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Self-assembled benzyl mercaptan monolayer as a coating in electromembrane surrounded solid-phase microextraction of antihistamines in urine and plasma samples

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Abstract

A new solid-phase microextraction (SPME) sorbent based on formation of self-assembled monolayer of benzyl mercaptan on a copper wire is used in electromembrane surrounded solid phase microextraction (EM-SPME) method. To evaluate the method performance, naphazoline (NAP) and antazoline (ANT) were selected and extracted as model analytes. An organic solvent was impregnated in the pores of the hollow fiber (HF) wall and the SPME fiber was placed in the HF lumen that was initially filled with the aqueous acceptor phase. Then, 100 V electrical potential was applied for 15 min to make the analytes migrate from the sample solution and adsorbed on the SPME fiber. Effective parameters on the extraction efficiency of the analytes such as composition of the organic liquid membrane, pH of the donor and acceptor phases, applied voltage, and extraction time were investigated and optimized. Extraction recoveries in the range of 10.1–20.9% and good detection limits (less than 1.5 ng mL^{-1}) were obtained. Linearity of the method was obtained in the range of 4.0–100.0 ng mL^{-1} and 3.0–100.0 ng mL^{-1} for ANT and NAP, respectively ($r^2 > 0.9947$). Finally, the optimal conditions were applied for analysis of human urine and plasma samples and acceptable results were obtained.

Keywords: Benzyl mercaptan; Copper wire; Electromembrane extraction; Gas chromatography; Self-assembled monolayers; Solid-phase microextraction.

1. Introduction

Sample preparation is one of the most important steps for analysis of complex samples and includes sample cleanup and enrichment of target compounds. Among the well-known sample preparation techniques, solid phase microextraction (SPME) and liquid phase microextraction (LPME) are miniaturized ones.[1,2]

SPME was introduced by Pawliszyn and co-workers in the 1990s.[1] During the recent years, SPME has been widely used in analytical fields such as analysis of environmental samples,[3,4] biological samples,[5,6] foods,[7,8] and pharmaceutical samples,[9,10] due to its many advantages such as ease of operation, saving of time, not need for organic solvent, and good compatibility with gas chromatography (GC) and high-performance liquid chromatography (HPLC). Up to now, many materials have been used as the coating of the extraction fibers.[11]

Headspace SPME (HS-SPME) and direct immersion SPME (DI-SPME) are two popular modes used for analysis of dirty matrices and nonvolatile analytes, respectively.[12,13] However, none of them are suitable for extraction of ionic species from complicated matrices.

In 2006, Pedersen-Bjergaard and Rasmussen demonstrated an electrical potential stimulated analytical extraction through a supported liquid membrane (SLM).[14,15] Such system was termed electromembrane extraction (EME). The analytes were extracted from an aqueous sample through an organic solvent into an aqueous acceptor solution via application of an electrical field. Since the EME acceptor phase is an aqueous solution and direct injection of water may cause some problems for GC instrument such as distraction of stationary phase, there are some difficulties in coupling EME with GC considering that GC is faster, simpler, and cheaper in comparison with HPLC and it can easily be coupled with different types of sensitive detectors.

Following new developments in the EME, electromembrane surrounded solid-phase microextraction (EM-SPME) was introduced as a simple and effective method to benefit from high extraction efficiency, sample cleanup, and fast kinetics related to EME technique as well as SPME compatibility with GC.[16,17] EM-SPME setup is the same as that of EME, unless one of the electrodes, located in the HF lumen, is substituted with a conductive sorbent. It was shown that EM-SPME is a suitable method for analysis of ionic analytes in complicated matrices.[16,17]

NAP and ANT are two imidazoline ligands, currently commercialized as nasal solution and both are commonly used for their anti-allergic properties. Due to their relatively narrow therapeutic/toxic index, monitoring their levels in biological fluids is necessary. To overcome the inherent complexity of biological samples, which limits the selectivity and sensitivity of the determinations, using some sample preparation techniques is needed prior to analysis.

