CHAPTER 9

Simultaneous Determination of Cadmium(II) and Mercury(II) in Aqueous, Urine and Soil Samples using pH-gradient Sequential Injection Extraction

9.1 Introduction

When looking at Mendeleev's Periodic Table of Elements, zinc, cadmium and mercury all belong to the same group (II b), which make one thinks that their chemical behaviour will in some extent be similar. Their most noticeable features compared with other metals are their low melting and boiling points, mercury being unique as a metal which is a liquid at room temperature. It is, however, a remarkable contrast that, whereas Zn is biologically one of the most important metals and is apparently necessary to all forms of life, Cd and Hg have no known biological role and are amongst the most toxic off all elements [1].

Cadmium as well as mercury are adsorbed on ground particles. This adsorption usually occurs when the ground pH is in the pH range 4.5 to 5.5. Lower pH values increase the solubility of these two species in groundwater and make them more mobile and available for uptake by plant roots or micro-organisms. Under the influence of low pH values or high chloride concentrations, mercury is converted into toxic organic forms, like methyl mercury, which plays a major role in studies on the toxicity of mercury.

The so-called Minamata and itai-itai deceases are serious conditions caused apparently by heavy metals, such as mercury [2] and cadmium [3], in the aquatic environment. Most patients were middle-aged or elderly females, who suffered from severe pains all over the body and died in pain. The first symptom of the decease was usually lumbago,

followed by later development of pseudofractures and a waddling gait. The etiology of the itai-itai decease is related to dietary cadmium and malnutrition. Cadmium inhibit renal function, increased loss of calcium due to depression of proximal reabsorption and lead finally to osteomalacia [3]. Other symptoms due to cadmium poisoning are: (i) emphysema of the lungs, (ii) dysfunction of the kidneys and (iii) low molar mass proteinuria.

Cadmium in the aquatic environment is generally taken into the body via the gastrointestinal tract as drinking water and food; the absorption rate from the gastrointestinal tract has been demonstrated to be 3 - 6%, while uptake of cadmium through the lungs is said to be as high as 10 - 40%. One third of the cadmium taken up by the body is accumulated in the kidneys. The blood cadmium level probably indicates the present degree of cadmium exposure, and the total burden of cadmium is roughly proportional to urinary cadmium excretion [3].

To diagnose cadmium poisoning it is, however, necessary to know the degree of cadmium exposure and total body burden of cadmium [3]. Environmental cadmium should therefore be checked first of all. Techniques such as anodic stripping analysis and/or flameless atomic spectroscopy were mainly used to determine cadmium. This study concentrated on finding more cost-effective, rapid methodologies to determine cadmium in urine and aqueous solutions. A simple sequential injection extraction method was proposed to solve this problem.

In nature, mercury occurs in several forms, e. g. metallic mercury, inorganic mercury and organic mercury compounds. All forms of mercury are considered poisonous, but methylmercury is of particular concern since it is extremely toxic and is frequently found in the environment. Through a very effective biomagnification mechanism, methylmercury is enriched in food chains which results in high levels in top predators, like fish such as northern pike and tuna. In Japan, methylmercury contamination caused severe brain damage to twenty two infants whose mothers had ingested contaminated fish during pregnancy. In Iraq the intake of wheat flour from seeds

treated with organic mercury has also led to large scale poisoning. In general, exposure to organic mercury can cause brain damage to the developing foetus. Exposure is also considered more dangerous for young children because their nervous system is still developing and thus are more sensitive towards these compounds [4].

In normal persons who has not been subjected to any particular exposure, the total mercury levels in the blood and in the urine are less than 5 µg/ℓ. It is considered that there is a long term risk of intoxication above 30 µg/ℓ and 50 µgℓ in urine. A part of the mercury in the blood is in an organic form, primarily as methylmercury [5]. As in the case of cadmium, the urinary excretion of mercury is also a measure of the levels of total body burden.

Dickinson Burrows [6] wrote a review of the status of total mercury analysis, wherein the most important analytical techniques were reported. According to the review, flameless or cold-vapour atomic absorption spectrometry is the method of choice for most laboratories. Since most mercury poisoning comes from environmental mercury, usually in the form of methylmercury, and because urinary excretion gives an good indication of the level of poisoning, it was decided to determine mercury by a sensitive colorimetric method, using Dithizone. Because mercury and cadmium required monitoring in the same type of samples it was decided to determine them simultaneously.

9.2 Choice of analytical method

9.2.1 Methods of determination

Several analytical methods to determine mercury exist which include cold vapour AAS [7 - 10], chromatography (GC and HPLC) [11], potentiometric stripping analysis [12], enzymatic determination [13], ion-selective electrodes [14], ICP-AES [15,16] and spectrometry [17 - 24]. All of these methods, except for the last, have the disadvantage that it is relatively expensive and it could not be used for on-site field analysis.

Spectrophotometric determinations are therefore essential as inexpensive on-site measuring tools.

Several reagents [18] are suitable to react with mercury to form coloured complexes that can be determined photometrically. Many of these photometric determinations were adapted to FIA systems - even those that needed an extraction step as part of the procedure. Photometric determinations include: kinetic methods [25] or indirect determinations where, for instance, the inhibitory effect of Hg(II) in the catalytic action of iodides on the As(III)-Ce(IV) reaction is measured [23].

The extraction method for methylmercury, as developed by Gage [26], is still widely used. The extraction is based on the addition of acid (hydrochloric, hydrobromic or hydroiodic) to a homogenised sample, the extraction of the methylmercury halide into an organic solvent (benzene or toluene), purification by stripping with a thiol compound (cysteine or thiosulphate) and re-extracted into benzene. A problem with the extraction method is the formation of often persistent emulsions which can be avoided by the use of cysteine impregnated paper instead of cysteine solution [4]. Because Hg is considered to be a "Dithizone metal", determinations of mercury using extraction with diphenylthiocarbazone (Dithizone) are well-known and widely used [6, 17, 27]. With Dithizone it is also possible to determine organic forms of mercury, like the methylmercury species [27].

Cadmium is usually determined using flame-AAS [28] or cold vapour AAS [29] and ion-selective electrodes [30]. A number of colorimetric reactions are adapted to FIA, this include a number of applications where Dithizone is used as extractant [17, 20, 21]. Existing methods for the determination of cadmium involve preconcentration which is very time-consuming. The determination of cadmium at low concentrations which may be of toxicological significance requires a highly sensitive method. A flow injection system designed to determine lead and cadmium using liquid-liquid extraction with Dithizone in chloroform was used by Klinghoffer [21] and was refined by Burguera *et al.* [17] to increase the selectivity and sensitivity of this procedure.

Since both mercury and cadmium can be extracted with Dithizone and because of the extreme sensitivity of the reagent, this extraction was chosen as analytical method. According to Irving [27] and Fries and Getrost [18] carbon tetrachloride or chloroform were the best organic solvents for the dissolution of Dithizone. Carbon tetrachloride was chosen as organic solvent for this study, not only because of the solubility of Dithizone in CCl_4, but also because of the ability of CCl_4 to form a sufficiently thick thin-layer against the Teflon walls of the tubing. Carbon tetrachloride is also used as solvent, since it also enables the separation of Cd/Zn [18].

9.2.2 Reactions with diphenylthiocarbazone

Depending on the reaction conditions, diphenylthiocarbazone (Dithizone) and mercury(II) ions form an orange-yellow dithizonate [$Hg(HDz)_2$] in the acidic range or a violet secondary dithizonate (HgDz) in the neutral to alkaline range [18]. Both complexes are insoluble in water, but readily soluble in chloroform and carbon tetrachloride. The primary dithizonate is of analytical importance, because it is not only stable in 5 mol/ℓ sulfuric acid solution, but once formed it is also very stable in dilute alkaline solutions. It is however less stable in hydrochloric acid, and decomposes completely at higher HCl concentrations.

Dithizone is the preferred reagent for photometric determination of cadmium, although this reagent is not specific and numerous other heavy metals also form dithizonates. Cadmium determinations with Dithizone give optimum results when carried out at a pH of approximately 9. To ensure this pH an alkaline buffer made up from sodium potassium tartrate and NaOH (or KOH) of pH of 10.5 was used. The cadmium-Dithizone complex has a pinkish colour and is very stable in the strong alkaline range. Although Cd-dithizonate is easily decomposed by weak acids, it experiences no interference from HCl, H_2SO_4 and HNO_3 [18].

9.3 The simultaneous extraction of mercury(II) and cadmium(II) with Dithizone

9.3.1 Experimental

9.3.1.1 Reagents and solutions

All solutions are prepared of analytical grade reagent unless specified otherwise. Deionised water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used to prepare all aqueous solutions and dilutions. The water used as carrier was degassed before use.

Extractant: 0.1 g of Dithizone (Hopkin & Williams Ltd.) was dissolved in 250 mℓ CCl_4 to produce an emerald green stock solution. This solution was stored in a dark bottle and overlaid with 10 mℓ water and 1 mℓ of a 0.5 mol/ℓ sulphuric acid solution. Stored in a cool place and protected from light this solution is stable for some months. Working solutions are obtained by suitable dilution of the stock solution with CCl_4. These solutions were saturated with water before use.

Mercury stock solution: A 100 mg/ℓ Hg^{2+} stock solution was prepared by dissolving 0.171 g $Hg(NO_3)_2.2H_2O$ (Merck) in 1ℓ of distilled water. Working solutions are obtained by suitable dilution of the stock solution.

Cadmium stock solution: A 100 mg/ℓ Cd^{2+} stock solution was prepared by dissolving 0.2744 g $Cd(NO_3)_2.4H_2O$ (Merck) in 1ℓ of distilled water. Working solutions are obtained by suitable dilution of the stock solution.

Alkaline buffer solution: A solution containing 5 g NaKtartrate and 2 g NaOH in 100 mℓ distilled water was used as buffer solution. This solution has an pH of ± 10.5.

H_2SO_4 solution: To ensure the correct pH for the Hg^{2+} extraction a 1 mol/ℓ H_2SO_4 solution was prepared by diluting 55.5 mℓ of concentrated acid (98%) to 1 ℓ with distilled water.

A 0.43 mol/ℓ *acetic acid* solution was used as eluent during the soil extractions.

9.3.1.2 Instrumentation

The sequential injection extraction (SIE) manifold is illustrated in Fig. 9.1. It was constructed from two Gilson Minipuls peristaltic pumps (both operating at 10 rpm), a 4 m long extraction coil (1.02 mm i.d.) made of Teflon (PTFE) tubing (SUPELCO) and a 10-port electrically actuated VICI selection valve (Model ECSD10P) (Valco Instruments, Houston, Texas). Acidflex pump tubing was used for both pumps. The reaction coil was constructed using 30 cm of 0.75 mm i.d. porous silicon tubing together with an h-shaped glass debubbler (D3) (Dermo Tech). A 50 cm (0.8 mm i.d.) piece of Teflon tubing connected the debubbler to pump 3 which was operating at 0.8 mℓ/min to pump away the air bubble. The holding coil was constructed of 1 m (0.8 mm i.d.) Teflon tubing. A Teflon pulsation coil of 6 m (0.25 mm i.d.), coiled around a 10 mm plastic rod, was used to eliminate pulsation of the CCl_4 carrier solution.

An Unicam 8652 UV-VIS spectrophotometer equipped with a 10 mm Hellma flow-through cell (volume 80 μℓ) was used to monitor the coloured product at 486 nm. Data acquisition and device control were achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (MINTEK, Randburg, South Africa). The FlowTEK [31] software package (obtainable from MINTEK) was used throughout the procedure.

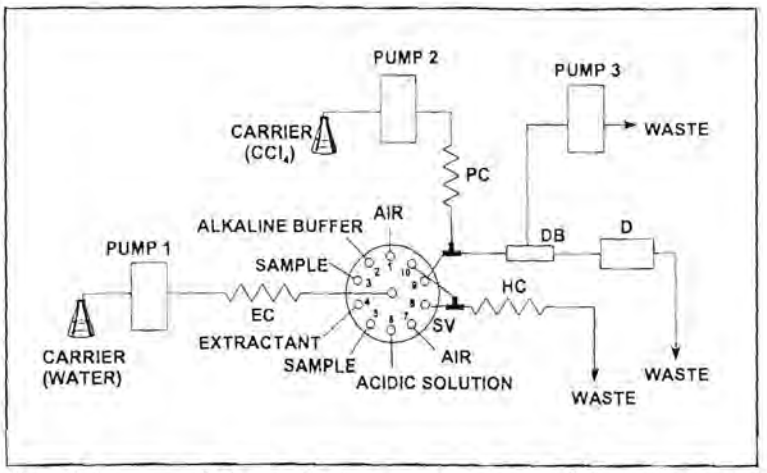

Fig. 9.1 Schematical representation of the sequential injection extraction manifold used for the simultaneous determination of cadmium(II) and mercury(II). EC - extraction coil, SV - selection valve, HC - holding coil, PC - pulsation coil, RC - reaction coil, DB - debubbler and D - detector.

9.3.1.3 Procedure

A small air bubble was drawn up (via port 1 of the selection valve) to separate the extraction zones from the aqueous carrier solution. Thereafter a zone containing alkaline buffer (port 2), a sample zone (containing both analytes) (port 3), the extractant zone (Dithizone in CCl_4) (port 4), a second sample zone (port 5) and a zone containing H_2SO_4 (port 6) were drawn up into the extraction coil. Another air bubble was drawn up (via port 7) to separate these zones from the aqueous carrier in the holding coil.

By switching pump 1 in the reverse direction, the H_2SO_4 and second sample zone mixed, ensuring the correct pH for the mercury extraction to take place. Extraction took place into the thin organic layer formed by the Dithizone zone, whose flow was impeded due to the hydrophobic interactions with the walls of the Teflon coil (Fig. 9.2). During this process an $Hg(HDz)_2$ complex was formed. This complex is stable in dilute alkaline solutions and therefore did not decompose when the flow was reversed to allow the alkaline extraction to take place in the same way as the acidic extraction.

The sequence of zones were propelled forward until the aqueous zones were pumped into the holding coil. The remaining cadmium and mercury dithizonates in the CCl_4, collected by the first bubble, was pumped into the reaction coil until the organic zone was just inside the reaction coil. The valve was switched to select port 10 which was connected via a T-piece to the holding coil. Pump 1 was then switched on (forward) to rinse the holding coil, while the product zone was propelled by the CCl_4 carrier solution using the forward motion of pump 2. The bubble was removed prior to detection using an h-shaped debubbler. Care was taken that the bubble was removed effectively, but that not too much of the product zone was removed.

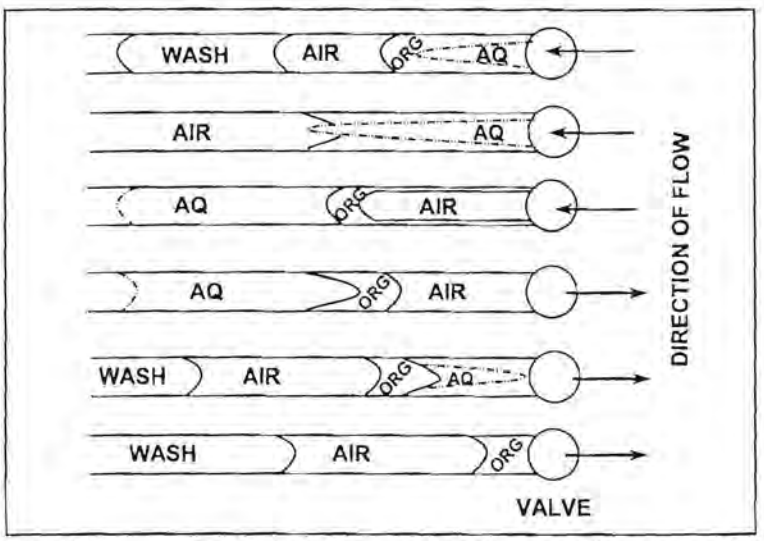

Fig. 9.2 Experimental protocol used in sequential injection wetting film extraction. AQ 1 and AQ 2 refer to the order in which the aqueous zones were drawn into the extraction coil and ORG represent the organic phase.

The instrumental procedure (as programmed in FlowTEK) is given in Table 9.1. The formed product peak was measured at 486 nm.

TABLE 9.1 Device sequence of the sequential injection system for determination of Hg and Cd.

Time (s)	Pump 1	Pump 2	Valve	Description
0	Off	Off	Air	Both pumps off, valve is turned to position 1 to select first air inlet.
4	Reverse			First air bubble is drawn up into the extraction coil.
5	Off			Pump 1 stops.
6			Alkaline buffer	Valve is turned to position 2 to select the alkaline buffer line.
7	Reverse			The alkaline buffer is drawn up into the extraction coil.
12	Off			Pump 1 stops.
13			Standard/ sample	Valve turned to position 3 to select the first standard or sample line.
14	Reverse			The first standard or sample zone is drawn up into the extraction coil.
15	Off			Pump 1 stops.
16			Extractant	Valve is turned to position 4 to select the extractant line.
17	Reverse			The extractant zone is drawn up into the extraction coil.
19	Off			Pump 1 stops.
20			Standard/sample	Valve is turned to position 5 to select the second sample or standard line.
21	Reverse			The second sample or standard zone is drawn up into the extraction coil.
22	Off			Pump 1 stops.
24			Acidic solution	Valve turned to position 6 to select the acidic solution line.
25	Reverse			The acidic solution is drawn up into the extraction coil.
26	Off			Pump 1 stops.
27			Air	Valve is turned to position 7 to select the second air inlet.
28	Reverse			Second air bubble is drawn up into the extraction coil.
29	Off			Pump 1 stops.
30			Holding coil	Valve is turned to position 8 to select the holding coil.

