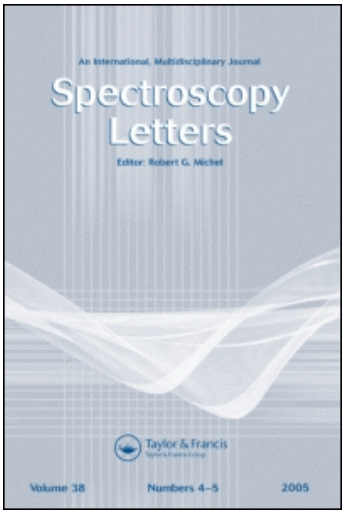

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Development of a Modified Thermospray for Coupling Reverse-Phase HPLC with GAAS for Highly Sensitive Cadmium Speciation in Human Urine

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ABSTRACT

The use of a Thermospray (TSP) in flame atomic absorption (FAA) increases sensitivity. Experimental data showed that a 75 μm orifice and 0.05 cm 1D capillary produced a sensitivity increase of about 10^4 compared to commercial flame atomizer. The signal was independent of power input over a considerable power range.

Based on this data a TSP nebulizer was built and used for interfacing HPLC and FAA. The instrument was used for cadmium speciation studies on urine. The presence of numerous cadmium compounds was revealed.

With further modification on TSP construction design, even higher analytical sensitivity for ultra trace metal analysis and better compatibility for interfacing HPLC with MS, ICP-MS, FAAS, and ICP-AES can be expected.

INTRODUCTION

In earlier cadmium speciation studies (1), a three fold absorption signal increases were observed using a thermospray interfaced HPLC-FAAS system. This unexpected sensitivity enhancement was then investigated.

Under a microscope it was found that a very narrow pinhole (roughly 0.007 cm ID) had formed by the accumulated deposit of impurtities from aqueous samples passed through the system. Based on this observation the orifice of the capillary was modified.

In related work 2-5 μm pinhole diaphragms used in the direct liquid introduction device (DLI) for LC-MS for finer solute droplet production have been found beneficial (**2, 3, 4**). Attachment of 25-50 μm pinhole nickel diaphragms to the TSP capillary tip (**5**) for LC-ICP sample introduction were studied for the purpose of improving the nebulization efficiency. Analytical sensitivity enhancement in the latter case was observed.

In order to study the relationship between the anaytical sensitivity and the tip aperture ID size, narrowed apertures were constructed on the tip of TSP capillaries with different ID sizes. Analytical sensitivity was systemmatically measured using those new TSP capillaries cadmium chloride standards of very low concentration (200 ppb) were used as standards. The absorbtion signals of the metal standards were measured as the functions of TSP heating energy power inputs, HPLC solvent volume flow rates, TSP capillary ID sizes of narrowd tip orifice. From the results the best parameters were selected to build an improved TSP nebulizer.

The sensitivities and reproductibilities of the metal analyses were calculated for TSP nebulizers of different ID capillaries equipped with 75 μm tip orifice, and compared with those of TSP nebulizers without tip narrowed orifice. The experimental results showed that, using TSP nebulizer equipped with tiny tip aperture (75μm) on thicker capillary (0.05 cm ID), significant sensitivity improvement (10^2-10^3fold) for metal cadmium analysis was achieved. Such. a.TSP whas then used for speciation studies of ultra trace cadmium compounds in human urine.

In previous cadmium speciation study (**1**), with regular capillary TSP interfaced HPLC-flame AAS, the extremely low total cadmium concentration in human urine (average 11-20ppb) (**6,7,8,9**) restricted the success of baseline separation of the metal compounds. A very noisy chromatogram was obtained with numerous cd compounds. It was anticipated that the improved TSP would give much better speciation identification of the metal compounds in human urine.

EXPERIMENTAL

A. Equipment Used

[1] Metal Detector: Perkin-Elmer Flame Atomic Absorption Spectrometer 370A with 10-cm single slot burner head.

[2] Radiation Source: Perkin-Elmer cadmium hollow cathode lamp operated at 5-mA and 12-V, with strongest cadmium resonance line at 228.8-nm monochromatic wavelength.

[3] Solvent Delivery System: Rainin Rabbit HPLC solvent delivery pump with 5.0-ml/min maximum flow rate single piston head.

[4] Pulse Dampener: Alltech Free Flow pump pulse dampener with 1/4" to 1/16" fitting converter for column-pump connection, and an old 150x4.6 mm, 3 μm HPLC column.

[5] HPLC Column: Rainin Dynanmax Shortone (50x4.6 mm), C-8, 3 μm, reverse-phase HPLC column, with 10x4.6 mm filled, C-8, 3 μm guard column.

[6] Sample Injector: Rheodyne 7125 injector with 100 μl sample loop except specified.

[7] Capillary Tubing Cutter: Alltech stainless steel tubing wheel-cutter with trapezoid-shaped cutting wheel modified in house.

