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Electromembrane extraction using deep eutectic solvents as the liquid membrane

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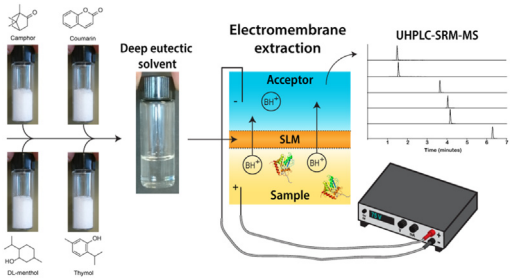
HIGHLIGHTS

- Natural deep eutectic solvents were used as SLM for EME for the first time.
- Coumarin and thymol-based SLMs were efficient for extraction most compounds.
- High extraction recoveries with good repeatability were obtained from human plasma.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we investigated for the first time hydrophobic deep eutectic solvents (DES) as supported liquid membrane (SLM) for electromembrane extraction (EME). Camphor, coumarin, DL-menthol, and thymol were used as non-ionic DES components. Different DESs compositions were tested, to study systematically the importance of hydrogen bonding and dispersion/aromatic interactions during mass transfer across the SLM. Unexpectedly, mixtures of coumarin and thymol were highly efficient SLMs, and provided exhaustive or near-exhaustive extraction of non-polar bases, non-polar acids, and polar bases. SLMs with such performance for both bases and acids, in a large polarity window, are not found in current literature. The SLMs were highly aromatic, very strong hydrogen bonding donors, and moderately strong hydrogen bonding acceptors. Aromatic (π type) interactions were apparently very important for transfer of bases, while hydrogen bonding were dominant for acids. EME of six polar basic drugs from plasma, with a coumarin and thymol mixture as SLM, and combined with UHPLC-MS/MS analysis, was evaluated to test the potential for analytical applications. Plasma was diluted 1:1 with phosphate buffer pH 2.0. Calibration curves were linear in the therapeutic ranges ($0.970 < R^2 < 0.999$), recoveries ranged between 47 and 93%, and repeatability was within 1.6–10.7% RSD. The clean-up efficiency was excellent and no matrix effects from plasma were seen. Presence of trace levels of coumarin in the acceptor phase was however found to cause some ion enhancement. Based on the current work, we foresee more research on the use of DES in EME.

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1. Introduction

In the modern world of analytical chemistry, the development of analytical instruments has improved sensitivity and selectivity dramatically. However, direct analysis of complex samples remains a challenge. Therefore, substantial research focuses on development of new and improved sample preparation procedures. This focus has particularly been directed towards miniaturization of extraction methods. The motivation has largely been a desire for more environmentally friendly and "green" methods, where smaller amounts of organic solvents and samples are required. Electromembrane extraction (EME) [1] is an example of a miniaturized sample preparation technique where only a few microliters of solvent is required. The solvent is immobilized in a porous membrane to make a supported liquid membrane (SLM), or alternatively as an unsupported free liquid membrane (FLM) [2]. The SLM is then used to separate a sample from a clean acceptor solution. Extraction is initiated by applying an electric field across the SLM to stimulate electrokinetic migration of charged analytes. EME is thus essentially electrophoresis across a hydrophobic membrane. Depending on the analytes of interest, EME can be tuned to provide high extraction selectivity. The main parameters are here the SLM solvent, and the polarity and magnitude of the applied voltage. As such, EME has been used for extraction of basic, acidic and zwitterionic analytes [3], including organic analytes covering a wide range of polarity with log P values from −5 to +5 [4–6], salts [7], heavy metals [8], and peptides [9–11], from biological fluids, food and environmental water samples.

Another trend towards greener sample preparation is the development of new extraction solvents as alternatives to toxic and volatile organic solvents [12,13]. Here ionic liquids (ILs) and deep eutectic solvents (DESs) are among the most promising ones. ILs are essentially molten salts composed of a bulky cation and a smaller anion. The size difference reduces the electrostatic interactions between the ions, and results in a relatively low melting point that for some ILs is below room temperature. The properties of ILs are dependent on the specific ions used, and may thus be tuned to suit the technical application and molecular interactions desired. ILs are thus considered as "designer solvents". However, they are generally known to suffer from problems with biodegradability, toxicity and cost of synthesis [14]. Additionally, many ILs are relatively viscous, which is unfavorable in terms of molecular diffusion. ILs have been used as SLM solvent in EME in two instances [15,16]. In both reports, the systems however had to be operated at a very low extraction voltage to avoid excessive electrolysis from high levels of current that destabilized the systems. The high current could be attributed to high conductivity of the IL SLM, a general characteristic of ILs that challenge their use in EME.

