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Temperature-response polymer coating for in-tube solid-phase microextraction coupled to high-performance liquid chromatography

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ABSTRACT

The silica nanoparticle (SiO_2 NP)-deposited capillary fabricated by liquid phase deposition (LPD) was bonded by 3-(triethoxysilyl) propyl methacrylate and then modified with poly(N-isopropylacrylamide) (PNIPAAm) by polymerization. The resulting PNIPAAm modified SiO_2 NP-deposited capillary was applied to in-tube solid-phase microextraction coupled to high-performance liquid chromatography (in-tube SPME–HPLC). To investigate the extraction performance of the prepared capillary, diethylstilbestrol (DES) with moderate polarity was selected as the model analyte. Results demonstrate that PNIPAAm modified SiO_2 NP-deposited capillary exhibited obvious temperature responsive character. Finally, the PNIPAAm modified SiO_2 NP-deposited capillary was applied to the analysis of three synthetical estrogens from milk samples. The detection limit of the method was found to be in the range 1.2–2.2 ng/g, and recovery was 71.7–98.9% with relative standard deviations in the range of 2.8–12.6%.

1. Introduction

Since it was proposed in 1997, in-tube solid-phase microextraction (in-tube SPME) technique has been widely utilized [1,2]. By integrating sample extraction, concentration, and injection into one step, in-tube SPME become an suitable sample preparation technique prior to HPLC and HPLC–MS [3]. Since it is fast to operate, easy to automate, solvent-free and need of small volume of the samples, in-tube SPME has been gained much attention.

Up to now, several kinds of capillaries have been developed for in-tube SPME [4,5]: inner-wall coated capillary [6,7], fiber inserted capillary [8,9], particle–packed capillary [10,11], and monolithic capillary [12–14]. Among them, coated capillary was the first one utilized in the in-tube SPME–HPLC. Due to its good reproducibility, as well as ideal mechanical stability and permeability to adopt high sampling flow rate, it is still attracting wide interests until now. However, its large dead volume and the small amount of coating in capillary result in low extraction efficiency. Therefore, the development of the coating with good extraction capacity for the coated capillary in in-tube SPME–HPLC method is of great importance [15].

To extract the analytes at low concentrations in complicated matrix, the materials with great sorption capacity or good selectivity towards concerned analytes could be applied as extraction media. For in-tube SPME, increasing the extraction efficiency can be achieved by enhancing the stationary phase loadings. A direct method is to increase the coating thickness [16]. However, a long equilibrium time was required. Another solution is to use thinner coatings with large available surface area, which will provide enhanced extraction efficiency and help faster mass transfer during extraction and desorption processes for analytes [17]. Therefore, increasing the surface area is one effective method to improve the extraction efficiency.

Ordered mesoporous coating has becoming a favorable extraction media for in-tube SPME because of its large surface area, which will provide improved extraction capacity [18]. Meanwhile, sol–gel technology was applied successfully to prepare different SPME coatings [6,7,19–25]. In our previous work, a titanium dioxide (TiO_2) nanoparticle-deposited capillary and a silica nanoparticle (NP)-deposited capillary were prepared by liquid-phase deposition (LPD) [15,26,27] for SPME; and LPD was demonstrated to be a new potential approach to prepare coatings with high extraction capacity for in-tube SPME.

Intelligent materials can respond to environmental conditions, such as pH [28], temperature [29], and light [30,31]. These materials possess great potential in many aspects, such as drug delivery [32–35], enzyme immobilization [36,37], biomaterials separation and purification [38,39].

As an extensively studied polymer, poly(N-isopropylacrylamide) (PNIPAAm) may undergo reversible phase transition when being exposed to different temperature environments. It exhibits a lower critical solution temperature (LCST) in aqueous solution which is about 32 °C [40,41]. The PNIPAAm polymer contains both hydrophilic imido groups and hydrophobic isopropyl groups. When the temperature is lower than LCST, the