[8] pH Meter: Chemcadet digital pH meter operated at 28°C.

[9] Recorder: Linear Instrument Chart recorder.

[10] Water Re-distiller: Pyrex condenser and flask.

[11] TSP Nebulizer: 150-W "firerod" electric heating cartridge by Watloo, with 1/16" OD, 0.005", 0.007", 0.01", 0.02", and 0.03" ID stainless steel capillary spray chamber by Alltech.

[12] TSP Constant Power Supply: The circuit was consisted of a constant high ampere transformer combined with two step-down transformers and a 1-100% variac. The variac gave a continuously fine voltage adjustment with 0.14-V and 0.01-A for 1% variac variation.

B. Samples and Reagents Used

[1] Sample: Fresh human urine samples collected immediately before testing.

[2] Standard Solution: 200., 100., and 50.0 ppb $CdCl_2$ freshly prepared from 1,000. ppm standard made by dissolving 1.000 gram cadmium metal in HCl acid and diluting to 1.000 liter.

[3] Solvents: Re-distilled lab deionized and distilled water to remove trace impurities.

[4] Washing Solutions: 10.% nitric acid and 10.% HCl acid (v/v) for burner assembly cleansing; 0.50 mM citric acid for TSP capillary regenerating; methanol, THF, and DCM for HPLC columns regeneration. 20.% nitric acid for HPLC assembly frits and fittings washing.

[5] Flame: Air-acetylene oxidizing flame was used.

C. Experimental Procedures

The same TSP nebulizer interfaced HPLC-flame AAS system used in preliminary cadmium speciation studies was optimized with the same procedures (**1**). Additional preparation procedures for the experiment are described as follows.

[1] Solvent Purification Process

The lab distilled and deionized water from the chemistry building actually contained trace impurities, arising from pipes and deionization columns. These inorganic or organic residues would accumulate on the internal wall and tip orifice of heated TSP capillary to cause capillary clogging and higher back pressure to the HPLC solvent delivery system. Thus, such water was re-distilled to further remove the impurities, degassed, and pH adjusted routinely.

[2] Solvent Delivery Pump Pulse Dampening System

The microbore (0.01-0.015 cm ID) capillary spray chamber of TSP operated constantly under high temperature (750-1,000°C). Extremely high vapor pressure of the HPLC effluent in the capillary would cause severe pressure fluctuation to the solvent delivery system. This result ed in a noisy chromatographic background in chromatogram which, for trace metal analysis, had to be eliminated.

The noisy background was only slightly reduced when a regular free flow pulse dampener was applied to the solvent delivery system, but was completely eliminated when an old 150x4.6 mm, 3 μm packing, HPLC column was further connected after the dampener.

[3] Preparation of Standards

Because of their instability, the metal standard solutions with concentrations lower than 1.0 ppm were freshly prepared daily from the 1,000 ppm standard. An hour period of homogenizing the low solute standards (<200 ppb) was applied. To ensure precise data acquisition from such dilute metal standards, the uncertainty of different pipettes were also taken into consideration.

[4] Modifications on Thermospray Nebulizer

(a) Construction of Capillary Replaceable Heating Cartridge

In previous TSP design, the capillary was completely silver soldered into the hollow rod of the heating cartridge (**1**). Whenever the capillary was clogged by solvent impurities, the nebulizer would no longer work. Regenerating a clogged capillary or replacing it was impossible. In most cases, a new nebulizer had to be installed, a process during which all the primed instrumental parameters would be altered, and the experimental data comparison would be meaningless.

Figure-1 shows a reusable TSP heating cartridge that was constructed in house. The 1/4" ID hollow rod of the "firerod" was completely blocked with a 1/4" OD brass rod in which a 1/16" ID through-hole was machine drilled. Then, the 1/16" OD capillaries were tightly inserted into the brass rod. The ID's and OD's of the three units were so precisely machined that optimum thermal contact was assured. In addition, top 1/4" unheated portion of the cartridge was removed to make the TSP nebulizer even smaller. A ring was threaded outside the cartridge to match the threaded base of flame burner stem.

Whenever the capillary in such new TSP nebulizer was clogged, it was pulled out of the brass rod, and replaced with a new one in minutes. This capillary replacement preserved other TSP operating parameters to generate reproducible results for meaningful comparison.

Furthermore, such configuration of the heating cartridge enabled us to conveniently change capillaries with different ID's or OD's at will.

