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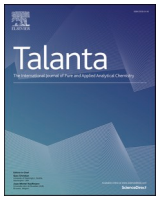

# Multi-extraction system with identical supported semi-liquid membrane: Enhanced stability for coextraction of acidic and basic drugs

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ABSTRACT

Coextraction of different groups of analytes is vital for saving sample volumes and simplifying analytical procedures in bioanalysis. Conventionally, coextraction was achieved by using multi-extraction systems with different supported liquid membranes (SLMs). However, the different membrane solvents tended to diffuse into the aqueous solutions and the other SLM to reach distribution equilibrium during extraction process, causing the system instability. In this work, a stable multi-extraction system (integration of liquid-phase microextraction and electromembrane extraction, LPME/EME) based on the identical supported semi-liquid membrane (SsLM) was developed. Principally, the state of distribution equilibrium of the membrane solvent (polypropylene glycol with molecular weight 4000) in SsLM could be reached at the beginning of extraction, which enhanced the coextraction stability. With this multi-extraction system, acidic and basic analytes were simultaneously extracted from practical biological samples. The extraction recoveries of the six model drugs in undiluted urine samples were over 70%. Followed by LC-MS/MS, the limits of quantification (LOQs) were in the range of 5–10 ng $mL^{-1}$. The multi-extraction system using the identical SsLM in this study shows promising potential in construction of other stable multi-extraction systems (e.g., LPME/LPME and EME/EME) in the future, which will greatly benefit the group separation of analytes in complicated biological samples.

## 1. Introduction

Sample preparation is a crucial step in bioanalysis due to the complexity of sample matrices [1–4]. Proper sample preparation techniques could not only enhance sample compatibility with subsequent instrument analysis, but also improve the sensitivity and accuracy of analytical methods. With the growing demand for small sample volume, low consumption of organic solvents and high efficiency, more and more miniaturized sample preparation techniques have been applied in practical analysis. Among these miniaturized techniques, liquid-phase microextraction (LPME) [5] and electromembrane extraction (EME) [6] are two of the most popular techniques, which are characterized by satisfactory sample clean-up ability, excellent selectivity and high extraction efficiency.

Both LPME and EME are supported liquid membrane (SLM) based extraction, but the fundamental extraction principles of LPME and EME are different. In LPME, by adjusting pH values of the aqueous solutions, the target analytes exist in neutral forms in donor solution and are ionized in acceptor solution. Due to the larger solubility of analytes in acceptor solution than that in donor solution, the analytes passively migrate from donor phase into acceptor phase [5]. While for EME, the target analytes exist in charged forms in both donor and acceptor solutions. With the external potential, ionized analytes migrate directionally from donor solution into the acceptor solution. Compared to the mass transfer process in LPME (passive diffusion), the mass transfer process in EME is mainly electrokinetic migration [7], which makes the extraction more rapid and achieve higher selectivity [8].

According to the above extraction principles, a single LPME or EME could only extract one group of analytes with close pKa values at an extraction batch. However, usually different groups of targets should be detected in one analytical procedure in many research fields involving public health, forensic medicine, pharmacology and other subjects

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concerning life and health. Theoretically, group separation of the analytes could be achieved by dividing the sample into several parts (each part was coupled with a different extraction approach). Obviously, this dividing method consumed a large sample volume. Unfortunately, biological samples are usually valuable, therefore concurrent separation and determination of different groups of target analytes are of great significance. So far, to meet the requirement of simultaneous analysis of various analytes, some combined multi-extraction techniques have been developed for sample preparation, mainly including dual EME system [9–16] and integrated LPME/EME system [17,18]. In dual EME, the pH value of the donor solution was adjusted between the pKa values of acidic and basic analytes to ensure that acidic and basic analytes were negatively and positively charged, respectively. Differ from that, in LPME/EME, the pH value of the donor solution was lower or higher than all the target analytes to ensure that one group of them was in molecular forms and the other group was in charged forms. The extraction principles of dual EME and LPME/EME are different and thus their proper application ranges are different. For example, the μ-LPME/EME microchip device was designed by using three polymethyl methacrylate layers. With diexyl ether and octanol as membrane solvents for SLMs in LPME and EME respectively, it accomplished the simultaneous extraction of five fluoroquinolones and four parabens with extraction recoveries over 77% [18]. As seen, the above combined techniques consisted of two extraction units. Since the affinity of membrane solvent differs to different analytes, one common characterization of these coextraction methods was that two or more membrane solvents were used for better extraction of different classes of substances. However, the reported membrane solvents were all low molecular organic compounds (e.g., 2-nitrophenyl octyl ether, 2-nitrophenyl octyl ether containing 5% (v/v) di-(2-ethylhexyl) phosphate, dihexyl ether, 1-octanol, etc.), these organic solvents were prone to leak from the SLMs to aqueous solutions under the function of agitation or vibration during the extraction [19–22]. To make matter worse, these different membrane solvents always showed high affinities to each other, since one of them had an affinity to acidic analytes and the other one to basic analytes. They would tend to distribute in equilibrium in the two SLMs during the extraction process. This behavior would reduce stability of the multi-extraction system and further influence the extraction efficiency. To reduce the adverse effects, Huang et al. [17] selected a shorter extraction time with relatively high voltage for EME unit in their LPME/EME system, which partly compromised the extraction recoveries of targets from plasma samples. Alternatively, Payán et al. [18] designed the microchip device using flow sample and acceptor solutions and tried to avoid the contact of two SLMs in design. But all in all, they still used two different membrane solvents and could not constantly guarantee no cross diffusion. To completely address the problem, it is considered to use the SLMs always in distribution equilibrium (of the membrane solvents), that is identical SLMs with the same membrane solvent.