Deep eutectic solvents (DESs) are a class of solvents similar to ILs regarding properties and potential as designer solvents. DESs are however composed of two (or more) solid components that when mixed form hydrogen bonds with each other, in addition to weak dispersion forces. This implies that one should be a hydrogen bond donor (HBD) and the other a hydrogen bond acceptor (HBA). The hydrogen bonding action results in a major depression in the melting point of the components, which is dependent on the molar ratio. The molar ratio with the lowest melting point is called the deep eutectic point. Compared to ILs, DESs are reported to be more biodegradable, less toxic and cheaper to purchase [17]. Additionally, the ratio of DES components may be further optimized to obtain the properties desired, as long as the resulting melting point does not exceed ambient temperature during extraction. Due to these reasons, the scientific interest in DESs is currently increasing [17,18]. The first DES was presented in 2003 [19], and was based on choline chloride as HBA. This has remained a very popular component for DES preparation; however, the majority of these DESs have been relatively polar and hence water-miscible. The first hydrophobic (water-immiscible) DESs (HDESs) were presented in 2015 [20], and were composed of quaternary ammonium salts with long alkyl chains as HBA and fatty acids as HBD. Many of the subsequent reports on new HDESs have likewise used quaternary ammonium salts as HBA. These are characterized as ionic DESs. A subclass of DES are the natural deep eutectic solvents (NADES) that are composed of components of natural origin. These have additional benefits of being readily available at low cost, and highly biodegradable [21].

Until now, the majority of extraction applications with HDESs as the extraction phase has been with methods derived from dispersive liquid-liquid microextraction (DLLME) [17,22]. In EME, on the other hand, DESs have not been tested yet. EME using SLMs based on DESs may be highly interesting for safety and environmental reasons. In addition, molecular interactions can be very strong with DESs and this may open for enhanced extraction of polar and large-molecule substances. In the present fundamental paper, we therefore report the first example of EME with HDESs as SLM solvent. For this, we selected four components of natural origin (NADES) that previously have been reported to form DESs at room temperature, and performed a systematic evaluation of their suitability for extraction of acids and bases, from water and biological samples. The main purpose was to identify suitable solvents and investigate how a solvent should be designed to provide optimal extraction performance for different classes of analytes, with respect to specific molecular interactions. Thus, the paper involves fundamental research, and at this stage, it is not intended to provide fully validated methods. We foresee great potential in DESs as a future platform for intelligent design of green and inexpensive SLM solvents for EME.

2. Experimental

2.1. Chemicals and reagents

Formic acid, camphor, DL-menthol, coumarin, thymol, 4-nitroaniline, 2-nitrophenyl octyl ether (NPOE), 1-octanol, phosphoric acid, pethidine hydrochloride, papaverine hydrochloride, promethazine hydrochloride, verapamil hydrochloride, amitriptyline hydrochloride, perphenazine, prochlorperazine dimaleate, ketoprofen, naproxen, flurbiprofen, fenoprofen calcium salt hydrate, diclofenac sodium salt, ibuprofen, tyramine, atenolol, metaraminol bitartrate, ephedrine hydrochloride, and metoprolol tartrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was from Merck (Darmstadt, Germany), and water was purified by a Milli-Q water purification system (Molsheim, France). Nile red (99%, ACROS Organics™) was purchased from Fisher Scientific (Pittsburgh, PA, USA), and *N*,*N*-diethyl-4-nitroaniline was purchased from Fluorochem (Derbyshire, UK). Drug-free plasma was obtained from Oslo University Hospital (Oslo, Norway) and stored at −32 °C.

2.2. Preparation of deep eutectic solvents

DESs were synthesized by weighing appropriate amounts of each component into a 5 mL Eppendorf-tube. The amounts were adjusted to get the desired molar ratio of components. After weighing, the tube was capped and the mixture was heated in an 80 °C oven for approximately 15 min to assist the melting process. After melting, the mixture was vortexed for 10 s to ensure a homogenous liquid.

2.3. Determination of Kamlet-Taft solvatochromic parameters

Kamlet-Taft parameter values of prepared solvents were determined based on the use of solvatochromic probes, i.e. dyes dissolved in the solvent that are subject to shifts in the maximum absorbance wavelength depending on the properties of the solvent. The dyes, Nile red (NR), *N*,*N*-diethyl-4-nitroaniline (DENA), and 4-nitroaniline (4NA) were first dissolved individually in methanol at 100–200 μg/mL. From each solution, 10 μL was pipetted into the bottom of individual 2 mL Eppendorf tubes, and left to evaporate in a fume hood for at least 3 h. Then, 300 μL DES was pipetted into the tubes and the dyes were dissolved assisted by brief vortexing. The solutions were transferred to a quartz cuvette with 10 mm light path, and absorbance spectra (300–700 nm) were recorded with a UV–Vis spectrophotometer (DU520, Beckman, CA, US). Dye-free solvent served as blind sample. Calculations of α, β and π* values were performed according to the following equations [23], where λ_{max} is the wavelength of maximum absorption and ν is the wavenumber:

