A Flow Injection On-Line Multiplexed Sorption Preconcentration Procedure Coupled with Flame Atomic Absorption Spectrometry for Determination of Trace Lead in Water, Tea, and Herb Medicines

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One of the limitations in previous flow injection (FI) sorption preconcentration procedures in a knotted reactor (KR), which have been carried out exclusively with a single continuous sample injection over a certain period, is the relatively low retention efficiency (typically 40−50%). Although the sensitivity of such systems could be improved by properly increasing sample preconcentration time, sample loading flow rate, or both, further improvement of the sensitivity has been limited by the narrow linearity of the relationship between signal intensity and preconcentration time or sample loading time. In this work, a novel on-line FI multiplexed sorption preconcentration procedure with repetitive sample injections was developed to overcome the above problems in the previous systems. In contrast to previous FI preconcentration systems, the proposed multiplexed preconcentration procedure evenly divides a single longer sample injection step into several shorter substeps while the total preconcentration time is still kept constant. To demonstrate its merits, the proposed FI on-line KR multiplexed sorption preconcentration system was combined with flame atomic absorption spectrometry (FAAS) for determination of trace lead in water, tea, and herb medicines. The lead in the sample solution on-line reacted with ammonium pyrrolidine dithiocarbamate, and the resultant analyte complex was sorbed on the inner walls of the KR. The residual sample solution was then removed from the KR with an air flow. The above two steps were repeated eight times with a total preconcentration time of 120 s. The sorbed analyte was eluted from the KR with 4.5 mol L^{-1} HCl for on-line FAAS detection. The present multiplexed preconcentration procedure with eight repetitive sample injections for a total preconcentration time of 120 s gave a retention efficiency of 92%, twice that obtained by one single sample injection preconcentration (47%). In addition, the linear ranges of the diagrams of absorbance against sample loading flow rate and sample loading time were extended, offering more potential for achieving high sensitivity by increasing sample loading rates or sample loading time compared to the previous one single continuous sample injection preconcentration procedure. At a sample loading flow rate of 3.6 mL min^{-1} for a total preconcentration period of 120 s, an enhancement factor of 57 and a detection limit (3σ) of 8 μg L^{-1} were obtained. The precision was 1.4% (RSD, $n = 11$) at the 200 μg L^{-1} level. The developed method was successfully applied to the determination of trace lead in various water samples, herb medicines, and a certified tea reference material.

Flow injection (FI) on-line preconcentration and separation coupled with flame atomic absorption spectrometry (FAAS) have been shown to be promising for determination of trace elements in view of the enhanced sensitivity, the efficient removal of matrix, the high sample throughput, and the low cost of the FAAS equipment.[1] Recently, FI on-line preconcentration and separation based on the sorption of hydrophobic organometallic complexes on the wall of the PTFE knotted reactor (KR) have been successfully coupled with atomic spectrometry for trace element analysis.[2−4] Compared with conventional microcolumn systems, the KR sorption system allows the analysis to be conducted at low cost owing to the nearly unlimited lifetime and the ease of construction of the KR, in no need of packing materials, and permits the use of higher sample loading rates for achieving higher sensitivity due to the low hydrodynamic impedance in the KR.[2−10]

All previous FI KR preconcentration procedures, however, have been achieved exclusively with a single continuous sample injection step in a certain period.[2−10] One of the limitations in such previous KR systems is the relatively low retention efficiency, typically 40−50%.[2] Proper increase in sample preconcentration time, sample loading flow rate, or both could improve the

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Table 1. Operational Sequence for the FI On-Line Multiplexed Sorption Preconcentration System for FAAS Determination of Lead

				flow rate/mL min^{-1}		
step	function	time/s	pumped medium	pump 1	pump 2	valve position
1 (Figure 1a)	sample loading	15	sample	off	3.6	fill
			APDC (0.01% m/m)		3.6	
2 (Figure 1a)	KR washing	15	air	3.0	off	fill
repeat steps 1 and 2 for eight times						
3 (Figure 1b)	analyte elution	25	HCl (4.5 mol L^{-1})	3.6	off	inject

sensitivity of the KR systems.[2-10] However, further improvement of the sensitivity has been limited by the narrow linearity of the relationship between signal intensity and preconcentration time or sample loading time.

