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Fabrication of a novel nanocomposite based on sol–gel process for hollow fiber-solid phase microextraction of aflatoxins: B_1 and B_2, in cereals combined with high performane liquid chromatography–diode array detection

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ABSTRACT

The new pre-concentration technique, hollow fiber-solid phase microextraction based on carbon nanotube reinforced sol–gel and liquid chromatography–photodiode array detection was applied to determination of aflatoxins B_1, B_2 (AFB_1, AFB_2) in rice, peanut and wheat samples. This research provides an overview of trends related to synthesis of solid phase microextraction (SPME) sorbnents that improves the assay of aflatoxins as the semi-polar compounds in several real samples. It mainly includes summary and a list of the results for a simple carbon nanotube reinforced sol–gel in-fiber device. This device was used for extraction, pre-concentration and determination of aflatoxins B1, B2 in real samples. In this technique carbon nanotube reinforced sol was prepared by the sol–gel method via the reaction of phenyl trimethoxysilane (PTMS) with a basic catalyst (tris hydroxymethyl aminomethan). The influences of microextraction parameters such as pH, ageing time, carbon nanotube contents, desorption conditions, desorption solvent and agitation speed were investigated. Optimal HPLC conditions were: C_{18} reversed phase column for separation, water–acetonitril–methanol (35:10:55) as the mobile phase and maximum wavelength for detection was 370 nm. The method was evaluated statistically and under optimized conditions, the detection limits for the analytes were 0.074 and 0.061 ng/mL for B1 and B2 respectively. Limit of quantification for B1 and B2 was 0.1 ng/mL too ($n = 7$). The precisions were in the range of 2.829–2.976% ($n = 3$), and linear ranges were within 0.1 and 400 ng/mL. The method was successfully applied to the analysis of cereals (peanut, wheat, rice) with the relative recoveries from 47.43% to 106.83%.

1. Introduction

Aflatoxins belong to a group of fungal toxins known as mycotoxins. Many of these mycotoxins can cause serious problems in livestock resulting in substantial economic losses. The most common mycotoxins are aflatoxins, ochratoxins, trichothecenes, fumonisins, zearalenone and ergot alkaloids. These mycotoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive [1]. The structures of AFB_1 and AFB_2 were shown in Fig. 1.

Degree of toxicity of these mycotoxins is as follows: $FB_1 > AFB_2 > AFG_1 > AFG_2$ [2]. Aflatoxins are found generally in feed and foodstuffs. Due to their toxicity, the European Union in the Commission Regulation (EC) No. 1881/2006 has established the maximum residue limits (MRLs) of aflatoxins in cereal and their derivatives: 2 μg/kg for AFB_1, 4 μg/kg for the sum of the four aflatoxins, and 0.1 μg/kg AFB_1 for baby foods [3]. High toxicity of aflatoxins is causing serious global health concern. Thus, detection and assessment these poisons even in low doses are critical.

These toxins, even in very small quantities can cause irreparable and serious effects, so methods used to measure these toxins should be able to detect the trace and ultra-trace amounts of them in natural samples.

Therefore, sensitive and expensive methods such as HPLC with *fluorescence* detection (HPLC–FD) combined with pre-column or post-column derivatization, and immune affinity column/HPLC are currently the most widely used for determination aflatoxins due to its great versatility in the analysis of complex matrices [4–6].

Despite the many advantages, they present several disadvantages. For example, pre-column derivatization is time consuming and requires the use of harmful reagents, poor stability of derivatized forms of AFB_1. Post-column derivatization presents several drawbacks such as: long time to prepare the mobile phase and dilution caused by the adding reagents [7–9].

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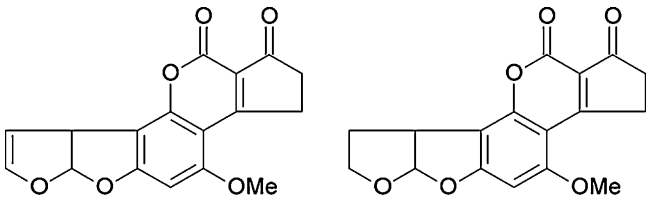

Aflatoxin B1 Aflatoxin B2

Fig. 1. Structures of AFB_1 and AFB_2.

Aflatoxins analysis by fluorescence techniques need to significant increase of the fluorescence signal of AFB_1. And immune affinity procedure is time and solvent consuming and requires a high expertise level and the use of expensive disposable cartridges [8,10,11].

