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DETERMINATION OF CADMIUM IN BIOLOGICAL MATERIALS BY FLAME ATOMIC ABSORPTION SPECTROMETRY WITH FLOW-INJECTION ON-LINE SORPTION PRECONCENTRATION

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Summary—A new on-line preconcentration flame atomic absorption spectrometry (FAAS) system for trace element determination was developed based on sorption of soluble metal complexes on the walls of a PTFE knotted reactor using flow injection techniques. The system was applied to the determination of cadmium in biological materials. Cadmium complexed with sodium diethyldithiocarbamate was sorbed on the inner walls of the reactor and eluted on-line by isobutyl methyl ketone. The retention efficiency was 81% at a sampling loading rate of 5.2 ml/min. The enhancement factor was 66 and the concentration efficiency was 61/min with a 50 sec preconcentration period, consuming 4.2 ml sample. A detection limit of 0.1 μg/l. Cd (3σ) was obtained with a sampling frequency of 55/hr. The precisions were 1.2% RSD for 20 μg/l. Cd ($N = 11$). Thiourea and ascorbic acid/phenanthroline were used to overcome interferences from copper and iron, respectively. The analytical results obtained for powdered rice and human hair standard reference materials were in good agreement with the certified values.

Flow injection (FI) techniques for the on-line preconcentration of heavy metals and subsequent determination with flame atomic absorption spectrometry (FAAS) have made great progress in recent years,[1] including preconcentration with sorption columns,[2] liquid–liquid extraction[3] and co-precipitation preconcentration.[4] On-line column preconcentration techniques, based on sorbent extraction using C_{18} columns were shown to provide better selectivity for the determination of cadmium in seawater[5] compared to those based on ion-exchange and was later extended to determine cadmium in biological materials.[6] The diethylammonium-*N*,*N*-diethyldithiocarbamate cadmium complex was sorbed on a 100 μl micro column and eluted into the AA spectrometer by ethanol or methanol. In spite of these successful applications, the technique was limited by not being able to use higher sample loading rates for achieving better enrichment factors owing to high hydrodynamic impedance in the columns. The technique also suffers interferences from large concentrations of transition metals in the sample matrix making it impossible to apply it to biological and environmental samples with relatively complex matrices. In this work, attempts were made to overcome these drawbacks by using a knotted reactor made from open-ended tubing to sorb the DDC-complex and by using thiourea and ascorbic acid/phenanthroline as masking agents. A more efficient system was developed, capable of application to cadmium in biological samples such as hair and rice.

EXPERIMENTAL

Apparatus and reagents

Perkin–Elmer Model 2100 and 4100 atomic absorption spectrometers with deuterium lamp background correctors, equipped with a cadmium hollow cathode lamp were used at 228.8 nm with 5 mA current. Flame conditions were set at 1.8 l./min acetylene and 8.0 l./min air, which were slightly leaner than recommended. This measure was taken to compensate for the effect of the organic solvent introduced during elution, which served as additional fuel. The spectrometer was set to work in the FI mode

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with a time constant of 0.5 sec for signal evaluation. Peak profiles, peak height absorbances and statistical data were printed with an Epson EX-800 printer.

A Perkin–Elmer model FIAS-200 FI system was connected to the flame atomic absorption spectrometer for automated processing of the preconcentration. The injector of the instrument was modified as described in Ref. 4. The actuation of the valve and pumps were programmed on the computer of the spectrometer.

A displacement bottle as described in Ref. 1 was used for delivery of the organic solvent isobutyl methyl ketone (IBMK).

Knotted reactors (KR) were made as described in Ref. 4 and used for collection of the Cd–sodium diethyldithiocarbamate (DCC) complexes.

All conduits used for connection of the various components were of 0.5 mm i.d. PTFE tubing.

All reagents were of analytical grade except those indicated otherwise and deionized water was used throughout.

DCC (0.5%) was prepared by dissolving 0.5 g sodium diethyldithiocarbamate (Xinzhong Chemicals Co., Shanghai, China) in a pH 9.2 buffer solution (0.01*M* acetic acid and 0.02*M* ammonia in water). IBMK (Beijing Chemicals Co., Beijing, China). Nitric, hydrochloric and perchloric acids (Beijing Chemicals Co., Beijing, China) were of ultrapure grade.