Recently, we reported that polypropylene glycol with molecular weight 4000 (PPG4000) possessed high affinities to both acidic and basic drugs, and the supported semi-liquid membrane (SsLM) using PPG4000 as membrane solvent had high stability and versatility during the extraction [23,24]. Inspired by these findings, a new LPME/EME set-up for coextraction of acidic and basic drugs was developed based on the novel SsLM in this work. The employment of the identical SsLM provided the multi-extraction system with high stability in a relatively long extraction time, which shows great potential in construction of other stable multi-extraction systems (e.g., LPME/LPME and EME/EME) for group separation.

## 2. Experimental

### 2.1. Chemicals and materials

The acidic model analytes naproxen (NAP), flurbiprofen (FLB) and diclofenac sodium (DIC), the basic model analytes haloperidol (HAL), fluoxetine (FLU) and sertraline (SER), the internal standard (IS) gemfibrozil (GEM) for acidic drugs, the membrane solvent PPG4000, 2-nitrophenyl octyl ether (NPOE) and dihexyl ether (DHE) were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). The internal standard fluoxetine-$d_5$ (FLU-$d_5$) for basic drugs was obtained from Sigma-Aldrich (Shanghai, China). Trifluoroacetic acid (TFA), hydrochloric acid (HCl), sodium hydroxide (NaOH) and formic acid were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetonitrile and methanol were supplied by Tedia (Fairfield, OH, USA). Formic acid, methanol and acetonitrile were of chromatographic pure grade and other reagents were of analytical pure grade. The supported membrane material polypropylene (PP) 1E (R/P) membrane (with the thickness of 100 μm, the maximum pore size of 0.36 μm, and the porosity of 69%) was purchased from Membrana (Wuppertal, Germany).

### 2.2. Apparatus

LPME/EME was carried out on a temperature-adjustable vortexer (Miulab Instrument Co., Ltd., China). HPLC-UV analysis was conducted using the Ultimate 3000 system (Dionex, Germering, Germany) comprising a VWD-3400RS UV–Vis detector. LC-MS/MS analysis was conducted using Ultimate 3000 UHPLC system interfaced with a TSQ Quantum Access MAX triple quadrupole Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). UV–Vis spectra of solutions were recorded with the UV–Vis spectrophotometer (UV-1800, Shimadzu, China). Fourier transform infrared (FT-IR) spectra, water droplet angles and scanning electron microscope (SEM) of the membranes were measured using the FT-IR spectrometer (VERTEX 80, Bruker, Germany), contact angle measuring system (IC2000D, Zhongchen, China) and a field emission SEM (SIGMA 500, Zeiss, Germany), respectively.

### 2.3. Standard solutions and real samples

The stock solution of acidic and basic drugs was obtained by dissolving the six model analytes (HAL, FLU, SER, NAP, FLB and DIC) in methanol at 200 μg $mL^{-1}$ and stored at 4 °C without light. The standard solution was prepared by diluting the stock solution to 5 μg $mL^{-1}$ with 10 mM TFA or HCl solution. The urine and plasma samples were collected from healthy volunteers without any treatment in the last three months and stored at 4 °C.

### 2.4. Set-up and procedure of LPME/EME

The integrated LPME/EME set-up was demonstrated in Fig. 1. The acceptor compartments for LPME and EME units were two homemade 1000 μL pipette tips sealed with PP membranes. The attached PP membranes were then interpenetrated with PPG4000 under the function

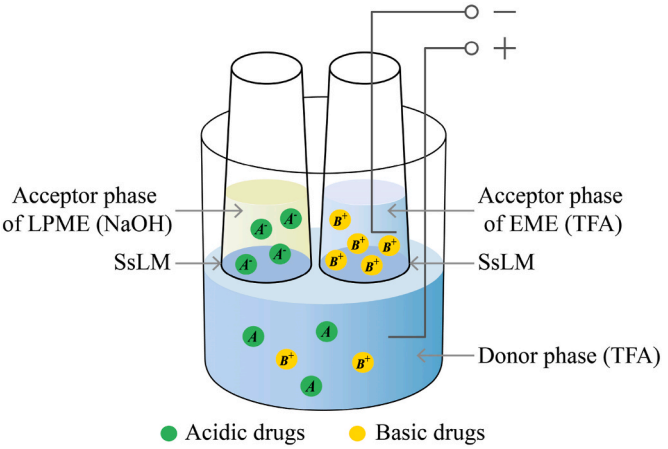


**Fig. 1.** Schematic illustration of set-up and principle of LPME/EME system.

of ultrasound to form the SsLM. The fabrication of acceptor compartments and the preparation of SsLM were the same as that in our previous work [24]. For extraction, 200 μL of acceptor solutions were added into the acceptor compartments. The donor compartment was a 5 mL beaker, and in this beaker 800 μL of donor solution was added. In this study, LPME and EME were used to extract acidic and basic drugs, respectively. To achieve the extraction, two L-shaped platinum wires were placed into the EME acceptor and donor phases as the cathode and anode, respectively. Subsequently, the two acceptor compartments were put into the donor compartment and made the SsLMs just contact with the liquid level of donor solution. After that, the LPME/EME set-up was placed on a temperature-adjustable vortexer and the electrodes were connected to a power supply. After extraction, drugs in the donor and acceptor solutions were detected using HPLC-UV or LC-MS/MS analysis, and the analytical conditions were provided in supporting information.