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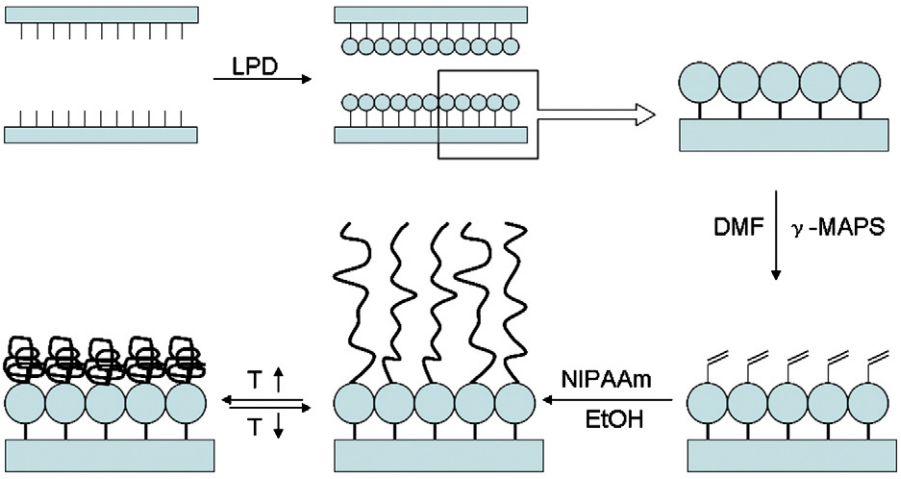

Fig. 1. Schematic illustration for the preparation of temperature-responsive polymer grafted onto silica nanoparticle-deposited capillary and the relationship between the structure change of poly NIPAAm and temperature change.

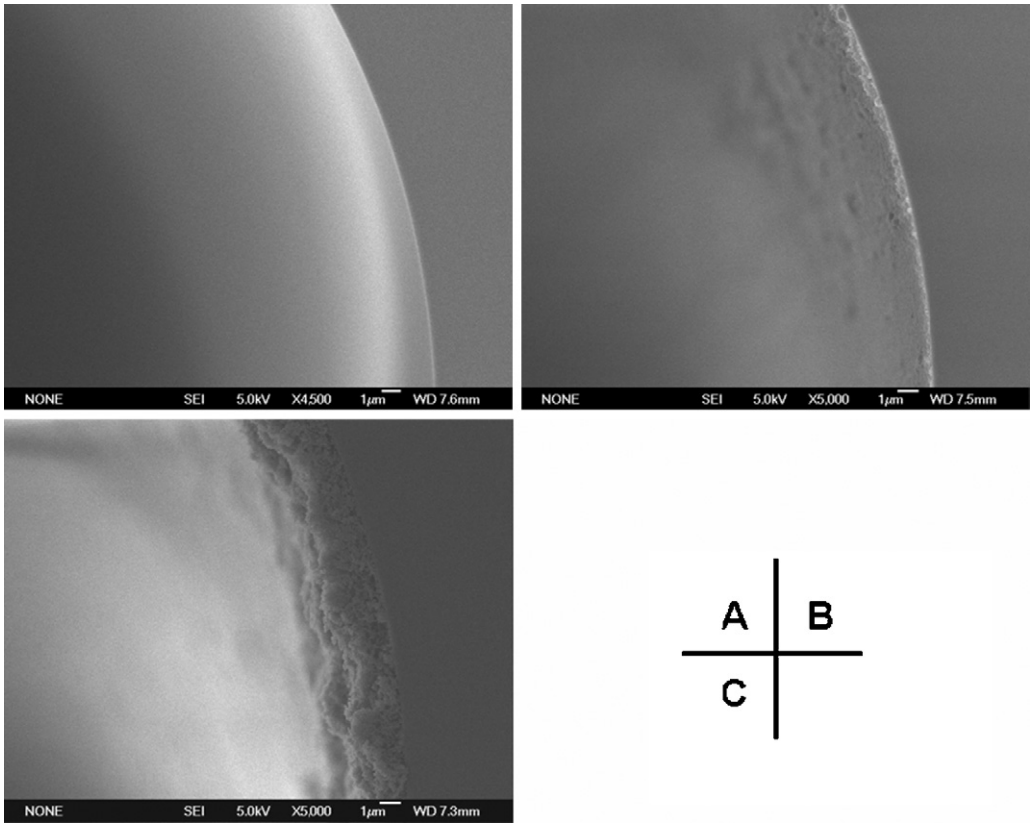

Fig. 2. Scanning electron microscopy images of the inner surfaces of capillaries: (A) without deposition of SiO_2; (B) after deposition; (C) after modified with PNIPAAm.

hydrophilic groups in PNIPAAm can combine with water through hydrogen bonding and the polymer chains stretch, endowing the PNIPAAm hydrophilic property. However, when the temperature is higher than LCST, the hydrophobic interaction between polymer chains increases, leading to the polymer chains curl up. So, the resultant material which is prepared with PNIPAAm as modifier would possess switchable hydrophobic–hydrophilic characteristic just by simply altering the environmental temperature.

Poly(N-isopropylacrylamide) (PNIPAAm) has been coated on the surface of silica gel [42] and grafted onto monolithic material as HPLC stationary phases [43,44]. Simultaneously, NIPAAm was also used as a functional monomer to prepare acrylamide based monoliths [45–47]. However, to the best of our knowledge, the application of temperature-response polymer coated capillary to in-tube SPME has not been reported.

