of planar concentration gradients in a sequential injection system for cell perfusion studies [31]. The group [32, 33] also innovated and designed a novel jet ring cell which was incorporated into a sequential injection system for automated immunoassays and for preconcentration of analytes on sorbents with in situ spectroscopic detection. The jet ring cell with a renewable solid support was connected to a sequential injection system to determine glucose amperometrically [34]. A renewable gas sampling interface (liquid droplet) coupled with a SIA analyser was used to determine ammonia [35].

SIA was also applied for the determination of total ammonium nitrogen and free ammonia in a fermentation medium [16], nitrites and nitrates [36 - 38], D-lactic acid in pork [39], glucose using sensor injection and amperometric detection [40] and cyanide using ion-selective electrodes [41]. Wine [42] and sugar [43] analysis were done using sequential injection (SI)-FTIR spectrometry. Sequential injection manifolds were also
used to handle reagents for fluorescence microscopic measurements [3].

Coupling of sequential injection analysis with inductively-coupled plasma mass spectrometry as an analytical tool for trace element detection was used by Al-Swaidan [44]. The technique was applied for the determination of lead, nickel and vanadium at the part per billion level in sample solutions of Saudi Arabian crude oils. Hydride-forming elements were determined by direct current plasma atomic emission spectrometry based on a modified version of the sequential injection technique [45].

SIA was also employed as a sample preparation device for especially high-performance liquid chromatography [46, 47]. Lakkari et al. [46] used solid-phase extraction on Al oxide in a sequential injection system to purify pyrocatechol, protocatechuic acid, pyrogallol and gallic acid in black liquor. Sequential injection systems for the determination of mercury by cold-vapour atomic absorption spectroscopy [48, 49] used special gas-liquid separation units for effective analysis.

Application of the SIA technique to anodic stripping voltammetry (ASV) allowed the online plating of the mercury film and therefore substantially reduced the generation of mercury waste [50]. Other potentiometric applications include the determination of glycerol and 2,3-butanediol in wine [51]. Primary explosive azides in environmental samples were determined amperometrically using a SIA system [52]. A sequential injection system used in speciation studies employed two detectors in series, namely a potassium ion-selective electrode and a flame emission spectrometer [53].

SIA was also extensively used for the monitoring of bioprocesses [14, 54 - 56], enzyme activity [57, 58] and fermentation processes [59, 60]. Immobilized enzyme reactors played a great part in sequential injection analysis [39, 61 - 65] as well as systems for medical and pharmaceutical use [66 - 72]. Other SIA methods were also used for medical and pharmaceutical uses [73, 74]. Vitamin C was monitored photometrically in a kinetic application involving an iron(II)-iron(III) reaction [75], while morphine was determined with a SIA system employing chemiluminescence detection [76]. Van
Staden and McCormack [77] used a SIA system to determine amino acids spectrophotometrically. Chemiluminescence detection was employed in the preliminary analytical evaluation of novel reagents using a SIA system [78]. A method for determining the bromine (Br) number by coulometric flow-injection titrations, using sequential injection with sinusoidal flow is described by Taylor [79]. SIA was even applied to determine 90Sr in nuclear waste [80].

### 2.3 Basic principles

Following the first step of zone sequencing, during which the sample and reagent zones are stacked in the holding coil conduit adjacent to each other, the valve is switched to the detector position (Fig. 2 A). In the next step, the flow is reversed so that the stacked zones are propelled through the valve and the reactor to the detector (Fig. 2 B). As the central streamline moves at a rate twice the speed of the mean flow velocity, whereas the elements of fluid more adjacent to the walls move at lesser rates, the cores of the sequenced zones penetrate each other [8]. During this movement the flow reversal creates a complex region within which the analyte is transformed into a detectable specie (Fig. 2 C). The fundamental requirement for SI to succeed is to achieve maximum zone penetration through a deliberate increase in axial dispersion, obtained by means of the flow reversal and channel design [1, 13, 81].

Reproducible dispersion is the basis for analysis by flow injection methods. Dispersion is the result of all the physical forces acting on the injected zones. It is the process by which the zones transform from homogeneous, geometrically well defined zones at the moment of injection to the final zone that is detected downstream. The dispersion coefficient is the ratio of the detector response of the injected solution in the absence of these forces to that of the solution due to these forces. Růžička and Hansen [8] defined the conceptually simple and practically useful dispersion coefficient, $D = C^0/C$, where $C^0$ is the detector response of the undispersed solution zone and $C$ is the detector response of the dispersed element of fluid that yields the analytical readout. Because there is generally a direct relationship between the property used for
detection, the magnitude of the transduced signal recorded and the concentration of
the sample or its reaction product, the dispersion coefficient can be taken as the signal
height ratio [8, 9]. The random walk model was also used by Růžička and Marshall [1]
to describe dispersion in the SIA analyser channel.

Fig. 2.2 Flow profiles of the sequenced (A) and injected
zones (B - immediately after flow reversal and C -
in reaction coil 2). S - sample, R - reagent and P -
formed product zone.

Although Růžička and Gubeli [13] stated that “for a rational design of the sequential
injection analyser, the degree of sample dispersion must be considered as main design
guideline”, zone penetration (related to dispersion) is found to be the key parameter,
the control of which is essential to the successful execution of sequential injection [14].
The importance of zone penetration can be ascribed to the fact that this influence has
a dramatic impact on the surface area over which a concentration gradient exists and
therefore over which axial mixing takes place. It follows from the foregoing that, for
reagent-based chemistries, a region of mutually interdispersed sample and reagent
zones must be identified, within which $D_s$ is larger than 2 and where at the same time
sufficient excess of reagent is present.
Analogous to the definition of resolution, zone penetration is defined as

$$P = \frac{2W_d}{W_s + W_r}$$  \hspace{1cm} (2.1)

Complete overlap is obtained for $P = 1$, zero overlap for $P = 0$ and for values in between partial overlap will be obtained. This approach yields useful results although it is difficult to determine the value automatically [11]. An isodispersion point, $I_0$, is observed in cases where $1 > P > 0$ (Fig. 2.3). At this point the dispersion of the sample and reagent zones are identical and the ratio of sample and reagent concentrations is the same as their ratio prior to injection ($C_S/C_r = C_S^0/C_r^0$).

Fig. 2.3 Schematic representation of zone penetration showing the isodispersion point ($I_0$). $R$ - reagent zone, $S$ - sample zone, $D$ - dispersion coefficient, $W_R$ - baseline width of reagent zone, $W_S$ - baseline width of sample zone and $W_O$ - baseline of the overlap.

The isodispersion point is independent of concentration, but studies done by van Staden et al. [82] illustrate the shift of the isodispersion point due to the difference in concentration gradients when different volume ratios of sample and reagent were employed. These studies also showed that the position of penetration and the sequence of introduction of samples and reagents for different sample and reagent volume ratios in a total constant volume has a major influence on the response of the
final peak profile. This is illustrated in Figs. 2.4 and 2.5, where Fig. 2.4 represents the injection order of first the metal followed by the ligand and Fig. 2.5 represents the reversed injection order. The response of the final peak profile depends largely on the kinetics involved in both the ascending and tailing parts of the sample and reagent zones at the isodispersion point. Zable [4] found mathematical equations to calculate the maximum zone overlap. Marshall and van Staden [11] measured zone penetration by integrating the area of overlap. The larger this number, the greater the degree of zone penetration. This approach too suffers from certain limitations as it does not indicate the sensitivity of the measurement, because it does not take the concentration of the sample and reagent into account.

