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Development of polyacrylic acid-functionalized porous zinc sulfide nanospheres for a non-aqueous solid phase extraction procedure toward alkaloids

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Polymer-based cation exchanger and silica-based sorbents are generally used for the conventional solid phase extraction (SPE) toward alkaloids, because they provide both ion exchange interactions and strong hydrophobic interactions between the stationary phase and samples. However, strong hydrophobic interactions could cause the retention of some non-alkaloid compounds, to reduce the selectivity for alkaloids. In this paper, a non-aqueous solid phase extraction (SPE) procedure was developed and optimized utilizing novel polyacrylic acid-functionalized porous zinc sulfide nanospheres (PAA-PZNs) as the sorbents for the enrichment of alkaloids. The SPE sorbents were fabricated by the amidation reaction of poly-(acrylic acid) homopolymer with amino groups modified PZNs, which afforded an abundance of carboxyl groups, to effectively eliminate non-alkaloid compounds and concentrate alkaloids from the extracts. They exhibited not only high extraction efficiency, high selectivity and high recoveries for alkaloids, but also good chemical and mechanical stability. Therefore, PAA functionalized porous zinc sulfide nanospheres and subsequently prepared non-aqueous solid phase extraction (SPE) procedure may prove to be a strong tool for selective enrichment of alkaloids from extracts.

Introduction

Alkaloids, naturally occurring basic organic compounds, have attracted considerable attention, because a multitude of them are biologically active compounds used in the development of new drugs.[1, 2] However, acquisition of alkaloids is usually not easy owing to the low levels (often less than 1%) in crude plant extracts.[2] Furthermore, alkaloid extracts obtained from plants also contain many undesirable compounds co-eluted with the alkaloids during chromatographic procedures. Thus, a well-designed extraction procedure preceding is usually necessary to remove these co-eluted compounds from the alkaloids and enrich alkaloids.

Solid phase extraction (SPE) has proved to be an effective and powerful tool for sample pre-treatment due to several advantages, such as high recovery, short extraction times, high enrichment factor, facile elimination of solvents, and ease of automation.3 The extraction efficiency of SPE is directly related to the sorbent material, which also determines the selectivity and sensitivity of the method. The widely explored sorbents for SPE included C18 matrix,[4-6] graphene,[7] fullerenes,[8,9] carbon nanotubes,[10] glassy carbon,[11] and magnetic nanoparticles [12, 13]. However, these sorbents is lack of highly selectivity for the enrichment of alkaloids based on "like dissolves like" principle at trace levels especially in complex samples.[14,15] In crude extracts, alkaloids usually exist as positively charged compounds, while most other compounds are neutral or negatively charged, and therefore are barely retained by cation exchange interactions.[14] So, selectivity for the enrichment of alkaloids could be greatly improved in cartridges with electrostatic interactions between the sorbents and alkaloids.[16-18] At present, organic polymer-based strong cation exchanger (SCX) and silica-based SCX are already explored for alkaloids enrichment.[14,19] These exchangers offer both ion exchange interactions and strong hydrophobic interactions between the stationary phase and samples.[19] However, the strong hydrophobic interactions could cause the retention of some non-alkaloid compounds, thus the reduced selectivity for alkaloids could be occurred. Therefore, the development of a SPE sorbent possessed strong ion exchange interactions and relatively weak hydrophobic interactions is critical for the further enrichment of alkaloids.

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† Electronic supplementary information (ESI) available: The XRD and FT-IR spectra of PZNs.

Recently, zinc sulfide (ZnS) nanoparticles have attracted considerable attention for many applications such as phosphors, bioimaging, sensing, and photocatalysis.[20-25] Porous ZnS nanospheres (PZNs) exhibited hierarchical structure with porous features, no swelling and no shrinkage in solvents, and high mechanical strength,[26,27] thus, they should be possible an excellent sorbent for the enrichment of alkaloids from extracts. However, ZnS nanospheres, and especially porous nanostructures, remain relatively under-explored in terms of SPE until now.

