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Functionalized carbon nanotube/ionic liquid-coated wire as a new fiber assembly for determination of methamphetamine and ephedrine by gas chromatography-mass spectrometry

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Abstract

A new ionic liquid (IL), 1-methyl 3-[(3-octylamino) propyl] imidazolium bis (trifluoro methane sulphonyl) imide, [OAPMIM][NTf_2] was synthesized and characterized by ^{1}H-NMR. The multi-walled carbon nanotubes' were functionalized by diethyl amine (FMWCNT) and dispersed in this ionic liquid. Then gel-like solution, once formed, was immobilized on stainless steel wire to act as a novel solid phase microextraction fiber. This fiber was applied for the purpose of extraction/preconcentration of methamphetamine (MAP) and ephedrine (EP) prior to determination by gas chromatography-mass spectrometry. The efficiency of fiber coating in the extraction of methamphetamine and ephedrine was compared with that of 1-octyl-3-methylimidazolium bis(trifluoromethanesulphonyl) imide [OMIM][NTf_2], N-octylpyrdinuim hexaflourophosphate [OPY][PF_6] and non-functionalized multi-wall carbon nanotubes have/had different polarities and physical properties. The highest efficiency and most desirable results were achieved using [OAPMIM][NTf_2]-FMWCNT as fiber coating. The calibration curves were linear in the ranges of 1 to 150 and 0.5 to 250 μg L^{-1} for MAP and EP, respectively. The limits of detection (LODs) were obtained as 0.1 and 0.07μg $L^{-1}$for MAP and EP, respectively. The developed method was successfully applied to the determination of MAP and EP in urine samples, and the recoveries were found to be 99.0(±5.0)% at the spiking level of 0.1 μg mL^{-1}.

Keywords: Solid phase microextraction, Ephedrine, Methamphetamine, Carbon nanotube-ionic liquid coating

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Introduction

Ionic liquids are very attractive to be used as SPME coating because they can be custom-designed according to target analytical system, and hence improve the selectivity and sensitivity of the extraction. Although IL-based SPME methods have been reported by Liu et al. [1] and Hsieh et al. [2], fiber preparation in Liu's work was tedious and time-consuming, limiting the practical use of the technique.
Hsieh et al. developed Nafion-membrane-supported IL SPME fibers for analyzing ultra trace PAHs in water samples. Both techniques had certain drawbacks such as: (i) re-coating of the fiber before the next extraction. (ii) the presence of ILs in liquid state on the fiber, causing inconvenience in fiber handling. (iii) low precision due to difficulty in the control of the reproducibility of the coating thickness. In order to overcome such drawbacks, the IL must be immobilized on the surface of the fiber. Carbon nanotubes (CNTs) are known to possess a strong adsorption affinity for a wide variety of organic compounds. Also, their large adsorption surfaces (high surface to volume ratio) make them excellent materials for solid-phase extraction (SPE) of metal ions [3-6] and solid-phase microextraction (SPME) of organic analytes. [7-9] However, CNTs are very prone to aggregating, which limits their excellent properties, mainly the ability to adsorb several compounds on their surface. Over the past decade, a variety of strategies have been devised to improve their solubility and dispersibility, mainly in strong acids or volatile organic solvents. In recent years, ILs have emerged as ‘‘green’’ alternatives to volatile organic solvents for this purpose. [10-12]They are able to disperse CNTs by themselves, affording readily processed gelatinous substances. For that reason, ILs provide an excellent way to increase CNT dispersibility without the need for solid substances or organic solvents. CNTs dispersed in ILs have been described in several works [13,14] In some cases, CNTs could be directly dispersed in the IL; and, in others, a stable aqueous or organic solution of an IL was necessary to carry out the total dispersion of CNTs. Combinations of these two types of materials [7-13] have been widely used in chemical, physical and biological applications. Worth special note in this respect are chemical functionalization of CNTs in ILs, the use of ILs as a new class of CNT dispersant and the development of new (soft) materials. It should be noted that not all IL/CNT combinations lead to the formation of a physical gel, as this requires using a minimum concentration of CNTs known as ‘‘critical gel concentration’’ in the mixture.[15]Above such a critical concentration, CNTs tend to aggregate. Furthermore, CNTs do not disperse in all ionic liquids, thus choice of IL type is important for special applications. For this reason, recent researches on the interaction of CNTs and ILs have attracted great interests.[16-18] CNTs have been effectively dispersed in some ILs and formed gel upon grinding attributed to the Vander Waals' interaction between CNTs and ILs.[19]Moreover, the cations of the IL interact with the π electrons of CNTs via a kind of ionic charge force known as cation-π interaction force.[20] This force anchors the polar hydrophilic cationic parts upon the CNT walls. The non-polar hydrophobic groups are alkyl hydrocarbon long chains that interact with each other to form the steric isolation barriers between CNTs to prevent their aggregation, hence be readily dispersed in ILs. The

