

Preparation of the diphenylcarbazone-functionalized silica gel and its application to on-line selective solid-phase extraction and determination of mercury by flow-injection spectrophotometry

Jing Fan^{a,b,*}, Yuxia Qin^b, Cunling Ye^{a,b}, Pingan Peng^a, Chunlai Wu^b

^a State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, PR China

^b School of Chemistry and Environmental Science, Henan Key Laboratory for Environmental Pollution Control, Henan Normal University, Xinxiang, Henan 453007, PR China

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Abstract

A new solid-phase extractor, diphenylcarbazone-functionalized silica gel has been synthesized and confirmed by IR and Raman spectrometry. The new solid-phase extractor is found to be stable in 1–6 mol L⁻¹ HCl or H₂SO₄, and also in common organic solvents. It can be used to separate and enrich Hg(II) selectively from eight metal ions with similar characteristics such as Cd(II), Ni(II), Co(II), Mn(II), Pb(II), Zn(II), Cu(II) and Fe(III). The pre-concentration factor is as high as 500. The results show that this new solid extractor has a good stability and can be reused for many times without decreasing its extraction percentage. The micro-column packed with diphenylcarbazone-functionalized silica gel was used for on-line solid-phase extraction and determination of mercury in real samples by flow-injection spectrophotometry. At the optimal conditions, the linear range and the detection limit for the determination of Hg(II) is found to be 1–1500 and 0.90 ng mL⁻¹, respectively. The relative standard deviation of 11 replicate measurements is less than 3%. The proposed method was demonstrated to be simple, fast, selective, low cost and less pollution.

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1. Introduction

It is well known that mercury is one of the most toxic heavy metals. In contaminated environment, different forms of mercury can be accumulated in animals and plants, and also enter into human body by food cycle, resulting in the damage of central nerve [1]. Mercury is also a kind of environmental hormone, which can destroy the functions of natural metabolism, increment and procreate for organism, leading to maladjustment in hormone secretion of organism. At the same time, mercury is

widely dispersed in natural world because of its extensive applications. Therefore, determination of trace of mercury in real samples is very important for environmental pollution monitoring. The commonly used methods for the determination of trace of mercury mainly include atomic absorption spectrometry (AAS) [2–4] and spectrophotometry [5,6]. The spectrophotometry is widely used as a detection technique due to low cost of the instruments and simplicity of the procedures, but its sensitivity and selectivity are low. Although AAS has higher selectivity and sensitivity, there is a crucial need for the pre-concentration of trace mercury before their determination due to their frequent low concentration in numerous samples. Additionally, since high levels of non-toxic components usually accompany analytes, a clean-up step is often required [7].

Real samples have the characteristics of complicated composition, various interfering materials and very low concentration

* Corresponding author at: School of Chemistry and Environmental Science, Henan Key Laboratory for Environmental Pollution Control, Henan Normal University, Xinxiang, Henan 453007, PR China.

E-mail address: fanjing@henannu.edu.cn (J. Fan).

of the component to be determined. Usually, these samples can only be analyzed after complicated pre-treatment. Thus, sample pre-treatment is a key step in real sample analysis. Traditional sample pre-treatment techniques include liquid–liquid extraction [8,9], precipitation separation [10,11], membrane filtration [12,13] and floatation separation [14,15] and among others. These methods are usually time-consuming, labor-intensive and result in the pollution. In addition, they require strict control of extraction conditions, such as pH and temperature. For all of these reasons, some new techniques have been rapidly developed for pre-treatment of samples in these years, solid-phase extraction (SPE) is one of them [16,17]. This technique is attractive as it reduces consumption of and exposure to solvents, their disposed costs and extraction time. It also allows the achievement of high recoveries, along with possible elevated enrichment factors. In addition, SPE can be interfaced on-line with analytical detection techniques [7]. Some sorbents have been used for pre-concentration of mercury [18–20].

In the present work, a new selective solid-phase extractor for Hg(II), diphenylcarbazone-functionalized silica gel, was synthesized for the first time. This new solid-phase extractor was used to separate and enrich Hg(II) selectively from Cd(II), Ni(II), Co(II), Mn(II), Pb(II), Zn(II), Cu(II) and Fe(III). A micro-column packed with diphenylcarbazone-functionalized silica gel was coupled with flow-injection (FI) spectrophotometry for the selective separation, pre-concentration and determination of Hg(II) in six different cigarette samples. The proposed method is simple, rapid, sensitive, selective, low sample and reagent consumption.