### 2.5. Stability characterizations

To investigate the diffusion of membrane solvents in conventional SLMs and the novel SsLM, the UV–Vis absorption spectra of acceptor and donor solutions after the simulated LPME/EME process were recorded in the range of 250–600 nm. FT-IR spectra of blank PP membrane, SLMs before and after the simulated extraction process were recorded in the range of 4000-500 $cm^{-1}$ with 16 scans at room temperature. To further confirm the stability of SsLM with PPG4000, water droplet contact angles and SEM images of blank PP membrane, SsLM before and after LPME/EME were recorded at room temperature.

### 2.6. Calculations

The extraction recovery of the drug was calculated by equation (1):

$$Recovery = \frac{C_A \times V_A}{C_D^0 \times V_D} \times 100\% \quad (1)$$

The matrix effect (ME) was calculated by equation (2):

$$ME(\%) = (\frac{A_{presence}}{A_{absence}} - 1) \times 100\% \quad (2)$$

The method accuracy was calculated by equation (3):

$$Accuracy = \frac{C_{calculated}}{C_{spiked}} \times 100\% \quad (3)$$

Here, $C_A$ was concentration of the drug in acceptor solution after LPME/EME. $C_D^0$ was the initial concentration of the drug in donor solution. $V_A$ and $V_D$ were volumes of the acceptor and donor solutions for extraction, respectively. $A_{presence}$ was the IS normalized peak area in the presence of matrix (the analytes-spiked acceptor solution after LPME/EME from blank urine sample), $A_{absence}$ was the IS normalized peak area in the absence of matrix (pure standard solution of drugs). $C_{calculated}$ was the calculated concentration according to the calibration curve, $C_{spiked}$ was the spiked concentration.

## 3. Results and discussion

### 3.1. Evaluation of the SsLM based LPME/EME

The concept of the SsLM based LPME/EME method for coextraction of acidic and basic drugs was proved here. In this work, NAP (pKa = 4.2), FLB (pKa = 4.4), DIC (pKa = 4.0) and HAL (pKa = 8.0), FLU (pKa = 10.1) and SER (pKa = 9.5) were used as model analytes for the following experiments. As shown in Fig. 1, the supported membranes in both of LPME and EME units were the versatile SsLM with PPG4000, the donor solution was 800 μL of 10 mM TFA containing the six drugs at the concentration of 5 μg $mL^{-1}$, the acceptor solutions for LPME and EME were 200 μL of 10 mM NaOH and 10 mM TFA solution, respectively. As illustrated in Fig. 1, NAP, FLB and DIC molecules in donor solution were extracted to the LPME acceptor phase by passive distribution. While HAL, FLU and SER in donor solution existed in positive ions and migrated to the EME acceptor phase under the external electric field.

To validate the feasibility of the SsLM, single LPME for acidic drugs (NAP, FLB, DIC) and single EME for basic drugs (HAL, FLU, SER) were conducted respectively. After extraction, the target analytes in acceptor and donor solutions were detected by HPLC-UV and the extraction recoveries were calculated. The chromatograms of drugs in donor and acceptor solutions after single LPME and EME were presented in Fig. S1. As presented in Table S4, the average recoveries of acidic and basic drugs were 87% and 94%, respectively, which were in accordance with our previous works [23,25]. The satisfactory extraction efficiencies verified that SsLM could be used to extract both acidic and basic drugs. This indicated that the novel SsLM has great potential for coextraction of different classes of analytes. On this basis, SsLM was employed to construct the multi-extraction system in this work.

To evaluate the extraction performance of the multi-extraction system, the SsLM based LPME/EME was operated for 30 min to simultaneously extract acidic and basic drugs. The recoveries of six target drugs and chromatograms of drugs in donor and acceptor phases after LPME/EME were demonstrated in Fig. 2. The average recoveries of acidic and basic analytes were 89% ± 3% and 101% ± 2%, respectively, which were in accordance with the extraction efficiencies of the single LPME and single EME. Consequently, the SsLM based LPME/EME could achieve coextraction of acidic and basic drugs with high efficiency and the extraction efficiency of LPME and EME would not be affected by each other in this multi-extraction system.

The supported liquid membrane, which separates the donor and acceptor solutions, is one of the most significant parts in both LPME and EME [26]. Principally, the compositions of SLM influence the extraction selectivity and efficiency, and the stability of SLM partly determines the robustness of extraction system. In the previous LPME/EME system [17] which used two different SLMs, the purity of group separation would deteriorate when prolonged the extraction time. As presented in Fig. S2, a small proportion of the acidic drugs appeared in the acceptor phase of EME (EME was used to extract basic drugs) when time exceeded 15 min, although the average recovery of acidic and basic drugs in diluted plasma samples increased from 39% to 68% when time prolonged from 15 min to 45 min [17]. This indicated that if a clear group separation was preferable, the extraction efficiency had to be compromised in the previous LPME/EME system using different SLMs. However, in this work, extremely pure group separation could still be achieved even after 30 min (Fig. 2b). Compared to previous multi-extraction systems, the newly SsLM based LPME/EME method in this work showed enhanced stability.

### 3.2. Enhanced stability of the multi-extraction system

In previous multi-extraction systems, two SLMs with different membrane solvents were used. As shown in Fig. 3, under the function of agitation or vibration, the two organic solvents would leak from the membrane pores and diffuse to the other SLM to reach distribution equilibrium. This behavior of cross diffusion would decrease the system stability and thus influenced the extraction efficiency. On the contrary, if two identical SsLMs were used in the multi-extraction system, the distribution equilibrium of the membrane solvent would be quickly achieved at the very beginning of the extraction. After that, the diffusion of membrane solvent would not occur. Hence, the stable multi-extraction system was obtained. To investigate the above supposition, the following experiments were conducted.

