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Novel fiber coated with amide bridged-calix[4]arene used for solid-phase microextraction of aliphatic amines

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Abstract

Solid-phase microextraction (SPME) using a novel fiber coated with 25,27-dihydroxy-26, 28-oxy(2′,7′-dioxo-3′,6′-diazaoctyl)oxy-*p*-*tert*-butylcalix[4]arene/hydroxy-terminated silicone oil has been introduced as a rapid and sensitive pretreatment technique coupled to gas chromatography–flame ionization detection (GC–FID) for the detection of aliphatic amines without derivatization. Due to the introduction of the polar amide bridge in calix[4]arene, the new fiber shows good selectivity and sensitivity to the polar aliphatic amines in addition to its high thermal stability (380 °C), solvent stability and good reproducibility between fibers. The extraction temperature, extraction time, pH, and ionic strength of the matrix sample were modified to allow for maximum sorption of the analytes onto the fiber. The method proposed in this study showed satisfactory linearity, precision and detection limits. Practical applicability was demonstrated through the determination of trimethylamine (TMA) in fish tissue. Mean recovery of 92.5% ($n = 5$) was obtained for the fish extracts and the relative standard deviation was 4.9% ($n = 5$). The results of fish freshness assay indicate the present method is a validated and simple procedure for the simultaneous determination of TMA in fish.

Keywords: Fish; Solid-phase microextraction; Calix[4]arene; Aliphatic amines

1. Introduction

Amines may occur as biodegradation products of organic matter like proteins, amino acids and other nitrogen-containing organic compounds [1]. In addition, they are used as raw materials or intermediate products in the manufacturing of a wide range of industrial chemicals [2]. Many amines have an unpleasant smell and are harmful to health because of their toxicity [3]. Moreover, they may react with nitrosylating agents, leading to the formation of potentially carcinogenic *N*-nitrosamines [3]. The occurrence and determination of amines have received an ever-growing interest due to global environmental changes.

Analysis of low-molecular-weight amines has been traditionally difficult because of their particular physicochemical properties, i.e. high aqueous solubility, volatility, polarity and basic character [4]. They have been determined using various methods including gas chromatography (GC) [5–8], gas sensitive resistor [9], liquid chromatography [10–13], capillary electrophoresis (CE) [14], spectrofluorimetry [15], spectrophotometry [16], quartz crystal microbalance (QCM) sensor [17]. All these methods mentioned above usually need expensive instruments, or involve a number of complicated steps such as derivatization, which is a popular method for overcoming some of the above problems by the formation of less polar compounds, but results in an increase of analysis time and easily leads to side effects.

Solid-phase microextraction (SPME) [18] is an attractive alternative to conventional preconcentration techniques for the determination of volatile and semi-volatile compounds from aqueous samples because of its fast, simple, low-cost, solvent-free properties. It has been used in the determination of aromatic amines without derivatization coupled to GC [3,19–21] and high-performance liquid chromatography (HPLC) [22,23]. In order to achieve high selectivity and sensitivity, different kinds of fibers are used, such as carbowax–divinylbenzene (CW–DVB) [3], CW–templated resin (CW–TPR) [19,22,23], poly(dimethylpolysiloxane)

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(PDMS)–DVB [19,20,22], and crown ether [21]. However, only a few papers deal with the determination of aliphatic amines without derivatization by SPME/GC. A headspace SPME/GC–NPD procedure was developed for the determination of free volatile amines in wastewater and sewage-polluted waters [1]. Limits of detection (LODs) with a 100 μm PDMS fiber are in the low- to mid-microgram-per-litre level, but the linearities are comparatively narrow. Namieśnik et al. [2] concluded that SPME can be used for the isolation and preconcentration of volatile aliphatic amines in non-derivatized form in air and the entire analysis can be completed in a relatively short time. However, the upper desorption temperature for the custom-made fibers is only 200 °C.

Calixarenes are macrocyclic molecules in which phenolic units are linked via methylene bridges. Analytical chemists are studying calixarenes because they provide a route to molecules with well-defined cavities, which offer simultaneous polar (lower-rim) and non-polar (upper-rim) features. Owing to these properties, calixarenes can form inclusion complexes with a wide range of guest species, depending on the binding groups substituted at each rim and the number of repeat units in the macrocycle [24]. They have been widely used in many fields [25–29]. Most of all, their applications as HPLC stationary phase [29] and gas chromatography open-tubular capillary coating [30] have interpreted special selectivity. A 5,11,17,23-tetra-*tert*-butyl-25,27-diethoxy-26,28-dihydroxycalix[4]arene/hydroxy-terminated silicone oil-coated fiber (C[4]/OH-TSO) was first prepared previously in our laboratory [31]. It showed wonderful selectivity and sensitivity to aromatic amines, particularly for the less polar ones owing to the π–π interactions, hydrophobic interactions, cavity-shaped cyclic molecular structure and the outstanding material properties of sol–gel coating [32] as well. In order to enhance the sensitivity to the polar aliphatic amines, it is necessary to modify with polar functional groups on calixarene at the lower-rim, and the cyclic hydroxyl groups provide excellent sites for bridged modification by bifunctional or polyfunctional reagents [24].