Diethylstilbestrol, dienoestrol and hexestrol are a group of estrogens with similar structures and widely applied in livestock production to promote growth rate or used as oral contraceptives. Diethylstilbestrol and hexestrol in animal husbandry are often used as feed additives to increase milk production. The dienoestrol has been confirmed to be the metabolite of diethylstilbestrol. Because of their carcinogenic effect, the application has been banned in EU and limited in such countries as United States and Canada. Their content in milk has been regulated to limited quantities.

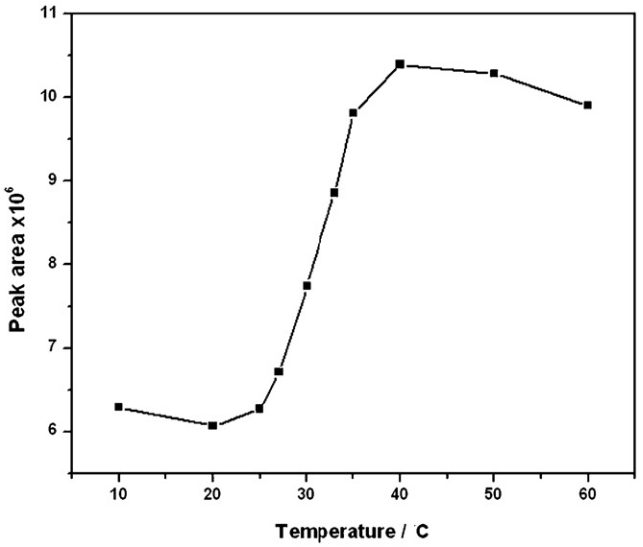

Fig. 3. Effect of temperature on the extraction efficiency of diethylstilbestrol.

Therefore, the detection of these three estrogens in milk is necessary.

In the present paper, a silica nanoparticle (NP)-deposited capillary prepared by LPD, followed by the modification with poly(N-isopropylacrylamide) (PNIPAAm), was used as an extraction medium for in-tube SPME. A simple, rapid and automated method for the analysis of three estrogens in milk sample was established using PNIPAAm modified SiO_2 NP-deposited capillary as an extraction media for in-tube SPME–HPLC system.

2. Experimental

2.1. Chemicals and materials

3-(Triethoxysilyl)propyl methacrylate (γ-MAPS) and tetraethoxysilane (TEOS, 98%) were both from Wuhan University Chemical Plant (Wuhan, China). N-Isopropylacrylamide (NIPAAm, 98% pure) purchasing from TCI (Tokyo, Japan) was purified by recrystallization from hexane and was dried at room temperature in vacuum. Azobisisobutyronitrile (AIBN) was purchased from Sigma (St. Louis, MO, USA). N,N-dimethylformamide (DMF), methanol (MeOH), sodium hydroxide (NaOH), hydrofluosilicic acid (H_2SiF_6, 35% weight), irregular silica (50–74 μm), boric acid (H_3BO_3), hydrochloric acid (HCl), ethanol, and acetonitrile (MeCN) were all purchased from Sinopharm Chemical Reagent Co. (Shanghai, China) and were of analytical reagent grade. Purified water was obtained using an Aike water purification system (Chengdu, China). Diethylstilbestrol (DES), dienestrol (DIEN) and hexestrol (Hex) were obtained from the Aladdin reagent Co. (Shanghai, China).

A stock solution of 1 mg mL^{-1} for each analyte was prepared in MeCN, and each set of standard mixture containing 50 μg/mL of each analyte was prepared by diluting the stock solution with MeCN. All the standard solutions were stored at 4 °C in the dark. The sample solution was spiked with the standard solution to the desired concentration for experiments. Fused-silica capillaries with 100 μm i.d. × 365 μm o.d. were obtained from Yongnian Fiber Plant (Hebei, China).

2.2. Preparation of PNIPAAm-grafted silica NP-deposited capillary

Schematic illustrations for the preparation of PNIPAAm-grafted silica NP-deposited capillary and the relationship between the structure change of the prepared capillary and temperature change were shown in Fig. 1. Briefly, the inner surface of the capillary was first deposited with the film consisting of silica nanoparticles by LPD and then chemically modified with 3-(triethoxysilyl)propyl methacrylate (γ-MAPS). At last, the γ-MAPS-bonded silica nanoparticles was modified with poly(N-isopropylacrylamide) (PNIPAAm) through the radical initiating polymerization. The temperature responsive effect of the polymer chains is also displayed in Fig. 1.