(b) Construction of Narrowed Pinhole and Guiding Tunnel on Capillary Tip

Capillary tip cut with a tubing wheel-cutter normally formed an orifice with an ID much smaller than that of the capillary. However, the rough internal surface around the tip cut usually resulted in an irregular orifice opening, and in turn an uneven spray to every directions with split branches and large droplets. In addition, with the tubing cutter, it was hardly possible to reproduce orifices with desired ID, which made the comparison of analytical data generated with different capillaries impossible. Thus, smooth edge on a perfectly round orifice with tiny aperture followed by a coaxial tunnel to guide the jet into straight orientation was required. The generation of a very thin, straight, and single thread jet spray with unheated TSP capillary, and of a perfectly coaxial, cone-shaped, thermospray aerosol of the HPLC effluents from the tip of a TSP capillary with such narrowed tip orifice was expected to be delivered into the flame.

After many trials with electrochemical and mechanical construction methods, finally, the first tip pinhole was made manually, with a 75 μm brass wire inserted through the TSP capillary. **Figure-2** shows how a conical tip of the capillary was carefully made with a trapezoid-shaped cutting wheel, and then gradually altered the tip into a dome-shaped end by slightly tapping the rough cut with a small wrench while rotating the capillary evenly and slowly by hand. Under a 50X magnifier, the center of the pinhole was constantly adjusted during tapping, and the tunnel inside the orifice was smoothed by repeat insertion of a 75 μm stainless steel needle. The aperture size of

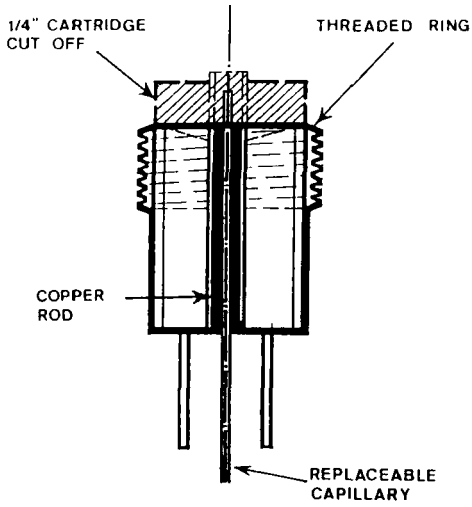

Figure-1. Diagram of the reusable TSP nebulizer. Note that the 1/4" OD brass rod with a 1/16" ID through-hole transfer thermal energy to the 1/16" OD capillary effectively. The capillary can be very easily replaced with new one whenever it is clogged. Top 1/4" unheated portion of the cartridge was cut off for compact size.

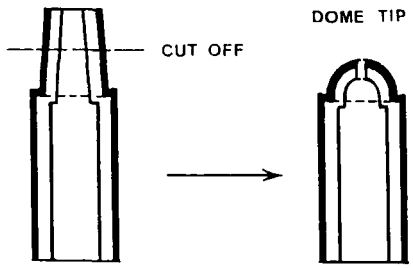

Figure-2. A dome-shaped capillary tip with a 75 μm orifice was made by cutting the capillary with wheel-cutter, and tapping into a dome shape tip with a straight coaxial internal guiding tunnel.

the pinhole and the tunnel was measured and adjusted with the needle and the cutter under the microscope.

Finally, the same dome-shaped tips with 75 μm ID pinhole were made on capillaries of 0.012 cm, 0.015 cm, 0.025 cm, 0.05 cm, and 0.075 cm ID. Examination under a 3-dimensional microscope showed that all these orifices had an perfectly round orifice with approximately the same aperture size.

[5] Evaluation on Analytical Performances of Modified Thermospray

A number of experimental variables affecting the analytical performance of the modified thermospray was tested for operational parameter optimization. These include the performance of narrow tip orifice at room temperature, the performance of heated capillary with various electrical energy inputs and at different solvent flow rates. All these tests were conducted on capillaries of different ID sizes with and without narrowed tip orifices.

(a) Performance of Capillaries with 75 μm Tip Orifice at Room Temperature

Five 1/16" OD stainless steel capillaries of different ID sizes, i.e. 0.012, 0.015, 0.025, 0.05, and 0.075 cm ID, but equipped with the same 75 μm aperture tip orifice were directly connected to the HPLC solvent delivery pump. Re-distilled lab deionized distilled water was pumped through the capillaries to generate a thin, thread jet. The solvent (water) volume flow rate was increased stepwise from 0.50 to 3.00 ml/min at 0.25 ml/min intervals. The vertical height of the thin thread jet versus each flow rate and pump pressure was measured. The formation of a coaxial, straight, thin thread jet should verify the perfection of a round orifice and smooth guiding tunnel, and the height of jet should confirm the aperture sizes of identical orifices on different capillaries.

(b) Performance of Heated Thermospray in Cadmium Standard Calibration

To evaluate the performance of modified TSP nebulizer with standard cadmium calibration, HPLC separation column was eliminated. The TSP nebulizer was directly connected to the HPLC pump through the pulse dampening system. For analytical performance comparison, parallel tests were performed on TSP nebulizer equipped with capillaries of different ID sizes but the same 75 μm orifice. For best absorption measurement with least relative error, according to the Ringbom plot theory (10), the percent absorption signals (Abs.%) of 100 μl 200 ppb cadmium chloride standards were measured. The Abs.% signals were plotted as the function of the heating energy inputs, solvent flow rates, capillaries ID sizes, capillary with or without narrowed tip orifice.