On the other hand, many biologically active alkaloids are hydrophobic in nature. They have low solubility in aqueous, nevertheless, these lipophilic alkaloids have usually fine solubility in organic solvents. Thus, non-aqueous SPE methods are desired for the selective enrichment of the alkaloids. Moreover, non-aqueous also can reinforce unique ion-exchange selectivity based on the extent of ion-pair formation and the solvation states of solute ions in most cases.[28-31] In non-aqueous solvents, neutral species cannot be retained since hydrophobic interactions between the mobile phase and neutral species are sharply increased, far more than interactions between the stationary phase and samples. So, neutral or negatively charged species would be eluted during the sample loading procedure. Therefore, non-aqueous SPE methods based on cation-exchange could provide additional selectivity for the enrichment of alkaloids and minimize co-eluted. At present, non-aqueous ion exchange-based SPE methods have been successfully applied to the enrichment of acidic compounds, [30, 32] few studies have reported their application in alkaloids.[14]

This paper elaborates on the first successfully developed PAA-functionalized porous zinc sulfide nanospheres for a non-aqueous SPE procedure toward alkaloid enrichment, presented as shown in Figure 1(A). The SPE sorbents were fabricated by the amidation reaction of poly-(acrylic acid) (PAA) homopolymer with amino groups modified PZNs, which afforded an abundance of carboxyl groups, to effectively load the alkaloids. The PAA-functionalized PZNs (PAA-PZNs) is supposed to show high extraction efficiency for the alkaloids via the electrostatic interaction between samples and functional carboxy groups. To verify the hypothesis, two alkaloids and two neutral species (the structures see Figure 1(B)) were selected as model compounds to investigate the extract performance. The recoveries and chemical and mechanical stability of the SPE sorbent were also evaluated. The PZNs' application as an extract sorbent in an SPE column was validated in the selective enrichment of alkaloids.

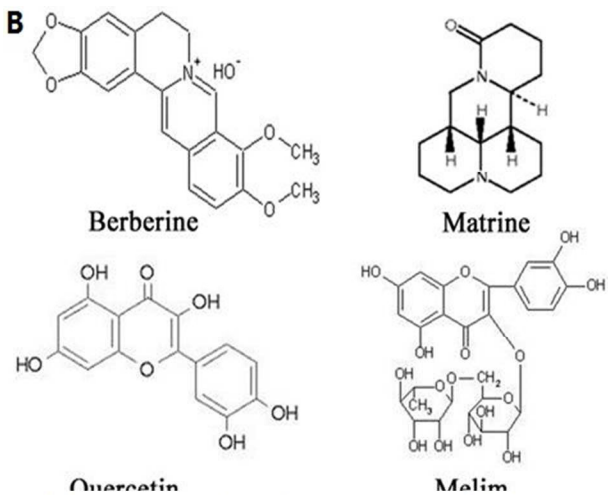


Figure. 1(A) Schematic mechanism for a non-aqueous solid phase extraction procedure based on functionalized porous zinc sulfide nanospheres toward alkaloid. (B) Structures of the selected alkaloids (upper row) and neutral species (lower row).

Experimental Section

Chemicals.

Cysteamine and gum arabic (GA) were purchased from Shanghai Aladdin-reagent Chemical Reagent Co., Ltd. Poly(acrylic acid) (PAA, Mw = 2000) and dimethylformamide (DMF) were obtained from Shanghai Aladdin reagent Co., Ltd. $ZnAc_2 \cdot 2H_2O$, thioacetamide (TAA) and other chemical reagents were purchased from Nanjing Chemical Reagent Co., Ltd. Matrine, berberine, quercetin and melim were obtained from Shanghai Sangon. Biotech.Co. Acetonitrile (ACN) and methanol were purchased from Merck (KGaA, Germany) and trifluoroacetic acid (TFA) was obtained from J&K (Hebei, China). Solvents were all of HPLC gradient grade. Water was prepared with a Milli-Q system (Billerica, MA, USA). Ammonium acetate was obtained from Shanghai Aladdin-reagent Chemical Reagent Co., Ltd.

Apparatus and Characterization

Transmission electron microscopy (TEM) images were recorded on a JEOL JEM-2010 CX with an accelerating voltage of 100 kV. Field emission scanning electron microscopy (FE-SEM) images were obtained using a JEOL 6701F field emission electron microscope at an accelerating voltage of 3.0 kV. TEM samples were prepared by dropping the samples dispersed in water onto carbon-coated copper grids with excess solvent evaporated. X-ray diffraction (XRD) measurements were performed on a Shimadzu XRD-6000 powder X-ray diffractometer, using Cu Ka (λ=1.5405 Å) as the incident radiation. The surface analysis was performed by nitrogen sorption isotherms at 77 K with a micromeritics ASAP2020 sorptometer. The surface areas were calculated by the Brunauer–Emmett–Teller (BET) method, and the pore size distributions were calculated by the Barrett–Joyner–Halenda (BJH) method. The measurement of the infrared spectroscopy

was performed using a Nicolet IR100 infrared spectrometer. Thermo-gravimetric analysis (TGA) was performed on a NETZSCH STA 449 C TGA instrument at a heating rate of 20 ℃• min^{-1} in a nitrogen flow from 100 to 750 ℃. UV-vis absorption spectra were obtained by a UV-3600 spectrophotometer (Shimadzu).

