Simultaneous determination of cadmium(II) and mercury(II) in aqueous, urine and soil samples using sequential injection extraction (SIE)

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This paper introduces a flow-based extraction system in which aqueous sample solutions and an organic extractant solution are injected sequentially into an extraction coil, and are mixed and separated due to the differential flow velocities of the aqueous and organic phases. An orange-yellow mercury dithizonate is formed during acidic extraction with dithizone (λ<sub>max</sub> = 485 nm) and a reddish-orange cadmium dithizonate is formed during alkaline extraction (λ<sub>max</sub> = 488 nm). In order to determine both analytes in the same sample without prior separation, a double extraction method was used. Two sample zones containing both mercury(II) and cadmium(II) ions were sandwiched around a CCl<sub>4</sub> zone in which the colour reagent dithizone was dissolved. The sequence of zones was as follows: an alkaline zone (2% NaOH solution), a 220 μl aqueous sample zone, an organic (dithizone dissolved in CCl<sub>4</sub>) zone, another aqueous sample zone (90 μl) followed by an acidic zone (1 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>). Acidic extraction occurred first during the reverse movement of the pump. During this step the aqueous sample was mixed with H<sub>2</sub>SO<sub>4</sub> to ensure that the correct pH was obtained and then extracted into the organic dithizonate zone where flow was impeded due to the hydrophobic interactions with the walls of the Teflon coil. Once formed [Hg(HDZ)<sub>2</sub>]<sup>2+</sup> 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 organic phase containing both analytes was then measured spectrophotometrically at 486 nm. Detection limits of 60 μg l<sup>-1</sup> Hg<sup>2+</sup> and 50 μg l<sup>-1</sup> Cd<sup>2+</sup> were calculated with a %RSD of 2.43 and 1.07, respectively.

The so-called Minamata and Itai-itai diseases are serious conditions apparently caused by heavy metals, such as mercury and cadmium, 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 disease was usually lumbago, followed by the development of pseudofractures and a waddling gait. The etiology of the itai-itai disease is related to dietary cadmium and malnutrition. Cadmium inhibits renal function, increases loss of calcium due to the depression of proximal reabsorption, and leads finally to osteomalacia. Cadmium is toxic to every system in the body, whether ingested, injected or inhaled. 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 lung is said to be as high as 10–40%. One third of the cadmium taken up by the body accumulates 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.
In nature, mercury occurs in several forms, for example, metallic mercury, inorganic mercury and organic mercury compounds. 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. Through a very effective biomagnification mechanism, methyl mercury is enriched in food chains, which results in high levels in top predators such as fish, e.g. northern pike and tuna. In Japan (Minamata), methyl mercury 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 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 to young children because the nervous system is still developing and is more sensitive to these compounds.

In normal persons, who have not been subjected to any particular exposure, the total mercury levels in the blood and urine are less than 5 μg l⁻¹. It is thought that there is a long-term risk of intoxication when levels above 30 μg l⁻¹ in blood and 50 μg l⁻¹ in urine are detected. Part of the mercury in the blood is in an organic form, primarily as methyl mercury. It is necessary to develop a method to determine mercury and cadmium ions in drinking water, sewage water, soil and urine samples. Soil samples are of particular interest since high concentrations of the heavy metals in soil could be absorbed by plants destined to be eaten. In such cases vegetables can be a hidden source of heavy metal intoxication.

Several analytical methods are available for the determination of mercury. These include cold vapour AAS, chromatography (GC and HPLC), potentiometric stripping analysis, enzymatic determination, ion selective electrodes, ICP-AES and UV/Vis spectrophotometry. All these methods, except ion selective electrodes and UV/Vis spectrophotometry, have the disadvantage that they are relatively expensive and cannot be used for on-site field analysis. Spectrophotometric determinations are therefore essential as inexpensive on-site measuring tools. These systems usually involve extraction procedures. The extraction method for methyl mercury, as developed by Gage, 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 methyl mercury halide into an organic solvent (benzene or toluene), purification by stripping with a thiol compound (cysteine or thiourea) and re-extraction into benzene. A problem with the extraction method is the regular formation of persistent emulsions. This can be avoided with the use of cysteine impregnated paper instead of cysteine solution. This option makes it impossible to adapt this method to any flow-based application.