Typical preparation procedure is as follows:

The fused-silica capillary was activated at ambient temperature by rinsing sequentially with 1.0 mol L^{-1} NaOH for 2 h, water for 30 min, 1.0 mol L^{-1} HCl for 4 h and water for 2 h, then dried at 160 °C with nitrogen gas for 10 h.

The precursor solution for LPD was prepared according to the procedure reported by Li et al. [15]. Briefly, 3.5 g silica powder was added to 100 mL of the 35% H_2SiF_6 to saturate the solution. After stirring at 35 °C for 16 h, it was centrifuged to remove the undissolved silica. Subsequently, 1.0 mL of the saturated solution was measured into a clean centrifuge tube, and then 0.5 mL of water and 0.6 mL of 0.1 mol L^{-1} H_3BO_3 was added one after the other. The mixture was vortexed for about 1 min and thus used as the precursor solution. Then the solution was filled into the activated capillary. Both ends of the capillary were sealed by silicon rubber for LPD at 40 °C controlled in a thermostat water bath for 16 h. After deposition, the capillary was washed with water and dried at 120 °C for 4 h under constant N_2 flow. Then the second and third depositions were performed in the same way. Finally, the deposited capillaries were calcined in a muffle furnace by heating at a rate of 1 °C/min to 300 °C and holding for 2 h to age the silica film.

The functionalization of the silica NP-deposited capillaries was performed as follows [15]: The capillaries were activated by 1.0 mol/L HCl at 60 °C for 8 h, and then rinsed with water and dried at 160 °C under N_2 flow for 10 h. The 3-(triethoxysilyl) propyl methacrylate dissolved in DMF was filled in the capillary with both ends sealed with silicon rubber immediately. The reaction was performed at 70 °C for 12 h, followed by rinsing the capillary with MeOH to remove the residual components. A mixture of NIPAAm (0.3 g) and AIBN (3 mg) in 1 mL anhydrous ethanol was completely mixed by vortexing and ultrasonication to form a homogeneous solution. Then the solution was filled into the capillary. Both ends of the capillary were sealed by silicon rubber for polymerization at 60 °C for 12 h. Finally the capillary was washed with methanol and then purified water.

For comparison, the capillary without treatment by LPD (blank capillary) was modified according to the same procedures above and named to be PNIPAAm-modified fused-silica capillary.

Though the preparation procedures of PNIPAAm-grafted silica NP-deposited capillary seemed tedious and time-consuming, the procedures are easy-to-manipulated.

2.3. Apparatus and analytical conditions

The morphology of the inner surfaces of capillaries was displayed by a JSM-6700F field emission scanning election microscope (JEOL, Japan). As described in the previous paper [48], the in-tube SPME–HPLC system is an on-line extraction process that is achieved through the use of two six-port valves and two groups of liquid chromatographic pumps. The whole system (Shimadzu, Kyoto, Japan), which consisted of a pre-extraction segment and an analytical segment, was manipulated automatically via the LCMS solution Ver 3 software and a CBM-20A communications bus module. The pre-extraction segment included a FCV-12AH valve unit (valve 1), a LC-20AD pump and a sample loop (2.0 mL); the analytical segment was composed of a FCV-12AH valve unit (valve 2, on which a 100 cm long piece of the extraction capillary was installed), a

LC-20AD pump, a DGU-20A3 degasser, a CTO-20AC column oven and a SPD-20A UV-detector. Valve 1 and valve 2 were connected by one peek tube. In desorption procedure, mobile phase for HPLC was used as desorption solution, and the desorption flow rate was set at 0.02 mL min^{-1}. The analytical column was an Ultimmate XB-C18 column (250 mm × 4.6 mm i.d.; 5 μm). The mobile phase was MeOH–water (65:35, v/v) with a flow rate of 1.0 mL/min. Detection wavelength was set at 280 nm.

2.4. Preparation of milk samples

Sample preparation was done using slightly modified methods by Lin et al. [49]. Milk samples were purchased from the local retail market and were stored in frozen −20 °C before use. The milk samples were diluted with 4.0 mL MeCN in centrifuge tubes. After mixing with a ultrasonic bath (KS-3000 Ultrasonic cleaner, Ningbo, China) for 5 min, the samples were centrifuged at −4 °C for 10 min at 10,000 rpm (Anting Scientific Instrument Co., Shanghai, China). 4 mL of the supernatant was filtered through a 0.22 μm pore filter and dried with N_2. The residues were dissolved with deionized water before injection.