As an effective sample preparation method, solid phase microextraction (SPME) has gained extensive applications in many areas. This technique was introduced by Arthur and Pawliszyn in early 1990 [12,13]. SPME similar to some other microextraction methods, combines extraction and concentration of analytes in a single step, thus the time required for sample preparation is reduced. But, conventional SPME fibers have some draw-backs such as: fiber breakage; stripping of coatings and memory effects. SPME fibers are relatively expensive and their useful lifetime is limited.

Meanwhile, we welcome the numerous advantages of this valuable sample preparation technique; we decided that developed it and reduce its disadvantages.

According to the above cases, we have focused on the different category and introduced a novel SPME technique namely; hollow fiber-solid phase microextraction (HF-SPME). In this new SPME format, a functionalized multi-walled carbon nanotubes reinforced nanocomposite was prepared based on sol–gel technique. The sol solution was placed inside a piece of treated polypropylene hollow fiber and was authorized the gel formed in situ, within the fiber [14,15]. Nowadays, carbon nanotubes have attracted much interest that was directed toward unique thermal, mechanical, electronic and chemical properties [16].

Apart from these excellent properties, as well as their highest tensile strength among known materials, some characteristics which will play a crucial role in their sorption capacity can be noted; they granted non-polar bonds meanwhile have a high ratio of length to diameter. This leads to a low solvent solubility (in the water and commonly used organic solvents) and affects the effective contact surface area available for interactions with analytes. But they can be activated via functionalization. In the other words, they can incorporate hydroxylic or carboxylic groups on their side walls under strong chemical conditions. It permits the immobilization of carbon nanotubes on solid supports such as pours silica. They can confirm non-covalent interactions with analytes, including hydrogen bond, π–π stacking, dispersion forces, dipole–dipole interactions and hydrophobic effect [17]. As was mentioned in our previous works, carbon nanotube reinforcement cased a dramatic increase in the active sites of adsorbent [14,15,18].

This novel format of SPME was used for cleanup and preconcentration of aflatoxins B_1 and B_2 in rice, peanut and wheat samples followed by HPLC–PDA analysis.

In conclude handling of this SPME device was more convenient than the other traditional SPME fibers. In addition, it is in-expensive and thus was used disposable, so there was no possibility of carry over effect. Entirely it is superior to the SPME fibers in these senses. Herein, the new method of HF-SPME was designed for the microextraction of aflatoxins that included AFB_1 and AFB_2 in cereals.

Aflatoxin determinations can be approached many ways. The HPLC and TLC methods combined with liquid–liquid extraction as conventional methods are not more often recommended only because they have two major disadvantages: (1) it is expensive because it uses large amounts of solvents which create a disposal problem, and (2) the major solvent used is chloroform which may be a hazard to workers. Reverse-phase HPLC separations of aflatoxins are more widely used than normal-phase HPLC separations. However, the fluorescence intensities of AFB_1 and AFB_2 are diminished in reverse-phase solvent mixtures so the derivatives B are diminished in reverse-phase solvent mixtures so the derivatives B are generally prepared before injection. The derivatives B are not stable in methanol.

ELISA or affinity column techniques are more suited to field use than the above mentioned techniques and will probably be extensively developed and utilized. The disadvantages of these methods include different an antibody specificities for B1 and cross reactivity with other aflatoxins. ELISA procedures are qualitative or semi-quantitative at best and are temperature sensitive. More development is needed before immunochemical techniques will be generally useful for applications where quantification is critical. Methods also need to be developed that will distinguish between B1, B2, G1, and G2 individually or collectively. Meanwhile, the above mentioned methods are expensive and time consuming and require special equipment. While compared with the old methods to measure these toxins, the novel method has many advantages. HF-SPME combined with HPLC–DAD is simple, sensitive, rapid, cost-effective and highly affordable.

2. Experimental

2.1. Chemicals and reagents

The reference standards of aflatoxins containing AFB_1 and AFB_2 were obtained from Food and Drug Administration, Khorasane Razavi (Mashhad, Iran). HPLC-grade acetonitrile, methanol and other chemicals were purchased from Merck (Darmstadt, Germany) and used without further purification.

Daily standard sample solutions of aflatoxins B_1 and B_2 were provided by dilution of stock solution (10 μg/mL in acetonitrile) in de-ionized water which was purchased from Samen Pharmacy Co. (Mashhad, Iran) and stored at $4 \pm 0.5\,^{\circ}C$.