HPLC analytical experiments were conducted on a Waters Technologies 2695/2996 series system. SPE cartridge tubes (3 mL) were obtained from Beijing Q&Q Technologies Co.LTD. A Tigerkin C18 column (150 mm×4.6 mm, 5 μm) was used for the separation of alkaloids, and was purchased from Sipore Co. Ltd (Dalian, China).

Preparation of SPE sorbents based on functionalized PZNs

Porous ZnS nanospheres were firstly synthesized and functionalized via a hydrothermal route, similar to previous report.[21] In a typical procedure, first, 1 mmol $ZnAc_2$•$2H_2O$ was dissolved in 40 mL of water, and then 100 mg GA, 1.0 mmol TAA were added to prepare a clear mixed solution. The mixed solution was transferred into a Teflon-line autoclave and maintained at 120 ℃ for 12 h. The white solid products were collected by centrifugation and washed at least three times with ethanol and water. They were then dried in a vacuum at 40 ℃ for 10 h and obtained white powders. For the further surface modification, 100 mg of PZNs was re-dispersed in 50 mL of dehydrated ethanol and then purged with dry nitrogen for 30 min to exclude the oxygen in the ethanol. And then, 2.5 mmol of cysteamine was added into the above solution, and the mixture was gently stirred for 24 h in a sealed vessel. In the period, mercapto groups (SH) from cysteamine would tightly attach onto the surface of the PZNs due to the excess of metal ions with respect to sulfide ions at the surface of the nanospheres. The prepared amine-capped PZNs (NH_2-PZNs) were centrifuged and washed with ethanol three times to remove the residue of cysteamine. They were then dried in a vacuum at 40 ℃ for 10 h and obtained white powders. Subsequently, 120 mg of the prepared NH_2-PZNs powders were dispersed in 40 mL of DMF, and then 40 mg of PAA (Mw=2000) was added into the mixture. The reaction mixture was stirred at 140 ℃ for 2 h. After the reaction, the mixture was centrifuged and washed with copious ethanol. The washing procedure was repeated many times to completely remove the PAA physically adsorbed on the PZNs until the weight loss of PAA-PZNs (calculated by TGA) did not change. The resultant product was dried overnight in a vacuum at 45 ℃. The preparation of SPE sorbents based on functionalized PZNs was also shown in Figure 1(A).

SPE procedures

First, one of 3 mL solid-phase extraction cartridge was prepared by packing with 100 mg functionalized PAA-PZNs. An upper frit and a lower frit were used to avoid the loss of PAA-PZNs adsorbents. Then, the sorbents were packed in the cartridges by a special tool.

10 mg matrine and 10 mg berberine were each dissolved in 2 mL acetonitrile (ACN), and 10 mg quercetin and 10 mg melim were each dissolved in 2 mL methanol. Then, the standard solutions were placed in two 10 mL measuring flasks. The stock solution and working solutions were stored at 4 ℃ and brought to room temperature before use. The SPE cartridge was first conditioned with 3 mL ACN. After the application of ACN, a 2 mm layer of the solvent was allowed to remain above the column. Then, the standard solutions dissolved in ACN were loaded onto the columns, and allowed to interact for 5 min. After the loading of samples, the cartridge was eluted from the stationary phase with 3 mL of 100 mM CH_3COONH_4/ACN. This elution was performed at a rate of 1 drop of eluate/3 s, which corresponds to a flow rate of 0.6 mL/min, and the alkaloid-enriched fraction was collected for HPLC analysis.

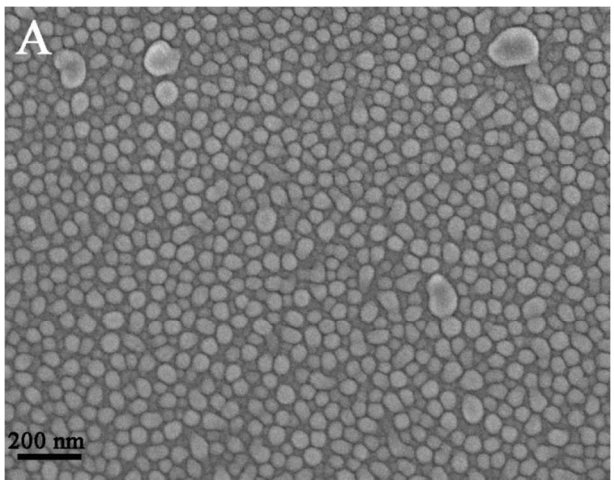
HPLC Analysis

The mobile phase for alkaloid separation was the mixture of ACN and 0.2% phosphoric acid solution (7: 3, v/v). The separation was performed on a Tigerkin C18 column with a flow rate of 1.0 mL•min^{-1}. The injection volume was 10 μL. The column temperature was controlled and held constant at 30 ℃.