compatibility of interaction between CNTs and ILs is expected to be improved by functionalization with polar groups, and hence to expand their scope.[21]Thus, the amounts of dispersed CNTs in ILs are increased and the homogeneity of gel is improved by these strong interactions. Monotony and gel formation of such mixture makes it a good candidate as SPME fibers coating, because ILs and CNTs are excellent sorbents for many materials. So we assessed the sorption potentiality of FMWCNTs and [OAPMIM][NTf_2] for extraction of polar drugs by SPME method for the first time. It is noteworthy that the IL [OAPMIM][NTf_2] has played dual role, *i.e.* dispersion of FMWCNT and synergic ability for extraction of MAP and EP(as polar drugs) due to its polar functional structure. Although fused silica coated with different materials is a widely used fiber, being fragile, its mechanical strength and reusability is limited. [22] Hence, a metallic wire such as stainless steel is a suitable choice with high mechanical strength. It should be noted that CNTs/IL cannot be well coated directly on the stainless steel fiber, because there is no binding between the surface of stainless steel wire and the coating material. Consequently, Nafion membrane has been used as the supporting media to increase the amount of coated MWCNTs/IL on the SPME fiber.

Nafion is a cation exchange polymer which possesses SO_3^- functional group. It would enhance the quantity and stability of IL adsorbed on the fiber surface through the electrostatic interaction between IL and the nafion-supported membrane.[2] In addition, Nafion could also play a role as a sorbent due to its high affinity for methamphetamine and ephedrine as polar compounds.[23]

Drug abuse is a serious global problem that can only be solved through international cooperation. In Asian countries, the abuse of methamphetamine is one of the most pressing problems. Methamphetamine utility has a high association with depression and suicide as well as heart disease, psychosis, anxiety and violent behaviors. Methamphetamine also has a very high addiction risk. Ephedrine is often abused for its stimulant effect. It has a chemical structure similar to amphetamines. Because of this similarity, it can be used to produce methamphetamine using a chemical reduction. This makes ephedrine a highly sought-after chemical precursor in the illicit manufacture of methamphetamine. As for polar and basic compounds like ephedrine and methamphetamine, reports continue to indicate that the selectivity and hence efficiency obtained in SPME are still poor, owing to a limited number of commercially available coatings. Hence, most SPME processes dealing with amphetamines involved additional derivatization to reduce the polarity and/or improve the volatility of the analytes.[24-27]Considering the aforementioned advantages of MWCNTs, [OAPMIM][NTf_2] and Nafion, the present work attempts to overcome the existing drawbacks[28, 29]by introducing a novel stainless steel fiber coated with Nafion/FMWCNT/IL. This fabrication possessed a porous and homogeneous structure with high extraction efficiency towards methamphetamine and ephedrine.

Experimental

It is to be stated that all experiments were performed in compliance with the relevant laws and guidelines of the University, and the research committees have approved the experiments.