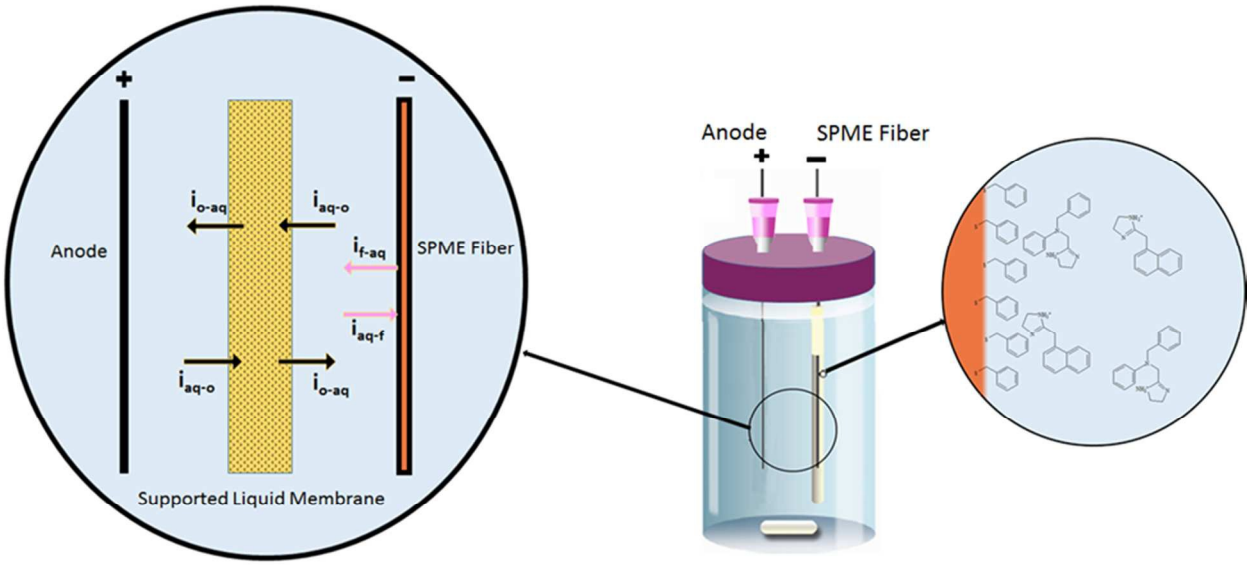
In the present work, the application of a self-assembled monolayer of benzyl mercaptan on a copper wire was investigated as the sorbent in EM-SPME approach. Self-assembled monolayer (SAM) is a layer formed by self-organization of molecules in an ordered manner by chemisorption on a solid surface. SAM is the most elementary form of nanometer-scale organic thin-film material.[18] The self-assembly technique is one of the prevalent techniques for surface modification in electrochemistry and a great attention has been paid to this technique because of its simplicity, versatility, and the high level of order on a molecular scale.[19-23] Due to the high affinity of SH groups to the metals, thiol-terminated SAMs have attracted tremendous attention for modification of electrode surfaces. Thiols form SAMs on gold, silver, copper, platinum, and palladium. A proper sorbent for EM-SPME technique should have a conductive nature and also proper characterizations for efficient adsorption of the analytes. Limiting the SPME sorbents to those with conductive natures makes the selection of the sorbent difficult. Therefore, SAMs can

be proper selection in these methods. SAMs offer many of the attributes needed for this application; nanometer-scale organic thin-film of benzyl mercaptan makes the surface suitable for adsorption of the analytes and random bare surfaces of the electrode could create the electrical field in EM-SPME methods. It is expected that, the fiber demonstrates higher extraction efficiency with high mechanical, electrical and thermal stability under high voltage and temperature conditions. Therefore, a self-assembled monolayer of benzyl mercaptan on a copper wire was used as the conductive sorbent for application of electrical field as well as analytes adsorption in EM-SPME. Then, the extraction capability of the prepared fiber was considered for extraction of ANT and NAP from human plasma and urine samples. After completion of the extraction, the sorbent was directly introduced into GC-FID injection port. The analytes were desorbed and transferred into the GC column for separation and analysis.

2. Experimental

2.1. EM-SPME equipment

The equipment used for the extraction procedure is shown in Fig. 1. A 10 mL vial with an internal diameter of 2.5 cm and a height of 5.5 cm was used. The platinum electrode used in this work, with diameters of 0.25 mm, was obtained from Pars Platin (Tehran, Iran). During the extraction, the EM-SPME unit was stirred at a stirring speed in the range of 0–1250 rpm by a heater magnetic stirrer model 3001 from Heidolph (Kelheim, Germany) using a 1.5 cm × 0.3 cm magnetic bar. The electrodes were coupled to a power supply model 8760T3 with a programmable voltage in the range of 0-600 V and with a current output in the range of 0–500 mA from Paya Pajoohesh Pars (Tehran, Iran).



115

116 **Fig. 1.** Equipment used for the EM-SPME method and mechanism of transport across liquid-liquid-liquid-solid
117 boundaries. The flux of the analytes is presented by "i" and o, aq, and f represent the organic, the aqueous and the
118 SPME fiber, respectively.

119 **2.2. Chemicals and materials**

120 ANT and NAP with purity more than 99% were purchased from Sina Darou Company
121 (Tehran, Iran). The chemical structure and physicochemical properties of the drugs are provided
122 in Table S1. Dihexyl ether, methanol, 1-octanol, and octanoic acid were obtained from Merck
123 (Darmstadt, Germany). 2-Nitrophenyl octyl ether (NPOE), tris-(2-ethylhexyl) phosphate
124 (TEHP), and di-(2 ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs,
125 Switzerland). All of the chemicals used were analytical reagent grades. The porous HF used for
126 the SLM was a PPQ3/2 polypropylene HF from Membrana (Wuppertal, Germany) with inner
127 diameter of 0.6 mm, wall thickness of 200 µm, and pore size of 0.2 µm. Ultrapure water was
128 obtained from a Young Lin 370 series aqua MAX purification instrument (Kyounggi-do, Korea).
129 Benzyl mercaptan was taken from Merck (Darmstadt, Germany).