$$\nu = 1 \Big/ \left(\lambda_{max}\, 10^{-4}\right) \quad (1)$$

$$\alpha = (19.9657 - 1.0241\pi^{*} - \nu_{NR}) / 1.6078 \quad (2)$$

$$\beta = (1.035\nu_{DENA} + 2.64 - \nu_{4NA}) / 2.80 \quad (3)$$

$$\pi^{*} = 0.314(27.51 - \nu_{DENA}) \quad (4)$$

2.4. EME procedure

All EME experiments were performed in 96-well format that allowed high throughput. The equipment is shown in Fig. S1. The sample plate (laboratory built) was constructed of stainless steel with 96 wells each holding 100 μL. A commercially available 96-well MultiScreen-IP filter plate with polyvinylidene fluoride (PVDF) filter membranes of 0.45 μm pore size (Merck Millipore Ltd., Carrigtwohill, Ireland) served as the SLM support and held the acceptor solution. Prior to extraction, 100 μL of samples were loaded into the sample plate. At corresponding positions on the filter plate, 4 μL aliquots of solvent was pipetted onto of each filter to make the SLM. The filter plate was subsequently clamped with the sample plate, and the sample solutions came into contact with the SLM. 100 μL acceptor solution was pipetted into the filter plate in the reservoir above the SLM, and an aluminum lid (laboratory built) with 96 electrode rods was attached (electrode plate). The entire clamped 96-well device (sample plate, filter plate, and electrode plate) was placed onto a shaking board (Vibramax 100, Heidolph, Kellheim, Germany), and the sample and electrode plates were connected to a power supply (model ES 0300e0.45, Delta Elektronika BV, Zierikzee, Netherlands). Extraction was initiated by simultaneous application of voltage and 900 RPM shaking. The electrode plate was cathode for extraction of bases, and anode for extraction of acids. The extraction current was recorded using a Fluke 287 multi-meter (Everett, Washington, USA) at an acquisition rate of 8 Hz. When the extraction was terminated, the acceptor solutions were directly transferred for UHPLC analysis.

2.5. UHPLC-UV/MS methods

Multiple chromatographic methods were employed for quantitation. Details of each method are given in Supplementary information 2. All methods were performed on an UHPLC system (Dionex UltiMate 3000 RS, Thermo Scientific, San Jose, CA, USA) comprising a pump, an auto-sampler, and a temperature controlled column compartment. Detection was by UV in sections 3.1-3.4, and with an LTQ XL linear ion-trap mass spectrometer (Thermo Scientific, San Jose, CA, USA) in section 3.5. Mobile phases were composed of (A) 95:5 v/v purified water and methanol containing 20 mM formic acid, and (B) 5:95 v/v purified water and methanol containing 20 mM formic acid, and the column was an Acquity UPLC® HSS T3 column (100 × 2.1 mm ID, 1.8 μm, Waters, Wexford, Ireland).

2.6. Calculations

Recovery (R) was calculated according to equation (5) for each analyte:

$$R = \frac{n_{a,\ final}}{n_{s,\ initial}} \times 100\ \% = \frac{C_{a,final}}{C_{s,initial}} \times \frac{V_a}{V_s} \times 100\ \% \quad (5)$$

Here $n_{a,\ final}$ and $n_{s,\ initial}$ are the number of moles of analyte finally collected in the acceptor solution and the number of moles of analyte originally present in the sample, respectively. $C_{a,final}$ is the final concentration of analyte in the acceptor solution, $C_{s,initial}$ is the initial analyte concentration in the sample, V_a is the acceptor volume, and V_s is the sample volume. For all experiments, the sample and acceptor solution volumes were 100 μL.

Matrix effect (ME) represents the difference in signal due to ion suppression or enhancement effects. ME was calculated according to equation (6):

$$ME = \frac{AUC_{post-extraction\ spiked\ matrix}}{AUC_{standard}} \times 100\ \% \quad (6)$$

Here $AUC_{post-extraction\ spiked\ matrix}$ is the peak area of a blank matrix sample spiked after extraction, and $AUC_{standard}$ is the peak area of a pure standard solution at the same concentration.