Equipments and chemicals

Surface characteristic studies of the prepared Nafion/CNT/IL coated fiber were performed using the scanning electron microscope (SEM, model LEO1530, Oberkochen, Germany). A homemade SPME device was prepared as follows: a 0.2 mm-diameter×10.0 cm-long prepared stainless steel wire was mounted into the SPME device (fabricated by Azarelectrode Co. Urmia, Iran), and the exposed fiber was trimmed to 1.5 cm. An ultrasound bath with temperature control (50 Hz Soner 203H, Rocker, Taiwan) was used for dispersion of MWCNTs. A Metrohm digital pH/ion-meter (model 692, Herisau, Switzerland), equipped with a combined glass-calomel electrode was used for pH adjustment. A circulating thermostatic water bath (JULABO MP5, Seelbach, Germany) was used for temperature control. The absorption spectrophotometry was carried out on a computer-controlled spectrophotometer (Helios Alpha, Heλlosα, England) with the scanning wavelength ranging from 200 to 800 nm.

The ^{1}H NMR spectra were recorded on a 250 MHz spectrometer (Brucker DRX, Germany) using DMSO-d6 as the solvent and tetramethylsilane as the internal standard. A gas chromatograph model HP6890N (Agilent Technologies, Wilmington, USA), equipped with split/splitless injector, a Hp-5 capillary column (30 m×0.32 mm i.d., 0.25 μm film thickness) and mass detector, was employed for the analyses. The instrumental temperature program used for this procedure was as follows: initial oven temperature 80 ˚C (held for 0.1 min), increased to 240 ˚C at a rate of 20 ˚C min^{-1} (held for 3 min), and then increased to 280˚C (held for 2 min). The injector temperature and GC–MS interface were both set at 275˚C. All injections were performed in splitless mode. Some of the optimization peaks were recorded by GC-flame ionization detector (FID).

All chemicals and reagents were of the highest available purity, and all aqueous solutions were prepared in double distilled de-ionized water. MAP and EP (in solid form with >95% purity and a liquid form with 5000 mgL^{-1}concentration, respectively) were purchased from Iranian National drug control headquarters (Tehran, Iran). MWCNTs (95% purity) with less than 10 nm in diameter and 5-15μm in length were provided from IO.Li.Tec. (Denzlingen, Germany). NaOH, NaCl and the solvents with highest available purity were obtained from Merck (Darmstadt, Germany). Ammoniumhexafluorophosphate, lithium bis(trifluoromethane) sulfonylimide, 1-methylimidazole, n-octyl iodide, Nafion (5% w/w solution in a mixture of lower aliphatic alcohols and water), and 3-bromopropylamine hydrobromide were obtained from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions of MAP at 1000 mgL^{-1}and EP

at 500 mgL^{-1} were prepared in deionised water. Working solutions were prepared freshly by appropriate dilution of the stock solutions.10 mL sample vials were used for all extractions.

Synthesis of ionic liquids

1-methyl 3-[(3-octylamino)propyl]imidazoliumbis(trifluoromethanesulphonyl)imide [OAPMIM][NTf_2]:

[OAPMIM][NTf_2] was synthesized as shown in Scheme 1. Briefly a solution of N-methylimidazole (0.011 mol) and 3-Bromopropylamine hydrobromide (0.01 mol) in acetonitrile (CH_3CN) was stirred at 80°C for 8 h. After completion, the solvent was removed by distillation, and the residue was neutralized by NaOH and recrystallized in EtOH to afford the 3-methyl 1-propylamine imidazolium bromide [MPI][Br] (>90%). 1-Iodooctane (0.00936 mol) was added slowly to a solution of [MPI][Br] (2.0 g) in CH_3CN (20 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was heated to 80 °C under reflux for 48 h. This mixture was allowed to cool and the solvent was evaporated and the remaining product was washed three times with ethyl acetate. The solvent was evaporated under reduced pressure and the product was dried under vacuum at 60 ˚C for 24 h. The remaining viscous clear yellow liquid was 1-methyl 3-(N-octylaminopropyl) imidazoliumiodide [OAPMIM][I]. A mixture of [OAPMIM][I](0.009 mol), NTf_2 (0.009 mol) and 150 mL of CH_3CN/H_2O (1:1) was stirred at room temperature for 20 h. Solvents of the mixture were evaporated under vacuum. The residue was then dissolved in ethanol to allow the inorganic salts to be precipitated and filtered off. After evaporation of the solvent, the desired [OAPMIM][NTf_2](97%) was obtained.