Fig. 2.4 Schematic representation of the influence of different sample : reagent ratios in a total constant volume. The figure represents the injection order of first the metal followed by the ligand.

Fig. 2.5 Schematic representation of the influence of different sample : reagent ratios in a total constant volume. The figure represents the injection order of first the ligand followed by the metal (reversed order).

2.4 Operational parameters

Although some would argue that SIA is simply a variation of FIA, there are certain fundamental differences in the use and control of the operational parameters used in SIA. Many researchers generally set up a system without regard to the dispersion of the individual components or some of the general rules for optimising the system. There are a number of publications describing different techniques of optimisation [83-86] and standardization [87] as well as systems able to diagnose multivariate
responses, with the aim of detecting faulty responses [88, 89]. The greatest challenge is the theory of flow dynamics, which will lead to optimisation of flow systems based on flow stopping and reversal. When applying the sequential injection technique, it is imperative to understand the principles on which it is based in order to do subsequent analysis. The extent of dispersion that the product peak will undergo is essentially influenced by the operational parameters that govern the SIA flow conduit.

A number of papers were published that described the most important parameters to be optimised [4, 9, 11, 14, 90, 91]. Almost without exception the following parameters had been shown to have a marked effect on zone dispersion in an SIA system: the volumetric flow rate, tube diameter, length of flow path from injection to detection, sample and reagent volumes, order of sample and reagent injection, flow reversal and to a lesser extent reactor geometry. The use of mixing chambers in the flow conduit and their influence on dispersion was studied by van Staden and Botha [90]. To evaluate the influence of every parameter, a non-reactive dye was used as sample and reagent zone respectively in a series of experiments.

The volumetric flow rate includes both the loading and forward flow rates and is also referred to as the combined effect of pump speed and the internal diameter of the pump tubing when using a peristaltic pump [4, 9, 90]. In correlation with the Vanderslice expression, \( D = k'\sqrt{q} \), where \( q \) is the flow rate in ml/min, the dispersion of the different zones decreases as the flow rate is increased [92]. The dispersion coefficient decreases with increasing flow rate because the residence time decreases, in a nonlinear fashion, with increasing flow rate [9]. A linear relationship consists between the pump speed and flow rate; therefore the flow rate can be altered by changing the pump speed [9, 11, 90]. At high flow rates a deterioration in sensitivity and reproducibility is experienced due to the higher back-pressure [9, 93]. It is preferred that the loading flow rate should be faster than the forward flow rate to ensure higher sample throughput, provided that the pump will allow this [11].
The length of the tubing is dictated by the experimental requirements [9]. Longer tubing leads to longer residence times and therefore larger dispersion. Zable [4] stated that the dispersion must be proportional to the square root of the tube length, but experimental data had shown that there was a linear relationship between dispersion and path length. The mixing height (number of plates or tanks) is defined as the average length of tubing used for each mixing stage [8].

In SIA the manifold tubing is divided into two parts: the holding coil which is the tubing between the liquid device (pump) and the selection valve and the reaction coil which is the part connecting the selection valve with the detector. The holding coil primarily acts as a reservoir and should be large enough to prevent the sample and reagents from entering the pump conduit. The reaction coils should not exceed one-third of the volume of the washing solution, thereby ensuring that they are adequately flushed during every experiment [11]. Reaction coils are usually kept as short as possible to avoid excessive dilution of the formed product zone. The length is governed by the physical distance between the valve and the detector [9, 13].

A knowledge of the reaction rate is of particular value when adapting a method to sequential injection analysis, because the time spent in the manifold can be too short to ensure complete colour development. Due to the discontinuous nature of SIA, stopped-flow periods can easily be incorporated to enlarge reaction times [94 - 96]. Van Staden and Taljaard [94] used a stopped-flow period of 80 seconds to ensure adequate formation of indophenol during the determination of ammonia.

Related to the Dean number the tubing diameter had a dramatic influence on the dispersion of the different zones [4]. The dispersion is found to be proportional to the fourth root of the coil diameter. Several factors should come to mind when considering the optimum tube diameter. These include the resultant back-pressure in a length of tubing, the vulnerability to blockage and the degree of radial dispersion attainable [9, 11]. Wider tubing is usually used for the holding coil, because of its promotion of axial dispersion and, therefore, zone penetration. Narrower tubing is used for the reaction
coils to prevent excessive dilution of the formed product zone. Wider tubing is used for the uptake tubes to prevent any back-pressure.

Gübeli et al. [14] have conducted an in depth study on the effect of sample and reagent volume on zone penetration and sensitivity. Their conclusions can be summarized in three rules:

1. Changing of the sample zone volume is an effective way of changing the sensitivity of the measurement. Dilution of concentrated samples is best achieved by reducing the injected sample volume.

2. Injecting at least twice as large reagent zone volume as sample zone volume, while keeping the volume of the sample zone less or equal to 0.5 $S_{1/2}$, allows the optimum conditions for single based chemistries to be met. ($S_{1/2}$ is defined as the sample volume required to yield a dispersion factor of two in the manifold).

3. Two reagent chemistries can be accommodated provided that the sample volume is kept below the $S_{1/2}$ value, so that the sample zone is surrounded by the reagent zones and that the concentration of the injected reagents are sufficiently high.

Van Staden et al. [82] found that the best sensitivity was obtained when a 1:1 sample to reagent ratio was used. At this ratio, the two zones experienced almost the same axial dispersion and penetration occurred almost at the maximum of the descending sample zone as well as the maximum of the ascending reagent zones. Gübeli et al. [14] found that increasing zone volumes at equal volume ratios caused zone overlap to decrease from nearly complete overlap (with small equal volumes) to a partial one (with relatively large equal volumes). While keeping the reagent volume constant, the authors [14] also varied the zone volume ratios by increasing the sample volume from less than the reagent volume to one where the sample volume was in excess of the reagent zone. This also resulted in a decrease in zone overlap.

Optimum sample and reagent zone volumes can be determined by plotting log $[1 - (A_{\text{max}}/A_0)]$ versus sample volume ($V_t$), where $A_0$ is the absorbance corresponding to the
case where the element of fluid undergoes no dispersion. The $S_{1/2}$ value can be
determined from the slope of the linear relationship. Experimental results gave good
correlation with calculated values [90]. $S_{1/2}$ values are, however, influenced by a
number of experimental parameters. Cladera et al. [97] and Araujo et al. [98] showed
that the ionic strength or electrolyte concentration of the medium influenced the $S_{1/2}$
value. The flow rate (slower flow rates result in higher $S_{1/2}$ values), the number of flow
reversals and the dimensions of the reactor loop also influence the value [97].

A simple and convenient method for the determination of injection volumes in
sequential injection analysis is presented by van Staden and Malan [99]. It is based
on comparing the dilution of the injected dye with a standard calibration curve. The
proposed colour method gave the volume of the whole injection device more accurately
than methods where the inner dimensions of the injection device are not precisely
known. The colour method is within the 95% confidence level with an RSD of 0.8%.
Sampling strategies in sequential injection analysis were also investigated by Vieira et
al. [100]. These techniques were exploited using a monosegmented-flow approach.

In a publication of Mas-Torres et al. [101], the authors discussed a new approach to
sequential injection analysis. This approach involved the use of the sample as carrier
stream. Although this technique may render good results, it takes away one of the
main advantages of sequential injection analysis - the fact that it uses minimized
amounts of sample [9].