TABLE 9.1 continued Device sequence of the sequential injection system for determination of Hg and Cd.

Time (s)	Pump 1	Pump 2	Valve	Description
31	Reverse		Holding coil	Extraction step 1: the acidic solution and sample 2 are mixed to ensure correct pH for Hg extraction and then extracted into the thin layer of CCl_4 (with Dithizone) which coated the Teflon walls of the tubing.
66	Forward			Extraction step 2: the flow is reversed and the acidic mixture moves back through the organic thin layer. The alkaline solution and sample 1 was also mixed during the previous step and this mixture is now also propelled through the organic layers and the analyte is extracted into the CCl_4 (with Dithizone). The second air bubble collect the organic layer again and the aqueous phase together with the first air bubble are pumped into the holding coil.
105	Off			Pump 1 stops.
106			Detector	Valve is turned to position 9 to select the detector line.
107	Forward			The organic zone with the extracted product and the second air bubble are propelled into the detector line.
110	Off			Pump 1 is stopped immediately after the bubble entered the detector line.
110		Forward		Pump 2 is switched on and the CCl_4 carrier is used to propelled the organic zone through the detector.
111			Holding coil	Valve is turned to position 10. This port of the valve is connected (by means of a T-piece) with a short piece of Teflon to the holding coil. This means that the holding coil is now selected.
112	Forward			The holding coil is now flushed with clean water to eliminate any sample carry-over.
186	Off	Off		Both pumps are stopped. End of analytical cycle.

9.3.1.4 Sample preparation

Sample collection: Urine samples were collected in polypropylene flasks which had previously been cleaned by rinsing with dilute nitric acid and water. The samples were quickly frozen after collection with minimum air space above the urine. Soil samples

were taken from a maize farm in the northern Free State and stored in polypropylene containers.

Before analysis, the frozen urine was allowed to reach room temperature and thoroughly mixed. All water samples were analysed directly. Representative soil samples of 20.00 ± 0.05 g were dried at 30°C for 8 - 10 hours. 5.00 ± 0.01 g of the air-dried soil was weighed into a beaker and 50 mℓ of a 0.43 mol/ℓ CH_3COOH solution was added. The suspension was stirred for 30 min and then filtered. The filtrate was analysed directly (pH corrections were done on-line).

9.4 Method optimisation

9.4.1 Physical parameters

A number of physical parameters can influence the degree of dispersion and extraction in the manifold. To obtain the highest sensitivity and precision it was necessary to optimise these parameters.

9.4.1.1 Zone sequence

Since two different reactions at different pH-values were taking place the zone sequence was of utmost importance. Different zone sequences were evaluate to find the sequence resulting in less interference and optimum extraction. The following sequence was first evaluated: extractant zone followed by the acidic zone, then the sample zone and finally the alkaline buffer. This sequence resulted in a neutralisation reaction when the acidic and basic zones overlap. This happened because the sample zone separating the two buffer zones was not large enough to prevent overlapping of the acidic and basic zones. When the organic extractant zone was placed behind the aqueous zones insufficient extraction took place as the aqueous zones only moved through the organic zone after the flow was reversed. The optimum zone sequence was found to be: an air bubble (separating extraction zones from carrier), the alkaline

buffer solution, sample zone 1, extractant zone (Dithizone reagent), a second sample zone, the acidic zone followed by another air bubble.

9.4.1.2 Introduction of air bubbles

Although it was feared that the introduction of air bubbles into a flow system would have led to irreproducible results, this was to a major extent not the case. The first bubble separated the extraction zones from the surrounding carrier solution. Preventing, in the first place, excessive dilution of the sample zones and secondly, it was used to 'collect' the thin layer of organic solvent again. This bubble was also pumped into the reaction coil and was removed by the debubbler prior to detection. The second bubble was used to separate the extraction zones from the solution in the holding coil, thereby preventing any carry-over between successive samples. In this way, a total recovery of the organic phase and the dispensing of the use of a separation device were achieved.

9.4.1.3 Removal of air bubbles

As important it was to introduce the air bubbles, it was even more important to remove them effectively prior to detection. Initially a debubbler in the form of a dialyser with a Spectrapor 1 membrane was used. The membrane (vol/cm = 8 mℓ, MW cutoff = 6 000 - 8 000, dry cylinder diameter = 31.8 mm and dry thickness = 0.030 mm) was insoluble in CCl_4. The membrane was mounted on a 16 cm x 5 cm perspex block with a flow path of 10 cm x 30 mm x 25 mm. The extraction product together with a small residue of aqueous solution and the first air bubble were pumped through the dialyser. This was aimed to achieve the removal of the excess water as well as the air bubble. Unfortunately, the lifetime of the membrane was not that long (maximum of two days) and deterioration of the membrane resulted in small leakages. The CCl_4 leaking through the membrane caused the iron screws to rust and that contaminated the system.

A piece of porous silicon tubing (length 30 cm, i.d. 0.75 mm and o.d. 1.25 mm) was then

introduced as debubbler. This would be effective, provided that there was ample back pressure on the air bubble, caused by the use of a flow restricter, or by simply elevating the outlet tube. The pressure placed on the bubble is then greater than atmospheric pressure and this difference force the air bubble through the porous tubing [32]. The pressure inside the SIA manifold was however not sufficiently high to push the air bubble through the pores. Eventually an h-shaped glass debubbler was placed in the reaction coil. A 50 cm (0.8 mm i.d.) piece of Teflon tubing connected the debubbler to a pump, operating at 0.8 mℓ/min, to pump away the air bubbles. Care was taken that the bubble was remove effectively, but that not too much of the product zone was removed. This debubbler proved to be the most effective and was incorporated into the SIA manifold.

9.4.1.4 Flow rate

Since two pumps were used in these application two different flow rates were to be considered. The first pump controlled the volumes of the different zones as well as the extraction process, whilst the second pump was used to propel the formed product zone to the detector. Table 9.2 and Fig. 9.3 illustrate the results obtained for the various flow rates evaluated. It shows clearly that lower flow rates needed to be used during the extraction process and faster flow rates to propel the product zone through the detector. Initially both pumps were set on the same speed. At flow rates higher than 2.64 mℓ/min the organic zone tended to break up and formed small organic bubbles in the aqueous phase. This lead to very irreproducible results. The optimum flow rate for pump 1 (using water as carrier) was therefore chosen to be 2.40 mℓ/min.

Faster flow rates result in less dispersion and it was therefore necessary to optimise this parameter further. An optimum flow rate of 3.8 mℓ/min was obtained for pump 2 (using carbon tetrachloride as carrier). The faster flow rate was also needed to flush out the fine suspension of water droplets inside the flow cell. These droplets resulted from the small amount of aqueous phase still present in the organic phase when it was pumped to the detector. The faster rinsing time also favoured shorter analysis time and

therefore a higher sample throughput.

TABLE 9.2 Influence of the individual flow rates of the two pumps on the sensitivity and precision

Flow rate (mℓ/min)		Relative peak height		%RSD	
Pump 1	Pump 2	Pump 1	Pump 2	Pump 1	Pump 2
1.35	1.35	2.65	2.65	5.75	5.75
1.50	1.50	2.96	2.96	6.21	6.21
1.75	1.75	3.42	3.42	5.20	5.20
2.40	2.40	4.52	4.52	1.79	1.79
2.64	2.64	5.28	5.28	2.79	2.79
3.00	3.00	6.77	6.77	10.3	10.3
2.40	3.00	4.52	7.13	-	2.08
2.40	3.50	4.52	7.58	-	1.98
2.40	3.80	4.52	8.25	-	1.85
2.40	4.20	4.52	8.13	-	2.06
2.40	4.50	4.52	8.06	-	3.26

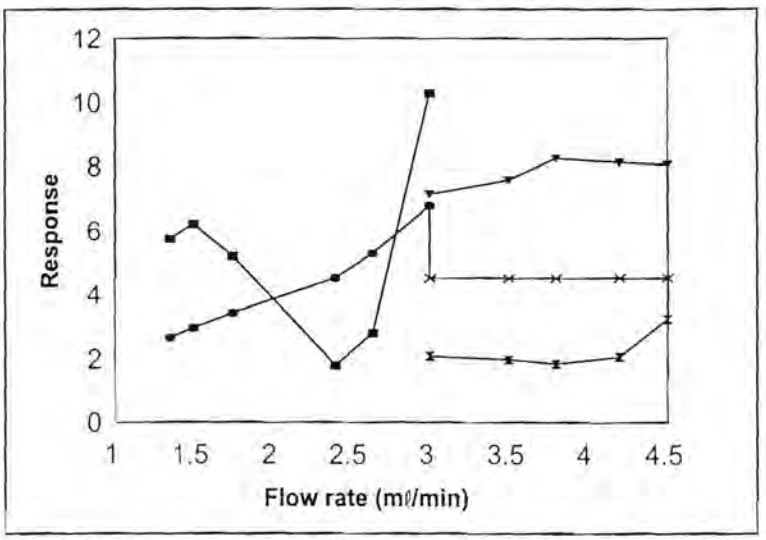

Fig. 9.3 Influence of flow rate on sensitivity and precision for both pumps. -●- = relative peak height when both pumps operate at the same flow rate, -■- = %RSD when both pumps operate at the same flow rate. To show the effect of the flow rate of pump 2 on sensitivity, -▼- = relative peak height of pump 2 alone, while pump 1 was kept constant (-✕-). The %RSD of pump 2 is represented by -✕-.

9.4.1.5 Sample volume

Sample zones were present on both sides of the organic zone. It was important to optimise this parameter to ensure that effective mixing with the buffer solutions was obtained as well as to ensure maximum analyte to be extracted into the organic phase. The two sample zones were evaluated independently. Table 9.3 and Fig. 9.4 shows the influence of different sample volumes on the sensitivity and reproducibility of the different test runs. For sample 1 (the zone between the alkaline solution and the organic phase) an optimum volume of 220 $\mu\ell$ (%RSD - 0.79) was established. It seemed from Fig. 9.4 that even bigger sample zones would led to higher sensitivity. These values were however ruled out due to their poor reproducibility. The volume of sample 1 was significantly higher than the optimum volume of 90 $\mu\ell$ (%RSD - 0.96) used for the second sample zone (the zone between the organic phase and the acidic solution).

The different results can easily been explained in terms of the different dispersions that the zones undergo. Smaller zones result in bigger dispersion, and therefore in better zone overlap [33, 34]. This explains why the second sample zone, which spends a shorter residence time in the extraction coil, needs to have a smaller volume than the first sample zone which spends a longer time in the extraction coil.

TABLE 9.3 Influence of the two sample zones on the precision and reproducibility of the SIE system

Sample volume ($\mu\ell$)		Relative peak height		%RSD	
Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
45	45	2.66	3.25	3.54	2.05
90	90	3.49	2.03	3.92	1.79
135	135	3.92	1.79	3.63	2.11
180	180	4.17	1.20	3.35	2.51
225	-	4.31	0.79	-	-
270	-	4.42	2.12	-	-

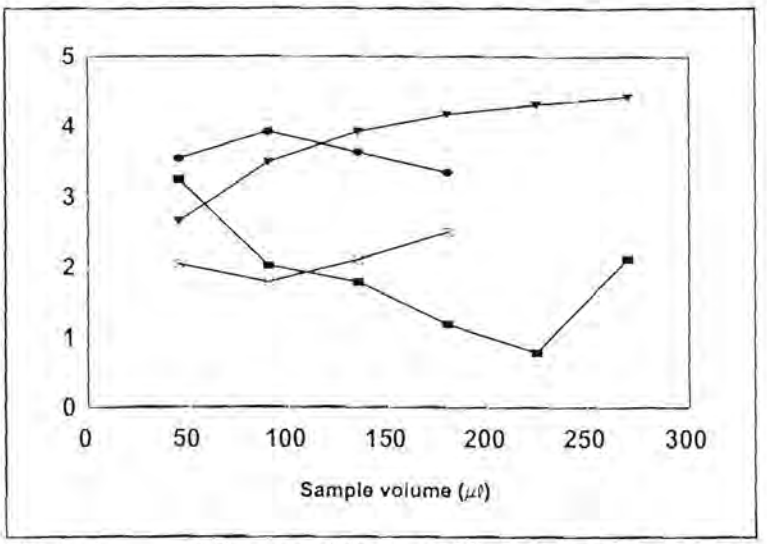

Fig. 9.4 Influence of the two different sample zones on the precision and sensitivity of the determination. ■ = %RSD for sample volume 1, ▼ = relative peak height for sample volume 1, ● = relative peak height for sample volume 2 and ⧖ = %RSD for sample volume 2

9.4.1.6 Extractant volume and extraction time

This volume is dependant on a critical parameter called the zone inversion length. Zone inversion length refers to the distance travelled by the zones from the load position (where the organic zone was situated in the extraction coil after aspiration), to the position where the aqueous phase (AQ 2) has moved through the organic phase and reached the tail of AQ 1 (refer to Fig. 9.2). At this point the organic zone has been deposited on the tubing as a film, allowing maximum contact between the two phases. The volume of the extractant zone influence both the time of the extraction as well as the length of the extraction coil.

Different extractant volumes were evaluated. Larger volumes not only produced an excess of reagent, which greenish colour interfered in the determination, but also lead to unnecessary long extraction times. Taking the 50 $\mu\ell$ used by Peterson *et al.* [35] as guideline a volume of 45 $\mu\ell$ was found to be the optimum. Smaller volumes gave very irreproducible results due to the fact that extractant volume was not reproducibly drawn into the extraction coil. For volumes smaller than 45 $\mu\ell$, aspiration times of less than

a second were needed. Because of the imperfect flow dynamics of the pump (start up and stopping are not instantaneous) [34], these small volumes were not aspirated reproducibly.

It took 35 s for zone inversion to take place whereafter the flow was reversed and the aqueous sample moved through the organic film into the holding coil. This step took 39 s, which accounted for a total extraction time of 74 s. During this step the organic phase was collected again by the second air bubble. Shorter extraction times lead to incomplete zone inversion and lower sensitivity as can be seen in Table 9.4.

Table 9.4 The effect of various extraction times on the sensitivity and reproducibility of the SIE method.

Time needed for zone inversion (s)	Time needed to collect organic phase (s)	Total extraction time (s)	Relative peak height	%RSD
23	29	52	1.80	2.67
25	31	56	2.15	3.63
27	33	60	2.29	3.05
29	34	63	2.80	3.59
31	36	67	3.02	3.12
33	37	70	3.61	2.33
35	39	74	4.26	1.53
37	41	78	4.27	3.77

9.4.1.7 Volumes of acidic and alkaline buffer solutions

Due to the pH dependancy of the different extraction procedures, the volumes of the buffer zones were evaluated using sample to buffer volume ratios. A known volume of sample was taken and known amounts of buffer solution was added till the correct pH was reached. These ratios were then used to estimate the volume of buffer to be drawn into the extraction coil. The following ratios were found manually: Alkaline buffer : sample = 1 : 5 and sample : acidic solution = 2 : 1. Due to the difference in dispersion

which the two buffer zones undergo, these parameters were also evaluated by aspirating different volumes of each buffer zone individually. The optimum volumes were found to be 45 $\mu\ell$ for both buffer solutions (Table 9.5), which corresponded excellently with the predicted ratios.

Table 9.5 Influence of buffer zone volumes on the extraction efficiency and sensitivity of the method.

Volume of buffer solution ($\mu\ell$)	Ratio Buffer : sample* (alkaline)	Ratio Buffer : sample# (acidic)	Relative peak height	%RSD
45	1 : 5	-	3.44	2.96
90	1 : 2.5	-	3.13	3.39
135	1 : 1.6	-	2.98	3.24
45	-	1 : 2	3.44	2.96
90	-	1 : 1	3.29	3.18
135	-	1 : 0.67	3.04	3.78

The optimum sample volume was used to determine the buffer to sample ratios.
* sample volume = 220 $\mu\ell$
# sample volume = 90 $\mu\ell$

9.4.1.8 Organic film thickness

The thickness of the organic film influences extraction by affecting the mass transfer of analytes into the film [36]. Relative film thickness per unit length (d_f) can be predicted using the equation [35]

$$d_f = kd_t(u\eta/\gamma)^a \qquad 9.1$$

where u represents flow rate (velocity) and d_t tubing diameter. The solvent characteristics also play an important role and are included in the equation. Viscosity of the solvent is represented by η and surface tension by γ, k and a are constants between ~1/2 and ~ 2/3. From the equation it can be seen that film thickness is directly proportional to viscosity and inversely proportional to surface tension. As colligative

properties, it is appropriate to consider either interfacial or surface tension to viscosity as a film thickness parameter, η/γ [35]. The film thickness parameter for CCl_4 was calculated to be 3.26×10^{-2} (η = 0.88 cP and γ = 27.00) [37].

Slower flow rates resulted in longer zone inversion times and therefore thinner films. Faster flow rates, on the other hand, resulted in thick films with high extraction capacity, but analyte recovery was only partly because the contact time with the extractant was shortened. Under optimum running conditions the relative film thickness was calculated to be 7.5 μm.