Freshness is one of the most important criteria for quality of fish products. But fish undergoes irreversible changes (color, texture, smell) soon after harvest, resulting in quality loss. A remarkable post-mortem deteriorating chemical change is the gradual accumulation of certain volatile amine compounds in the flesh. Traditionally, the evaluation of fish quality has been based on organoleptic tests. Since the determination of the total volatile base-nitrogen (TVB-N) was published as standard method for the inspection of fish in Germany [33], it has been widely applied to assess the quality of fish and seafood. Trimethylamine (TMA) is produced in the greatest quantities (95% of the overall content of amines in fish), and is therefore an indicator of spoilage [34]. Several methods to estimate the freshness have been proposed based on such substances. GC [35], LC [36] and flow injection analysis (FIA) [37] procedures were early described for the determination of volatile amines in fish. Analysis with these methods is relatively time consuming, requiring samples pretreatments. Recently, attention has been focused on alternative sensor-based approaches as they are very rapid and relatively inexpensive, and could potentially be used on-line at many points in the distribution network [38–40]. Interesting results have been obtained with different sensor technologies, such as electrochemical sensors [38], thickness shear mode quartz resonators [39] and metal-oxide semiconductor gas sensors [40]. Fish freshness sensors were developed based on metal-oxide semiconductors technology [40], with improved selectivity and sensitivity towards compounds such as DMA and TMA and this technique is promising because all sensor testing and modifications were designed with multiple fish samples and not with chemicals that are believed to be fish degradation markers.

Another method for the rapid determination of fish freshness is based on GC preceded by SPME preconcentration techniques [41]. The fish sample for SPME requires no other pretreatments. The methodology allows for the rapid quantification of either the volatile amines or the non-amine volatiles of fish. Result with the new method is in good agreement with that with the TVB-N reference method.

In this paper, 25,27-dihydroxy-26,28-oxy(2′,7′-dioxo-3′,6′-diazaoctyl)oxy-*p*-*tert*-butylcalix[4]arene (amide bridged-C[4]) was synthesized and was used for preparation of SPME fiber blending with OH-TSO by sol–gel coating technology. It was applied to analyze aliphatic amines using headspace SPME with no derivatization. Several extraction variables such as extraction time, extraction temperature, pH and ionic strength of the aqueous sample, and desorption time were optimized. Linearity, detection limits, and precision of the whole procedure were determined. Finally, the developed method was employed to determine TMA in fish tissue.

2. Experimental

2.1. Instrumentation

Melting points were recorded on a Gallenkamp melting point apparatus in open capillaries and were uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury VX300 instrument at ambient temperature. TMS was used as an internal standard for NMR. FAB-MS spectra were obtained from a Kratos MS80RF mass spectrometer, with *m*-nitrobenzyl alcohol as a matrix. IR spectra were done on IR instrument model FTIR-8201PC (Shimadzu).

To mix various solution ingredients thoroughly, an ultrasonator model SY-1200 (Shanghai Ultrasonic Instrument Factory) was used. A centrifuge model TGL-16C (Shanghai Anting Instrument Factory, Shanghai, China) was used to separate the sol solution from the precipitate. The fused-silica fiber (120 μm, o.d.) with protective poly-

imide coating was obtained from Academy of Post and Telecommunication, Wuhan, China.

The experiments were performed using a SP-6800A capillary GC system (Shandong, China) equipped with a capillary split/splitless injector system and flame ionization detector (FID), on a SE-54 capillary column (25 m × 0.32 mm i.d.). Online data collection and processing was done on chromatopac model SISC-SPS (Beijing, China). The oven temperature was programmed from 60 °C (held for 4 min) and increased at 25 °C/min to 230 °C (held for 6 min). The temperatures were 260 °C for the injection port and 260 °C for FID. Nitrogen was used as a carrier gas at linear velocity of 12–15 cm/s in the 1:100 split mode for all the analyses except that the split mode was 1:150 for comparison of fibers. A laboratory-made SPME with amide bridged-C[4]/OH-TSO fiber (30 mg, 60 μm) syringe was used to transfer the extracted sample to the GC injector for analysis. C[4]/OH-TSO (30 mg, 60 μm) and OH-TSO (60 μm) fibers were prepared for comparison. The commercially available polyacrylate (PA, 85 μm) and PDMS–DVB (65 μm) coated fibers were obtained from Supelco (Bellefonte, PA).

2.2. Reagents and standards

Hydroxy-terminated silicone oil (OH-TSO) was purchased from Chengdu Center for Applied Research of Silicone (Chengdu, China). 3-(2-Cyclooxypropoxyl)propyltrimethoxysilane (KH-560), tetraethoxysilane (TEOS), and poly(methylhydrosiloxane) (PMHS) were obtained from the Chemical Plant of Wuhan University. Trifluoroacetic acid (TFA) was purchased from Shanghai Chemical Factory, China. The synthesis of *N*,*N*′-*bis*(chloroacetyl)ethylenediamine was performed according to US patent [42]. The synthesis of *p*-*tert*-butylcalix[4]arene was according to Ref. [43].

