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Determination of Lead(II), Copper(II), Zinc(II), Cobalt(II), Cadmium(II), Iron(III), Mercury(II) using sequential injection extractions☆

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Abstract

Sequential injection analysis is still dominated by single component analysis. In this proposed robust, economical simple instrumental system seven different metal ions are determined simultaneously using thin-film sequential injection extraction (SIE) with multivariate calibration and multiwavelength detection. Dithizone, in ethanol, is used as extractant and the metal dithizonates' spectra are generated by a diode array spectrophotometer between 300 and 700 nm. The SI thin-film extraction using water miscible with ethanol works due to the hydrophobic interaction of ethanol with the Teflon wall to create a thin film. A sample frequency of 27 samples per hour was obtained with a sample carry-over of less than 1%. The results of the proposed sequential injection extraction system compare favourably with the results obtained by using standard atomic absorption spectrometry (AAS) methods on conventional extraction samples.

Keywords: Sequential injection extractions; SI thin-film extraction; Diode array spectrophotometer

1. Introduction

Metal pollution of water in a water scarce country like South Africa could become an economical hazard, since purification of polluted water are quite expensive. Although mining is a large part of the South African economy, together with the electroplating industry it is also one of the main producers of metal pollution of dams and rivers. Metals, which are captured in certain areas in soils, 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. It is therefore crucial that the amount of dissolved metals in effluent streams must be monitored carefully. Furthermore, these metals can have toxic effects on people, animals and plants when absorbed in excess amounts and should be screened on-site immediately when suspected.

Since its introduction in 1990, sequential injection analysis has grown in popularity [1,2] not only because of its economical use of sample and reagents, but surely because of its robustness and simple instrumental design. It is rather unfortunate that such a powerful technique is used mostly for single component analysis. A literature survey showed that more than 72% of all publications on SIA consist of single component analysis [3]. This proposed system surely fits into the other 28% of multi-component analysis.

Simultaneous determination of trace amounts of heavy metals usually employ one of the following methods: atomic absorption spectrometry (AAS), cold vapour AAS or flame-AAS-ETA (electrothermal atomisation) [4–6,7], inductively coupled plasma-optical emission spectroscopy (ICP-OES) [8], potentiometry (ion selective electrodes) [9], anodic stripping voltammetry [10], chromatography (usually HPLC) [11], gravimetric detection [7] or photometry [7,12–16].

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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 [4]. Many of the systems employ on-line separation of the analyte from specific interferences. These systems may include dialysis [9,17] or extractions [4,18–21].

Although atomic spectrometric techniques are normally used to determine several metals on a routine basis in large well-equipped laboratories, the instrumentation is not normally suited for on-site monitoring especially where the process analysing system should be moved continuously and where monitoring is done in inflammable surroundings. Furthermore, atomic spectrometric instrumentation is also too expensive for less equipped laboratories.

The first objective was to find a single cheaper detector for the multi-component determination of the seven metals (lead, zinc, copper, iron, cobalt, cadmium and mercury). All of these seven metals are so-called dithizone metals [4,22,23]. 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 and the different metals as dithizone complexes can be measured using multivariate calibration and multiwavelength detection.

SIA is a simple, robust, reliable and inexpensive technique with a low frequency of maintenance that should be capable of monitoring the contents of Pb, Zn, Cu, Fe, Co, Cd and Hg in certain soil and water areas. SIA with a diode array spectrophotometer as detector seemed to be an ideal system for such an analyser and this paper reports on a system that was optimised and developed for this purpose.

2. Experimental

2.1. Reagents and solutions

All solutions are prepared from analytical grade reagents 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.

2.1.1. Extractant

Dithizone (0.05 g) (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 10 days. Working solutions are obtained by suitable dilution of the stock solution with ethanol.