3. Results and discussion

3.1. Selection of deep eutectic solvents for SLMs and model system

Solvents used as SLM in EME should be non-volatile and water immiscible, to maintain SLM integrity during extraction. Melting point should be well below room temperature, viscosity should be low for rapid diffusion and electro-kinetic migration, and conductivity should be low, to avoid excessive current upon application of the electrical field. For EME of non-polar bases, NPOE or related nitro aromatic solvents are preferred. These are with zero Kamlet-Taft α value, moderately high values for β and π*, and are aromatic. They are hypothesized to operate mainly based on hydrogen bonding interactions, where the SLM is HBA and the protonated analytes are HBDs. For non-polar acids, the typical SLM is 1-octanol. This solvent has relatively high values for α and β, and a moderately high π* value. Hydrogen bonding interactions are expected to be dominant, where the SLM is HBD and the deprotonated analytes are HBAs.

In the present work, we selected four non-ionic components forming hydrophobic deep eutectic solvents at room temperature [24,25]. Camphor and coumarin were selected as HBA components, while DL-menthol and thymol were selected as HBD components. Coumarin and thymol are aromatic, while camphor and menthol are non-aromatic. The chemical structures are shown in Fig. 1. The components are of natural origin, and the solvents may therefore be considered as natural deep eutectic solvents (NADES).

For each pair of HBA and HBD, mixtures were prepared in molar

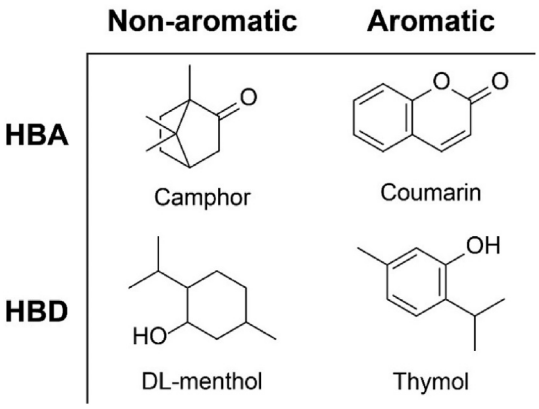

Fig. 1. Chemical structures of the selected DES components.

ratios of 2:1, 1:1, and 1:2 (HBA:HBD). The combinations forming a deep eutectic solvent are given in Table 1 (SLMs 1–11). The selected DES compositions were carefully designed to investigate interactions based on dispersion (cation-π-interactions and π-stacking) and hydrogen bonding. As such, SLMs 1 and 2 were non-aromatic, SLMs 3 to 8 were moderately aromatic, while SLMs 9 to 11 were highly aromatic. For each mixture, the molar ratio was varied to create DESs with different HBA versus HBD balance. Viscosity, density and melting point for the DESs have been reported previously [24,25]. Kamlet-Taft properties were determined experimentally in the present work, and these are summarized in Table 1. EME was performed with all 11 DESs, and performance was compared with NPOE and 1-octanol as typical reference SLMs for basic and acidic model analytes, respectively. Three different sets of model analytes were extracted. Pethidine, papaverine, promethazine, verapamil, amitriptyline, perphenazine and prochlorperazine were selected as representative non-polar basic drugs, while ketoprofen, naproxen, flurbiprofen, fenoprofen, diclofenac and ibuprofen were non-polar acidic model analytes (drug substances). The non-polar model analytes were within log P 2.5 to 5.0. Another set of drug substances, namely tyramine, metaraminol, sotalol, ephedrine, atenolol and metoprolol, were used as polar basic model analytes (log P −0.4 to +1.8). Chemical structures of all model analytes are provided in Fig. S2.

3.2. DES for extraction of non-polar bases and acids

For initial testing, four μL DES was applied as SLM. All DESs diffused into the PVDF membrane, and they were successfully immobilized by capillary forces. The extraction performance of each solvent was first tested for the non-polar analytes. 50 mM phosphoric acid pH 2.0 was used as sample and acceptor solution for extraction of bases, while 50 mM ammonium phosphate pH 9.0 was used for extraction of acids. These conditions provided complete ionization of the analytes. EME was conducted for 15 min with agitation at 900 rpm, and the results are shown in Fig. 2.

Table 1
SLM solvents selected for testing. Camphor:DL-menthol 2:1 mixture was not liquid at room temperature. The Kamlet-Taft properties were determined experimentally.

	Composition (molar ratio)	Aromaticity	α	β	π*
SLM 1	Camphor:DL-menthol 1:1	None	0.37	0.68	0.60
SLM 2	Camphor:DL-menthol 1:2		0.47	0.73	0.59
SLM 3	Camphor:thymol 2:1	Moderate	0.77	0.55	0.66
SLM 4	Camphor:thymol 1:1		0.94	0.51	0.72
SLM 5	Camphor:thymol 1:2		0.87	0.29	0.95
SLM 6	Menthol:thymol 2:1	Moderate	0.75	0.46	0.75
SLM 7	Menthol:thymol 1:1		0.74	0.34	0.89
SLM 8	Menthol:thymol 1:2		0.79	0.22	1.01
SLM 9	Coumarin:thymol 2:1	Very high	0.98	<0.67[a]	0.98
SLM 10	Coumarin:thymol 1:1		1.00	<0.62[a]	1.03
SLM 11	Coumarin:thymol 1:2		0.99	<0.53[a]	1.10
SLM 12	NPOE	High	~0.0[a]	High[a]	High[a]
SLM 13	1-octanol	None	0.66	0.83	0.57

[a] Exact values could not be determined due to high background absorbance of the solvents. Approximate values for NPOE have been discussed previously [26].