2.3. Biological samples and standard solutions

Drug-free human plasma (blood group A+) was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran). Urine samples were collected from two persons who were treated with the eye drops containing both of ANT and NAP (eye drops include naphazoline Hcl 0.05% + antazoline phosphate 0.5%) and one person who did not consume the drugs. The urine samples were collected according to the national and institutional guidelines and approved by the institutional committee. Also consent was obtained for any experimentation with human subjects. The samples were stored at -4 ºC, thawed, and shaken before extraction. A stock solution containing 1 mg mL^{-1} of each analyte was prepared in methanol and stored at -4 ºC protected from light. Working standard solutions were prepared by dilution of the stock solution in methanol.

2.4. Apparatus

Separation and detection of ANT and NAP were performed using an Agilent 7890A gas chromatograph (Palo Alto, CA, USA) equipped with a split-splitless injection port and a flame ionization detector (FID). A 30 m HP-5 Agilent fused-silica capillary column (0.32 mm i.d. and 0.25 μm film thickness) was applied for separation of the target compounds. Helium (purity 99.999%) was used as the carrier gas at the constant flow rate of 0.6 mL min^{-1}. The temperatures of injector and detector were set at 280 and 300 ºC, respectively. The injection port was operated at the splitless mode. Oven temperature program was 160 ºC for 3 min, increased to 280 ºC with a ramp of 20 ºC min^{-1}, and held at 280 ºC for 3 min.

2.5. SPME fiber preparation

For fiber preparation of benzyl mercaptan monolayers on copper, the following approach was followed. Mechanically abraded and degreased Cu electrodes were etched in a 7.0 mol L^{-1} HNO_3 solution for 30 s to obtain a fresh, oxide-free copper surface, rinsed with deionized water and absolute methanol as quickly as possible, and immersed immediately into a solution of methanol containing benzyl mercaptan (deoxygenated or exposed to air) for 48 h. Deoxygenation of solutions was ensured by a flow of N_2 (99.99%). The schematic of self-assembling process was shown in Fig. S1. At the end of the designated times, the electrodes were taken out, rinsed with absolute methanol, and dried in the nitrogen atmosphere.

2.6. EM-SPME procedure

Ten milliliters of the sample solution containing the model analytes in pure water was transferred into the sample vial. To impregnate the organic liquid membrane in the pores of the HF wall, a 2.8-cm piece of the HF was cut out and dipped in the organic solvent for 5 s and then the excess of the organic solvent was gently wiped away by blowing air with a Hamilton syringe. Also, pure water was introduced into the HF lumen as the acceptor phase by a microsyringe and then the lower end of the HF was mechanically sealed. The prepared copper fiber (the cathode) was introduced into the HF lumen. The HF, containing the cathode together with the SLM and the acceptor solution, was afterward directed into the sample solution. The platinum anode was led directly into the sample solution. The electrodes were subsequently coupled to the power supply and the extraction unit was placed on a stirrer with stirring rate of 700 rpm. When the extraction was completed, the copper fiber was inserted into the GC injection port for thermal desorption of the analytes at 280 ºC for 2 min.

3. Results and discussion

The effects of variables that influence the extraction efficiency, including composition of the supported liquid membrane (SLM), extraction time, applied voltage, donor and acceptor phases' compositions, desorption time, salt%, and stirring rate were investigated and optimized. Extraction time and applied voltage, affect the extraction efficiency concurrently.[24-26] Increase in extraction time limits the voltage and vice versa. On the other hand, the total ionic concentration of the donor phase to that of the acceptor phase, which is mainly determined by the pH values of donor and acceptor phases, influences the flux through the membrane.[27] Since there is an antagonistic effect among these parameters, they were simultaneously considered and the interaction of time–voltage and the pH values of the acceptor and donor phases were investigated.

3.1. Characterization of fiber coating

Fig. S2-A shows the cyclic voltammograms of 1 mM ferrocyanide measured at a bare copper and copper coated electrodes with the benzyl mercaptan in a 40 mM phosphate buffer solution, recorded at a scan rate of 100 mV s^{-1}. At the bare copper electrode the quasi-reversible response for ferrocyanide is observed, with a formal potential of E_0= 0.262 V and a peak separation of 100 mV; whereas in the presence of the $C_{12}SH$ monolayer, ferrocyanide can't contact the electrode surface and its apparent ET rate, governed either by tunneling through the layer or by reaction at pinholes, decreases strongly. However at higher potentials the benzyl mercaptan modified electrode shows small current, due to pinholes and tunneling current (Fig. S2-B). Existence of the holes on coated wire and tunneling current at higher potential, provide suitable using of benzyl mercaptan coating in EM-SPME procedure. In comparison with the commercial fibers, the fiber developed showed better extraction efficiency, higher mechanical and thermal stability (up to 320 ^{0}C, that was tested in GC injection port), longer life span (over

15 times), and lower production cost. Life time of 15 for SPME fibers is very small. But in EM-SPME techniques, fiber lifetime may reduce due to using higher extraction voltages. So, life time of 15 for SPME fibers in EM-SPME is suitable. The fiber to fiber reproducibility was assessed by calculating the relative standard deviation (RSD %) for drugs extraction. The intra-day and inter-day RSD% were in the range of 7.3–7.9% and 9.8–11.3%, respectively.