9.4.1.9 Diameter and length of tubing

Extraction coil: The length of the extraction coil depends on the zone inversion length. Thickness of the wetting film is directly proportional to the inner diameter of the coil [38]. As a result, the extraction capacity (volume of the wetting film) is larger for wider and longer extraction coils. Using an extractant volume of 45 $\mu\ell$ an extraction coil of 2.8 m was needed. To ensure that none of the zones reached the pump conduit and became deformed, an extraction coil of 4 m was used. An inner diameter of 1.02 mm ensured good axial dispersion and zone overlap.

Holding coil: A 1 m long (0.8 mm i.d.) Teflon coil was used to allow the flow of the aqueous carrier to propel the extraction zones inside the extraction and holding coils. The relative large diameter was chosen to ensure minimum back pressure when the aqueous zones were pumped from the extraction coil to waste. It was important to rinse the holding coil after every analysis to avoid sample carry-over.

Reaction coil: Porous silicon tubing (30 cm, 0.75 mm i.d.) was used as reaction coil. This was done mainly to debubble the carrier solution prior to entering the flow cell. Unfortunately, this did not have the desired result and an h-shaped glass debubbler was installed. The debubbler was connected to pump 3 by means of a 50 cm (0.8 mm i.d.) piece of Teflon tubing. The pump was operating at a flow rate of 0.8 mℓ/min to ensure

effective removal of the air bubbles. The spectrophotometer should be positioned as close to the debubbler as possible. Longer distances between the debubbler and detector led to higher dispersion, longer rinsing times and ultimately lower sample throughput.

9.4.2 Chemical parameters

9.4.2.1 pH

Dithizone forms complexes with all the heavy metals as well as some other metals, such as Fe and Al [18]. To ensure high selectivity in determinations using Dithizone, pH control is of the utmost importance. Mercury forms a primary dithizonate in the acidic range (pH 1 - 4) [18], while cadmium forms a orange-red dithizonate in the alkaline range (pH 8.5 - 10.5) [30]. Preliminary experiments showed that the optimum extraction pH for mercury would be 3.5 and for cadmium extraction about 9. To ensure that these pH values were achieved in the flow system a sample to buffer volume ratio was used.

It was also important that the acidic extraction should have taken place first due to the stability of the $Hg(HDz)_2$. The cadmium dithizonate is however not very stable in acidic solutions [18] and the alkaline buffer was therefore carefully evaluated. Ammonia and sodium hydroxide were chosen as possible alkaline buffer solutions. Results obtained with ammonia proved to be not only very irreproducible (%RSD = 7.68), but also less sensitive than the same NaOH concentration. Various NaOH concentrations were then evaluated and a 2% m/v solution was chosen as optimum (relative peak height - 3.44 and %RSD - 3.67). Addition of this solution did improve the sensitivity with 14.8%, but reproducibility was still not satisfactory. Addition of 2% m/v NaKtartrate solution to the NaOH solution solved the problem to a great extend. Although the sensitivity did not improve much, the relative standard deviation dropped to 1.66%.

9.4.2.2 Choice of organic solvent

The choice of solvent or the solvent composition is a critical parameter for the successful application of sequential injection extraction, since it determines the difference in flow velocity between the organic and aqueous phases, the chemical selectivity and efficiency of the extraction [35]. Solvents with low viscosities do not offer a sufficient difference in flow velocity when compared to water and make SIE less effective as it requires more time and longer extraction coils. On the other hand, highly viscous solvents are difficult to wash out of the tubing.

CCl_4, with an intermediate viscosity of 0.88 cP, was chosen as solvent not only because of its film forming ability, but also because of Dithizone's high solubility and stability in the organic solvent. Dithizone, as well as its metal dithizonates are all highly soluble in chlorinated solvents [27]. The visible absorption spectrum of Dithizone is very sensitive to the organic solvent in which it is dissolved [39]. Dissolved in CCl_4, $Hg(HDz)_2$ and $Cd(HDz)_2$ absorbed at almost the same λ_{max}. Experimental values for λ_{max} are 485 nm and 488 nm for $Hg(HDz)_2$ and $Cd(HDz)_2$ respectively. Detection was done at 486 nm.

One disadvantage about CCl_4 is that it is highly carcinogenic and has ozone depleting properties [40]. Using the solvent in a SIE system ensured a closed system and no atmospheric contact. The CCl_4 waste was collected and recycled by adsorption on charcoal, water washing and distillation.

9.4.2.3 Concentration of Dithizone

Since solutions of Dithizone of any but the lowest concentration are deeply coloured, and often almost opaque, it is quite difficult to be certain whether excess solid is present in contact with a saturated solution. Special care is needed to ensure that metallic impurities are not introduced by the filtering medium, especially when the concentration is to be calculated afterwards from the absorbance of a suitably diluted aliquot and a

knowledge of the molar (decadic) absorption coefficient, ϵ [27]. A 100 mg/ℓ stock solution of Dithizone in CCl_4 was prepared. Several dilutions, using CCl_4, were made and evaluated. Most of these solutions could not been used due to the deep green colour of the unreacted Dithizone. A solution containing 0.10 mℓ of the stock solution, made up to 50 mℓ with CCl_4, produced a extractant solution with an absorbance in the range 0.4 - 0.6 (10 mm cell). This solution was used throughout the whole procedure as optimum Dithizone concentration. The solution had to be prepared daily.

9.5 Evaluation of the system

The proposed sequential extraction method was evaluated under optimum running conditions with regard to linearity of the two analytes respectively, sample frequency, reproducibility, sample interaction, detection limits, accuracy and major interferences.

9.5.1 Linearity

Evaluation under optimum running conditions delivered analytical curves for the extraction of Cd(II) and Hg(II) with Dithizone as shown in Table 9.6 and Fig. 9.5. The curves for the individual metals are linear between 50 μg/ℓ and 3 mg/ℓ for both metals. For cadmium r^2 = 0.9914 and for mercury r^2 = 0.9875. Peak height was used to evaluate the analytical signal.

TABLE 9.6 Calibration values for the individual metals analysed with the SIE system

Concentration (mg/ℓ) of both analytes	Relative peak height measured for Hg^{2+}	Relative peak height measured for Cd^{2+}
0.025	0.001	0.002
0.05	0.011	0.039
0.10	0.019	0.078
0.50	1.02	0.59
1.0	2.01	1.22
2.0	3.99	2.39
3.0	6.02	3.58
4.0	7.41	4.43
5.0	8.52	4.82
10.0	9.40	6.02

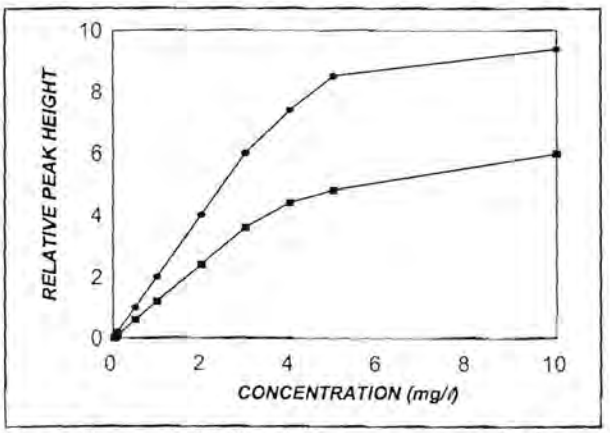

Fig. 9.5 Calibration curves for both Hg^{2+} and Cd^{2+} individually. -●- = Hg^{2+} and -■- = Cd^{2+}.

9.5.2 Accuracy

To investigate the accuracy of the SIE system a calibration curve for each individual metal were constructed. Because absorbance is additive two equations are obtained which should yield the required unknown concentrations when solved simultaneously [41]. The equations for the calibration curves obtained experimentally were:

Calibration curve for Hg(II): $A = 1.37[Hg] + 0.021$

Calibration curve for Cd(II): $A = 0.602[Cd] + 0.017$

Addition of the two equations gave:

$$A = 1.37[Hg] + 0.602[Cd] + 0.038$$

The same procedure was followed as described in Chapter 8. The above data represents the value of the peak maximum as determined by the FlowTEK program (refer to it as A). The other value was found by retrieving the data points of the specific peak profile into Quattro Pro and using the absorbance value recorded one second before the peak maximum was reached (refer to it as A'). The calibration data at this time were as follow:

For mercury: $A' = 1.24[Hg] + 0.001$ $r^2 = 0.9878$

For cadmium: $A' = 0.427[Cd] + 0.008$ $r^2 = 0.9789$

Addition of the equations give: $A' = 1.24[Hg] + 0.427[Cd] + 0.009$

Solving these two equations (A and A') simultaneously give the desired concentrations.

Three different aqueous mixtures, three urine mixtures and two soil extracts were analysed. The results are listed in Table 9.7. The percentage recovery was calculated for the aqueous samples and soil extracts, whilst standard addition to the urine samples showed that the urine did not contain any Cd(II) or Hg(II). The percentage recovery yielded satisfactory results in most cases.

Table 9.7 Evaluation of the accuracy of the proposed SIE method.

Sample	Known concentration in mg/ℓ		Concentration obtained with SIE in mg/ℓ		% Recovery	
	$[Cd^{2+}]$	$[Hg^{2+}]$	$[Cd^{2+}]$	$[Hg^{2+}]$	$[Cd^{2+}]$	$[Hg^{2+}]$
Aqueous 1	0.25	2.0	0.23	2.15	92	108
Aqueous 2	0.75	0.75	0.70	0.67	93.3	89.3
Aqueous 3	1.5	0.5	1.65	0.45	110.5	90
Urine 1	0.1	1.0	0.12	0.84	120	84
Urine 2	1.0	0.5	0.94	0.58	94	116
Urine 3	2.0	2.5	2.21	2.32	110.5	92.8
Soil 1	1.0	2.5	0.82	2.45	82	98
Soil 2	2.5	0.5	2.40	0.6	96	120

9.5.3 Reproducibility

The results obtained (Table 9.8) when using the proposed system revealed surprisingly good reproducibility. It is expected when using air bubbles in the flow system, that the reproducibility will deteriorate due to the irregular stretching and compressing of the bubble. Signals obtained for the two metals, in the standard solutions as well as the samples analysed, show relative standard deviations lower than 2.5% for ten measurements at each concentration or sample.

TABLE 9.8 Reproducibility of the proposed sequential injection extraction system

Standard/sample ([ ] in mg/ℓ)	%RSD
0.05	2.41
0.5	2.10
1	1.57
2	1.91
3	1.51
Aqueous 1	2.15
Aqueous 2	2.12
Aqueous 3	2.11
Urine 1	1.22
Urine 2	2.10
Urine 3	1.85
Soil 1	1.63
Soil 2	1.58

9.5.4 Sample frequency

It took 186 s to complete one whole extraction cycle, including the time needed for detection and to rinse the extraction coil. This resulted in a sample frequency of 19 samples per hour. Although the sample frequency seemed a little low, the fact that two analytes were determined during the analytical cycle, increased the importance of the system.

9.5.5 Sample interaction

Some carry-over between more concentrated samples was experienced. The sample interaction was calculated to be about 2% using the equation

$$Interaction = \frac{(A_3 - A_1)}{A_2} \times 100$$

where

A_1 = the true peak height of a sample with a low analyte concentration (0.1 mg/ℓ),

A_2 = the true peak height of a sample containing ten times more analyte (1 mg/ℓ), and

A_3 = the peak height for an interacted sample containing the same amount of analyte as A_1.

Rinsing of the extraction coil with a small amount of CCl_4, after every twentieth run, eliminate this problem.

9.5.6 Detection limits

The detection limits, estimated as three times the signal-to-noise ratio [42], was equal to 50 and 60 μg/ℓ for Cd(II) and Hg(II), respectively. These detection limits are acceptable, since a concentration of 50 μg/ℓ for both metals individually gave clearly recognisable peaks.

9.5.7 Interferences

Possible interferents were tested using a solution containing 1 mg/ℓ of both analytes. The following substances did not interfere with the determination of cadmium and mercury ions: 250 mg/ℓ SO_4^{2-}, 10 mg/ℓ PO_4^{3-}, 0.5 mg/ℓ Al^{3+}, 50 mg/ℓ Mg^{2+} and 400 mg/ℓ Ca^{2+}.

Chloride up to 14 g/ℓ (0.4 mol/ℓ) did not interfere in the mercury determination as long as the H_2SO_4 concentration did not exceed 2 mol/ℓ, while chloride concentrations of up to 20 g/ℓ could be tolerated in the cadmium determination. Bromide, cyanide and thiocyanate interfere in the mercury determination, since they complex mercury more strongly than Dithizone. These anions could be tolerated up to 10 mg/ℓ. Anions could

be removed by using anion exchange columns in the sample uptake tubes.

The metals normally present in urine, *viz.* Co(II), Fe(II), Ni(II), Pb(II) and Zn(II), were also tested as possible interferents. The metals were individually added to two different urine samples which contained 1 mg/ℓ Cd(II) and 1 mg/ℓ Hg(II) respectively. Although variation in pH usually is the most important way to eliminate interferences from metal cations, the cations still interfere at the optimum pH for the extractions. The interferences were less for the mercury determination. The extent of interference for the different metals are listed in Table 9.9.

TABLE 9.9 Effect of metal ions normally present in urine sample on the determination of Hg(II) and Cd(II)

Interferent	Influence on cadmium signal	Influence on mercury signal
Co(II)	Increase peak height with 5.2%	No interference
Fe(II)	Increase peak height with 10.8%	Increase peak height with 1.8%
Ni(II)	Increase peak height with 3%	No interference
Pb(II)	Increase peak height with 11.5%	Increase peak height with 2.5%
Zn(II)	Increase peak height with 7.5%	Increase peak height with 0.8%

These interferences can however be masked by addition of a mixture of hydroxylamine (1 mol/ℓ), sodium potassium tartrate (0.75 mol/ℓ and potassium hexacyanoferrate(II) (1.5 mol/ℓ) [17]. 2 mℓ of this solution was added to 5 mℓ urine samples. These mixtures were then diluted to 10 mℓ with distilled water prior to introduction into the sequential injection extraction system.

9.6 Conclusion

The sequential injection extraction system described allows the automated extraction of aqueous samples while keeping the organic solvent volume to a minimum and does not compromise repeatability or recovery as compared to conventional glassware

extraction with the same volumes. It operates without phase separators or segmenters by stacking the reagents in such a way that the faster moving phase overcome the slower one as the two travel through the tubing. In addition, as a completely enclosed system, SIE isolates potentially hazardous organic solvents from the operator and reduces contact with biohazardous samples. The robustness of design and ease of changing reagents make SIE a useful technique for automating batch analysis of liquid-liquid extractions and is therefore applicable to many fields.

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CHAPTER 10

Determination of Lead(II), Copper(II), Zinc(II), Cobalt(II), Cadmium(II), Iron(III) and Mercury(II) using Sequential Injection Extractions

10.1 Introduction

The use of certain metals has increased tremendously in our modern society. An increase in population density has inevitably led to ecological problems, due to the increased availability of these metals in the environment. These metals can have toxic effects on plants, animals and eventually men when absorbed in excess amounts. According to Adriano [1] metals could be present in one or more of the following species in soil or sediments:

1. Dissolved in soil solutions
2. On exchange positions of organic solids or inorganic compounds
3. Included in ground minerals
4. Precipitated together with other compounds in soil and sediments
5. Included in biological matter

The first two species are mobile and available to plants, while the last three are non mobile. They can however become mobile and plant available with time and other external factors, like leaching.

Metals, which are captured in river or dam sediments, are potentially dangerous as it can influence the quality of the water with which they are in contact. These metals can be released into the water when certain changes in the water environment take place.

These changes include pH changes, change in redox potential and/or oxygen deficiency, the presence of complexing agents (e. g. EDTA) or surfactants and microbiological transformations [2].

There are several sources of metals in nature, but metals are also added to soils as part of fertilisers, pesticides, fungicides and sewage. Metal pollution of water in a water scarce country like South Africa could become an economical hazard, since purification of water and solving problems associated with the procedures are quite expensive. Although mining is a huge part of the South African economy, it is also one of the main producers of metal pollution of dams and rivers. It is therefore crucial that the amount of dissolved metals in effluent streams must be monitored carefully.

Metals are never found in isolation in nature. Cobalt is invariably associated with nickel, and often also with copper and lead, and is usually obtained as a byproduct or co-product of the recovery of these metals. The production of cobalt is usually subsidiary to that of copper, nickel or lead and the details of its extraction depends on which of these it is associated with [3]. The bulk production of copper is from sulphide ores containing iron [4]. Cadmium on the other hand is closely related to zinc and is usually found to be associated with zinc and lead [2, 3]. Zinc is also associated with minerals that are rich in iron [2]. Mercury is seldom present in nature in metallic form, but occurs in several other forms, e.g. inorganic mercury and organic mercury compounds [5]. Lead and mercury form methyl compounds which are more toxic than the metals itself [2].