Results and Discussion

Characterization of PAA-functionalized porous ZnS nanospheres.

As shown in the SEM and TEM image of Figure 2, the prepared PZNs present a well-defined and uniform spherical morphology with a mean diameter of approximately 60 nm. The high-resolution TEM image of one individual PZN in Figure 2 (C) exhibited detailed structural and crystallinity information. The distinct contrast within the PZNs was derived from the difference in electron density, indicating that the nanospheres exhibit a porous structure. The porous structure was apparently assembled by small primary particles with a diameter of about 4-5 nm. The primary particles display high crystallinity with clear lattice fringes. The adjacent lattice fringes spacing of 0.31 nm is analogous to the (111) plane of cubic ZnS.



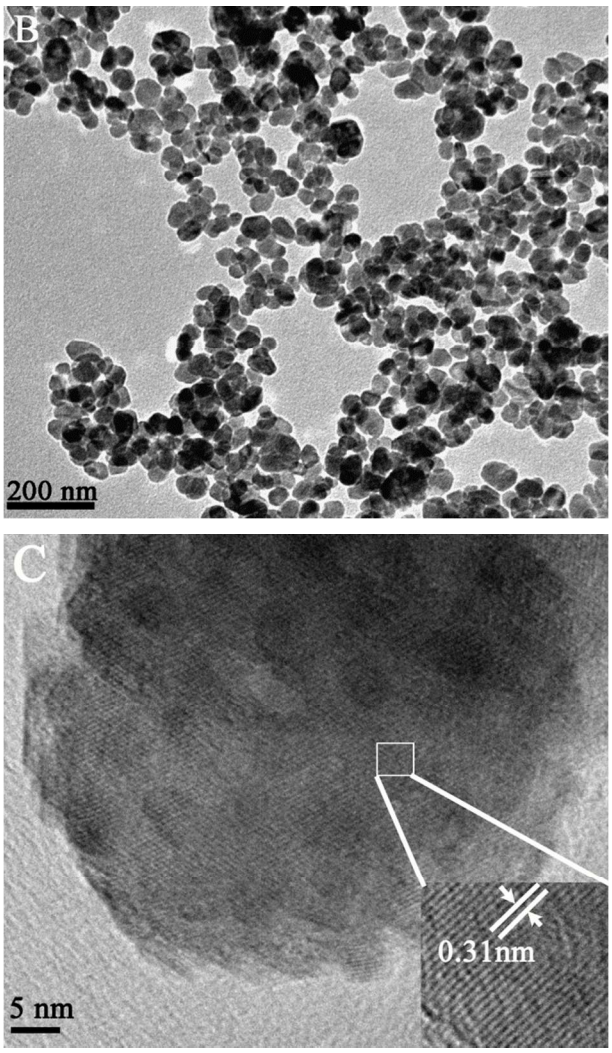


Figure. 2 (A) SEM image, (B) TEM image, (B) high-resolution image of PZNs.

The crystalline structure of the PZNs was determined with XRD as shown in Figure S1 (see the Supporting Information). All of the XRD peaks of the PZNs match well with the standard pattern of cubic zinc blend structure of ZnS (JCPDS file no. 01-0792). Calculations using the Debye–Scherrer formula for the strongest peak (111) showed grain sizes of 6.8 nm, which is in good agreement with the TEM result.

The porous structure of prepared PZNs was further confirmed by the N_2 absorption and desorption analysis. As shown in Figure 3 (A), the BET isotherms exhibited the characteristic type of I–V curves with a hysteresis loop generated by capillary condensation according to the IUPAC classification, which indicated that the PZNs possess uniform porous channels. The specific surface area and pore volume of the PZNs were calculated to be about 145 $m^2 \bullet g^{-1}$ and 0.47 $cm^3 \bullet g^{-1}$. As shown in the inset of Figure 3 (A), the average pore diameter of the nanospheres is about 5.5 nm. The relatively large surface area and pore volume strongly indicated that the nanospheres have a porous structure, which is attractive for loading samples and the following sustained release.

The successful grafing of PAA in ZnS nanospheres was confirmed by the Fourier transform infrared (FT-IR) spectra as shown in Supporting Information Figure S2. The detailed experimental data and discussion are in the Supporting Information. The grafted amount of PAA on PZNs was also estimated by Thermo-gravimetric analysis (TGA) in Figure 3 (B), and the graft ratio of PAA could be calculated to be about 11.5 wt%.