Standard solutions of 0, 4, 8, 12, 16 and 20 μg/l. Cd were prepared by appropriate dilution of 100 mg/l. cadmium stock solution [GBW (E) 08005] (Institute of Chemical Metallurgy, Beijing, China) with 0.1*M* HCl, all standard solutions contained 2% thiourea, 2% ascorbic acid and 0.2% phenanthroline.

The following standard reference materials were used: GBW 09101, human hair (Institute of Atomic Energy, Academia Sinica, Shanghai, China). GBW 08502, rice (Environmental Monitoring Center, Beijing, China).

Procedures

Samples were digested mainly following procedures described in Ref. 4. For rice samples, 2.0 g sample was weighed into a 50 ml glass beaker and 10 ml nitric acid was added. For human hair, 0.5 g sample was weighed into the beaker and 5 ml mixed acid (HNO_3/HCl, 1:3) was added. The samples were digested on a heating plate at 130–150°C until brown fumes disappeared. After cooling to about 50°, 100 μl perchloric acid was added to promote digestion. The digests were then heated nearly to dryness on the plate. The residue was washed into a 10 ml volumetric flask containing 2 ml 10% thiourea, 2 ml 10% ascorbic acid and 2 ml 1% phenanthroline, and diluted to volume with 0.1*M* HCl.

The FI manifold is shown in Fig. 1 with the main operation parameters. Details of the FIAS-200 program and the operation sequence are given in Table 1. A sample loading time of 50 sec and an elution time of 10 sec were used, unless stated otherwise. IBMK was used to elute the analyte into the flame.

In step 1 with valve position as shown in Fig. 1a and pump P1 stopped, the sample or standard solution was pumped by P2 and merged with the DDC reagent before passing through the KR. The DDC–Cd complexes formed were collected on the walls of the reactor. In the elution step shown in Fig. 1b, pump P2 was stopped and P1 was activated to deliver

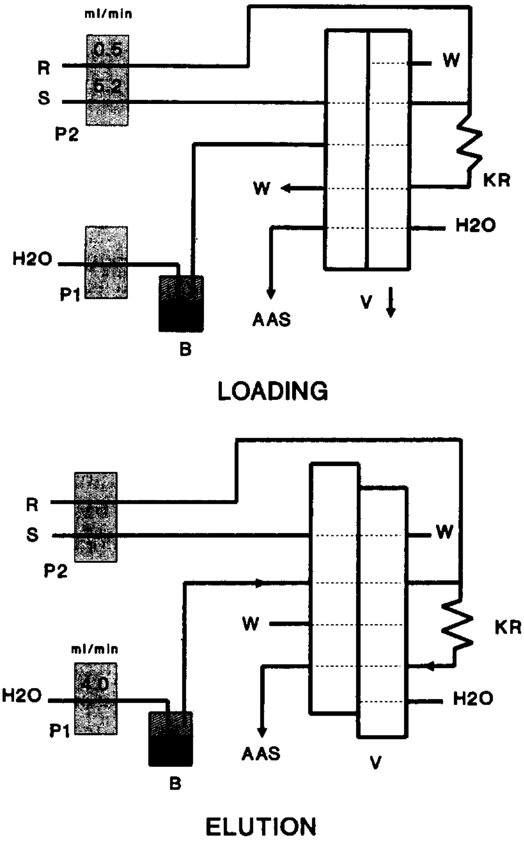

Fig. 1. FI manifold for on-line sorption preconcentration FAAS system. P1, P2, Peristaltic pumps; S, sample solution; R, 0.5% DDC solution; KR, knotted reactor; W, waste; B, displacement bottle; V, valve; AAS, atomic absorption spectrometer.

Table 1. FIAS program and sequence of operation for on-line sorption preconcentration and elution

Step	Function	Time (*sec*)	Medium delivered	Flow rate (*ml/min*) pump 1	pump 2	Valve position
1	Loading	50	Sample	0	5.2	Fill
			DDC	0	0.5	
2	Elution	10	IBMK	4.0	0	Inject

IBMK and elute the collected DDC–Cd complexes into the spectrometer.

Method development

The FI manifold used for method development was identical to that used for on-line co-precipitation with HMA–HMDTC[4] which also used a KR for analyte collection, and is shown in Fig. 1.