These associations between different metals usually complicate the analytical determination of individual metals. Metals associated or closely related to other metals are difficult to remove from samples prior to analysis and sometimes removal of interfering species is impossible. Cation exchange columns are useless when faced with these interferences, since they all carry positive charges in the ionic form and the analyte will therefore also be removed. To find the correct masking agent to eliminate interferences can also be cumbersome and may change the chemical environment of

the analyte and influence the determination. In this study a sequential injection extraction system with which seven metal ions can be detected without prior separation, is proposed. Lead, zinc, copper, iron, cobalt, cadmium and mercury can be quantitatively analysed by the SIE system. The procedure involves the extraction of these metal ions with dithizone and the subsequent detection of the formed dithizonates by diode array spectrophotometry. The bioavailability of the metals in soil and water samples as well as the use in clinical analysis of urine are highlighted.

10.2 Uses and biological importance of the different metals

The car and construction industries are the main consumers of lead. Lead is used in alloys, batteries, silencers, in paint, as additive to petrol and, for clinical applications, as a shield against radio active rays [2]. Lead is present in almost every sphere of our daily life. Humans ingest Pb from food and drink, and inhale it in the form of aerosols and particulates from air. Organo-lead compounds can also enter the body through the skin. Under normal conditions, food accounts for nearly 70% of the total Pb intake. There is no Pb-free food because there is a background Pb level in food according to the background Pb level in soil. Lead contamination of food can also occur through poorly soldered cans, food wrappers, and storage of food in lead-glazed pots. Other important non-food sources of Pb intake, particularly in children, are lead-containing paints, multicolored printed paper, cosmetics and household and playground dust [6].

Lead is generally considered to be a non-essential toxic element which accumulates in the organism. Although Pb in the skeleton is physiologically inert, the Pb in blood and soft tissues has identifiable toxic effects. The general order of susceptibility to the toxic effects of Pb is men < women < children < fetuses. According to the US Centre for Disease Control (CDC), a blood-Pb level < 240 µg/ℓ is 'normal' (Class I), while blood-Pb levels of 250 - 490 (Class II), 500 - 690 (Class III), and > 700 (Class IV) µg/ℓ fall into the 'moderate risk', 'high risk' and 'urgent risk' categories respectively [6].

Symptoms of lead poisoning include distinct neurobehavioral and learning disabilities in children. Lead also effects the hematopoetic system, peripheral nerve conduction, kidneys, gastrointestinal system, gingival tissues, brain, embryo and bone marrow [6].

Zinc is extensively used in the electrical, car and hardware manufacturing industries. It also found use in paints, domestic items, cosmetics, powders, ointments, antiseptic agents, linoleum, rubber, glass, tyres, television screens and dry batteries [2]. Other applications are as micro nutrients, fertilisers and pesticides for agricultural use, as well as hardeners in cements.

In contrast with lead, zinc is biologically one of the most important metals and is apparently necessary to all forms of life. The body of an adult human contains about 2 g of Zn, mainly as Zn enzymes which are present in most body cells [3]. The two Zn enzymes which have received most attention are carboxypeptidase A and carbonic anhydrase. Carboxypeptidase A catalyses the hydrolysis of the terminal peptide bond in proteins during the process of digestion, while carbonic anhydrase is used to catalyse the reaction:

$$CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$$

The forward (hydration) reaction occurs during the uptake of CO_2 by blood in tissue, while the backward (dehydration) reaction takes place when the CO_2 is subsequently released in the lungs [3].

Copper is mainly used in the manufacturing of wire and copper alloys. Electrical industries are major consumers of copper for the manufacturing of electrical wires and apparatus. Due to its high thermal conductivity and relative inertness, copper is used in stove pipes, car radiators and cooking devices. Copper is used in the agricultural industry as fertiliser, pesticide and fungicide [2].

Copper occurs in all bodily tissues. The distribution varies with age, species and diet. The liver, heart, brain, kidneys and hair contain high concentrations of copper compared to other tissues. A adult human contains around 100 mg of copper, mostly attached to

protein, an amount exceeded only by iron and zinc among transition metals. The required daily intake of copper is about 3 - 5 mg [4].

Abnormally high copper levels in the liver are characteristic of certain disease states in humans. Conditions that manifest increased copper content in the liver include tuberculosis, hemachromatosis, cirrhosis and yellow atrophy of liver. Wilson's disease is also characterized by abnormally high copper levels and is ascribed to the congenital inability to excrete copper, resulting in its accumulation.

Although copper occurs naturally in human organs, the ingestion of copper through other means can also result in the increase of its levels in humans and animals. An example is the ingestion of water. Drinking water that originates from natural sources could contain elevated concentrations of copper due to, e.g. the use of copper salts to control plant growth in reservoirs or from the corrosion of copper, brass or bronze pipe fittings. Drinking water that contains abnormal high amounts of copper could cause ptyalism, nausea, vomiting, epigastric burning and diarrhea [4].

Iron is used in various types of steels and alloys. Iron and its compounds are used in paints, colourants, in the electronic industry, as cover material for magnets and as catalysts. Iron salts are also used as flockulants in water purification [2, 3].

Biologically, iron plays crucial roles in the transport and storage of oxygen and also in electron transport and it is safe to say that, with only a few possible exceptions in the bacterial world, there would be no life without iron [3]. Iron is the most important transition element involved in living systems, being vital to both plants and animals. The stunted growth of the former is well known on soils which are either themselves deficient in iron, or in which high alkalinity renders the iron too insoluble to be accessible to the plants. In case of man, iron was the first minor element to be recognised as being essential when, in 1681, it was used to treat anaemia. The adult human body contains about 4 g of iron of which about 3 g are in the form of haemoglobin and this level is maintained by absorbing a mere 1 mg of iron per day - a remarkably economical

utilization [3].

Cobalt is mainly used as pigment in the ceramic and paint industries, but also serves, along with aluminium and nickel, in the manufactory of magnetic alloys [3].

Cobalt is essential for microorganisms fixing molecular N_2 and thus for higher plants relying on symbiotic nitrogen assimilation [7]. Cobalt is important for living species as complexed vitamin B_{12}. Vitamin B_{12} is present in human and animal cells in the forms of adenosylcobalamin(III) and methylcobalamin(IV). The deficiency of cobalt in ruminants usually results in different types of anaemia [3]. Despite of its essential biological role, high concentrations of cobalt can be very dangerous. Toxicological effects of large amounts of cobalt include vasodilation, flushing and cardiomyopathy in humans and animals [7].

Cadmium is used for electroplating, in alloys and in batteries (Ni/Cd batteries). Cadmium compounds are also used in glazing, as stabilisers in polyvinyl plastics and in the tubes of colour television sets [2]. Cadmium, as well as mercury, are extremely toxic and has no vital biological role to play [3]. Cadmium inhibits renal function, increases loss of calcium due to depression of proximal reabsorption and leads finally to osteomalacia [8]. Serious symptoms of cadmium poisoning include lumbago, followed by the later development of pseudo fractures and a waddling gait. Other symptoms due to cadmium poisoning are: (i) emphysema of the lungs, (ii) dysfunction of the kidneys and (iii) low molar mass proteinuria.

Mercury is widely used in the fields of science, agriculture and industry. The element is also used in paints, as catalyst, in pharmaceutical products and in the paper industry. Agricultural applications include the use of mercury as fungicide against plant diseases, which make pollution due to mercury unavoidable [2].

All forms of mercury are considered poisonous, but methyl-mercury is of particular concern since it is extremely toxic and is frequently found in the environment [5]. 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, 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.

10.3 Methods of determination

10.3.1 Conventional methods of detection

Simultaneous determination of trace amounts of heavy metals usually employ one of the following methods: AAS, cold vapour AAS or flame-AAS [11, 12, 15], ICP-OES [9], potentiometry (ion-selective electrodes) [10], anodic stripping voltammetry [19], chromatography (usually HPLC) [14], chemiluminescence detection, ETA [16], gravimetric detection [16] or photometry [4, 16 -18]. Several of these techniques were already adapted to FIA systems. Most of the systems already adapted included photometric determination of the analyte. Several reagents can be used to determine metal ions in various matrixes [11]. Many of the systems employ on-line separation of the analyte from specific interferences. These systems may include dialysis [20, 21] or extractions [11, 22 - 25].

All of the seven metals analysed with the proposed system, are so-called dithizone metals [11, 26]. They form intensely coloured complexes with the reagent dithizone. Extraction at a neutral pH of 7 - 7.5 with dithizone was therefore chosen for the quantitative determination of the seven metals.

10.3.2 Reactions with diphenylthiocarbazone (dithizone)

Distinction is made between primary and secondary dithizonates, according to whether the metal ion reacting with dithizone only replaces the imide hydrogen and forms the so-

called keto complex or also reacts with the hydrogen of the thiol compound and form a so-called enol complex [11]. All seven elements can be extracted at a pH 7 - 7.5. Most of the metals form primary dithizonates, but depending on reaction conditions cobalt, copper and mercury can form either primary or secondary dithizonates [11]. Table 10.1 lists the different metals, their resulting dithizonates, pH ranges for formation of the dithizonates and their absorption maxima. The absorption maxima of the dithizonates in ethanol were determine experimentally.

TABLE 10.1 Properties of the different metal dithizonates

Cation	Dithizonate complex	Primary/ secondary	pH range	Absorption maxima (λ_{max} in nm)
-	Dithizone (H_2Dz)	-	-	435 590
Cd	$Cd(HDz)_2$	primary	6 - 14	436
Co	$Co(HDz)_2$	primary	6.5 - 10.5	519
Cu	Cu(Dz)	secondary	7 - 14	447
Fe	$Fe(HDz)_3$	primary	7.5 - 8.5	402
Hg	Hg(Dz)	secondary	7 - 14	519
Pb	$Pb(HDz)_2$	primary	6.5 - 10.5	500
Zn	$Zn(HDz)_2$	primary	6.5 - 9.5	571

Primary dithizonates follow the reaction scheme represented in Fig. 10.1, while the secondary dithizonates follow the reaction scheme represented in Fig. 10.2 [27]. Dithizone also formed complexes with organometallic complexes, like methyl mercury or methyl lead [26]. This reaction scheme is represented in Fig. 10.3.

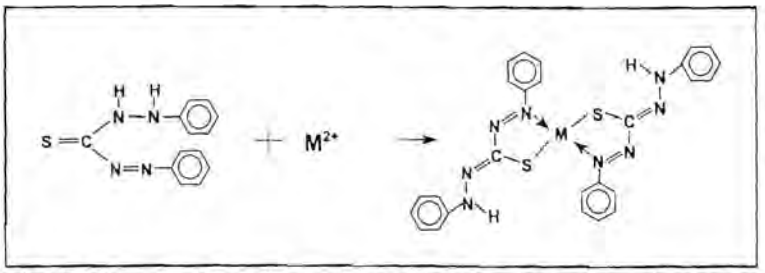

Fig. 10.1 Reaction scheme for the formation of primary dithizonates. M^{2+} - Pb, Zn, Co, Cd and Fe.

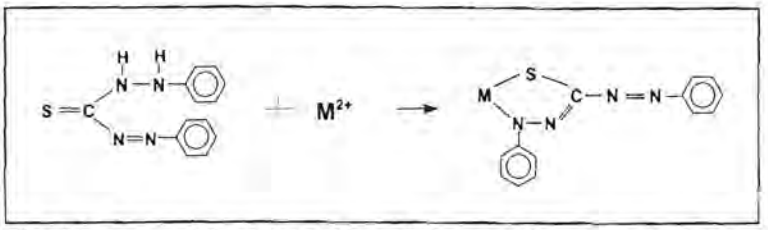

Fig. 10.2 Representation of the reaction scheme for the formation of secondary dithizonates. M^{2+} - Hg and Cu.

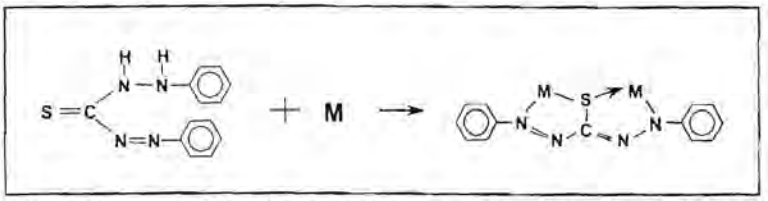

Fig. 10.3 Reaction scheme for the formation of organometallic dithizonates. M - HgR^+ or PbR^+. R = methyl or ethyl group.

10.4 Simultaneous determination of Pb(II), Cu(II), Zn(II), Co(II), Cd(II), Fe(III) and Hg(II) with sequential injection extraction

10.4.1 Experimental

10.4.1.1 Reagents and solutions

All solutions are prepared of analytical grade reagent unless specified otherwise. Deionised water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used to prepare all aqueous solutions and dilutions. The water used as carrier was degassed before use.

Extractant: 0.05 g of dithizone (Hopkin & Williams Ltd.) was dissolved in 250 mℓ ethanol to produce an emerald green stock solution. The solution was filtered using a Whatman no. 4 filter paper to remove all undissolved dithizone particles. Stored in a cool place (5°C) and protected from light this solution was stable for up to ten days. Working solutions are obtained by suitable dilution of the stock solution with ethanol.

Metal stock solutions: The following 1000 mg/ℓ metal stock solutions were prepared:

- *Lead(II):* 0.1609 g $Pb(NO_3)_2$ (PAL Chemicals) was dissolved in 1 ℓ deionised water.
- *Copper(II):* Pure Cu metal coarse chips were used in the preparation of the Cu(II) stock solution. The copper metal was cleaned to remove any oxides and dissolved by heating 1.0 g of the copper metal in 10 mℓ 55% (m/m) HNO_3 and *ca.* 10 mℓ of water. The resulting solution was cooled and then diluted to 1 ℓ with deionised water.
- *Zinc(II):* Pure Zn metal was cleaned with diluted HCl and the stock solution was prepared by dissolving 1.093 g Zn metal in 50 mℓ concentrated HCl. The solution was then diluted to 1 ℓ with deionised water.
- *Cobalt(II):* 0.4036 g $CoCl_2 \cdot 6H_2O$ (Riedel-de Haën AG) was dissolved in 1 ℓ deionised water.
- *Cadmium(II):* 2.744 g $Cd(NO_3)_2 \cdot 4H_2O$ (Merck) was dissolved in 1 ℓ deionised water.
- *Aluminium(III):* Pure Al metal was cleaned with a mixture of diluted HCl and HNO_3. The stock solution was prepared by dissolving 1.0833 g Al metal in 60 mℓ concentrated HCl and 10 mℓ concentrated HNO_3 and then diluting the solution to 1 ℓ with deionised water.
- *Iron(III):* 0.702 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was dissolved in *ca.* 20 mℓ of water together with 1.1 mℓ of 18.4 mol/ℓ H_2SO_4 (98%, m/m). The final solution was diluted to 100 mℓ with deionised water.
- *Mercury(II):* 1.71 g $Hg(NO_3)_2.2H_2O$ (Merck) was dissolved in 1 ℓ of deionised water.

Distilled water was used as carrier solution. A 0.43 mol/ℓ acetic acid solution was used as eluent during the soil extractions. For pH corrections either a 1 mol/ℓ NH_3 solution or a 0.5 mol/ℓ H_2SO_4 solution was used.

10.4.1.2 Instrumentation

The sequential injection extraction (SIE) manifold is illustrated in Fig. 10.4. It was constructed from a Gilson Minipuls peristaltic pump (operating at 18 rpm), a 4 m long extraction coil (1.02 mm i.d.) made of Teflon (PTFE) tubing (SUPELCO) and a 10-port electrically actuated VICI selection valve (Model ECSD10P) (Valco Instruments, Houston, Texas). Acidflex pump tubing was used. The reaction coil was constructed using 45 cm of 0.25 mm i.d. Teflon tubing. Device control were achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (MINTEK, Randburg, South Africa). The FlowTEK [28] software package (obtainable from MINTEK) was used throughout the procedure. An Hewlett Packard UV-VIS diode array spectrophotometer (HP 8453), equipped with a 10 mm Hellma flow-through cell (volume 80 $\mu\ell$), was used for measuring the absorbance and data acquisition. The single component analysis program was used to record the linear ranges of the analytes individually and the multi component analysis program was used to record spectra of the seven analytes in mixed standards as well as in the different samples. Spectra were recorded over a wavelength range from 340 - 750 nm with intervals of 5 nm each when using the sequential injection system and intervals of 1 nm each when hand extractions were done to confirm the performance of the system. The calibration program was used for data analysis.

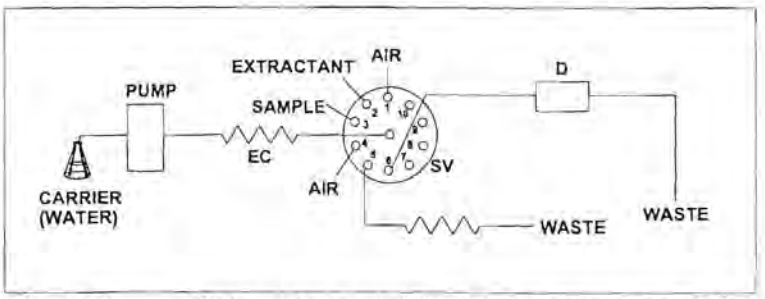

Fig. 10.4 SIA system used in the determination of the seven analytes. EC - extraction coil, SV - selection valve, HC - holding coil and D - detector.