Here, to investigate the diffusion of membrane solvents when two different SLMs were used in the conventional multi-extraction system, the diffusion process was observed using NPOE as an example since it was the most popular membrane solvent in LPME and EME [27]. Initially, LPME/EME using different SLMs (DHE and NPOE as membrane solvents, respectively) was simulated using ultrapure water as donor and

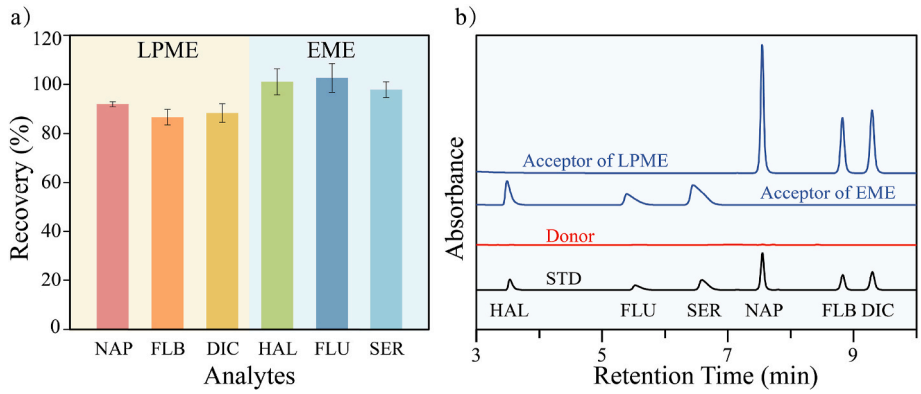


**Fig. 2.** LPME/EME for coextraction of acidic and basic drugs. (a) Extraction recoveries of the six drugs; (b) Chromatograms of drugs in donor and acceptor phases after LPME/EME.

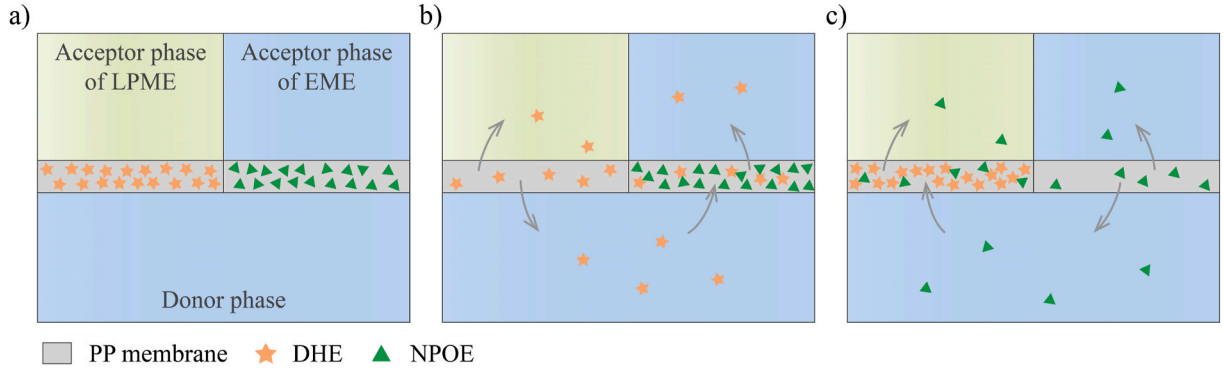


**Fig. 3.** Illustration of the cross diffusion of different membrane solvents in the multi-extraction system. (a) The distribution of DHE and NPOE in two SLMs before LPME/EME; (b) The diffusion of DHE during the LPME/EME; (c) The diffusion of NPOE during the LPME/EME.

acceptor solutions. To better observe the diffusion of NPOE, the extraction was proceeded for a relatively long time (90 min). To see whether NPOE distributed into the aqueous solutions, the donor and acceptor phases after extraction were examined by UV–Vis spectrophotometer. As demonstrated in Fig. 4a, for 0.5 mg $mL^{-1}$ NPOE solution, there was an absorbance in the range of 250–300 nm. And absorbances in the same place were observed for donor and acceptor phases, indicating that NOPE diffused from SLM to the solutions. To further investigate whether the diffusion of NPOE occurred, the SLM (DHE) before and after 90 min of extraction were tested by FT-IR analysis. As presented in Fig. 4b, the stretching vibration at 1569-1506 $cm^{-1}$ was corresponding to the nitro group of NPOE [28]. Apparently, the specific vibration of the nitro group was observed in the SLM (DHE) after extraction, indicating that NPOE in solutions distributed into the other SLM (DHE). From the above results, it is concluded that NPOE diffused into the other SLM during the multi-extraction process, which was harmful to the function of SLMs and might further influence the stability of the extraction system.

Afterwards, to confirm the stability of the SsLM used in this work, characterization experiments including UV–Vis spectrophotometry, FT-IR analysis, water droplet contact angle and SEM analysis were conducted to observe the changes of SsLM. LPME/EME based on the same

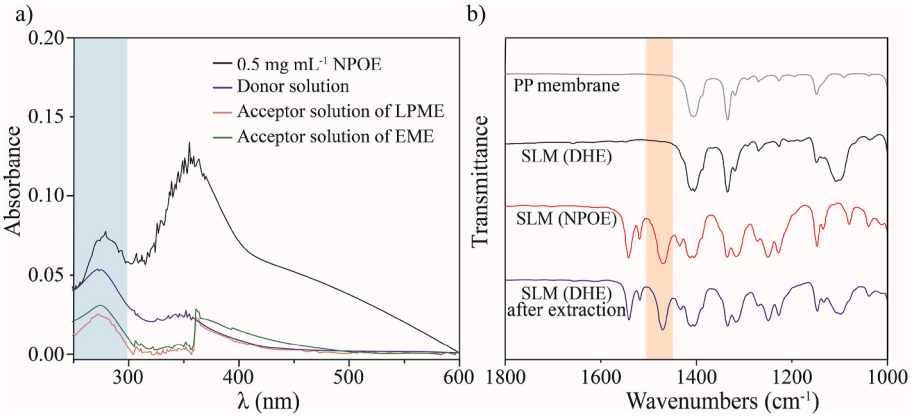


**Fig. 4.** Characterizations of the multi-extraction system based on two different SLMs. (a) UV–Vis spectra of 0.5 mg $mL^{-1}$ NPOE, donor and acceptor solutions after LPME/EME; (b) FT-IR spectra of the blank PP membrane, SLM (NPOE), SLM (DHE) before and after LPME/EME.