In this work, a novel FI on-line multiplexed sorption preconcentration procedure was developed to improve the retention efficiency of the KR system and to extend the linearity of the diagrams of signal intensity against preconcentration time and sample loading time. In contrast to conventional FI preconcentration, the proposed multiplexed preconcentration procedure evenly divided a single longer preconcentration step into several shorter substeps while the total preconcentration time is still kept constant. Taking lead as a model element, and ammonium pyrrolidine dithiocarbamate (APDC) as the complexing reagent, the proposed FI on-line KR multiplexed sorption preconcentration was coupled with FAAS for determination of trace lead in water, tea, and herb medicines. To demonstrate the advantages of the present procedure, potential factors affecting the preconcentration of trace lead were investigated in detail.

EXPERIMENTAL SECTION

Instrumentation. All measurements were carried out on a Hitachi 180-80 atomic absorption spectrometer. An Hitachi lead hollow cathode discharge lamp was used as the radiation source at 283.3 nm with a current of 7.5 mA and a 1.3-nm slit width. A flow spoiler was used in the spray chamber for all measurements. The recommended flame conditions were employed (2.2 L min^{-1} acetylene and 9.4 L min^{-1} air).

An FIA-3100 flow injection system (Vital Instruments Co. Ltd., Beijing, China) was connected to the flame atomic absorption spectrometer with the shortest possible length of 0.35-mm-i.d. PTFE tubing (∼20 cm). Ismaprene pump tubes were used to deliver the samples and reagents. Small-bore (0.35-mm i.d.) PTFE tubing was used for all connections, which were kept as short as possible to minimize the dead volumes. The FIA-3100 consists of two peristaltic pumps and a standard rotary injection valve (eight ports on the rotor and eight ports on the stator). The rotation speed of the two peristaltic pumps, their stop and go intervals, and the actuation of the injection valve were programmed as shown in Table 1.

Knotted reactors used for the preconcentration were made of hydrophobic micro-PTFE tubing (0.5-mm i.d.), which was tied into knots ends on ends with a 50-, 100-, 150-, 200-, and 250-cm-long PTFE tubing, respectively.

Reagents. All reagents used were of the highest available purity and at least of analytical grade. Doubly deionized water (DDW) was used throughout this work. The complexing agent

solution was prepared by dissolving APDC (Sigma-Aldrich, Ontario, Canada) in DDW. Concentrated nitric acid and perchloric acid (Tianjin Chemicals Co., Tianjin, China) were used for the digestion of tea and herb medicines. A 10% m/m ascorbic acid solution was prepared by dissolving ascorbic acid (Tianjin Pharmaceutical, Tianjin, China) in DDW. A 2.5% m/m phenanthroline solution was prepared by dissolving phenanthroline (Tianjin Pharmaceutical) in 0.2 mol L^{-1} HCl (Tianjin Pharmaceutical). Working standard solutions containing 10−400 μg L^{-1} Pb, 0.2% m/m ascorbic acid and 0.02% m/m phenanthroline were prepared by stepwise dilution of the stock solution of 1000 mg L^{-1} Pb (National Center for Standard Materials, Beijing, China) and by adding appropriate volumes of 10% m/m ascorbic acid solution and 2.5% m/m phenanthroline solution immediately before use.

Sample Pretreatment. Three river water, two lake water, and one tap water samples were collected locally. Three wastewater samples were collected from local chemical manufactories. Immediately after sampling, all water samples were filtered through 0.45-μm Supor filters (Gelman Sciences). The filtered samples were then acidified to pH 1.6 with nitric acid, and the resultant samples were stored at 4 °C in low-density polyethylene (LDPE) bottles, which were precleaned with sub-boiling 8 mol L^{-1} HNO$_3$ and rinsing with DDW. A certified reference material, GBW 08607 (River Water, National Center for Standard Materials) was used for quality control in the determination of lead in water samples.