2.1.2. Metal ion stock solutions

The following 1000 mg l^{-1} metal ion 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 1.000 g of the copper metal in 10 ml 55% (m/m) HNO_3 and approximately 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): 0.7020 g $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ was dissolved in approximately 20 ml of water together with 1.1 ml of 18.4 mol l^{-1} H_2SO_4 (98%, m/m). The final solution was diluted to 100 ml with deionised water.
- Mercury(II): 1.7100 g $Hg(NO_3)_2{\cdot}2H_2O$ (Merck) was dissolved in 1 l of deionised water.

The concentrations of these metal ion solutions was standardised using standard AAS methods.

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

2.2. Instrumentation

The sequential injection extraction (SIE) manifold is illustrated in Fig. 1. 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 (TFE) tubing (SUPELCO) and a 10-port electrically actuated VICI selection valve (Model ECSD10P) (Valco Instruments, Houston, Texas). Acidflex pump tubing was used. The holding coil was constructed of 1 m (0.8 mm i.d.) Teflon tubing and the reaction coil of 45 cm of 1.25 mm i.d. Teflon tubing. Device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an assembled distribution board (MINTEK, Randburg, South Africa). The FlowTEK [24] software package (Version 1.3a, obtainable from MINTEK) was used throughout the procedure. A Hewlett-Packard UV-vis diode array spectrophotometer (with HP 845395–97 Chem station software), equipped with a 10 mm Hellma flow-through cell (volume: 80 μl), was used for measuring the absorbance and data acquisition. The single component analysis program was used to record the linear

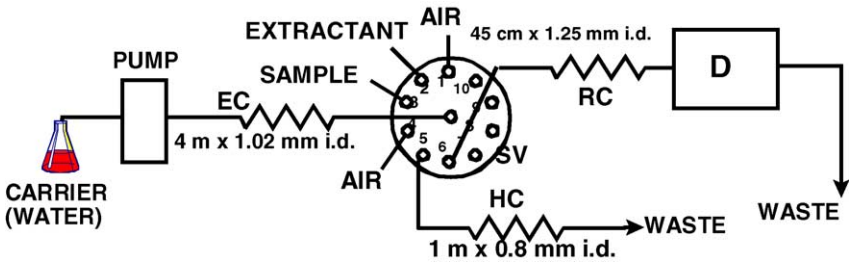

Fig. 1. SIA system used in the determination of the seven analytes. EC: extraction coil (4 m × 1.02 mm i.d.), SV: selection valve, HC: holding coil (1 m × 0.8 mm i.d.), RC: reaction coil (45 cm × 1.25 mm i.d.) and D: detector.

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. The absorption maxima of the different metal dithiozonates in ethanol were determined experimentally for the individual analytes as well as for the seven analytes in mixed standards as well as in the different samples in order to find the optimum working conditions for the proposed sequential injection system. Spectra were recorded over a wavelength range from 340 to 750 nm with intervals of 5 nm each when using the sequential injection system and intervals of 1 nm each when manual extractions were done to confirm the performance of the system. The calibration program was used for data analysis.

2.3. 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, therefore 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 it was therefore 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 that enabled the computer to read the signal coming from FlowTEK. The program used by FlowTEK to control the devices is given in Table 1.

2.4. Sample preparation

2.4.1. Sample collection

Urine samples were collected in polypropylene flasks that 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 then 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 h. 5.00 ± 0.01 g of the air-dried soil was weighed into a beaker and 250 ml of a 0.43 mol l^{-1} 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 mP using deionised water. Acetone (10 ml) (AR) was added to every working solution (standard) and sample prepared. Real samples with very low metal ion contents were spiked with standards when necessary.

3. Optimisation

3.1. 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. The optimisation was done with a standard solution containing 1 mg l^{-1} 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. 2).