2. Experimental

2.1. Apparatus

All analytical measurements were carried out on a Shanghai spectrophotometer (Model 722) with an 18 μL flow cell and on a Shanghai fluorophotometer (Model 930A). The flow assembly was provided with one injection valve and two peristaltic pumps. The reaction coils were made of PTFE tubing (i.d., 0.5 mm), and the self-made micro-column was made of organic glass. All pH measurements were performed by a Hangzhou digital pH-meter (Model pH-3C) with a combined glass-calomel electrode. Raman and IR spectra were recorded, respectively, on a Raman module of the Nicolet Nexus FT-IR/Raman Spectrometer and on a Bio-Rad FT-40 infrared spectrophotometer (KBr discs). A FIA lab sequential injection unit (Model 3500) was also used.

2.2. Reagents

All the reagents used were of analytical grade. Doubly distilled water was used throughout the experiments. HCl solution of 6.0 mol L⁻¹, borax solution of 0.5 mol L⁻¹, H₂SO₄ stock solution of 5.0 mol L⁻¹, silica (particle size, 0.18–0.30 mm, Qingdao Haiyang Chemical Factory, China), tetraethyl orthosilicate (Shanghai Silicon Mountain Macromolecular Materials Co., Ltd., China), toluene, ethanol, aether and pyridine were used

in the present work. Diphenylcarbazone (ShangHai SSS Reagent Co., Ltd., China) solution was prepared by dissolving 2.4000 g of the solid reagent in 100 mL of methylbenzene. A series of buffer solutions (0.2 mol L⁻¹) of pH 1.0–9.0 were used for pH adjustment: pH 1.0–2.0 (HCl–KCl), pH 3.0 (ClCH₂COOH), pH 4.0–6.0 (NaAc–HAc), pH 6.0–8.0 (KH₂PO₄–Na₂HPO₄), pH 6.0–9.0 (KH₂PO₄–NaOH). Standard solutions of 1 mg mL⁻¹ of Cd(II), Ni(II), Co(II), Mn(II), Pb(II), Zn(II), Cu(II), Fe(III) and Hg(II) were prepared from special grade reagents. Neutral red stock solution of 8 $\times 10^{-4}$ mol L⁻¹ was prepared by dissolving 0.1154 g of the solid in 500 mL water. Potassium bromate stock solution of 0.25 mol L⁻¹ was prepared by dissolving 41.7500 g of the salt in 1.0 L of water. All working solutions were prepared by stepwise dilution as required.

2.3. Preparation of the diphenylcarbazone-functionalized silica gel micro-column

2.3.1. Synthesis of the diphenylcarbazone-functionalized silica gel

In order to remove any adsorbed metal ions, silica gel was activated by dipping it in 6 mol L⁻¹ hydrochloric acid for 2 days, then filtered and washed repeatedly with distilled water until no detectable Cl⁻ can be found in the filtrate. Such treated silica gel was dried in an oven at 110 °C to remove any adsorbed water on the surface. Five grams of the activated silica gel was suspended in a mixture consisting of 75 mL of dry toluene and 15 mL of tetraethyl orthosilicate, and refluxed for 12 h. The product, silanized silica gel (Si–OC₂H₄), was filtered, washed with toluene, ethanol and ether, and dried at 70 °C for 6 h. The diphenylcarbazone-functionalized silica gel was synthesized according to the following procedure: 5.0 g dry Si–OC₂H₄ was added into a solution formed by dissolving 2.4 g of diphenylcarbazone in 100 mL of hot toluene in the presence of a few drops of pyridine; after the reaction mixture was refluxed for 6 h, the resulting solid phase was filtered, washed with toluene, ethanol and water until the filtrate showed no characteristic color of diphenylcarbazone. The solid was then dried at 70 °C for 6 h to obtain the diphenylcarbazone-functionalized silica gel.

2.3.2. Preparation of the micro-column

The micro-column (3.0 cm \times 2.0 mm) was prepared according to the method described by Fang [21]. It was packed with diphenylcarbazone-functionalized silica gel. The ends of the micro-column were fitted with nylon filter cloth (0.18–0.30 mm) to keep the functionalized silica gel inside the column.