Two herb medicine samples (donated by a pharmaceutical manufactory) and a certified tea reference material (GBW 07605, National Center for Standard Materials) were pretreated as follows. Amounts of the sample (0.1000 g for herb medicines, 1.000 g for tea) were weighed accurately into a 20-mL PTFE beaker, to which 5 mL of concentrated nitric acid and 5 mL of concentrated perchloric acid were added. The sample was gently heated on a hot plate until white fumes appeared. After the solution was cooled, the contents were transferred into a 100-mL calibrated flask containing 2 mL of 10% m/m ascorbic acid, and 0.8 mL of 2.5% m/m phenanthroline in 0.2 mol L^{-1} HCl.

Procedures. Details of the FIA-3100 program and the sequence of operation are given in Table 1. In step 1 (Figure 1a), the Pb −PDC complex was formed on-line and sorbed onto the inner walls of the KR. In step 2 (Figure 1a), an air flow pushes the effluent from the KR flowing to waste. Step 1 and step 2 were repeated eight times, while the total periods for sample loading and air washing were kept to 120 s, respectively. Finally, in step 3 (Figure 1b), a solution of 4.5 mol L^{-1} HCl was introduced into the KR to elute the sorbed analyte and, subsequently, to deliver
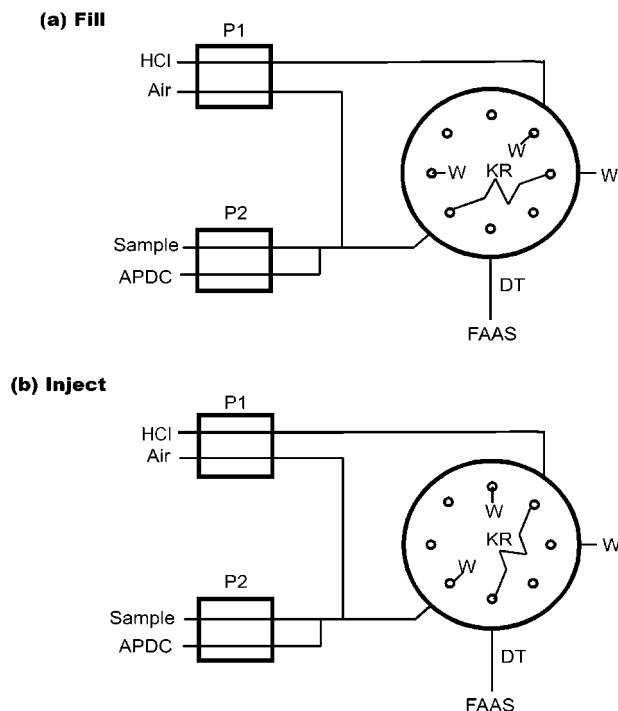
Figure 1. FI manifold for the KR sorption preconcentration for FAAS. For details, see text. Key: P1 and P2, peristaltic pumps; DT, delivery tubing; KR, knotted reactor (0.5-mm-i.d. × 250-cm-long PTFE tubing); W, waste. Valve position: (a) fill; (b) inject.

Figure 2. Effect of the number of repetitive sample injections for preconcentration of 200 μg L^{-1} Pb with a total preconcentration period of 120 s. All other conditions as in Figure 1 and Table 1.

the eluted analyte into the FAAS system. The total time for a single determination was 265 s containing 120 s for sample loading.

Method Development. A univariate approach was used for optimization studies. The main figure of merit used for optimization is the absorbance (peak height) with simultaneous consideration of precision (aiming at <3% RSD). The parameters studied included the pH of the APDC solution, concentration of APDC, sample loading flow rate and time, wash flow rate and time, KR tubing length, composition of the eluent, and elution flow rate. Three to four cycles of univariate studies were required to adjust the fixed parameters to optimal values in each univariate study.

RESULTS AND DISCUSSION

The FI on-line multiplexed sorption preconcentration system was optimized in order to determine the best chemical and flow conditions for lead determination with good sensitivity and precision. Considering that the acidity of the sample solution (especially for water samples) in routine analysis is often in the range of pH 1–2, the standard solutions and sample solutions were all adjusted to pH 1.6 with nitric acid so that the optimization of other parameters can be simplified.