3. Results and discussion

3.1. SEM characterization

In this paper, field emission scanning electron microscope was performed to examine the microscopic morphology of the PNIPAAm modified silica nanoparticle (SiO_2 NP)-deposited capillary. Prior to measurement, the capillary samples were cut into 2 mm long pieces, placed on an aluminum stub and then sputter-coated with gold. Fig. 2A shows a smooth inner surface of the bare fused-silica capillary, and Fig. 2B shows the deposited silica NP onto the inner surface of capillaries after 3 repetitions of the LPD process. It can be seen that uniform and dense silica nanoparticles (<100 nm) are formed on the originally smooth surface dispersedly after deposition (Fig. 2B). Fig. 2C shows the deposited silica NP after polymerization. 1 μm thickness layer of granular polymer attached to the capillary inner surfaces was observed in Fig. 2C.

3.2. Temperature responsive performance of PNIPAAm modified SiO_2 NP-deposited capillary

Because of PNIPAAm's temperature-responsive properties, it can show different hydrophobic or hydrophilic characters at different temperatures. In this paper, diethylstilbestrol was chosen as a modal analyte to investigate the extraction performance of PNIPAAm modified SiO_2 NP-deposited capillary at different temperatures.

The effect of temperature on extraction efficiency towards diethylstilbestrol in deionized water (200 ng/mL) was investigated using PNIPAAm modified nano-silica coated capillary as extraction medium for in-tube SPME. Fig. 3 showed the extraction efficiency towards diethylstilbestrol at various temperatures (10–60 °C). It demonstrated that the extraction efficiency towards diethylstilbestrol decreased slightly when the temperature was changed from 10 °C to 20 °C, while the extraction efficiency increase rapidly when temperature continued to rise from 20 °C to 40 °C. However, the temperature was increased above 40 °C, the extraction efficiency showed a slight decrease.

The underlying mechanism of this temperature-responsive extraction medium may be concerned with Lower Critical Solution Temperature (LCST) of PNIPAAm. Since the LCST of PNIPAAm is 32 °C, the isopropyl group with hydrophobility exposed and the hydrophobility of capillary inner surface increased when the temperature is higher than PNIPAAm's LSCT. The hydrophobic interaction between the diethylstilbestrol and the extraction media played a major role in the extraction, which resulted in rapid increase of the extraction efficiency from 20 °C to 40 °C. This demonstrated that PNIPAAm has been modified successfully to the inner surface of the capillary and exhibited obvious temperature responsive property.

Nanosized particles were reported to have high specific surface area [15] and, hence, relatively high enrichment capability was expected when they were employed in SPME. In order to further demonstrate the advantages of the PNIPAAm modified SiO_2 NP-deposited capillary, the extraction efficiency towards diethylstilbestrol was also investigated with the blank capillary and PNIPAAm-modified fused-silica capillary.

The extraction performances of blank capillary, PNIPAAm-modified fused-silica capillary and PNIPAAm modified SiO_2 NP-deposited capillary at 25 °C and 40 °C have been investigated. It has been reported that the extraction performance of extraction coatings can be characterized by the slopes of standard calibration curves [50]. Therefore, the extraction performance of the three capillary can be compared by the slopes of standard calibration curves of diethylstilbestrol. As can be seen from Table 1, the blank capillary did not exhibit remarkable difference in extraction efficiency for diethylstilbestrol at different temperatures, while two kinds of PNIPAAm modified capillaries at 40 °C showed higher extraction efficiency than that at 25 °C. These results demonstrated that the two PNIPAAm modified capillaries showed temperature-responsive performance. As shown in Table 1, PNIPAAm modified SiO_2 NP-deposited capillary exhibited higher extraction efficiency towards diethylstilbestrol than the PNIPAAm-modified fused-silica capillary whether under the low or high temperature. This result may be due to that nanosized particles on the inner-wall of the capillary can provide higher specific surface area and then more surface coverage of PNIPAAm, leading to higher enrichment capability towards diethylstilbestrol.

3.3. In-tube SPME performance of PNIPAAm modified SiO_2 NP-deposited capillary

In view of the importance of residue analysis of such estrogens as DES, DIEN and Hex, the in-tube SPME–HPLC system was applied to the simultaneous determination of the three estrogens from milk using the PNIPAAm modified SiO_2 NP-deposited capillary.

3.3.1. Profile of extraction volume

In order to assess the extraction capacity of PNIPAAm modified SiO_2 NP-deposited capillary, the extraction volume profile for DES, DIEN and Hex was obtained by extracting 200 ng/mL sample solution for progressively extraction volume. The flow rate of the carrier solution was kept at 0.05 mL/min, and the sample volume was increased from 100 μL to 2000 μL. The peak area of each analyte was recorded and the amount of extracted analyte increased greatly with the increasing sample volume until to 1500 μL. The results demonstrated that the PNIPAAm modified SiO_2 NP-deposited capillary exhibits satisfactory extraction capacity for the three estrogens. To achieve sufficient sensitivity within a short time, extraction volume of 800 μL was selected for the subsequent analysis.