Depending on the reaction conditions, 1,5-diphenylthiocarbazone (dithizone) and mercury(II) ions form an orange-yellow dithizonate [Hg(HDZ)]⁻ in the acidic range, or a violet secondary dithizonate (HgDZ-) in the neutral to alkaline range. Both complexes are insoluble in water, but readily soluble in carbon tetrachloride, chloroform and several other organic solvents. The primary dithizonate is of analytical importance, because it is not only stable in 5 mol l⁻¹ sulphuric 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.

Usually, existing methods for the determination of cadmium involve preconcentration which is time-consuming. The determination of cadmium at low concentrations, which may be of toxicological significance, requires a highly sensitive method, such as spectrophotometry using dithizone as reagent. A flow injection system designed to determine lead and cadmium using liquid–liquid extraction with dithizone in chloroform was used by Klinghoffer and was refined by Burguera et al. to increase the sensitivity of this procedure.

In this paper, a combination of the above-mentioned dithizonate extractions was used to determine cadmium and mercury in samples without prior separation. Two sample zones, containing both mercury(II) and cadmium(II), were sandwiched around a CCl₄ zone in which the colour reagent dithizone was dissolved. Extraction took place into the thin organic layer formed by the dithizone zone; the flow was impeded due to the hydrophobic interactions with the walls of the Teflon coil. While the faster moving aqueous phase was moving through the organic phase, extraction took place. This movement of the aqueous phase through the organic phase resulted in a reasonably good separation of the two phases. The organic phase was then collected by an air bubble introduced into the system for this purpose. This procedure followed the same experimental protocol as the sequential injection-wetting film extraction introduced by several authors, except that, no back extraction was needed, since the analytes formed CCl₄ soluble dithizones that are intensely coloured.

**Experimental**

**Reagents and solutions**

All solutions were prepared from analytical grade reagents unless specified otherwise. De-ionised water from a Modulab system (Continental Water Systems, San Antonio, Texas) was used to prepare all aqueous solutions and dilutions. The de-ionised water used as the carrier stream was degassed before use.
Extractant
0.1 g of dithizone (Hopkin & Williams Ltd) was dissolved in 250 ml CCl₄ to produce an emerald green stock solution. This solution was stored in a dark bottle and overlaid with 10 ml de-ionised water and 1 ml 0.5 mol l⁻¹ sulphuric acid. 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 CCl₄. These solutions were saturated with water before use.

Mercury stock solution
A 100-mg l⁻¹ Hg²⁺ stock solution was prepared by dissolving 0.171 g Hg(NO₃)₂·H₂O (Merck) in 1 l of de-ionised water. Working solutions are obtained by suitable dilution of the stock solution.

Cadmium stock solution
A 100-mg l⁻¹ Cd²⁺ stock solution was prepared by dissolving 0.2744 g Cd(NO₃)₂·4H₂O (Merck) in 1 l of de-ionised water. Working solutions are obtained by suitable dilution of the stock solution.

Alkaline buffer solution
A solution containing 5 g sodium potassium tartrate and 2 g NaOH in 100 ml de-ionised water was used as buffer solution. This solution has a pH of about 10.5.

H₂SO₄ solution
To ensure the correct pH for the Hg²⁺ extraction a 1-mol l⁻¹ H₂SO₄ solution was prepared by diluting 55.5 ml of concentrated acid (98%) to 1 l with de-ionised water. A 0.43 mol l⁻¹ acetic acid solution was used as eluent during the soil extractions.

Instrumentation
The sequential injection extraction (SIE) manifold is illustrated in Figure 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 (TFE) 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 (Dermo Tech). 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 CCl₄ carrier solution. A 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) and an assembled distribution board (MINTEK, Randburg). The FlowTEK™ software package (obtainable from MINTEK) was used throughout the procedure.

Procedure
A small air bubble was drawn up to separate the extraction zones from the aqueous carrier solution. Thereafter a zone containing alkaline buffer, a sample zone (containing both analytes), the extractant zone (dithizone in CCl₄), a second sample zone and a zone containing H₂SO₄ were drawn up into the extraction coil. Another air bubble was drawn up to separate these zones from the aqueous carrier in the holding coil. By reversing the flow, the H₂SO₄ and sample zones 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, which impeded the flow due to the hydrophobic interactions with the walls of the Teflon coil (Figure 2). During this process an [Hg(HDZ)₂] complex formed which 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

Figure 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, DB - debubbler and D - detector.

Figure 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 represents the organic phase.
in the CCl_4, collected by the first bubble, were pumped into the reaction coil until the organic zone was just inside the reaction coil. Pump 1 was then switched to reverse to rinse the extraction 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.