Advantages of the Proposed FI Multiplexed Sorption Preconcentration System. To demonstrate the merits of the present FI multiplexed preconcentration system, the influence of the number of repetitive sample injections for preconcentration on the absorbance was investigated with a total preconcentration period of 120 s. In this case, the duration for each sample injection was determined by dividing the total preconcentration period by the number of repetitions. As shown in Figure 2, the absorbance increased almost linearly as the number of repetitive sample injections increased from two to at least eight. When the sample
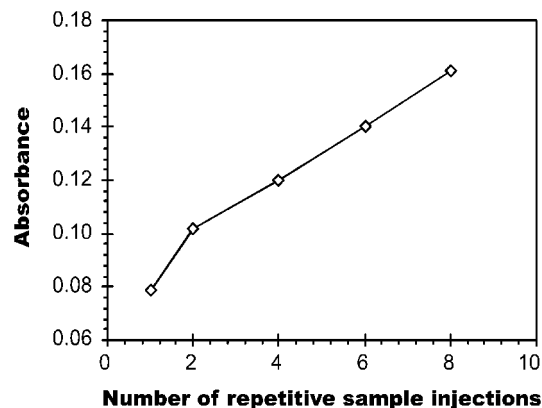
loading and air washing step was repeated eight times, excellent elution peaks were observed. Meanwhile, the absorbance increased to twice that obtained by a single continuous sample injection (i.e., the number of repetitions is one) for a total preconcentration time of 120 s. The retention efficiency, which is defined as the percentage of the amounts of the analyte adsorbed by the KR relative to the total amounts of the analyte introduced into the KR, increased from 47 to 92% on increasing the number of repetitive sample injections from one to eight. These results indicate that the observed sensitivity enhancement by the present multiple sample injections resulted from an improvement of the retention efficiency, probably because the first adsorption generated in the KR might act as "nucleation sites" for subsequent injections. It is also possible that the sorbed Pb–DPC complexes were more uniformly distributed onto the inner wall of the KR when the sample injection and the followed air-washing steps were repeated several times in comparison with the conventional one single continuous sample injection procedure. However, the exact reasons for the sensitivity enhancement still need further investigation.

The advantages of the proposed multiplexed preconcentration procedure over the conventional one single continuous sample injection preconcentration procedure were further demonstrated in terms of sensitivity and linear range in the diagrams of absorbance versus sample loading time and sample loading rate. For this purpose, the diagrams describing the dependence of absorbance on sample loading time (Figure 3) and on sample loading flow rate (Figure 4) between eight repetitive sample injections and one single continuous sample injection for a total preconcentration time of 120 s were compared. In addition to the obvious enhancement of sensitivity, the proposed multiplexed preconcentration procedure with eight repetitive sample injections offered a wider range of linearity in the diagrams of absorbance against sample loading time (at least up to 160 s; see Figure 3a) and absorbance against sample loading flow rate (at least up to 4.3 mL min^{-1}; see Figure 4a) compared to conventional preconcentration with one single continuous sample injection (up to 120 s in Figure 3b, 3.8 mL min^{-1} in Figure 4b). In addition, the present multiplexed preconcentration procedure exhibits a rapid increase in absorbance with increasing sample loading time and loading flow rate (Figure 3a vs Figure 3b, Figure 4a vs Figure 4b). All the above results indicate that higher sensitivity could be achieved
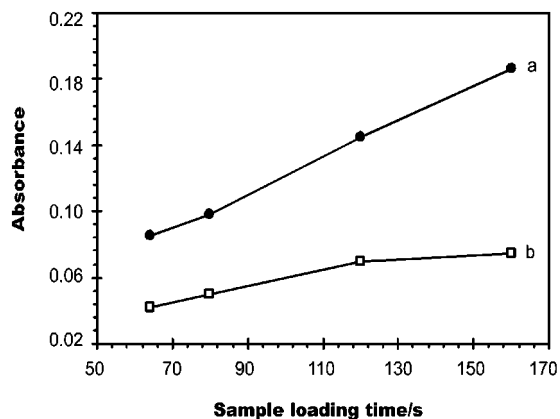
Figure 3. Effect of total sample loading time on the preconcentration of 200 μg L^{-1} Pb with (a) a multiplexed preconcentration procedure with eight repetitive sample injections and (b) a single continuous sample injection preconcentration. All other conditions as in Figure 1 and Table 1.
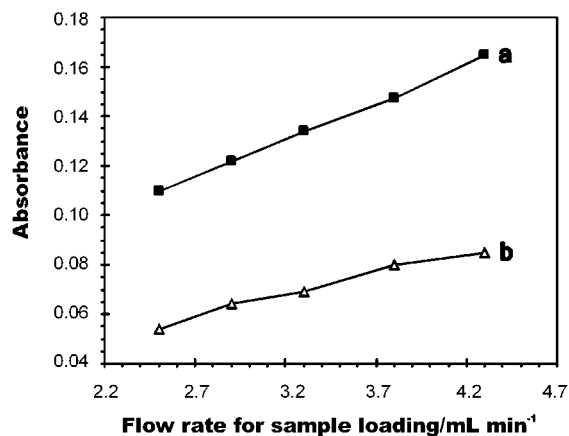