A univariate approach was used for optimization starting with conditions based on those used previously for on-line co-precipitation systems.[4] The main figure of merit used for optimizing the chemical parameters was the peak absorbance, but with simultaneous considerations on selectivity. For optimization of FI variables (sample loading time and loading rate), concentration efficiency (CE), *i.e.* enhancement factor achieved per min was used as the main figure of merit.

Three to four cycles of univariate studies were made to adjust the fixed parameters in each study to optimized values.

RESULTS AND DISCUSSION

Mechanism of DDC complex retention in the knotted reactor tube walls

In the sorbent extraction on-line preconcentration system[5] employing DDC as complexing agent, the metallic complexes were retained on a column packed with C_{18}. An observation was made in that system that the reaction coil length preceding the column significantly influenced the enrichment factor (EF) of the preconcentration, EF decreasing with an increase in coil length. This was rather unexpected, since the mixing of reagent and sample as well as the complexing reaction should have been more complete in longer reaction coils. This phenomenon was ascribed to a fast decomposition of the complex for lack of a better explanation, but remained mostly unexplained. In a recent unpublished study on preconcentration systems involving on-line co-precipitation using KRs we observed that analyte retention occurred in the absence of a co-precipitation carrier, in fact without visible evidence of precipitate formation. That a retention had actually occurred was proven by eluting KR with a suitable eluent after sample loading (merged with DDC) and rinsing. Excellent elution peaks were obtained with retention efficiencies of over 90% when KR lengths were sufficiently long. This observation is clear evidence of the capability of the KR, at least those constructed from PTFE, to sorb DCC metal complexes, and possibly other organic complex species. The finding may be readily used to explain the phenomenon observed in the column sorbent extraction system. Obviously a fraction of the DDC metal complexes has been retained on the walls of the PTFE mixing coil preceding the valve on which the sorption column was connected as a special form of sample loop. Since the mixing coil was not washed by the eluent, this fraction was lost during the elution. This loss would continue to occur until the walls of the mixing coil were saturated by the sorbed species. However, owing to the low concentration and small sample volume this would happen only after hundreds of injections.

Scanning electron microscopic observation of the KR wall surface were made to verify that the retention mechanism of the present system was different from that of a co-precipitation system by comparing the DDC system to a HMDTC–Fe(II) co-precipitation system using identical KR collection (Fig. 2). No observable particles were found on the KR walls for the DDC system under a 200-fold magnification, while precipitate particles were obvious for the HMDTC–Fe(II) system. It is assumed, therefore, that the retention of DDC–Cd complex on KR walls is achieved through molecular sorption.

Optimization of chemical and FI variables

Although ethanol and methanol have been used successfully as eluents in an on-line sorbent