3.3.2. Optimization of in-tube SPME

In most in-tube SPME, the configuration requires a mobile phase composition that provides complete desorption of the extracted analytes from the coating, while still providing the proper separation of the three analytes in the analytical column. It is necessary to optimize the mobile phase for separation and desorption by extracting 800 μL of 200 ng/mL sample solution at the extraction flow rate of 0.1 mL/min. Different methanol content and

Table 1
Comparison of the slopes of standard calibration curves of diethylstilbestrol at (a) 25 °C and (b) 40 °C.

Extraction media	25 °C	40 °C	Difference in slopes
Blank capillary	0.0038	0.0039	0.0001
PNIPAAm-modified fused-silica capillary	0.0109	0.0136	0.0027
PNIPAAm modified SiO_2 NP-deposited capillary	0.0131	0.0214	0.0084

The diethylstilbestrol was spiked at 200 ng/mL in water. The extraction volume was from 100 μL to 500 μL.

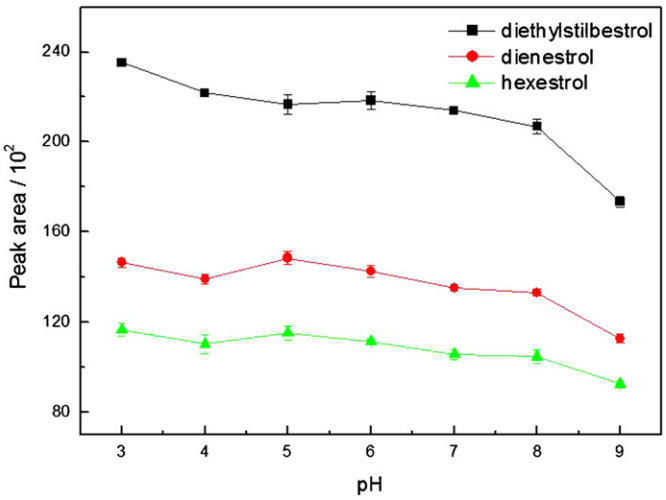

Fig. 4. Effect of the pH in the sample matrix on the extraction efficiency. Sample solutions of three estrogens spiked at 200 ng/mL were prepared with 5 mmol/L phosphate buffer at pH 3.0–9.0. Extraction time was 5 min.

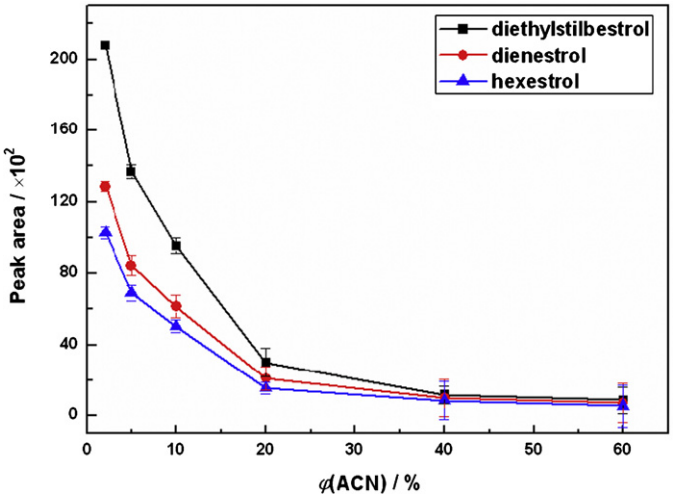

Fig. 5. Effect of the acetonitrile content in the sample matrix on the extraction efficiency.

various desorption volume (20–100 μL) were optimized for the chromatographic separation and the desorption of the three estrogens. Considering the retention time and the separation factor, the mobile phase consisting of 65% (v/v) methanol was selected for the experiments. The capillary was allowed to be flushed by the mobile phase during the whole chromatographic run to avoid any possible carryover. The three estrogens can be eluted completely using 40 μL of the mobile phase. Meanwhile, the extraction flow rate was optimized and the flow rate of 0.05 mL/min was selected for the following experiments, which will offer both relatively short analysis time and adequate extraction efficiency.

In order to further enhance the extraction efficiency of the in-tube SPME–HPLC system, the sample matrix including pH, the inorganic salt concentration and the content of organic solvent was also optimized.