2.2. Instrumentation

The HPLC system used in this work was a Knauer (Germany, d-14163) containing photo diode array detector; S2600, a port sample injection valves equipped with a 20-μL loop. Separation was accomplished using a 100/5-C_{18} column with; 4.6 mm diameter, 250 mm length; from Knauer (Germany). An RP-18 guard column was fitted upstream of the analytical column. The mobile phase was water:methanol:acetonitrile, optimized on (55:25:20, v/v) and was de-gassed by own system de-gasser and delivered two pumps S1000. The flow rate of the mobile phase was 0.8 mL min^{-1} and PDA detection wavelengths were set as 380, 265, 372 and 362 nm.

The system also was equipped to a computer system and software EZ-Chrom Elite with integration capability.

A Metrohm 780 pH-meter equipped with a combined glass electrode was used to determine pH values during the experiment (Herisau, Switzerland).

2.3. HF-SPME procedure

2.3.1. Carbon nanotube functionalization

Raw MWCNTs were heated in an oven at 350 °C for 30 min to remove amorphous carbon. After thermal treatment, a half-gram of CNTs was dispersed into a flask containing 20 mL of a 70% sodium

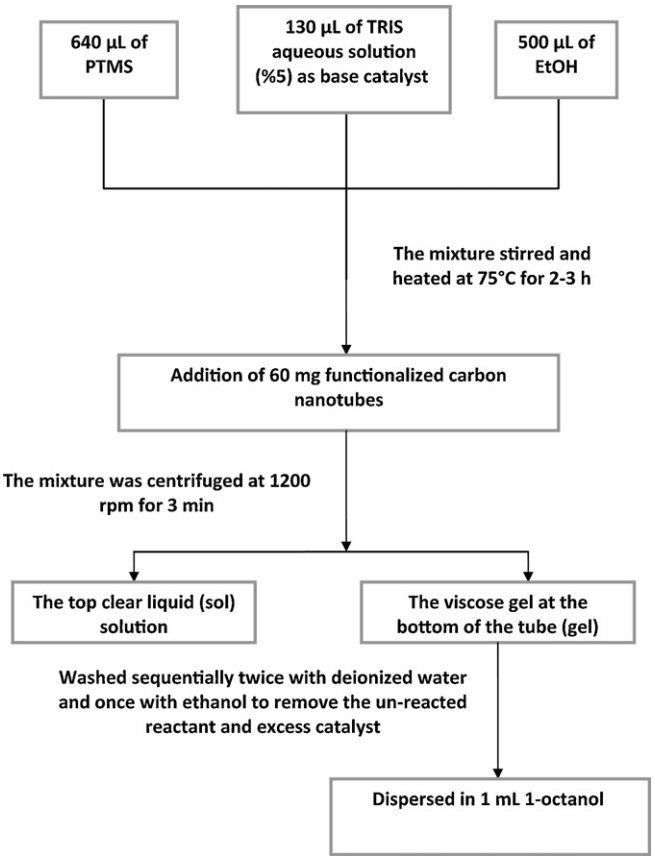

Fig. 2. The schematic of preparation of sol–gel.

hypochlorite solution (6 mL of H_2O + 14 mL of NaClO). The solution was then shaken in an ultrasonic cleaning bath for 20 min. The resulting solution was filtered through a 0.45-μm Nylon fiber filter, yielding the activated CNTs. Finally, the CNTs were washed thoroughly with re-distilled water several times until the pH of the filtrate was neutral. The filtered solid was dried in the oven at 85 °C, obtaining carboxylic acid-functionalized MWNTs (MWNT–COOH) [18].

2.3.2. Sol–gel preparation

The sol–gel solution based on a basic catalyst was prepared as follows: First to start the hydrolysis, 640 μL of phenyltrimethoxysilane (PTMS), 130 μL of 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS) aqueous solution (5%) as base catalyst and 500 μL of EtOH were added into a vial and the mixture stirred continually and heated at 75 °C for 2–3 h until hydrolysis was performed and turbidity appeared. In this step 60 mg functionalized carbon nanotube was added to the mixture. The mixture was stirred thoroughly and then centrifuged at 1200 rpm for 3 min. The top clear liquid solution was removed and the gel at the bottom of the tube was washed sequentially twice with deionized water and once with ethanol to remove the un-used reagents and excess catalyst. The resulting gel was transferred to a clean vial and dispersed in 1 mL 1-octanol.