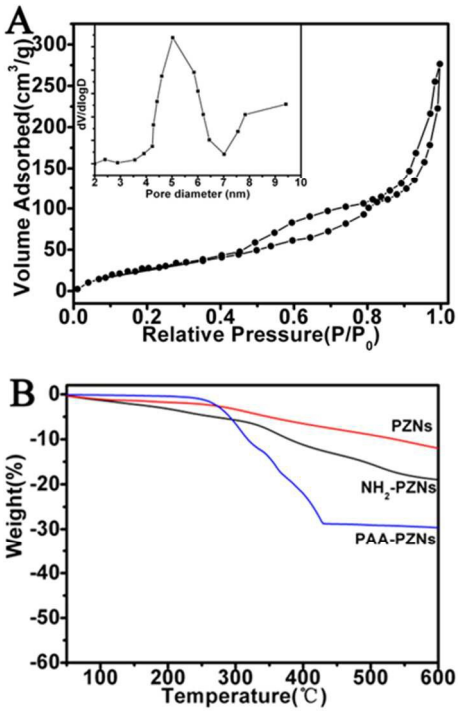


Figure.3 (A) Nitrogen adsorption–desorption isotherms and pore size distribution plot (inset) of PZNs. (B) TGA curves of PZNs, NH_2-PZNs, and PAA-PZNs.

Elution solvent effects.

Besides making use of suitable sorbents, another important step in the development of SPE procedure is the selection of an appropriate solvent to elute alkaloids retained on the stationary phase. Acetate salts are usually much more soluble in ACN than other salts, so we chose sodium acetate, potassium acetate and ammonium acetate as candidate elution salts, which are both frequently used for SCX.[33,34] A set of solvents including ACN, 100 mM CH_3COONa-ACN, 100 mM CH_3COOK-ACN and 100 mM CH_3COONH_4-ACN were employed for SPE elution. The results (See Figure. 4) showed that the elution efficiency for the two alkaloids (berberine and matrine) decreased directly in the following order: CH_3COONH_4-ACN, CH_3COOK-ACN, CH_3COONa-ACN, ACN, agreed well with previous reports.[14, 35] What's more, experimental results also showed that 4 mL of 100 mM CH_3COONH_4-ACN was sufficient to elute 2 mg each of berberine and matrine from the 100 mg PAA-PZNs SPE sorbents, and none of remnant alkaloids could be detected. However, for both CH_3COONa-ACN and CH_3COOK-ACN elution solvents, residual alkaloids were still detected on the stationary phase after the cartridge

was eluted with the same volume. Thus, ammonium acetate could be suitable for the elution of alkaloids from the PAA-PZNs stationary phase.

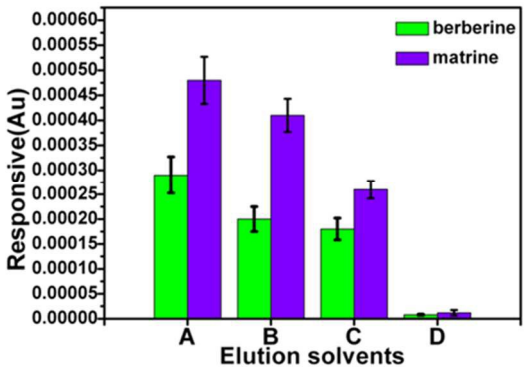


Figure.4 (A) The HPLC responsive of the fraction from the SPE eluted with different elution solvents. (Elution solvents: A. CH_3COONH_4-ACN; B. CH_3COOK-ACN; C. CH_3COONa-ACN; D. pure ACN.) Error bars mean the standard deviation. Each point was an average value of three independent measurements.

High extraction performance.

Two alkaloids (berberine and matrine) were selected as model compounds to investigate the extraction performance of the novel PZNs sorbents. Standard alkaloids were dissolved in ACN and loaded onto the PAA-PZNs cartridge. Then, the alkaloid-enriched cartridge was eluted with 100 mM CH_3COONH_4-ACN solution. All fractions obtained from the SPE procedure were analyzed by HPLC, and the chromatograms are shown in Figure 5. Berberine and matrine were only detected in the fraction eluted with 100 mM CH_3COONH_4-ACN, while both of them could not be found in the fraction during sample loading, which indicated that the PAA-PZNs sorbents posses strong adsorption and the CH_3COONH_4-ACN solution has excellent eluting power for alkaloid. Obvious enhancement of the peak heights was found in the SPE chromatograms compared with the direct injection of the two alkaloids, which almost could not be detected, also indicating the remarkable extraction efficiency of the PAA functionalized PZNs sorbents for the alkaloids (See Figure.5 (A), b, c and Figure.5 (B), b, c). Moreover, high recoveries were also obtained for both berberine (96.8%) and matrine (97.4%). Functional carboxy groups on the huge surface of the ZnS nanospheres enable the SPE stationary phases selectively extract alkaloid species containing amino groups. Electrostatic interactions are believed to be the primary interactions for this type of SPE cartridge.