10.4.1.3 Procedure

A small air bubble was drawn up to separate the extraction zones from the carrier solution (ethanol). Thereafter the extractant zone (dithizone in ethanol) and the sample zone (containing some of or all seven analytes) were drawn up into the extraction coil. Another air bubble was drawn up to separate these zones from the carrier in the holding coil. By reversing the flow, extraction took place into the thin organic layer formed by the dithizone zone whose flow was impeded due to the hydrophobic interactions with the walls of the Teflon coil. Since ethanol and water is miscible in all ratios no separation step was needed and after flow reversal the product peak was measured directly. No removal of the air bubbles was necessary, since the product zone was stopped inside the flow cell prior to detection. At this stage the second bubble (drawn up second, therefor reaching the detector first) was already propelled through the flow cell, while the first bubble did not yet entered the flow cell.

The spectrophotometer used three different files to store data (BLANK, STANDARD and SAMPLE) and therefore it was needed to construct three different programs which enable FlowTEK to sent the correct signal at the desired time. These programs were all basically the same and differ only in the command given to the spectrophotometer. This command was received by the spectrophotometer via a macro which enabled the computer to read the signal coming from FlowTEK. The program used by FlowTEK to control the devices is given in Table 10.2.

TABLE 10.2 Device sequence for the proposed sequential injection extraction system

Time (s)	Pump	Valve	Detector	Description
0	Off	Air ①		Pump off, valve is turned to select first air inlet.
4	Reverse			Draw up air bubble
4.5	Off			Pump stop
5.5		Extractant ②		Select extractant line
6.5	Reverse			Draw up extractant solution
10.5	Off			Pump stop
11.5		Standard/ sample ③		Select standard/sample line
12.5	Reverse			Draw up standard/sample solution
16.5	Off			Pump stop
17.5		Air ④		Select second air inlet
18.5	Reverse			Draw up second air bubble
19	Off			Pump stop
20		Holding coil ⑤		Select holding coil
21	Reverse			Extraction step 1: Zones are drawn back into extraction coil to ensure effective mixing and extraction.
26	Forward			Extraction step 2: Pump stack of zones forward until the bubble reached a position just in front of the valve.
31	Off			Pump stop
32		Detector ⑥		Select detector line
33	Forward			Pump stack of zones to detector until the second bubble is visible outside the flow cell. This ensure that the product zone is entirely inside the flow cell.
43	Off			Pump stop, a waiting period is now installed to ensure that there will be no interferences due to mirages that occur when ethanol and water are mixed.
93			BLANK/ STANDARD/ SAMPLE	A signal is sent to the diode array spectrophotometer to do either a blank, standard or sample spectra.
103	Forward			Stack of zones are pump to waste.
153	Off			Pump stop, end of analytical cycle.

10.4.1.4 Sample preparation

Sample collection: Urine samples were collected in polypropylene flasks which had previously been cleaned by rinsing with dilute nitric acid and water. The samples were quickly frozen after collection with minimum air space above the urine. Soil samples were taken from a maize farm in the northern Free State and stored in polypropylene containers.

Before analysis, the frozen urine was allowed to reach room temperature and thoroughly mixed. Urine samples were diluted 1:3 with deionised water. All water samples in the pH range 7 - 7.5 were analysed directly. For other water samples the pH was first corrected by using either NH_3 or H_2SO_4 solution. Representative soil samples of 20.00 ± 0.05 g were dried at 30°C for 8 - 10 hours. 5.00 ± 0.01 g of the air-dried soil was weighed into a beaker and 50 mℓ of a 0.43 mol/ℓ CH_3COOH solution was added. The suspension was stirred for 30 min and then filtered. Since the pH of the reaction mixture needed to be 7.5, a 25% (m/m) NH_3 solution, was used to correct an 50 mℓ aliquot of the filtrate. The solutions were then made up to 100 mℓ using deionised water. *10 mℓ acetone (AR) was added to every working solution (standard) and sample prepared.*

10.4.2 Optimisation

10.4.2.1 Physical parameters

A number of physical parameters can influence the degree of dispersion and extraction in the manifold. To obtain the highest sensitivity and precision it was necessary to optimise these parameters. Most of the optimization of the physical and chemical parameters was done using the same sequential injection system as in Chapter 8. This was done, because of the simplicity of the detection system employed. When using the diode array spectrophotometer, a BLANK run must be done every time a parameter is changed - even if the parameter is changed only slightly. Except for increasing the

analysis time to double the original time, it also make comparisons between different results obtained very difficult. The optimisation was done with a standard solution containing 1 mg/ℓ of every analyte. Absorption was measure at 500 nm, since most of the dithizonates show absorption at that wavelength and the reagent show minimum absorbance at 500 nm (Fig. 10.5). It was not surprisingly that almost exactly the same optimum conditions were found for the two systems. Only two parameters were optimize using the diode array spectrophotometer - the duration of the waiting period and the addition of acetone to the standard and sample solutions.

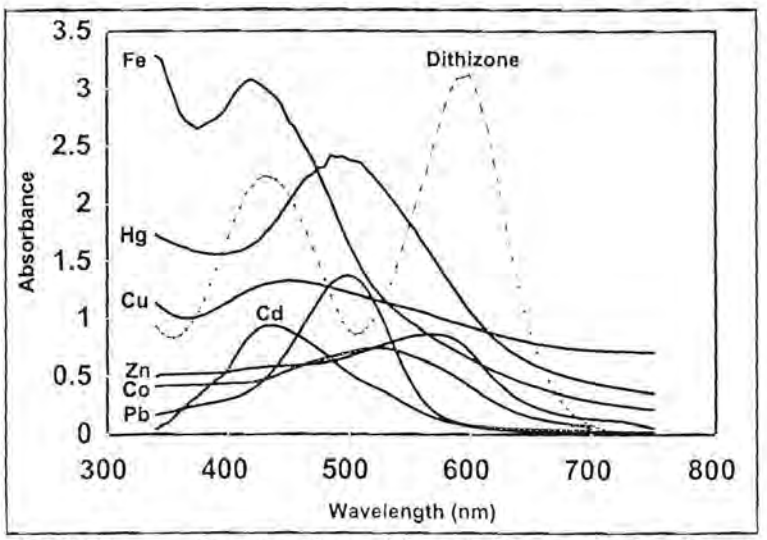

Fig. 10.5 Spectra of the different dithizonates in ethanol. Optimisation of the sequential injection system was done at 500 nm.

10.4.2.1.1 Introduction and removal of air bubbles

Air bubbles are highly undesirable in flow and sequential injection systems, not only because they led to spurious results, but also because it decreased the reproducibility of the procedure. Although it was feared that the introduction of air bubbles into a flow system would have led to irreproducible results, this was to a major extent not the case. The bubbles separated the extraction zones from the surrounding carrier solution, preventing excessive dilution of the extraction zones.

No removal of bubbles took place in this procedure, since the product zone was halted inside the flow cell during detection. Flow was stopped as soon as the second bubble was just outside the flow cell. Because the extraction zones were so big, the first bubble had at that stage not yet entered the flow cell. This ensured that the whole product zone was inside the flow cell when absorbance measurements were done. After measurements were completed, the product zone as well as the bubbles were flushed to waste.

10.4.2.1.2 Flow rate

From previous experiments (Chapters 8 and 9), it was clear that slower flow rates improve the mass transfer from aqueous to organic phase, while faster flow rates were needed to propel the formed product zone through the detector. The faster flow rates through the detector was necessary to eliminate excess dilution of the product zone as well as to prevent peak tailing in the detector. During this application absorbance measurements were not made while the product zones were propelled through the detector. The product zone was stopped inside the flow cell and after a waiting period of 50 seconds, the absorbance was measured. Thereafter the product zone was flushed to waste. Pump rate was therefore only optimise to ensure optimum extraction.

Slower flow rates result in thinner organic film and inevitably in longer extraction times. Thinner films are useful when back extractions are employed, but for single extractions, thicker films resulted in better extraction efficiency, since they have higher capacities [29]. Flow rates between 1.5 and 3 mℓ/min were evaluated and the results are listed in Table 10.3. Flow rate was determined as the mass of the volume of water delivered by the pump in one minute, divided by the density of water at 25°C. The average of three determinations was used.

TABLE 10.3 Influence of different flow rates on sensitivity (extraction efficiency) and precision

Flow rate (mℓ/min)	Sensitivity (Relative peak height)	Precision (%RSD)
1.51	2.96	2.21
1.73	3.42	2.63
1.97	3.65	1.75
2.41	4.29	2.01
2.64	5.28	1.79
2.97	5.77	2.30

An optimum flow rate of 2.4 mℓ/min was chosen, due to its sensitivity and good reproducibility. Although higher flow rates delivered better sensitivities, it was ruled out due to their poor precision.

10.4.2.1.3 Sample volume

Smaller sample zones lead to bigger dispersion and ultimately to lower sensitivity. The sample volume was therefore carefully evaluated to obtain maximum sensitivity and reproducibility. The results obtained in the evaluation are schematically represented in Fig. 10.6.

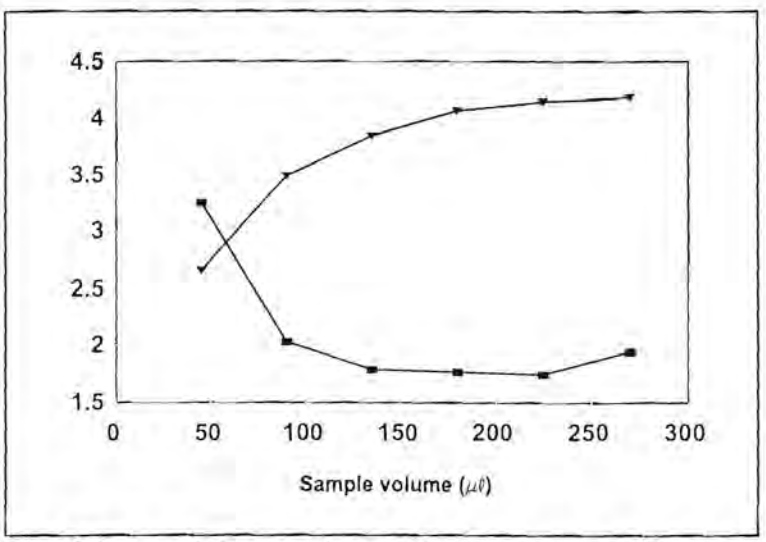

Fig. 10.6 Influence of sample volume on the sensitivity and reproducibility of the SIE method.

Larger sample volumes led to better sensitivity until a plateau was reached. Since the flow was stopped inside the flow cell, a large enough extraction zone must be created to allow that the whole product zone would be situated inside the flow cell during measurements. The reproducibility of the method decreased with volumes bigger than 180 $\mu\ell$. This is because of insufficient zone overlap, because larger volumes led to smaller axial dispersion which decrease the zone penetration [30, 31]. An optimum sample volume of 180 $\mu\ell$ was thus chosen for the system.

10.4.2.1.4 Extractant volume and extraction time

Although the extractant volume is dependant on a critical parameter called the zone inversion length, it also governs the linear ranges of the different analytes determined as well as the number of analytes that can be analysed. Because the organic solvent, ethanol, is miscible with water in all ratios, optimal mixing rather than film thickness will be optimised. Since extraction still has to take place, longer extraction time will be allowed for the reaction. The volume of the extractant zone influence both the time of the extraction as well as the length of the extraction coil.

Extractant volumes between 45 $\mu\ell$ and 180 $\mu\ell$ were evaluated (Fig. 10.7). Smaller volumes gave very irreproducible results, because of the imperfect flow dynamics of the pump (start up and stopping are not instantaneous) [30, 31], these small volumes were not aspirated reproducibly. A plateau is reached in peak height at volumes bigger than 135 $\mu\ell$. The optimum extractant volume was chosen to be 180 $\mu\ell$ due to the good precision and sensitivity it delivered. This also contributed towards the larger product zone inside the flow cell during measurements.

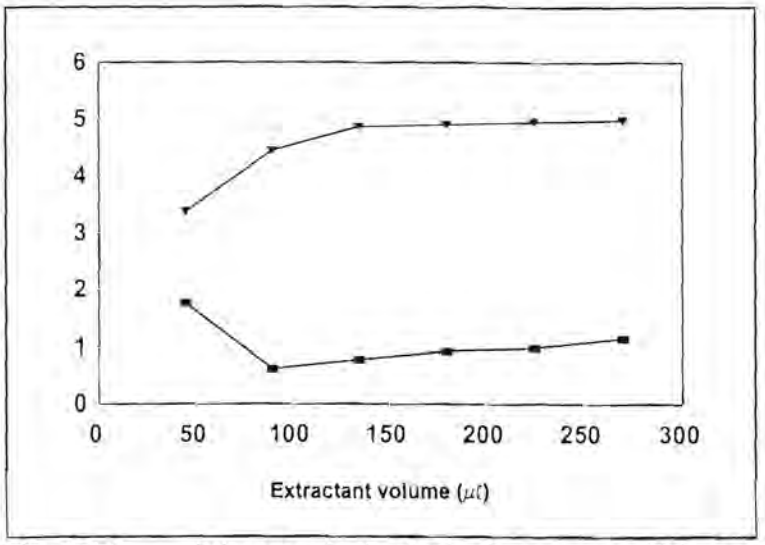

Fig. 10.7 Influence of extractant volume on sensitivity and precision. ▼ = relative peak height and ■ = %RSD.

Several extraction options, including multiple flow reversals to obtain thorough mixing of the zones were evaluated in Chapter 8. This study revealed that an extraction step consisting of only one flow reversal and extraction time of 17.5 seconds resulted in peaks with the desired Gaussian shapes. Longer extraction times were evaluated for the proposed SIE system, but did not have any effect on the sensitivity of the technique. This might be due to the waiting period incorporated prior to detection. This allow the reactions to reach a certain level of equilibrium. Extraction times shorter than 11 seconds led to a decrease in sensitivity and 11 seconds were therefor chosen as optimum extraction time.

10.4.2.1.5 Organic film thickness

Relative film thickness per unit length (d_f) can be predicted using the equation [29]

$$d_f = kd_t(u\eta/\gamma)^a$$

where u represents flow rate (velocity) and d_t tubing diameter. The solvent characteristics also play an important role and are included in the equation. Viscosity

of the solvent is represented by η and surface tension by γ, *k* and *a* are constants between ~1/2 and ~ 2/3. From the equation it can be seen that film thickness is directly proportional to viscosity and inversely proportional to surface tension. As colligative properties, it is appropriate to consider either interfacial or surface tension to viscosity as a film thickness parameter, η/γ [29]. The film thickness parameter for ethanol was calculated to be 4.64 x 10^{-2} (η = 1.06 cP and γ = 21.80) [32]. The thickness of the organic film is very important since it influences extraction by affecting the mass transfer of analytes into the film [33]. Under optimum running conditions the relative film thickness was calculated to be 9.4 μm.

10.4.2.1.6 Diameter and length of tubing

Extraction coil: The length of the extraction coil depends on the zone inversion length. It was found that during the extraction step a reverse step of 5 seconds was used. This time multiplied with the flow rate 5.38 x cm/s (2.64 mℓ/min), resulted in a zone inversion length of 26.9 cm. Due to dispersion in the flow conduit, these zones occupy about 9 times the inversion length. Thickness of the wetting film is directly proportional to the inner diameter of the coil [34]. As a result, the extraction capacity (volume of the wetting film) is larger for wider and longer extraction coils. Using an extractant volume of 180 μℓ an extraction coil of 3.8 m was needed. To ensure that none of the zones reached the pump conduit and became deformed, an extraction coil of 4 m was used. An inner diameter of 1.02 mm ensured good axial dispersion and zone overlap.

Reaction coil: The spectrophotometer should be positioned as close to the debubbler as possible. Longer distances between the debubbler and detector led to higher dispersion, longer rinsing times and ultimately lower sample throughput. Wider tubing also contribute to dispersion and undesired dilution of the product zone. Since no removal of bubbles was needed, a 45 cm 1.25 mm i.d. Teflon reaction coil was used. This length of tubing was needed as it represent the shortest distance between the valve and the spectrophotometer.

10.4.2.1.7 Waiting period and measuring intervals

Because the diode array spectrophotometer is so extremely sensitive, it was very difficult to obtain smooth analytical curves therewith. When the water and ethanol were mixed due to the flow in the manifold conduit, mirages were created in the process. These mirages led to very spurious peaks. To reduce the effect of the mirages to a great extent, a waiting period was incorporated into the flow programming. As it is known that stop and start in flow and sequential analysis systems are not instantaneous [30, 31], the waiting period allow the product peak to come to a standstill within the flow cell. It was difficult to evaluate the influence of the waiting period on sensitivity and precision, because of the high noise experience with none or short waiting periods. As this parameter was evaluated on the diode array spectrometer itself, different backgrounds were needed for different waiting periods. Initially the background value was very unstable, but with increasing waiting time length, it stabilised to give a background value as shown in Fig. 10.8. Waiting periods longer than 50 seconds did not improve the shape and smoothness of the peaks and only increased the analysis time. The waiting period was then taken as 50 seconds.