3.2. Selection of organic liquid membrane

The chemical characteristics of the supported liquid membrane (SLM) are highly critical for a successful electromembrane extraction. The analyte flux is affected by gradient of the analyte concentration across the SLM, and is partially determined by the sample-to-SLM distribution ratio. This parameter is controlled by the type of solvent used as the SLM. In addition, the type of solvent can affect the diffusion coefficient of the analyte[28] and it could be adjusted to increase the selectivity and sample cleanup.[29,30]

Based on the previously published results, NPOE is an efficient organic solvent for extraction of basic drugs.[31] Also, addition of some carriers to NPOE could improve phase transfer and electrokinetic migration of basic analytes.[30] For this purpose, experiments were run using different percentages of DEHP and TEHP in NPOE. Both carriers had positive effects on extractability of the model analytes. As demonstrated in Fig. S3, best results were obtained using NPOE together with DEHP. It was shown that DEHP could enhance the extractability by increasing the SLM polarity and it also could act as an anionic ion pairing reagent. Arrangement of DEHP molecules at the phase boundaries facilitates the analyte entrance into the SLM by formation of ion-pair complexes.[32-34] Thus, NPOE containing 5% DEHP was chosen as the SLM to reach the best extractability for both of the analytes.

3.3. The effect of sample solution and acceptor phase pH

Following the optimization procedure, the pH values of both acceptor and donor phases were optimized. Pedersen-Bjergaard et al. showed that the total ionic concentration of the donor phase to that of the acceptor phase, which is defined as the ion balance (χ), affect the flux over the membrane.[35] The flux may be decreased as this ratio increases according to theoretical models.[35] Ion balance is mainly determined by the concentrations of H^+ and OH^- in the acceptor phase and sample solution. Sample solution should be acidic enough, so that the basic analytes carry a net positive charge to migrate toward the cathode in an electrical field. The pH of acceptor phase can influence the extraction recovery in two ways. On one way, the acceptor solution should be acidic to enable the analytes releasing into this phase. On the other hand, H+ competition with the analytes for adsorption onto the sorbent decreases the extraction efficiency. To investigate the effect of ion balance, pH of the donor phase was changed in the range of 1.0–7.0, while the pH value in the acceptor phase was varied in the range of 1.0–13.0 by adding appropriate amounts of hydrochloric acid and/or sodium hydroxide solutions. Fig. 2A, B shows that the chromatographic signal decreases by decreasing the pH value of sample solution. Both model analytes are ionized at the neutral pH value and as the concentration of H^+ increases, competition between H^+ and cationic analytes decreases the extraction efficiency. This is while low extraction efficiencies were obtained at low pH values of the acceptor phase due to H^+ predomination in the electrostatic migration toward the SPME fiber electrode. At a relatively high pH value of the acceptor phase, extraction yield was severely reduced because the analytes were mainly present as their neutral form. This confirms that the extraction mechanism is electrokinetic migration of cationic species. Thus, neutral pH value was chosen as the pH of both acceptor and donor phases for the rest of the work.

3.4. Applied voltage and extraction time

In EM-SPME, the electrokinetic migration of the analytes across the SLM into the acceptor solution greatly depends on the applied voltage. Therefore, the applied voltage across the SLM is an important factor to be considered for efficient extraction of basic drugs. In three-phase microextraction, mass transfer is a time-dependent process; thus, time is another parameter that can affect the flux of analytes. Both time and voltage directly increase the flux of ions and so they could increase extraction recovery. Due to their antagonistic effects, simultaneous investigation of extraction time and applied voltage leads to a more accurate optimal point.

To investigate the effect of applied voltage and extraction time simultaneously, electrical potential differences in the range of 50–200 V were applied for extraction durations of 5–20 min. As demonstrated in Fig. 2C, D, the maximum amounts of the drugs were adsorbed on the SPME fiber when electrical potential of 100 V was applied for 15 min. Further increase in voltage and extraction time leads to a decrease in final response. It should be noted that EME is a non-exhaustive process. At the beginning of the process, recoveries increased rapidly by increasing the extraction time and applied voltage up to 15 min and 100 V, respectively; but declined thereafter. This showed that EME reached steady state at 15 min under voltage of 100 V. The decreased peak area after these time and voltage values may be caused by different factors. Increasing the thickness of ionic double layers at the interfaces at higher voltages and longer extraction durations was a very important effective factor. Also, saturation of the analyte in the acceptor phase may be another factor that results in decreasing the extraction efficiency at applied voltages and extraction times over 100 V and 15 min, respectively. The gradual suppression of analyte net transfer resulted from heat generation at longer time and higher voltages can also decrease the extraction efficiency.[36] Furthermore, a relatively low voltage leads

to extraction protraction. Therefore, the extraction efficiency is improved by increasing the extraction time. Finally, 100 V was applied for 15 min to obtain the best results.