The importance of the correct order of sample and reagent injection is highlighted in
a number of publications [11, 82, 94, 102 - 104]. The order in which the different
sequences of reagents are drawn up are very dependent on the reactions involved.
The residence time of a specific zone also depends on its position in the reagent
sequence. The zone that is drawn up first reaches the detector last due to the flow
reversal. This zone has the longest residence time of all the zones and is therefore
more dispersed [4, 9, 11]. The following must be considered: when sensitivity is
important, the reagent, at a sufficiently high concentration, should be introduced first.
and allowed to penetrate the sample zone, which will experience minimal dispersion. If buffering of the sample by the wash solution is required, the order must be reversed. If solubility considerations prevent the reagent concentration from being increased, sandwiching of the sample between two reagent zones is an option to be considered [11].

Although it is the first flow reversal and its duration that is the most effective in providing mutual zone penetration [11, 14], more than one flow reversal was needed in the determination of ammonia, due to insufficient mixing of the adjacent zones (because of their different viscosities). The stack of zones was subjected to three flow reversals before the product zone was propelled to the detector. Multiple flow reversals were also used to better the transfer of ammonia over the membrane in a coupled gas-diffusion-SIA system [18] as well as to improve iron dialysis through the membrane during the determination of iron(III) [93].

Various reactors have been described in the literature on FIA manifolds [8]. Where the reactor consists of a length of tubing, various geometries have been proposed. Three were evaluated to establish the effect of reactor geometry on zone penetration and geometry. Studies done by Taljaard [9] and Marshall and van Staden [11] showed that reactor geometry does not have a marked effect on sensitivity or precision. Straight tubes are, however, preferred in SIA manifolds due to the better axial dispersion obtained.

It should be noted that only the physical dispersion of all of the above-mentioned parameters were highlighted. The influence of a chemical reaction on dispersion is not even mentioned. The optimum values for each parameter will to a large extent depend on the specific reaction conditions and do not only depend on maximum sensitivity, but also on the reproducibility of measurements (%RSD). A set of parameters resulting in high sensitivity can be rejected if the relative standard deviation is too high, as shown by van Staden and du Plessis [105] and by Nakano et al. [106]. It is however surprising that such good precision is attained in SIA systems, because the reaction
takes place at an interface with steep concentration gradients [11].

2.5 Single zone sequential injection analysis

The analysis of chemical species that can be measured directly, such as those that have a high molar absorptivity of light at a specific wavelength (e.g. concentrated hexavalent chromium) can be analysed using single zone sequential injection analysis [4]. The technique can also be used for pH determinations or methods where detection is done using ion-selective electrodes or chemical sensors. In these types of analysis the sample is the only zone injected.

2.6 Two zone sequential injection analysis

Double zone sequential injection analysis depends on the addition of a single reagent. In this type of analysis, the sample and the reagent solution are the only two zones injected. Reaction stoichiometries of different complexes can easily be determined when using a two zone system [85, 102]. Van Staden et al. [102] described a system to determine the reaction stoichiometries of both the Fe(III)/Tyron complex (1:1) and the Fe(II)/1,10-phenanthroline (1:3) using the Yoe-Jones’ Method and Job’s Method. The main advantage of these methods above their FIA counterparts is that the tedious and time-consuming process of changing the sample loops for every different ligand : metal ratio, is eliminated. Sultan and Desai also described a SIA method for the concentration, stoichiometry and formation constant studies when promethazine hydrochloride complexed with palladium(II) in hydrochloric acid [107].

The turbidimetric determination of sulphate also employs in principle a two zone sequence. Due to the build up of barium sulphate in the manifold an alkaline buffer-EDTA solution was introduced to clean the tubing. This zone was separated from the acidified barium chloride reagent by means of a water zone, resulting in a sequence of a stack of four zones [108]. Two zone sequential injection analysis was also used by van Staden and Botha [139, 140] in the determination of Cu(II) with DDTC.
2.7 Three zone SIA systems

The order in which the different sequences of reagents are drawn up depends very much on the reactions involved. In the determination of ammonia using the indophenol blue method \[94\], a three zone sequence of sample, phenol reagent and hypochlorite reagent was used. This sequence is in contrast with the manual and flow injection methods \[109\], where the hypochlorite reagent is first added to the sample. The importance of the correct zone sequence is also highlighted in the determination of phosphate \[103\], where the sample had to be drawn up first to ensure minimum reaction time between the molybdenum and ascorbic acid reagents. This was done because of the rapid reduction of the molybdenum by the ascorbic acid prior to reaction with the phosphate in the aqueous samples. When the molybdenum reagent was sequenced between the sample and ascorbic acid zones, the phosphate already started to react with the molybdenum while the ascorbic acid was being drawn up. Munoz et al. \[110\] did an evaluation of spectrophotometric methods for the determination of orthophosphates using sequential injection analysis. Three zone SIA was also applied to determine calcium in water, urine and pharmaceutical samples using CPC \[104\] as well as in paper machine white water \[111, 112\].

2.8 More than three zone systems

Although it is stated that three zones were the maximum to ensure effective mixing \[3, 9, 13\], Guzman and Compton \[113\] published an article where six zones were used in the determination of rhFXIII fluorometric activity. The SI method simulated to a large extent the chronology of the manual procedure and a mixing chamber was incorporated into the manifold to ensure efficient mixing of the different zones.

2.9 Multi-component techniques in SIA

Only a limited number of papers considering multi-component techniques were published \[19, 114 - 119\]. A multi-linear regression program for the simultaneous
determination of calcium and magnesium in mixtures, was introduced by Gómez et al. [19]. Also using the chromogenic reagent 4-(2-pyridylazo)resorcinol, Taljaard and van Staden [114] described a procedure for the simultaneous kinetic determination of nickel and cobalt in water and soil extracts. The feasibility of large sample volumes in sequential injection analysis, in order to determine various analytical parameters in an unique sample injection, was investigated by Estela et al. [115]. This technique employs two reagents sandwiched around a large sample volume and allows the determination of two different parameters in the sample. The accuracy of the resolution of each resulting reaction peak and the linear working ranges are dependent on injected sample volumes, reagents and their concentrations.

A sequential flow injection method for the simultaneous determination chloride and fluoride in water with potentiometric detection using two ion selective electrodes in two serial flow through cells is described by Alpizar et al. [116]. The authors [120] also described the simultaneous determination of chloride and pH in waste waters by sequential injection analysis. This method uses a separate flow cell to measure pH by stopping the flow during the chloride determination. Simultaneous determination of calcium and magnesium in mineral waters was also done by Araujo et al. [118]. In this case, the sequential injection system was used as a sample preparation step and the analysis was done via flame atomic absorption spectrometry. Another simultaneous determination, these of phosphate and silicate in waste waters, using SIA is described by Mas-Torres et al. [119].

2.10 More complicated systems

2.10.1 Calibration

Baron et al. [40] described a single standard calibration technique using the same manifold as for dilution with a dilution coil. Through variations of the volume parameter sizes, conditions were found that generate four 'slices' from the tail of the gradient in the dilution conduit. This gave increasing responses at the detector. These values
were used for generating data required for system calibration. The response with the dilution conduit was compared with a volumetric calibration using the steady-state responses of four BTB standards. By using the previously calculated dispersion coefficients, the actual concentration at the SIA peak maximum for the four aliquots (slices) was calculated and plotted as concentration versus absorbance together with the responses for the volumetric standards. These values agree within 5%, which is sufficiently close to confirm the validity of the SIA method. It is also possible to cluster standards around the multi-position valve so that the system might be automatically recalibrated as required [12]. A multivariate statistical process control procedure, for sequential injection analysis systems, is developed by Rius et al. [89]. This multivariate calibration technique was evaluated for sulphate analysis.