The gel was very thick and there was no possibility of injection into the fiber, so it was diluted with 1-octanol. Due to 1-octanol completely consistent with the structure of the polypropylene fiber and in many other experiences of hollow fiber liquid phase or solid phase microextraction, 1-octanol was used [15,18–21] .The schematic of preparation of sol–gel was shown in Fig. 2.

2.3.3. Fabrication of HF-SPME device

The polypropylene hollow-fiber tubes were cut into 2.0-cm segments. Each piece was used only once to decrease the memory effect. The fiber segment was cleaned with acetone to remove impurities and directly dried in air. After that, 6.0 μL of mixture of functionalized MWCNTs and sol–gel which was dispersed in 1-octanol injected into the fiber segments with a 25-μL Hamilton syringe.

Fiber wall pores are channels through which the analytes molecules (in the feed solution) and the adsorbent (inside the fiber) were in contact with each other.

Meanwhile, the pores can cause a kind of dimensional selectivity to the analyte molecules. The fiber segments were sealed on both ends by flame and before use were left to dry at room temperature for 1.0 h. Thereupon the fiber segments were placed into the sample solution present in a proper vial (25 mL volume). The vial was covered and stirred for appropriate time. During the extraction time, the analytes from the sample solution diffuses through the porous polypropylene membrane into the sorbent. At the end of the extraction, the hollow fiber was taken out from the vial and transferred into a dry and clean glass vial and the analytes were back-extracted by 2.0 mL of MeOH with ultrasonic agitation for 15 min. Then the 10 μL of the extracted solution was injected into HPLC for analysis. The fiber segments were disposable.

2.4. Real sample treatment

Many investigations have been conducted to show that methanol/water extraction for rice and wheat and methanol/water plus hexane for peanut and pistachios gave recoveries at least as good if not better than other solvent systems for naturally contaminated materials. In the case of samples with high fat like peanut, it was required to prepare the extract, perform the clean-up using one immunoaffinity clean-up column and analyze the extracts by HPLC.

10 g of cereal samples finely ground by a mill were weighed into a blender jar. 1 g NaCl was added and the toxins were extracted with 100 mL methanol–water (80:20, v/v) by blending at high speed for 2 min.

The mixture extract was sonicated (Lind sonic 35, ISCO, Milan, Italy) for 20 min, centrifuged (ALC Centrifuge 4235-D, CHEMIE, Bari, Italy) at 2500 rpm for 10 min to ensure that the toxins completely extracted by methanol–water and the solid residue removed. The extract was evaporated under a N_2 stream for 10 min to eliminate MeOH, and then the remaining extract was diluted with 40 mL distilled water. The diluted extract was transferred into a dark flask and subjected to HF-SPME.

In the case of samples with high fat (peanut), 100 mL n-hexane was added to the sample and then it was filtrated. 2 mL of the extract was diluted with water up to 10 mL and eluted through immunoaffinity (IA) Afla-Clean column (LCTech, Dorfen, Germany) at a 3 mL min^{-1} flow rate. The IA column was then washed with distilled water (10 mL) and aflatoxins were finally eluted with MeOH (2 mL) in a dark flask. The eluted was dried under a stream of N_2 to evaporate the methanol and residue aflatoxins were dissolved in 1 mL of mobile phase, diluted to optimal feed volume with distilled water and subjected to HF-SPME.

Since fatty matrices are complex an immunoaffinity clean-up process is necessary. So, the method is suitable for peanut in terms of the lower limits at which validation has been carried out for aflatoxin B1 and total aflatoxins [22,23].

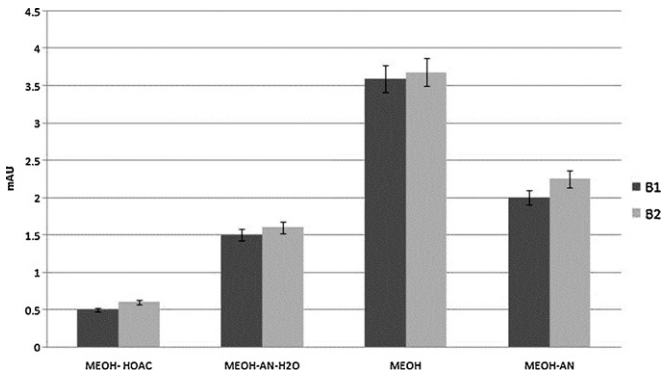

Fig. 3. Effect of type of the up-take solvent on the extraction. Conditions: analytes concentration, 1 μg/mL; donor phase volume, 20.0 mL; acceptor phase volume 6.0 μL; stirring speed, 1000 rpm; extraction time, 60 min; temperature 22 ± 0.5 °C.

