**Electrospun biocompatible Chitosan/MIL-101 (Fe) composite nanofibers for
solid-phase extraction of Δ^9 -tetrahydrocannabinol in whole blood samples
using Box-Behnken experimental design**

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Abstract

The nanofibers of biocompatible Chitosan/MIL-101 (Fe) composite were synthesized by a simple, cheap and accessible electrospinning method and applied for mat-based extraction of trace amount of Δ^9 -tetrahydrocannabinol (THC) from human whole blood sample following its combination by high performance liquid chromatography-ultraviolet detection. The composite nanofibres were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy, X-ray diffraction and N₂ adsorption-desorption experiments. The volume of eluting solvent, sorbent amount, pH and % NaCl (w/v) influencing on the responses were investigated using factorial experimental design. The optimum point was achieved by analysis of the results according to design expert (DX) software. The volume of eluting solvent, sorbent amount and pH were significant variables, and 150 μ L, 7 mg and 7.0 were respectively chosen for obtaining the best extraction response. Under the optimum conditions, the method was exhibited a linear range of 0.1-100 μ g L⁻¹ ($R^2 = 0.9943$) for THC with a detection limit of 0.04 μ g L⁻¹. Acceptable values for intra-day (3.2 %) and inter-day (4.8 %) relative standard deviations were obtained. The high preconcentration factor (970) and satisfactory recoveries (88.2 % to 92.4 %) in whole blood samples were achieved which proved the capability of the method for trace determination of THC in the human whole blood samples.

Keywords: Electrospinning; Chitosan/MIL-101 (Fe); Biocompatible composite nanofibers; Δ^9 -tetrahydrocannabinol; Central composite design

1. Introduction

Nowadays, researchers are focusing on fabricating composite nanofibers which exhibit specific functional properties for solid phase extraction (SPE) application [1-5]. Chitosan, as the second abundance of natural polysaccharide, is obtained from chitin and is well known for its biocompatible, nontoxic and biodegradable properties [6]. Chitosan has been used extensively as bio-adsorbent due to its low cost, ease of production, nontoxicity, high hydrophilicity and high contents of hydroxyl and amino functional groups, which possess high activity as adsorption sites. As an adsorbent, chitosan is commonly used in the form of powder, flakes or hydrogel beads. It is clear that these physical forms suffer from two major drawbacks: the low specific surface area and unavailability of the majority of active binding sites, which result in a relatively low adsorption capacity and slow adsorption kinetics. Therefore, a way is needed to increase the surface area of chitosan for more effective adsorption [7].

Nanoporous materials have attracted great attention with various applications. Development of metal organic frameworks (MOFs), which built from metal ions and organic ligands, is one of the most exciting recent developments in nanoporous material science [8]. These porous materials have unique properties such as high surface area with well-defined cavities or channels that can be used in various potential applications including gas storage, separation, drug delivery, catalysis and others [1, 9-12]. Among the tens of thousands of known MOFs, the MIL (Materials Institute Lavoisier) family, crafted from trivalent metal centers and carboxylic acid bridging ligands by Férey et al. [13], has particularly attracted a great deal of attention due to its enhanced stability, very large pores and enormous porosity. The MIL-101 materials have a rigid zeotype crystal structure, comprising of quasi-spherical cages of two modes. They have very large surface area and many transition metal sites, which can be activated by heating under vacuum

conditions [14]. MIL-101(Fe), a biocompatible porous iron-containing analog in the MIL-101 family, was synthesized by Pashow et al. [15] in 2009. It can be an appropriate material as SPE sorbent.

Current research activities mainly focus on the means of applying electrospun nanofibers in SPE. To increase the adsorption of different compounds on electrospun nanofibers, the following methods have been attracted the most attention: (1) increasing the surface area of nanofibers, (2) surface modification of nanofibers with functional groups and (3) fabricating of composite nanofibers [1, 16-18]. Composite nanofibers modified by the functional groups have been extensively used because of their specific properties such as: (1) high surface-to-volume ratio, (2) uniform pore-size distribution, and (3) high adsorption capacities of different target compounds. So, the composite nanofibers can be a kind of high efficient adsorbent for adsorption of different target compounds. Compared with the powder composite materials, electrospun nanofibrous materials are good candidates for SPE application because of their high surface-to-volume and length-to-diameter ratios that can achieve a higher specific surface area and more active sites for adsorption. Therefore, a small amount of nanofibers is sufficient for the extraction, and the volume of desorption solvent can be reduced greatly. It is consistent with the green chemistry concept [19-20].