Trimethylamine (33% (w/w)), triethylamine (TEA), tri-*n*-propylamine (TPA), *n*-butylamine (BA), di-*n*-butylamine (DBA), and tri-*n*-butylamine (TBA) were purchased from Shanghai Chemical Factory. The physicochemical properties of the six amines were shown in Table 1. Aliphatic amines were dissolved in methanol to make stock solution at a concentration of 1 mg/ml for TMA and TEA, 2 mg/ml for BA, 0.1 mg/ml for TPA, TEA, and DBA. All other chemicals were analytically pure grade.

Table 1
Basic physicochemical properties of analytes

Compounds	Molecular weight[a]	Water solubility (g/l)[a]	log K_{ow}[a]
TMA	59.11	890	0.16
BA	73.14	1000	0.97
TEA	101.19	73.70	1.45
TPA	143.27	0.75	2.79
DBA	129.25	3.50	2.83
TBA	185.36	0.14	4.46

[a] Data obtained from [44].

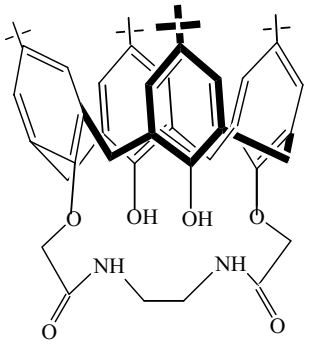

Fig. 1. The structure of amide bridged-calix[4]arene.

2.3. Synthesis of amide bridged-C[4]

To a suspension of anhydrous K_2CO_3 (0.9 g, 6 mmol) and *p*-*tert*-butylcalix[4]arene (2.6 g, 4 mmol) in 250 ml dry acetonitrile, *N*,*N*′-*bis*(chloroacetyl)ethylenediamine (0.95 g, 4.4 mmol) and KI (1.46 g, 8.8 mmol) were added. The reaction was stirred and refluxed under Ar for 24 h. After evaporation of the solvent, the residue was treated with HCl (10% (v/v)) and extracted with $CHCl_3$. The organic layer was separated, dried by $MgSO_4$ and filtered. The organic phase was evaporated again. The crude product was purified by column chromatography. The product was recrystallized in $CHCl_3$ and methanol. The yield was 71%. m.p. >310 °C. ^{1}H NMR ($CDCl_3$): 1.02 (s, 18H, $C(CH_3)_3$), 1.15 (s, 18H, $C(CH_3)_3$), 3.48 (d, 4H, $J = 12.2$ Hz, $ArCH_2Ar$), 3.82 (s, 4H, NCH_2CH_2N), 4.25 (d, 4H, $J = 12.2$ Hz, $ArCH_2Ar$), 4.83 (s, 4H, OCH_2CO), 6.31 (s, 4H, ArH), 7.02 (s, 4H, ArH), 8.53 (s, 2H, OH), 8.72 (s, 2H,NH). FAB-MS: $m/z = 788[M]^+$.

The structure of the final product is shown in Fig. 1.

2.4. Preparation of fiber

The fiber was prepared according to Ref. [31]. The 30 mg amide bridged-C[4]-contained fiber was selected to carry out all the experiments since this kind of fiber coating gave good results in the previous work [31]. A OH-TSO fiber was also coated for comparison by sol–gel technique with an almost identical preparation procedure except that C[4] was not added.

2.5. SPME procedure

Before the initial application, the fiber was conditioned in the hot port of the gas chromatograph at 260–380 °C for 1–3 h. After the conditioning process, a fiber blank was run to confirm that there were no extraneous peaks which could be assigned to compounds introduced during the manufacture of the fiber. For all analyses, a 10 μl portion of standard solution was diluted with 4 ml of pH 13 NaOH solutions in a 10 ml amber vial. The concentration of NaCl in the sample was 20% (w/w). Headspace SPME was carried out within 40 min under magnetic stirring of the liquid phase.

All the determinations were performed in duplicate except the evaluation of thermal stability and precision, which was performed in three and five replicates, respectively. The average values are reported. The relative standard deviations for these measurements were acceptable (R.S.D. $\leq$ 10.0%).

2.6. Fish samples

Chub was obtained from the local market. The fresh fillets were cut into portions placed in plastic bag. The finely ground fish fillet were divided into three parts. One was stored at 0 °C on ice, one was stored at 15 °C in a refrigerator, and the other was used for analysis of TMA in fish at room temperature (30 °C). The samples were analyzed at intervals of 1–3 days in order to follow the aging process.

The fish sample for headspace SPME was prepared according to Ref. [41] for the determination of TMA in fish. Fillet (1 g) was weighted in a 10 ml vial equipped with a magnetic stirrer bar, and then 4 ml of 40% NaOH and 1 g NaCl were added. The stirred sample was heated for 20 min at 70 °C to liberate the volatile bases and then cooled to 35 °C. For the measurement, the SPME fiber was exposed for 5 min in the headspace of the vial maintained at 35 °C and inserted in the measuring device equipped with FID.