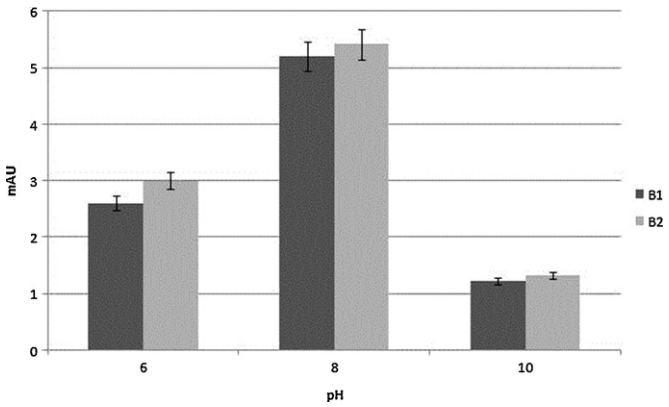

Fig. 4. Effect of feed solution pH on the extraction. Conditions: analytes concentration, 1 μg/mL; donor phase volume, 20.0 mL; acceptor phase volume 6.0 μL; stirring speed, 1000 rpm; extraction time, 60 min; temperature 22 ± 0.5 °C.

3. Results and discussion

3.1. Optimization

3.1.1. Up-take solvent selection

Choosing the most suitable up-take solvent is very important for achieving a good selectivity and extraction efficiency. The up-take phase must therefore be selective for extracting analytes with high enrichment factor. Also, the analytes should have high solubility into the back extracted solvent in order to achieve a high degree of recovery of analyte in the up-take phase.

Four different solvent systems were investigated namely: methanol, methanol–acetonitrile (55:45, v/v), methanol–acetonitrile–water (25:20:55, v/v) and methanol–acetic acid (90:10, v/v) (see Fig. 3) [14,15,18]. According to the results, methanol with high viscosity and other suitable characterizations was selected for this work.

3.1.2. Effect of the feed phase pH

The pH value of aqueous feed-phase plays an essential role in the extraction process. Considering that the feed solution pH is also one of the major factors that progress the transfer of analyte from the feed to the HF-SPME device. Therefore, after survey of the pH effect in the pH range 3–10, was observed that practical preconcentration factors of analytes increased for sample solution in pH 8 was the best and we used.

When the pH was lowered to 8, the amount of analyte extracted was significantly reduced. When the pH was adjusted to higher values, the amount extracted also decreased. These results indicate that the suitable pH for aflatoxins extraction was 8. The result was predictable, because these toxins in the strong acidic or basic conditions (3 > pH > 11) are decomposed [19,24]. The result was depicted in Fig. 4.

3.1.3. Effect of the stirring rate

Stirring speed affects the extraction efficiency because agitation of the sample is routinely applied to the mass transfer coefficient in aqueous solution and accelerates the extraction kinetics. Increasing the stirring rate can decrease the thickness of the diffusion film in the aqueous phase and improve the repeatability the extraction method [25,26]. Thus, the influence of the stirring rate in the range of 100–1500 rpm was surveyed and in stirring speed 1000 rpm as optimized rate, practical pre-concentration factor reached the highest. Faster stirring rates may lead to fiber collision with the wall of the vial and air bubbles formation around the fiber side wall.

3.1.4. Extraction time (T1)

In the first step, analytes were extracted from the aqueous sample into the HF-SPME device that is a slow equilibrium process, and mass transfer is depended on time [14,15,27]. SPME is an equilibrium process and extraction time affects the equilibrium conditions. Over the extraction time solute molecules have sufficient chance for transfer from donor phase to interface between the feed and HF-SPME device and for collection in it. Therefore, extraction time was a significant factor that was affects the method efficiency.

In this work, different extraction times of 30–90 min were evaluated at room temperature, and the extraction efficiency for AFB1 and AFB2 increased up to 60 min. After this optimum time the efficiency showed a soft decline. Therefore, an extraction time of 60 min was chosen as the optimal time. The result was shown in Fig. 5.