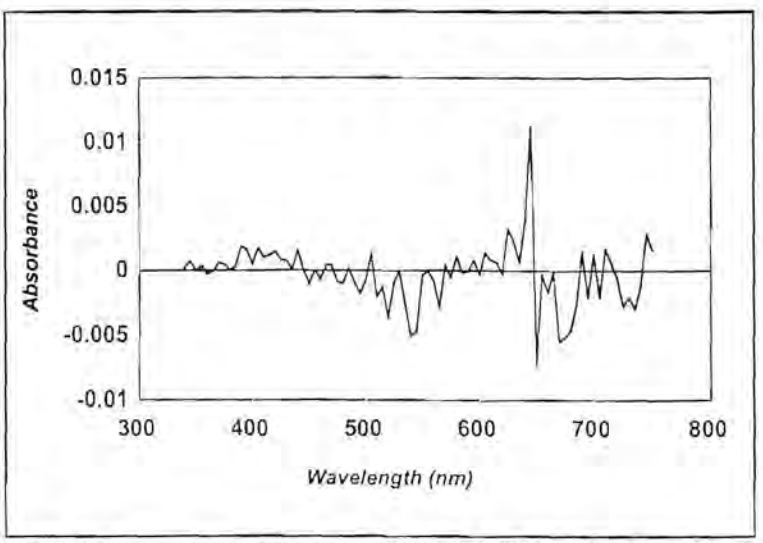

Fig. 10.8 Background obtained with a blank solution containing 10% acetone when employing a 50 second waiting period into the flow programming.

It was necessary to do a blank measurement after every ten runs, to ensure smooth peaks. The necessity of a new blank became visible in especially the region between 600 and 750 nm, when very 'spiky' peaks were obtained. This was probably due to the difference in the refractive index, n_r, of the different phases. The refractive index of a medium is the ratio of the speed of light in a vacuum to its speed in the medium [32]:

$$n_r = \frac{c}{v}$$

The refractive index is related to the molecular polarizability because the propagation of light through a medium can be imagined as occurring by the incident light inducing an oscillating dipole moment, which then radiates light of the same frequency. The newly formed radiation is delayed in phase relative to the incident radiation, so the light propagated more slowly through the medium as through a vacuum. Since photons of high frequency light carry more energy than those of low frequency light, they can distort the electronic distributions of the molecules in their path more effectively. Therefore, it is expected that the polarizabilities of molecules, and hence the refraction index, will increase with increasing frequency [32].

The different refractive indices for both ethanol and water are given in Table 10.4. Although the refractive indices of the two solvent do not vary much at the respective wavelengths, light moves faster through ethanol than water, which is the reason for the mirages obtained.

TABLE 10.4 Refractive indices relative to air at 20°C [32]

Solvent	434 nm	589 nm	656 nm
Ethanol	1.3700	1.3618	1.3605
Water	1.3404	1.3330	1.3312

Δ refractive indices = 0.03

Another option to smooth the analytical curve was to reduce the number of measurements. Initially measurements were done at every wavelength (1 nm intervals). This was reduced to measurements at every fifth nanometre. Experiments with measurements at intervals of 10 nm, resulted in very poor reproducibility and sensitivity. Measurements at intervals of five nanometres were chosen for measurements done by the sequential system. Stationary measurements (hand extractions) were still taken with measurements at 1 nm intervals.

10.4.2.2 Chemical parameters

10.4.2.2.1 pH

The formation of all dithizonates is pH dependant and when employing different pH values, differentiation between different metals is possible. In Table 10.1 it was shown that a pH between 7 and 7.5 allowed the extraction of all seven metal ions as dithizonates. The pH of clean drinking water falls into this range. The pH of all samples that were analysed with this system were therefore measured and corrected to pH 7.5 with 1 mol/ℓ ammonia solution or 0.5 mol/ℓ sulphuric acid solution. Ammonia was chosen to eliminate any interference of NaOH due to the formation of less soluble hydroxides [35].

10.4.2.2.2 Choice of organic solvent

Except for the flow rate, the viscosity and surface tension of the organic solvent also play a major role in the film thickness and therefore in the extraction efficiency. Solvents with low viscosities do not offer a sufficient difference in flow velocity when compared to water and make SIE less effective as it requires more time and longer extraction coils. On the other hand, highly viscous solvents are difficult to wash out of the tubing. With less dense solvents, phase separation may present problems, although there may be cases where the use of a diluent not much less dense than water has special advantages [26]. As described earlier, there are several factors, including

economical factors, that influence the choice of organic solvent used.

Dithizone, as well as its metal dithizonates are all highly soluble in chlorinated solvents and the most common solvent used in extractions are carbon tetrachloride and chloroform [26, 36]. One disadvantage about CCl_4 is that it is highly carcinogenic and has ozone depleting properties [37]. To avoid the use of toxic organic solvents such as CCl_4 and $CHCl_3$ [24], ethanol was used as solvent for the dithizone reagent.

The major reasons used to justify the use of ethanol as solvent were highlighted in Chapter 8. Firstly, since ethanol and water are miscible in all ratios, the use of phase separators were omitted and the mixture of aqueous and organic liquids containing the reaction product was determined directly. Secondly, according to the physical properties of ethanol, it was calculated that it would produce the thickest extraction film, when compared to a few other solvents. The film thickness parameter, as calculated for ethanol was 4.64×10^{-2}.

The visible absorption spectrum of dithizone is, however, very sensitive to the organic solvent in which it is dissolved [36]. The absorption maxima for the seven metal dithizonates in ethanol was determined experimentally and are listed in Table 10.1 and represented in Fig. 10.5.

10.4.2.2.3 Concentration of dithizone

Dithizone is only sparingly soluble in ethanol, with a solubility of 0.3 g/ℓ at 20°C [26]. Since solutions of dithizone of any but the lowest concentration are deeply coloured, and often almost opaque, it is quite difficult to be certain whether excess solid is present in contact with a saturated solution. Special care is needed to ensure that metallic impurities are not introduced by the filtering medium, especially when the concentration is to be calculated afterwards from the absorbance of a suitably diluted aliquot and a knowledge of the molar (decadic) absorption coefficient, ε [26].

A stock solution containing 0.05 g of dithizone in 250 mℓ ethanol was prepared. Since most of the primary dithizonates favoured a 1:2 metal:dithizone ratio, excess reagent was needed in the determinations. The solution was first used undiluted, but this resulted in very 'spiky' peaks. Because of the sensitivity of the detector, the undissolved dithizone reagent interfered in the determination. Filtering of the reagent through a Whatman no. 4 filter paper solved this problem. The reagent was however too concentrated and resulted in deformed peaks. Several dilutions, using ethanol, were made and evaluate. A 1:1 dilution gave smooth peaks and allow the determination of slightly more concentrated samples. This solution was used throughout the whole procedure as optimum dithizone concentration. The solution had to be prepared daily, but the stock solution was stable for up to two weeks when stored in the refrigerator and protected from light.

10.4.2.2.4 Addition of acetone

As stated earlier, the mirages originating from the mixing of ethanol and water, created huge problems during measurements. The difference in surface tension between ethanol and water are relatively big (Δ surface tension = 0.05 N/m), which could have an influence on the mixing of the two phases. The addition of acetone to the aqueous sample lower the surface tension of the water which resulted in better mixing and reduction of the effect of the mirages. Different acetone concentrations were evaluated. For concentrations up to 10% the smoothness of the peak was enhanced, while for concentrations higher than 10% no additional enhancement was observed. Preparation of sample and standard solutions therefore included the addition of 10% acetone.

10.4.3 Evaluation of the system

The proposed sequential extraction method was evaluated under optimum running conditions with regard to linearity of the individual analytes, sample frequency, reproducibility, sample interaction, detection limits, accuracy and major interferences.

10.4.3.1 Linearity

Analytical curves for the different metals were difficult to obtain, since the unreacted dithizone reagent interfere at low concentrations. At these concentrations the shape of the dithizone reagent was very recognisable. The spectra obtained for different concentrations of the individual metals as well as the calibration curves obtained are given in Figs. 10.9 to 10.22 Concentrations up to 20 mg/ℓ were evaluated. At 20 mg/ℓ there was still excess reagent present, since most dithizonates react in a 1:2 metal:reagent ratio.

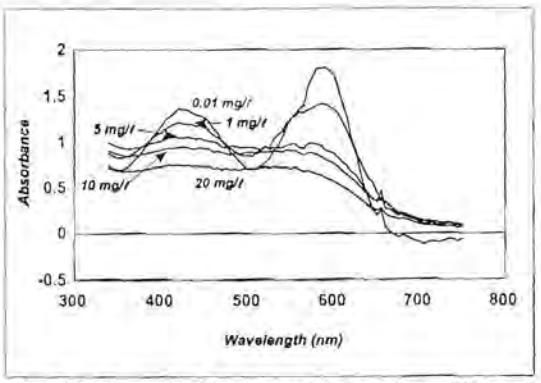

Fig. 10.9 Spectra obtained for different Co concentrations.

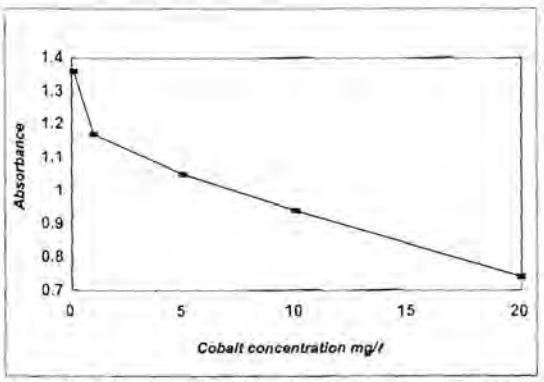

Fig. 10.10 Calibration curve for cobalt at 420 nm.

At 519 nm, the absorbance maximum for cobalt, difficulties were experience in finding the correct absorbance value for the cobalt dithizonate. This was mainly because of the excess reagent present when smaller concentrations were determined. The calibration curve was constructed from the absorbance values at 420 nm (Fig. 10.10). This represent the decrease in reagent with increasing cobalt concentration. The calibration curve was linear between 1 and 20 mg/ℓ Co. The equation of the line was:

$$A_{Co} = -0.022[Co] + 1.169; \quad r^2 = 0.9955$$

For mercury it was even more difficult to set up an calibration curve, since the peak shape changed and the absorbance maximum shifted with increasing mercury concentration (Fig. 10.11). The decrease in reagent concentration, with increasing analyte concentration, was monitored at 430 nm. This plot gave a linear range for

mercury between 1 and 10 mg/ℓ (Fig. 10.12).

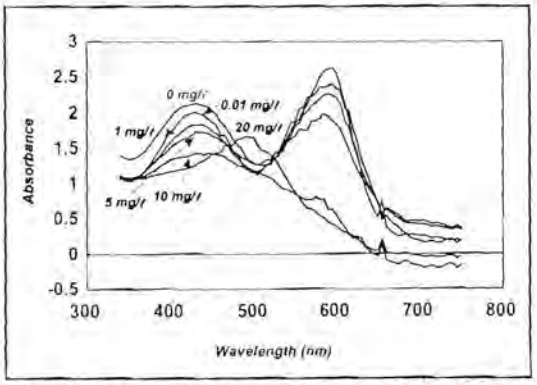

Fig. 10.11 Spectra obtained for different mercury concentrations.

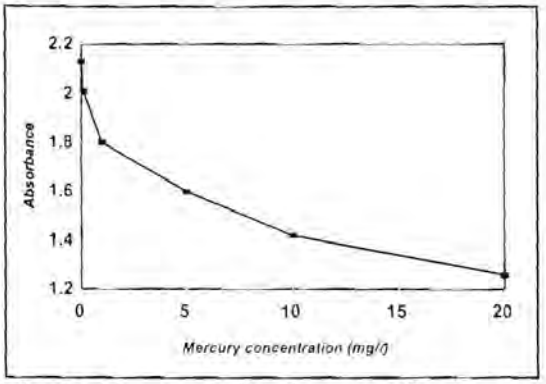

Fig. 10.12 Calibration curve for mercury at 430 nm.

As with mercury, the iron peak form changed when higher concentrations were analysed. It seems as if there were different linear sections on the calibration curve which corresponded with a certain peak form (Figs. 10.13 and 10.14). The linear range for iron was taken between 1 and 10 mg/ℓ. The small analytical range can be ascribed to the fact that the iron-dithizone complex favours a 1:3 metal:reagent ratio.

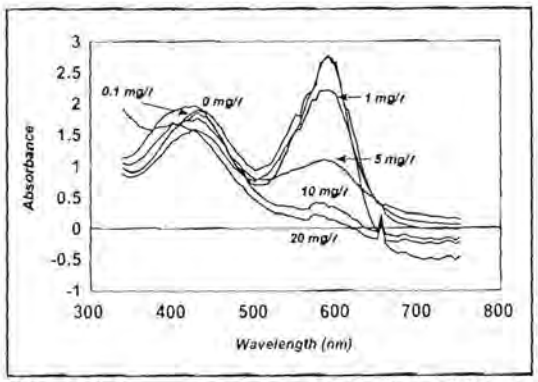

Fig. 10.13 Spectra obtained for different iron concentrations.

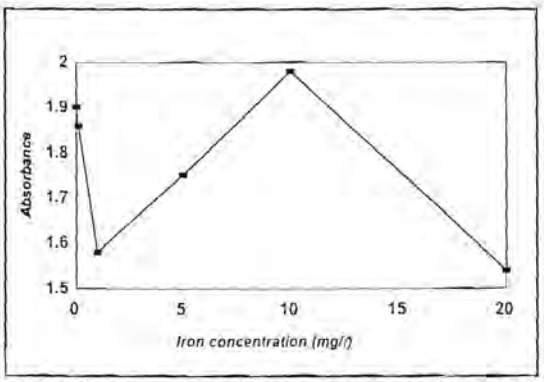

Fig. 10.14 Calibration curve(s) for iron at 430 nm.

For copper it was impossible to use decrease of reagent to construct the calibration curve, because the spectra of the different concentrations overlap tremendously, especially at the wavelengths were dithizone absorb. The calibration curve was therefore constructed from the absorbance readings at 490 nm. This resulted in a linear range between 1 and 20 mg/ℓ copper. The equation of the line was:

$$A_{Co} = 0.028[Cu] + 1.009; \quad r^2 = 0.9976$$

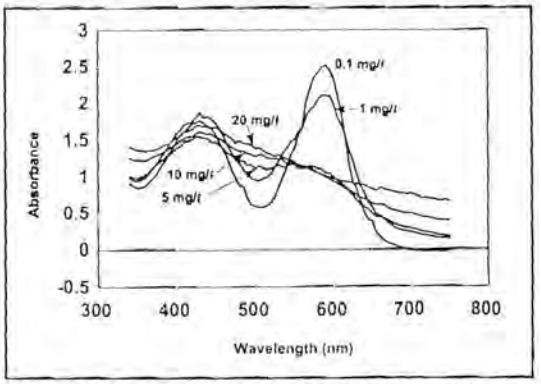

Fig. 10.15 Spectra obtained for different copper concentrations.

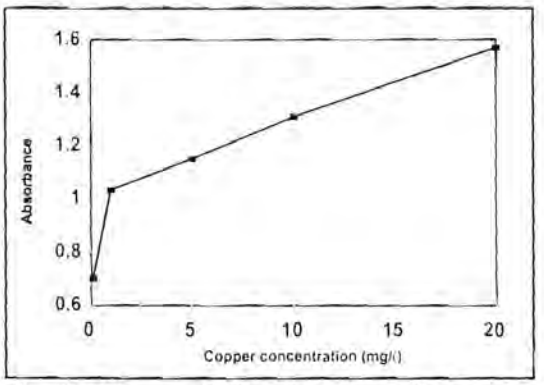

Fig. 10.16 Calibration curve for copper at 490 nm.

Again the peak shape seemed to determine the linear range. Lower concentrations of zinc showed peaks with an dithizone shape, due to the excess of the reagent and for this peak shape the calibration curve was linear between 1 and 10 mg/ℓ. Higher concentrations of zinc showed peak profiles with maximum absorbance close to 571 nm. The calibration curve was linear between 10 and 20 mg/ℓ for these peak profiles.

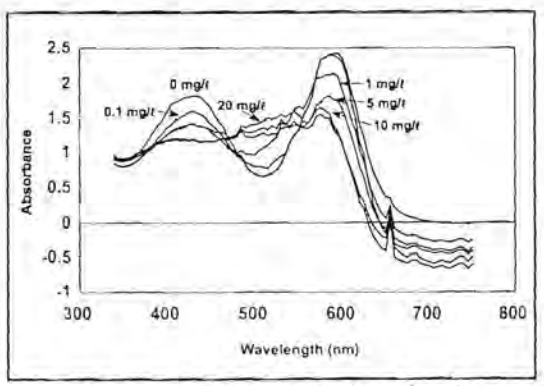

Fig. 10.17 Spectra obtained for different concentrations of zinc.

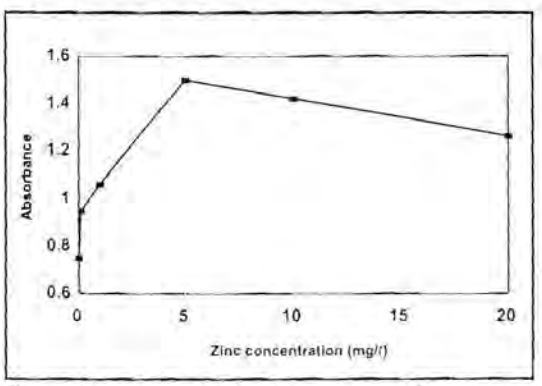

Fig. 10.18 Calibration curve for zinc at 525 nm.

