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Microextraction of mebendazole across supported liquid membrane forced by pH gradient and electrical field

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ABSTRACT

In the present study, extraction of mebendazole across a supported-liquid membrane (SLM) was performed based on two different driving forces: (1) pH gradient over the SLM, and (2) electrical field sustained over the SLM. The extracted drug concentration was studied using reversed-phase HPLC–UV. At passive extraction conditions, mebendazole was extracted from alkaline samples (0.01 mmol L^{-1} NaOH) into 1-undecanol immobilized in the pores of a porous hollow fiber of polypropylene (SLM), and then transported into 25 μL of 100 mM HCl as the acceptor solution. Under electrokinetic migration conditions, mebendazole transported under applied voltage from acidic solutions (100 mmol L^{-1} HCl) through 2-nitrophenyl octyl ether (NPOE) immobilized in the pores of hollow fiber, into 25 μL of 100 mmol L^{-1} HCl as the acceptor solution. The effects of several factors including the nature of organic solvent, pH of donor and acceptor solutions, extraction time and stirring speed on the extraction efficiency of the drug were investigated and optimized. Under optimal conditions, preconcentration factors (PF) of 211 and 190 were obtained for the drug based on passive transport and electromembrane extraction (EME), respectively. Also, linear range of 0.5–1000 μg L^{-1} with estimation of coefficient higher than 0.994 was obtained for both of the proposed methods. The results showed that EME has higher speed in comparison with simple passive transport. The methods were successfully applied to extract mebendazole from plasma and urine samples and satisfactory results were obtained.

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1. Introduction

Methyl-5-benzyl-2-benzimidazole carbamate (mebendazole) is used as an anthelmintic drug. The drug is known to act through irreversible inhibition of glucose uptake in the parasite, leading to depletion of glycogen store which results in a decrease in adenosine triphosphate activity. Only 5–10% of the ingested drug is absorbed from the human gastrointestinal tract [1].

In recent years, a miniaturized approach to supported-liquid membranes (SLM) extraction is liquid-phase microextraction (LPME). The SLM is an organic solvent immobilized in the pores of a porous polymeric membrane. Hollow fiber liquid-phase microextraction is based on passive diffusion of analytes from sample solution, through a water-immiscible organic solvent immobilized as a SLM in the pores of the wall of a porous hollow fiber into micro-liter volume of acceptor solution filled inside the lumen of the hollow fiber. This configuration creates a three-phase extraction system compatible with HPLC–UV [2,3]. The extraction based on passive diffusion (pH gradient) is limited to basic or acidic analytes. For basic compounds, pH of sample has to be adjusted at alkaline region to form neutral analyte and ensure efficient distribution of uncharged analytes into the SLM, whereas pH in the acceptor solution should be low for efficient trapping of the analytes. In this manner, the basic compounds may be easily extracted into the organic phase and finally into the acceptor phase, which is directly compatible with HPLC [4]. Extraction is further promoted by strong agitation of the extraction system to reduce the stagnant boundary layer in the vicinity of the SLM and to induce convection in the sample [5]. The porous hollow fiber with SLM prevents migration of salts, biological macromolecules, acids, hydrophilic compounds and neutral substances into the acceptor solution providing very clean extracts [6]. In most of LPME applications, high preconcentration factor without the need for solvent evaporation and reconstitution is common, since the analytes are extracted from relatively large sample volumes into a very small volume of acceptor solution (typically 25 μL) [7–9]. Although hollow fiber LPME (HF-LPME) is a very simple and effective sample preparation method, it is relatively a time-consuming technique, typically taking 15–120 min [10]. In order to increase the extraction speed, an electrical potential difference is applied over the SLM as the driving force. Application of voltage (150 V, DC) over a SLM has been found to enable very fast extractions from small sample volumes

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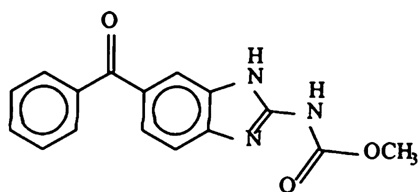

Fig. 1. The chemical structure of mebendazole.

[10]. This system is termed electromembrane extraction (EME), and has been performed with exactly the same equipment as for LPME besides the use of a DC power supply and two electrodes to sustain the voltage across the SLM [10]. Extraction principle is the major difference between EME and HF-LPME. In EME, electrical field is the driving force and the analyte is extracted by electrokinetic migration [11,12]. The pH on each side of the SLM is adjusted to ensure full ionization of the analyte in the EME system. Thus, in this technique, the only driving force is the applied voltage with no contribution from pH gradient as in HF-LPME. The use of an electrical potential difference as the driving force shortens the extraction time to approximately 5–15 min [13]. EME has been demonstrated for extraction of different basic [10,12] and acidic compounds [13,14] from human urine and plasma samples. The selectivity of the extraction depends on the choice of organic solvent for the SLM. For basic analytes, a negative electrode is placed in the acceptor solution, whereas the potential is reversed for acidic compounds. In the present work, three-phase LPME mode was used for extraction and preconcentration of mebendazole (Fig. 1) from aqueous, plasma and urine samples. The preconcentration factor (PF), defined as the final concentration of analyte in the acceptor phase divided by the initial analyte concentration in the donor phase, was studied as a function of the nature of organic solvent, pH of solutions, extraction time, stirring speed and salt concentration.