3.5. Effect of stirring rate

Stirring the sample solution increases the mass transfer of the analytes and reduces the thickness of double layer around the SLM. Hence, it could increase the kinetics and efficiency of extraction. The effect of stirring rate on extractability was surveyed up to 1250 rpm. A stirring rate of 700 rpm was selected owing to formation of extreme whirlpool into the sample solution and bubble formation around the HF at higher rates.

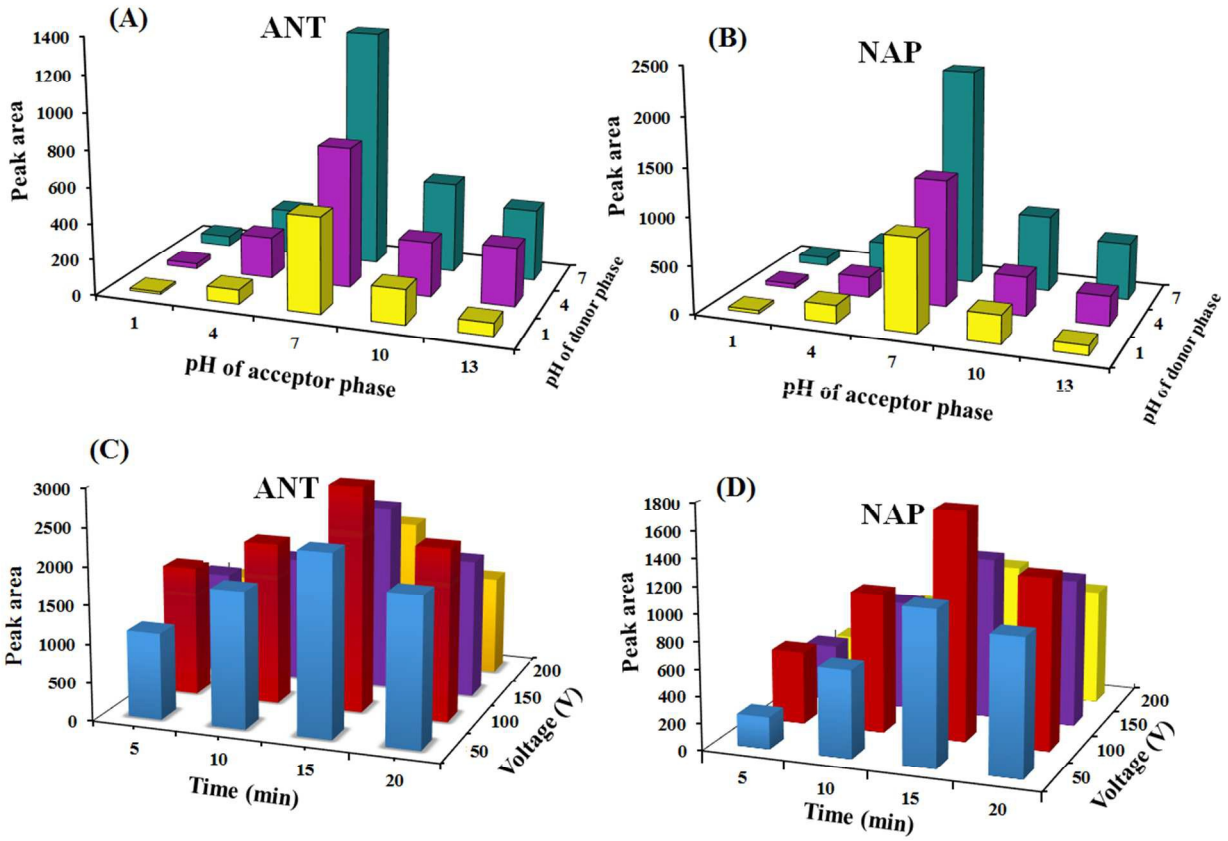


Fig. 2. Effect of pH values of the acceptor and donor solutions on extraction efficiencies of ANT (A), and NAP (B); Simultaneous investigation of time and voltage effects on extraction efficiency of ANT (C) and NAP (D).

3.6. Salt effect

Presence of large amounts of ionic species increases the value of the ion balance (χ), which is defined as the ratio of the total ionic concentration in the sample solution to that in the acceptor solution.[27] This in turn decreases the flux of analytes across the SLM. By increasing the concentration of other ions in the sample solution, competition between these ions and target analytes for migration toward electrodes through the SLM increases. Also, increasing in salt content resulted in increase in the number of ions migrating through the SLM, which caused an increase in the Joule heating and instability of the SLM. The effect of χ was investigated using the solution contained 2.5% NaCl. In the presence of salt, extraction recoveries of ANT and NAP decreased significantly. Since salt addition has a negative effect on extraction efficiency, all of the experiments were performed in the absence of salt.