The product was characterized by ^{1}H-NMR spectrometry (250 MHz; DMSO-d_6; Me_4Si). In [OAPMIM][NTf_2], the protons of imidazolium showed characteristic peaks at δ_H 9.04, 7.63-7.7, and 7.56-7.60 ppm because imidazole had been converted to the salt form of imidazolium, which acts as an electron-withdrawing group producing a down-field shift. The propyl protons of Ha, Hb, and Hc of aminopropyl were observed at δ_H 4.16- 4.30, 2.83-2.93, and 4.11-4.16 ppm, respectively. The proton attached to nitrogen was observed at δ_H 6.06-6.13 ppm. The protons of methyl group attached to imidazolium showed characteristic peak at δ_H 3.81 ppm. The octyl protons were observed at δ_H 2.46-2.54, 2.11-2.17, 1.81-1.78, and 1.20-1.57 ppm for 4 protons and 0.81-0.84 ppm for other protons, respectively.

[OPY][PF_6] and [OMIM] [NTf_2] were synthesized similar to those reported in the literature. [30, 31]Briefly, the syntheses were as follows:

[OPY][PF_6]:

The ionic liquid 1-n-octyl-pyridinium hexafluorophosphate was prepared according to a modified version of the procedure described by Safavi.[30]To neat 1-iodooctane (2.0 mL, 11.0 mmol) pyridine was added (0.89 mL, 11.0 mmol) and the resulting mixture was stirred at room temperature for 72 h. To the residue was added CH_3CN (0.5 mL) and the resulting solution was washed with diethyl ether (Et_2O) (3×5 mL). The Et_2O washings were discarded and the product was dried under vacuum at room temperature to give 1-n-octyl-pyridinium iodide as a clear yellow viscous oily product (3.50 g, 10.85 mmol) which was used without further purification. To a stirred solution of 1-n-octyl-pyridinium iodide (1.0 g, 3.10 mmol) in H_2O (1.5 mL) was added a solution of ammonium hexafluorophosphate (0.51 g, 3.10 mmol) in H_2O (1.5 mL) drop-wise at room temperature. After stirring at room temperature for 2 h, the resulting white precipitate was isolated by filtration and washed with H_2O (3×5 mL) followed by Et_2O (2×5 mL) and dried under vacuum at room temperature for 24 h to give 1-n-octyl-pyridiniumhexafluorophosphate as a white powder (0.91 g, 87%) with a melting point of 64–65 ˚C.

[OMIM] [NTf_2]:

The [OMIM] [NTf_2] was prepared according to the synthesis method described earlier.[31] Here, equimolar amounts of 1-iodooctane and 1-methylimidazole were added to a round bottomed flask fitted with a reflux condenser for 4 h at 70 ˚C with stirring under nitrogen atmosphere until two phases formed. After cooling to room temperature, the reaction mixture was washed four times using ethyl acetate in order to remove any un-reacted material. The product was dried under vacuum at 70 ˚C for 8 h. 1-octyl-3-methylimidazolium iodide ([OMIM][I]), was obtained as a pure product. To a solution of 20.6 g [OMIM][I] dissolved in 40 mL distilled water, 18.2 g of NTf_2 dissolved in 25 mL distilled water was added and the mixture was stirred for about 5.5 h at room temperature.