2. Experimental

2.1. Equipment for LPME and EME

The setup used for EME was identical to the HF-LPME unit except for the electrode system. The sample compartments with different volumes, internal diameters, and different heights were used in HF-LPME and EME methods. The porous hollow fiber used for immobilization of the SLM and for housing the acceptor solution was a PPQ 3/2 polypropylene hollow fiber from Membrana (Wuppertal, Germany) with an internal diameter of 600 μm, wall thickness of 200 μm, and a pore size of 0.2 μm. During the experiments, the extraction unit was agitated on a magnetic stirrer model MR 3001 from Heidolph (Kelheim, Germany). The DC power supply in EME was a model PTS 1002 with programmable voltage in the range of 0–300 V, and with a current output in the range of 0–2.5 A from Akhtarian (Tehran, Iran). Platinum wires with diameters of 250 μm and 100 μm from Pars electrode (Tehran, Iran) were used as electrodes in the sample and acceptor solutions, respectively. They were both connected to the power supply. A 25 μL syringe model 702 NR from Hamilton (Bonaduz, Switzerland) was employed to introduce the acceptor phase into the lumen of hollow fiber, to suspend the hollow fiber and also to inject the extracted analyte at the end of the extraction into the HPLC loop.

2.2. Procedure for LPME

The extractions were carried out according to the following procedure: 10 mL of alkaline donor solution (containing 0.01 mmol L^{-1} NaOH) was filled into the 15 mL glass vial containing a 10 mm × 4 mm magnetic stirring bar. The vial was placed on the magnetic stirrer.

The whole fiber was cut into small segments with length of 8.8 cm. The fiber was dipped for 5 s in the organic liquid to immobilize the SLM in the pores of the wall of the hollow fiber. 1-Undecanol was used as the SLM, which has been found to be efficient in HF-LPME. Excess of organic solvent from lumen of hollow fiber was removed by air blowing by a medical syringe (1 mL). A 25 μL syringe was employed to introduce the acceptor solution into the lumen of hollow fiber. The outer diameter of the needle was 800 μm and thus had to be inserted into the hollow fiber by applying some force. The end of each resulting hollow fiber was sealed by a piece of aluminum foil. The fiber was bent into U-shape and submerged in the sample solution together with a small part of the supporting syringe needle. The vial was stirred for a prescribed time period. At the end of the extraction time, the hollow fiber was removed from the sample solution and its closed end was opened. The acceptor phase was subsequently withdrawn into microsyringe and injected into the HPLC loop. In the initial experiments, the volumes of donor and acceptor solutions were 10 mL and 25 μL, respectively, and relatively high concentration of aqueous solution of mebendazole (100 μg L^{-1}) was used. All the experiments were done at room temperature and the donor solution was stirred at a rate of 700 rpm for 1 h.

2.3. Procedure of EME

The extraction was carried out according to the following steps: 7 mL of acidified donor solution (containing 100 mmol L^{-1} HCl) was filled into 8 mL glass vial containing a 5 mm × 3 mm magnetic stirring bar and the vial was placed on the magnetic stirrer. The hollow fiber was cut into small segments with the length of 8.8 cm. The fiber was dipped for 5 s into the 2-nitrophenyl octyl ether (NPOE) to immobilize the SLM in the pores of the wall of hollow fiber. Excess of liquid in the SLM was removed by air blowing by a medical syringe. Twenty five μL of 100 mM HCl serving as the acceptor solution was introduced into the lumen of hollow fiber via a 25 μL syringe and its end was closed by a small piece of aluminum foil, similar to HF-LPME. The fiber and the positive electrode were then directed into the donor solution, and the negative electrode was placed into the acceptor solution. Afterwards, the voltage (150 V, DC) was applied while the compartment was stirred for a predetermined period of time. After the extraction was completed and the voltage turned off, the acceptor solution was collected with the microsyringe and injected to the HPLC loop.

2.4. HPLC system

Chromatographic separations were performed on a Cecil HPLC containing a CE4100 HPLC pump (Cambridge, England), a six-port Rheodyne HPLC valve (Oak Harbor, Washington, USA) equipped with a 20 μL injection loop and a CE 4300 HPLC UV–visible detector (version R0050). Chromatographic data were recorded and analyzed using Power Stream software (version 3.2). The separations were accomplished on a Hichrom C_{18} column (25 cm × 4.6 mm) with 5 μm particle sizes from Hichrom (Berkshire, England). A mixture of 10 mmol L^{-1} phosphate buffer with pH 4.5 (solvent A), methanol and acetonitrile (2:3, v/v) (solvent B) with a flow rate of 1 mL min^{-1} was used as the mobile phase. The injection volume was 20 μL for all of the standards and samples, and detection was performed at wavelength of 250 nm. All of pH measurements were fulfilled using an 827 Metrohm pH meter (Herisau, Switzerland).