3.7. Effect of desorption time

The effect of desorption time on final chromatographic signal was investigated. Desorption time was verified in the range of 0.5–5.0 min whereas desorption temperature was 280 ºC. The final signals of both of the drugs were improved by increasing desorption time from 0.5 to 2.0 min. The GC signal was slightly increased by further increase in desorption time. Therefore, 2.0 min was selected as the optimized time to diminish the fiber degradation probability.

3.8. Method validation

In order to evaluate the practical applicability of the proposed EM-SPME method, the optimized extraction conditions were adopted to evaluate its quantitative performance**.** The optimal extraction conditions include, NPOE with 5% DEHP as SLM, 100 V potential difference, an acceptor solution and a sample solution with pH 7, and 15 min as extraction time. Figures of merit of the method including limits of detection (LODs), square of correlation coefficients (R^2), dynamic linear ranges (DLRs), and enrichment factors were evaluated under

these conditions. Also, the extraction recovery (ER) was defined as the percentage of the number of moles of the analyte adsorbed on the sorbent (n_f) to those that originally was presented in the sample solution (n_i).

$$ER\% = \frac{n_f}{n_i} \times 100 \quad (1)$$

The relative recovery (RR%) and accuracy (Error%) were calculated by the following equations:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (2)$$

$$Error\% = 100 - RR\% \quad (3)$$

where C_{found}, C_{real}, and C_{added} are the concentrations (ng mL^{-1}) of the analyte after addition of known amount of standard into the real sample, the concentration of the analyte in real sample, and the concentration of known amount of standard which was spiked into the real sample, respectively. The performance of the EM-SPME is shown in Table 1. The results obtained show that EM-SPME could effectively be employed for analysis of model drugs even in complexe matrices such as biological fluids. To improve mass transfer of the analytes, human plasma and urine samples were diluted 1:10 and 1:1, respectively, with pure water, and the pH value was adjusted to 7.0 by addition of appropriate amounts of hydrochloric acid and/or sodium hydroxide solutions. Calibration plots were obtained over the range of 4.0–100 ng mL^{-1} for ANT and 3.0–100 ng mL^{-1} for NAP and the coefficients of determinations (R^2) between 0.9947 and 0.9985 were obtained for the two analytes. The limits of quantification (LOQs) were estimated to be 4.0 ng mL^{-1} for ANT and 3.0 ng mL^{-1} for NAP, while the LOD was 2.5 ng mL^{-1} for ANT and 1.5 ng mL^{-1} for NAP. Precision of the method was determined from five successive analyses at the same operational parameters and presented as relative standard deviations (RSDs), which were between 3.2% and 8.8%, showing good repeatability of the method. The model drugs were

effectively extracted with ER% in ranges of 14.8%–18.6%, 10.1%-20.9% and 12.0%-16.9% in water, urine and plasma media, respectively. According to literatures, a common extraction recovery (absolute recovery) for SPME technique is often less than 2%.[37,38] The main reason for these results is that no effective driving force is used in conventional SPME. Also, in the HS-SPME mode, which is performed for complicated matrices, enhances the limitations for non-volatile compounds. Therefore, obtained recoveries in the present work are noticeably greater than previously reported ones. Moreover, result show that extraction recoveries obtained for the proposed sorbent were comparable and in many cases better than those of the published sorbents.[16] Intra- and inter-assay precisions ranged between 3.2–8.8% and 5.9–11.7%, respectively (Table 2). Also, calculated Error% for the analytes in the range of −9.5% to +9.7% for different matrices demonstrates that the presented method offers reasonable accuracy even in complicated matrices such as human plasma and urine samples.

Table 1
Figures of merit of EM-SPME-GC-FID for analysis of ANT and NAP in water, urine, and plasma samples.

Sample	Analyte	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Linearity (ng mL^{-1})	ER%	R^2
Water	ANT	2.5	4.0	4.0-100	18.6	0.9957
	NAP	1.5	3.0	3.0-100	14.8	0.9985
Urine	ANT	4.0	5.5	5.5-100	10.1	0.9982
	NAP	2.5	4.0	4.0-100	20.9	0.9969
Plasma	ANT	8.0	10.0	10.0-100	16.9	0.9956
	NAP	7.0	9.0	9.0-100	12.0	0.9947

Table 2
Accuracy and precision of the proposed method for determination of ANT and NAP in pure water and drug-free urine and plasma samples.

Analyte	Conc. (ng mL^{-1})	Accuracy (Error %)						Precision (RSD %)					
		Intra-assay (n = 3)			Inter-assay (n = 3)			Intra-assay (n = 5)			Inter-assay (n = 3)		
		W[a]	U[b]	P[c]	W	U	P	W	U	P	W	U	P
ANT	10	+0.5	-2.7	-1.5	-2.0	+3.8	-1.4	3.9	4.2	6.4	8.2	5.9	8.0
	40	+7.1	-3.8	+2.5	-3.6	+6.7	+4.2	5.7	6.5	7.3	6.9	8.7	11.2
	80	-1.9	+2.1	-0.7	+7.3	+1.9	-9.5	4.0	7.9	8.3	6.8	9.9	10.1
NAP	10	-5.3	+6.3	-1.3	+1.8	-3.5	+9.7	6.3	8.8	4.9	9.5	10.2	7.9
	40	+2.8	-3.5	-0.9	+5.2	+5.1	-5.7	7.8	3.2	6.4	11.7	10.3	9.8
	80	+1.9	-4.1	-5.7	-3.2	+8.1	-2.5	5.6	5.4	4.9	11.1	8.9	10.7

[a, b, c] W: Water, U: Urine, P: Plasma.