SsLM with PPG4000 as membrane solvent was carried out using ultrapure water as simulative donor and acceptor phases. After 90 min of extraction, both solutions and SsLMs were analyzed. As shown in Fig. 5a, there was no obvious absorbance in donor and acceptor solutions just as that in 1.0 mg $mL^{-1}$ PPG4000 solution, which demonstrated that the membrane solvent had hardly diffused into the solution from the SsLM. On the other hand, there's no clear difference between the FT-IR spectra of SsLM before and after extraction (Fig. 5b), indicating almost no loss of PPG4000 during the extraction. What's more, water droplet contact angles and SEM images further directly confirmed the stability of SsLM. It could be seen in Fig. 5c–j that the contact angles and surface morphologies of the SsLM before and after LPME/EME were basically the same, proving that the two identical SsLMs for LPME and EME units could maintain unchanged and stable within a long extraction process. This could be considered that the PPG4000 in two SsLMs was in the state of equilibrium at the beginning and would not leak from the membrane, which means no diffusion and high stability of the multi-extraction system.

### 3.3. Optimization of LPME/EME

Generally, the optimal operational parameters were considered as experimental conditions that achieved high efficiency and meanwhile maintained both reproducible and stable extractions [29]. In this section, experiments were carried out to optimize some crucial parameters and finally build an optimal LPME/EME system for coextraction of acidic and basic drugs in this study. According to our previous works [23,25], the optimal conditions of SsLM based single LPME for acidic drugs and single EME for basic drugs have been obtained and summarized in Table 1. In our previous work, the extraction temperature of EME was directly set at room temperature (25 °C), while the optimal temperature of LPME was 60 °C. This might be because that the viscosity of PPG4000 would decrease with increasing the temperature, and thus facilitated the diffusivity of target analytes [30]. While the extraction principles of LPME and EME are passive diffusion and mainly electrokinetic migration respectively, therefore the influence of temperature was more critical for LPME than that for EME. Given by these reasons, the extraction temperature for LPME/EME was initially set at 60 °C. Based on the available parameters, the composition of donor phase and extraction time for LPME/EME were further optimized.

#### 3.3.1. Composition of donor phase

To achieve the coextraction of acidic and basic drugs using LPME/EME, the donor solution should maintain a proper pH level which could make the acidic drugs in neutral molecules and basic drugs in charged forms. According to Table 1, the pH values of optimal donor solutions for single LPME and EME were equal (pH = 2), only the background electrolytes were different. Hence, LPME/EME for the six analytes was