2.5. Chemical

All the reagents were of analytical grade. Mebendazole was kindly donated by the Department of Chemistry, Tehran Univer-

sity (Tehran, Iran). HPLC-grade methanol and acetonitrile were purchased from Caledon (Georgetown, Ont., Canada). *n*-Dodecane, 1-undecanol, benzyl alcohol, methyl isobutyl ketone, 1-octanol, dihexyl ether, HCl, NaOH, NaCl, di(2-ethylhexyl) phthalate (DEHP), and ortho-phosphoric acid were purchased from Merck (Darmstadt, Germany) and NPOE (98%, w/w) was purchased from Fluka (Buchs, Switzerland). Phosphate buffers were prepared from phosphoric acid and NaOH solutions. The water used in the experiment was purified on a Milli-Q ultra-pure water purification system purchased from Millipore (Bedford, MA, USA). Urine sample was collected from a healthy volunteer and the sample preparation was performed immediately. Plasma sample was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran) and stored at −20 °C prior to use.

2.6. Standard solution and real samples

Mebendazole stock solution with concentration of 1 mg mL^{-1} was prepared in 0.1 mol L^{-1} methanolic hydrochloric acid. Standard solutions were freshly prepared from the stock solution by proper dilution with ultra-pure water. All of the standard solutions were stored at 4 °C. The concentration of the drug in the experiments was 0.1 μg mL^{-1}. Urine samples were collected from two woman volunteers. One of these volunteers had taken 100 mg chewable mebendazole tablets from Sobhan Pharmaceutical Co. (Tehran, Iran) during three days (one tablet twice daily) before sampling. Ten and 15 h after taking last tablet, urine sampling was done. Urine samples were stored at −4 °C, thawed and shaken before extraction. For preparation of human urine, 1 mL of each urine sample was transferred into a 10 mL volumetric flask and diluted to the mark with ultra-pure water. Its pH was adjusted at 1 by dropwise addition of 4 mol L^{-1} of HCl and/or NaOH. Drug-free human plasma (blood group O+) was obtained from Iranian Blood Transfusion Organization (Tehran, Iran). Frozen plasma sample was thawed and allowed to reach room temperature. To precipitate the protein contents of the plasma, 1 mL of plasma with a 500 μL of 60% trichloroacetic acid was centrifuged for 5 min at the rate of 8000 rpm. After decantation of the plasma sample, it was diluted at 1:10 ratio with ultra-pure water. By dropwise addition of 4 mol L^{-1} NaOH and/or HCl solutions, pH of the real samples was adjusted at 9.0 and 1.0 in HF-LPME and EME experiments, respectively. The working standards for real sample analysis were prepared by spiking the target in the water and biological samples.

2.7. Calculations

The preconcentration factors (PF) and extraction recovery (ER) of the drug were calculated based on the following equations:

$$\mathrm{PF} = \frac{C_{\mathrm{a,final}}}{C_{\mathrm{d,initial}}} \quad (1)$$

$$\mathrm{ER}\% = \frac{n_{\mathrm{a,final}}}{n_{\mathrm{d,final}}} \times 100 = \frac{C_{\mathrm{a,final}}V_{\mathrm{a}}}{C_{\mathrm{a,final}}V_{\mathrm{d}}} \times 100 \quad (2)$$

where $n_{\mathrm{d,initial}}$ and $n_{\mathrm{a,final}}$ are the number of moles of the analyte initially present in the donor solution and the number of analyte moles finally collected in the acceptor solution, respectively. V_{a} and V_{d} are the volumes of acceptor and donor phases, respectively. $C_{\mathrm{a,final}}$ is the final concentration of analyte in the acceptor phase and $C_{\mathrm{d,initial}}$ is the initial analyte concentration in the donor phase [3].

3. Results and discussion

Mebendazole as a basic drug was selected as model compound. It is a relatively non-polar drug which was extracted successfully using both of HF-LPME and EME techniques. In order to generate comparable data for EME and HF-LPME, extractions were accomplished with similar equipment, and with the same stirring speeds. Except for the electrodes and the power supply used in EME, the only difference between the two extraction systems was the composition of sample solution. The alkalized sample solution in HF-LPME contained unionized analytes, promoting distribution into the organic liquid [15,16]. In EME, the sample solution was acidified to ensure ionization of the basic analytes; this was necessary to promote electrokinetic migration in the system.