The results obtained show that EM-SPME offers higher preconcentration factors and better limits of detection, which confirm the advantages of this technique compared to conventional EME or SPME. Comparison of the proposed method with different available methods for extraction and determination of ANT and NAP is provided in Table 3. The results show an excellent applicability of the proposed new method for determination of ANT and NAP in biological samples. This technique provides high sample cleanup for complex biological matrices, which make analysis of these drugs by common instruments like GC-FID possible. It is

shown that this technique demonstrates wide linearity range, high sensitivity, and an acceptable reproducibility with an important emphasis on the extraction time, which seems to be short. Also, in comparison with other methods, the technique eliminates possible carry-over problems because the hollow fiber is not expensive and can be discarded after each extraction and the consumption of organic solvents in this technique is at the minimum amount. Since the organic liquid membrane increases the electrical resistance of the system, it is possible to apply high voltages, which reinforce the extraction recoveries. Also, solitude chromatograms are obtained even by extraction from biological fluids. No need for extra sample pretreatment steps is one of the most interesting advantages of the proposed method and it is assumed that the electrical field contributes to break of the bonds between proteins and analytes.[28] Therefore, EM-SPME could be introduced as a novel and simple technique for helpful extraction of analytes from complicated matrices and the proposed method can reduce the risk of working with biological fluids and loss of sample during therapy steps.

Table 3

Comparison of figures of merit of EM-SPME with other analytical techniques for determination of ANT and NAP.

Analytical method[a]	Analyte	Matrix	LOD (ng mL^{-1})	LOQ[b] (ng mL^{-1})	Extraction time (min)	ER %	RSD %	Reference
HPLC	NAP	Eye drop	10	-	40	100.32	0.87	39
CEP	NAP	Eye drop	20	-	7	100.13	0.69	39
UV-Vis	ANT	Eye drop	-	800.0	-	98.5–103.8	0.90-4.32	40
UV-Vis	NAP	Eye drop	-	1000.0	-	97.4–105.8	2.96-6.60	40
CE	NAP	bulk drug	250.0	500.0	25	-	3.99	41
FL-CL	NAP	Raw Drops	210.3	1051.5	0.5	-	2.8	42
LLE-RPIPC	ANT	Plasma	-	100	-	83.54	20	36
LLE-RPIPC	ANT	Urine	-	100	-	85.65	20	36
D-SPC-PT	ANT	Eye drop	1000	1000	-	-	0.82	43
D-SPC-PT	NAP	Eye drop	200	200	-	-	0.96	43
EM-SPME	ANT	Water	2.5	4.0	15	18.6	5.7[c]	This work
EM-SPME	ANT	Urine	4.0	5.5	15	14.8	6.5[c]	This work
EM-SPME	ANT	Plasma	8.0	10.0	15	10.1	7.3[c]	This work
EM-SPME	NAP	Water	1.5	3.0	15	20.9	7.8[c]	This work
EM-SPME	NAP	Urine	2.5	4.0	15	16.9	3.2[c]	This work
EM-SPME	NAP	Plasma	7.0	9.0	15	12.0	6.4[c]	This work

[a] High performance liquid chromatography (HPLC), capillary electrophoresis (CEP), ultraviolet detector (UV), (HPLC-UV), UV–vis spectrophotometer (UV-Vis), capillary electrophoretic (CE), flow injection chemiluminescence (FI-CL), reversed-phase ion pair chromatography method with liquid–liquid extraction (LLE-RPIPC), derivative spectrophotometry (D-SPCPT).

[b] Limit of quantification.

[c] For five-replicate measurements at 40 ng mL^{-1}.

366

367 **3.9. Analysis of real samples**

368 To investigate the applicability of the EM-SPME technique using a self-assembled SPME
369 fiber, final experiments were implemented on different human plasma and urine samples. The
370 sampling procedure was performed according to the guidelines for research ethics. The protocol
371 was approved by an Internal Review Board. To this end, plasma and urine samples were diluted
372 1:10 and 1:1, respectively, with pure water and the pH values were adjusted to 7.0 by addition of
373 proper amounts of hydrochloric acid 0.05 M and/or sodium hydroxide 0.05 M solutions. Then,
374 10 mL of each solution was transferred into the sample vial and the extraction process was
375 performed three times for each sample under optimal conditions. To reduce the matrix effects,
376 external calibration curve was plotted in drug free urine and plasma samples. The results showed
377 that plasma and urine 2 samples were free of the drugs. Urine 1 sample was obtained after 6
378 hours of consumption eye drops by a patient. The results confirmed that urine 1 sample
379 contained ANT and NAP. According to the results, urine 1 sample contain 7.9 and 11.3 ng mL^{-1}
380 of ANT and NAP, respectively. Thereafter, to determine the method accuracy, each sample was
381 spiked at 20 ng mL^{-1} of the drugs and EM-SPME was carried out to calculate extraction error.
382 Table 4 demonstrates that results of three-replicate analyses of each sample obtained by the
383 proposed technique are in satisfactory agreement with the spiking amounts. Fig. 3 demonstrates
384 chromatograms of extractions from drug-free and spiked urine and plasma samples. Excellent