After the mixture was left for 30 min, the aqueous phase was separated from the organic phase. The aqueous phase was then washed twice with dichloromethane, each time with 50 mL volume. The combined organic phase was then added to the IL phase. The organic phase was washed three times with distilled water, each time with 50 mL and was dried over magnesium

sulfate. The suspension was filtered and its solvent was evaporated. The final product was dried completely at 70 ˚C under vacuum to give (27 g, 90%).

Fiber coating

After the treatment of MWCNT with acid and amine, [32] 0.005 g of it (FMWCNTs) was dispersed in 0.5 mL of IL by ultrsonication for 5 min to obtain homogenous IL/FMWCNT gel. The stainless steel fiber was cleaned and dipped into Nafion solution for 1 min, then pulled out and immersed in homogenous IL/FMWCNT gel solution. A thin homogenous layer of IL/FMWCNT with 27μm thickness was immobilized on the fiber. Finally the fiber was placed in an oven at 200˚C for 30 min to be conditioned.

Sample preparation

The informed consents were obtained from the subjects at the beginning of the study.
The participants were asked to fill out a questionnaire (a copy of this questionnaire is available upon request from the corresponding author) in order to collect detailed information on the age, occupational history, job description, socioeconomic status, lifestyle, food habits and family and personal medical history.
Urine samples were collected from two 30-year-old healthy voluntary men. In order to avoid any contamination, the subjects were asked to avoid eating, drinking (except water) and especially taking any medicine for 6 h (during night) before sample collection (early morning). The samples were filtered through a 0.25 μm pore size syringe filter to remove any suspended matter. 5 mL of each sample was analyzed by the proposed method after dilution with water.

HS-SPME procedure

After conditioning the fibre in oven, the sample vial was prepared as follows:
5 mL deionised water was placed in a 10 mL sample vial then exact amount of NaCl, NaOH and analytes were added to it. Magnetic stirring with a Teflon-coated stir bar was used to agitate the solution at certain temperature controlled by a circulating thermostatic water bath. To perform the extraction, certain coated SPME fiber was exposed to the headspace above the solution for a given time. After finishing extraction time the fiber was withdrawn back into the needle of the SPME device and inserted into GC inlet for thermal desorption.

Results and discussion

Nafion/IL/FMWCNTs coated SPME fiber and its extraction ability

At reported works often fused-silica was applied as SPME fibers [22]but this fiber is fragile and unavoidable breakage of the fiber occurred during the stirring and sampling procedure. Stainless steel wire is a better choice because of its excellent mechanical strength and low cost. Hence it was selected as fiber; and Nafion, FMWCNTs and a new IL ([OAPMIM][NTf2]) have been applied as coating. Some applications have exploited the individual properties of each material;[33-37] but we have exploited their synergistic effects to develop new coating for determination of MAP and EP in urine sample. The highest sorption capacity obtained was suggestive of a synergistic effect of the coating materials. Subsequently, extraction efficiency of each material was compared. The results for such comparison are shown in Fig.1. Nafion have played a role as sorbent for MAP and EP, due to its affinity for polar compounds. But extraction ability of the Nafion coated fiber was low. Another advantage of Nafion membrane was the ability of electrostatic interactions with [OAPMIM][NTf2]. This interaction has increased the amount of [OAPMIM][NTf2] coated on fused-silica-based SPME fibers. Nafion/[OAPMIM][NTf2] exhibited some degree of extraction efficiency for EP and MAP, but high temperatures can lead to the IL bleeding from the SPME fiber and into the liner assembly. As a result, chromatograms may show IL impurities while the liner assembly may require frequent swapping or cleaning. In addition, this will likely result in the need to recoat the fiber after every run. Nafion/MWCNTs fiber has same extraction efficiency with Nafion/IL, but coating was not homogeneous, therefore reproducibility of this fiber was low. Extraction efficiency for IL/FMWCNTs/Nafion was higher than IL/MCNTs/Nafion because the NH_2 functional group (in FMWCNT) could have an interaction with polar atom in EP and MAP structure. SEM images of the fiber as shown in Fig. 2 indicate that the coating possessed a porous and homogeneous structure, and its average thickness was 14.5μm.