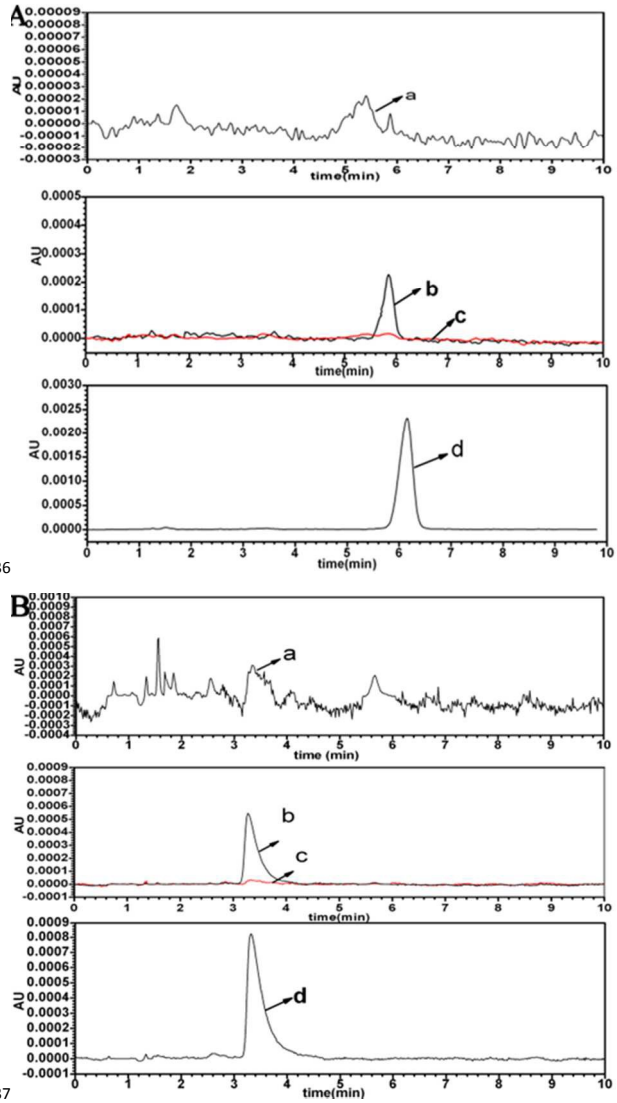


Figure. 5 (A) Comparison of chromatograms between (a) the fraction obtained from the SPE during sample loading of berberine at 0.01 μg/mL; (b) the fraction eluted with 100 mM CH_3COONH_4-ACN; (c) the direct injection of 10 μL of berberine at 0.01μg/mL and (d) the direct injection of 10 μL of berberine at 0.5 μg/mL. The mobile phase for matrine separation was the mixture of ACN and 0.2% phosphoric acid solution (7:3, v/v). The separation was performed on a 5 μm Tigerkin C18 column, 150 mm×4.6 mm I.D. with a flow rate of 1.0 mL•min^{-1}. The injection volume was 10 μL. The column temperature was controlled and held constant at 30 ℃. Wavelength was 275 nm. (B) Comparison of chromatograms between (a) the fraction obtained from SPE during sample loading of matrine at 0.1μg/mL; (b) the fraction eluted with 100 mM CH_3COONH_4-ACN; (c) the direct injection of 10 μL of matrine at 0.1μg/mL and (d) the direct injection of 10 μL of berberine at 1 μg/mL. The mobile phase for matrine separation was the mixture of ACN and 0.2% phosphoric acid solution (8:2, v/v). The separation was performed on a 5 μm Tigerkin C18 column, 150 mm×4.6 mm I.D with a flow rate of 1.0 mL•min^{-1}. The injection volume was 10 μL. The column temperature was controlled and held constant at 30 ℃. Wavelength was 210 nm.

The non-aqueous SPE procedure based on PAA-PZNs could also offer an additional advantage, to a relatively large volume of solvent extract can be injected directly onto the SPE column with no loss of efficiency. Berberine and matrine were dissolved in different volumes of ACN and transferred onto a 100 mg PAA-PZNs cartridge. Table 1 shows recoveries

obtained for different large sample volumes. Even though a 500-fold column volume of solvent extract was loaded directly onto the SPE column, high recoveries were still maintained from 96.4% to 102.3% both for berberine and matrine. Clearly, this SPE procedure of PAA-PZNs can be utilized to concentrate alkaloids from large sample volumes.