2.4. Analytical procedure

The FIA system is shown in Fig. 1. The manifold was connected by PTFE tubes (i.d., 0.7 mm). The analytical procedure is described as follows. The first step was the on-line separation and pre-concentration of mercury by the micro-column, as shown in Fig. 1a. For this purpose, the peristaltic pump 1 (P₁) was opened with pump 2 (P₂) closed, and sample solution was injected into the sampling loop (flow rate: 1.2 mL min⁻¹). Mercury was enriched by passing through the micro-column for 5 min. Then

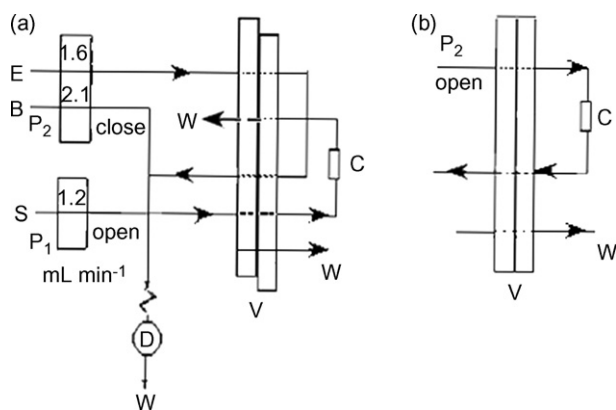


Fig. 1. Manifold used for the determination of mercury(II): (a) sampling system; (b) elution system; S, sample; B, reagent; E, eluent; C, micro-column packed by diphenylcarbazone-functionalized silica gel; P₁ and P₂, peristaltic pumps; V, sampling valve; D, detector; W, waste.

a doubly distilled water stream was passed to remove the sample still being present in the line and in the column. The second step was the elution of the separated and enriched target metal from the micro-column (flow rate: 1.6 mL min⁻¹). In doing so, the peristaltic P₂ was opened with P₁ closed, 0.27 mol L⁻¹ of H₂SO₄ (as an eluent) and solutions of neutral red and KBrO₃ were pumped into the flow cell to form the reaction baseline for measurement of the blank absorbance (A₀). Next, the injection valve was switched on and the H₂SO₄ (0.27 mol L⁻¹) eluent solution was pumped inversely through the column to elute the mercury (Fig. 1b). Then the eluent solution containing mercury(II) flowed into the reaction coil, where the reaction occurred among neutral red, KBrO₃ and mercury(II). When the reaction mixtures flowed through the flow cell, the absorbance (A) was detected by a spectrophotometer at $\lambda = 560$ nm. The values of $\Delta A (=A_0 - A)$ were found to be directly proportional to the concentration of the mercury(II).

3. Results and discussion

3.1. Spectroscopic characterization of the diphenylcarbazone-functionalized silica gel

3.1.1. Infrared spectroscopy measurements

The main characteristic groups of silica gel are –Si–O– and –OH. Diphenylcarbazone is a kind of organic dye containing azo-groups. Its main characteristic groups include –N=N–, –C=C– and –C–H. Infrared spectroscopy measurements of silica gel, diphenylcarbazone and diphenylcarbazone-functionalized silica gel are carried out by using potassium bromide technique. The results are summarized below.

Absorption spectroscopy of the silica gel is listed in Table 1.

Absorption spectroscopy of the diphenylcarbazone:

2858 and 2933 cm ⁻¹	Absorption bands of –C–H
1453 cm ⁻¹	Absorption band of –C=C– in benzene ring
3312 cm ⁻¹	Absorption band of –N–H

Absorption spectroscopy of the diphenylcarbazone-functionalized silica gel:

Table 1
The absorption of silica gel

Wavelengths (cm ⁻¹)	Absorption
1104	Absorption band for –Si–O–
473, 803 and 970	Absorption bands for the background of silica gel
1635	Bending vibration for –OH
3458	Stretching vibration for –OH

Besides the absorption bands mentioned above for silica gel and diphenylcarbazone, two new bands are observed at 1559 cm⁻¹, it is the bending vibration absorption of –N–H or the absorption of –CONH, 1508 cm⁻¹ is the absorption band of –C=C– in benzene ring or the absorption band of –CONHR.

3.1.2. Raman spectroscopy measurements

Raman spectra were determined for silica gel, diphenylcarbazone and the diphenylcarbazone-functionalized silica gel. Two new bands are found at 1402.0 and 1143.21 cm⁻¹, they are the absorption of –N=N– or –Si–ph and –Si–O–Si– or –Si–O–R, respectively.