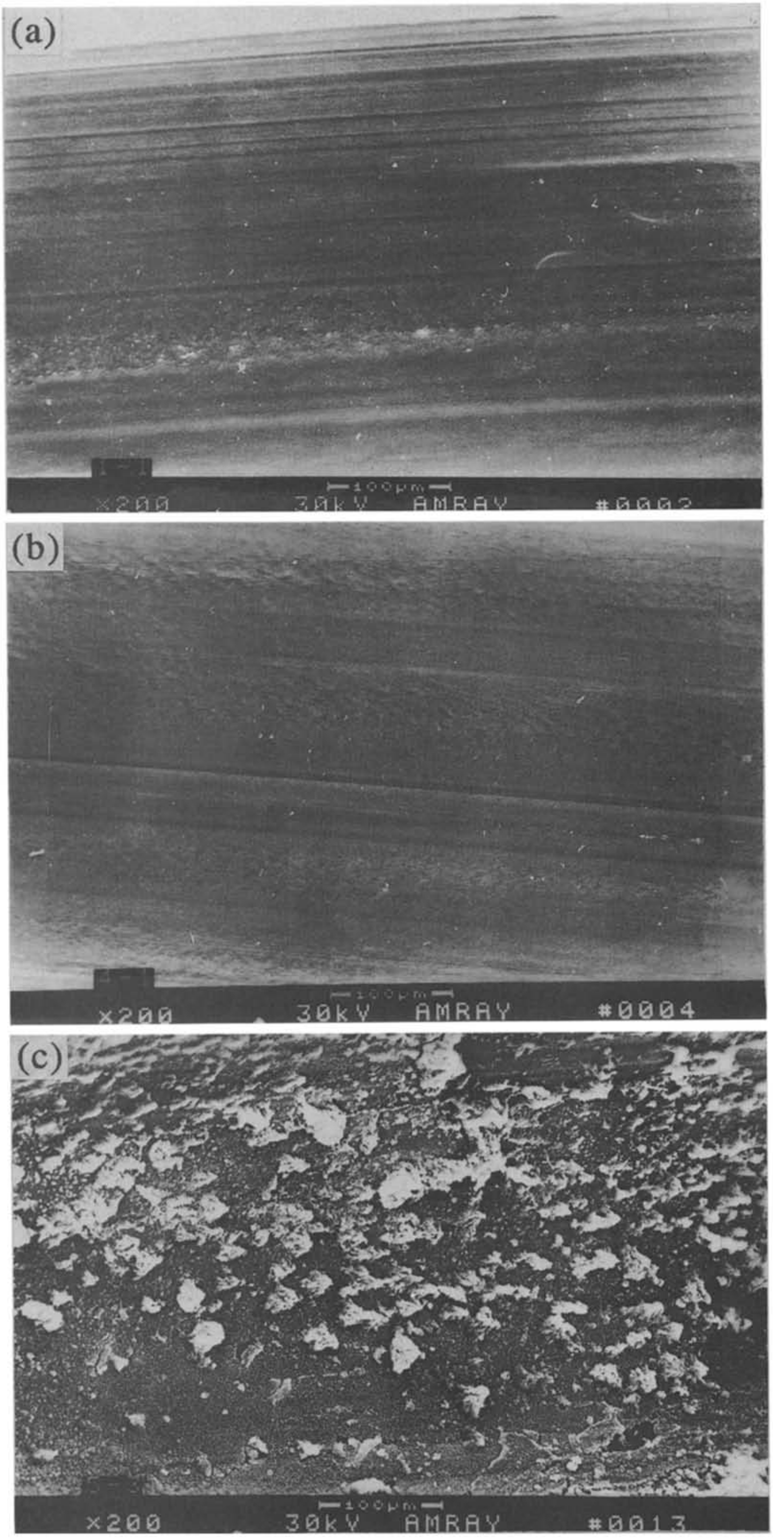

Fig. 2. Scanning electron micrograph of KR PTFE tubing inner walls. (a), Untreated walls; (b), DDC–Cd sorbed on walls after 50 sec collection; (c), Cd–HMDTC–Fe(II) precipitates collected on walls (200-fold magnification) using procedure in Ref. 8.

extraction preconcentration AAS system,[5] IBMK, when used as dissolution agent was shown to produce higher enhancement effects in on-line co-precipitation with AA detection.[4] Since IBMK could potentially also act as an eluent for the sorption system, the effects of ethanol and IBMK were compared in preliminary studies. The superior performance of IBMK is shown in Fig. 3. IBMK produced 3–4-fold higher signals when compared to those produced by ethanol under identical collection conditions (Fig. 3a, c). The gain is partially due to a more intensive organic solvent effect and partially to a decrease in dispersion owing to the use of a water immiscible solvent. The dispersion of eluted analyte into the preceding sample waste during transport to the detector was impeded at the organic/aqueous phase boundary producing narrower and taller peaks, analogous to the effect of introducing a small air segment between two aqueous phases used in some systems.[6] This was verified by introducing a small air segment with ethanol elution and the segment preceding the eluent. A narrower peak was obtained and the sensitivity was enhanced by a factor of two (compare Fig. 3a, b). The advantages of using IBMK as eluent by far outweighed the disadvantages of using an organic solvent unsuitable to be directly delivered through peristaltic pump tubings (*i.e.* a displacement bottle had to be implemented). Therefore, IBMK was used in all further studies.

The optimum pH range for chemical reactions were occasionally found to be narrower for FI systems when compared to the original batch procedures. In the present system additional effects might appear owing to the sorption process which might also be pH dependent. A detailed study on pH effects (acid strength) was undertaken in order to optimize the system. The results shown in Fig. 4 indicate a rather narrow pH range for obtaining optimum sensitivity or collection efficiency. It is particularly interesting to see the decrease in collection efficiency at pH higher than 2, which is in contrast to the high collection efficiency obtained within the range pH 2–6 in on-line sorbent extraction systems employing a C_{18} sorbent column.[5] Presumably, this is the effect of a difference in surface properties and retention