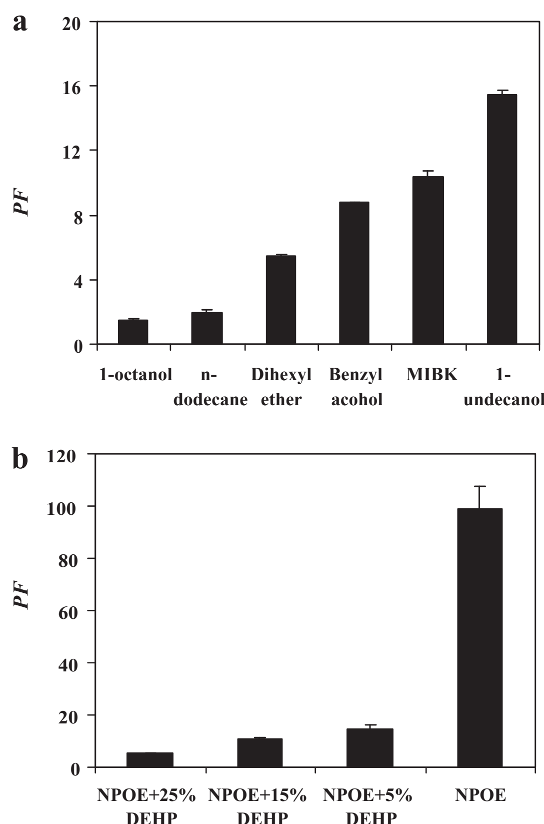

Fig. 2. Effect of impregnation solvent on PF of mebendazole in, (a) HF-LPME and (b) EME. Conditions of (a): V_{donor}, 10 mL of 1 mmol L^{-1} NaOH (pH: 11.0); C_{MEB}, 100 μg L^{-1}; V_{acceptor}, 25 μL of 100 mmol L^{-1} HCl (pH: 1.0); stirring rate, 700 rpm; time, 60 min. Conditions of (b): V_{donor}, 7 mL of 10 mmol L^{-1} HCl (pH: 2.0); C_{MEB}, 100 μg L^{-1}; V_{acceptor}, 25 μL of 100 mmol L^{-1} HCl (pH: 1.0); stirring rate, 700 rpm; time, 15 min; voltage, 150 V.

3.1. Selection of organic solvent

In the first series of experiments, PF versus different organic solvents was evaluated in HF-LPME. The polarity of the organic phase should be similar to that of the polypropylene fiber so that it can be easily immobilized within the pores of the fiber [17–19]. Six different organic solvents including i.e. *n*-dodecane, 1-octanol, methyl isobutyl ketone (MIBK), 1-undecanol, benzyl alcohol, and dihexyl ether were investigated as organic membrane solvents in the preliminary experiments (Fig. 2a). Because of these results, 1-undecanol was chosen for the subsequent experiments. In recent studies of EME of basic drugs, 2-nitrophenyl octyl ether (NPOE) has been utilized as the SLM in combination with voltages in the range of 50–300 V [12]. Among the tested solvents, NPOE was found to be efficient for extraction of mebendazole (Fig. 2b).

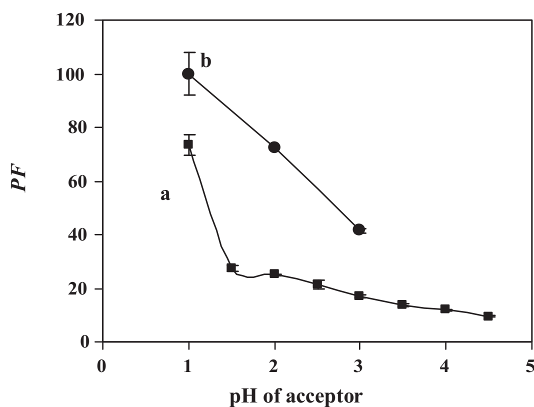

Fig. 3. Effect of pH on the PF of mebendazole in acceptor solution. Conditions as in Fig. 2, except different pH values of acceptor phase, (a) HF-LPME and (b) EME.

3.2. *Influence of pH in donor and acceptor solutions*

The pHs of the donor and acceptor phases play important roles in the three-phase HF-LPME. In this method, the ionized analytes should be in their neutral form in the donor solution, so that they can be transferred into the organic phase, while in the acceptor solution they should exist as their ionized form and, therefore, they cannot be back-extracted into the organic phase. pK_a of mebendazole is 3.27 [20]. Accordingly, to investigate the effect of pH of both acceptor and donor solutions, the pH values in the acceptor and donor solutions were varied in the range of 1–4.5 and 6–11, respectively. In the case of acceptor solution, by keeping the pH of donor phase constant at 11.0 higher recoveries were obtained at lower pH values (Fig. 3a). In donor phase, the PFs increased as the pH increased from 6.0 to 9.0, whereas it decreased as the pH increased above this level (Fig. 4a). Therefore, for HF-LPME, pH values of 9.0 and 1.0 were selected for the donor and acceptor solutions, respectively. In EME, the effect of different pH values in the acceptor solution was investigated. By increasing pH, the electrokinetic migration into the acceptor solution was reduced due to partial deprotonation of the model analytes, and back-diffusion based on passive transport from the acceptor to the artificial liquid membrane was accelerated for the same reason [11]. The pH of the donor phase was kept constant at 2.0 and the results are shown in Fig. 3b. The highest recovery was obtained at pH 1.0 in acceptor solution. Also, the effect of different pH values in the donor solution was studied. By keeping the composition of acceptor phase constant (pH 1.0), the highest extraction recovery was obtained at pH 1.0 in the donor solution (Fig. 4b). Therefore, pH of both acceptor and donor solutions was adjusted at pH 1.0.

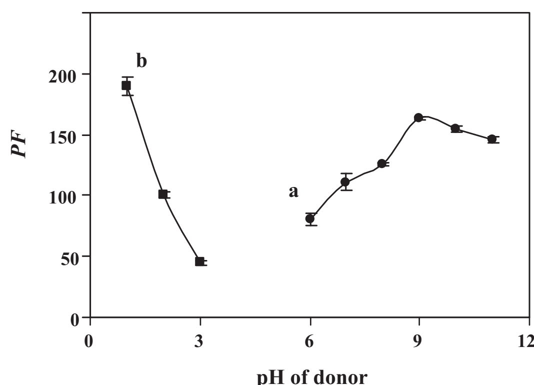

Fig. 4. Effect of pH on the PF of mebendazole in donor solution. Conditions as in Fig. 3, except different pH values of donor phase, (a) HF-LPME and (b) EME.