Selection of the IL

The effects of three different synthesized ILs on the extraction efficiency of MAP and EP were tested. As shown in Fig. 3, among the three synthesized ILs, [OAPMIM][NTf_2] was the best of all for MAP and EP preconcentration and possessed the best extractability for them. The extraction efficiencies were in the order [OAPMIM][NTf_2] > [Omim][NTf_2] > [OPY][PF_6]. This might be related to the difference in polarity and diffusion rate of tested ILs. It was anticipated that the polar amine linkage in [OAPMIM][NTf_2] would provide a particular interaction with polar amine group in MAP and EP to enhance extraction.

Dispersion of MWCNTs in IL

Fig. 4A illustrates that MWCNTs are hardly suspended in pure water, even after sonication. However, after small amount of MWCNTs was added into [OAPMIM][NTf_2] solutions as a dispersant and ultrasonicated for 10 min, a macroscopic homogenous black dispersion was obtained(Fig. 4B). This obviously indicates that IL has a superior ability to disperse MWCNTs. Upon adding MWCNTs to the IL solution, some IL molecules interact with MWCNTs by hydrophobic interaction of alkyl chain, and the parts of hydrophilic head ordinate avoid MWCNTs, which makes MWCNTs disperse in IL solution (Fig. 5A).When amine treated carbon nanotube (FMWCNT) added to [OAPMIM][NTf_2], interaction between amine group in alkyl chain and NH_2 group on FMWNT helped in better dispersion (Fig. 5B), furthermore this interaction and polarity of nitrogen group in FMWCNT and [OAPMIM][NTf_2] increased the extraction efficiency of MAP and EP compared to un-functionalized MWCNT and other IL.

The stability of MWCNT suspensions in IL was examined by comparison of their UV–Vis spectra after sonication (with no standing) and after standing for 1 month. Fig. 6 shows that there is no significant variation in the absorbance, which indicates that MWCNTs are still stable after a period of time.

Optimization of extraction parameters

The most important factors related to this extraction technique such as the salting effect, the amount of NaOH, temperature, agitation rate, extraction time and amount of FMWNT and IL were investigated and optimized. The salt effect was evaluated by variation of NaCl concentration (from salt-less up to saturation) in aqueous sample solution. Results showed that the extraction efficiency increased with the salt content up to 10%w/v for EP and 35%w/v for MAP; hence they were selected as optimum salt concentrations.

The influence of NaOH was investigated by varying its concentration in the range 0.5 to 14 mol L^{-1}. The optimum value was found to be 2and 10 mol $L^{-1}$for MAP and EP, respectively. Since both MAP and EP are basic drugs (pK_a = 10.1 for both drugs), [38] they were present in solution as undissociated forms in the investigated concentration range. However, compared with a 0.5 mol L^{-1}concentration, the extraction of MAP and EP was improved for each analyte with the increase of NaOH concentration. Since NaOH further increased the ionic strength of sample solution, it additionally acted as a salting-out reagent. [39]

Increasing operational temperature could increase the headspace/sample partition coefficient of the analyte. On the other hand increase in the temperature leads to decrease of fiber coating /headspace partition coefficient. The first phenomenon leads to a higher concentration of the analyte in the headspace, and the second one results in the lowering of the equilibrium amount of the analyte that the fiber coating is able to extract. The optimal extraction temperature was investigated by exposing the fiber to the sample headspace at temperature ranging from 20 to 100 °C. Consequently, 60 and 85°C were

chosen for the subsequent extraction of MA and EP, respectively. The difference in optimum temperatures is most probably due to the different volatility of the analytes.