For TSP capillaries with different ID sizes, the analytical sensitivities, i.e. the limit of detection (LOD), and the reproducibilities, i.e. the relative standard deviation (RSD) achieved in the metal calibration were calculated from the experimental data. The optimum parameters for the new TSP operating conditions and for the TSP design modifications were selected from the results of above analytical ability comparison.

[6] Speciation Study on Ultra-trace Cadmium in Human Urine

A TSP nebulizer composed of a 0.05 cm ID capillary with 75 μm tip orifice and a 150-W heating cartridge was constructed to couple HPLC with FAAS for the metal speciation study. A series of 100 μl of fresh human urine samples were injected into the C-8 reverse-phase separation column. A 2.50 ml/min aqueous solvent flow rate was chosen to maximize the sample introduction rate, in turn the absorption signal. The nebulizer was heated with 130-W power input, the lowest point where the energy-independent range started with minimum decomposition power toward biological sample components, but with the maximum nebulization and desolvation efficiencies to achieve the maximum atomization efficiency.

The preliminary speciation of cadmium in human urine was successful. Two inorganic and many complexed organic cadmium compounds were detected in the chromatogram. The chromatographic separation pattern of the metal species in human urine was studied with the results from the parallel study previously performed on the metal complexation in metallothionein in *in vitro* simulation (**11**).

RESULTS AND DISCUSSION

[1] Results using a Capillary with a Narrowed Tip Orifice at Room Temperature

Under room temperature, according to hydrodynamics, the linear velocity of liquid flowing through a pipe is inversely proportional to the pipe internal diameter at a given volume flow rate (**12**). With a capillary equipped with narrowed pinhole orifice at the end tip, the solvent should undergo a linear acceleration at the tip exit to generate a very fast-moving jet. With the coaxial guiding tunnel, a straight, thin, liquid thread jet should shoot out to a considerable distance.

Table-1 shows the results of the testing. At flow rate below 0.45" ml/min, only droplets emerged from the tip of the capillaries, but at flow rate of 0.50 ml/min, all the capillaries generated a 6" straight thread jet coaxially. When the flow rate increased up to 3.00 ml/min, five capillaries all produced a straight, coaxial, thin thread water jet shooting up to 52" maximum height.

Table-1. Vertical Height of Jet vs. Flow Rate and Pump Pressure

Flow Rate (ml/min)	Pump Pressure (psi)	Jet Height (inch)
0.40	200	0
0.45	250	4
0.50	350	6
0.75	550	15
1.00	750	23
1.25	1050	30
1.50	1250	35
1.75	1500	40
2.00	1700	42
2.25	1900	44
2.50	2100	46
2.75	2300	48
3.00	2500	52

This indicated that, at the same volume flow rate, the capillaries with different ID's and identical orifices behaved identically. Such results ensured the comparison study on TSP nebulizer performances responding to different parameters under high temperature operating conditions.

At high operating temperature, very high solvent vapor pressures were created inside the microbore capillary. A huge pressure drop between vapor and the atmosphere at the tip exit created a fast expansion environment The generation of a coaxially conical shape thermospray aerosol/vapor jet with very fine droplets were formed This type of spray is highly desirable for efficient nebulization and atomization.

[2] Performance of Heated Thermosprays in Metal Standard Calibration

With a certain solvent passing through the TSP capillary at a given volume flow rate, the temperature of the capillary was directly proportional to the heating energy input to the nebulizer. The temperature controlled the efficiencies of vaporization and desolvation of the sample solution, as well as other physical properties of the aerosol, such as the droplet size distribution, which in

turn controlled the efficiency of atomization. These factors directly influenced the signal responses in TSP interfaced LC-MS (**13**) and ICP (**14**, **15**) and FAAS (**16**).

In previous studies, the relationship between the heating energy input to the TSP nebulizer and the flame AAS signal were studied with different metals and solvents (**8**), however with high metal concentrations (>0.5-2.0 ppm) (**17**), lower energy inputs (<40-150-W) (**13**), lower solvent flow rates (<2.50 ml/min)(**18**), and the same capillary ID size (0.005") without narrowed tip orifice (**19**).

This present study focused on the analytical functions of modified TSP, in terms of AAS signal from cadmium detection of much lower metal concentration (<200 ppb), with higher energy input (100 to 180-W), at wider flow rate range (1.00 to 3.00 ml/min), on different capillaries with different ID sizes (0.005"-0.03") but the same tip orifice diameter (75 μm).