GC peak identification was conducted by comparing the gas chromatographic retention time with that of an authentic standard. Only a chromatographic peak of TMA was found in chromatograms of the fish samples. The quantity of TMA in real samples was calculated using the calibration curves obtained in fresh fish matrix (TMA spiking level 2.01–201 μg/g of fish sample) after subtraction of the blank value.

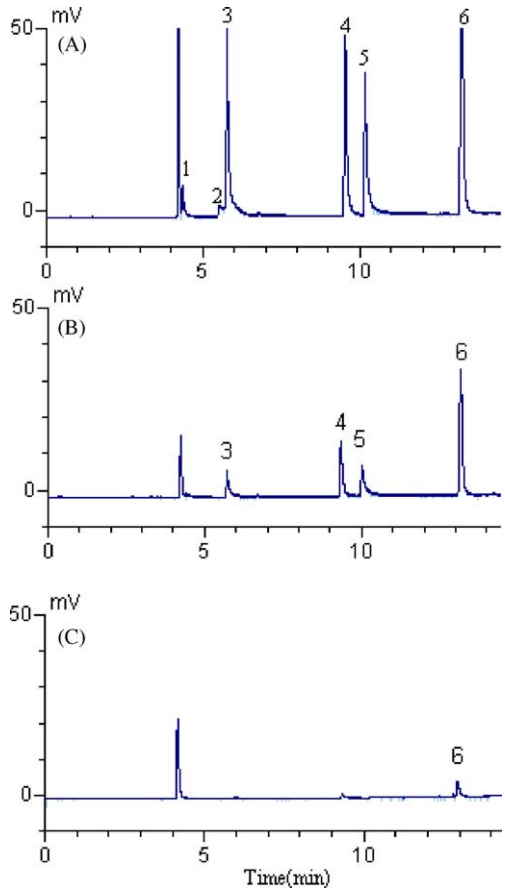

Fig. 2. Comparison of the SPME–GC–FID chromatograms for extraction of six aliphatic amines with three sol–gel-coated fibers at the same conditions. Extraction time, 10 min; extraction temperature, 30 °C; desorption time, 3 min; pH, 13; 20% NaCl. (A) Amide bridged-C[4]/OH-TSO (30 mg, 60 μm); (B) C[4]/OH-TSO (30 mg, 60 μm); (C) OH-TSO (60 μm). Peaks: (1) TMA; (2) BA; (3) TEA; (4) TPA; (5) DBA; (6) TBA.

3. Results and discussion

3.1. Characteristics of amide bridged-C[4]/OH-TSO fiber

In sol–gel chemistry, a gel can be formed by the simultaneous hydrolysis and polycondensation of a precursor followed by aging and drying under ambient atmosphere [45]. Characterized by the interactions of hydroxyl groups in all steps, amide bridged-C[4] containing hydroxyl groups can be chemically bonded with other components by ring-opening polymerization with KH-560 [31].

The IR spectra of the stationary phase having been dipped in methylene chloride for 2 h were used. The feature identified by amide bridged-C[4]: 1201.73, 1166.70 cm^{-1} (ν_{Ar-O-C}), 1536.43 cm^{-1} (ν_{Ar-C-C}), 1781.62 cm^{-1} ($\nu_{C=O}$), 1688.00 cm^{-1} (ν_{N-H}) also appeared in amide bridged-C[4]/OH-TSO, which confirmed the successful binding of amide bridged-C[4] to the stationary phase.

Fig. 2 compares the SPME–GC–FID chromatograms for extraction of six aliphatic amines with amide bridged-C[4]/OH-TSO (30 mg, 60 μm), C[4]/OH-TSO (30 mg, 60 μm) and OH-TSO (60 μm) fibers under the same conditions. The extraction ability of the amide bridged-C[4]/OH-TSO fiber to these amines is much better than that of OH-TSO fiber, which confirmed the important role of amide bridged-C[4] during the extraction. Furthermore, it is also superior to that of C[4]/OH-TSO fiber due to the introduction of the polar amide bridge in calix[4]arene molecules, which obviously increases the polarity of the fiber coating and results in the highest affinity for the aliphatic amines.

Table 2 illustrates the thermal stability of sol–gel-coated amide bridged-C[4]/OH-TSO SPME fiber. Before experiments, the fiber was conditioned for 1 h at 260, 300, 340, 360 and 380 °C, respectively. As shown in the table, the peak areas did not significantly change after the fiber was conditioned at high temperatures up to 380 °C. Moreover, it did not show any sign of crack on the surface of the fiber. Being chemically bonded to the substrate, sol–gel coatings are inherently stable in operations requiring their exposure to high temperatures. The introduction of calixarene into the fiber coating may also increase its thermostability.