Figure 4. Effect of sample loading flow rate on the preconcentration of 200 μg L^{-1} Pb with a total preconcentration period of 120 s by (a) a multiplexed preconcentration procedure with eight repetitive sample injections and (b) one single continuous sample injection for preconcentration. All other conditions as in Figure 1 and Table 1.

with the proposed multiplexed preconcentration procedure compared to the conventional procedure. The wider linearity in the diagrams of the absorbance versus sample loading time and sample loading flow rate suggests that the proposed multiplexed preconcentration with eight repetitive sample injections present more potential for achieving high sensitivity by increasing sample loading rates, sample loading time, or both. For the rest of this work, a multiplexed preconcentration procedure with eight repetitive sample injections at a sample loading flow rate of 3.6 mL min^{-1} for a total preconcentration time of 120 s was employed. To our knowledge, no such multiplexed sorption preconcentration procedure, to date, has been reported.

pH of the System. To find the optimal conditions for the preconcentration of trace lead in aqueous solutions, a detailed study on pH effect was undertaken. The on-line pH adjustment of the reaction mixture in step 1 was achieved through adjusting the pH of the APDC solution with ammonia solution while the acidity of the sample solution was kept at pH 1.6. The pH of the effluent in step 1 was monitored as the indication of the pH of the system. It was found that the absorbance of lead increased with increasing pH of the effluent up to 2.5 and then leveled off

until pH 9.2. The wide optimal pH range (2.5−9.2) is an obvious advantage for easy adjustment of the pH of the system. For further work, a pH of 4 in the effluent, corresponding to pH 10.8 in the APDC solution, was used.

APDC Concentration. The optimal APDC concentrations were found in the range of 0.005−0.02% m/m. Below the APDC concentration of 0.005% m/m, the absorbance significantly increased with concentration of APDC. This result indicates that the formation of the Pb−PDC complex and its subsequent sorption onto the inner walls of the KR played an important role for the preconcentration. However, even in the absence of APDC, ~15% of the lead was collected, suggesting that not all of the lead was preconcentrated through sorption of the Pb−PDC complexes onto the inner walls of the KR. The sorption of the neutral lead hydroxide by the KR at such acidity (pH 4) might partially account for the preconcentration of trace lead. However, as the APDC concentration increased from 0.02 to 0.1% m/m, the absorbance reduced by ~20%, probably due to the competition of excessive complexing reagent for the active sites on the KR walls.[3,5−7] For further studies, an APDC concentration of 0.01% m/m was used.

Removal of Residual Sample Solution. To obtain high sensitivity, dispersion in the manifold should be minimized. To this end, the residual solution in the KR and in the eluate delivery tube was removed by air as completely as possible prior to elution. The removal of residue sample solution could also facilitate the elution of the Pb−PDC complex from the inner walls of the KR and improve the absorbance signal.

