

On-line Pre-concentration for the Measurement of Cadmium in Urine by Flame Atomic Absorption Spectrometry

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The sensitivity for the measurement of cadmium in urine by flame atomic absorption spectrometry was increased 27.7-fold by the use of an on-line alumina microcolumn and a slotted quartz tube. Cadmium in 2.5 ml of sample was trapped on to basic alumina and rapidly eluted with 8% V/V HNO₃. The effects of sample pH and column size on performance were investigated. Results obtained with a series of urine specimens compared well with those obtained by electrothermal atomisation atomic absorption spectrometry.

Keywords: *On-line pre-concentration; slotted quartz tube; cadmium in urine; flame atomic absorption spectrometry*

Increased exposure to cadmium from occupational or other sources can be difficult to detect. Concentrations of cadmium in blood or urine are grossly increased only after recent exposure or if there is renal failure.¹ In other situations the cadmium is maintained within tissues (kidney and liver) and can be detected only with sophisticated procedures (*e.g.*, *in vivo* neutron activation analysis) which are not widely available.^{2,3} Other possible procedures to monitor exposure include the measurement of urinary proteins and cadmium. Low molecular mass proteins (*e.g.*, β_2 microglobulin, retinol binding protein and α_1 microglobulin) appear in urine in increased concentrations if renal tubular re-absorption is impaired.⁴ Small increases in the concentration of cadmium in urine can indicate increased exposure or be an early sign of tubular damage.

Measurement of cadmium in urine at concentrations of about 1 $\mu\text{g l}^{-1}$ is not easy. Atomic absorption using electrothermal atomisation (ETAAS) affords the necessary sensitivity but can be subject to interferences and if not set up properly can give erroneous results.⁵ Flame atomic absorption spectrometry (FAAS) is generally simpler to carry out and is less susceptible to the effects of urine composition. However, sensitivity is not as good as can be achieved by ETAAS.

Two recent developments have provided for increased sensitivity and suggest that measurement of cadmium in urine at concentrations below 1 $\mu\text{g l}^{-1}$ may be possible by FAAS. These developments are the flame atom trapping devices which prolong the residence time of the atomic vapour in the light path⁶ and column pre-concentration.⁷

The pre-concentration techniques have generally been applied to the analysis of simple standard solutions and water samples by inductively coupled plasma atomic emission spectrometry (ICP-AES).⁷ In this study we have applied these developments to measurements in urine by FAAS.

Experimental

A Pye Unicam SP9 atomic absorption spectrometer was used with a slotted quartz tube mounted on a 5-cm burner.⁶ The operating conditions used were: wavelength 228.8 nm, band width 0.5 nm, lamp current 4 mA, air - acetylene flame and sample uptake rate 7.5 ml min⁻¹. The quartz tube was coated with lanthanum to prevent devitrification⁸ by continuous aspiration of a 1% *m/V* lanthanum solution for 15 min.

The sampling manifold is illustrated in Fig. 1. The proportioning pump, Carlo Erba Model 1512/20, was fitted with a pump tube having a nominal delivery rate of 2.5 ml min⁻¹. Although this rate of delivery is less than that which would normally occur by aspiration, there was no marked effect on flame appearance or stability. Columns, 2.5, 5.0 and 7.5 cm long \times 1.5 or 2.0 mm i.d., were obtained from Omnifit, Cambridge, UK and were filled with alumina.

Reagents

The cadmium nitrate standard solution for atomic absorption spectrometry (1 mg ml⁻¹ of cadmium), nitric acid, ammonia solution, 10% *m/V* lanthanum solution and basic aluminium oxide (alumina) were of the highest available purity and were obtained from BDH Chemicals, Poole, Dorset, UK. Alumina was prepared for use as follows: 5 g were soaked in de-ionised water for 1 h, the fine particles were decanted off and the remaining mixture was filtered. The alumina was washed with water until the filtrate was clear after standing for 1 min, and dried at 105 °C.

Methods

The experiments to investigate on-line pre-concentration are described in the next section but the general procedure was to place the capillary in the test specimen and allow the sample to be pumped for a pre-determined time. The capillary was then placed in the eluent solution for a specific period of time and then in the wash solution to restore the basal condition of the column. These transfers were carried out manually but could be performed with an automatic sampler which would also provide reproducible control over the timed periods. The parameters investigated were: column size, eluent solution, sampling times and sample pH. All measurements were carried out in duplicate unless stated otherwise.