Similar to the C[4]/OH-TSO fiber, amide bridged-C[4]/OH-TSO fiber also can be dipped in methylene chloride or

Table 2
The effect of desorption temperature on the extraction efficiency of amide bridged-C[4]/OH-TSO

Injection temperature (°C)	Peak area ($n = 3$)					
	TMA	BA	TEA	TPA	DBA	TBA
260	1935588	5705683	12081174	6401582	15645005	16811410
300	2064796	5464118	13135630	7045726	16813980	17242393
340	1883642	6478804	11199498	6393804	14436320	15905858
360	1967540	5938806	13845735	6846259	14570815	17555818
380	1880110	6438385	12548218	6085650	13631765	16397510

SPME–GC conditions are the same as in Fig. 2 except that the injection temperature is variable.

water for 12 h without loss of extraction efficiency. The average lifetime of the fiber was above 200 uses for headspace SPME. The fiber-to-fiber reproducibility (R.S.D., $n = 5$) was 1.1–5.3% for the aliphatic amines.

3.2. Optimization of SPME operating conditions

Experimental conditions, like extraction temperature and time, pH, ionic strength of the aqueous sample, and desorption time were optimized before validating the analytical method. All the experiments were done with constant stirring (600 rpm).

3.2.1. Extraction temperature

By increasing the temperature of the sample solution, the vapor pressure of the analyte is increased, allowing the partitioning of the analyte between the sample and the headspace to reach equilibrium more quickly. However, the distribution constant for the analyte between the headspace and fiber coating is also temperature-dependent. At excessively high temperatures, the affinity of the analyte for the fiber coating diminishes. An optimum temperature exists for the partitioning of the analyte among the sample matrix, headspace, and fiber coating, with the maximum loading onto the fiber. The effect of temperature on the amount of aliphatic amines sorbed by the new SPME fiber was investigated in 25–65 °C temperature range, as shown in Fig. 3. For the compounds studied, an increase in the extraction temperature decreased the extraction yield except for TMA. In this study, all experiments were performed at room temperature (about 30 °C).

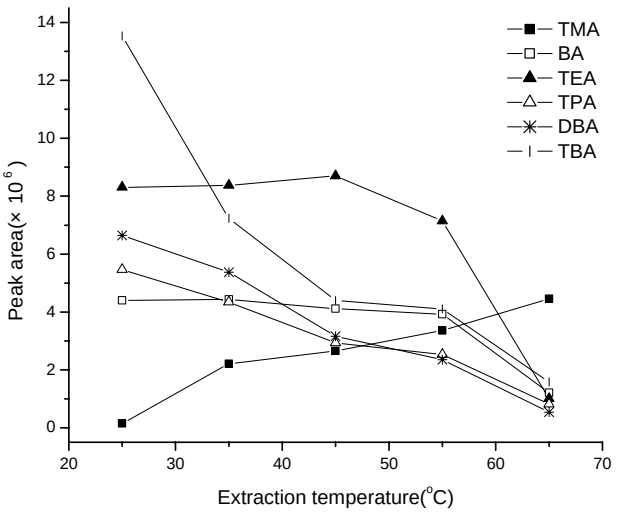

Fig. 3. The extraction temperature profile for aliphatic amines. Extraction time, 10 min; desorption time, 3 min; pH, 13; 20% NaCl.

3.2.2. Extraction time

The effect of extraction time on extraction efficiency was determined (Fig. 4). The maximum peak amounts were obtained by amide bridged-C[4]/OH-TSO fiber only in 3 min for TPA and TBA, 5 min for DBA and TMA. The extraction amounts did not significantly increase after 30 min for BA and TEA. An extraction time of 10 min was selected for subsequent analysis so the response and the relative standard deviation were acceptable.

3.2.3. Effect of pH and ionic strength

The influence of pH in the extraction extent, within the range 11–14, was evaluated (Fig. 5). Experiments were done with extraction temperature of 30 °C and extraction time of 10 min. The change in pH has slight effect on TPA and TBA, but it dramatically affects the selectivity and sensitivity for the analysis of TMA, BA, TEA, and DBA when pH <12. For this reason, pH 13 was chosen.

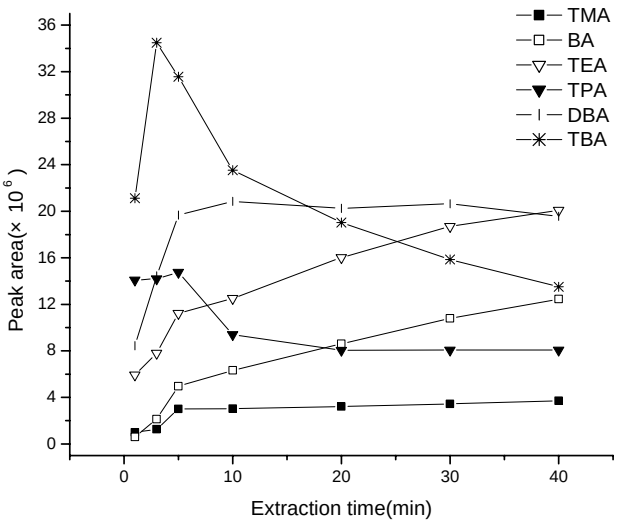

Fig. 4. The extraction time profile for aliphatic amines. Extraction temperature, 30 °C; desorption time, 3 min; pH, 13; 20% NaCl.