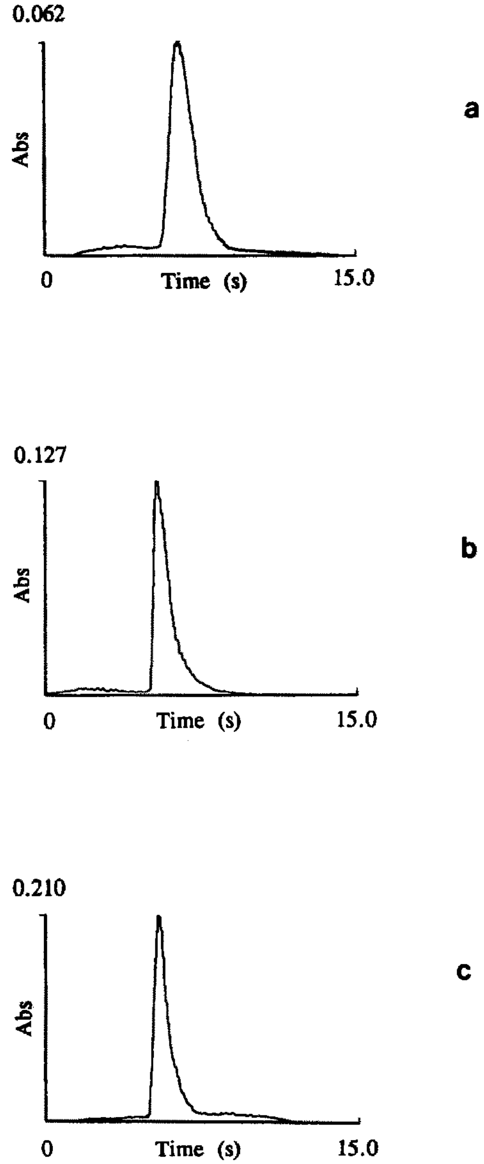

Fig. 3. Effects of eluents on the peak forms of 20 μg/l. Cd. Recordings obtained with the system shown in Fig. 1. 200 cm, 0.5 mm i.d. KR. (a), Ethanol, normal elution (without displacement bottle); (b), ethanol, elution with air segment introduced between sample and eluent and (c), IBMK, normal elution.

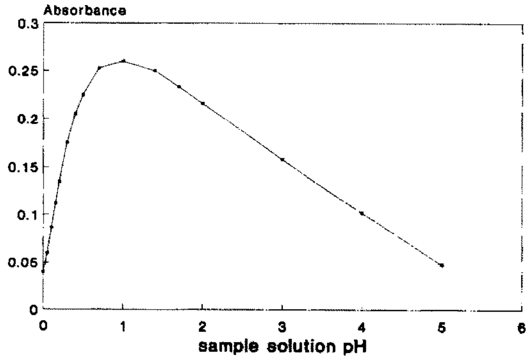

Fig. 4. Effect of HCl concentration on peak height of 20 μg/l. cadmium for 50 sec preconcentration, 270 cm 0.5 mm i.d. KR. Other parameters as in Fig. 1.

mechanism of the sorption media. While further studies are required to fully explain the phenomenon, the pH range was wide enough to be readily controlled in routine analysis and 0.1M HCl was used as sample acidity and reaction medium in further studies.

Since the collection mechanism in this preconcentration system was somewhat different from the co-precipitation system, it was considered necessary to re-optimize the geometrical dimensions of the collector. Among the three configurations tested (all made from 100 cm long 0.5 mm i.d. PTFE tubing) the KR still produced the higher sensitivity. With 20 μg/l. Cd and a 50 sec loading period the peak absorbances produced by KR, a coiled reactor with 7 cm coil diameter and a straight conduit using the same piece of PTFE tubing were 0.200, 0.107 and 0.089, respectively.

The effects of the KR length on the peak response are shown in Fig. 5. The peak absorbance increased almost linearly with an increase of shorter KR lengths, implying breakthroughs probably owing to insufficient contact of the complex with the reactor, but leveled off at about 250 cm. A KR length of 300 cm which was about a factor of two longer than that used for co-precipitation[4] was used in further studies. The difference in KR length for the two systems may imply that precipitates are more readily collected than complexes in molecular form.