(a) Absorption Signals using TSP Capillary without a Narrowed Tip Orifice

Figure-3 shows that, without the narrowed tip orifice, an energy-developed AAS signal relationship was observed. The AAS signals were inversely proportional to the ID sizes of regular capillaries. At 2.25 ml/min solvent flow rate and under optimum power input, the maximum abs.% signal of 100 μl 200 ppb cadmium obtained from TSP nebulizer with 0.005"ID capillary was five times stronger than that with 0.02"ID.

Without a reduced aperture the vaporization of the sample was incomplete, and the liquid/aerosol mixture was not shattered by high pressure drop to fine droplets. The larger liquid sample droplets caused the slowest sample transportation rate, worst analyte desolvation, highest sample loss, least atomization efficiency and lowest AAS signals.

(b) Effect of TSP Capillary Tip Orifice Size on Absorption Signals

With the 75 μm tip aperture on TSP capillary of 0.01" ID, the energy-dependent absorption signal was only observed with lower power input (<120 watts). However, compared to the Abs.% signals obtained from two TSP capillaries of 0.01" ID, **Figure-4** shows an approximate 3-fold of Abs.% increase in 200 ppb cadmium detection with the one equipped with a 75 μm tip aperture. This confirmed the inverse proportional relationship between the Abs.% signal and the tip orifice size on TSP capillary, observed in previously.

With the capillary tip restricted by narrowed aperture, the very high sample vapor pressure which built inside the capillary was only released when the vapor/aerosol mixture exited the tip.

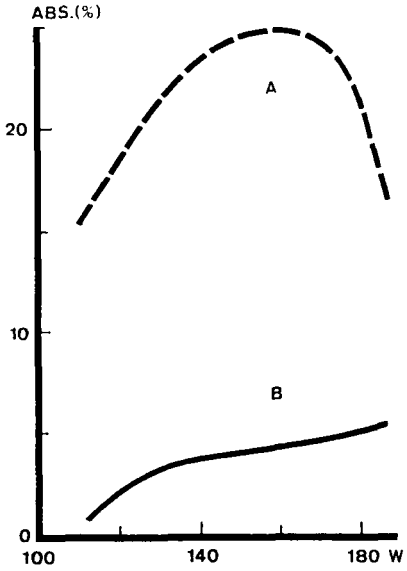

Figure-3. Absorption signals versus TSP heating energy input, with two capillary: (A) 0.005" ID without tip orifice, (B) 0.02" ID without tip orifice. Cadmium: 200 ppb. Flow rate: 2.25 ml/min. Note the signal drop after a maximum with energy input increase.

The huge pressure drop generated a large amount of finer sample droplets. The droplets were accelerated, according to the hydrodynamics, by restricted tip aperture at the exit of the capillary, and delivered into the flame at a high sample transportation rate. Finer sample droplets resulted in better sample desolvation, and in turn higher atomization efficiency and hence better Abs.% signals.

(c) Effect of Energy Input to Narrowed Tip TSP on Absorption Signals

In **Figure-3**, without narrowed tip orifice, the energy-dependent absorption response was increasing with the increase of TSP heating energy input upto a certain maximum. Beyond a critical value of the input, the response started to decline with the input increase until completely disappeared at a certain value. The analyte deposition inside the TSP capillary under a superheated condition was the cause which was presumably believed (**20**).

A very interesting phenomenon was noticed when the TSP capillary equipped with 75 μm tip aperture. Below 100-W of TSP energy input, the directly proportional energy-dependent signal

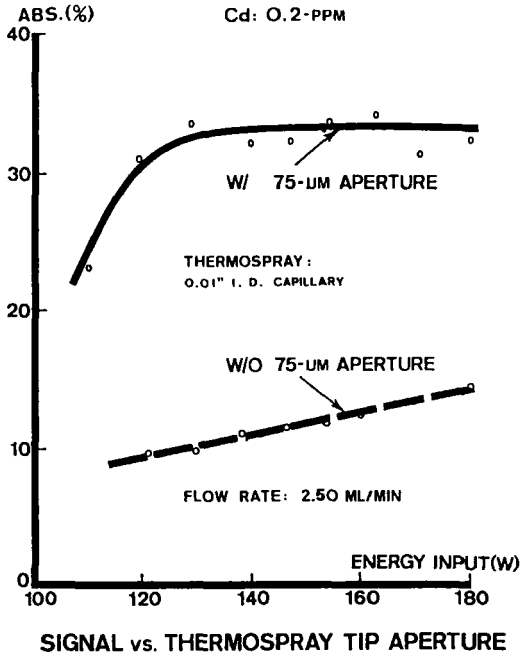

Figure-4. The absorption signal comparison between two 0.01" ID TSP capillaries with and without 75 μm ID tip orifice. A about three fold sensitivity increase in cadmium detection to a three fold decrease in capillary tip aperture ID size was seen.

responses still existed. However, **Figure-4 and 5** shows that, beyond 100-W, the energy-dependent signal responses were almost completely disappeared with three TSP capillaries having the same orifices. Such energy-independent Abs.% signal response extended over wide power and flow rate ranges (100-180 watts and 1.0-3.0 ml/min).