Magnetic stirring is commonly used to improve the extraction efficiency of SPME fiber. As expected, the chromatographic peak areas of the analytes increased as the stirring rate increased from 200 to 1250 rpm. However, at stirring rates above 1000 rpm the magnet fluttered and created air bubbles. Thus, an optimum stirring rate of 1000 rpm was chosen for all subsequent experiments.

The extraction time was varied from 5 to 70 min for determination of the optimum value. Extraction efficiencies for two analytes under investigation increased up to 40 min, after which the extraction efficiency did not change significantly for analytes. Since the analyte concentration equilibrium between headspace and fiber is reached at 40 min, it was chosen as optimum time of operation.

The optimum amount of FMWNTs dispersed in IL by ultrasonic agitation for about 30 min was obtained as 6 mg mL^{-1}. The coated fiber was prepared by immersing the stainless steel fiber in this solution (less than 6 mg mL^{-1} decreases the extraction efficiency and more amounts lead to a heterogeneous coating).

Analytical performance

The analytical characteristics of the proposed method such as calibration curve equations, correlation coefficients, and linear dynamic ranges (LDR) were studied and the results are summarized in Table 1. Good linear relationship between the corresponding peak areas and the concentrations were obtained for both the analytes (R^2> 0.9932). The limits of detection (LODs) and quantification (LOQs), based on signal-to-noise ratio(S/N) of 3:1 and 10:1, were determined. Excellent results were obtained with LOD values in the low μg L^{-1}range, thus proving the potential of the method for determination of MAP and EP compounds at ultra-trace levels. LODs and LOQs were obtained in the range of 0.07–0.1 and 0.5–1μg L^{-1}, respectively. In order to access the repeatability of the method, five replicate determinations (spiked at 50 ng $mL^{-1}$with each target analyte) were carried out using a single fiber and the relative standard deviations (RSDs) were calculated. The observed repeatability ranged in 4.6–4.8% depending on the compound considered. The fiber-to-fiber reproducibility of three different fibers coated under the same conditions was also investigated, which ranged from 5.8 to 6.1%.The stability and reusability of the present SPME fiber is better than previous reported fibers that used only ionic liquids. [40] Because of using FMWCNT and nafion together with the IL, this coating's bleeding at high temperature occurred much later and the extraction efficiency did not decline even after 30 cycles of usage, beyond which the peak area for analytes was decreased by 5%, and thus re-coating of the fiber is required. Therefore it is considered to be stable and reusable for at least 30 replicate extractions.

Application to urine sample and method validation

In order to access the applicability of the proposed method, it was applied to the recovery and determination of MAP and EP from urine sample. 5 mL of each urine sample was diluted in the ratio of 1:3 (urine: water) as the working solutions (to reduce viscosity and also ionic strength), prior to extractions. To access matrix effects, the sample was spiked with 0.1μgmL^{-1} MA and EP, and then extraction recoveries were obtained. The recovery values were obtained in the range of 91.5–109% for all the studied samples, which indicates that the matrix effect is negligible. The related chromatograms are shown in Fig. 7.

Comparison with other related SPME fibers

Some statistical data of the proposed method were compared with some previously reported SPME methods in literature (Table 1). The results showed that relative standard deviations values of the proposed method using Nafion/IL/FMWCNTs coating fiber were lower than the other ones. It can be seen that this method has comparable LOD with those of the other reported works. Most of applied coating fibers for determination of MAP and EP used derivatization technique. Derivatization requires a great deal of time and reagent. In present work derivatization was not used and figures of merit were similar or even better than the others.

These advanced performances are mainly due to high surface area of MWCNTs as a sorbent modifier. Indeed MWCNTs are able to provide the enhanced adsorption efficiency for the analytes. On the other hand, nitrogen atoms in the structure of FMWCNTs and [OAPMIM][NTf_2] enhanced the polarity of the coating and could interact with amine groups in MAP and EP structures by hydrogen bonding.