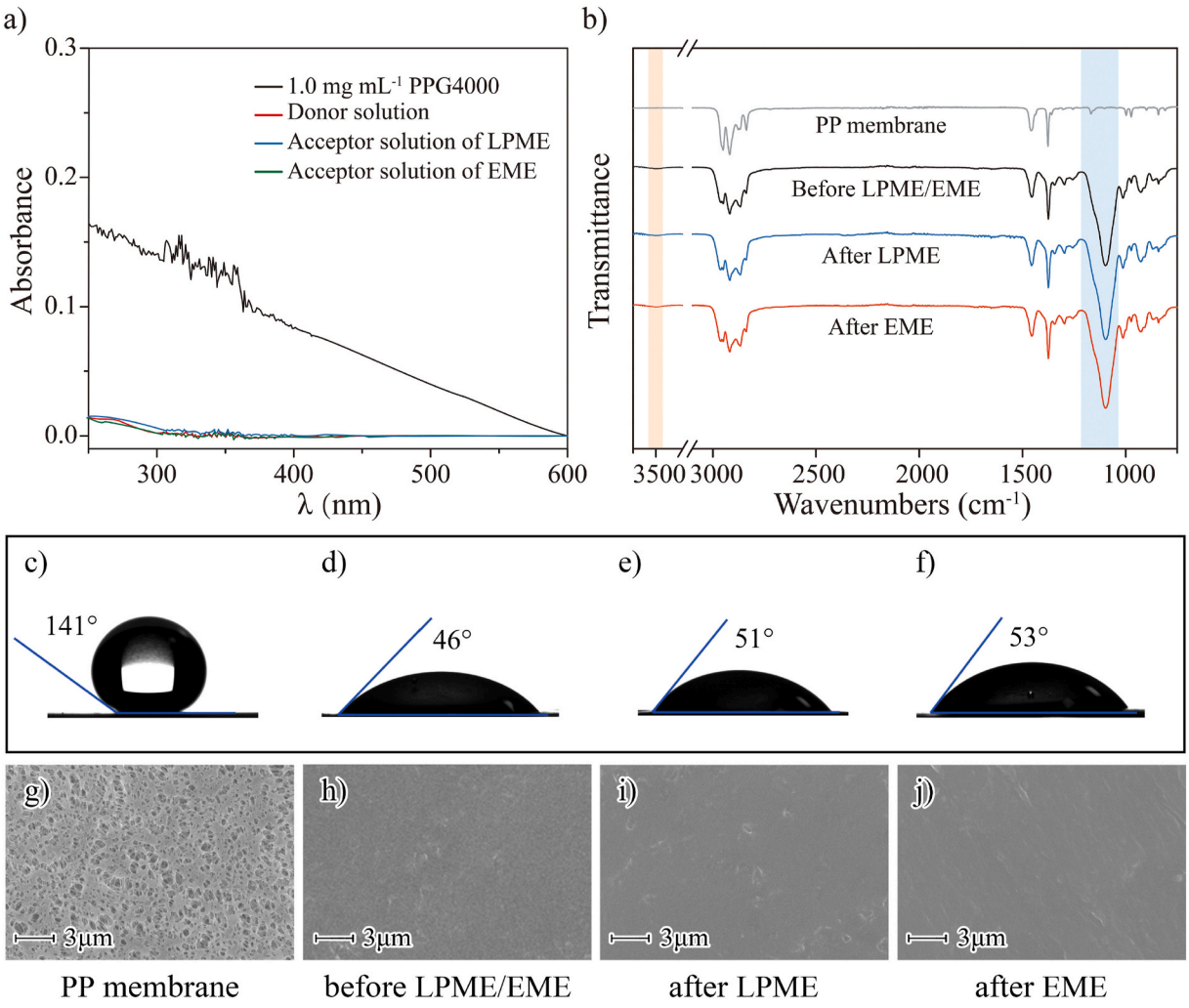


**Fig. 5.** Characterizations of the multi-system extraction based on the identical SsLMs. (a) UV–Vis spectra of 1.0 mg $mL^{-1}$ PPG4000, donor and acceptor solutions after LPME/EME; (b) FT-IR spectra of the blank PP membrane, SsLMs before and after LPME/EME; (c–j) Contact angles and SEM images of blank PP membrane, SsLMs before and after LPME/EME.

**Table 1**
The optimal parameters for SsLM based single LPME and single EME.

| Extraction method | Analytes | Donor phase | Acceptor phase | Time (min) | Voltage (V) | Temperature (°C) |
|---|---|---|---|---|---|---|
| LPME | NAP, FLB, DIC | 10 mM HCl | 10 mM NaOH | 30 | / | 60 |
| EME | HAL, FLU, SER | 10 mM TFA | 10 mM TFA | 20 | 100 | 25 |

carried out using 10 mM HCl and 10 mM TFA as background donor solutions, respectively. As presented in Fig. 6a, the average recoveries of acidic drugs (91% and 89%) had no clear difference whether 10 mM HCl or TFA was used, but the average recovery of basic drugs using 10 mM HCl (61%) was much lower than that in the latter condition (101%). In LPME unit, all the acidic drugs existed in neutral molecule forms in strong acidic environment and thus their partition efficiency and recoveries were similar when used the two donor solutions. While in EME for basic drugs, organic acid electrolytes in donor solution often provided higher extraction efficiency because TFA could act as an ion pair regent during the extraction [23]. Consequently, 10 mM TFA was used for the background donor solution in this study.

#### 3.3.2. Extraction time

According to the available data (Table 1), the optimal extraction time for single LPME and single EME were 30 min and 20 min, respectively. Based on the mass transfer kinetics of SLM based extraction, properly prolonging the extraction time could increase the recoveries of analytes in both LPME [31] and EME [4]. Nevertheless, it should be noted that the equilibrium time in EME is relatively short and excessive time might be adverse to the stability of extraction system because of the employment of electric field [32]. Here, extraction time of LPME/EME was further optimized to obtain the optimal combined performance of coextraction of acidic and basic drugs. As shown in Fig. 6b, the equilibrium time for LPME and EME systems were 30 min and 20 min, respectively. The results were in accordance with previous studies for single-extraction systems. But the recoveries of basic analytes in EME did not reduce when time increased up to 30 min. Therefore, 30 min was selected as the optimal extraction time for the SsLM based LPME/EME.

In summary, under the optimal extraction conditions (background donor solution: 10 mM TFA, acceptor solution of LPME: 10 mM NaOH, acceptor solution of EME: 10 mM TFA, extraction temperature: 60 °C, extraction time: 30 min, applied voltage for EME: 100 V), recoveries of 5 μg $mL^{-1}$ NAP, FLB, DIC, HAL, FLU and SER were 92%, 87%, 88%, 101%, 103% and 98%, respectively.

### 3.4. Coextraction of two groups of analytes with different concentrations

Generally, the concentration of target analytes in real samples varies in a wide range. To confirm the practical ability of SsLM based LPME/EME method, the coextraction of two groups of analytes with different concentrations were studied. In donor phase, the concentration of one group of drugs was kept as 1 μg $mL^{-1}$ and the other group was set as the three levels (0 μg $mL^{-1}$, 1 μg $mL^{-1}$ and 20 μg $mL^{-1}$). The LPME/EME was conducted under the optimal conditions. As shown in Fig. 7a, when the acidic drugs were kept at 1 μg $mL^{-1}$ in donor solution, their extraction recoveries did not be affected by the presence of basic drugs with different concentrations, and vice versa (Fig. 7b). This confirmed that the extraction of the two groups of analytes was not affected by each other even when they were at different concentrations. This also showed great potential of the SsLM based LPME/EME method in analysis of the complicated biological samples.

### 3.5. LPME/EME for coextraction of acidic and basic drugs from real samples

To further confirm the applicability of the SsLM based LPME/EME method in real samples, separation of the six model drugs from the spiked human urine and plasma samples was carried out. The collected biological samples were spiked with the six model drugs with the concentration of 5 μg $mL^{-1}$ (which was the concentration of the standard solution). Before extraction, the pH values of biological samples were adjusted to 2 using 1 M TFA. In this case, acidic drugs exited in molecular forms while basic drugs were protonated to positive ions in donor solution.