Δ^9 -tetrahydrocannabinol (THC) is the main psychoactive constituent of Cannabis Sativa L., the most commonly abused drug after alcohol and nicotine [21]. For most forensic toxicology laboratories, the confirmation and quantification of THC and its metabolites in the blood samples are on the top of list of requests from the enforcement agencies, such as police departments and district attorneys. So, the ability of efficiently extract and quickly analyze of the trace amount of

THC in biological samples, submitted by such agencies, is seen as a definite benefit to the laboratory in question.

Various analytical techniques for the quantification of THC are already reported [21-24]. Usually, gas chromatography-mass spectrometry (GC-MS) technique was used for the routine analysis of THC [24-28]. However, this method is demanding and time consuming, since it requires derivatization of the THC. Among the most recent developments, GC-MS is rapidly replaced by liquid chromatography (LC) combined with mass spectrometry (MS) due to its relative methodological simplicity for THC quantification [23, 29-33]. Although the specificity and sensitivity of these techniques are good, the required equipments are expensive and usually not available in most of the laboratories.

In this work, a biocompatible Chitosan/MIL-101 (Fe) composite nanofibers was synthesized by electrospinning, and a simple method for determination of trace amount of THC in human whole blood samples was developed with a simplified sample clean-up procedure. The sensitivity and throughput of the method without requiring derivatization was significantly improved, and it allows quantitation of trace amount of THC in biological samples by high-performance liquid chromatography with UV detection (HPLC/UV).

2. Experimental

2.1. Materials

All reagents were of analytical grade. Double distilled water was used throughout the experiments. Glacial acetic acid, methanol (HPLC grade), ethyl acetate, acetonitrile (HPLC grade), n-hexane, hydrochloric acid, sodium hydroxide, dimethylformamide (DMF), terephthalic

acid (H_2BDC) and Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) were obtained from Merck (Darmstadt, Germany). Medium molecular weight chitosan powder and THC were purchased from Sigma-Aldrich (Shanghai, China). The stock solution of THC (1000 mg L^{-1}) was prepared in methanol and stored in the refrigerator. Working solutions were prepared daily by appropriate dilutions of the stock solution with methanol. The sample solutions of THC for method validation step, were prepared in the diluted drug free human whole blood samples by spiking with the concentrated standards.

2.2. Instrumentation

All chromatographic measurements were performed using an Agilent 1100 high performance liquid chromatography (Santa Clara, CA, USA) with a C18 column ($250 \text{ mm} \times 4.6 \text{ mm i.d.}$, $5 \mu\text{m}$ particle size) and a UV/Vis detector which is set at 215 nm . The mobile phase was a mixture of acetonitrile and ultra-pure water (90:10). Electrospinning machine (ANSTCO-RN/X, Asian Nanostructures Technology Co., Iran) was used for preparation of the composite nanofibers. Nano-sized MIL-101 (Fe) was synthesized by microwave (ETHOS 1, Milestone Inc., USA). The pH values were adjusted with a WTW 3310 pH meter (Germany) combined with a glass electrode. The nanostructures were characterized by SEM (KYKY EM-3200). XRD analysis was performed using a Philips-PW 17C diffractometer with Cu-K α radiation. FT-IR spectra of KBr powder-pressed pellets were recorded on an ABB BOMEM model FTLA 200-100 instrument. Brunauer–Emmett–Teller (BET) analysis surface area was measured at 77 K with a Belsorp mini II (Japan) using nitrogen adsorption at 77 K .

2.3. Synthesis of MIL-101 (Fe)

128 The nanoporous MIL-101 (Fe) was synthesized using a facile microwave irradiation method ^[34].
129 Equimolar (0.346 mmol) of FeCl₃.6H₂O and H₂BDC were dissolved in 15 mL DMF. The
130 mixture was then heated to 150 °C for 15 min via microwave irradiation. After cooling to room
131 temperature, the product was filtered and washed with 20 mL DMF and methanol, and dried at
132 150 °C under vacuum for 3 h.

133 2.4. Preparation of Chitosan nanofibers

134 The chitosan powder (7% w/v) was dissolved in 90% aqueous acetic acid solution and stirred
135 with a magnetic stirrer at room temperature for 12 h to make even spinning solution, and
136 continuously supplied into a glass syringe (volume; 5 mL). The syringe, used in the experiments,
137 had 20 gauge stainless needle (diameter; 0.58 mm) with a flat tip connected to the anode. A
138 grounded aluminum foil was served as the collection screen (cathode). An electric field of 4
139 kV/cm and a flow rate of 1.2 mL h⁻¹ were used to produce bead-free chitosan nanofiber. Finally,
140 the resulting electrospun nanofiber layers were dried at 60 °C overnight to remove possibly
141 remaining acetic acid and water after the electrospinning [35].