The experiments identified SLM1, SLM5, SLM9, SLM10, and SLM11 as highly efficient for EME of non-polar bases. They all provided average recoveries higher than 75% for the model analytes. Interestingly, they represented three different eutectic systems, and they differed substantially from the typical EME solvents in terms of Kamlet-Taft properties. SLM1 was non-aromatic, with low α and moderately high values for β and π*. We hypothesize this solvent principally operated based on hydrogen bonding and dipole interactions. SLM5 was moderately aromatic, with high values for α and π*, and with a relatively low β value. Due to the latter, this SLM probably operated based on dipole and π-type interactions. SLMs9-11 were mixtures of coumarin and thymol with high values for α and π*, and with moderately high β values. These SLMs operated principally based on dipole and π-type interactions. SLMs6-8 was inefficient and provided much less current than the other DESs, and we have currently no explanation for this. Interestingly, within each eutectic system relatively small changes in the balance between α, β, and π affected extraction efficiency substantially.

For EME of the non-polar acids, only SLM9, SLM10, and SLM11 provided high efficiency. Based on their high α values, this finding was expected and it is in agreement with previous work and current understanding of operational principles. SLMs1-2 were inefficient due to low α values, while SLMs3-5 were moderately efficient due to somewhat higher α values.

The mixtures of coumarin and thymol represent an important step forward, and they appear to be the first SLMs highly efficient for EME of both non-polar bases and acids. Such SLMs may simplify method development, and may open for simultaneous cationic and anionic extraction with a single SLM. Non-polar acids are transferred primarily based on hydrogen bonding interactions, while dipole and π-type interactions are more dominating for the transfer of non-polar bases. Recoveries for bases and acids were affected by the balance between coumarin and thymol, and this may be considered during method development. The coumarin and thymol composition 2:1 was prone to recrystallization in less than 24 h, but gentle heating and mixing reestablished the eutectic solvent.

3.3. Extraction of polar bases

In a new set of experiments, the same DESs were tested for EME of polar basic drugs in the log P range −0.4 to 1.8. The results are shown in Fig. 3.

As seen from the data, SLMs1-8 were inefficient, while SLMs9-11 provided high efficiency even for the polar basic drugs. The high efficiency of the mixtures of coumarin and thymol was attributed to strong dipole and π-type interactions. This is a very interesting observation, as all previous EME of polar bases have involved an ionic carrier in the SLM. Such systems, based on ionic interactions, are sensitive to high current and instability, and the use of non-ionic membranes such as SLM9-11 therefore represent an important step forward.

Among the six model analytes, the four with highest log P, namely sotalol, ephedrine, atenolol and metoprolol, were not sensitive to the ratio of coumarin and thymol. On the other hand, the two compounds with lowest log P (tyramine and metaraminol) were strongly affected by the SLM composition, and recoveries increased with increased content of coumarin. This may be