2.10.2 Dialysis

Dialysis is a valuable tool that fulfils a very important role for on-line separations as part of segmented auto analysers, flow injection and sequential injection systems [121]. Iron(III) was separated from a sample matrix by dialysis in a sequential injection system [93]. The dialysed iron was complexed with Tyron and the resulting complex was monitored spectrophotometrically at 667 nm. In this work, the influence of various parameters, including pump speed of both donor and recipient streams, sample volume, reagent volume, dialysis time and the effect of multiple flow reversals, on dialysis efficiency was studied. A sample frequency of 8 samples per hour and a detection limit of 45 mg/l Fe(III) were obtained.

To ensure different flow rates for the donor and recipient streams respectively, two pumps were used in the application [93]. A slower flow rate was used for the donor stream, because of the longer dialysis time needed and a faster flow rate for the recipient stream, resulting in better penetration of the zones and shorter rinsing times. The dialyser unit used consisted of a 160 x 30 x 25 mm single dialyser. The path length of both the donor and recipient streams was 300 mm. The grooves of the dialyser had an inner diameter of 0.5 mm. A Technicon type C membrane was used.
The coil connecting the outlet of the recipient stream to the carrier container must be kept as short as possible to avoid the introduction of bubbles via the inlet of the dialyser.

With the incorporation of a passive neutral semi-permeable dialysis membrane into the conduits of the sequential injection system, the contact time of the sample zone with the membrane had a marked influence on the quantity of iron dialysed through the membrane. Using the feature of multiple flow reversals, the percentage dialysis was improved to 4.5%, which compares well with the 4% obtained with conventional flow analysis [122]. In the optimized system a dialysis time of 180 seconds was used.

Dialysis was also used in the spectrophotometric determination of L(+)-lactate in wines [123]. For the determination of total ammonium-nitrogen and free ammonia in a fermentation medium, a two channel sequential injection system was used [16]. The streams were propelled by an Altea S2-V two channel piston sinusoidal flow pump equipped with two cam driven parallel syringes. Two electrically actuated multi-position valves, a six port valve on the donor line and an eight port valve on the acceptor line, were used to direct the flow streams. A Celgard 2400 hydrophobic membrane was used in the combined gas diffusion unit/flow cell. Luo et al. [124] described the determination of gaseous ammonia using a glass diffusion denuder in a sequential injection system.

Coupling of gas-diffusion separation and sequential injection analysis is applied to determine ammonia in aqueous environmental samples [18]. The sample and an alkaline solution are sequentially aspirated using an automatic burette and mixed by flow reversal while being propelled to a gas-diffusion unit. Here the ammonia formed diffuses through a hydrophobic membrane into an acid-base indicator solution used as acceptor stream. The dispersion (broadening of the zones) is dependent on the travelled length (l) and on the number of flow reversals (N). This effect can be favourable in GD-SIA because zone broadening will result in longer residence times for the sample in the diffusion unit, without changing the flow rate, which could favour
ammonium diffusion.

Differences in pressure between both sides of the membrane reduced the gas transfer efficiency and caused large sample dispersion and long-tailed, undefined peaks. Maximum preconcentration was achieved when both the inlet and outlet of the acceptor channels were closed during the preconcentration step. Placing an injection valve in the manifold ensured that the pressure at both sides of the membrane almost stayed the same. Increased travel distance \( l \) meant larger washing volumes and longer rinsing times and that reduced the sample frequency. It was therefore concluded that longer travel times was not an effective way of increasing sensitivity.

The inclusion of a second holding coil, providing a reservoir of a ready-to-use indicator solution, allowed the minimization of the dispersion of the indicator acceptor zone. This limited the sample frequency, because the holding coil had to be refilled after every measuring cycle. To ensure limited dispersion and dilution of the indicator zone, an air segment was inserted between the indicator zone and the water carrier in the holding coil. The sensitivity improved about three times for \( N = 3 - 5 \). The limit of detection was 2 mg/l and the transfer efficiency of ammonia through the membrane was calculated to be 15%.

### 2.10.3 Titrations

Van Staden and du Plessis [105] described a sequential injection titration system for the titration of a strong acid with a strong base. The concept is based on the sequential injection of a base titrant, acid analyte and a second base titrant zone into a distilled water carrier stream. Using this zone sequence close resemblance with flow-injection titrations was obtained. The stack of zones is swept by flow reversal through a reaction coil to the detector. The base zones contain Bromothymol Blue as indicator and the endpoint is monitored spectrophotometrically at 620 nm. A straight reactor with a length of 90 cm and a diameter of 0.51 mm was used as gradient device (instead of a mixing chamber) for the creation of the concentration profile of the acid
sample. This was done because the use of a mixing chamber resulted in excessive dilution of all the zones and a dramatic decrease in sample throughput.

A linear relationship between peak width and logarithm of acid concentration is obtained in the range 0.01 to 0.1 mol/l of HCl with $1 \times 10^{-3}$ mol/l NaOH as titrant. Other linear ranges are possible at different titrant concentrations. The results obtained for the sequential injection titration compare excellently with those obtained with standard potentiometric titrations with an RSD < 0.30%.

A titration method without mixing or dilution is described by Holman et al. [125]. This method also involves the use of chemical sensing membranes. A method for determining the bromine (Br) number by coulometric flow-injection titrations, using sequential injection with sinusoidal flow is described by Taylor [79].

2.10.4 Dilution

On-line dilution with sequential injection analysis has been evaluated using a dilution coil in the conduits of the manifold system and a dilution step as part of the timing sequence [9, 40, 126, 127]. The manifold of the SIA system with the dilution coil is more complicated than the system including the dilution step. The former method needs more complicated programming as well. Control over the magnitude and range of dilution is effected by three volume parameters: Sample volume, $V_s$, transfer volume, $V_T$, and analysis volume, $V_A$ [40]. The sample volume is the amount of sample or standard which is drawn into the holding coil via the sample port. The transfer volume describes the volume of sample plus accompanying wash solution in the holding coil and tubing which is transferred into the dilution conduit from the holding coil. The analysis volume is the volume of the aliquot taken from the dilution conduit to the holding coil and then pushed through the detector.

Shorter analysis time favours the dilution with the dilution step, but the limited linear range of this method is a large drawback. Dilution is obtained by introducing a well-
defined water zone between the barium chloride reagent zone and the sample zone. The degree of dilution obtained depended on the timing sequence allowed for the dilution step. Timing sequences between 0 and 15 seconds were evaluated and the calibration curves associated with the different linear regions are illustrated in Fig. 2.6. It is clear from the curves that a linear calibration curve (Fig. 2.6 D) was obtained between 750 and 5000 mg/l sulphate with a timing sequence of 15 seconds.

In the determination of phosphate in bioprocesses, dilution (when required) was performed in a mixing chamber connected to the selector valve [128]. In the spectrophotometric catalytic determination of iodide in nutrition salts, Lima et al. [127] used a SIA system with mixing chamber for handling high concentrated solutions.
2.10.5 Extraction

Peterson et al. [129] described a flow-based extraction method where an aqueous sample and organic solvent were sequentially injected into an extraction coil, mixed and separated due to the differential flow velocities of the aqueous and organic phases. A 500 \( \mu l \) aqueous sample is propelled through a 50 \( \mu l \) segment of organic solvent whose flow is impeded due to hydrophobic interactions with the walls of a Teflon extraction coil. This wall drag allows the faster moving aqueous sample to penetrate through and ultimately separated from the slower organic solvent. These steps are repeated with a back extraction into a second aqueous segment (100 \( \mu l \)) that is collected and analysed with high-pressure liquid chromatography (HPLC).