Conclusions

In the present work, an innovative coating was introduced to determination of MAP and EP by SPME-GC-MS method. A new IL ([OAPMIM][NTf_2]) was synthesized and applied to disperse the FMWCNT and produce a gel form. This gel was stable for at least one month and immobilized on stainless steel wire with the aid of Nafion. It is to be mentioned that Nafion played the roles of binder as well as sorbent. The SEM image showed that this coating possessed a porous and homogeneous structure which significantly increased the surface area availability on the fiber. The novel fiber exhibited especially higher extraction efficiency in comparison with commercial PDMS, PA and other fibers applied to determination of these analytes. Taking into account the advantage of the aforementioned properties, the as-prepared coated

fibers could be expected to be widely used in complex matrix sample preparation. Based on these features, in this article, a rapid and facile method for routine ultra-trace analysis of MAP and EP in urine samples was introduced. Therefore, it could be expected to have a potential use in the other complex matrix samples. On the other hand, there are still some areas of research left to modify MWCNTs with other functional groups to increase fiber coating selectivity for highly complex samples.

Further improvements to the methodology may still be worthy of investigation, with application to analysis of other analogs of EP and MA contained in the international Olympic committee list of prohibited substances involving more viscous and/or interfering biological matrices such as blood.

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Figure captions

Scheme 1. The synthesis route and chemical structure of the ionic liquid [OAPMIM][NTf2]**.**

Fig. 1. Comparison of the extraction efficiency of various materials coated fiber for determination of MAP and EP. SPME conditions for EP (1.0 μg mL^{-1}): extraction time, 40 min; extraction temperature, 85˚C; NaOH, 10 mol L^{-1}; NaCl, 10%w/v; with constant stirring. SPME conditions for MA (1.0 μg mL^{-1}): extraction time, 40 min; extraction temperature, 60˚C; NaOH 2 mol L^{-1}; NaCl 35%w/v; with constant stirring.

Fig. 2. SEM images of stainless steel wire and Nafion/ FMWCNTs/IL coated wire. (A) Bare (insert) and coated (main figure) stainless steel wire surfaces at 100 magnification; (B) Nafion/ FMWCNTs/IL coated fiber surface at 7000 magnification, illustrating the coating thickness and surface morphology.

Fig. 3. Comparison of 3 types coatings efficiency (Nafion/FMWCNTs combination with various ionic liquids) for extraction of MAP and EP. SPME conditions for EP (1.0 μg mL^{-1}): extraction time, 40 min; extraction temperature, 85˚C; NaOH, 10 mol L^{-1}; NaCl, 10% w/v; with constant stirring. SPME conditions for MA (1.0 μg mL^{-1}): extraction time, 40 min; extraction temperature, 60˚C; NaOH, 2 mol L^{-1}; NaCl, 35%w/v; with constant stirring**.**

Fig. 4. Vials containing MWCNTs suspensions: (A) with 1.0 mg MWCNTs added into pure water, (B) 1.0 mg MWCNTs were added into the IL ([OAPMIM][NTf_2]) solution.

Fig. 5. Schematic illustration of how MWCNT were dispersed in IL (A) and in FMWCNT/[OAPMIM][NTf_2] solution (B).

Fig. 6. UV–Vis spectra of MWCNTs suspensions in IL, (A) immediately after ultrasonic dispersion for 1 h and (B)1 month after dispersion.

Fig. 7. (a) Chromatogram of the urine sample before spiking standard solution after performing SPME under optimum conditions, (b) Chromatogram of the urine sample after spiking standard solution (1.0 μg mL^{-1}) and performing SPME under optimum conditions, (c) Chromatogram of the standard solution (1.0 μg mL^{-1}) of EP and MAP after performing SPME under optimum conditions.