The phenomenon might be interpreted with energy related sample solution desolvation process. Within a power input range, certain solvent passing through the capillary at a given flow rate, the energy consumption for complete sample vaporization, desolvation, and decomposition should be fixed. Excess thermal energy only enhanced the kinetic movement of solvent and solute molecules or ions inside the capillary. Partial or complete analyte deposition onto the hot wall of TSP capillary was difficult with the solvent vapor being restricted in the capillary longer by the

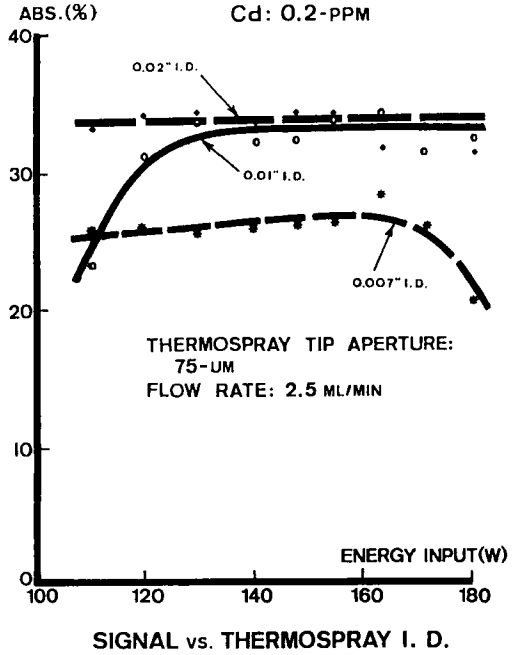

Figure-5. The absorption signals versus TSP nebulizer heating energy input using TSP with three capillaries of different ID sizes but equipped with the same 75 μm ID tip orifice. Note that the 0.02" ID capillary resulted in the highest sensitivity, widest energy-independent response range, and best reproducibility.

narrowed tip exit. Therefore, the signal decrease or disappearance did not occur. After a sudden acceleration at the exit, consequently, the equally well vaporized and desolvated fine analyte droplets should result in identical signal responses.

(d) Effect of Capillary ID with Narrowed Tip TSP on Absorption Signals

Figure-5 also reveals that, with the same 75 μm orifice on the capillary, the width of the energy-independent signal range varied and seemed directly proportional to the capillary ID size. It started from 120 watts for the 0.01" capillary, and stretched over 110-160 watts for the 0.007" ID, but almost extended over 110-180 watts for the 0.02" ID.

More importantly, with the same metal concentration, the 0.05 cm ID capillary TSP yielded the highest absorption signal, but the 0.007" capillary resulted in the lowest. It was then believed that, with the same tip aperture, the thicker the capillary ID, the better the analytical sensitivity in terms of absorption signals, and the wider the energy-independent Abs.% signal range.

The understanding of the above phenomenon could be related to the linear velocity inside the capillary. At a given solvent volume flow rate, the thicker the capillary internal diameter, the slower the linear velocity inside the capillary, the better the thermal energy transfer efficiency, and the higher the sample solution vaporization and desolvation. Therefore, the better the atomization efficiency, and the higher the Abs.% signal response.

(e) Effect of Solvent Flow Rate with Modified TSP on Absorption Signals

Figure-6 illustrates the effect of solvent volume flow rate on absorption signals of 200 ppb cadmium detection using TSP nebulizer with 75 mm tip orifice on 0.02" capillary. A very wide dynamic range covered flow rate from 1.0 ml/min to 3.0 ml/min, with a TSP heating power input range extended over 128 to 140 watts.

It was believed that, with the effective sample vaporization and desolvation processes inside the capillary with sufficient thermal energy, the higher the solvent volume flow rate, the faster the vapor sample transportation rate in unit time into the flame, the higher the atom concentration, and the stronger the absorption signal.

The identical responses within a wide range of TSP power input revealed the possibility of using lowest TSP heating energy input for the metal speciation study on thermally unstable human biological samples. The absorption response was directly proportional to the flow rate indicating that the application of high speed chromatography for the ultra trace cadmium speciation in human urine might be necessary.

(f) Analytical Reproducibility with Modified TSP Nebulizer

Table-2 lists the standard deviations calculated from the data collected in the calibration. The performance of TSP nebulizers with different capillaries were compared at 2.25 ml/min flow rate, 140 watt energy input, with 200 ppb cadmium sample detection signals, in terms of metal absorption signals (Abs.), standard deviation (SD) and relative standard deviation (RSD).

The data shows that the overall analytical performance of the TSP nebulizers with 75 μm tip orifice were much better than those without the tip orifice. Among the TSP with the tip orifices, the 0.02" ID capillary gave the best analytical performance.