Barbiturates (phenobarbital, amobarbital, pentobarbital and secobarbital) and serotonin re-uptake inhibitors (SRIs) - venlafaxine, paroxetine, sertraline and norsertraline - were extracted as model acidic and basic compounds from urine into a 1 : 4 (v : v) mixture of 1-octanol and butyl chloride and back extracted into 0.45 mol/l NaOH (acidic sample) and 0.18 mol/l \( \text{H}_3\text{PO}_4 \) (basic sample), respectively. The sample throughput, including extraction and back extraction, was 20 samples per hour. This extraction procedure was mainly used for sample preparation and air instead of water was used as propellant. A volume of 400 \( \mu l \) acetonitrile, loaded initially, washed the organic film to waste - leaving the extraction coil ready for the next extraction cycle. The solvent composition was found to be a critical parameter for successful application of SIE (sequential injection extraction), because it determined the difference in flow velocity between the organic and aqueous phases, the chemical selectivity and extraction efficiency.

Nakano et al. [106] combined wetting film extraction with colorimetry to determine nanogram amounts of molybdenum(VI). Using a very simple manifold and almost the same procedure as described earlier a highly sensitive and selective sequential injection system was developed. Molybdenum (which reacted with thiocyanate) was extracted in the first step into a toluene film as an ion paired complex. The thiocyanate
ligands were displaced by 1,5-diphenylcarbazone (DPC) to form an intensely coloured product which was measured at 540 nm. Wetting film extraction was also used in the photometric determination of vanadium(IV) and vanadium(V) [130] and chromium(VI) and chromium(III) in water [131].

Grate and Taylor [132] described an on-line soil extraction procedure employing SIA. On-line soil extraction was performed with the soil placed in an open-ended column attached to the sample line.

2.10.6 Preconcentration and separation

Rubi et al. [133, 134] described a sequential injection assembly for the determination of Fe(II) in natural waters. Fe was preconcentrated on a microcolumn packed with a chelating resin (Chelex 100) that was inserted into the manifold. The sample was passed through the column and the Fe, retained by the resin was subsequently eluted with 2 mol/l HNO₃. The SIA system offers automatic preconcentration, elution, detection and data acquisition. Using a simple sequential injection method, ammonium was determined with conductometric detection. NH₃ permeated through a gas permeable membrane and was collected (preconcentrated) in a static acceptor stream [135].

In the determination of 90Sr, the 90Sr was separated from other radionuclides using a sorbent minicolumn containing a resin that selectively binds 90Sr as a crown ether under acidic conditions [80]. The isolated 90Sr was then detected on-line with a flow-through liquid scintillation counter.

2.10.7 Mixing chambers

Despite the fact that mixing chambers have certain undesired properties such as large dead volumes and that it causes a hold up effect, it offers some distinct advantages, for example, in the use of mixing of liquids with different viscosities [136]. Mixing
chambers were initially used in sequential injection analysis to dilute highly concentrated samples [40, 127, 128] or to ensure adequate mixing when three or more zones were involved [81, 113, 114, 137]. Accordingly, in an SI process control application, a mixing chamber connected to a fibre optic detector has proven to be successful for the determination of total biomass [24, 138]. This mixing chamber or cell was used both as a dilution chamber and detection cell. Recently, mixing chambers were used to improve the degree of mixing - even for cases where only two zones were used [102].

Depending on the application of the mixing chamber, it can be placed either between the holding coil and the valve or between the valve and the reaction coil. When placing the mixing chamber between the holding coil and valve, the zones will be drawn up through the mixing chamber into the holding coil and then propelled through the mixing chamber to the detector after flow reversal took place. For the mixing chamber positioned between the valve and the reaction coil, the zones will be drawn into the holding coil and then propelled through the mixing chamber. A lot more dispersion is expected for the former situation, because the zones enter the mixing chamber twice and because of the increased mixing, an increase in dispersion is experienced [90, 140].

The volume of the mixing chamber is also an important parameter which could be used to control the amount of dispersion needed. Larger volumes resulted in larger dispersion, which is not always desirable [90, 114, 140]. In the simultaneous determination of cobalt and nickel, a mixing chamber with a volume of 500 µl was used to ensure adequate mixing of the reagents before splitting the product zone in two [114].
2.11 Conclusion

Although the sample throughput frequency of an SIA system is normally less than that of the conventional FIA system [13, 14], the major advantage of SIA is the more cost effective use of reagents. SIA methodology has important advantages over conventional FIA as it is simple in equipment and the manifold does not have to be changed if flow parameters or injection volumes are modified. It is also a very versatile approach as a single SIA configuration can be adapted for multi-reagent techniques and multi-detection systems without the need of reconfiguring the manifold.

Different sample-handling techniques were successfully adapted to sequential injection analysis. Calibration using a dilution conduit is particularly suited to sensor injection in a situation where the sensor is susceptible to fouling or degradation over time. Flow detector fouling does not occur in SIA manifolds as the detector is in contact with water between analysis [103, 128]. The use of a dilution conduit in the SIA mode allows for simple and precise dilution of analytes and for easy and rapid generation of a set of calibration samples from a single standard solution. It is also possible to cluster standards around the multi-position valve so that the system might be automatically recalibrated as required.

Sequential injection analysis has reached the point where a manifold that does not need changing can be designed. Further developments concentrate on using versatile controlling software to manipulate sample and reagents in novel ways to achieve desired sample-handling procedures. In comparison with flow injection analysis it is more flexible for applying stopped-flow and reversed-flow operations [138]. Although this does not mean that sequential injection analysis will replace flow injection analysis, SIA surely has a very useful future laying ahead.
2.12 References

3.1 Introduction

When choosing an analytical method, three factors should be considered in the selection process: Cost, Speed and Accuracy. The ideal method will count the last atom in no time and cost nothing. In practice, higher speed reduces accuracy, and higher speed and accuracy cost more. The best compromise should be adequately accurate, at the highest affordable speed.

One of the simplest ways of increasing speed without increasing cost or compromising accuracy is to do simultaneous determinations. Simultaneous determinations are defined as the determination of the concentration of two or more chemical species in a single sample using only a single set of sample preparation steps and a single presentation of the sample to the instrument.

The need to measure several parameters rapidly in the same sample in areas such as clinical chemistry, environmental pollution and industrial control has urged the development of automated methods of analysis which in both the continuous and discrete modes offer the possibility of carrying out simultaneous determinations [1]. In the discrete mode, the same sample must be present in as many locations as there are parameters that are going to be determined, there being a single detection system. In the continuous mode, the sample is split up into as many channels as there are parameters that are going to be determined. Reaction and detection units exist for each of them.

The most elementary classification of simultaneous methods of analysis can be made on the basis of taking the correlation between the detection unit and the species to be
determined. Thus, there is one group of methods in which a detector for each species to be analysed exists, while another group consists of methods in which the number of detectors is lower than the number of species to be determined.

Although simultaneous determinations are quite common in flow injection analysis, only a few articles were published which described these techniques in sequential injection systems (refer to Chapter 2). Since sequential injection analysis developed from the mother technique, flow injection analysis, techniques used in FIA are adopted for use in SIA systems. It is therefore appropriate to highlight a few of these techniques employed in flow injection analysis.

3.2 Simultaneous determinations in FIA

Despite the obvious importance of developing FIA manifolds for simultaneous determinations, only two reviews [1, 2] on this subject have appeared since the inception of FIA. The main simultaneous determinations developed for FIA are summarised in Table 3.1.