The effects of sample loading flow rate and loading time on the peak absorbance are shown in Figs 6 and 7, respectively. Substantially higher loading flow rates are feasible compared to those used in sorbent extraction systems employing on-line columns packed with C_{18} owing to the lower hydrodynamic impedance in the KR. This allowed more sample to be loaded within a defined time period, resulting in higher concentration efficiencies. The peak absorbance increased linearly with increase in loading rates below 3 ml/min but gradually leveled off with further increase, implying insufficient contact time for achieving complete sorption. However, a flow rate of 5.2 ml/min was chosen for better sensitivity which could at the same time maintain a reasonable degree of retention efficiency to ensure good reproducibility. For the effects of loading time, the peak height increased almost linearly up to 20 sec using a 300 cm, 0.5 mm i.d. KR (Fig. 7) after which the slope decreased gradually, presumably as the result of a chromatographic effect with the sorbed analyte being partially leached by further sample.[1] However, about 80% of the analyte was retained with 50 sec loading at 5.2 ml/min.

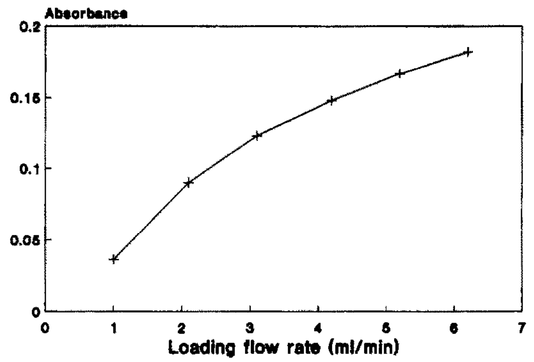

Fig. 6. Effect of loading flow rate on peak height of 20 μg/l. cadmium for 30 sec preconcentration, 0.1M HCl medium, 0.5 mm i.d. 170 mm KR. Other conditions as in Fig. 4.

Interference studies

DDC being a strong complexing agent for a large number of metals, interferences from

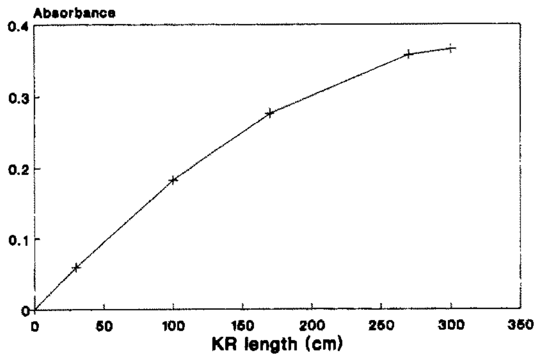

Fig. 5. Effects of KR length (i.d. 0.5 mm) on the peak height of 20 μg/l. Cd with 50 sec preconcentration. FI parameters as in Fig. 1.

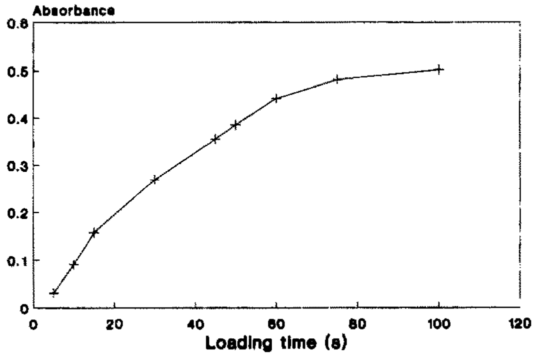

Fig. 7. Effects of loading time on peak height in 0.1M HCl medium. Other parameters as in Fig. 4.

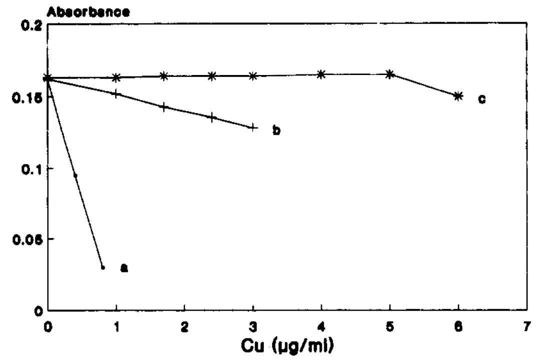

Fig. 8. Effects of copper concentration on the peak height of 20 μg/l. Cd for 50 sec preconcentration. Other parameters as in Fig. 6. a, Without masking agent; b, with 1% thiourea; c, with 2% thiourea.

coexisting metallic ions always have to be considered seriously in order to achieve a desired degree of selectivity. This reagent has been used successfully for preconcentration of cadmium in seawater matrix which does not contain large amounts of transition metals,[5] but serious interference problems may arise with biological and geological samples containing appreciable amounts of copper and iron, which compete with cadmium in the formation of DDC complexes. In this work attempts were made to mask these ions in order to allow the preconcentration of cadmium in such samples.