Infrared and Raman spectra data indicated that the reaction took place between silica gel and diphenylcarbazone, and the diphenylcarbazone-functionalized silica gel was formed.

3.2. Properties of the diphenylcarbazone-functionalized silica gel

3.2.1. Density

Densities of silica gel and the diphenylcarbazone-functionalized silica gel were determined by the method described by El-shahat et al. [22]. Their values are found to be 476.8 and 792.7 kg m⁻³, respectively. These data suggest that silica gel has been modified by diphenylcarbazone.

3.2.2. Stability of the diphenylcarbazone-functionalized silica gels

In order to examine the stability of the diphenylcarbazone-functionalized silica gels in different solvents, 0.3 g of the functionalized silica gel was suspended in 25 mL of the acetone, ethanol, butanol, chloroform, carbon tetrachloride, 1–6 mol L⁻¹ of HCl, 1–6 mol L⁻¹ of H₂SO₄ and doubly distilled water, respectively. After the systems were stirred for 120 min, the absorbances of diphenylcarbazone in the leaching solutions were determined. The results showed that no detectable diphenylcarbazone can be washed out from the functionalized silica gel. This suggested that the functionalized silica gel was highly stable in acids and in common organic solvents.

3.2.3. Adsorption of metal ions by silica gel and the diphenylcarbazone-functionalized silica gel

0.3 g of the silica gel and the functionalized silica gel were respectively put into 20 mL of the solution containing Fe(III), Pb(II), Cd(II), Co(II), Hg(II), Ni(II), Mn(II), Cu(II) and Zn(II). Concentration for each of the metal ions is 4 μ g mL⁻¹, and pH value of the solution is at 7.0. After the systems were stirred for 40 min, concentration of metal ions in the solution

Table 2
Adsorption of metal ions by silica gel and the diphenylcarbazone-functionalized silica gel

Metal	Quantity added (μg)	Quantity adsorbed on silica gel (μg)	Quantity adsorbed on functionalized silica gel (μg)
Co(II)	80.0	57.6	2.10
Cd(II)	80.0	53.5	12.8
Pb(II)	80.0	69.7	0.80
Fe(III)	80.0	24.9	0.00
Hg(II)	80.0	16.2	80.0
Ni(II)	80.0	26.9	0.70
Mn(II)	80.0	29.1	1.60
Cu(II)	80.0	51.8	31.7
Zn(II)	80.0	64.3	63.5

was determined by spectrophotometric method [23]. The results are summarized in Table 2. As shown from these data, silica gel has no selectivity for the adsorption of the metal ions investigated, and can adsorb different kinds of metal ions simultaneously. However, metal ions can be adsorbed selectively by the diphenylcarbazone-functionalized silica gel under given experimental conditions. Therefore, selectivity of the stationary phase was enhanced greatly by using the functionalized silica gel.

3.3. The pre-concentration behavior of the diphenylcarbazone-functionalized silica gel for mercury(II)

3.3.1. pH effect on the pre-concentration of Hg(II) by the functionalized silica gel

In separated beakers, 25 mL of aliquots containing $5 \mu\text{g mL}^{-1}$ of Hg(II) was added and their pHs were adjusted at different levels. These solutions were mixed with 0.3 g of the functionalized silica gel, and then stirred for 1 h. After the functionalized silica gel was deposited and separated, the amount of Hg(II) remained in the aqueous solution was determined using suitable spectrophotometric procedure. The extraction percentage (E) can be calculated by the equation:

$$E = \frac{(C_0 - C) \times 100}{C_0} \quad (1)$$

where C_0 and C stand for the initial and final concentrations of Hg(II) in the tested solution. It is evident from Fig. 2 that the percentage of extraction was 90–100% in the pH range of 6.0–9.0. At low pHs, because of the protonation of the functionalized silica gel, few of Hg(II) in solution can chelate with diphenylcarbazone, the absorption percentage is low. With the increase of the pH, the protonation of the functionalized silica gel becomes weak, most of Hg(II) in solution can chelate with diphenylcarbazone, hence, the phenomenon of the increase of the absorption was observed. Therefore, pH 7.0 was selected for the pre-concentration of Hg(II) by the diphenylcarbazone-functionalized silica gel.