No change in peak shape was observed for lead. The difference in the absorbance between the different concentrations were noticeable, but very small. Virtually no distinction could be made between the blank and a 0.1 mg/ℓ solution. The graph shows linearity between 1 and 20 mg/ℓ when reading the absorbance at 525 nm. The calibration graph's equation was as follow:

$$A_{Pb} = 0.015[Pb] + 0.807;\ r^2 = 0.9974.$$

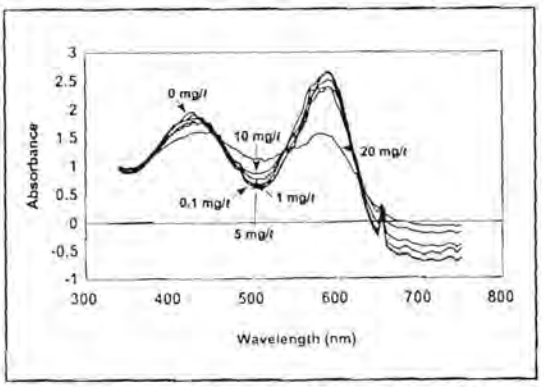

Fig. 10.19 Spectra obtained for different concentrations of lead.

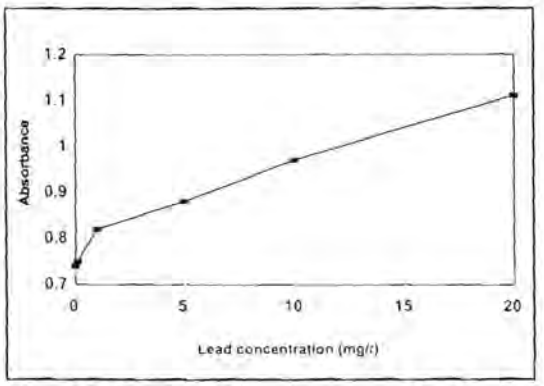

Fig. 10.20 Calibration curve for lead at 525 nm.

The calibration curve for cadmium was constructed of measurement values taken at 435 nm. Two different linear ranges could be found: between 1 and 5 mg/ℓ and between 5 and 20 mg/ℓ. As in the case with the other metals, the peak shape were different for higher concentrations of the analyte.

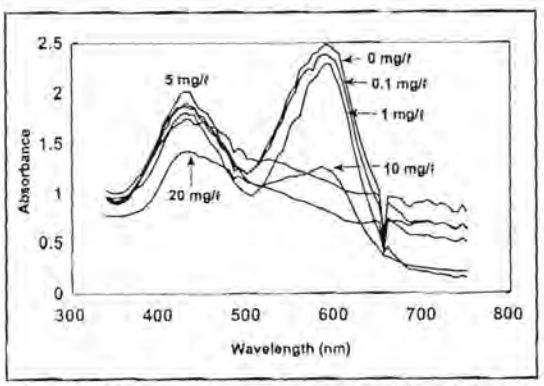

Fig. 10.21 Spectra obtained for different cadmium concentrations.

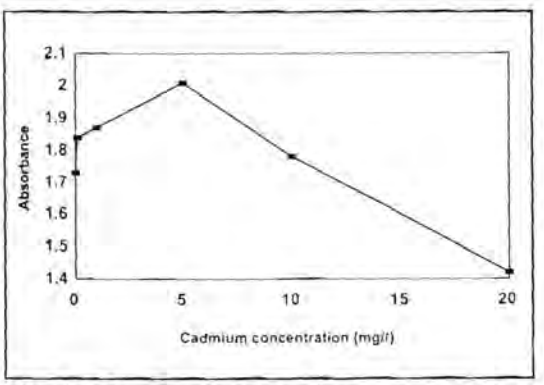

Fig. 10.22 Calibration curve for cadmium at 435 nm.

10.4.3.2 Accuracy and precision

To evaluate the accuracy of the sequential injection system, three soil samples, three urine samples, two tap water samples and one synthetically prepared aqueous test solution were evaluated. Seven standard which contain six of the seven analytes as

well as one standard containing all seven analytes were prepared. The standard solutions prepared are listed in Table 10. 5.

TABLE 10.5 Standard solutions prepared to construct calibration data

Standard	Analyte (mg/l)						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
1	7	6	5	4	3	1	0
2	6	5	4	3	2	0	7
3	5	4	3	2	1	7	6
4	4	3	2	1	0	6	5
5	3	2	1	0	7	5	4
6	2	1	0	7	6	4	3
7	1	0	7	6	5	3	2
8	5	5	5	5	5	5	5

The results were verified using results obtained by conventional hand extractions at the same pH. Figs. 10.23 and 10.24 show the spectra obtained for the standard solutions using the conventional extraction (Fig. 10.23) and using the sequential injection extraction system (Fig. 10.24).

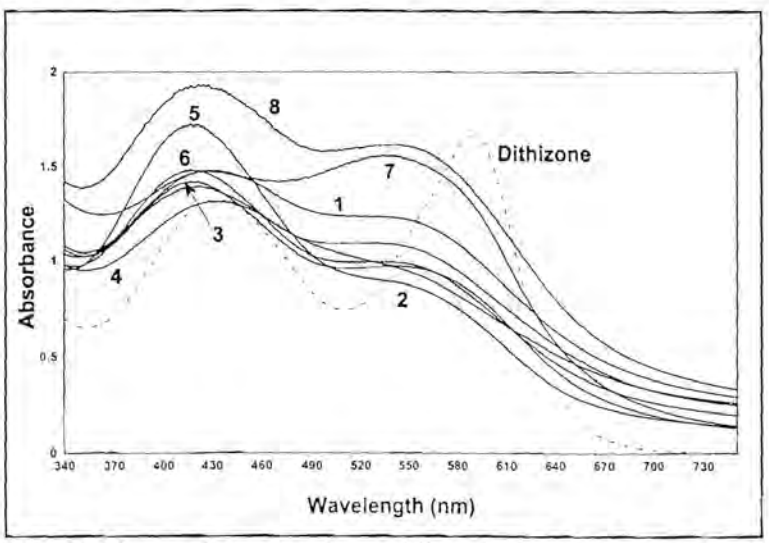

Fig. 10.23 Spectra of the different standard solutions to create calibration data. Analyses were done by traditional hand extractions.

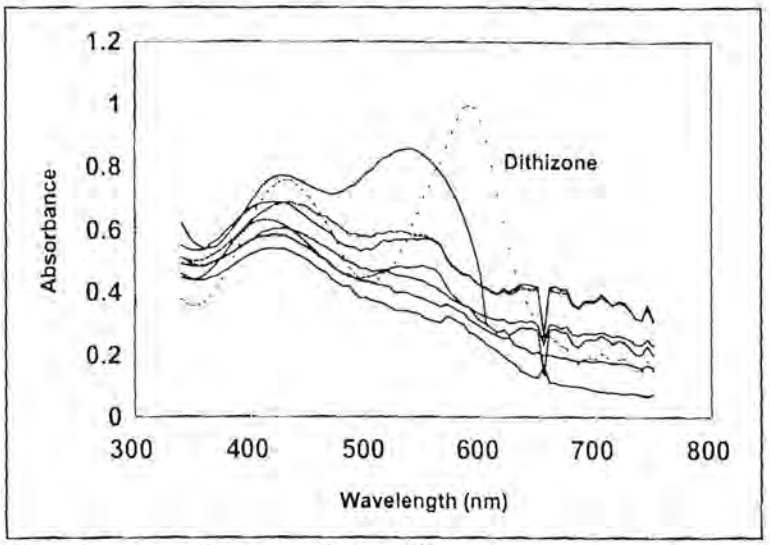

Fig. 10.24 Spectra of the different standard solutions to create calibration data. Analyses were done by using the sequential injection analyser.

It can be observed that the sensitivity of the spectra obtained with the sequential injection system was much lower compared to the spectra obtained with traditional hand extractions. Because the sensitivity of the SIE system was so low, it was difficult to distinguish clearly between the different spectra of the standards. Figs. 10.25 to 10.33 were therefore created to show the peak shapes and the difference in sensitivity for every standard solution.

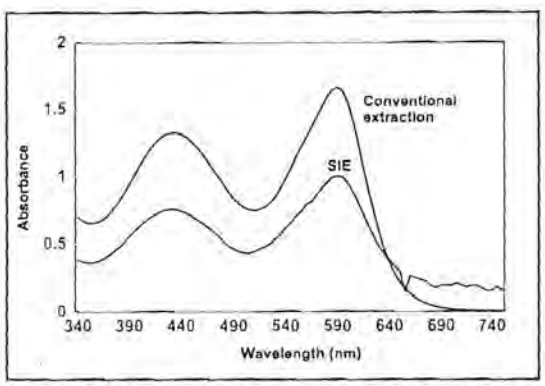

Fig. 10.25 Blank spectra (only dithizone) using conventional extraction and sequential injection extraction (SIE).

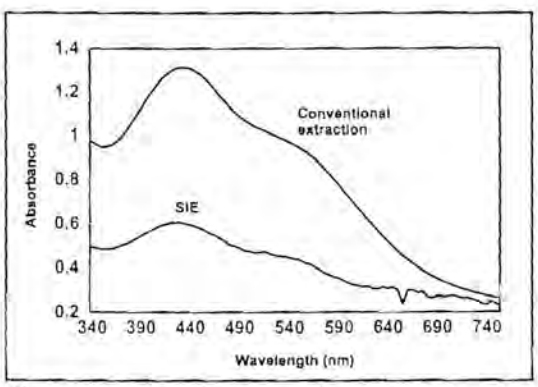

Fig. 10.26 Spectra of standard 1 using conventional extraction and sequential injection extraction (SIE).

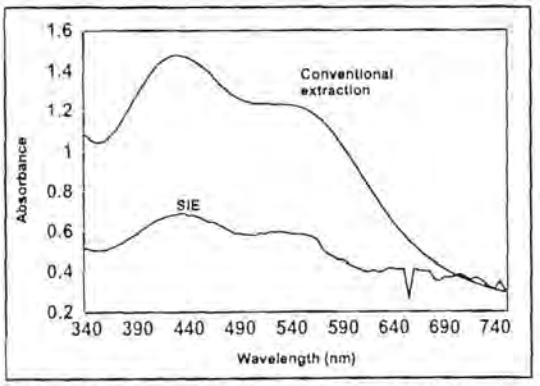

Fig. 10.27 Spectra of standard 2 using conventional extraction and sequential injection extraction (SIE).

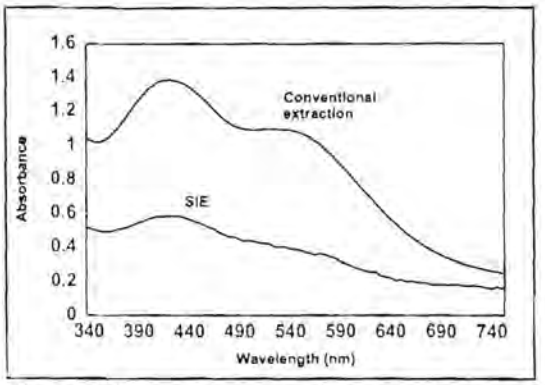

Fig. 10.28 Spectra of standard 3 using conventional extraction and sequential injection extraction (SIE).

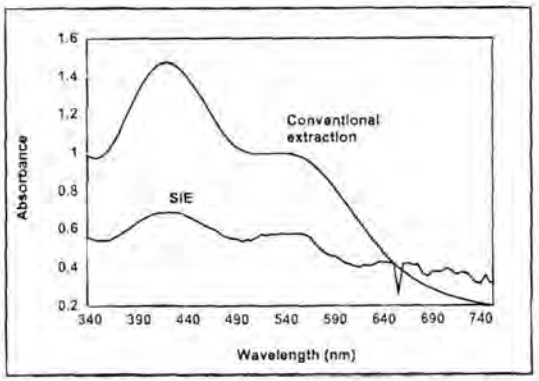

Fig. 10.29 Spectra of standard 4 using conventional extraction and sequential injection extraction (SIE).

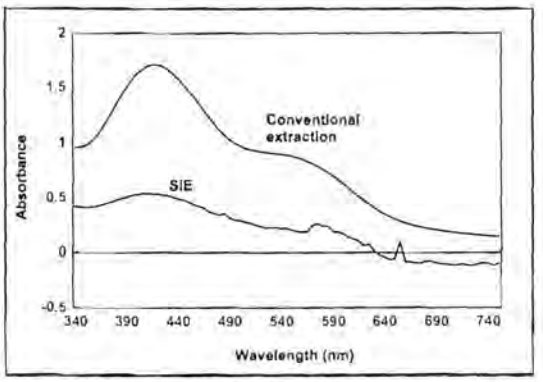

Fig. 10.30 Spectra of standard 5 using conventional extraction and sequential injection extraction (SIE).

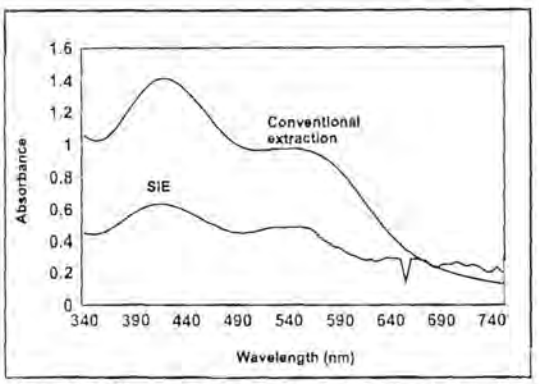

Fig. 10.31 Spectra of standard 6 using conventional extraction and sequential injection extraction (SIE).

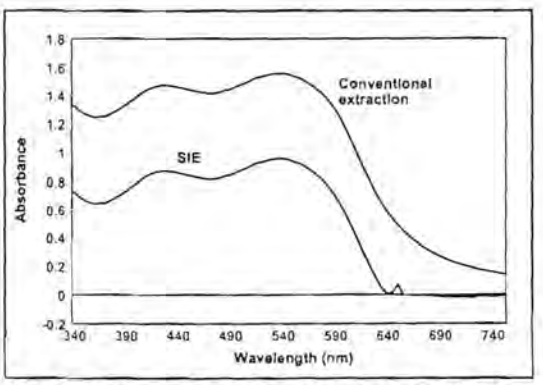

Fig. 10.32 Spectra of standard 7 using conventional extraction and sequential injection extraction (SIE).

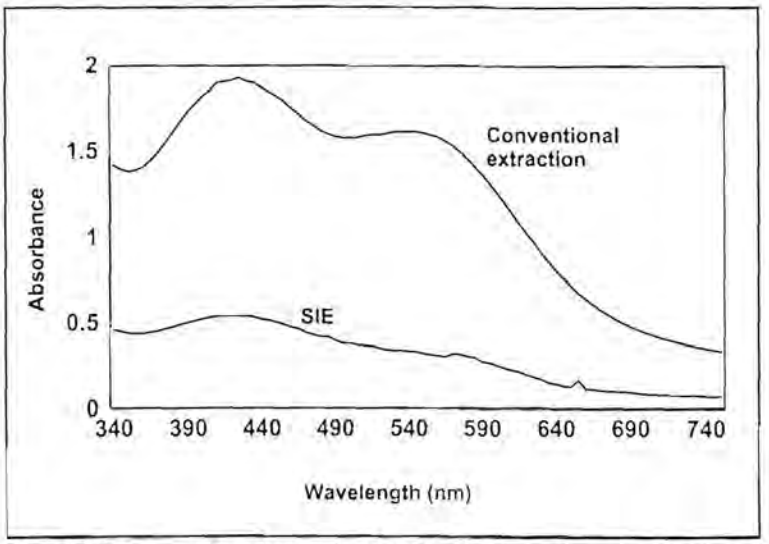

Fig. 10.33 Spectra of standard 8 using conventional extraction and sequential injection extraction (SIE).

The reproducibility of the two systems were evaluated and the percentage relative standard deviation as calculated for each individual analyte, are given in Tables 10.6 and 10.7. Calculations were done by the calibration program of the diode array spectrophotometer.

TABLE 10.6 Relative standard deviation obtained for each individual analyte in a standard solution. Results were obtained with the sequential injection system.

Standard	%RSD						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
1	1.43	2.07	0.17	0.97	0.34	1.93	0.49
2	1.92	2.06	0.81	0.69	0.53	0.92	0.64
3	1.74	1.51	0.31	0.62	0.62	0.51	0.32
4	0.65	2.41	0.21	0.25	0.52	1.70	0.43
5	1.57	1.83	0.47	0.86	1.46	0.75	1.86
6	1.62	1.47	0.76	0.78	1.51	1.42	1.91
7	2.03	1.65	0.95	0.77	1.09	1.54	1.40
8	2.09	1.67	0.89	0.87	0.94	1.61	1.64

TABLE 10.7 Relative standard deviation for each individual analyte in the standard solutions. %RSD obtained when employing conventional hand extraction methods.

Standard	%RSD						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
1	1.44	0.78	1.94	1.21	0.31	1.31	1.08
2	0.42	0.83	1.41	1.30	0.97	0.62	1.16
3	0.68	0.58	1.17	0.91	1.82	0.67	0.81
4	0.46	0.75	1.61	0.88	1.76	1.05	0.78
5	1.15	1.37	1.91	1.25	1.28	1.71	1.66
6	1.28	1.47	1.95	1.41	1.34	1.58	1.80
7	1.95	1.36	1.54	0.41	1.34	1.78	1.06
8	1.79	1.48	1.58	0.44	1.23	0.61	1.60

The samples that were analysed with the sequential injection extraction system are listed in Table 10.8. Standard additions are indicated as well.