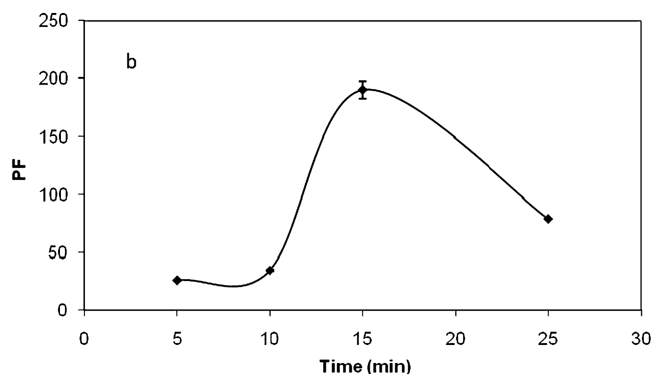

Fig. 5. Effect of stirring rate on the PF of mebendazole. Conditions as in Fig. 4, except different stirring rate, (a) HF-LPME and (b) EME.

3.3. *Influence of stirring speed*

The importance of stirring the whole extraction assembly in HF-LPME has been highlighted in several publications [5]. The principal effect of stirring is to reduce the thickness of the boundary layer at the interface between the sample solution and the SLM where mass transfer is only promoted by diffusion. Stirring was important also in EME to promote the mass transfer and reduce the thickness of boundary layer. To study the effect of this parameter in more details, the effect of stirring on the extraction recovery of HF-LPME (60 min extraction) and EME (15 min extraction) was evaluated. The obtained results for HF-LPME experiments are illustrated in Fig. 5a. By increasing the stirring rate from 300 to 700 rpm, the PF of mebendazole in HF-LPME method increased but at higher stirring rates (700–1250 rpm) the PF decreased. Accordingly, the stirring rate of 700 rpm was selected as the optimum value for the subsequent experiments. In EME, there is no passive diffusion of the analyte from the bulk solution into the SLM. When the stirring speed increased from 500 to 700 rpm, the recovery increased because of the convection effects, while recoveries decreased at higher rates (700–1250 rpm) because of bubble formation [21]. As seen in Fig. 5b, 700 rpm was selected as the optimum value.

3.4. *Extraction time*

In a series of experiments, PF versus time was evaluated in HF-LPME and EME methods. As can be observed in Fig. 6, recoveries increased with increasing the extraction time until a certain level, where equilibrium was obtained for HF-LPME after 60 min and steady state was obtained for EME after 15 min [3]. For HF-LPME, a small decrease in the recovery at higher extraction times (higher

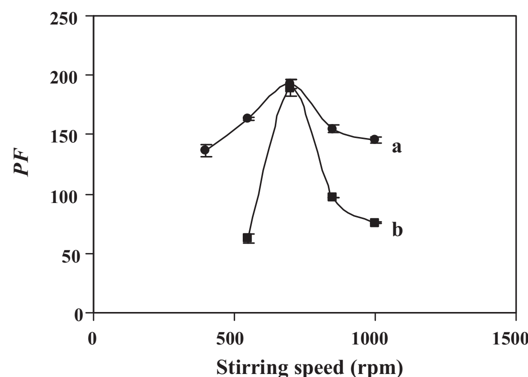

Fig. 6. Effect of extraction time on the PF of mebendazole. Conditions as in Fig. 5, except different extraction time, (a) HF-LPME and (b) EME.

than 60 min) is due to a slight loss of the organic solvent which occurs at longer extraction times. In EME, the extraction recovery increased rapidly with increasing time due to the high flux of target analytes across the membrane. At extraction times above 15 min, the recovery decreased. The possible causes for the slight decrease in recoveries after more than 15 min of EME are probably the instability of the electrical current in the system, back-diffusion of analyte toward donor phase due to increase of pH resulted from electrolysis into acceptor phase, and experimental inaccuracies or small losses of artificial liquid membrane [3,11,12,14]. To summarize the experiments, EME was found to provide significantly improved extraction kinetics as compared with HF-LPME.

3.5. Salt effect

For HF-LMPE in aqueous solutions, the addition of salt (such as NaCl or Na_2SO_4) can decrease the solubility of the analytes and enhance their partitioning into the organic phase (salting out effects) [6]. The effect of salt addition on recovery was examined by adding sodium chloride to aqueous samples at concentration levels of 0, 1, 2 and 3 mol L^{-1}. The recovery was decreased by increasing the salt concentration in HF-LPME. Such interactions would tend to restrict movement of the analyte from the donor solution to the membrane solvent. So, all the subsequent experiments in HF-LPME were performed in the absence of salt. According to previous studies [22,23], the presence of high content of ionic substances causes an increase in the value of the ion balance (χ) in the system, which in turn decreases the flux of analytes across the SLM. Our observations are in full agreement with previous studies [22,23]. Thus, migration of the analytes would be more efficient in the absence of salt.