3.3.2. Effect of stirring time on retention of Hg(II)

The stirring time taken for the adsorption of the metal ion by the functionalized silica gel and the attainment of the equilib-

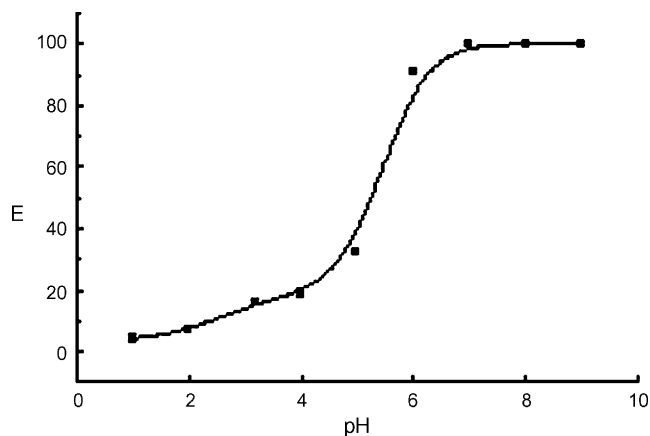


Fig. 2. Effect of pH on the mercury(II) extraction: 0.3 g of functionalized silica gel, 25 mL of aliquots containing $5 \mu\text{g mL}^{-1}$ of Hg(II), stirring time 1 h.

rium conditions is of considerable importance. Effect of stirring time on the retention of Hg(II) by the functionalized silica gel was studied by the batch experiments. At pH 7.0, 0.2 g of functionalized silica gel was stirred with $80 \mu\text{g}$ of Hg(II) solution. With the increase of the stirring time, the extraction percentage increases, it will attain to equilibrium until 20 min and the extraction percentage is more than 95% for Hg(II).

3.3.3. Adsorption isotherm of mercury(II) on the functionalized silica gel

0.2 g of the functionalized silica gel and different amounts of mercury(II) were added into 25 mL aliquots at the optimized pH conditions, then these systems were stirred for 30 min. Concentration of the remained mercury(II) in the aqueous phase was determined by spectrophotometry. Then the amounts of mercury(II) adsorbed by the functionalized silica gel (Q) were calculated at different Hg(II) concentrations. Thus, obtained results are shown in Fig. 3. It can be seen that the amount of Hg(II) adsorbed by the functionalized silica gel increased linearly with increasing concentration of mercury(II) in aqueous solutions over a wider range of concentration. From this

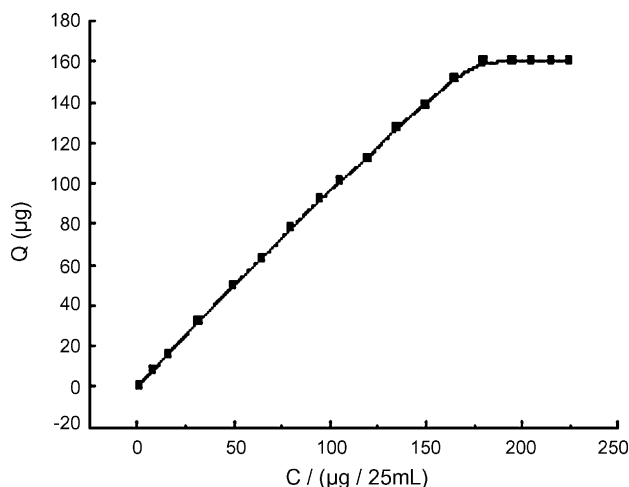


Fig. 3. Adsorption isotherm of mercury(II): 0.2 g of the functionalized silica gel, pH 7.0.

adsorption curve, the maximum adsorption capacity of Hg(II) by the functionalized silica gel was found to be $3.99 \times 10^{-3} \text{ mmol g}^{-1}$.

3.3.4. Breakthrough capacity of the functionalized silica gel micro-column

The micro-column was packed with 0.2 g of the functionalized silica gel by using overflowing liquid method. A solution containing $8 \mu\text{g mL}^{-1}$ of mercury was allowed to pass through the column at the optimized conditions, the flow rate is 1 mL min^{-1} , and the percentage of mercury in the effluent was determined. From the variation of mercury(II) percentage in the effluents with the solution volumes passed through the column, the breakthrough volume and the volume needed to reach the saturation for the mercury can be determined. It is found that the breakthrough volume of the column is generally reached after passing 10 mL of the solution containing $8 \mu\text{g mL}^{-1}$ of mercury. The breakthrough capacity of the functionalized silica gel column for mercury was calculated to be $1.99 \times 10^{-3} \text{ mmol g}^{-1}$ of dry functionalized silica gel.