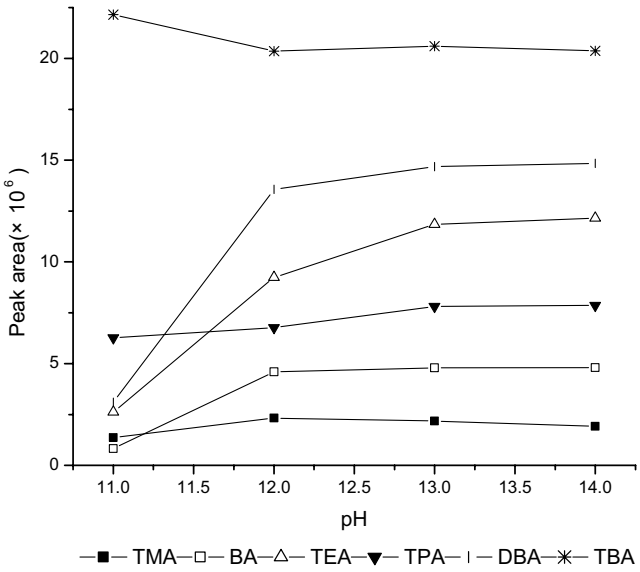

Fig. 5. The influence of pH on the peak areas of aliphatic amines. Extraction time, 10 min; extraction temperature, 30 °C; desorption time, 3 min; 20% NaCl.

Usually, depending on the solubility of the target analytes, adding salt to the sample decreases the solubility of compounds in water, and enhances extraction, especially for the more polar analytes. Fig. 6 studied the effect of ionic strength. Experiments were done with different NaCl concentrations, i.e. 0, 20% (w/w), and saturated salt solution. As shown in Fig. 6, the maximum peak area of BA and TEA was obtained at a saturated salt solution. The extraction amount of TMA, BA, and TEA increases with increasing the salt concentration, while it decreases slightly for others

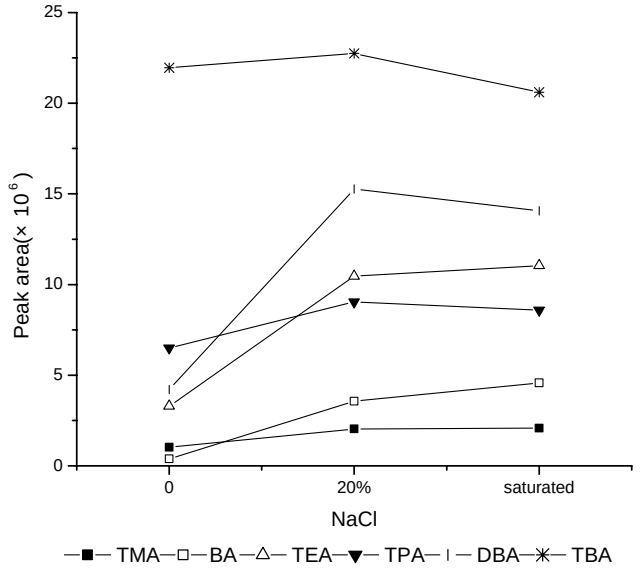

Fig. 6. The effect of ionic strength on the peak areas of aliphatic amines. Extraction time, 10 min; extraction temperature, 30 °C; desorption time, 3 min; pH, 13.

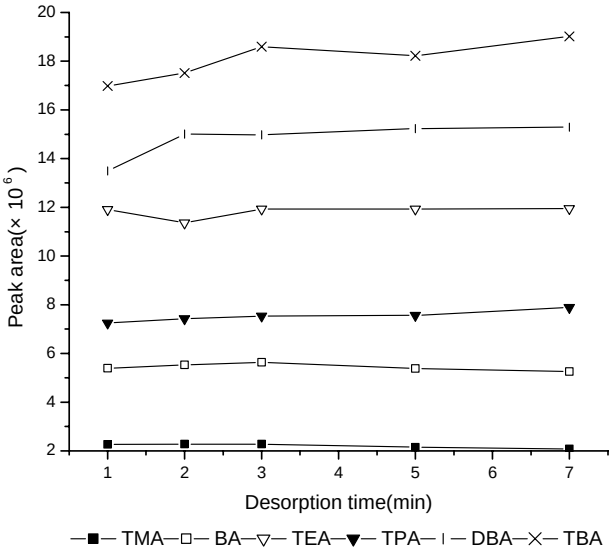

Fig. 7. The effect of desorption time on the amounts of aliphatic amines. Extraction time, 10 min; extraction temperature, 30 °C; pH, 13; 20% NaCl.

when the NaCl concentration is above 20%. Taking all considerations into account, a concentration of 20% NaCl was set in subsequent extractions.

3.2.4. Desorption time

Fig. 7 represents the effect of desorption time on the amounts of aliphatic amines extracted. The results indicate that the minimum time for quantitative transfer of the analytes from the SPME fiber to the GC column depends on an amine, but it is never longer than 3 min for all the compounds tested at 260 °C. Sol–gel coatings possess a porous structure that provides faster mass transfer during extraction as well as analyte desorption processes during sample introduction, which shortens the desorption time of analytes.