142 2.5. Preparation of Chitosan/MIL-101 (Fe) composite nanofibers

143 The composite of chitosan nanofibers containing highly nanoporous MIL-101 (Fe) were
144 fabricated by electrospinning according to the following procedures. First, 1.4 g chitosan powder
145 was dissolved in 93 g aqueous acetic acid solution (90 % v/v). Then, 5.6 g MIL-101 (Fe) powder
146 was added and stirred with a magnetic stirrer for at least 30 min. The appropriate amount of this
147 slurry was loaded into a glass syringe (volume, 5 mL). The syringe was fitted to a stainless
148 needle (diameter 0.5 mm) with a flat tip connected to the anode. With an interval of 15 cm, a
149 grounded 10 × 10 cm of aluminum foil was served as the collection screen, and a voltage of 18

kV was applied between the tip and aluminum foil, and the flow rate was 2 mL h⁻¹. All procedures were carried out at room temperature for 30 min. So, a dense layer of composite nanofibers was collected on the aluminum foil. Finally, the resulting composite nanofiber layers were dried at 60 °C overnight to remove possible acetic acid and water remaining in the material after electrospinning.

2.6. Extraction procedure for nanofibers-based SPE

In order to enrich the target compound by the mat-based SPE method, a homemade SPE device was made. One piece of composite nanofibers mat (7 mg) was accurately cut in a circular form with a diameter of approximately 30 mm and attached tightly to the filter. Both ends of the filter were screwed down with a sample tube on the top and a collecting tube on the bottom. Prior to use, SPE column was preconditioned by the sequential addition of 1 × 500 µL of methanol and 1 × 500 µL of deionized water. 5 mL cold acetonitrile was added drop-wise to the whole blood sample (5 mL) during vortexing sample at a moderate speed. Following addition of the acetonitrile, the whole blood sample was further vortex and mixed for approximately 1 min. Any precipitated material in the blood sample was fully removed by centrifuging the sample at 10000 rpm for 10 min. Following centrifugation, the acetonitrile phase, supernatant of the blood sample, was transferred into the clean glass tubes. The solvent was evaporated to approximately 1 mL with N₂ gas stream. 50 mL deionized water was added to each tube, and the pH of solution was adjusted to 7.0. The mixtures were passed through the SPE column and allowed to flow through the sorbent using the gravitational flow. The column was washed with 200 µL water. The analyte was quantitatively eluted with 150 µL methanol. The elution solvent was allowed to flow through the sorbent with the aid of gravity and collected in silanized glass tubes for evaporation. The elution solvent was filtered by 0.22 µm PTFE syringe filter, and then

evaporated to dryness with N₂ gas stream, after that the extract was dissolved in the mobile phase (50 µL) for analysis by HPLC-UV.

2.7. Extraction procedure for C18 cartridges SPE

The extraction using C18 cartridge was performed by the optimized experimental procedure. Three mL methanol was used as the eluent solvent and the pH of the sample solution (50 mL) was 7.0. The column was conditioned with sequential elution with 3 mL water and 3 mL methanol. Then, 50 mL sample was successively passed through the cartridges. Each solvent was allowed to percolate through the sorbent using gravity. The analyte was quantitatively eluted with 3 mL methanol, and then evaporated to dryness with N₂ gas stream, after that the extract was dissolved in mobile phase (50 µL) for analysis by HPLC-UV.

3. Results and Discussion

3.1. Electrospinning

Successful electrospinning of MIL-101 (Fe) nanoparticles requires finding a suitable polymeric carrier/binder and a suitable solvent. Based on the preliminary experiments and considering the specifications, chitosan was selected as the polymeric carrier/binder, and 90% aqueous acetic acid solution was used as the solvent. The balance between suitable viscosity, high MOF nanoparticles loading, and also high ratio of polymer in the slurry is very important to guarantee the spin ability. A number of electrospinning slurries containing different MIL-101 (Fe) to chitosan weight ratios (0.7:6.3, 1.4:5.6, 2.8:4.2, 4.2:2.8, 5.6:1.4, 6.3:0.7) were prepared. For every slurry composition, the electrospinning conditions were optimized individually to obtain the fibers. The initial attempts for electrospinning were failed to give fabrics with a uniform

distribution of nanoparticle, due to the low content (< 20 wt %) of MIL-101 (Fe) in the slurry. When the weight ratio of MIL-101 (Fe) to chitosan was low (< 20 wt %), most of the fibers content were chitosan with a small amount of MIL-101 (Fe) agglomerates. On the other hand, the weight ratio more than 90 wt % for MIL-101 (Fe) was not possible, because the slurry was not stable during the electrospinning process. The slurry containing 80 wt % MIL-101 (Fe) nanoparticles (weight ratio = 5.6:1.4) showed good quality chitosan fabrics with relatively uniform distribution of the nanoparticles. In general, good fibers were prepared from the slurry containing a high concentration of MIL-101 (Fe) nanoparticles.