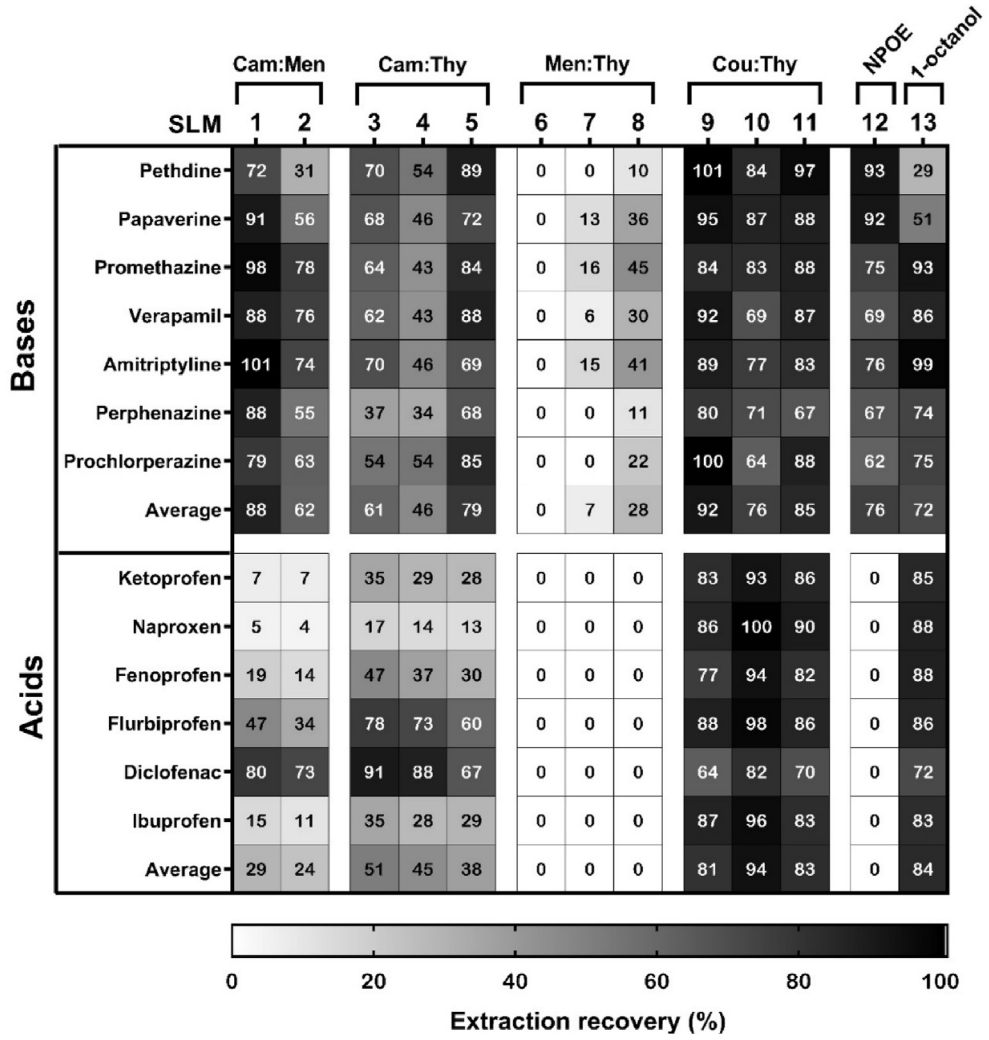

Fig. 2. Extraction recoveries (%) obtained after 15 min of extraction with different SLM compositions. All extraction were performed in triplicate. The voltage was 75 V for bases and 35 V for acids. The sample solution was 10 μg/mL for both acids and bases.

attributed to stronger π-type interactions with the conjugated system in coumarin, as well as increased HBA properties.

From the current experiments, SLMs of coumarin and thymol provided exhaustive or near-exhaustive extraction of bases in the range $-0.4 < \log P < 5.0$ (as well as acids in the range $2.5 < \log P < 5.0$). To the best of our knowledge, such capabilities have not been reported for any previous EME system.

3.4. SLM stability during extraction

In a next series of experiments, attention was focused on leakage of DESs into the sample and acceptor during EME. Samples and acceptor solutions were analyzed by LC-UV after EME. This enabled detection of traces of camphor, coumarin, and thymol. DL-menthol was not measured due to lack of UV absorbance. Leakage from SLMs1, 4, 9, and 11 is shown in Fig. 4.

Leakage was observed for all SLMs, corresponding to 2–8% loss of SLM. As illustrated in Fig. 2, SLMs with DL-menthol were less stable than with thymol, and increasing the molar ratio of thymol increased stability. This was likely due to the strong HBD properties of thymol. Thymol thus stabilized the DES. Leakage occurred both to the sample and acceptor solutions (data not shown); though fouling of the sample usually is of less concern. In general, leakage of the DES components was at the same level as expected for 1-octanol, which was calculated to approximately 6% based on previous experience [27]. We consider this acceptable since the current during EME was stable, and supported that the integrity of the SLM was maintained during extraction. In addition, the chromatographic peaks of DES components were separated from analyte peaks in the chromatograms. Future research should however investigate more hydrophobic DES components to reduce leakage to a minimum.

3.5. Evaluation of analytical performance and matrix effects from human plasma

Lastly, an initial test on data reliability was conducted, with DES-based EME from human plasma combined with UHPLC-MS. This was conducted with the polar base model analytes only, and should not be considered a complete validation. The latter was outside the scope of the current fundamental research. We selected the polar bases tyramine, metaraminol, sotalol, ephedrine, atenolol, and metoprolol as model analytes, since polar analytes are considered challenging and prone to poor extraction efficiency from complex samples [4,28]. Based on the results in Fig. 3, SLM 9 was chosen as the best solvent.