Table 1
Device sequence for the proposed sequential injection extraction system

Time (s)	Pump	Valve	Detector	Description
0	Off	Air		Pump off, valve is turned to select first air inlet
4	Reverse			Draw up air bubble
4.5	Off			Pump stop
5.5		Extractant		Select extractant line
6.5	Reverse			Draw up extractant solution
10.5	Off			Pump stop
11.5		Standard/sample		Select standard/sample line
12.5	Reverse			Draw up standard/sample solution
16.5	Off			Pump stop
17.5		Air		Select second air inlet
18.5	Reverse			Draw up second air bubble
19	Off			Pump stop
20		Holding coil		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		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

3.1.1. 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 was flushed to waste.

3.1.2. Flow rate

From previous experiments [3], 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 s, the absorbance was measured. Thereafter, the product zone was flushed to waste. Pump rate was therefore only optimised to ensure optimum extraction.

Slower flow rates result in thinner organic film and inevitably in longer extraction times. Thinner film is useful when back extractions are employed, but for single extractions, thicker films resulted in better extraction efficiency, since they have higher capacities [25]. Flow rates between

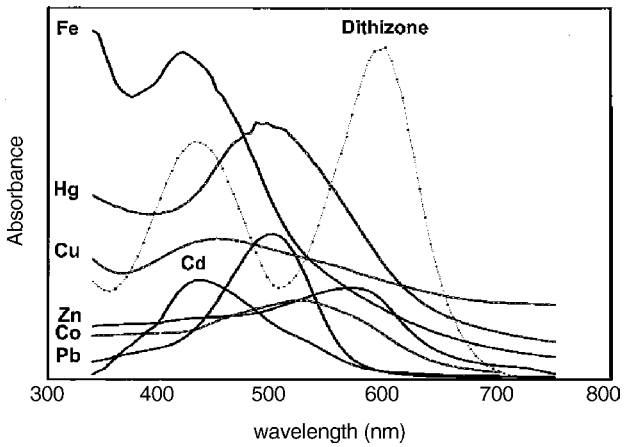

Fig. 2. Spectra of the different dithizonates in ethanol. Optimisation of the sequential injection system was done at 500 nm.

1.5 and 3 ml min^{-1} were evaluated. An optimum flow rate of 2.4 ml min^{-1} was chosen, due to its sensitivity and good reproducibility.

3.1.3. 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. 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 will be situated inside the flow cell during measurements. The reproducibility of the method decreased with volumes larger than 180 μl. This is due to insufficient zone overlap, because larger volumes led to smaller axial dispersion which decrease the zone penetration [26,27]. An optimum sample volume of 180 μl was thus chosen for the system.

3.1.4. 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. The organic solvent, ethanol, is miscible with water in all ratios, therefore optimal mixing rather than film thickness will be optimised. Since extraction still has to take place, longer extraction will be allowed for the reaction. The volume of the extractant zone influenced both the time of the extraction as well as the length of the extraction coil.

Extractant volumes between 45 and 180 μl were evaluated. Smaller volumes gave very irreproducible results, because of the imperfect flow dynamics of the pump (start up and stopping are not instantaneous) [26,27], and these small volumes were not aspirated reproducibly. A plateau is reached in peak height at volumes larger than 135 μl. An optimum extractant volume of 180 μl was chosen due to the good precision and sensitivity. This also contributed towards the larger product zone inside the flow cell during measurements.

Several extraction options, including multiple flow reversals to obtain thorough mixing of the zones were evaluated. 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 allows the reactions to reach a certain level of equilibrium. Extraction times shorter than 11 s led to a decrease in sensitivity and 11 s were therefore chosen as optimum extraction time.

3.1.5. Organic film thickness

Relative film thickness per unit length (d_f) can be predicted using the equation [25]

$$d_f = k d_t \left(\frac{u\eta}{\gamma} \right)^a$$

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 [25]. The film thickness parameter for ethanol was calculated to be 4.64×10^{-2} (η = 1.06 cP and γ = 21.80) [28]. The thickness of the organic film is very important since it influences extraction by affecting the mass transfer of analytes into the film [29]. Under optimum running conditions the relative film thickness was calculated to be 9.4 μm.