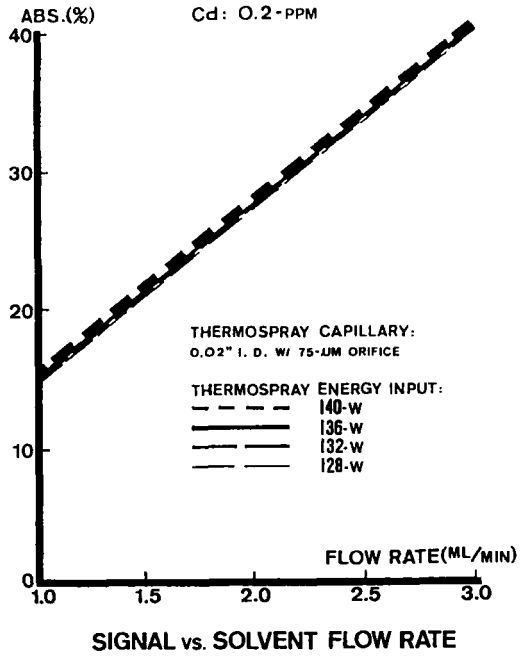

Figure-6. The linear relationship between absorption signal and solvent flow rate with a 0.02" ID TSP capillary equipped with 75 µm tip orifice. Note the wide dynamic range with flow rate and the perfect energy-independent response range (128 to 140 w).

Table-2. Sensitivity and Reproducibility of 8 TSP capillaries

Capillary ID(cm)	75 µm Tip	Absorbance	Std. Dev.	Rel. Std. Dev.
0.05	No	1.3×10^{-2}	2.0×10^{-3}	15.0%
0.025	No	4.8×10^{-3}	1.8×10^{-3}	4.0%
0.02	No	6.7×10^{-3}	1.4×10^{-3}	2.0%
0.012	No	1.0×10^{-1}	1.4×10^{-3}	1.0%
0.012	Yes	1.1×10^{-1}	1.0×10^{-3}	0.9%
0.002	Yes	1.2×10^{-1}	1.4×10^{-3}	1.2%
0.023	Yes	1.5×10^{-1}	2.4×10^{-3}	1.5%
0.05	Yes	1.6×10^{-1}	9.0×10^{-4}	0.6%

A TSP nebulizer with 0.02" ID capillary equipped with a 75 μm tip orifice was used for the speciation study of ultra trace cadmium in human urine.

[3] Speciation of Cadmium Compounds in Human Urine

From previous studies, the total average concentration of cadmium in human urine was in the range of 11-20 ppb. With such a low concentration, the commercial (Perkin-Elmer) flame atomic absorption spectrometer with a 26 ppb sensitivity (**21**) was unable to perform the speciation experiment, since the average concentration of each cadmium components separated from the HPLC might be as 1 ppb or less.

With a regular TSP interfaced HPLC-FAAS system, in our previous cadmium speciation study, more than dozens of cadmium compounds were detected, however the detection sensitivity was very low (<0.035 absorbance), with severe chromatographic background noise (**1**).

Figure-7 shows the a chromatogram of a successful speciation separation of cadmium compounds in human urine, using the reverse-phase HPLC-FAAS system interfaced with a TSP nebulizer with 0.02" ID capillary and equipped with 75 μm tip orifice. A remarkable sensitivity increase was observed. A doublet peak of absorbance of 0.265 and 0.227, which later were confirmed as the peaks of cadmium chloride and cadmium sulfate, was recorded at retention times of 28 and 38 seconds. The intensity of the signals were almost eight times stronger compared with the results obtained in pervious cadmium speciation in urine from the same individual (**1**).

Following the doublet, several tiny peaks were recorded. Comparing the retention times of these peaks in the chromatogram with those in the previous study of zinc and cadmium metallothionein complexation (**11**), the similar pattern of the chromatograms confirmed that those peaks belonged to the complexed organic cadmium compounds in human renal system.

The absorbance of one of the two peaks in the doublet, 0.265 absorbance, was equivalent to 46% absorption. This resulted in a concentration sensitivity (1% absorption) of 0.24 ppb, for the average total cadmium concentration as 11 ppb in human urine, which was about 110 times more sensitive than the commercial flame AAS system. Considering the sample introduction was 0.10 ml injection for our study, compared with the 6.5 ml/min continuous sample introduction with the commercial flame AAS system, the absolute sensitivity resulted from this cadmium speciation reached 0.0037 ppb (or 3.7 parts per trillion), i.e. 3.7 pg/ml. This result revealed a 7.0 X 10^3 fold of sensitivity increase using our advanced TSP interfaced HPLC-flame AAS system compared to the sensitivity with the commercial system for cadmium detection.