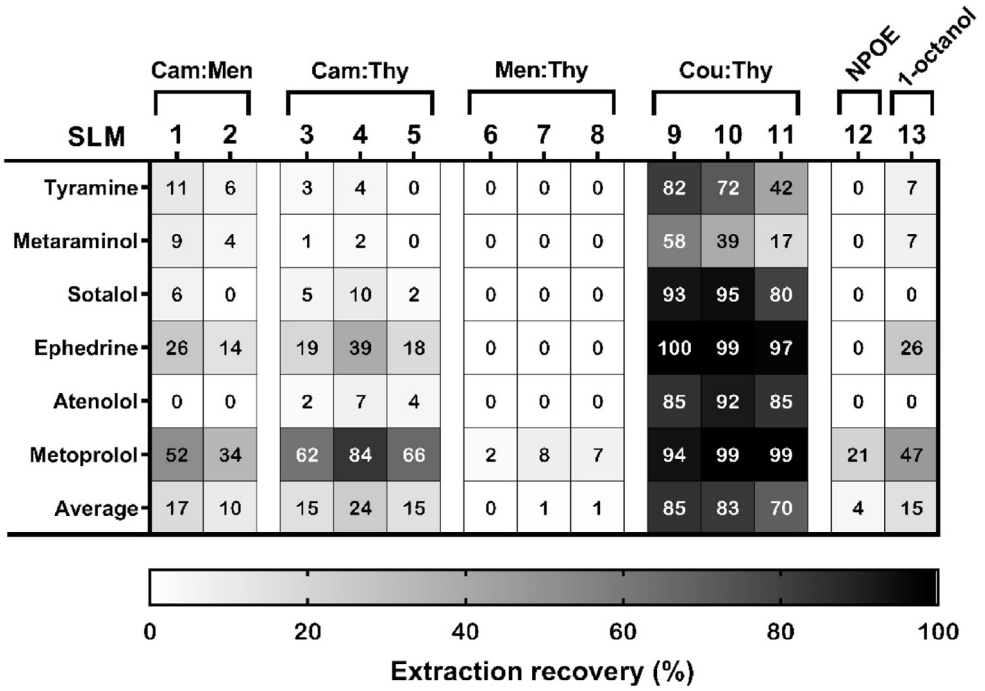

Fig. 3. Extraction recoveries (%) obtained after 15 min of extraction with different SLM compositions. All extraction were performed in triplicate. The voltage was 75 V, and the sample solution was 10 μg/mL in 50 mM phosphoric acid pH 2.0.

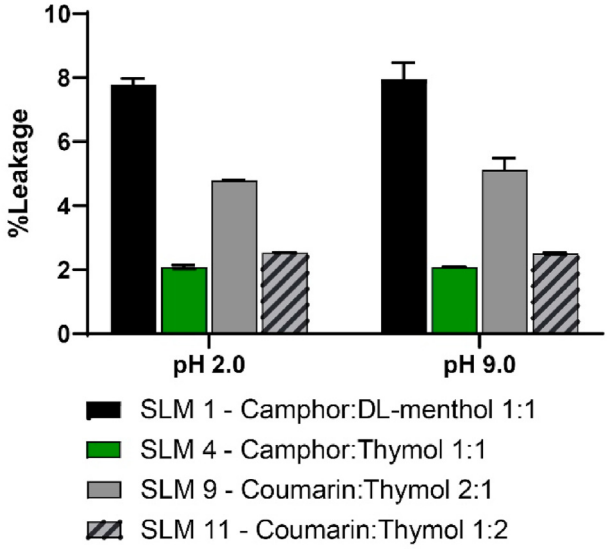

Fig. 4. Percentage leakage of selected SLMs during extraction at pH 2.0 and pH 9.0. Note that for SLM 1, the leakage was only based on data for camphor since DL-menthol could not be quantified. Error bars represent the standard deviation (n = 3).

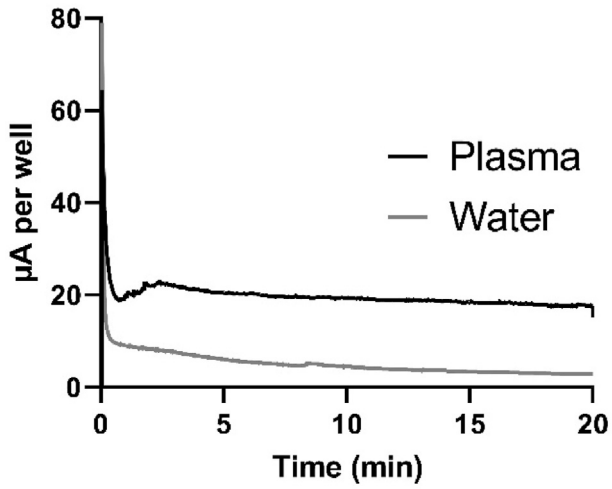

Fig. 5. Representative profiles of the extraction current during extraction from human plasma and buffered water samples. Initially, a spike is observed as the SLM acts like a charging capacitor, before the current settles onto a slowly decreasing level associated with electrophoretic transport.