3.2. Characterization

The structure of MIL-101 (Fe) was confirmed by X-ray diffraction (XRD) (Fig. 1a). The relative intensity and peak positions of the sample are in agreement with the previous works [34], and confirm the formation of MIL-101 (Fe). To confirm stability of the sorbent, the XRD patterns of MIL-101 (Fe), soaked in methanol and water, were taken. As shown in Fig. 1b, the peaks shape was found similar to pure phase MIL-101 (Fe), it make sure that the crystalline MIL-101 (Fe) structure was still intact after immersing in water and methanol, and therefore, it is suitable for the extraction of analytes from the biological samples. The comparison of XRD patterns of MIL-101 (Fe) and the prepared chitosan/MIL-101 (Fe) composite nanofibers (Fig. 1c) clearly confirms that MIL-101 (Fe) crystals remain intact during the electrospinning process when they are exposed to the fiber surface. It is found that MIL-101 (Fe) crystals are tightly embedded in the chitosan matrix, and peeling off the nanoparticles from the nanofibers surface through the water and methanol was not observed (Fig. 1d).

The investigation of the specific surface area of pure chitosan in comparison to the chitosan/MIL-101 (Fe) composite nanofibers was also performed by nitrogen sorption

measurements at 77 K. Prior to the analysis, the samples were degassed overnight at 50 °C. The specific surface area (BET, $p/p_0 = 0.2$) is increased from 14.3 to 3300 m²g⁻¹. As a result, compared with pure chitosan nanofiber, chitosan/MIL-101 (Fe) composite nanofibers are good candidates for SPE application because of their high surface-to-volume ratio that can achieve a higher specific surface area and more active sites for adsorption.

The morphologies of MIL-101 (Fe) nanoparticles, chitosan and chitosan/MIL-101 (Fe) nanofibers were studied by scanning electron microscopy (SEM) after gold deposition. It can be seen that the crystals of MIL-101 (Fe) have a well-shaped and high-quality octahedron shape (Fig. 2). As can be seen, the electrospun chitosan nanofibers (Fig. 3a) and chitosan/MIL-101 (Fe) composite nanofibers (Fig. 3b) exhibited a well-defined morphology. Homogeneous distribution of MIL-101 (Fe) nanoparticles on the surface of the chitosan nanofibers has formed a necklace-like structure. It has also led to a material sorbent with a high surface area.

Further confirmation of the successful incorporation of MIL-101 (Fe) nanoparticles into the chitosan monolith was verified by the Fourier transform infrared (FT-IR) spectroscopy, presented in Fig. 4. Strong bands in the region of 1300–1750 cm⁻¹ correspond to ν (C–C), ν_s (COO) and ν_{as} (COO) vibrations, implying the presence of dicarboxylate linker in MIL-101 (Fe). The sharp band at 749 cm⁻¹ and weak band at 1117 cm⁻¹ are attributed to γ (C–H) and σ (C–H) vibrations of the aromatic rings, respectively. The band at 1635 cm⁻¹ is assigned to the vibration of C=O group. Also, the Fe–O vibration peak in MIL-101 (Fe) framework appeared at 540 cm⁻¹ (Fig. 4a). The FT-IR spectrum of the chitosan/MIL-101 (Fe) composite nanofibers is also shown in Fig. 4b. The chitosan exhibited characteristic broad bands of OH group at 3400–3500 cm⁻¹. The band of NH₂ group can be observed at 1638 cm⁻¹. The absorption bands at 1164 cm⁻¹ (ν_{as} (COOOC bridge)), 1092, and 1042 cm⁻¹ (ν_s (COO)) are characteristic of chitosan's

saccharine structure. Therefore, it was a stronger proof that the MIL-101 (Fe) was indeed loaded into the chitosan monolith.

3.3. Optimization of the parameters influencing on extraction efficiency

3.3.1. Optimization of parameters using fractional central composite design

Optimization of the parameters influencing on extraction efficiency was performed by using fractional central composite design. Before the optimization of extraction procedure by response surface methodology (RSM), single factor experiments were performed to select the type of eluting solvent. An efficient eluting solvent is a solvent which can break the interactions between the target compounds and the sorbent quickly at the minimum possible volume. For selection of the best eluent, five different organic solvents with various polarities were used to investigate the optimum elution condition. n-Hexane, n-hexane:ethyl acetate (1:1), methanol, acetonitrile and 1% (v/v) glacial acetic acid in acetonitrile were tested for eluting of the analyte. The results indicated that methanol had the highest efficiency as the eluting solvent for THC. It can be attributed to good solubility of THC in methanol.