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 with minimum air spaces 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. All water samples were analysed directly. Representative soil samples of 20.00 ± 0.05 g were dried at 30°C for 8 hours. 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. The filtrate was analysed directly.

Results and Discussion
Optimisation
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.

Zone sequence. Different zone sequences were evaluated. Using a sequence of the organic zone followed by the acidic zone, sample zone, and basic solution resulted in a neutralisation reaction when the acidic and basic zones overlapped. This parameter to ensure that effective mixing with the buffer solutions was obtained, as well as to ensure maximal recovery of the acidic and basic zones only moved through the organic zone after the flow was reversed. The optimum zone sequence was found to be: basic solution, sample zone, organic zone, a second sample zone followed by the acidic zone.

Introduction of air bubbles. Although it was feared that the introduction of air bubbles into a flow system would lead to irreproducible results, this was to a major extent not the case. The first bubble separated the extraction zones from the surrounding carrier solution. In the first place it prevented excessive dilution of the sample zones and, secondly, it was used to 'collect' the thin layer of organic solvent again. These bubbles were also pumped into the reaction coil and were 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.

Flow rate. Because two pumps were used in these applications, two different flow rates had to be considered. The first pump controlled the volumes of the different zones as well as the extraction process, whereas the second pump was used to propel the formed product zone to the detector. Figure 3 illustrates the results obtained for the various flow rates. It shows clearly that lower flow rates were more successful during the extraction process and faster flow rates to propel the product zone. Initially, both pumps were set on the same speed. At flow rates higher than 2.64 ml min\(^{-1}\) the organic zone tended to break up and formed small organic bubbles in the aqueous phase. This led to irreproducible results. The optimum flow rate for pump 1 was therefore chosen to be 2.40 ml min\(^{-1}\).

Faster flow rates resulted in less dispersion and it was therefore necessary to optimise this parameter further. An optimum flow rate of 3.8 ml min\(^{-1}\) was obtained for pump 2. This 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. Faster rinsing time also favoured shorter analysis time and, therefore, a higher sample throughput.

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 maximal recovery of the sample zones, insufficient extraction occurred as the aqueous zones moved through the organic zone after the flow was reversed. Therefore, a higher sample throughput was necessary to optimise this parameter further. The optimum zone sequence was found to be: basic solution, sample zone, organic zone, a second sample zone followed by the acidic zone.

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Extractant volume and extraction time. Different extractant volumes were evaluated. Larger volumes not only produced an excess of reagent, when the greenish colour interfered with the determination, but also led to unnecessarily long extraction times. Taking the 50 μl used by Peterson et al.\(^6\) as a guideline, a volume of 45 μl was found to be the optimum. Smaller volumes gave irreproducible results because the extractant volume was not reproducibly drawn into the extraction coil. For volumes smaller than 45 μl, 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), these small volumes were not aspirated reproducibly.

It took 35 s for zone inversion to take place, whereas 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 air bubble. Shorter extraction times led to incomplete zone inversion and lower sensitivity as can be seen in Table 1.

### Volumes of acidic and alkaline buffer solutions

Due to the pH dependency 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 solutions were added until 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: 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 μl for both buffer solutions (Table 2), which corresponded excellently with the predicted ratios.

### Organic film thickness
The thickness of the organic film influences extraction by affecting the mass transfer of analytes into the film.\(^2\) Relative film thickness per unit length (\(d_f\)) can be predicted using equation\(^2\)

\[
d_f = k_d(\mu vr)^{1/3}
\]

where \(\mu\) represents flow rate (velocity) and \(d_j\) tubing diameter. The solvent characteristics also play an important role and are included in the equation. Viscos-

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### Table 1 The effect of various extraction times on the sensitivity and reproducibility of the SIE method

<table>
<thead>
<tr>
<th>Time needed for zone inversion(s)</th>
<th>Time needed to collect organic phase(s)</th>
<th>Total extraction time(s)</th>
<th>Relative peak height %RSD</th>
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<tbody>
<tr>
<td>23</td>
<td>29</td>
<td>52</td>
<td>1.80</td>
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<td>25</td>
<td>31</td>
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<tr>
<td>37</td>
<td>41</td>
<td>78</td>
<td>4.27</td>
</tr>
</tbody>
</table>
mainly to debubble the carrier prior to entering the flow path. The carrier (aqueous, 0.75 mm i.d.) was used as reaction coil. This was done to allow the flow of the aqueous carrier to propel the extraction zones inside the extraction coil. The 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil.

(ii) 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 coil. 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil. 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 extraction zones inside the extraction coil.