Cadmium was measured by ETAAS in urine specimens. Nitric acid was added to the samples, to give a final

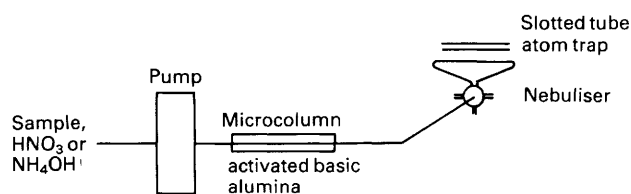


Fig. 1. Sampling manifold for use of on-line microcolumn

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concentration of 2% V/V and calibration was achieved by standard additions.

Results

Eluent and Wash Reagents

Preliminary experiments with larger off-line columns (3.0 cm \times 8 mm i.d.) indicated that basic alumina had better retention - elution responses than either Chelex 100 (100–200 mesh) ion-exchange resin or neutral alumina. Recovery of 100% was obtained with 8% V/V nitric acid eluent. The conditions were re-assessed with the on-line columns and the 8% V/V nitric acid was again found to give the greatest elution of cadmium (Table 1). With 8% V/V nitric acid, restoration of basal conditions was efficiently achieved when 0.03 M ammonia solution was pumped as the wash reagent. A wash period of 1.5 min was necessary in order to obtain reproducible results.

Sample pH

Deposition of cadmium on to the basic alumina columns at pH values of 4.0–8.0 was measured with aqueous standards and with specimens of urine. Very little cadmium deposited from acid standards but at pH 6 and higher there was good retention with little effect of changed H^+ concentrations (Table 2). A similar response was obtained with the urine samples, and specimens with pH at, or adjusted to, 5.8–7.0 could be used.

Column Sizes

Deposition of cadmium was less efficient with the shorter and narrower columns. Signals obtained with the 1.5 mm i.d. columns were reduced by 83–92% compared with their 2.0 mm i.d. counterparts. With the larger (7.5 cm) columns the metal did not elute cleanly and there was some initial retention which caused the appearance of double peaks. The best results were obtained with a column of dimensions 5.0 cm \times 2 mm i.d. and this was used for subsequent work.

Sampling Time

Cadmium was collected for periods of 10–120 s and released by pumping for 5 or 10 s. Peak heights increased with longer sampling times but with volumes greater than 2.5 ml (60-s sampling) the efficiency started to reduce (Fig. 2). Sample peaks were of similar size with both 5- and 10-s elution times. A combination of long sampling times and 10-s elution tended

to produce double peaks. A working procedure of 60 s for analyte collection with 5-s elution was adopted.

Column Performance

The reproducibility of the procedure was determined from five replicate measurements at a concentration of $20 \mu\text{g l}^{-1}$ and the relative standard deviation was 2.2%.

Recovery of cadmium from the column was assessed by measurement of the peak-absorbance values from urine

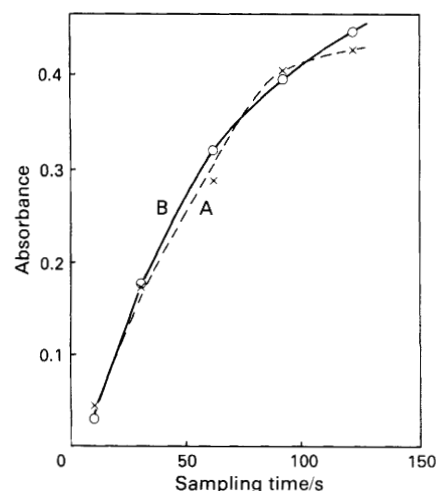


Fig. 2. Effect of sample collection times on peak height with: A, elution for 5 s and B, for 10 s. Test solution, $100 \mu\text{g l}^{-1}$ of cadmium

Table 3. Absorbance values obtained for $50 \mu\text{g l}^{-1}$ of cadmium, measured with and without slotted quartz tube, with and without alumina microcolumn

Sampling conditions	Absorbance
Conventional aspiration*	0.016
Via pump,† for 5 s	0.008
Conventional aspiration + slotted quartz tube	0.043
Via pump, for 5 s + slotted quartz tube	0.015
Via pump, with 60 s concentration on to column	0.092
Via pump, with 60 s concentration on to column + slotted quartz tube	0.225

*rate = 7.5 ml min^{-1} .
†rate = 2.5 ml min^{-1} .