Prior to extraction, human plasma samples were thawed, spiked and diluted 1:1 with 250 mM phosphoric acid to adjust pH to 2.0. Initially, the voltage and time was optimized for EME from plasma (Fig. S3 and Fig. S4). The highest recoveries were obtained at 75 V, whereas higher voltage lead to instability and reduced recovery. At 75 V, most analytes approached steady-state after 20 min, though slightly higher recoveries were obtained at 30 min. Kinetics were slower compared to pure buffered water samples, which is consistent with previous literature [29,30]. To maintain high throughput, the extraction time was set to 20 min. A representative current profile for plasma and buffered water samples is shown in Fig. 5.

With buffered water, the average current was 5.2 μA, while for plasma the average current was 19.5 μA. Though the current was higher for plasma, it was still well below 50 μA, which we normally recommend as limit EME [5]. The analytical evaluation data is provided in Table 2.

Linear calibration curves were obtained for all analytes with $R^2 > 0.970$. Extraction recoveries were in the range 47%–93% from plasma, and these were close to recoveries obtained from buffered water samples. This represents an improvement of extraction efficiency compared to previous literature [4,31–33]. The repeatability was acceptable with RSDs ranging from 1.6 to 10.7%, and no matrix effects were found. This indicated that the cleanup from plasma

Table 2
Evaluation data of polar bases extracted for 20 min at 75 V from human plasma diluted 1:1 with 250 mM phosphoric acid. Repeatability (RSD) and matrix effects (%ME) were evaluated at 50 ng mL^{-1}.

Analyte	Linear range (ng mL^{-1}) (n = 4)	R^2	Recovery	RSD (n = 6)	%ME ± SD (n = 4)
Tyramine	1–100	0.982	84%	6.9%	105 ± 6
Metaraminol	1–100	0.970	47%	10.7%	103 ± 2
Sotalol	0.05–75	0.999	86%	1.6%	101 ± 2
Ephedrine	0.05–75	0.996	93%	4.2%	101 ± 3
Atenolol	0.2–75	0.993	77%	5.9%	102 ± 1
Metoprolol	0.05–100	0.995	90%	3.9%	98 ± 2

was excellent. With the current LC-gradient, the DES components coumarin and thymol eluted after the analytes, and the DESs were therefore directed to the waste. A post-column infusion experiment was also performed to check for ion suppression caused by these components. For this, a blank plasma sample was extracted according to the same procedure as above, and injected onto the UHPLC-MS. A 5 μg/mL mixture of polar analytes was infused post-column via a T-union at 5 μL min^{-1} to 0.4 mL min^{-1} of mobile phase. The eluate was directed to the MS source during the entire gradient. Chromatograms are provided in Fig. S5. Except during the elution front (0.6 min), no ion suppression was observed during the gradient. Some ion enhancement was however observed when coumarin eluted (5.6 min), while the elution of thymol (approximately 13 min) did not produce any change in the signal. The DES was thus fully compatible with mass spectrometric detection, though the use of an internal standard is recommended. This represents a major benefit of the current DESs compared to ILs that tend to cause ion suppression [34,35]. Interestingly, despite that SLM 9 extracted near-exhaustively in a wide window of analyte polarity, the SLM still provided very low permeability for endogenous components in the plasma sample.

4. Conclusion

In the present work, the application of deep eutectic solvents for EME was studied for the first time. The four DES components tested were of natural origin, and the solvents belonged to the class of natural deep eutectic solvents (NADES). These are generally considered green, and are readily available and inexpensive. The nature of the eutectic mixtures made it possible to prepare solvents with different hydrogen bonding and dispersion/aromatic interactions capabilities. Unexpectedly, mixtures of coumarin and thymol were highly efficient SLMs, and provided exhaustive or near-exhaustive extraction of non-polar bases, non-polar acids, and polar bases. SLMs with such performance for both bases and acids, in a large polarity window, are not found in current literature. The SLMs were highly aromatic, very strong hydrogen bonding donors, and moderately strong hydrogen bonding acceptors. Aromatic (π type) interactions were apparently very important for extraction of bases, while hydrogen bonding were dominant for acids.

The current conceptual data and experiences are very important for several reasons. First, we demonstrate that DESs are well suited for fundamental studies of SLM properties. This may be very important for future development of new applications. Up to date, optimization of the SLM for new applications has been by trial and error type of experiments. However, using a limited number of DES, the ideal combination of molecular interactions may be derived fast and systematically. Second, the current work indicates that EME potentially can be operated with a single or a very few very general SLMs. This may be important for routine implementation of EME, where robust generic methods will be requested.

CRediT 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.11.044.

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