Table 1 Recoveries against the ratio of sample volume to SPE column volume for berberine and matrine

Ratio of sample volume to SPE column volume	Recoveries for berberine	Recoveries for matrine
10	97.4%	96.4%
20	98.6%	95.2%
50	97.5%	98.9%
100	98.3%	99.2%
150	99.4%	98.1%
200	100.9%	102.3%
500	96.8%	98.7%

Two neutral species, quercetin and melim (with structures as shown in Figure. 1(B)), were selected as the reference compounds to investigate the selectivity of the PAA-PZNs SPE. As shown in Figure 6 (a), the peak heights for berberine and each of the two reference compounds, quercetin and melim, in the direct injection were comparable. However, after extraction by the PAA-PZNs SPE procedure, peak height of berberin was much higher than those of the direct injection of the sample, while reference compounds, both quercetin and melim could not be detected in the fraction eluted solution as shown Figure 6 (c), which indicated that the PAA-PZNs sorbents revealed high selectivity to the berberine due to effectively eliminate non-alkaloid compounds and concentrate alkaloids from the extracts. The peaks owing to the reference compounds were detected from the fraction during SPE sample loading in Figure 6 (b), to indicate the PAA-PZNs sorbents possess no adsorption for the two neutral species, which also confirmed the result of high selectivity. High selectivity is attributed mainly to the strong electrostatic interaction between amino groups in alkaloid and carboxy groups in the PAA-PZNs sorbents.

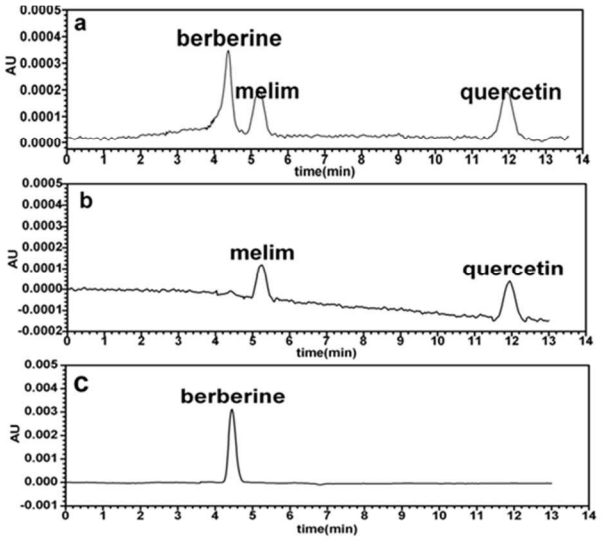


Figure. 6 Comparison of chromatograms between (a) the direct injection of 10 μL mixture solution of berberine at 0.1μg/mL and each of two reference compounds, melim and quercetin at 0.1μg/mL; (b) the fraction obtained from the SPE during sample loading; (c) the fraction eluted with 100 mM CH_3COONH_4-Methanol. The mobile phase for separation was the mixture of Methanol and 0.2% phosphoric acid solution (55: 45, v/v). The separation was performed on a 5 μm Tigerkin C18 column, 150mm×4.6mm I.D with a flow rate of 1.0 mL•min^{-1}. The injection volume was 10 μL. The column temperature was controlled and held constant at 30 ℃. Wavelength was 280 nm.

Chemical and mechanical stability of the PAA-PZNs SPE sorbents.

Chemical and mechanical stability of the PAA-PZNs SPE sorbents were also investigated by comparison of extraction efficiency after the PAA-PZNs SPE sorbents were immersed into distilled water, methanol, acetonitrile, hexane and dichloromethane for 2 h, together with a solvent free control. As shown in Figure 7, peak areas of extract are almost no difference after in the treatment of above different solvent, to indicate the sorbents exhibit good endurance ability towards both the organic solvent and water. What's more, obvious decrease in extraction ability was also not observed using PAA-PZNs sorbents more than 120 times. The resistance of the prepared PAA-PZNs SPE sorbents to water and the organic solvents indicates the high chemical and mechanical stability. This remarkable chemical stability makes the PAA-PZNs a suitable alternative for SPE coupled to HPLC, since desorption of analytes from the SPE sorbents in HPLC generally involves various liquid solvents.