Table 1. Influence of nitric acid concentration on release of cadmium from alumina on-line columns. Test solutions, $20 \mu\text{g l}^{-1}$ of cadmium

Nitric acid concentration, % V/V	Absorbance
6	0.066
8	0.090
10	0.088
12	0.068

Table 2. Influence of sample pH on trapping and release of cadmium from alumina on-line columns. Test solutions, $20 \mu\text{g l}^{-1}$ of cadmium and urine with $10 \mu\text{g l}^{-1}$ of cadmium added

pH	Absorbance	
	Water	Urine
4.0	0.010	0.019
5.0	0.099	0.050
6.0	0.107	0.055
7.0	0.104	0.050
8.0	0.100	0.028

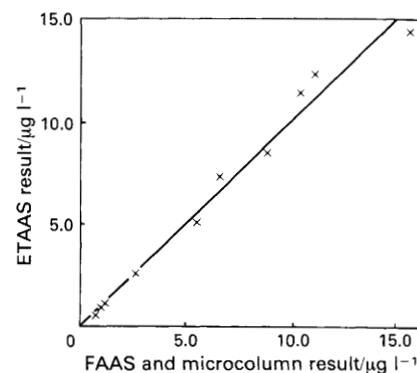


Fig. 3. Comparison of results obtained with ten specimens of urine analysed by ETAAS and by FAAS using the microcolumn and slotted quartz tube

samples to which cadmium had been added to increase the concentration by 0, 2 and 5 $\mu\text{g l}^{-1}$. These were compared with absorbance levels obtained for standard solutions sampled for 5 s via the pump. The recovery was calculated from the mass of cadmium delivered to the flame as a transient (5 s) pulse and values of 93 and 98% were obtained.

The linear range was examined with a series of standard solutions up to a concentration of 100 $\mu\text{g l}^{-1}$. Linearity was observed with cadmium added to water and to a "low-cadmium" urine specimen.

The limit of detection, defined as the concentration equivalent to twice the background noise, was 0.43 $\mu\text{g l}^{-1}$. The increase in sensitivity afforded by the microcolumn and slotted quartz tube compared with conventional aspiration is shown in Table 3 and represents a 27.7-fold improvement.

Measurement of Cadmium in Urine

Specimens of urine obtained from a group of subjects with occupational exposure to cadmium were analysed by the pre-concentration procedure and by ETAAS. The agreement between the results from the two techniques is shown in Fig. 3.

Discussion

Despite developments with electrothermal atomisers, conventional FAAS with sample introduction via a pneumatic nebuliser is still very widely used. Measurements are rapid, reproducible and in many circumstances are free from interference. Analyses are cheap and do not require highly trained staff to achieve good results. The major limitation to FAAS is the poor sensitivity in comparison with ETAAS, a factor that will always outweigh the many advantages. However, where sensitivity can be increased, FAAS would be the preferred technique.

Sensitivity for the measurement of cadmium by conventional FAAS is specified by the manufacturers of instrumentation to be 10 $\mu\text{g l}^{-1}$, which is far too high a concentration to measure the metal in normal specimens of urine. However, with the simple accessories described in this paper it was possible to determine cadmium at these normal concentrations.

Previous reports of on-line pre-concentration devices have used equipment designated for flow injection (FI) techniques.^{7,9} We have shown that acceptable results can be obtained with much less sophisticated equipment although further improvements in performance might be possible with FI components. Although excellent results were obtained with columns prepared with basic alumina, other materials might also be suitable or even superior. If more efficient columns could be developed, with enhanced capacity for collection of the cadmium, the detection limit for measurements would be further reduced.

The results given here substantiate previous reports of work with samples such as water in demonstrating that the sensitivity limitations of techniques such as ICP-AES and FAAS can be overcome. The devices described could easily be automated and further developments with different columns and with other sample types would be expected to show that a wide range of applications are possible with this technique.

We are grateful to the British Council and the European Science Foundation for financial support and to Dr. A. A. Brown for helpful discussion of the work.

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Paper 8/03209B

Received August 5th, 1988

Accepted December 20th, 1988