TABLE 10.8 Sample solutions analysed with the SIE system and conventional hand extraction methods to evaluate the accuracy of the SIE system

Sample	Analyte added (mg/ℓ)						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
Soil 1	-	-	-	-	7	5	2
Soil 2	5	6	-	-	4	-	4
Soil 3	-	-	5	5	1	-	1
Tap water 1	-	-	-	-	-	-	-
Tap water 2	-	2	-	2	-	5	4
Urine 1	-	-	-	-	-	-	-
Urine 2	-	-	-	-	5	-	2
Urine 3	-	-	-	-	-	5	7
Control	5	5	5	5	5	5	5

The results obtained with the conventional hand extractions are shown in Fig. 10.34, while the results obtained using the SIE system are highlighted in Fig. 10.35.

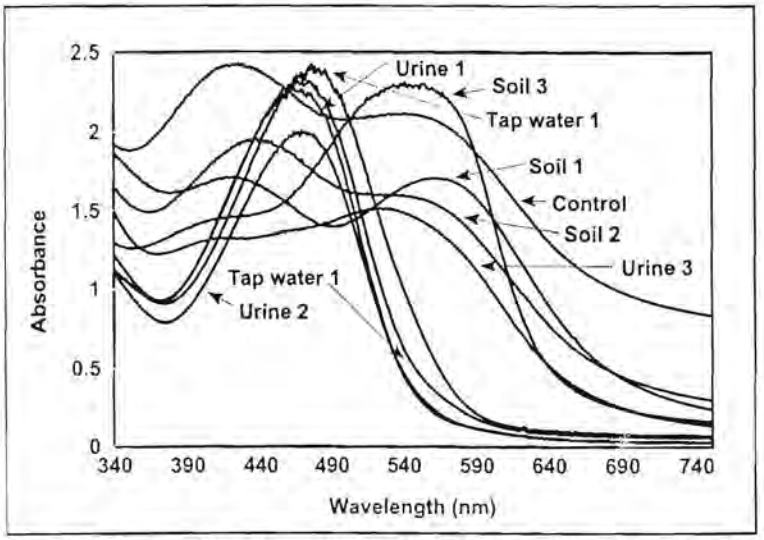

Fig. 10.34 Spectra obtained for the different samples analysed. Conventional extractions were used to obtain the dithizonates.

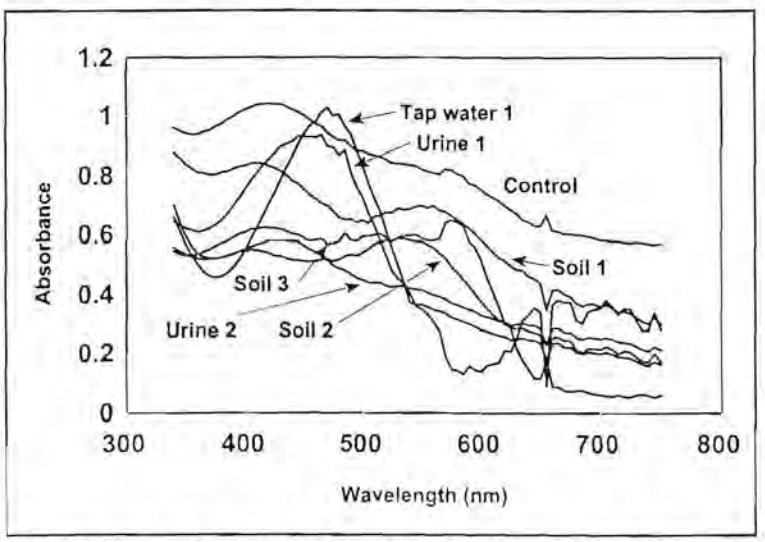

Fig. 10.35 Spectra obtained for the different samples analysed. The sequential injection extraction system were used to obtain the dithizonates.

Using the multivariate calibration option of the diode array spectrophotometer, the concentration of the various analytes as well as the percentage relative standard

deviation of each determination could be obtained. These values are listed in Table 10.9 and 10.10, while the results obtained with the conventional hand extractions are listed in Table 10.11 and 10.12.

TABLE 10.9 Results obtained employing sequential injection extraction system

Sample	Analyte concentration found (mg/ℓ)						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
Soil 1	4.60	3.18	0.66	22.56	1.30	9.69	2.52
Soil 2	7.29	5.99	5.58	17.37	5.83	8.67	6.25
Soil 3	3.74	0	4.74	17.56	4.74	9.30	1.22
Tap water 1	8.49	7.67	11.08	23.62	12.93	46.82	0.29
Tap water 2	4.96	5.62	17.49	27.00	13.20	46.20	0.47
Urine 1	8.34	6.43	5.31	19.54	13.48	4.93	0.74
Urine 2	7.05	5.19	5.80	25.36	11.96	439	2.40
Urine 3	0	2.61	3.58	14.05	13.19	4.28	6.43
Control	4.91	5.01	4.89	5.42	5.04	5.11	4.79

TABLE 10.10 Relative standard deviations obtained for samples analysed with the sequential injection extraction system

Sample	%RSD						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
Soil 1	0.81	0.19	0.34	0.49	0.78	0.41	1.08
Soil 2	0.21	0.14	0.26	0.32	0.58	0.31	0.81
Soil 3	0.76	0.80	1.46	1.86	1.37	1.76	1.66
Tap water 1	0.98	1.04	1.90	1.94	1.36	1.28	1.06
Tap water 2	0.78	0.85	1.54	1.97	1.54	1.85	1.88
Urine 1	0.96	1.07	1.93	1.44	1.44	1.34	1.13
Urine 2	0.87	0.94	1.70	1.15	1.91	1.95	1.40
Urine 3	0.52	0.28	0.50	0.68	1.17	0.62	1.63
Control	0.64	0.75	0.67	0.47	0.28	0.58	0.84

TABLE 10.11 Results obtained using the conventional hand extractions

Sample	Analyte concentration found (mg/ℓ)						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
Soil 1	4.26	3.64	0.31	23.17	1.09	10.59	2.40
Soil 2	7.15	5.99	5.52	17.02	5.33	8.85	5.61
Soil 3	3.74	2.12	4.74	18.21	5.45	11.19	1.46
Tap water 1	8.60	7.67	1.18	13.09	13.00	47.80	0.74
Tap water 2	5.56	5.24	1.74	27.88	13.41	47.23	0.58
Urine 1	8.45	6.40	5.31	3.97	13.56	4.92	1.46
Urine 2	7.05	5.19	5.80	3.56	12.01	4.38	1.32
Urine 3	0	2.62	3.58	1.48	10.70	2.87	5.73
Control	4.95	5.09	4.99	4.87	5.01	5.18	5.08

TABLE 10.10 Relative standard deviations obtained when using conventional hand extractions

Sample	%RSD						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
Soil 1	0.17	0.20	0.35	0.46	0.83	0.44	1.16
Soil 2	0.13	0.14	0.25	0.31	0.57	0.30	0.78
Soil 3	0.74	0.83	1.51	1.91	1.47	1.82	1.80
Tap water 1	0.95	1.07	1.94	1.46	1.48	1.34	1.18
Tap water 2	0.77	0.86	1.56	1.97	1.58	1.87	1.95
Urine 1	0.97	1.06	1.92	1.42	1.41	1.31	1.08
Urine 2	0.86	0.94	1.70	1.15	1.91	1.05	1.40
Urine 3	0.26	0.28	0.50	0.64	1.16	0.61	1.60
Control	0.18	0.17	0.19	0.34	0.49	0.78	0.41

10.4.3.3 Sample frequency

To complete the whole analytical cycle, including the extraction and detection, took 135 seconds. This resulted in a sample frequency of 27 samples per hour. When

considering that seven analytes were determined simultaneously, the sample frequency is quite remarkable for a sequential injection system.

10.4.3.4 Sample interaction

Sample carry-over was evaluated employing a Unicam 8625 spectrometer, to avoid the effect of deteriorating blank signals. Negligible carry-over between samples was experienced when employing this system. A sample with low analyte concentration (1 mg/ℓ) was analysed, followed by a sample with analyte concentration five times higher than the first. To evaluate sample interaction the first sample was analysed again. Sample carry-over was then calculated according to the difference between the two peak height values [18]. The percentage carry-over calculated were low enough to be ignored. The sample interaction was calculated to be about 0.05%.

10.4.3.5 Detection limits

The detection limit is both a function of sensitivity and noise. The lowest concentration, that could be determined without doubt, would be considered the detection limit of the system. Due to the excess of reagent, detection limits were difficult to obtain. Low concentrations of the analyte showed peak profiles similar to those of the reagent alone, while the peak shape changed with higher analyte concentrations. Since the most of the linear ranges started at 1 mg/ℓ, this concentration was taken as limit of detection for all seven analytes.

10.4.3.6 Interferences

Possible interferents were tested using a solution containing 1 mg/ℓ of all seven analytes. The following substances did not interfere in the determination: 500 mg/ℓ SO_4^{2-}, 10 mg/ℓ PO_4^{3-}, 2 mg/ℓ Al^{3+} (Al do not react with dithizone under neutral to alkaline conditions), 50 mg/ℓ Mg^{2+} and 400 mg/ℓ Ca^{2+}.

The mercury determination was more vulnerable to interferences. Chloride up to 14 g/ℓ (0.4 mol/ℓ) did not interfere in the mercury determination as long as the H_2SO_4 concentration did not exceed 2 mol/ℓ. Bromide, cyanide and thiocyanate also interfered seriously in the mercury determination, since they complex mercury more strongly than dithizone. These anions could be tolerated up to 10 mg/ℓ. Thiocyanate and cyanate were used to mask interferences due to cobalt and interfered seriously in the determination of cobalt. Anions could be removed by using anion exchange columns in the sample uptake tubes. The other metals did not experience any interference due to the anions present in the solution.

Since the metals normally present in urine, *viz.* Fe(II), Ni(II), Pb(II) and Zn(II), were also analysed with the SIE system, they were ruled out as interferents. Variation in pH could be used to separate the different metals from one another to allow individual analysis, but at the pH used all the analytes were extracted into the organic layer.

10.5 Conclusion

A simplified, automated extraction system is described which employ a non toxic solvent, ethanol. This makes the technique much more environmental and operator friendly. The technique can be applied without the use of phase separators or segmenters. This fact highlights the durability and robustness of the technique, since less maintenance will be needed. The sequential injection system is fully computerised and allow the determination of seven metal ions (lead, zinc, copper, iron, cobalt, cadmium and mercury) in the same sample without prior separation. A sample frequency of 27 samples per hour place it ahead of other SIA systems, where the main drawback usually is the low sample throughput. Coupling sequential injection extraction with a diode array spectrophotometer resulted in high sample throughput and a sensitive method to determine related species without the need of tedious analyte separations.

10.6 References

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CHAPTER 11

Summary

The development of analysers capable of on-line monitoring, with a high sample throughput, minimum sample and reagent consumption that are robust, reliable and requires a low frequency of maintenance became a necessity in many laboratories. Flow injection analysis was only moderate successful in complying the strict requirements of precise timing and low sample and reagent consumption. Furthermore, FIA manifolds require physical manipulation of the manifold when changing from one configuration to another. Very often multiple lines are required that make the system inconvenient to operate and more prone to failures. Simply changing a chemistry can be very time consuming.

The attributes of flow-injection analysis (FIA): speed, solution containment, capability of miniaturization, automated standardization, sample dilution, matrix removal, analyte preconcentration and the ability to control the time/concentration domain of any solution chemistry under investigation, are, however, well recognized and exploited by the world wide community of flow injection enthusiasts.

The introduction of SIA was an attempt to make injection techniques more rugged for process control application. This single-line system that is completely computer controlled, can be configured to perform most operations of conventional flow injection analysis with no or minimal physical reconfiguration of the manifold. This system contributes considerably to reducing reagent and sample configuration which is one of its greatest attributes. The simplicity of the SIA system is attributed to the multi-port selection valve by which the samples, reagents, reactions lines and detector line(s) are accessed. Adaption of chemistries to SIA is also (in most cases) an easy task, as it can be based on the practice and the theory of flow injection methodology, which provide guidelines for the design of sequential injection systems.

The inherent flexibility of SIA makes it an ideal tool for monitoring bioprocesses where sample matrix changes with time and different conditions of analysis may be required. Another advantage of SIA is the possibility of clustering standards around the multi-position valve, so that the system might be automatically recalibrated as required. The detector is only exposed to the potentially harsh sample for a short time, the rest of the time, the detector is exposed to the wash solution. There is also the possibility of two assays being performed with an unique SIA system.

It is unlikely that SIA in its present form will replace FIA. Few sample manipulation techniques match FIA in flexibility. The only requirement for successful implementation in FIA is a repeatable flow pattern. In SIA careful planning and method design is required. Attention must be given to ensure that zones are contained within the reaction coil and that device events are carefully synchronized. This requires closer attention during the method development phase. Nevertheless, once a method has been developed, SIA tirelessly and slavishly repeats the device sequence which generates the desired analytical results.

Important guidelines were established to assist in the design of SIA manifolds. Of course, these guidelines must be interpreted in the light of the application at hand; it may not always be desirable to maximize sensitivity.

The development of sequential injection analysis is aimed at providing the industrial, agricultural, clinical and pharmaceutical fields with reliable, precise and cost-effective instrumentation for performing the analyses required. The increasing awareness regarding environmental pollution and the regulation of the effluents that are released into the environment, places an enormous responsibility on analysts to develop methods that can be effectively applied to determine the actual amount of polluting elements that are discharged.

A wide range of successful applications of SIA are already described by various researchers. Unfortunately, most of these applications involved the determination of

only one analyte at a time. Simultaneous analysis (or multi-component analysis) employing sequential injection analysis seems to be more complicated or less feasible than single-component analyses. This might be described to the fact that most multi-component determinations require extractions 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. Systems that only use one detector for multi-component detection are more complex to program and manipulate, but are much more cost effective than multi-detector systems. In this study, various single detector systems for multi-component analysis were illustrated and evaluated.

The methodology developed during this study demonstrates the suitability of sequential injection analysis for sequential and simultaneous determinations. A novel tandem technique for the determination of iron and sulphate in the same or different samples were described. It was possible by using an optimised sequence of samples and reagents and the kinetics of the two reactions to determine to analytes in one analytical cycle. The zones of the slower turbidimetric determination of sulphate were first introduced into the holding coil, followed by the zones of the faster iron-tiron reaction. This arrangement allowed ample time for both reactions to develop to a desired stage before photometric detection at the same wavelength took place.

The same reactions were also used to evaluate a sandwich technique based on the introduction of a very large sample zone sandwiched between the two reagents. The volume of the sample zone was crucial to eliminate any interference of the two reactions on one another and to ensure sufficient resolution between the two reagent peaks obtained. The latter was necessary for convenient measurement of the respective peak heights.

Due to the discontinuous nature of sequential injection analysis, a multi-component determination based on differential kinetics were successfully adapted from FIA. The simultaneous determination of nickel(II) and cobalt(II) were based on the different

reaction rates of the two analytes with a non-selective reagent, PAR. Since SIA is a discontinuous technique, stopped flow periods are easily incorporated to ensure longer reaction times for slower reactions.

Three different extraction procedures were developed for determination of two or more metal ions in soil extracts, clinical samples and aqueous samples. The first sequential injection extraction (SIE) system described a common extraction method. The sample in the aqueous phase was mixed with the reagent (dithizone) in the organic phase. Since the organic solvent used was ethanol and because ethanol and water are miscible in all ratios, no separation of the phases was necessary prior to detection. Cobalt(II) and mercury(II) were determined with this SIE method with acceptable accuracy and reproducibility.

Since no phase segmenters or separators are needed in a sequential injection extraction system, the manifold is much simpler and easier to construct than those of flow injection extraction systems. SIE is a flow-based extraction method where an aqueous sample and organic solvent are injected sequentially into an extraction coil, then mixed and separated due to the differential flow velocities of the aqueous and organic phases.

Dithizone was also used for the second extraction process, but this time it was dissolved in carbon tetrachloride. Specific metal dithizonates are formed at specific pH values. For the extraction of mercury(II) and cadmium(II) a pH gradient was established in the extraction coil to ensure maximum extraction of both analytes into the organic phase. The acidic extraction product ($Hg(HDz)_2$) was formed first, due to its stability in dilute alkaline solutions and therefore did not decompose when the flow was reversed to allow the alkaline extraction of the cadmium dithizonate. The organic phase containing both analytes was determined photometrically employing only one detector.

In the third extraction method, seven metal ions (Pb(II), Zn(II), Cu(II), Fe(III), Co(II), Cd(II) and Hg(II)) were determined using a single detector. The metal ions were extracted into an organic phase (ethanol) containing dithizone. No separation of phases was necessary due to miscibility of the two phases. The flow was stopped in the flow-through cell in the detector and the spectra were recorded with a diode array spectrophotometer. Spectra were recorded between 350 and 750 nm. The method proved to be accurate, sensitive and reproducible.

Since simultaneous or multi-component analysis allow the measurement of more than one analyte or parameter at a time, it increases the sample throughput of SIA systems tremendously. This study also proved that the sequential injection analysers developed, are compatible with analysis of samples in different forms and from different application fields. It can therefore be expected that multi-component sequential injection analysers will be proliferated in modern chemical, biochemical, metallurgical and environmental applications.