The ratio of sample loading time to air-washing time on the preconcentration was investigated at 1:2, 1:1, and 2:1, respectively. The absorbance slowly increased with the ratio of sample loading time to air-washing time. Even so, considering the analytical efficiency and sample loading volume overall, the ratio of 1:1 was chosen as optimal in the further work. The air flow rate should be slower than the sample loading rate for efficient KR washing. To keep a sufficient air interval between two sample loadings, an air flow rate of 3.0 mL min^{-1} was chosen.

KR Tubing Length. The influence of KR tubing length on the preconcentration of lead was investigated in the range of 50−250-cm-long PTFE tubing for a period of 120-s preconcentration. The absorbance was found to increase linearly with increasing in KR tubing length up to at least 250 cm. For further optimization studies, a KR with a tubing length of 250 cm was employed.

Elution. Choice of a suitable eluent is important for the analytical performance of an FI on-line preconcentration system. Isobutyl methyl ketone was exclusively employed as the eluent in all previous FI on-line KR sorption preconcentration systems for FAAS owing to its effective elution of the sorbed analyte complex and its extra enhancement effect for FAAS detection.[2−4] For obvious reasons, however, it would be preferable if an inorganic solvent, such as a mineral acid, could be employed, because many organic solvents exhibit toxic or unpleasant properties.[11] In addition, organic solvents cannot be handled by most pump tubes and, therefore, require the use of displacement facilities, which makes the system more complicated.[11]

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Table 2. Analytical Results for the Determination of Trace Lead in Water, Tea, and Herb Medicines

sample	concentration (mean ± σ, n = 3)/μg L⁻¹ certified	concentration (mean ± σ, n = 3)/μg L⁻¹ determined	recovery of 100 μg L⁻¹ Pb spiking (%) without masking reagents[a]	recovery of 100 μg L⁻¹ Pb spiking (%) with masking reagents
GBW 08607 (River Water)	4.4 ± 0.2^b	4.6 ± 0.2^b	-	
GBW 07605 (Tea)	1.00 ± 0.02^b	0.99 ± 0.06^b		-
river water 1		nd^c	61	98
river water 2		nd	66	99
river water 3		nd	59	103
lake water 1		nd	37	97
lake water 2		nd	58	97
wastewater 1		27 ± 2	63	98
wastewater 2		97 ± 1	85	99
wastewater 3		44 ± 2	39	102
tap water		nd	46	103
herb medicine 1		15 ± 2^b	65	103
herb medicine 2		14 ± 1^b	71	97

^{a} 0.2% m/m ascorbic acid with 0.02% m/m phenanthroline. ^{b} Concentration, μg g⁻¹. ^{c} nd, not detectable.

In this work, ethanol and diluted HNO₃ and HCl were tested as the eluent. The use of ethanol as the eluent produced an absorbance signal with many shoulder peaks. When diluted HNO₃ was employed as the eluent, the KR tubing and even the nebulizer were clogged very often, probably from solid sulfur in the tubing. This fact likely resulted from the strong oxidation nature of HNO₃ and the liability of APDC to be oxidized and decomposed under such a condition, thus leading to a decrease in the preconcentration efficiency of the KR. In contrast, the use of diluted HCl as the eluent not only produced much earlier and sharper absorbance signals with higher sensitivity and better precision but also resulted in no clogging. The results indicate that dilute HCl was very efficient for the elution of sorbed analyte complexes from the KR and, therefore, was chosen as the eluent.

The effect of HCl concentration on analyte elution was tested at the elution flow rate of 3.6 mL min⁻¹. In the absence of HCl, there was no appreciable adsorbance signal. However, the signal intensity increased linearly with concentrations of HCl below 4.0 mol L⁻¹, but tended to be stable with further increase in the concentration of HCl up to at least 6 mol L⁻¹, suggesting a wide range of HCl concentration could be employed for optimal elution. For further experiments, a 4.5 mol L⁻¹ HCl solution was used for analyte elution.