The Box-Behnken design was used for the optimization of extraction process through the fractional central composite design (FCCD). The CCD consists of fractional factorial points ($N_f = 2^{f-1}$), axial points ($N_a = 2f$), and a set of center points (N_0). The center points are usually repeated to obtain a good estimate of the experimental errors. The axial points are located at $+\alpha$ and $-\alpha$ from the center of the experimental domain. An axial distance $\pm\alpha$ was calculated equal to ± 1.682 by using Eq. (1) in order to establish the orthogonally and rotatability conditions [36].

$$\alpha^2 = \sqrt{N_f} \quad (1)$$

N_0 was obtained equal to 7 using Eq. (2).

$$\alpha^2 = \frac{\sqrt{(N_f + N_a + N_o)N_f} - N_f}{2} \quad (2)$$

Based on the preliminary studies, four parameters including volume of eluting solvent (μL) (A), sorbent amount (mg) (B), pH of sample solution (C) and % NaCl (w/v) (D) were considered. Therefore, the total number of experiments (N) was obtained equal to 23 by using Eq. (3):

$$N = 2^{f-1} + 2f + N_0 \quad (3)$$

Where, f is the number of variables. The main variables, and their symbols and levels are shown in Table 1. Also, the coded and uncoded values of the designed experiments based on FCCD methodology are shown in Table 1. All experiments were run in a random manner, using 2 mL of the sample solution containing $500 \mu\text{g L}^{-1}$ of THC, in order to nullify the effect of uncontrolled variables. According to the experimental design with four factors, a quadratic polynomial model was generated as Eq. (4):

$$\begin{aligned} R = & -1.17326\text{E}+008 + 1.33662\text{E}+006 \times A + 9.80344\text{E}+006 \times B + 6.74036\text{E}+006 \times C - \\ & 2.94241\text{E}+006 \times D - 33806.13884 \times AB - 24375.22826 \times AC + 44065.70607 \times AD + \\ & 6.46470\text{E}+005 \times BC - 8.47127\text{E}+005 \times BD + 95059.59421 \times CD - 3638.17019 \times A^2 + \\ & 2.18888\text{E}+005 \times B^2 - 5.11035\text{E}+005 \times C^2 + 2924.13845 \times D^2 \end{aligned} \quad (4)$$

Where R is the peak area of THC.

An analysis of variance (ANOVA) was developed to evaluate the significance and suitability of the proposed model (Table 2). The determination coefficient ($R^2 = 0.9971$) and the adjusted determination coefficient ($R^2_{\text{adj}} = 0.9920$) of the model indicated a good fit of the experimental data with the second-order polynomial equations. It supports the hypothesis that the proposed model shows high accuracy and good fit [36]. The “Lack of Fit (LOF) p-value” of 0.9289

implies the LOF is not significant relative to the pure error, manifesting a good response to the model. A p-value less than 0.05 in the ANOVA table indicates the statistical significance of an effect at each confidence level [36]. In the proposed model, A, B, C, AB, AC, AD, BC, BD, A^2 and C^2 were found significant for the experimental response. Fig. 5 illustrates the relationship between the response and experimental levels of factors in a three-dimensional representation of the response surface and two-dimensional contour plots.

The effect of volume of eluting solvent (methanol) on the quantitative elution of the target analyte was investigated by varying the elution volume from 66 to 234 μL , and the results are shown in Fig. 5. The results show that the peak area is dramatically increased by increasing the elution volume from 66 to 150 μL and then remained constant. Therefore, 150 μL of methanol was enough to obtain a satisfactory efficiency and also complete elution of the target analyte.

The effect of sorbent amount in the range of 3 to 8 mg was examined. According to Fig. 5, by increasing the amount of the sorbent, the peak area was increased because of the complete extraction of THC. The amount of sorbent higher than 7 mg may lead to an increase in the column pressure, and also the volume of eluting solvent. Therefore, 7 mg of nanofibrous sorbent was selected as the optimum value for the sorbent amount in order to obtain effective extraction.

The effect of pH in the range of 1–11 on the extraction efficiency of THC was investigated. As can be seen, the highest peak area was observed at pH 7. So, a pH of 7 was selected for the following experiments (Fig. 5). Also, the influence of ionic strength was studied by adding different amounts of NaCl, ranging from 2–12 % (w/v). However, the high concentration of salt was not favorable, because it can cause high pressure on the column as a result of high viscosity of the sample solution. According to the results, no obvious differences were observed among the peak areas of the target analyte by adding various amounts of NaCl.

Finally, the optimized conditions were obtained using the desirability function of the statistical software. As a result, the optimum working conditions to obtain the best response were as: 150 μ L for volume of eluting solvent; 7 mg for sorbent amount and 7.0 for pH of the sample solution.