3.1.5. Back-extraction time (T2)

In order to analytes up-take from the fiber and its transfer to the back-extraction solvent, should be given enough time. An appropriate up-take time causes increase extraction and leads to progressed pre-concentration factor. However, longer back-extraction time will result in the evaporation of up-take solvent especially under stirring. We have tested different back-extraction times from 5 min to 25 min. On this basis, 15 min was selected as optimal back-extraction time.

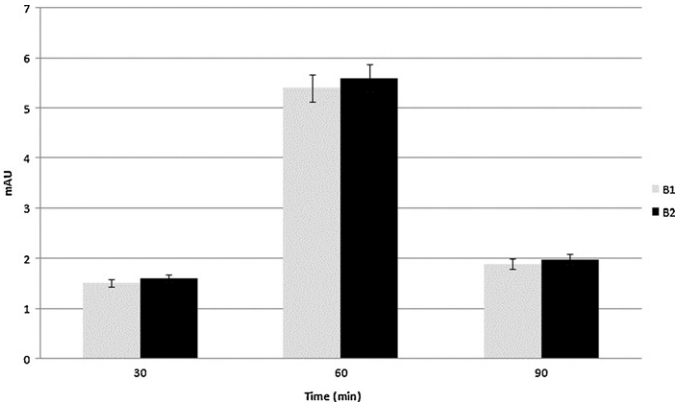

Fig. 5. Effect of extraction time on the extraction. Conditions: analytes concentration, 1 μg/mL; donor phase volume, 20.0 mL; acceptor phase volume 6.0 μL; stirring speed, 1000 rpm; pH 8.0; temperature 22 ± 0.5 °C.

3.1.6. Effect of the donor phase volume

The length of the hollow fiber segment (indeed, volume of the acceptor phase) was fixed to 2.0 cm and the reduced length was compatible with small sample volumes, which are highly relevant in some analytes in the biomedical and environmental applications. In addition, enrichment of the analyte increases with increasing the volume ratio of sample solution to acceptor solution. The pre-concentration factor in HF-SPME basically depends upon the phase's volume of the sample and the acceptor. As the volume of the sample increases, the pre-concentration factor also increases.

The effect of sample volume on the extraction efficiency of analytes was also studied. In the present work, the phase ratio of donor and acceptor solutions was optimized by changing the volume of the donor phase between 10 and 30 mL while the volume of acceptor phase was kept constant at 6.0 μL. The pre-concentration factor can be improved by the increasing the volume ratio of donor and acceptor phases [25–28]. According to the results, the optimum donor phase volume was 20 mL for pre-concentration of the analytes in question.

3.1.7. Role of the amount of MWCNTs in extraction

The extraction efficiency dramatically increased with increasing carboxylic functionalized MWCNTs, because amount of carbon nanotubes is fundamental for obtaining a satisfactory pre-concentration and extraction process. But increasing the amount of functionalized MWCNTs (up to 50 mg per 1 mL sol) to sol solution causes the solution to come thick. This solution hardly is drawn with the syringe to inject the right inside the hollow fiber segment. Thus, 50 mg of nanotubes per 1 mL of sol solution was chosen as the optimal amount.

3.1.8. Ageing time of sol–gel

Surface morphology of the sorbent was directly related to the age of the sol. Also, the sorbent properties were correlated to the amount of polymerization of the precursors dissolved in solution. Ageing for an extended period, however, led to a gradual decrease in the viscoelasticity of the gel, likely driven by the expulsion of liquid from the gel, a phenomenon called syneresis [29].

The average sorption properties of the nanocomposites show an increase at around 72 min. It should also be noted that the ageing times depend dramatically on the precursor concentration. It is expected that the increase in the viscosity might cause the formation of the network structure of silica particles.

The times of the gelling strongly depend on the concentration of precursor, excessive value of catalyses and temperature.

Fig. 5 shows the effect of ageing time on the extraction efficiency. Different ageing times (25–120 min) were studied at room temperature. The pre-concentration for analytes increased up to 72 min, and then decreased with additional ageing time (see Fig. 6).

After the optimal ageing time, viscosity increased due to multi-dimensional network formation and polymer growths in a limited volume (inside the fiber), led to polymer network distortion, and pores disruption [14]. Thus extraction efficiency decreased. Therefore, 72 min was selected as the optimal ageing time.

4. Method validation

4.1. Analytical performance

Stock solutions containing 10 mg mL^{-1} of aflatoxins B1, B2 were prepared, in acetonitrile and stored at 4 °C. Standard solutions were obtained by spiking calculated amounts of the stock solution into the real sample solutions which were prepared and described previously. These working samples were used for experimental and plotting the calibration curves under optimal conditions; donor phase volume, 20.0 mL; acceptor phase volume 6.0 μL; stirring speed, 1000 rpm; pH 8.0 and temperature 22 ± 0.5 °C.