Under the optimal conditions for water samples, LPME/EME was initially conducted from the undiluted urine samples. As presented in Fig. 8, recoveries of NAP, FLB, DIC, HAL, FLU and SER were 94%, 86%, 85%, 70%, 74% and 75%, respectively. The average recovery (81%) was a little lower than that (95%) from water samples, which might be due to the presence of complicated matrix components in urine samples [33]. Interestingly, the average recovery of basic drugs had a pronounced reduction (from 101% to 73%) while that of acidic drugs was nearly unchanged (from 89% to 88%). This might be due to the fact that the matrix components increased the ion balance value ($\chi$, ratio of the total ions concentration in donor solution to that in acceptor solution), which could reduce the flux of drugs across the membrane in EME unit [34]. However, the ion balance had no obvious effect on LPME performance and even the mass transfer capacity in LPME increased with the increasing concentration of drugs in donor solution [35]. Nevertheless, the extraction recoveries have already been adequate and thus the operational conditions were not further be optimized. That was to say,

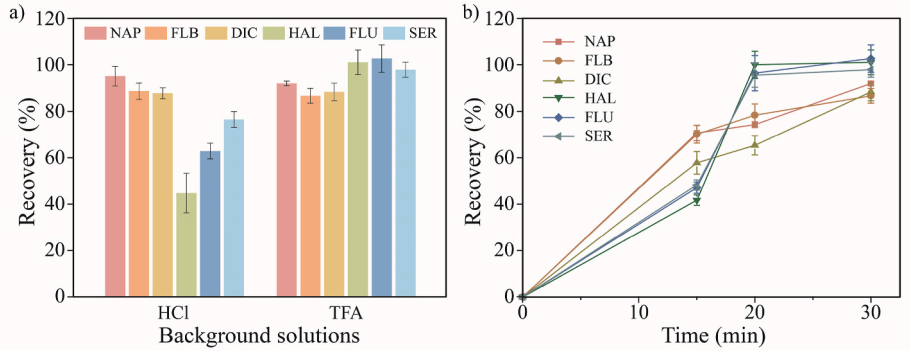


**Fig. 6.** Optimization of crucial parameters. (a) Effect of the composition of donor phase on the recoveries (acceptor solutions of LPME and EME: 10 mM NaOH and 10 mM TFA, 60 °C, 30 min, 100 V for EME); (b) Effect of the extraction time on the recoveries (acceptor solutions of LPME and EME: 10 mM NaOH and 10 mM TFA, background donor solution: 10 mM TFA; 60 °C, 100 V for EME).

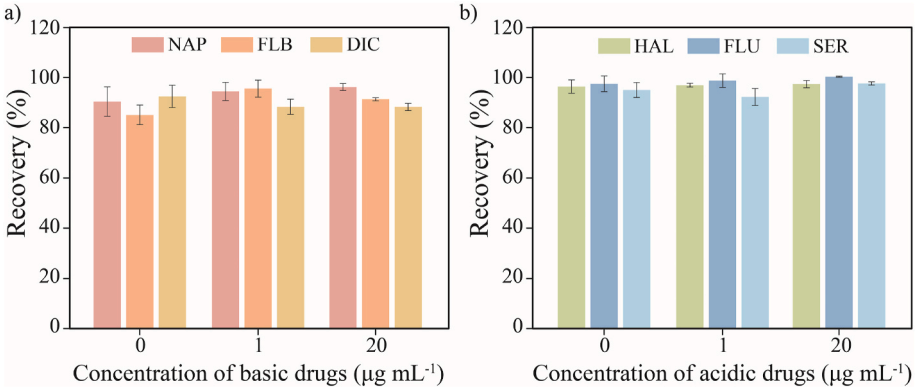


**Fig. 7.** Interference effects of concentration. (a) Effect of the basic drugs' concentration on recoveries of acidic drugs; (b) Effect of the acidic drugs' concentration on recoveries of basic drugs.

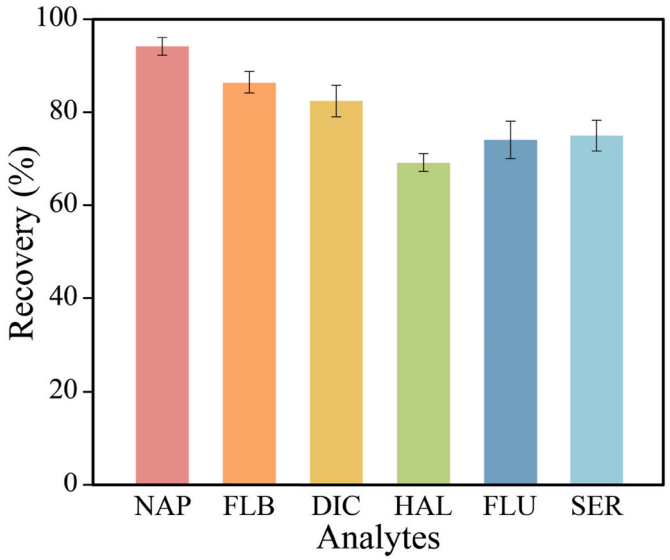


**Fig. 8.** Extraction recoveries of the six drugs from urine samples using LPME/EME.

the SsLM based LPME/EME method could be directly applied to undiluted urine samples and achieve satisfying extraction performance. Subsequently, LPME/EME was carried out from 3-fold diluted plasma samples for extracting the six drugs, acceptable recoveries (31%–72%) were obtained. This demonstrated that the SsLM based multi-extraction system performed well in coextraction of acidic and basic analytes from real biological samples.