3.3.2 Effect of sample volume

In order to obtain the maximum applicable sample solution loading volume on the cartridge, reproducible results and high extraction efficiency, breakthrough volume was investigated in the range of 1–100 mL. If the volume of the sample exceeds that of the breakthrough volume, the analyte get flushed away by the sample solution. All procedures were carried out in the presence of a constant amount of THC (1 μ g) under the optimized conditions. The results indicated that the sample volume did not affect quantitative recoveries in the range of 1–50 mL for the investigated analyte. Above 50 mL, the recoveries decreased for the target compound. In order to obtain a high enrichment factor, 50 mL was adopted for further experiments. The high ratio of sample volume (50 mL) to amount of composite nanofibers (7 mg) indicates the high extraction capability of the introduced biocompatible composite sorbent. Also, the LOD would be lower with increase of the sample volume.

3.3.3. Reusability of Chitosan/MIL-101 (Fe) composite nanofibers

Reusability of the composite nanofibrous mat can improve their availability compared with the other SPE sorbents. 200 μ L of methanol and water were passed through the same nanofibrous mat before the next SPE extraction in order to get rid of the possible residue of the target compound. It was found that the recovery changes of the target compound were not significant after ten extraction times. Therefore, we could conclude that the nanofibrous mat can be used ten times. Also, the stability of the sorbent after ten cycles of the SPE procedure was

investigated by SEM (Fig. 3c) and XRD (Fig. 1e). It was found that no obvious changes in the sorbent structure was observed, which also indicated that Chitosan/MIL-101 (Fe) composite nanofibers were suitable for the extraction of target the compound up to ten times.

3.4. Comparison of extraction efficiencies of Chitosan/MIL-101 (Fe) and Chitosan nanofibers

In order to evaluate the composite nanofibers mat, the extraction efficiencies of chitosan nanofibers and chitosan/MIL-101 (Fe) composite nanofibers were compared for THC in the same extraction conditions (n=3). The extraction conditions included: methanol as an eluting solvent, 7 mg of sorbent, pH value of 7 and 2 mL of the sample volume. The concentration of THC was $500 \mu\text{g L}^{-1}$. The results revealed that the chitosan/MIL-101 (Fe) composite nanofibers showed higher extraction efficiency ($91.8 \% \pm 3.1 \%$) than that of chitosan ($21.9 \% \pm 3.9 \%$). The presence of MOF nanoparticles can increase the surface active sites of the chitosan nanofibers for the adsorption of THC. On the other hand, the presence of MIL-101 (Fe) nanoparticles introduces another excellent merit to the mat-based sorbent. However, MIL-101 (Fe) nanoparticles may provide higher surface area for the nanofibrous sorbent and also more interactions with the target analyte than the pure chitosan nanofibers.

3.5. Method validation

The linearity, limit of detection (LOD) and repeatability were further investigated under the optimized conditions. The calibration curve of the analyte showed good linearity in the range of $0.1 - 100 \mu\text{g L}^{-1}$ ($R^2 = 0.9943$). To estimate the LOD, THC was extracted from the spiked diluted blank human whole blood sample to get distinguishable signals from the noise (signal to noise

ratio of 3). The LOD ($S/N = 3$) and LOQ ($S/N = 10$) was $0.04 \mu\text{g L}^{-1}$ and $0.10 \mu\text{g L}^{-1}$, respectively.

The repeatability of the presented method was assessed by analyzing six replicates of the diluted blank human whole blood samples spiked with THC at three different concentrations (1, 30, $90 \mu\text{g L}^{-1}$). The average coefficients of variation of intra-day and inter-day assays were 3.2 % and 4.8 %, respectively which were assessed by analyzing duplicate injections for each concentration for six consecutive days.

The preconcentration factor (PF) was calculated as the ratio of the slopes of calibration curves obtained with and without preconcentration, and the PF value was found 970 for 50 mL sample solution.

Table 3 shows a comparison between the figures of merit of the presented method and some reported methods in the literature for the extraction and determination of THC in different matrices. It seems that the method based on the composite nanofibers sorbent has comparable or better linearity, LOD, PF and RSDs in comparison to the other methods in the literature.

A comparative study was also performed between the extraction efficiencies of chitosan/MIL-101 (Fe) composite nanofibers and a commercial C18 SPE sorbent. A recovery study was carried out by spiking the blood samples at the concentration level of $10 \mu\text{g L}^{-1}$. In contrast to C18, the chitosan/MIL-101 (Fe) composite nanofibers showed better sorption properties. The new method provided a higher absolute recovery ($92.4\% \pm 2.9\%$) than that of the C18 sorbent ($61.3\% \pm 4.1\%$), and the detection limit of the new method ($0.04 \mu\text{g L}^{-1}$) were lower than that of the C18 ($0.5 \mu\text{g L}^{-1}$). On the other hand, since the mass of the sorbent in C18 SPE column (200 mg) was more than that of the composite nanofibrous mat (7 mg), large volumes of toxic organic solvents

would be required for the C18 sorbent (6 mL), and also the evaporation is necessary after the extraction. Also, the SPE with biocompatible nanofibers mat saves the time and is more environmental friendly than SPE with C18. Therefore, the chitosan/MIL-101 (Fe) composite nanofibers mat could be as an ideal substitute for the traditional SPE columns.