Thiourea was found to be quite effective for reducing the interference from copper. The results in Fig. 8 show strong interference from copper without masking even at the 10^{-1} mg/l. Cu level, but as much as 5 mg/l. Cu could be tolerated in the presence of 2% thiourea. However, thiourea was not sufficiently effective for the masking of iron(II) or iron(III), which also had strong interferences on cadmium (Fig. 9). Several other masking agents were tested, with a combination of ascorbic acid and phenanthroline being the most effective. In the presence of 2% ascorbic acid and 0.5% phenanthroline, 300 mg/l. iron could be tolerated.

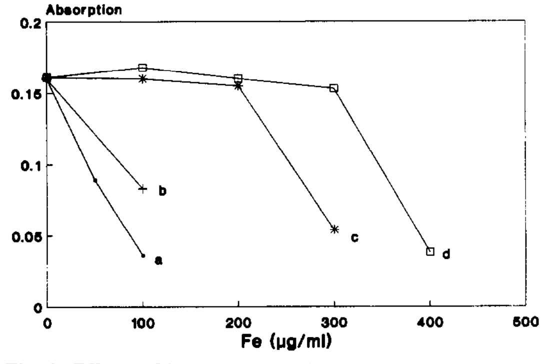

Fig. 9. Effects of iron concentration on the peak height of 20 μg/l. Cd. FI parameters as in Fig. 6. a, Fe(III); b, Fe(II) without phenanthroline; c, Fe(II) with 0.25% phenanthroline and d, Fe(II) with 0.5% phenanthroline.

Table 2. Performance of the on-line sorption preconcentration system (50 sec collection with 0.5 i.d., 167 cm KR)

Loading time (*sec*)	50
Concentration range (*µg/l.*)	4–20
Enhancement factor	66
Enrichment factor	30
Sampling frequency (*hr*$^{-1}$)	55
Sampling volume (*ml*)	4.2
Concentration efficiency (CE) (*min*$^{-1}$)	61
RSD (%, $N = 11$, 20 *µg/l*)	1.2
Detection limit (*µg/l.*, 3σ)	0.1
Regression equation* ($N = 5$)	$A = 0.012C - 0.008$
Correlation coefficient	0.9992

*A, peak height absorbance; C, *µg/l.* Cd.

Performance of the on-line sorption preconcentration system

The performance of the on-line sorption preconcentration system with 50 sec sample loading is shown in Table 2. The enhancement and enrichment factors and the concentration efficiency were evaluated as recommended in Ref. 1. The retention efficiencies for 20 μg/l. cadmium at loading flow rates of 5.2 and 3.2 ml/min were 81 and 91% respectively. The results for the determination of cadmium in powdered human hair and rice powder standard reference materials shown in Table 3 agreed well with the certified values.

CONCLUSIONS

Knotted reactors produced from PTFE are shown to be capable of retaining metal complexes of DDC under appropriate experimental parameters through sorption. This phenomenon was exploited to develop a novel on-line preconcentration system which permitted higher sample loading rates to achieve higher concentration efficiencies. Good results were also obtained for the determination of copper in water samples.[7] Although only cadmium was tested in this work, preliminary studies have already

Table 3. Results for the determination of cadmium in standard reference materials

Sample	Certified (*µg/g*)	Found (*µg/g*)	*N*
GBW 09101 powdered hair	0.095 ± 0.012	0.093 ± 0.012*	3
GBW 08502 powdered rice	0.020 ± 0.003	0.022 ± 0.004*	3

*Uncertainty at the 95% confidence level.

shown that other DDC complexes behave similarly. This advantage plus that of using a better eluent (IBMK) producing higher enhancement effects resulted in a preconcentration system which yielded the highest concentration efficiency[4] achieved thus far for cadmium. The addition of thiourea and ascorbic acid/phenanthroline was shown to be effective in improving the sensitivity of the preconcentration system using DDC as complexing agent, making it possible to determine cadmium in biological and environmental samples with high copper and iron contents. This measure might also be effective for other sorbent extraction preconcentration systems employing DDC and its derivatives for complexation.

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