It is known that changing the pH of the sample matrix results in change of the existing form of the hydroxyl groups of the three estrogens, which might lead to the change in extraction efficiency. As can be seen from Fig. 4, a slight decrease of extraction efficiency was found when the pH increase to above 8. This could be attributed to the deprotonation of the analytes and the resulted electrostatic repulsion between the analytes and the coatings. However, there was also no significant influence on the extraction efficiency when varying the pH of the sample matrix from 3 to 8 since the p*K*a value of the three estrogens was reported in the range of 7.2–10.4 [51] and thus the molecule forms of the three estrogens was not likely to be affected very much. Therefore, a neutral pH of the sample matrix was applied for extraction.

Generally, the addition of inorganic salt to the aqueous samples could result in an increase in the extraction efficiency of neutral organic molecules. The effect of the NaCl content on the extraction efficiency was experimented in the range from 0 to 0.1 mol min^{-1}. No obvious change in extraction efficiency was found. Probably the salting-out effect is supposed to be responsible for this phenomenon. So, the sample solution was prepared without adding NaCl.

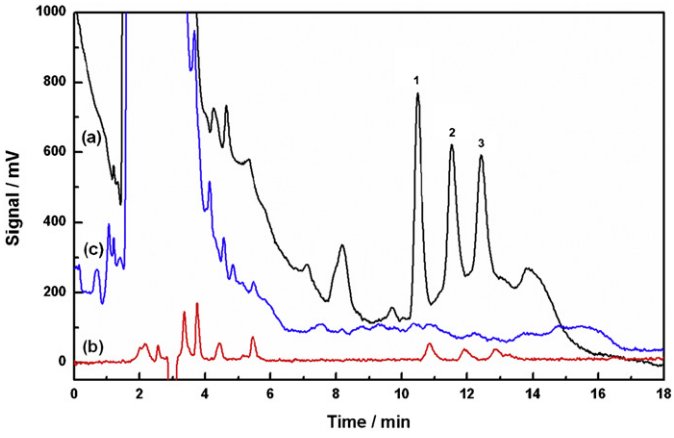

Fig. 6. HPLC chromatograms of (1) diethylstilbestrol, (2) dienestrol and (3) hexestrol obtained by (a) in-tube SPME–HPLC from spiked milk sample, (b) direct injection and (c) in-tube SPME–HPLC from blank milk sample. The analytes were spiked at 50 ng/g. The volume for direct injection was 20 μL; the extraction volume was 800 μL; Column: Ultimmate XB-C18 250 mm × 4.6 mm i.d.; mobile phase: MeOH:water = 65:35 (v/v); wavelength: 280 nm.

Next, we investigated the effect of the organic solvent content of the sample solution on the extraction efficiency. Because the hydrophobic interaction between the analytes and PNIPAAm modified SiO_2 NP-deposited capillary played a major role in the extraction, increasing the concentration of organic solvent will lead to the decline of the extraction efficiency. Fig. 5 showed that the extraction efficiency towards the three analytes decreased sharply when increasing the acetonitrile content. The analytes were not retained in extraction media until that the acetonitrile content was higher than 40%. Therefore, the

Table 2
In-tube SPME–HPLC linearity characteristics, sensitivity characteristics, precisions of estrogens from milk.

Compound	Concentration range (ng/g)	Regression line			LOD (ng/g)	LOQ (ng/g)	Intra-day RSD/(%, $N=5$)		Inter-day RSD/(%, $N=4$)	
		Slope	Intercept	R^2 value			10 ng/g	50 ng/g	10 ng/g	50 ng/g
Diethylstilbestrol	5–200	188.1	2326.6	0.9926	1.2	4.0	11.3	7.0	12.6	5.9
Dienestrol	10–200	144.1	59.2	0.9993	1.9	6.1	5.1	4.7	5.9	3.1
Hexestrol	10–200	117.3	83.8	0.9987	2.2	7.3	2.8	5.4	2.7	6.3

Table 3
Determination of estrogens in milk samples by the in-tube SPME–HPLC method.

Compound	Sample 1			Sample 2		
	Found (ng/g)	Added (ng/g)	Recovery (RSD)% ($N=4$)	Found (ng/g)	Added (ng/g)	Recovery (RSD)% ($N=4$)
Diethylstilbestrol	N.D.	10	71.7/(10.7)	N.D.	10	73.8/(12.3)
Dienestrol	N.D.	10	81.0/(5.0)	N.D.	10	85.2/(6.2)
Hexestrol	N.D.	10	89.9/(6.5)	N.D.	10	98.9/(6.8)

Table 4
Comparison of LOD and recovery obtained by different sample preparation.