### 3.6. Method evaluation

In this section, the SsLM based LPME/EME method combined with LC-MS/MS for analysis of acidic and basic drugs in urine samples was evaluated. LPME/EME was conducted under the optimal conditions using urine samples spiked with NAP, FLB, DIC, HAL, FLU, SER, GEM (internal standard for acidic drugs) and FLU-$d_5$ (internal standard for basic drugs). The concentration of model analytes in samples varies from 1 to 200 ng mL$^{-1}$, and the internal standards were kept at 10 ng mL$^{-1}$ in all urine samples. After LPME/EME, drugs in the acceptor solutions were analyzed by LC-MS/MS. The mass spectra and extracted ion chromatograms of the six drugs and two internal standards were presented in Fig. S3 and Fig. S4, respectively. The obtained results of method evaluation were presented in Table 2. Linear ranges of LPME/EME-LC-MS/MS for NAP, DIC and FLB, HAL, FLU, SER were 10–200 ng mL$^{-1}$ and 5–200 ng mL$^{-1}$ with $R^2 \geq 0.9971$, respectively. In literatures, two approaches could be used for calculating the LOQ value of the analytical method. Most of the reported LOQs were calculated by 10 S/N (signal to noise ratio) [13–15,17,18], which sometimes could not reflect the actual sensitivity of the method. Recently, a more reasonable LOQ has been established at the lowest concentration level with acceptable accuracy and precision (usually the low LOQ in spiking studies) using sample solution [36,37]. In this work, the second approach was used to obtain the LOQ values. It could be seen from Table 2 that LOQs were in the range of 5–10 ng mL$^{-1}$. Matrix effect was calculated according to equation (2) and ranged from −7% to 17%. The quantification accuracy (n = 4) of the LPME/EME-LC-MS/MS method for all analytes at four concentration levels ranged from 87% to 118%, which verified the applicability of the obtained calibration curves. Moreover, the precision (n = 4) for determining the six drugs was evaluated at four concentration levels and all the RSDs were below 9%, which demonstrated good repeatability of this analytical method. Accordingly, the evaluation data of LPME/EME-LC-MS/MS method from urine samples meet the demands for analysis of biological fluids.

## 4. Conclusions

In practical analysis of biological samples, coextraction of different groups of analytes is of great significance for saving sample volumes and simplifying analytical procedures. Conventional strategies usually achieved the coextraction by multi-extraction with two different SLMs, but the application of different membrane solvents would cause cross diffusion for distribution equilibrium, deteriorating the robustness of the multi-extraction system when it lasted a relatively long time. To completely avoid this problem, a stable LPME/EME set-up based on the identical SsLM was developed for coextraction of acidic and basic drugs in this study. The stability of the identical SsLM based multi-extraction system during the extraction process was investigated and compared to that of multi-extraction system using different SLMs. For the identical SsLM, the distribution of membrane solvent rapidly reached distribution equilibrium, which greatly reduced the diffusion motivation. Besides, the high molecular weight of PPG4000 also made it interpenetrate with the supported membrane more firmly. Followed by LC-MS/MS, the SsLM based LPME/EME method was successfully applied to the concurrent analysis of acidic and basic drugs in urine samples with high efficiency. Undoubtedly, the employment of identical SsLM in multi-extraction system enhanced the stability for coextraction of different groups of analytes, which improved its practical ability in analysis of complicated biological samples. We believe that the multi-extraction systems (e.g., LPME/EME, LPME/LPME and EME/EME) based on the same supported liquid membrane will be a trend in the future, and the stable and versatile SsLM could be the prior selection.

**Table 2**
Evaluation of the LPME/EME-LC-MS/MS method using spiked urine samples.

| Analytes | Concentration (ng $mL^{-1}$) | Linear range (ng $mL^{-1}$) | $R^2$ | LOQ (ng $mL^{-1}$) | ME±SD (%) | Accuracy (%, n = 4) | Precision (%, n = 4) |
|---|---|---|---|---|---|---|---|
| NAP | 10 | 10–200 | 0.9971 | 10 | −6±2 | 117 | 3 |
| | 20 | | | | | 104 | 8 |
| | 100 | | | | 5 ± 4 | 92 | 1 |
| | 200 | | | | | 101 | 7 |
| FLB | 5 | 5–200 | 0.9986 | 5 | | 118 | 3 |
| | 10 | | | | −2±6 | 88 | 8 |
| | 100 | | | | 7 ± 5 | 100 | 3 |
| | 200 | | | | | 105 | 9 |
| DIC | 10 | 10–200 | 0.9991 | 10 | −7±3 | 106 | 4 |
| | 20 | | | | | 95 | 2 |
| | 100 | | | | −4±6 | 104 | 1 |
| | 200 | | | | | 99 | 2 |
| HAL | 5 | 5–200 | 0.9998 | 5 | | 87 | 2 |
| | 10 | | | | 17 ± 2 | 99 | 4 |
| | 100 | | | | −3±6 | 102 | 1 |
| | 200 | | | | | 100 | 1 |
| FLU | 5 | 5–200 | 0.9999 | 5 | | 95 | 2 |
| | 10 | | | | 9 ± 8 | 99 | 3 |
| | 100 | | | | −4±2 | 101 | 1 |
| | 200 | | | | | 100 | 2 |
| SER | 5 | 5–200 | 0.9996 | 5 | | 118 | 2 |
| | 10 | | | | 15 ± 4 | 102 | 4 |
| | 100 | | | | 2 ± 4 | 97 | 1 |
| | 200 | | | | | 101 | 5 |

## Credit author statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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