<table>
<thead>
<tr>
<th>Detection systems</th>
<th>Injection system</th>
<th>Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional FIA methods</td>
<td>In series</td>
<td>Single injection</td>
</tr>
<tr>
<td>With several detectors</td>
<td>In parallel</td>
<td>Single injection</td>
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<tr>
<td></td>
<td></td>
<td>Multi-injection</td>
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<td></td>
<td></td>
<td>Zone sampling</td>
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<tr>
<td>Single multi-detector</td>
<td>Sequential injection</td>
<td>Use of different reagents for different samples according to the parameter to be determined.</td>
</tr>
</tbody>
</table>
| | Single injection | Splitting up of the flow with two flow cells aligned in the same optical path. pH gradient ion-exchange.
Luque de Castro and Valcárcel [1] stated that the word “simultaneous” in analytical terms means measurements of several species in the same sample or in different portions of the same samples carried out at the same time. Other methods that employ measurements of samples at different times were referred to as sequential measurements. Sequential measurements are included into this discussion, since it also allow multi-component detection.

The review of Kubañ [2] also highlights the difficulties in the classification of multi-component FIA systems. This is because of the wide selection of methods and tricks involved and the inconsistent nomenclature used by different research groups. It is, therefore, also important to understand the difference between multi-detection and multi-determination [1].

**Multi-detection:**
Two or more signals are obtained from a single injected sample. It can be performed with a single detector at different times, at different instrumental conditions (wavelength, potential applied, etc.) or with several detection points located in series or parallel. It may be,
(i) **Sequential**, using one or several detection points to obtain several signals at different times per injected sample; or

(ii) **Simultaneous**, using a single detection point performing simultaneous measurements at different instrumental conditions.

**Multi-determination:**

Determination of two or more analytes in a sample. It can be,

(i) **Sequential**, determining \( n \) analytes from \( n \) injections of the same sample.

(ii) **Simultaneous**, determining several analytes from a single sample injection.

It must be noted that multi-detection does not always involve multi-determination, since multi-detection can be applied to measure physical constants such as diffusion constants.

### 3.2.1 Simultaneous determinations with several detectors

The relative location of the detectors in simultaneous FIA systems permits a sub-classification according to whether their configuration is in series or in parallel. Their characteristics differ considerably.

#### 3.2.1.1 Detectors in series

Fig. 3.1 shows a typical manifold where detectors are arranged in series. The most common examples are the use of ion-selective electrodes placed sequentially in the manifold. Other non-destructive detection methods can also be applied, provided that the manifold configurations prevent excessive dilution of formed products.
3.2.1.2 Detectors in parallel

It is possible to distinguish between the FIA manifolds by comparing the manner in which the sample reaches each detector. The most common division is where the sample is split up in a regular and reproducible manner, sending each part of the sample to a different detector (Fig. 3.2) [3]. The length of the reactors of each subsystem is a function of the intrinsic characteristic of the employed reactions. Suitable diameter and length of tubing used, are also needed to compensate for the resulted decrease in pressure in the sub-systems. This is done to ensure that homogeneous division of the sample is obtained.
Samples can also be propelled to different detectors by using several valves operating simultaneously (simultaneous multi-injection). Fig. 3.3 shows a typical FIA manifold employing multiple valves. Each valve injects the corresponding sample into an aqueous stream that merges with a reagent suitable for the determination of the species to be analysed in the sub-system.

![Fig. 3.3 Schematic diagram of a FIA system where multi-injection is used. (D - detector and W - waste)](image)

Less usual is the technique of zone sampling where a part of the original injected sample is extracted and sent to one detector, while the rest of the sample is sent to another detector. This technique is used in simultaneous determinations where the parameters (or species) being analysed require different degrees of sample dispersion [4].

An advantage of having detectors in parallel arrangement is the fact that destructive detection systems can be used, since the same sample does not need to pass through a series of detectors. These destructive detectors include atomic-absorption spectrophotometers, flame photometers, ICP and ETA.
3.2.2 Simultaneous determinations with a single detector

FIA manifolds utilizing only one detector usually make use of photometric detection, where the two (or more) analytes absorb at the same wavelength after being reacted with a suitable reagent. Single multi-detectors, like ICP and AAS are also included under this heading. These detectors are unfortunately very expensive. Figs. 3.4 to 3.6 show FIA systems for multi-component analysis utilising only one detector.

**Fig. 3.4** Schematic diagrams of a FIA manifold for simultaneous determinations with a single detector. This manifold is based on the establishment of a concentration gradient. (D - detector and W - waste).

**Fig. 3.5** Schematic diagram of a FIA manifold with simultaneous double injection and an asymmetric merging configuration. (D - detector and W - waste).
3.2.2.1 Two flow cells

A FIA manifold designed by Steward and Růžička [5] allows the detection of two compounds using two flow-through cells aligned in the same optical path. After injection, the sample is split into two channels and allows to merge with the appropriate reagents, where after it is propelled through the two flow-through cells. A longer transmission line (tubing) is used to delay one part of the sample, ensuring that the peaks corresponding to the two species do not overlap. The flow cells used must be carefully constructed, since the background value depends on the type of material as well as the thickness of the walls of the cell. Too high background values lead to small linear ranges and low sensitivity.

3.2.2.2 pH gradients

This method involves a single detector and a single injection and is based on the use of a carrier with a pH value that is different to that of the sample. If the volume of the latter is sufficiently large, two zones of different characteristics exist that are close to the interfaces with pH values different of that of the central zone plug. The characteristics of these regions can be used to determine several species in the same
sample. Since this technique was used in Chapter 9, it will be discussed in greater detail in the following paragraph.

The complexation of metal ions with most spectrophotometric reagents, and especially with those ligands that might be protonated, is dependant on the pH of the reaction medium as well as the nature and the concentration of the ligand considered. Plots of pH versus absorbance are characteristic for any given metal ion-reagent combination and they are additive if the reagent is in large excess [6]. The shape of a curve obtained for a mixture of metal ions takes the form of a series of stepwise increases in absorbance towards higher pH, as each metal reaches the pH at which complexation took place. Provided that the pH regions of increasing absorbance do not overlap, the concentration of each metal may be determined from the absorbance change at each step and each element may be identified by the pH at the point of inflection [6].

3.2.2.2.1 Establishment of pH gradients [6]

The pH at any point along the tube when a sample of strong acid has been injected into a carrier stream of weak base will be governed by the extent of chemical reaction which has taken place between the acid and the base, and by the physical distribution of the acid and base along the tube. The effect of the chemical reaction follows from elementary acid-base theory. If an acid of concentration $C_A$ and acidity constant $K_a$ reacts with a base of concentration $C_b$, and the fraction of acid present after the reaction, $f_A$, is defined as $C_A/(C_A + C_b)$, then

$$pH = pK_a - \log (f_A/(1 - f_A)).$$

(3.1)

Within the limits of buffering action, $0.1 < f_A < 0.9$, there is an approximately linear relationship between $f_A$ and pH.

In a flowing system the physical distribution of the injected acid will follow a dispersion pattern, which in its most general form is described by

$$C_0 = \left[2(\pi DL)^{\frac{1}{3}}\right]^{-1} \exp \left[-(1 - \theta)^2(4D/L)^{\frac{1}{3}}\right]$$

(3.2)

where $C$ is the concentration, $\theta = t/T$ ($t$ is the time of the measurement and $T$ is the
mean residence time), \( D \) is the diffusion coefficient, \( u \) is the flow rate and \( L \) is the length of the tube. Fig. 3.7 outlines such a dispersion profile.