3.1.6. Diameter and length of tubing

3.1.6.1. Extraction coil. The length of the extraction coil depends on the zone inversion length. Results showed that during the extraction step a reverse step of 5 s should be used. This time multiplied with the flow rate 5.38 × cm s^{-1} (2.64 ml min^{-1}), resulted in a zone inversion length of 26.9 cm. Due to dispersion in the flow conduit, these zones occupy about nine times the inversion length. Thickness of the wetting film is directly proportional to the inner diameter of the coil [29]. 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.

3.1.6.2. 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 contributes 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 represents the shortest distance between the valve and the spectrophotometer.

3.1.7. 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, variations in the refractive index within the flow cell were created in the process. These variations led to very spurious peaks. To reduce the effect of the variation to a great extent, a waiting period was incorporated into the flow programming, to 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. Waiting periods longer than 50 s did not improve the shape and smoothness of the peaks and only increased the analysis time. The waiting period was then taken as 50 s.

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 10 nm apart resulted in very poor reproducibility and sensitivity. Measurements at intervals of five nanometres were chosen for measurements done by the sequential system. Manual measurements (hand extractions) were still taken with measurements at 1 nm intervals.

3.2. Chemical parameters

3.2.1. pH

The formation of all dithizonates is pH dependant and when employing different pH values differentiation between different metals is possible. 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^{-1} ammonia solution or 0.5 mol l^{-1} sulphuric acid solution.

3.2.2. 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 [22]. 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 [22,30]. One disadvantage about CCl_4 is that it is highly carcinogenic and has ozone-depleting properties [31]. To avoid the use of toxic organic solvents such as CCl_4 and $CHCl_3$ [20], ethanol was used as solvent for the dithizone reagent.

The major reasons to justify the use of ethanol as solvent are as follows. 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 were 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}.

3.2.3. Concentration of dithizone

Dithizone is only sparingly soluble in ethanol, with a solubility of 0.3 g l^{-1} at 20 °C[22]. 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 [22].

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 the presence of undissolved dithizone reagent resulted in very ‘spiky’ peaks and together with the sensitivity of the detector ultimately 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 evaluated. A 1:1 dilution gave smooth peaks and allows 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.

4. Evaluation of the system

The proposed sequential extraction method was evaluated under optimum running conditions with regard to first linearity of the individual analytes, then accuracy and precision using standard solutions of the seven analytes in mixed standards and also for standard solutions of the seven analytes in

the different real samples, sample frequency, sample interaction and major interferences.

4.1. Linearity

Originally 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. Calibration graphs and their linearity were first examined by using a series of single metal ion standard solutions. Concentrations up to 20 mg l^{-1} were evaluated where there was still excess reagent present, since most dithizonates react in a 1:2 metal:reagent ratio. Filtering of the concentrated dithizone reagent solution and a 1:1 dilution of the filtrate with ethanol gave smooth peaks and allows the determination of the more concentrated samples. The following were, however, some of the problems for each analyte originally encountered with before establishing the final proposed operating SIA analyser.

At 519 nm, the absorbance maximum for cobalt, difficulties were experience in finding the correct absorbance value due to 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. This represents the decrease in reagent with increasing cobalt concentration. The calibration curve was linear between 1 and 20 mg l^{-1} Co. The equation of the line was:

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

For mercury it was even more difficult to set up a calibration curve, since the peak shape changed and absorbance maximum shifted with increasing mercury concentration. 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^{-1}.

As with mercury, the peak form for iron changed when higher concentrations were analysed. It seems as if there were linear sections on the calibration curve that corresponded with a certain peak form. The linear range for iron was taken between 1 and 10 mg l^{-1}. The calibration curve for iron was constructed from the absorbance readings at 425 nm. The small analytical range can be ascribed to the fact that the iron-dithizone complex favours a 1:3 ratio.