3.3.5. Separation and pre-concentration of mercury(II) using the functionalized silica gel column

Twenty micrograms of mercury(II) was dissolved in different volumes of water. These solutions were passed through the functionalized silica gel column in sequence at the optimized experimental conditions. Then Hg(II) was eluted from the column with aqueous HCl solution at pH 2.0. Concentration of Hg(II) in the eluting solutions was determined by spectrophotometry. The extraction percentage and the pre-concentration factor were calculated. The results are presented in Table 3. These data suggested that Hg(II) can be selectively enriched by the functionalized silica gel column from large volumes of dilute aqueous solutions. Furthermore, we prepared a solution formed by dissolving $1 \mu\text{g}$ of Hg(II) in 1.0 L of water, and this solution was allowed to pass through the functionalized silica gel column in the optimized conditions. Then, Hg(II) was eluted from the column by aqueous HCl solutions. Concentration of mercury(II) in the eluent was determined by fluorometry [21]. The percentage of extraction for Hg(II) was found to be 96.5%.

3.4. Establishment of FIA method for the determination of Hg(II)

A new flow-injection catalytic kinetic spectrophotometric method was proposed for the determination of trace of mer-

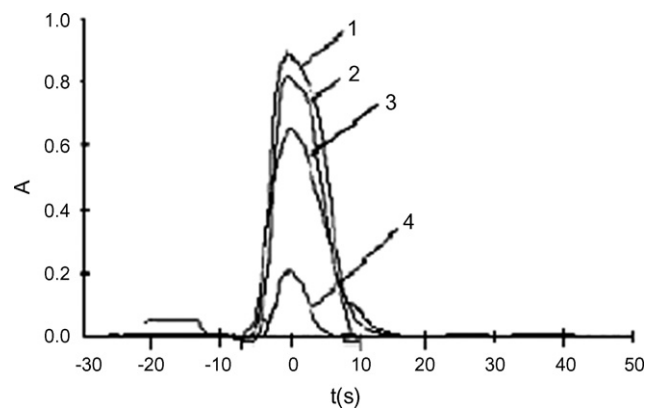


Fig. 4. Absorbance signals obtained for different reaction systems as a function of reaction time: 1, neutral red; 2, neutral red + potassium bromate; 3, neutral red + potassium bromate + sulfuric acid; 4, neutral red + potassium bromate + sulfuric acid + Hg(II).

cury, which is based on the catalytic effect of mercury(II) on the oxidation of neutral red by potassium bromate in sulfuric acid medium. The absorbance–time curves determined for different reaction systems are shown in Fig. 4. It can be seen that the reaction occurs between neutral red and potassium bromate, and this reaction was accelerated significantly by Hg(II) in sulfuric acid medium as evidenced by the greatly decreased absorbance of this reaction system in the presence of Hg(II). This indicates that Hg(II) has catalytic effect on the reaction. In addition, the difference in absorbance signal (ΔA) between (neutral red + $\text{KBrO}_3 + \text{H}_2\text{SO}_4$) and (neutral red + $\text{KBrO}_3 + \text{H}_2\text{SO}_4 + \text{Hg(II)}$) is found to be proportional to the concentration of Hg(II).

3.4.1. Optimization of the physical parameters for flow-injection analysis

Effect of Hg(II) concentration on the ΔA was investigated experimentally. It is shown that the concentration of Hg(II) should be more than 0.3 ng mL^{-1} for the effective enrichment of Hg(II) by the functionalized silica gel. In order to reduce the relative error of the determinations, 800 ng mL^{-1} of Hg(II) solution was chosen for the optimization of the physical parameters for flow-injection analysis.

The pre-concentration time ranging from 10 to 800 s was examined. It is shown that the adsorption quantity of Hg(II) and the analytical signal (ΔA) increased with increasing pre-concentration time. The ΔA values become constant after pre-concentration for 680 s. It means that the micro-column used for pre-concentration of Hg(II) is saturated when the pre-concentration time is 680 s. In order to completely absorb the Hg(II) in the solution, the pre-concentration time must be less than 680 s. It is known that sampling frequency will be very small if the pre-concentration time is too long. In order to achieve a compromise between the sampling frequency and the sensitivity, 300 s was adopted as the pre-concentration time in this work.