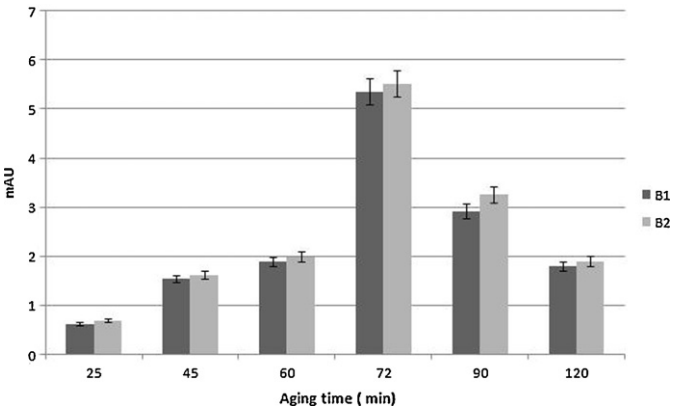

Fig. 6. Effect of ageing time on the extraction. Conditions: analytes concentration, 1 μg/mL; donor phase volume, 20.0 mL; acceptor phase volume 6.0 μL; stirring speed, 1000 rpm; pH 8.0; temperature 22 ± 0.5 °C.

In concentration range between 0.05 and 500 ng/mL linear calibration curves were plotted. The obtained calibration equation for B1 was $Y = 0.0336X + 1.33$ and for B2 was $Y = 0.0412X + 1.478$ respectively. Linearity was observed with $R = 0.9928$ for B1, and $r^2 = 0.9961$ for B2. Practical limit of detection (LOD) and limit of quantification (LOQ) were calculated as the minimum concentration providing chromatographic signals three times and ten times higher than background noise, respectively. Thus, obtained LOD for B1 was 0.074 and for B2; 0.061 ng/mL. LOQ for B1 and B2 were 0.1 ng/mL too.

For calculation the practical pre-concentration factor, the chromatogram peak area after extraction of 1 μg/mL of analyte was divided by the peak area before extraction under the same conditions. Specifically, after extraction of the analyte into the fiber, it was desorbed with methanol, 2.0 mL of up-take solvent. The peak area after this extraction was divided by the peak area of the sample before extraction and multiplied by a dilution factor (2000/6). The volume of donor phase was 20.0 mL, and 6 μL was the volume of the solid sorbent spaces that were occupied by analyte molecules (i.e., the acceptor phase volume).

Pre-concentration factor for B1 was 3996.9 and for B2 3677.06 respectively. Repeatability (RSD%) evaluated with three replicated experiments. Mean RSD% for B1, is 2.92 and for B2 is 2.68 respectively for the measured toxin concentrations 1.0, 200.0 and 250.0 ng/mL (see Table 1).

The review of some methods which were used for determination of aflatoxins B1, B2 in the different samples was demonstrated in Table 2.

4.2. Real sample analysis

To investigate matrix effects and applicability of the technique to real samples, final experiments were carried out on two samples containing aflatoxins B1, B2 (rice and wheat samples). Concentration of AFB1 in a contaminated rice sample was 10 (ng/mg) with RSD% 2.45 ($n = 3$). In the wheat sample, AFB1 was 15 ng/mg with RSD% 2.80 ($n = 3$) and concentration of aflatoxin B2 in these two contaminated samples was lower than detection limit.

The standard solution of aflatoxins B1, B2 was spiked into real samples to demonstrate the potential of this method as a viable extraction technique for cereal samples.

Relative recovery defined as the ratio of the concentrations found in the investigated matrix to those in distilled or deionized water spiked with the same amounts of analytes. It's generally used

Table 1
Figures of merit of the proposed method in AFB1 and AFB2 determination.

Analyte	LR (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	*R*	Pre-concentration factor	RSD% (*n* = 3)	RR% (in rice)	RR% (in wheat)
B1	0.1–400	0.074	0.1	0.9912	3996.9	2.928	47.43 ± 6.4%	82.83 ± 4.9
B2	0.1–400	0.061	0.1	0.9921	3677.06	2.679	53.68 ± 5.5	103.15 ± 6.1

Table 2
Comparison of some methods which were used for determination of AFB1 and AFB2.