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 where 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^{-1} copper. The equation of the line was:

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

With zinc the peak shape seemed to determine the linear range. Lower concentrations of zinc showed peaks with a 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^{-1}. 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^{-1} for these peak profiles.

For lead no change in peak shape or form was observed. The difference in the absorbance between the different concentrations was noticeable, but very small. Virtually no distinction could be made between the blank and a 0.1 mg l^{-1} solution. The graph shows linearity between 1 and 20 mg l^{-1} when measuring absorbance at 525 nm. The calibration graph(s) equation was as follow:

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

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

4.2. Accuracy and precision

To evaluate the accuracy of the proposed sequential injection system, three soil samples, three urine samples, two tap water samples and one synthetically prepared aqueous test solution were evaluated. Seven standards, which contain six of the seven analytes as well as one standard containing all seven analytes were prepared. The standard mixed solutions of the seven analytes to construct the calibration data are listed in Table 2. The results of real samples spiked with standards are as follows. The diode array spectra obtained for the different samples using the conventional manual extraction procedure are shown in Fig. 3 and those for the proposed SIA system in Fig. 4. The concentrations of the various analytes as well as the precision of each determination were obtained using the multivariate calibration option and multiwavelength detection of the diode array detector.

The results and the relative standard deviations obtained with the proposed SIA system are given in Tables 3 and 4. The results of the proposed SIA system were verified using results obtained by conventional manual extractions at the same pH with diode array detection. The results and the relative standard deviations obtained with conventional

Table 2
Standard mixed solutions prepared to construct calibration data

Standard	Analyte (mg l^{-1})						
	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

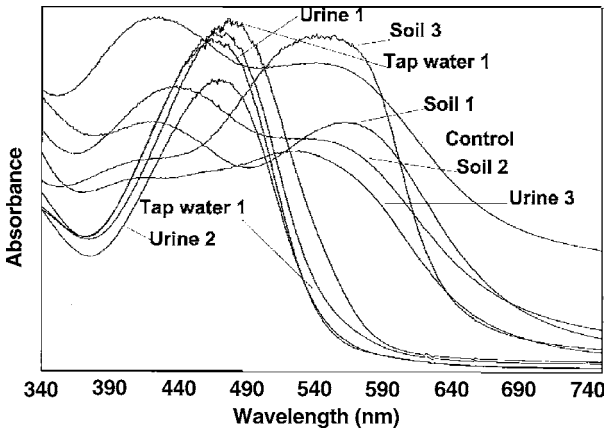

Fig. 3. Spectra obtained for the different samples analysed. Conventional manual extractions were used to obtain the dithizonates.

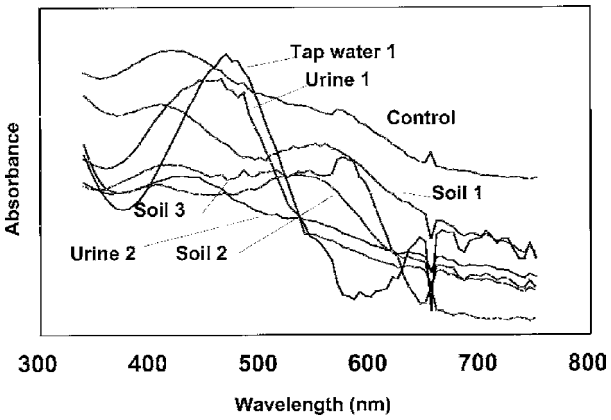

Fig. 4. Spectra obtained for the different samples analysed. The proposed injection extraction system was used to obtain the dithizonates.

manual extractions are given in Tables 5 and 6. The accuracy of the proposed sequential injection extraction system was also evaluated by comparing the results of the seven metal ions of the different spiked real samples (Table 3) with the results obtained by using standard AAS methods (Table 7) on conventional manual extraction samples. The results between the proposed sequential injection extraction system (Table 3), conventional manual extractions (Table 5) and standard AAS