3.6. Analysis of whole blood samples

In order to assess the application of the method, it was applied to determine THC in real whole blood sample. Whole blood samples were obtained from a clinical research participant before and after use of a 6.8% THC cigarette.

In order to reduce the matrix effects, whole blood samples were diluted ten times with deionized water prior to use. THC was detected in whole blood sample after smoking of the participant. The results also revealed that the whole blood sample obtained before smoking of the participant did not show the target analyte. A recovery study was carried out by spiking the blood samples at the concentration levels of 1 and 10 $\mu\text{g L}^{-1}$. Each treatment was done in triplicates, and the results are shown in Table 4. The detected amount of THC in the whole blood sample of the participant after smoking was 1.8 $\mu\text{g L}^{-1}$. Fig. 6 shows the HPLC chromatograms of the whole blood samples before smoking (blank blood sample), after smoking, spiked blank blood sample and spiked blood sample after smoking at the concentration of 1 $\mu\text{g L}^{-1}$. The obtained results clearly proved that the suggested method is sensitive enough and applicable for determination of THC in biological samples.

4. Conclusions

The synthesis and characterization of biocompatible chitosan nanofibers containing high loadings of MIL-101 (Fe) nanoparticles were performed. The advantages of both types of

materials were combined to achieve a new class of nonporous composite materials. The chitosan/MIL-101 (Fe) composite nanofibers were used for the extraction and preconcentration of THC in the biological samples. The high specific surface of the prepared composite nanofibers ensured strong interaction between the adsorbent and the analyte. As a result, THC was quantitatively adsorbed on a small amount of mat-based SPE sorbent and also quantitatively desorbed with a small volume of an organic solvent with satisfactory recovery, high PF value and repeatability. As compared to C18 cartridge, the prepared new sorbent could truly offer better analytical performance in terms of recovery, reproducibility and detection limit with low consumption of hazardous organic solvents and sorbent material. The method was successfully applied for determination of THC in human whole blood samples.

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Figure Captions:

Fig. 1. XRD patterns of MIL-101 (Fe) (a), MIL-101 (Fe) after exposure to methanol and water (b), Chitosan/MIL-101 (Fe) composite nanofibers (c) Chitosan/MIL-101 (Fe) composite nanofibers after exposure to methanol and water (d) and Chitosan/MIL-101 (Fe) composite nanofibers after ten cycles of SPE procedure (e).

Fig. 2. SEM images of MIL-101 (Fe) crystals synthesized by microwave heating.

Fig. 3. SEM images of Chitosan nanofibers (a) Chitosan/MIL-101 (Fe) composite nanofibers (b) and Chitosan/MIL-101 (Fe) composite nanofibers after ten cycles of SPE procedure (e).

Fig. 4. FT-IR spectra of MIL-101 (Fe) (a) and Chitosan/MIL-101 (Fe) composite nanofibers (b).

Fig. 5. Response surface estimated from the central composite design for: (a) sorbent amount (mg) vs. volume of eluting solvent (μL); (b) pH vs. % NaCl (w/v). All experiments were done in triplicates, using 2 mL of the sample solution containing $500 \mu\text{g L}^{-1}$ of THC

Fig. 6. HPLC chromatograms of the SPE extract of the blank whole blood sample (a), spiked blank blood sample at the concentration of $1 \mu\text{g L}^{-1}$ (b), the whole blood sample obtained from a participant after smoking (c) and blood sample obtained after participant smoking spiked at the

551 concentration of 1 $\mu\text{g L}^{-1}$ (d). Condition: 150 μL for volume of eluting solvent; 7 mg for sorbent
552 amount and 7.0 for pH
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Table 1 Independent factors, their symbols, levels, design matrix and responses for the central composite design

Factor	Symbol	Level				
		$-\alpha$	-1	0	+1	$+\alpha$
Volume of eluting solvent (μL)	<i>A</i>	65.9	100	150	200	234.10
sorbent amount (mg)	<i>B</i>	2.977	4	5.5	7	8.0230
pH	<i>C</i>	0.954	3	6	9	11.046
% NaCl (w/v)	<i>D</i>	1.954	4	7	10	12.046