(iii) 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 prior to entering the flow path. Unfortunately, this did not have the desired result and a glass debubbler was installed. The spectrophotometer should be positioned as close to the debubbler as possible. Longer distances between the debubbler and detector led to higher dispersion and lower sample throughput.

### Chemical parameters

**pH.** Dithizone forms complexes with all the heavy metals as well as some other metals, such as Fe and Al. 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), while cadmium forms an orange-red dithizonate in the alkaline range (pH 8.5–10.5). 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's 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 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 of a sodium potassium tartrate solution to the NaOH solution solved the problem to a great extent. Although the sensitivity did not improve much, the relative standard deviation dropped to 1.66%.

### Choice of organic reagent

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#### Choice of organic reagent

The choice of solvent or the solvent composition is a critical parameter for the successful application of SIE, determining the difference in flow velocity between the organic and aqueous phases, the chemical selectivity and efficiency of the extraction. Solvents with low viscosities do not offer a sufficient difference in flow velocity when compared to water, making 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.

\[\text{CCL}_4, \text{an intermediate viscosity of 0.88 cP, was chosen as solvent not only because of its film forming ability, but also because of dithizone's high solubility and stability in the organic solvent. Dithizone, as well as its metal dithizonates are all highly soluble in chlorinated solvents.} \]

The visible absorption spectrum of dithizone is very sensitive to the organic solvent in which it is dissolved. Dissolved in \text{CCL}_4, [Hg(HDZ)₂]⁻ and [Cd(HDZ)₂]⁻ absorbed at almost the same wavelength.
Experimental values for \( \lambda_{	ext{abs}} \) are 485 nm and 488 nm for \([\text{Hg(HDZ)}]\) and \([\text{Cd(HDZ)}]\) respectively. Detection was done at 486 nm.

One disadvantage of CCl\(_4\) is that it is highly carcinogenic and has ozone depleting properties.\(^{15}\) 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.

**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, \( e.\)\(^{21}\) 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 be used due to the deep green colour of the unreacted dithizone. Solutions containing these solutions could not be used due to the deep green colour.

**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.

**Linearity.** Analytical curves for the extraction of Cd(II) and Hg(II) with dithizone are shown in Figure 5. The curves are linear for both metals between 50 \( \mu \)g/l and 3 mg/l. For cadmium \( r^2 = 0.9914 \) and for mercury \( r^2 = 0.9875. \) Peak height was used to evaluate the analytical signal. A good reproducibility was obtained. 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 at 1 and 2 mg/l show relative standard deviations lower than 2.5% for ten measurements at each concentration.

It took 186 s to complete one whole extraction cycle, including the time needed for detection. This resulted in a sample frequency of 19 samples per hour. Some carry-over between more concentrated samples was experienced. The sample interaction was calculated to be about 2%. Rinsing of the extraction coil with a small amount of CCl\(_4\) eliminates this problem. The detection limit, estimated as three times the signal-to-noise ratio,\(^{20}\) was equal to 50 and 60 \( \mu \)g/l for Cd(II) and Hg(II), respectively.

**Accuracy.** To investigate the accuracy of the SIE system a calibration curve for each individual metal was constructed. Because absorbance is additive two equations are obtained which should yield the required unknown concentrations when solved simultaneously. Three different aqueous mixtures, three urine mixtures and two soil extracts were analysed. The results are listed in Table 3. 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.

**Interferences.** Possible interferences 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 \( \text{SO}_4^{2-} \), 10 mg/l \( \text{PO}_4^{3-} \), 0.5 mg/l \( \text{Al}^{3+} \), 50 mg/l \( \text{Mg}^{2+} \) and 400 mg/l \( \text{Ca}^{2+} \).

Chloride up to 14 g/l (0.4 mol/l) did not interfere in the mercury determination as long as the \( \text{H}_2\text{SO}_4 \) concentration did not exceed 2 mol/l, while chloride concentrations of up to 20 g/l could be tolerated in the cadmium

**Table 3 Evaluation of the accuracy of the proposed SIE method**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration in mg/l</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Cd(^{2+})]</td>
<td>[Hg(^{2+})]</td>
</tr>
<tr>
<td>Aqueous 1</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Aqueous 2</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Aqueous 3</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Urine 1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Urine 2</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Urine 3</td>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Soil 1</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Soil 2</td>
<td>2.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
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.

Conclusions

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 volume. It operates without phase separators or segmentors by stacking the reagents in such a way that the faster moving phase overcomes 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 therefore applicable to many fields.

References