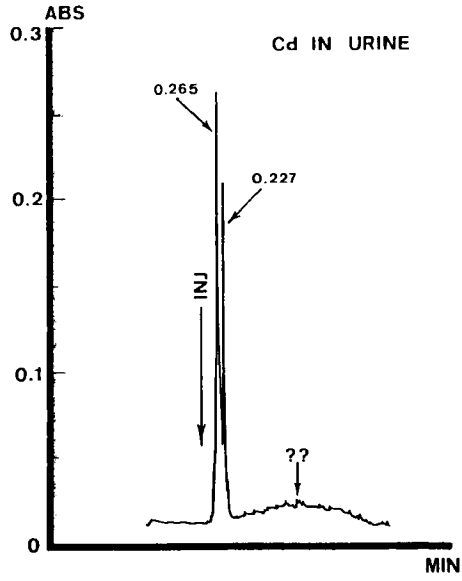

Figure-7. The HPLC chromatogram of the first speciation separation of trace cadmium compounds in human urine with the advanced TSP nebulizer interfaced HPLC-flame AAS system. The 0.265 absorbance of the first peaks revealed a 10^3 fold sensitivity increase. Note many complexed cadmium compounds.

CONCLUSIONS

A. The relationship of absorption signal was inversely proportional to the ID size of tip aperture on TSP capillary. This confirmed that the sensitivity increase in previous metal speciation was indeed caused by the formation of narrowed tip orifice on capillary from solvent impurity accumulation.

B. With narrowed tip aperture on the TSP nebulizer, regardless the capillary ID difference, an energy-independent absorption response always existed within a wide TSP heating energy input range with various solvent flow rates. This enabled us the generation of reliable analytical data with the modified TSP nebulizer interfaced instruments under much simpler operating conditions.

C. The experimental data revealed that the narrower the tip aperture produced the higher the analytical sensitivity and the better the reproducibility. By shrinking the tip aperture further (<25

μm) on even thicker ID TSP capillary (>0.03"), future modified TSP nebulizer can be expected to bring even higher sensitivity and much better reproducibility for even tougher ultra trace chemical analysis.

D. The chromatogram of cadmium speciation in human urine revealed that there are many different organic cadmium compounds complexed in human renal system and excreted with urine, which might explain the metal detoxification function of human renal system.

E. The concentration sensitivity for cadmium detection using the modified TSP interfaced HPLC-flame AAS system reached 0.24 ppb, which accounted for a 110 fold of sensitivity increase compared to that of the commercial system. The absolute sensitivity for the metal detection was calculated as 3.7 pg/ml, which was 7.0×10^3 times more sensitive than that of commercial system result.

F. Further modification on TSP design and construction materials, such as a smaller heating cartridge should be able to improve the compatibility for TSP coupling HPLC with MS, ICP, ICP-MS, and Graphite AAS.

BIBLIOGRAPHY

1. P. Chang, J. Robinson, J. of Environ. Sci. & Health, 1993, A, Vol. 28, (10)
2. P. J. Arpino, G. Guiochon, Anal. Chem., 1979, 51, 683A
3. A. P. Bruins, J. Chromatogr., 1985, 323, 99-111
4. P. J. Arpino, Trends in Anal. Chem., 1982, 1(7), 154
5. J. K. Koropchak, H. Aryamanya-Mugisha, Anal. Chem., 1988, 60, 1838
6. M. Webb, The Chem./Biochem. and Bio. of Cd: Topic in Environ. Health, 1979, 2, 255-271
7. J. H. Mennear, Cd Toxicity, Modern Pharm-toxicology, 1979, 15, 5-14
8. S. D. Weiss, Ph. D. Dissertation, LSU, Aug. 1981
9. E. Skelly, Ph. D. Dissertation, LSU, 1982
10. G. Horlick, Anal. Chem., 1984, 56, 278R
11. P. Chang, J. Robinson, J. of Environ. Sci. & Health, 1993, A, Vol. 28, (6)
12. R. Resnick, D. Halliday, Physics, John Wiley, 1977
13. M. L. Vestal, G. J. Fergusson, Anal. Chem., 1985, 57, 2373
14. J. A. Koropchak, D. H. Winn, Anal. Chem., 1986, 58, 2558

15. J. A. Koropchak, D. H. Winn, Trends in Anal. Chem., 1987, 6(7), 171

16. S. S. Mo, D. S. Choi, J. Robinson, J. of Environ. Sci. & Health, 1988, A23(2), 139

17. D. S. Choi, J. Robinson, 42rd ACS Southeastern Regional Meeting, 1986, Houston, TX

18. D. S. Choi, J. Robinson, Spectroscopy Letters, 1987, 20(4), 375

19. D. S. Choi, J. Robinson, Spectroscopy Letters, 1988, 21, 337

20. D. S. Choi, Ph. D. Dissertation, LSU, Aug. 1989

21. Perkin-Elmer, Owner's Manual, for Model 370A, 1975

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