The effect of the reaction coil length on ΔA was also investigated. The results indicated that ΔA values increased as the reaction coil length increased in the range of 240–270 cm, then

Table 3
Pre-concentration and recoveries of Hg(II)

Solution volume (mL)	Hg(II) in eluting solution (μg)	Recovery (%)	Pre-concentration factor
10	20.0	100	2.5
50	20.0	100	12.5
250	19.8	99.0	62.5
500	19.7	98.5	125
1000	19.7	98.5	250
2000	19.5	97.5	500

Table 4
Tolerance limit of interfering ions

Coexisting ion	Ratio [coexisting ions]/[Hg]	Relative error (%)
Na ⁺	2750	+3.4
K ⁺	2250	+4.7
Pb ²⁺	1375	+3.7
Mn ²⁺	1225	-3.9
Cd ²⁺	1150	-4.0
Co ²⁺	1350	-4.5
Bi ³⁺	215	-4.0
MoO ₄ ⁴⁺	200	+4.0
Fe ³⁺	1200	-4.0
Si ²⁺	595	-4.5
Ni ²⁺	1225	-4.7
NH ₄ ⁺	850	+4.4
NO ₃ ⁻	2500	+2.7
Zn ²⁺	1175	+4.3
As ³⁺	45	-3.4
CO ₃ ²⁻	1500	+4.2
SCN ⁻	375	+4.2
CH ₃ COO ⁻	3500	+3.9
Al ³⁺	1200	+4.4
VO ₃ ⁻	12.5	+4.2
Cu ²⁺	1000	+1.7
Mg ²⁺	1550	+2.5
Ag ⁺	175	+4.2

they decreased with increasing reaction coil length. Hence, the reaction coil length of 270 cm was selected.

Flow rate has great effect on the adsorption quantity and elution efficiency of Hg(II) and on the sensitivity of the method. The flow rate can be varied by using different inner diameters of the pumping tubes. If flow rate of the sample and eluent solutions was too fast, Hg(II) could not be adsorbed completely and the elution efficiency will be decreased. This results in the decreased sensitivity of the proposed analytical method. It is found that ΔA achieved the maximum when the flow rate of sample, eluent and reagents was 1.2, 1.6 and 2.1 mL min⁻¹, respectively.

Influence of reaction temperature on the catalytic reactions was studied in detail. ΔA was decreased with the increasing of the reaction temperature. In order to enhance the sensitivity of the determinations and to realize the convenient operation, room temperature was selected in the determinations.

3.4.2. Optimization of the reagent concentrations

3.4.2.1. Effect of eluent and reaction medium. Effect of the following reaction media on the analytical signal has been tried: hydrochloric acid, phosphoric acid and sulfuric acid. The results

indicated that among these media, the catalytic effect of Hg(II) was the strongest in sulfuric acid. At the same time, as a kind of eluent, the eluting power of sulfuric acid was better than the others. Therefore, sulfuric acid was used as both the reaction media and the eluent in the subsequent experiments.

Influence of sulfuric acid concentration was investigated in the range of 0.25–0.30 mol L⁻¹. The results showed that in the case of micro-column pre-concentration, the highest ΔA value was obtained in 0.27 mol L⁻¹ of sulfuric acid with good eluting effect.

3.4.2.2. Concentration effect of neutral red and potassium bromate. Influence of neutral red concentration on ΔA was studied in the range of 2.50–3.40 × 10⁻⁴ mol L⁻¹. It was found that 3.20 × 10⁻⁴ mol L⁻¹ is the optimum concentration of the chromogenic reagent.

The concentration of potassium bromate was also an important factor affecting the sensitivity. Different concentrations of potassium bromate were examined in the range of 0.15–0.20 mol L⁻¹. It is shown that 0.180 mol L⁻¹ of potassium bromate was the optimized concentration for the oxidizer.

3.4.3. Calibration curve, precision and detection limit for the determinations

Under the optimum experimental conditions, linear relationships between ΔA and the Hg(II) concentration were obtained in the range of 1–1500 ng mL⁻¹ for the determinations with the micro-column pre-concentration. The calibration curve was found to be $\Delta A = 0.079 + 3.851 \times 10^{-4} C$ with a correlation coefficient of 0.9997, where C is the concentration of Hg(II) in ng mL⁻¹. The relative standard deviation of 11 replicate determinations for 60 and 800 ng mL⁻¹ of Hg(II) was 3.0 and 2.1%, respectively. The detection limit, taken as three times the standard deviation of the reagent blank/slope, was found to be 0.9 ng mL⁻¹.