No.	Volume of eluting solvent (μL)	sorbent amount (mg)	pH	% NaCl (w/v)	Total peak area
1	150.00	5.50	6.00	9.60	5.2E+007
2	150.00	2.98	6.00	7.00	4.1E+007
3	150.00	5.50	6.00	7.00	5.2E+007
4	150.00	5.50	6.00	7.00	5.1E+007
5	200.00	7.00	3.00	10.00	5.3E+007
6	200.00	7.00	3.00	4.00	5.2E+007
7	150.00	5.50	6.00	7.00	5.4E+007
8	100.00	4.00	3.00	4.00	1.9E+007
9	150.00	5.50	6.00	2.58	5.4E+007
10	150.00	5.50	0.95	7.00	3.4E+007
11	150.00	5.50	6.00	7.00	5.1E+007
12	150.00	5.50	11.04	7.00	4.6E+007
13	150.00	5.50	6.00	7.00	5.2E+007
14	100.00	4.00	9.00	7.00	2.2E+007
15	150.00	5.50	6.00	7.00	5.3E+007
16	200.00	4.00	3.00	10.00	5.7E+007
17	200.00	4.00	9.00	10.00	5.2E+007
18	234.10	5.50	6.00	7.00	4.6E+007
19	200.00	7.00	9.00	4.00	5.6E+007
20	150.00	5.50	6.00	7.00	5.5E+007
21	100.00	7.00	9.00	10.00	3.8E+007
22	65.90	5.50	6.00	7.00	8.2E+006

23 150.00 8.02 6.00 7.00 6.7E+007

Table 2 Analysis of variance table (ANOVA) for response surface quadratic model.

Source	Sum of square	d.f. ^a	Mean square	F-Ratio	P-Value	Effect ^b
Model	4.157E+015	14	2.969E+014	196.16	< 0.0001	Significant
A- Volume of eluting solvent	8.414E+014	1	8.414E+014	555.82	< 0.0001	
B- sorbent amount	3.992E+014	1	3.992E+014	263.72	< 0.0001	
C- pH	9.947E+013	1	9.947E+013	65.71	< 0.0001	
D- % NaCl	3.868E+012	1	3.868E+012	2.56	0.1486	
AB	1.516E+013	1	1.516E+013	10.01	0.0133	
AC	4.585E+013	1	4.585E+013	30.29	0.0006	
AD	8.186E+013	1	8.186E+013	54.07	< 0.0001	
BC	2.768E+013	1	2.768E+013	18.28	0.0027	
BD	4.179E+013	1	4.179E+013	27.61	0.0008	
CD	2.360E+012	1	2.360E+012	1.56	0.2471	
A ²	1.167E+015	1	1.167E+015	770.81	< 0.0001	
B ²	3.406E+012	1	3.406E+012	2.25	0.1720	
C ²	2.983E+014	1	2.983E+014	197.06	< 0.0001	
D ²	3.834E+009	1	3.834E+009	2.533E-003	0.9611	
Residual	1.211E+013	8	1.514E+014			
Lack of Fit	2.941E+011	2	1.471E+011	0.075	0.9289	Not significant
Pure Error	1.182E+013	6	1.969E+012			
Cor Total	4.169E+015	22				

^adegree of freedom.

Table 3 Comparison of the introduced method and other methods used for preconcentration and determination of THC.

Ref.	RSD (%)	Recovery (%)	PF	LOQ ($\mu\text{g L}^{-1}$)	LOD ($\mu\text{g L}^{-1}$)	Linear rang ($\mu\text{g L}^{-1}$)	Analytical methods
[21]	< 9.3	> 85.0	-	5.000	-	5–100	SPE - HPLC/UV
[37]	4.0	85.0	-	0.250	0.100	0.25-50	SPE – LC/MS–MS
[23]	< 9.8	78.0	-	0.440	0.180	0.50-20	SPE – LC/MS–MS
[38]	< 10.4	> 93.8	-	1.000	-	1.00-100	SPE – LC/MS–MS
[39]	< 14.1	> 86.0	-	0.125	0.125	0.125-25 25-100	SPE - 2D-GC/EI- MS
[40]	< 7.9	> 91.3	-	1.00	0.500	1.00-100	SPE – LC/MS–MS
This work	< 3.2	> 88.2	970	0.100	0.040	0.10-100	SPE - HPLC/UV

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Table 4 The recovery and RSD values obtained from analysis of THC in whole blood samples after dilution.

RSD % (n = 3)	Recovery %	C _{found} (µg L ⁻¹)	C _{added} (µg L ⁻¹)	C _{initial} (µg L ⁻¹)	whole blood samples
3.7	89.0	0.89	1.00	n.d. ^a	1h before smoking a 6.8% THC cigarette
2.9	92.4	9.24	10.0		
3.8	88.2	2.47	1.00	1.8	1 h after smoking a 6.8% THC cigarette
3.0	90.7	10.7	10.0		
^a not detected					

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6