3.6. Effect of applied voltage in EME

In order to find the optimal potential, the voltage applied across the SLM was varied between 50 and 300 V [10,11]. Recovery increased up to 150 V. At higher voltages up to 300 V, recoveries decreased (Fig. 7). In this case, the system suffered from bubble formation at the electrodes and corresponding instability problems. In one occasion, sparking was observed. Thus, electrical potential differences above 150 V were found to be inappropriate, and during the rest of this work, 150 V was used.

3.7. Evaluation of the method performance

The calibration curves of mebendazole were plotted in the concentration range of 0.5–1000 μg L^{-1} in aqueous solutions for both of HF-LPME and EME methods. The correlation coefficient (R^2), dynamic linear range (DLR), the limit of detection (LOD), PF and ER% of mebendazole in real samples by both methods were calculated whose results are summarized in Table 1. The PFs of 211 and 190, and the LODs of 0.05 and 0.10 μg L^{-1} were calculated at a

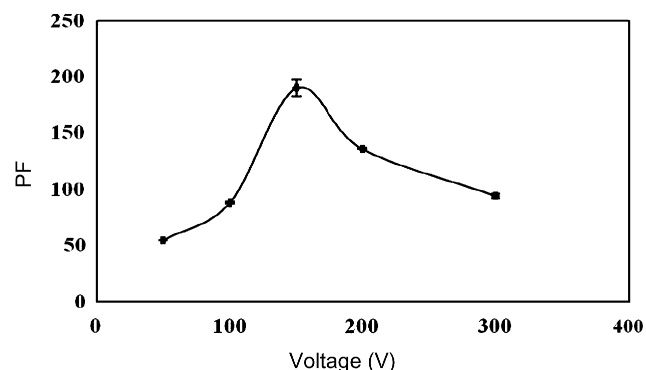

Fig. 7. Effect of voltage on the PF of mebendazole in EME. Conditions as in Fig. 6(b), except different voltage.

signal-to-noise ratio of 3 for HF-LPME and EME methods, respectively.

3.8. Extraction of mebendazole from plasma and urine samples

Applicability of the proposed LPME and EME methods to real samples was evaluated by extraction and determination of mebendazole in human plasma and urine samples. Gjelstad and coworkers have described the first systematic study of EME from biological matrices under physiological conditions and obtained satisfactory results [24]. But there is no report concerning HF-LPME of the drugs from biological samples without sample preparation (i.e. precipitation of proteins, etc.) especially in plasma samples. In EME, electrical potential can act as a power force for breaking and reduction of analyte-protein binding [24]. In HF-LPME, pH gradient has not enough potential for extraction of analyte. A comparison between EME and HF-LPME has been made in several papers [3] which show the important role of electrical potential in reduction of protein binding, whereas HF-LPME has shown very low and negligible extraction. On the other hand, the mentioned papers for comparison of EME and HF-LPME which indicate low potential of HF-LPME have been applied to drugs with moderate percentage of protein binding whereas mebendazole has more than 90% protein binding [25]. In this work, protein precipitation was fulfilled for both HF-LPME and EME for showing ability and performance of both methods under the same conditions, i.e. without protein binding for both techniques. It seems that true criteria for comparison of HF-LPME and EME cannot be obtained if EME and HF-LPME were carried out without and with precipitation of protein, respectively. Therefore, regarding high percentage of protein binding for mebendazole and the mentioned points, protein precipitation was done for both techniques. The preparation steps of real samples were performed according to Section 2.6. At first, non-spiked plasma and urine samples were extracted by HF-LPME and EME under optimal conditions obtained for each method. Afterwards, differ-

Table 1
Figures of merit of the proposed methods HF-LPME and EME for analysis of mebendazole.

	HF-LPME[a]			EME[b]		
	Aqueous	Plasma	Urine	Aqueous	Plasma	Urine
LOD (μg L^{-1})	0.05	1.00	0.50	0.10	0.10	0.10
DLR (μg L^{-1})	0.5–1000	10–1000	1–500	0.5–1000	0.5–1000	0.5–1000
R^2	0.997	0.994	0.999	0.999	0.996	0.995
PF	211	155	175	190	144	156
ER%	53	39	44	68	51	56
R.S.D%[c]	2.5	3.6	7.3	2.7	4.3	5.6

[a] All of the parameters were obtained based on V_{donor} = 10 mL.
[b] All of the parameters were obtained based on V_{donor} = 7 mL.
[c] Standard deviation for three-replicate measurements.

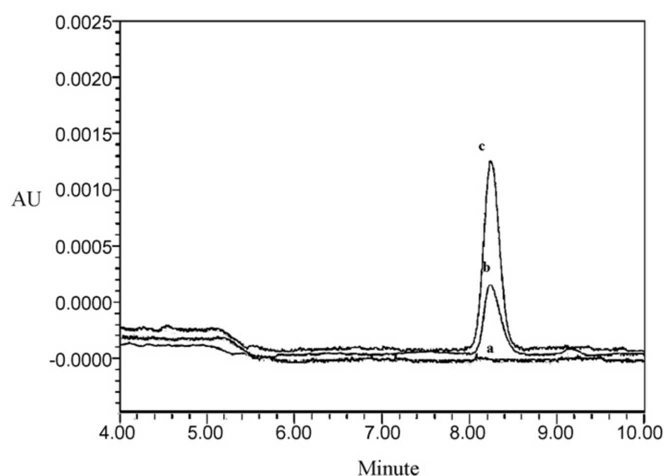

Fig. 8. The chromatograms corresponding to (a) non-spiked urine sample, (b) the urine sample of a volunteer treated with mebendazole, and (c) the spiked urine sample with 20 μg L^{-1} mebendazole that were extracted under optimal conditions of the EME.