3.3. Coating evaluation

Table 3 compares the equilibrium distribution constants (*K*) [46] of the analytes between different solid-phase coatings and water. A coating with a high affinity for an analyte will have a high *K* value associated with it,

Table 3
Comparison of the equilibrium distribution constants (*K*) of amide bridged-C[4]/OH-TSO and commercial PDMS–DVB and PA fibers

Compounds	Amide bridged-C[4]/OH-TSO (60 μm)	PDMS–DVB (65 μm)	PA (85 μm)
TMA	89.5	39.0	n.d.[a]
BA	8.6	40.2	n.d.
TEA	124.4	126.4	2.1
TPA	792.4	721.9	57.0
DBA	2520.3	1976.0	n.d.
TBA	3388.2	2354.5	117.0

SPME–GC conditions are the same as in Fig. 2.
[a] Not detected.

which will result in good sensitivity. As indicated by the K values in Table 3, the PA coating was not suitable for aliphatic amines analysis. TMA, BA and DBA could not be extracted in large enough amounts to be detected by GC–FID under the experimental conditions. Both amide bridged-C[4]/OH-TSO and PDMS–DVB fibers show high affinity to the tested compounds. With the exception of BA, the amide bridged-C[4]/OH-TSO fiber are more suitable for the analysis of these compounds. Firstly, the good sensitivity of amide bridged-C[4]/OH-TSO fiber to the aliphatic amines should be attributed to the introduction of the polar amide bridge in calixarene molecules, which enhances the polarity of the sol–gel coating and increases the hydrogen bonding and dipole–dipole interactions. Secondly, it is also due to the cavity-shaped cyclic molecular structure and hydrophobic interactions between analytes and calixarene resulting from the cavity and the hydrophobic *tert*-butyl groups on its upper rim. Thirdly, this becomes possible also thanks to the outstanding material properties of sol–gel coating. The organic-inorganic nature of the sol–gel coating provides sorption sites for both the polar and non-polar analytes.

3.4. Method validation

Table 4 summarizes the detection limits (LODs), relative standard deviations, and linear ranges for the analysis of six aliphatic amines in deionized water with amide bridged-C[4]/OH-TSO fiber. The linearity of the method was tested by extracting aqueous standards with increasing concentrations. The investigated aliphatic amines showed three orders of magnitude of linear ranges. LODs were calculated based on three times the average background noise divided by the detection sensitivity. Owing to the high extraction ability of this fiber, the SPME procedure showed low LODs (μg/l), good linearity with correlation coefficient 0.9986–1. The precision of the method was determined by performing five consecutive fiber extractions from the aqueous solution. The R.S.D. values obtained were ≤5.1%.

Fig. 8 is a typical chromatogram of extraction of six aliphatic amines in deionized water.

Table 4
Precision, limits of detection, linearity, and correlation coefficients (r) for the analysis of aliphatic amines

Compounds	Precision (%) ($n = 5$)	Linearity (μg/l)	LOD (μg/l)[a]	r
TMA	5.1	500–80000	25.89	0.9995
BA	4.7	500–50000	39.51	0.9986
TEA	1.4	50–5000	7.37	0.9999
TPA	3.2	2.5–250	0.41	0.9999
DBA	3.0	2.5–250	0.43	1
TBA	3.4	2.5–250	0.19	0.9996

[a] Detection limits were estimated on the basis of 3:1 signal to noise ratios.

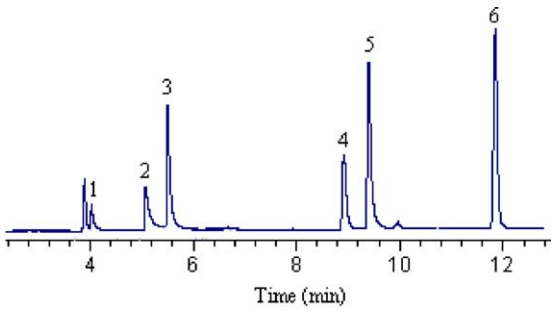

Fig. 8. A typical chromatogram of extraction of six aliphatic amines in deionized water. SPME–GC conditions are the same as in Fig. 2. Peaks: (1) TMA; (2) BA; (3) TEA; (4) TPA; (5) DBA; (6) TBA.

3.5. Application to real samples

It is of concern that the consumption of amines in human diets may play a significant role in the incidence of certain cancers [36]. In fish, TMA is formed from the bacterial reduction of osmoregulatory substances such as trimethylamine oxide (TMAO) in marine fish muscle [33]. TMA is of particular interest because it is used as an indicator of spoiling flavor or texture since the TVB-N methods are rather time-consuming and require pretreatment of fish samples [36,47]. Therefore, a rapid, selective method is required for the determination of TMA in fish. The performance of the SPME/GC method with amide bridged-C[4]/OH-TSO fiber was tested by monitoring the freshness of chub by determination of TMA content after the death of the fish.