sample clean-up was obtained and the method evaluation indicated that the system provided reliable results and it is feasible to detect and quantify the anions in biological fluids.

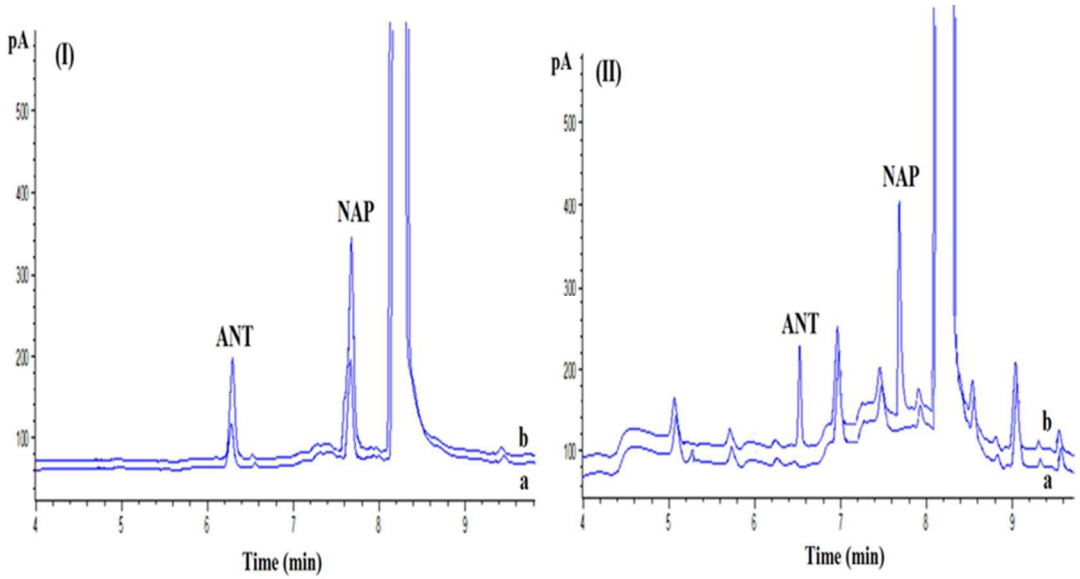


Fig. 3. Chromatograms obtained after extraction of drugs from: (I) human urine, (II) human plasma (non-spiked (a) and spiked (b) samples).

Table 4
Determination of ANT and NAP in different urine and plasma samples.

Sample	Analyte	C_{real} (ng mL^{-1})	C_{added} (ng mL^{-1})	C_{found} (ng mL^{-1})	RSD% (n = 3)	Error%
Plasma 1	ANT	Nd[a]	20.0	18.9	6.0	-5.5
	NAP	Nd	20.0	19.7	4.7	-4.4
Plasma 2	ANT	Nd	20.0	20.7	7.6	+3.5
	NAP	Nd	20.0	19.5	3.6	-2.5
Urine 1	ANT	7.9	20.0	28.4	6.2	+2.5
	NAP	11.3	20.0	30.7	5.8	-3.0
Urine 2	ANT	Nd	20.0	19.8	7.2	-1.0
	NAP	Nd	20.0	20.4	4.8	+2.0

[a] Not detected

4. Conclusions

This study presents the application of a new synthesized sorbent, based on self-assembly of benzyl mercaptan on copper wire, in electromembrane surrounded solid phase microextraction method for determination of naphazoline and antazoline in biological samples. The technique demonstrated several advantages over other available extraction methods including high sample cleanup and preconcentration factors, particularly good limits of detection as well as good linearity and acceptable repeatability. In comparison with the commercial fibers, the fiber developed showed better extraction efficiency, higher mechanical and thermal stability, longer life span, and lower production cost. Using a membrane protected solid phase microextraction fiber in electromembrane surrounded solid phase microextraction noticeably increases the selectivity and reduces the probability of extraction of interferences. Thus, the method could be directly used for analysis of complex matrices, overcoming the shortcomings of conventional solid phase microextraction. Due to the application of electrical potential as the driving force, which facilitates mass transfer kinetics, electromembrane surrounded solid phase microextraction can provide excellent recoveries in a relatively short time. The suggested method may become a very powerful and innovative sample preparation technique for drug analysis in different complex biological matrices in the future, especially in miniaturized formats.

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