Table 2
Determination of mebendazole in different spiked samples.

	HF-LPME[a]		EME[b]	
	Plasma	Urine[d]	Plasma	Urine[e]
$C_{initial}$	–	4.3	–	8.7
C_{add} (μg L^{-1})	20.0	20.0	20.0	20.0
C_{found} (μg L^{1})	19.6	24.1	21.7	29.7
R^c%	98	99	108	105
R.S.D% (n = 3)	3.5	4.0	5.1	5.6

[a] All of the parameters were obtained based on V_{donor} = 10 mL.
[b] All of the parameters were obtained based on V_{donor} = 7 mL.
[c] Relative recovery.
[d] The sample was taken after 15 h.
[e] The sample was taken after 10 h.

ent amounts of mebendazole were added to the real samples and extraction procedure was repeated again. In the case of urine taken from the volunteer who had consumed mebendazole tablet, it was observed at concentration level of 4.3 (for the sample that was taken after 15 h) and 8.7 μg L^{-1} (for the sample that was taken after 10 h) using HF-LPME and EME, respectively. Fig. 8 shows the chromatograms corresponding to (a) non-spiked urine sample, (b) the urine sample of a volunteer treated with mebendazole, and (c) the spiked urine sample with 20 μg L^{-1} mebendazole that were extracted under optimal conditions of the EME. Table 2 shows that, the results of each real sample obtained by the proposed methods are in satisfactory agreement with the spiking amount.

Table 3
Comparison of the proposed methods with other methods for extraction and determination of mebendazole.

Method/ instrumentation	DLR (μg L^{-1})	LOD	ER%	Refs.
SPE-LC-DAD[a] (in bovine liver)	–	12 (μg Kg^{-1})	53	[26]
LPE/HPLC–UV[b] (in eel tissue)	1.61–64.21 (nmol 100 mg^{-1})	0.32 (nmol 100 mg^{-1})	72	[27]
HF-LPME/HPLC–UV	0.5–1000 (μg L^{-1})	0.05 (μg L^{-1})	51	This work
EME/HPLC–UV	0.5–1000 (μg L^{-1})	0.1 (μg L^{-1})	56	

[a] Solid-phase extraction liquid chromatography diode array.
[b] Liquid-phase extraction/high performance liquid chromatography ultraviolet.

3.9. Comparison of the applied methods with other reported methods

The present method was compared with other methods in terms of validation and precision (Table 3). As can be deducted, the method is quite comparable to those mentioned in Table 3. The proposed HF-LPME and EME methods have some advantages in comparison with other extraction methods, including low consumption of organic solvents and reagents, simplicity and low cost of the extraction device, and producing a clean extracting phase for the analysis.

4. Conclusion

The present work has compared extraction of a basic analyte across a supported liquid membrane based on (1) passive diffusion based on a pH gradient sustained over the SLM (HF-LPME), and (2) electrokinetic migration in an electrical field sustained over the SLM (EME). The proposed methods were successfully developed for the extraction and analysis of mebendazole in biological fluids. Whereas HF-LPME to a large extent occurs only under diffusion condition, mass transfer in EME always includes electrokinetic migration in an electrical field. These techniques demonstrated several advantages over the other extraction methods, such as high clean-up in the case of complex samples. Also, only microliter amounts of the extraction solvent were needed, the needed equipment is very simple and inexpensive so the hollow fiber can be discarded after each extraction to eliminate possible carry-over problems as compared to the solid-phase microextraction. The proposed methods provided high preconcentration factor (211, 190) without the need for solvent evaporation after the extraction and the extract is directly injected into the HPLC loop. In EME system, applied potential was crucial to avoid excessive current in the system. Because of short extraction time, EME may have a strong potential as a future sample preparation technique.