The elution flow rate is another important parameter in the FI on-line preconcentration and separation systems. The effect of elution flow rate on the peak height absorbance was investigated in the range of 1.9−4.6 mL min⁻¹. It was found that the absorbance increased with the elution flow rate of 1.9−2.5 mL min⁻¹ and then leveled off in the range of 2.5−3.8 mL min⁻¹. Over the flow rate of 3.8 mL min⁻¹, the absorbance decreased slowly. Therefore, a flow rate of 3.6 mL min⁻¹ was chosen for the elution in the rest of this work.

Interference Studies. Since APDC is a potential complexing agent for a large number of metals, interferences from coexisting metal ions always have to be considered seriously in order to achieve a desired degree of selectivity. Table 2 shows the recovery

Table 3. Analytical Performance of the FI On-Line Multiplexed Sorption Preconcentration with Eight Repetitive Sample Injections for FAAS Determination of Lead under the Conditions in Table 1

preconcentration time/s	120
enrichment factor	57
sample consumption/mL	7.2
reagent consumption/mL	
0.01% m/m APDC	7.2
4.5 mol L⁻¹ HCl	1.5
precision (RSD, $n = 11$)/%	1.4 (200 μg L⁻¹)
retention efficiency/%	92%
detection limit (3σ)/μg L⁻¹	8.0
regression equation (A, peak height absorbance; C, μg L⁻¹; 5 standards)	$A = 0.0008C - 0.00082$
correlation coefficient	0.999

of the spiked lead (100 μg L⁻¹) in the river water, lake water, and wastewater, as well as the digest of two herb medicines. In the absence of masking reagents, the recovery ranged from 37 to 85%, indicating serious interferences in these matrixes. A combination of 0.2% m/m ascorbic acid with 0.02% m/m phenanthroline was found quite effective for eliminating the matrix interference and, therefore, was employed as a masking reagent. As shown in Table 2, quantitative recovery of the spiked lead from these samples was achieved in the presence of a mixture of 0.2% m/m ascorbic acid and 0.02% m/m phenanthroline.

Analytical Performance. Analytical characteristic data for the performance of the FI on-line multiplexed sorption preconcentration system for FAAS are shown in Table 3. The enrichment factor (EF) is defined as the ratio of the analyte concentrations after and before preconcentration.[1] In practice, however, such evaluation is not feasible because the actual concentrations are unknown. Therefore, in this work, the EF was approximated as the ratio of the slopes of the linear section of the calibration curves after and before preconcentration. With the proposed multiplexed sorption preconcentration procedure as shown in Figure 1 and Table 1, an enrichment factor of 57 and a detection limit (3σ) of 8 μg L⁻¹ were obtained. The precision (RSD) for 11 replicate measurements of 200 μg L⁻¹ Pb was 1.4%. The retention efficiency was evaluated to be 92%.

The developed procedure was applied to the determination of lead in two certified reference materials, GBW 08607 (River Water) and GBW 07605 (Tea), to evaluate the accuracy. As shown in Table 2, the concentrations of lead in these two CRMs obtained by the present method using simple aqueous standard calibration agreed well with the certified values. The analytical results for the local water samples and herb medicines are also given in Table 2. The concentrations of trace lead in these samples determined by the present method were found to be in good agreement with those obtained by an independent ETAAS technique.

CONCLUSIONS

In this work, a novel FI on-line multiplexed sorption preconcentration procedure was developed for the preconcentration of trace metals. The present multiplexed sorption preconcentration procedure has been successfully combined on-line with FAAS for determination of trace lead in various water samples, tea, and herb

medicines. Besides significant increase of the retention efficiency, the proposed multiplexed preconcentration procedure offers a wider range of linearity in the diagrams of absorbance versus sample loading time and sample loading flow rate. In addition, the present method presents more potential for achieving high sensitivity by increasing sample loading rates and sample loading time than the conventional one single continuous sample injection preconcentration procedure. We expect this new preconcentration protocol to be effective and judicious for the preconcentration of various analytes instead of conventional FI sorption preconcentration for other potential detectors.