Analytes	Matrix	Sample preparation	Extraction media	Detection	LOD	Recovery	Reference
Diethylstilbestrol (DES), dienestrol (DIEN) and hexestrol (Hex)	Milk	Matrix solid-phase dispersion	C_{18}, Florisil and PSA	Ultraviolet detection	4–6 ng/g	84.1–93.5%	[52]
Diethylstilbestrol (DES), dienestrol (DIEN) and hexestrol (Hex)	Milk	liquid-phase microextraction	DES-imprinted polymer-coated polypropylene hollow fiber tube (MIP-HFT)	Ultraviolet detection	2.5–3.3 μg/L	83.7–90.6%	[53]
Diethylstilbestrol (DES)	Urine	Stir bar sorptive extraction	Poly (methacrylic acid stearyl ester–ethylene dimethacrylate)	Diode array detection (DAD)	0.062 μg/L	48.0 ± 5.6%	[54]
Diethylstilbestrol (DES)	Milk	SPE	C_{30}-silica	Mass spectrometry	0.05 ng/mL	83.8–84.9%	[49]
Diethylstilbestrol (DES), dienestrol (DIEN) and hexestrol (Hex)	Milk	Supported liquid membrane (SLM)	Liquid membrane	Mass spectrometry	2.5–4.3 ng/L	60–70%	[55]
Diethylstilbestrol (DES), dienestrol (DIEN) and hexestrol (Hex)	Milk	SPE	Oasis HLB	Mass spectrometry	2.0–6.0 ng/kg	86–103.9%	[56]
Diethylstilbestrol (DES), dienestrol (DIEN) and hexestrol (Hex)	Milk	SPME	PNIPAAm modified SiO_2 NP-deposited capillary	Ultraviolet detection	1.2–2.2 ng/g	71.7–98.9%	This work

actual sample was directly dissolved in deionized water and then extracted.

The above investigation on the effect of pH, the inorganic salt concentration, and the content of organic solvent confirmed that the hydrophobic interaction dominated extraction process.

3.3.3. Analysis of estrogens in milk samples

Under the optimized extraction conditions, analyte-free milk samples were spiked with the three estrogens and then extracted. The chromatograms of spiked milk samples after extraction with PNIPAAm modified SiO_2 NP-deposited capillary are shown in Fig. 6. Compared with the direct HPLC analysis (chromatogram b in Fig. 6), the sensitivities of the three estrogens in milk were greatly enhanced after the PNIPAAm modified SiO_2 NP-deposited capillary.

In order to validate the linearity of the method, calibration curves were constructed using three estrogens from the milk samples by comparing peak area counts against analyte concentrations over a range of 50–5000 ng/mL. As shown in Table 2, good linearities for all compounds were obtained with the linear coefficient R^2 values above 0.9926. Detection limits (LODs) and quantification limits (LOQs) were calculated as the concentration corresponding to a signal 3 and 10 times the standard deviation of the baseline noise, respectively. As listed in Table 2, the LODs and the LOQs for the analytes were 1.2–2.2 ng/g and 4.0–7.3 ng/g, respectively.

In addition, the method reproducibility was investigated. The analyte-free milk samples were spiked with three estrogens at concentration level of 10 ng/g and 50 ng/g, respectively, and analyzed under optimum condition. Method reproducibility was estimated by intra and interday precisions, yielding RSDs of less than 11.3 and 12.6%, respectively (Table 2). Moreover, the PNIPAAm modified SiO_2 NP-deposited capillary exhibited high stability since no significant changes in temperature responsive property and extraction performance were found after more than 200 times of extractions.

Furthermore, the recoveries (relative to spiked standard samples) of the spiked analytes from milk samples were found to be 71.7–98.9% (Table 3). The method was applied to the analysis of two different brands of milk samples, no residues of the three estrogens were found.

The proposed method was compared to some methods published previously for the determination of the three estrogens. As

shown in Table 4, the LOD of the methods except LC–MS was at the same level, while the proposed method in this study required less sample and solvents.

4. Conclusions

PNIPAAm was successfully grafted to the inner surface of silica NP-deposited capillary and was introduced into in-tube SPME–HPLC system as an extraction media. Nanosized silica particles fabricated by LPD onto the inner-wall of fused-silica capillary were confirmed to be able to increase the specific surface area and improve the extraction capacity. PNIPAAm modified SiO_2 NP-deposited capillary was proved to be temperature responsive by comparing the extraction efficiency towards diethylstilbestrol at different temperatures. Finally, the PNIPAAm modified SiO_2 NP-deposited capillary was successfully applied to the extraction of three synthetical estrogens from milk samples.

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