Table 3
Results obtained for spiked real samples employing the proposed sequential injection extraction system

Sample	Analyte concentration found (mg l^{-1})						
	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	2.03	4.74	17.56	4.74	9.30	1.22
Tap water 1	8.49	7.67	1.10	23.62	12.93	46.82	0.29
Tap water 2	4.96	5.62	1.75	27.00	13.20	46.20	0.47
Urine 1	8.34	6.43	5.31	3.66	13.48	4.93	0.74
Urine 2	7.05	5.19	5.80	3.61	11.96	4.39	2.40
Urine 3	0	2.61	3.58	1.45	13.19	3.28	6.43
Control samples	4.91	5.01	4.89	5.42	5.04	5.11	4.79

Table 4
Relative standard deviations obtained for spiked real samples analysed with the proposed sequential injection extraction system

Sample	%R.S.D.						
	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 samples	0.64	0.75	0.67	0.47	0.28	0.58	0.84

Table 5
Results obtained for spiked real samples using conventional manual (hand) extractions

Sample	Analyte concentration found (mg l^{-1})						
	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	23.09	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 samples	4.95	5.09	4.99	4.87	5.01	5.18	5.08

Table 6
Relative standard deviations obtained for spiked real samples when using conventional manual (hand) extractions

Sample	%R.S.D.						
	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 samples	0.18	0.17	0.19	0.34	0.49	0.78	0.41

Table 7
Results obtained with standard AAS methods on conventional manual extraction samples

Sample	Analyte concentration found (mg l^{-1})						
	Pb^{2+}	Cu^{2+}	Zn^{2+}	Co^{2+}	Cd^{2+}	Fe^{3+}	Hg^{2+}
Soil 1	4.31	3.29	0.49	23.32	1.17	9.76	2.49
Soil 2	7.20	6.05	5.61	17.40	5.51	8.71	6.09
Soil 3	3.77	2.21	4.66	17.99	4.68	10.55	1.31
Tap water 1	8.40	7.63	1.21	23.86	13.32	46.99	0.59
Tap water 2	5.61	5.71	1.75	27.45	13.28	46.27	0.53
Urine 1	8.32	6.28	5.36	3.50	13.75	4.95	1.10
Urine 2	7.07	5.31	5.69	3.44	11.97	4.40	2.05
Urine 3	0	2.59	3.65	1.40	12.84	2.48	6.04
Control samples	4.91	5.01	5.06	5.12	5.06	5.15	4.95

methods (Table 7) compared favourably. The precision of the proposed SIE system (Table 4) and conventional manual extraction (Table 5) were evaluated and both gave a %R.S.D. < 2.0%.

4.3. Sample frequency

To complete the whole analytical cycle, including the extraction and detection, took 135 s. This resulted in a sample frequency of 27 samples per hour representing 189 determinations per hour. When considering that seven analytes were determined simultaneously, the sample frequency is quite remarkable for a sequential injection system.

4.4. 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^{-1}) 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 [12,15,17]. The percentage carry-over calculated was low enough to be ignored. The sample interaction was calculated to be about 0.05%.

4.5. Interferences

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

The mercury determination was more vulnerable to interferences. Chloride up to 14 g l^{-1} (0.4 mol l^{-1}) did not interfere in the mercury determination as long as the H_2SO_4 concentration did not exceed 2 mol l^{-1}. 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^{-1}. Thiocyanate and cyanate were used to mask interferences due to cobalt and interfered seriously in the determination of cobalt. The interfering anions can be exchange to less interfering ions 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.

5. Conclusions

A simplified, automated extraction system is described which employ ethanol as solvent. The technique can be applied without the use of phase separators or segmentors. This fact highlights the durability and robustness of the technique, since less maintenance will be needed. The sequential injection system is fully computerised and allows 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 (189 measurements 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.

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