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References

[1] Z. Al-Kurdi, T. Al-Jallad, A. Badwan, A.M.Y. Jaber, High performance liquid chromatography method for determination of methyl-5-benzoyl-2-benzimidazole carbamate (mebendazole) and its main degradation product in pharmaceutical dosage forms, Talanta 50 (1999) 1089–1097.
[2] M. Rezaee, Y. Yamini, M. Faraji, Evolution of dispersive liquid–liquid microextraction method, J. Chromatogr. A 1217 (2010) 2342–2357.
[3] A. Gjelstad, T.M. Andersen, K.E. Rasmussen, S. Pedersen-Bjergaard, Microextraction across supported liquid membranes forced by pH gradients and electrical fields, J. Chromatogr. A 1157 (2007) 38–45.
[4] M. Balchen, T.G. Halvorsen, L. Reubsaet, S. Pedersen-Bjergaard, Rapid isolation of angiotensin peptides from plasma by electromembrane extraction, J. Chromatogr. A 1216 (2009) 6900–6905.
[5] K.M. Al-Azzam, A. Makahleah, B. Saad, S.M. Mansor, Hollow fiber liquid-phase microextraction for the determination of trace amounts of rosiglitazone (antidiabetic drug) in biological fluids using capillary electrophoresis and high performance liquid chromatographic methods, J. Chromatogr. A 1217 (2010) 3654–3659.
[6] S. Shariati, Y. Yamini, A. Esrafili, Carrier mediated hollow fiber liquid phase microextraction combined with HPLC–UV for preconcentration and determination of some tetracycline antibiotics, J. Chromatogr. B 877 (2009) 393–400.
[7] C. Basheer, S.H. Tan, H.K. Lee, Extraction of lead ions by electromembrane isolation, J. Chromatogr. A 1213 (2008) 14–18.
[8] T.G. Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaet, K.E. Rasmussen, Liquid-phase microextraction combined with flow-injection tandem mass spectrometry Rapid screening of amphetamines from biological matrices, J. Sep. Sci. 24 (2001) 615–622.
[9] T.G. Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaet, K.E. Rasmussen, Liquid-phase microextraction combined with liquid chromatography–mass spectrometry. Extraction from small volumes of biological samples, J. Sep. Sci. 26 (2003) 1520–1526.

[10] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Electrokinetic migration across artificial liquid membranes: tuning the membrane chemistry to different types of drug substances, J. Chromatogr. A 1124 (2006) 29–34.
[11] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrokinetic migration across artificial liquid membranes: new concept for rapid sample preparation of biological fluids, J. Chromatogr. A 1109 (2006) 183–190.
[12] I.J. Kjelsen, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Low-voltage electromembrane extraction of basic drugs from biological samples, J. Chromatogr. A 1180 (2008) 1–9.
[13] M. Balchen, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Electrokinetic migration of acidic drugs across a supported liquid membrane, J. Chromatogr. A 1152 (2007) 220–225.
[14] M. Balchen, L. Reubsaet, S. Pedersen-Bjergaard, Electromembrane extraction of peptides, J. Chromatogr. A 1194 (2008) 143–149.
[15] A. Esrafili, Y. Yamini, S. Shariati, Hollow fiber-based liquid phase microextraction combined with high-performance liquid chromatography for extraction and determination of some antidepressant drugs in biological fluids, Anal. Chim. Acta 604 (2007) 127–133.
[16] A. Sarafraz-Yazdi, F. Mofazzeli, Z. Es'hsghi, A new high-speed hollow fiber based liquid phase microextraction method using volatile organic solvent for determination of aromatic amines in environmental water samples prior to high-performance liquid chromatography, Talanta 79 (2009) 472–478.
[17] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid-phase microextraction and capillary electrophoresis of citalopram, an antidepressant drug, J. Chromatogr. A 909 (2001) 87–93.
[18] S. Pedersen-Bjergaard, K.E. Rasmussen, Extraction across supported liquid membranes by use of electrical fields, Anal. Bioanal. Chem. 388 (2007) 521–523.
[19] C. Basheer, H.K. Lee, Analysis of endocrine disrupting alkylphenols, chlorophenols and bisphenol-A using hollow fiber-protected liquid-phase microextraction coupled with injection port-derivatization gas chromatography–mass spectrometry, J. Chromatogr. A 1057 (2004) 163–169.
[20] M. Shalaeva, J. Kenseth, F. Lombardo, A. Bastin, Measurement of dissociation constants (pK_a values) of organic compounds by multiplexed capillary electrophoresis using aqueous and cosolvent buffers, J. Pharm. Sci. 97 (2008) 2581–2606.
[21] S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid–liquid extraction, J. Chromatogr. A 1184 (2008) 132–142.
[22] J. Lee, F. Khalilian, H. Bagheri, H.K. Lee, Optimization of some experimental parameters in the electro membrane extraction of chlorophenols from seawater, J. Chromatogr. A 1216 (2009) 7687–7693.
[23] T.M. Middelthon-Bruer, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Parameters affecting electro membrane extraction of basic drugs, J. Sep. Sci. 31 (2008) 753–759.
[24] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Electromembrane extraction of basic drugs from untreated human plasma and whole blood under physiological pH conditions, Anal. Bioanal. Chem. 393 (2009) 921–928.
[25] H. Allgayer, J. Zähringer, P. Bach, J. Bircher, Lack of effect of haemodialysis on mebendazole kinetics: studies in a patient with echinococcosis and renal failure, Eur. J. Clin. Pharmacol. 27 (1984) 243–245.
[26] G. Caprioli, G. Cristalli, R. Galarinib, D. Giacobbea, M. Ricciutelli, S. Vittori, Y. Zuoa, G. Sagratini, Comparison of two different isolation methods of benzimidazoles and their metabolites in the bovine liver by solid-phase extraction and liquid chromatography–diode array detection, J. Chromatogr. A 1217 (2010) 1779–1785.
[27] L. Mottier, L. Alvarez, C. Lanusse, Quantitative chromatographic determination of several benzimidazole anthelmintic molecules in parasite material, J. Chromatogr. B 798 (2003) 117–125.