Linear ranges, precision and recovery of TMA standard in fresh fish matrix were determined. It was 2.01–201 μg/g of fish sample, 4.9% ($n = 5$) and 92.5% ($n = 5$), respectively. LOD for TMA is 0.075 μg/g. The best mean recoveries ranging from 97.2 to 99.1% were obtained with the gas chromatographic method by Veciana-Nogues et al. [35] who tested two addition levels by the standard addition method. In agreement with our results, the ion chromatography method by Yao et al. [48] yielded recoveries varying from 92 to 108%.

Fig. 9 monitored TMA in fish at room temperature (30 ± 2 °C) after 0–12 h of storage. TMA value increased significantly all the time at a high temperature of 30 °C, from 10.4 to 92.5 μg/g. The error bars were taken as the relative standard deviation of two measurements. As the fish sample aged, the uncertainty of the measurement was observed to increase. Such behavior may be due to microbial spoilage [41]. Béné et al. [41] reported TMA could only be measured from the estimated fifth day after harvesting and storage under normal conditions. But in this study, TMA could be measured immediately after the finely ground fish fillet was prepared. That may be attributed to the high room temperature, which accelerates the spoilage of the fish. Fig. 10 showed the plot of TMA versus storage days at 15 °C. From the beginning to the fifth day of storage, there

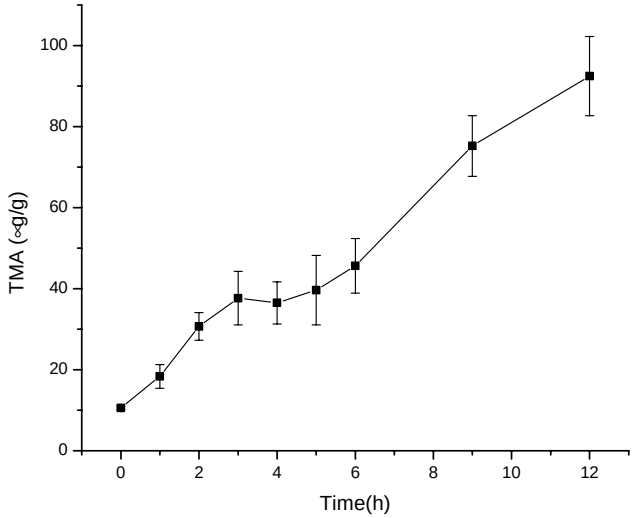

Fig. 9. Profile of TMA content upon storage of the chub at room temperature (30 °C). Extraction time, 5 min; extraction temperature, 30 °C; desorption time, 3 min; 40% NaOH; 20% NaCl.

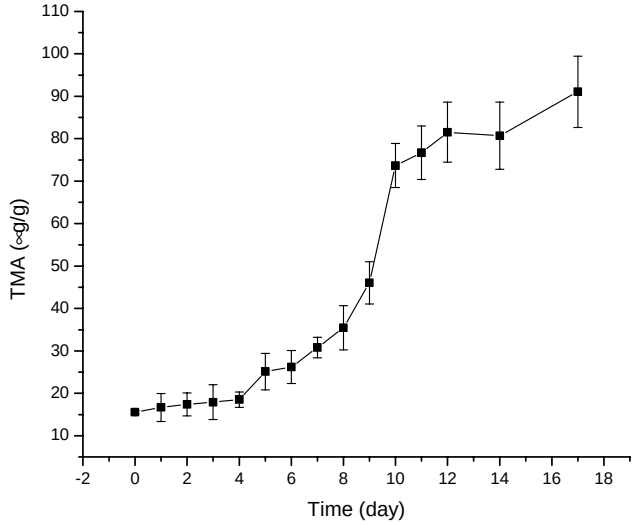

Fig. 10. Profile of TMA content upon storage of the chub at 15 °C. Extraction time, 5 min; extraction temperature, 30 °C; desorption time, 3 min; 40% NaOH; 20% NaCl.

was a slight variation in the spoilage rate. But after 5 days, the spoilage became serious consecutively. Contents of TMA in control at frozen temperature were always very low (10.4 μg/g). These results confirmed that a low temperature resists microbial growth, therefore prevents TMA formation. Experiments showed that TMA does not increase after long storage periods (2 years) at −20 °C [35]. According to Castell's criterion, fish samples showing TMA lower than 42 μg/g (TMA-N 10 μg/g of fish sample) could be rated as excellent quality grade [35], which suggests the present method is a validated, simple and rapid procedure for the simultaneous determination of TMA in fish.

4. Conclusion

A simple and rapid solid-phase microextraction/GC–FID method using amide bridged-C[4]/OH-TSO sol–gel-coated novel fiber is presented for analysis of aliphatic amines without derivitazition. Owing to the introduction of the polar amide bridge in calixarene molecules, the polarity of the coating is increased. It exhibits better sensitivity to most of the investigated aliphatic amines compared to commercial PDMS–DVB and PA fibers. The fiber also shows other advantages because of its high thermal stability (380 °C), solvent resistance and satisfactory fiber-to-fiber reproducibility. The application of the fiber has been shown to be a feasible technique for the determination of fish freshness.

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