These considerations do not take into account the mode of mixing, which is of course crucial. In narrow tubes at low flow rates, radial molecular transport plays an important role in bringing about mixing of sample and carrier and indeed may predominate at very low flow velocities. If the tube was considered as a series of segments, in any one of which the concentration of acid and base were given by equation 3.2 and if mixing was by radial diffusion, a pH gradient would be established across the sample plug, the gradient being alkaline at the circumference and acidic at the centre. Whatever the mechanism of mixing, the process is efficient and the net effect of physical dispersion and chemical reaction is that the predicted pH gradient follows the line described by equation 3.2, with the pH at the points of inflection corresponding to the pK\(_a\) of the weak base if \( C_A^0 \) and \( C_B^0 \) are equal.

Insofar as the physical dispersion is easy to control and vary, and is reproducible, it is a straightforward matter to alter the pH gradient to suit requirements. The range of pH over the gradient is governed by the choice of weak base. A simple buffer is used if the pH range is to be narrow, but an universal buffer is used if the pH range is to be great. The argument is the same for a strong base injected into a weak acid or series of weak acids.
Care must be taken that the injected acid (or base) zone undergoes sufficient dispersion to allow gradual pH changes. Acid-base boundaries that are too steep resulted in overlapping of peaks.

3.2.2.3 Ion exchange

FIA and ion exchange association provide the possibility of carrying out simultaneous determinations. These reactions are usually indirect methods of determinations and are based on inhibitory effects on metal catalysed reactions [1].

3.3 Commutation in flow injection analysis

The application of intermittent and alternating streams without stopping the peristaltic pump, the sequential injection process and the concept of mono segmented flow injection systems have all been achieved successfully with manual commutation. Time controlled commutation greatly expanded the possibilities of FIA, allowing the development of new processes such as zone sampling, zone trapping and time based injection [7].

A commutator is usually made of perspex and consists of two fixed external plates with a movable central bar, all held tightly together by two screws with springs. Holes (1 mm bore) are made through the pieces of the commutator in accordance with the related flow diagram. For insertion of the polyethylene connecting tubes, either Tygon bushings were used or the holes were drilled so as to be slightly conical near the surface. Silicone rubber sheets with holes corresponding to those of the external plates were placed between the commutator pieces to avoid leakage. A metal lever, which can be operated manually or electronically, is used to move the central bar of the commutator between the two resting positions associated with the two (or more) states of the manifold. A simple commutator is illustrated in Figs. 3.8 A and B.
In Fig. 3.8 A the sample flow through the commutator to waste. In this way the loop (L) inside the commutator is filled with a precise volume of sample. The movable part of the commutator is now moved downwards (Fig. 3.8 B). This result in the insertion of the sample loop into the flowing carrier stream. The ‘injected’ sample is now propelled to the detector, while the flow of the sample stream is either stopped or is still flowing to waste through the third channel in the commutator. When the movable part of the commutator is moved back to the original position, the sample loop is filled for the next analytical cycle.

Commutators with more channels can allow the sequential or time-based ‘injection’ of different samples and reagents. It can also allow stopped-flow periods by blocking the flow in through a certain channel, without actually stopping the peristaltic pump. In this way sequential or simultaneous determinations can be performed.

3.4 Methods based on differential kinetics

One of the advantages of kinetic analysis methods over equilibrium methods is the possibility of carrying out simultaneous determinations based on the different rates of their reactions with a common reagent. In spite of this being a promising aspect, it has
several important limitations that restrict its applicability. Firstly, it should be emphasised that it is not easy to find chemical systems in which significantly important differences can be established in the experimental conditions between two or more reaction rates. On the other hand, the differential kinetic methods described do not have a very high level of accuracy and/or reproducibility, because slight disturbances produced by the diversity of samples or by slight changes in the working conditions lead to a low precision in comparison with other manual or kinetic techniques [1].

The principle of the kinetic methods is that for pseudo-first order reactions of different rates straight line calibration curves may be obtained for samples for any time \( t \) after injection for various metal concentrations. For different metals (analytes) which have different rates, the calibration curve will have different slopes, slower reaction components having lower slopes [9].

Few differential kinetic methods have been developed for FIA. For a description of the most significant contributions the systematisation in Table 3.1 has been adopted, according to the number of detectors that the system contains.

### 3.4.1 Systems with two detectors

This consists of a differential kinetic mode with a simple principle. The signal produced by the reacting species is measured at two different times, \( t_1 \) and \( t_2 \), in each detector [1]. The FIA manifold employs the two detectors in series (Fig. 3.1), which means that no splitting of zones take place. The detectors are situated sufficiently apart to allow ample time for the slower reaction to develop.

### 3.4.2 Systems with multi-detection

Hooley and Dessy [8] described a kinetic determination based on multiple measurements. The multiple detection system consists of a quartz reactor tube with a series of independent detection units with a LED (light emitting diode) and a
photodetector. Its signal is monitored by an electronic data-processing system. A plug of reactant sample passes successively through each measurement point at different times in such a matter that it is possible to process as many types of kinetic data as there are detectors. The relative locations of these detectors depends on the flow and the rate of the reactions considered. It is an ideal system for kinetic determinations and very useful for the simple determination of rate constants.

3.4.3 Systems with a single detector

Employing these manifolds, the detector must provide two different signals, or a signal increase, or both, at two times (or at a time increase) that coincide(s) with different reaction times. Several different manifold configurations for carrying out these determinations exist. Distinction can be made between systems with double and single injection.

3.4.3.1 Single injection

3.4.3.1.1 Combination of conventional FIA and stopped flow

These determinations depend on the fact that the first analyte complex is completely formed when it reached the detector, while the slower reaction develop during the delay time (stopped flow period) in the flow cell. These methods are only successful if the reaction of the more reactive component is essentially complete before the reaction of the less reactive component reaches one half-life [9].

3.4.3.1.2 Splitting of the flow

Fig. 3.6 shows a typical manifold where the sample stream is split into two parts and propelled through reactors with different lengths and diameters. The different geometrical and hydrodynamic properties of the two channels provide different residence times for each of them and two analyte peaks are obtained. The one part
of the sample travels through a shorter reactor and reaches the detector first (time = \( t_1 \)), allowing the detection of mainly the more reactive component, while the second peak (at \( t_2 \)) allow detection of the less reactive compound. Adaptation of this method to SIA is described in Chapter 7.

Detection is done either by using a double path flow cell [9, 11] or by stopping the flow when the zone reaches the flow cell. Data collection is done by monitoring of the change in absorbance as the reaction develops [10]. The ratio in which the sample is split is an important aspect when accuracy, sensitivity and reproducibility are concerned. The variables influencing the splitting are thoroughly described by Fernandez et al. [11].

### 3.4.3.2 Double injection

The principle of this method is that two zones of different composition are simultaneously injected and are propelled through tubing of different lengths (which coincide prior to detection) so that they reach the detector sequentially, resulting in two analyte peaks [1].

### 3.4.3.3 Adaptation to SIA systems

Since sequential injection analysis has the advantage that stopped flow periods can be easily incorporated, adaption of kinetic determination to SIA has many positive features. Unfortunately, it also has a few disadvantages. Because of the discontinuous nature of SIA, splitting of the sample zone can not be done by simply propelling the zone through two reactors with different geometrical and hydrodynamic properties. The zone is split by pumping the first part of the reacting sequence of zones into a second holding coil and leave it there to allow time for the less reactive compound to form. The rest of the zone which is still inside the first holding coil is then propelled through the detector and measured. The selection valve is then turned in such a way to select the second holding coil again. The portion of the zone "stored"
there is then drawn back into the first holding coil where after it is propelled through the
detector to be measured. Fig. 3.9 shows the SIA manifold used for the kinetic
determination of Ni(II) and Co(II) in Chapter 7.