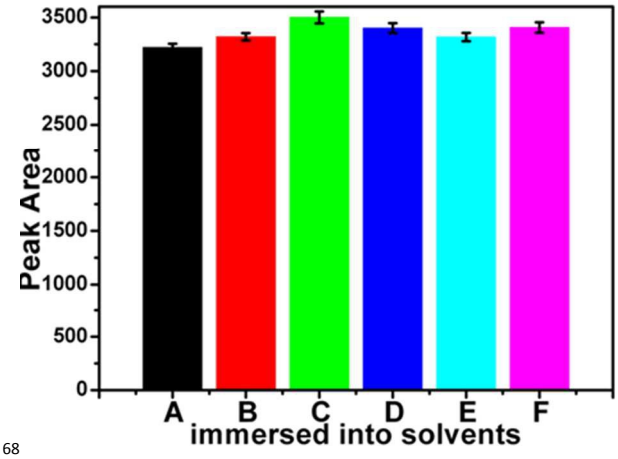


Figure. 7 Comparison on the peak area of berberine extract on the PAA-PZNs sorbents after in the treatment of different solvent including water (A), methanol (B), acetonitrile (C), hexane (D), dichloromethane (E) for 2 h and (F) without treatment. Error bars mean the standard deviation. Each point was an average value of three independent measurements.

Adsorption capacity.

The adsorption capacity of the PZNs SPE sorbents was also determined. The berberine was diluted in acetonitrile to obtain a set of eighteen standard solutions ranged from 0.1 to 50 mg·mL^{-1}. Then, the PZNs-SPE protocol was applied to each standard solution. The sample volume loaded was fixed (1 mL). The fraction recovered at each elution step was collected and analysed by HPLC-UV. Then, the amount of berberine

recovered during the elution step was plotted against the amount of berberine loaded on SPE cartridge as shown in Figure 8. At low amounts of berberine (0.1–2 mg), the capacity curve was linear with a slope close to unity (0.997), which indicated that the recovery of berberine was then almost approximated in 99%, and the adsorption sites having the highest affinity are occupied first by target molecules. When the amount of loaded analyte increased, recoveries decreased steadily, indicating gradual saturation of the PZNs sorbents. The capacity curve reached a limit amount when loaded-berberine was higher than 24 mg, and then the maximum amount of berberine retained by the PZNs was estimated at 5.6 mg. So, the adsorption capacity of the PZNs was calculated as 56 $mg \cdot g^{-1}$ by specific interactions on a 100 mg PZNs-cartridge.

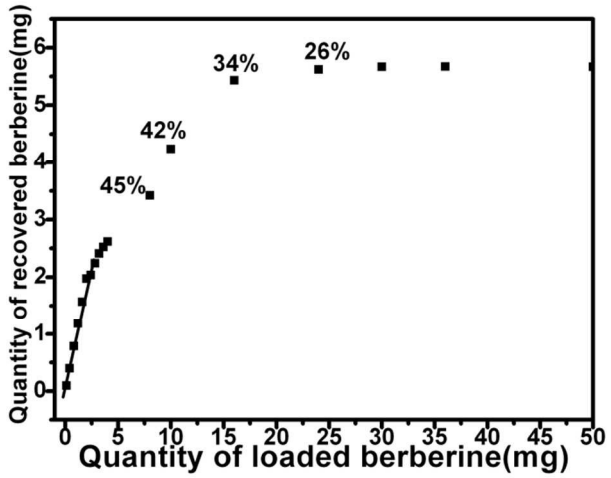


Figure 8. Capacity curve obtained after loading increasing amounts of berberine on the PZNs SPE cartridges packed with 100 mg-amount of sorbent. Loaded volume of berberine solution in acetonitrile: 1mL. The partial recoveries (%) obtained after elution step are given in the graph

In addition, its performance was compared with that of commonly used reserved-phase sorbent materials of C18 silica. Comparing the peak areas of the two alkaloids, quercetin and melim, the quantitative values of the alkaloids calculated using the current method were higher (2.3 times to 2.5 times) than those obtained using C18 silica sorbents. The higher adsorption capacity of the two alkaloids can be attributed to the the strong electrostatic adsorption of the PZNs, while C18 silica provide strong hydrophobic interactions.

Conclusions

In this study, we developed a highly selective, chemically and mechanically robust PAA-PZNs sorbents for a non-aqueous SPE procedure. The proposed sorbents exhibited high extraction efficiency for alkaloids owing to highly functionalized molecules containing plenty of carboxy groups via electrostatic interactions. Moreover, high recoveries and chemical and mechanical stability were obtained for enrichment of alkaloids. This non-aqueous SPE procedure has the potential to be utilized to enrich alkaloids from plants and extracts.

Acknowledgements

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