3.4.4. Effect of foreign ions

Effect of foreign ions on the determination of 0.8 μg mL⁻¹ of Hg(II) was investigated. Each substance was considered a non-interfere if it caused a relative error of less than ±5%, the permissible ratio of coexisting substance is summarized in Table 4. Obviously, the influence of many kinds of interfering ions is very small by use of the micro-column separation technique.

Table 5
Determination of Hg(II) in cigarette samples

Sample number	This method (μg g ⁻¹)	R.S.D. (%) (n = 5)	Recovery (%)	Fluorimetric method [21] (μg g ⁻¹)	t ^a
1	11.8	1.3	96.0	11.6	2.48
2	6.80	3.8	96.0	6.70	0.85
3	11.4	1.3	108	11.3	1.23
4	10.6	2.7	106	10.4	1.53
5	9.30	3.3	96.2	9.20	1.11
6	7.20	3.3	98.0	6.90	2.16

^a Theoretical value = 2.78, n = 5 with 95% confidence value.

Table 6
Other methods used for pre-concentration and determination of Hg(II)

LOD ($\mu\text{g L}^{-1}$)	Line range ($\mu\text{g L}^{-1}$)	Instrument	Pre-concentration factor	Reference
0.006	0.015–1.5	CVAAS	32	[25]
10–25 ng g^{-1}	10–2000	HPLC	80	[26]
0.05	0.13–25	CVAAS	200	[27]
0.2	–	GFAAS	100	[28]
0.39	–	ICP-OES	150	[29]
0.01	0.05–85.6, 90–9600	CVAAS	300	[30]
0.9	1–1500	Spectrophotometry	500	This work

3.5. Determination of Hg(II) in cigarettes

A quantity of 0.5000 g of six different cigarette samples, for which the filter tip was removed, was dipped in 5 mol L^{-1} of HNO_3 for 24 h, then filtrated and evaporated near to 4 mL. After 2 mL of 1.0 mol L^{-1} H_2SO_4 was added, the system was further evaporated until emitting white smog to remove HNO_3 . The solution was adjusted to be neutral pH by using 2.0 mol L^{-1} of NaOH and diluted to 100.00 mL with water. The reagent blank was treated in the same way. Finally, the mercury(II) in the cigarette samples was determined by the proposed analytical method. The results are summarized in Table 5, and compared with those determined by fluorimetric method [24]. The *t*-test shows no significant difference between results obtained by the two methods.

3.6. Comparison to other methods used for pre-concentration of mercury

Table 6 shows the sorbents reported in the literature for the pre-concentration and determination of Hg(II). Although they can be used for the effective adsorption and determination of Hg(II), there is scope for further improvement. The diphenylcarbazone-functionalized silica gel and flow-injection analysis described in this work showed high pre-concentration factor, regenerability and high selectivity.

4. Conclusion

The results obtained in this work show that compared with silica gel, the diphenylcarbazone-functionalized silica gel has the following advantages: (i) high stability in acids and many kinds of organic solvents; (ii) good selectivity for Hg(II) in the presence of other metals; (iii) good stability, the recovery percentage for Hg(II) was still more than 95% after reuse for 35 times; (iv) high pre-concentration factor of 500; (v) quite good enrichment capability for Hg(II).

The micro-column packed with the diphenylcarbazone-functionalized silica gel can be coupled with FIA. Therefore, a new method for the on-line separation, pre-concentration and determination of trace Hg(II) was established with the following main characteristics: (i) the enhanced sensitivity, by using the micro-column pre-concentration procedure, detection limit of the method has been decreased more than 40 times; (ii) improved selectivity, the tolerance ratio of most of interfering

ions has been increased several times; (iii) on-line operation, the whole procedure can be finished within 6 min from injecting sample to separation, pre-concentration and determination for each sample; (iv) reduced pollution, the reagent consumption is only 6.0 mL once a determination, and the possibility of the sample, reagents and environment being polluted was greatly reduced.

The proposed method in this work has been applied successfully to the determination of mercury in different kinds of cigarettes. It is suggested that this new method could also be applied to the determination of Hg(II) in other complicated samples.

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