The system can be largely simplified if the selection valve could move in both
directions, viz. clockwise and counter-clockwise. If this is, however, not the case,
different procedures are needed. In the manifold used in Chapter 7, the VICI selection
valve was able to move only in the counter-clockwise direction. Since the port in the
middle of the valve is connected to only one other port at a time, it was needed to
connect the second holding coil to ports 5, 8 and 1 by means of a perspex connector.
When the first holding coil (port in the middle of the valve) was connected to port 5
(second holding coil) half of the stack of zones was pumped into the second holding
coil. The remainder of the zones in the first holding coil was reacted with EDTA (port
6) and propelled through the detector (port 7).

Since it was impossible to move back to port 5 to collect the part of the zones in the
second holding coil, the second holding coil was connected via the perspex connecter
to port 8. Because port 8 was connected to the first holding coil via the port in the
middle of the valve, this connection allowed the movement of the zones from the
second holding coil back into the first holding coil. The zone was then reacted with EDTA (port 9) and propelled through the detector (port 10 was connected to the detector line via a T-piece perspex connector). To avoid any sample carry-over, it was needed to rinse the second holding coil prior to the next analysis. To do this, port 1 (which was connected to the first holding coil via the port in the middle of the valve) was connected with a short piece of Tygon tubing to the perspex connector and doing so it was connected to the second holding coil. Forward movement of the pump ensured effective rinsing of the second holding coil.

3.5 Multi-component techniques in sequential injection analysis

When excluding the articles published in speciation analysis, multi-component analysis in sequential injection analysis is virtually a novelty. The first publication on multi-component techniques in SIA was, however, published in 1995 [12]. A multi-linear regression program for the simultaneous determination of calcium and magnesium in mixtures was described. The assay relied on the formation of the Ca and Mg complexes with the chromogenic reagent 4-(2-pyridylazo)resorcinol (PAR); the complex mixture could be readily resolved, notwithstanding the extensive spectral overlap involved. Data were recorded with a diode array spectrophotometer and spectra were corrected in order to avoid the effect of changes in the refractive index inside the sensing microcell.

Estela et al. [13] described the simultaneous determination of ionic species by SIA using a sandwich technique with large sample volumes. Iron(II) and nitrite were determined using a large sample volume (2 ml) with different reagents at each end of the plug. Minimum zone overlap (good resolution between the two analyte peaks) was obtained with this large sample zone, which lead to minimum mutual interference. The optimisation of the method is slightly more difficult than for others and the sample and reagent consumption are much higher, since higher concentration and bigger reagent zones were needed to obtain the desired sensitivity. This technique was also evaluated in Chapter 6 together with a tandem SIA application.
Multi-component techniques involving detectors such as HPLC [14, 15], ion-selective electrodes [16, 17], ICP [18], immobilized enzymes [19] and hydride generation direct plasma AAS [20] are also described.

3.6 Other techniques that can do simultaneous determinations [21]

Separational (chromatographic) techniques are inherently simultaneous. The components of a sample are separated and the same attribute of every component measured. If this technique is combined with a scanning spectrometric technique, identification and quantification can be done simultaneously.

All techniques that involves scanning (monitoring the effects of varying levels of energy on the sample) are in principle capable of simultaneous determinations. The main problem with this is the great number of possible interferences, that require complex calibration procedures.

Thermal techniques are capable of simultaneous determinations, but in general resolution is poor. It is the only family of non-spectroscopic techniques that can analyse solid samples.

Of the electrochemical techniques, the scanning voltammetric techniques are the main candidates for simultaneous determinations. Non-scanning electrochemical techniques are not inherently simultaneous.

The usefulness of instruments that are in principle capable of simultaneous determinations is often limited by the time it takes to gather the information. Slow scans and lengthy separations are usually the culprits. This is why the development of new instrumentation that gather information faster are often used in simultaneous determinations. Examples of these, mostly expensive, detectors are diode-array spectrophotometers, FT-IR and ED-XRF.
Several simultaneous determinations also involve the use of a dialyser to separate the analytes from interferences as well as to isolate them from the matrix [22 - 25]. Although most speciation studies are also in effect simultaneous determinations, they were not discussed, since they do not really fit the term multi-component analysis.

3.7 Concepts in simultaneous determinations

A problem that arises in simultaneous determinations, not found in single determinations, is that of mutual interference. Chemical interference takes place when a species other than the analyte present in the sample changes the response of the instrument to a value different from the one when only the analyte is present. This is a problem in any kind of analysis. Mutual interference takes place when the concentration of two or more species are to be determined, and the instrumental response to at least one of them is influenced by one or more of the other species to be determined. The higher the selectivity, the smaller the problem of interference.

In separational techniques, the problem of mutual interferences is overcome by increasing the separation (number of plates).

Ordinary interferences can be eliminated; mutual interferences cannot, because to do so would destroy information. If the action of the interferences is well understood, measuring both interfering and interfered analytes can give enough information to separate the mutual effects. From this, the concentration of the analytes can be determined. A simple example of this is the simultaneous spectrophotometric determination of titanium, vanadium, and molybdenum in steel [26].

The absorbance (A) at a certain wavelength and light path length depends on the molar absorptivity (c) and the concentration (c) of a chemical species. According to “Beer’s law” [27] the dependence is linear:

\[ A = cc \]
If two species (x and y) absorb at the same wavelength, the total absorbance is equal to the sum of the individual absorbances:

\[ A = \varepsilon_x c_x + \varepsilon_y c_y \]

When the absorbances at two wavelengths (\(\lambda_1\) and \(\lambda_2\)) are measured and the molar absorptivities are known, a system of two simultaneous equations in two unknowns is generated:

\[ A^{\lambda_1} = \varepsilon_x^{\lambda_1} c_x + \varepsilon_y^{\lambda_1} c_y \]
\[ A^{\lambda_2} = \varepsilon_x^{\lambda_2} c_x + \varepsilon_y^{\lambda_2} c_y \]

The two unknowns can be determined by simple algebra, or (preferably) by multiple linear regression. In principle, measuring at \(n\) wavelengths make determinations of \(n\) analytes possible.

Another example is the use of the Nikolskii-Eisenman equation to separate the effects of mutually interfering ions on an array of electrodes [21].

\[ E_{ij} = E_{ij}^0 + S_j \log (a_{ik} + \sum_{n} K_{jk} z_{ij}^{z/n}) \]

The potentials \(E\) of \(j\) electrodes are measured in \(i\) samples. Using the known activities \(a_{ik}\) of the primary ions and the interfering ions (\(a_i\)), the slope \(S_j\), the standard potentials \(E_{ij}^0\), and the selectivity coefficients \(K_{jk}\) of the electrodes are determined by non-linear curve fitting. Knowing the parameters of the electrodes, predictions of activity can be made from the measured potentials of unknown samples.

In his review on simultaneous determinations in FIA, Kubán [2] mentions another problem of simultaneous determinations: redundant information. More data are collected than is needed for the determination of the analytes in question. Storage space (physical and/or electronic) costs makes this an expensive excercise.
3.8 Conclusion

Simultaneous analysis are of great value in the fields of clinical analysis, environmental analysis and also in control laboratories. Multi-component SIA (and FIA) systems have the advantage that automation lead to less errors due to human imperfectness and that precise timing, splitting of samples and multiple measurements can be accomplished. Although it is true that increasing the number of detectors increases the overall cost of the manifold, there are several relatively inexpensive detectors that can perform the detection just as well. In a world where efficiency needs to be high, while cost needs to stay low, development of multi-component techniques employing only one detector will be the road to the future.
3.9 References