Matrix	Extraction method	Detection system	LOD (ng/mL)	LOQ (ng/mL)	Linear range	*R*	RSD% (*n* = 3)	RR%	Ref.
Peanut	–	HPLC–FL	0.03–0.09	0.04–0.3	–	0.985–0.995	3.27–14.84%	77.16–98.51	[30]
Peanuts	Nanogold probe-immunochromatography		0.03–0.25	–	–	–	–	–	[31]
Pistachio nuts	SPE	HPLC–DAD	0.03–0.22	0.1–0.68	–	–	–	–	[32]
Cereal flours	SPME	HPLC–FL	0.065–0.212	0.103–0.21	B1, G1 up to 20 (ng/g) B2, G2 up to 6 (ng/g)	0.989–0.998	0.99–2.27%	–	[33]
Food	–	MS	2.1–2.8 (pg/mL)	–	0.05–2 (ng/mL)	0.999	1.9–3.3%	94.5–102	[34]

instead of absolute recovery. The recovery values close to 100% indicate the lack of matrix effect and good accuracy of the procedure.

The relative recovery of aflatoxins from real samples was determined as the ratio of the concentration in the real sample and distilled water samples spiked at the same concentration level (1 μg/mL) [14,15]. Relative recovery percent of 47.43 ± 6.4% (mean ± S.D.) and 53.68 ± 5.5 were obtained for B1 and B2 in the rice sample. 82.83 ± 4.9 and 103.15 ± 6.1 were calculated for B1 and B2 in the wheat sample respectively. HPLC chromatogram of a rice sample after spiking with 1 μg/mL of aflatoxins B1, B2 was shown in Fig. 7.

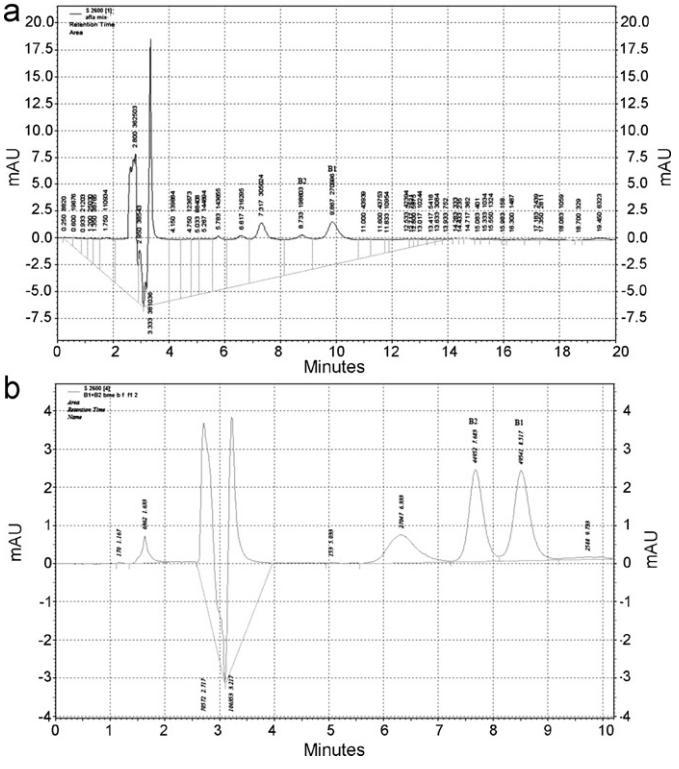

Fig. 7. HPLC chromatogram of (a) rice sample and (b) rice sample after spiking with 1 μg/mL of aflatoxins B1, B2.

5. Conclusion

A new affordable, rapid and compatible method of hollow fiber solid phase microextraction compared to most conventional procedures, was introduced in this work. This extraction technique requires very little expensive and toxic organic solvents. It is a promising pre-treatment method for the fast, trace analysis in many complicated matrixes such as cereal samples.

The method has a high pre-concentration factor and excellent selective clean-up of aflatoxins samples as the very complicated analytes. Good linearity and reasonable relative recovery were also obtained. We used the method to isolate aflatoxins B1 and B2 from real samples and found it to have many advantages over conventional methods.

The experimental operations involved in HF-SPME are very simple. Moreover, this procedure offers several advantages over traditional extraction techniques such as; a reduction in extraction time, also this method is economical and easy to use. In our method, we introduced a reliable qualitative and quantitative technique for determination aflatoxins B1, B2 at low level of concentration in real samples.

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