Effect of Aliquat®336 on supported liquid membrane on electromembrane extraction of non-steroidal anti-inflammatory drugs.

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Abstract

Up to now, most electromembrane extraction methods describe the use of pure organic solvents or mixtures as supported liquid membrane. However, the need to incorporate carriers in the supported liquid membrane to achieve the extraction of high polar compounds, seems to indicate that the presence of certain additives in the organic solvent may improve the extraction yield. For this reason, some studies have tried to enhance electrokinetic migration in different ways, modifying either the supported liquid membrane or even the donor solution. In this work, it has been studied and optimized an electromembrane extraction of five widely used non-steroidal anti-inflammatory drugs: salicylic acid, ketoprofen, naproxen, diclofenac and ibuprofen. The thickness and porosity of the support, the supported liquid membrane composition, the donor and acceptor phase pH, the voltage, the extraction time and the electrode configuration were optimized. supported liquid membrane was modified by adding different amounts of Aliquat®336, a cationic carrier commonly used in electromembrane extraction procedure for anionic compounds. The results compared with those obtained in the same extraction conditions using the pure organic solvent as supported liquid membrane, showed better extraction recoveries. The highest recoveries were achieved using a pH 5 donor phase and an acceptor phase at pH 12. The recoveries were within the range of 39 and 53% after 12 minutes extraction, using a voltage of 80V, a stirring speed of 400 rpm and 1-nonanol modified with Aliquat®336 2.5% (w/v) as support liquid membrane. Detection and quantitation limits were within 0.02-1.0 ng mL^{-1} and 0.05-3.0 ng mL^{-1}, respectively.

The selected analytes were extracted by electromembrane extraction using a home-made device designed with a flat configuration. The analyses were carried out by

high performance liquid chromatography with diode array and fluorescence detection and finally, applied to the analysis of human urine samples.

Keywords: electromembrane extraction; supported liquid membrane; cationic carrier; non-steroidal anti-inflammatory; urine samples

1. Introduction

Despite the outstanding evolution in analytical instrumentation in the last decades, especially in terms of efficiency, selectivity and sensitivity, several factors, such as the presence of interferences, matrix effects and incompatibility with analytical instruments prevent direct analysis of analytes at low or trace levels in complex samples [1, 2].

New sample treatment methodologies look for faster, simpler, more sensitive, selective, powerful and versatile [3-5] methods. In addition, many investigations have been focused on eliminating or minimizing the disadvantages related to traditional techniques [6]. In this sense, membrane-based microextraction methods have become in an interesting alternative because they allow to reach high selectivity and enrichment factors and the resulting extracts are usually compatible with analytical methods [1, 7].

One of the most popular membrane-based extraction techniques is electromembrane extraction, (EME), a liquid phase microextraction (LPME) that was proposed for the first time in 2006 by Pedersen-Bjergaard and Rasmussen for the extraction of charged analytes from aqueous samples [8]. One of the most interesting aspects is the control of the extraction selectivity depending on the nature of the SLM and the direction of the supplied electric field [8, 9], as well as, fast extraction simple and low-cost instrumentation, small sample volumes, minimization of organic solvents volumes, and an excellent clean-up even for complex biological and environmental matrices [10, 11].

Most EME systems employ a three-phase configuration consisting of a donor aqueous sample, a supported liquid membrane (SLM) that should be water-immiscible, and an acceptor aqueous solution. Two platinum wires, connected to an external power supply, are placed, respectively, in each aqueous solution. Charged analytes are extracted from donor to acceptor solution by applying an adequate potential difference between the electrodes. Aqueous solutions are separated by an organic water-

immiscible solvent that is immobilized in the pores of a polymeric support acting as SLM.

In SLM-EME, charged analytes are driven to the SLM interface by migration, where they move through the SLM by a diffusive mechanism and reach the acceptor phase by the reverse process at the internal side of the SLM.

The selection of the organic solvent is one of the key steps in EME, since chemical composition of the SLM determines, to a large extent, the extraction selectivity and efficiency [12, 13]. The solvent should have very low water-solubility, low viscosity to promote the mass transfer, low volatility to keep the stability of the liquid membrane, a minimal electrical conductivity, low toxicity, and an adequate affinity for the analytes to maximize the partition process from donor to acceptor solutions [9, 14, 15].

Most EME publications to the date describe the use of pure organic solvents or mixtures thereof as supported liquid membrane. However, the use of carriers or mixtures of organic solvents seems to indicate that the presence of additives in the SLM is the best way to improve the extraction yield due to an increase in SLM permeability, produced by a change in viscosity and a decrease in conductivity [16-22]. The use of dissolved carriers into the SLM, such us di-(2-ethylhexyl) phosphate (D2EHP) [19], tris(2-ethylhexyl) phosphate (TEHP) [20] and Aliquat®336 [21], amines such us hexadecyl trimethyl ammonium bromide (CTAB) [22] or some crown ethers such as 15-crown-5 ether and 18-crown-6 ether [23] have been reported. Some authors described even the use of a non-ionic carrier (bis(2-ethylhexyl) phosphite (DEHPi)) as a pure solvent for the extraction of polar basic analytes [24]. Also modified solvents containing solid additives such us carbon nanotubes (CNTs) [25], graphene nanosorbents [26], metal nanoparticles [27] and molecularly imprinted polymers [28], have been tested as SLM for the analysis of acidic and basic compounds (polar and non-polar) in wide variety of matrices.

Although carriers are generally employed to enhance the mass transfer of highly polar compounds (log P < 2.5), the present work aims to modify the SLM with a carrier to improve the extraction efficiency of a drug family that includes polar and non-polar compounds (log P > 2.5).

Five non-steroidal anti-inflammatory drugs (NSAIDs) were selected as model analytes: salicylic acid (SAC), ketoprofen (KTP) naproxen (NAX), diclofenac (DIC) and ibuprofen (IBU), owing to our previous experience in the application of different

extraction procedures to these compounds [29]. All of them are weak acids, derived from aromatic carboxylic acids with pKa values within 3 and 5. Table 1, shows the main chemical properties of the five selected NSAIDs as well as their molecular structure.

Most works related to EME of acidic substances describe the use of 1-octanol as the most efficient SLM. However, some authors point this solvent is not enough stable

as SLM and further research in the development of new and more stable liquid membranes is necessary [13]. Therefore, aliphatic alcohols with more than eight carbons were investigated and discussed in order to study their suitability as supported liquid membrane. On the other hand, to improve the extraction efficiency, SLM was modified by adding different amounts of Aliquat®336, a cationic carrier commonly used in EME procedure for anionic compounds [15, 30, 31]. The results were compared with those obtained in the same extraction conditions using the pure organic solvent as SLM, resulting better extraction recoveries for all compounds when the carrier was present in the organic medium. Finally, the optimized method was satisfactorily applied to the determination of the target analytes in human urine.

2. Experimental

2.1 Chemical and reagents

Non-steroidal anti-inflammatory drugs (salicylic acid (SAC), naproxen (NAX), ketoprofen (KTP), diclofenac (DIC) and ibuprofen (IBU)) and Aliquat®336 were purchased from Fluka-Sigma-Aldrich (Madrid, Spain). Sodium hydroxide, potassium hydroxide, 1-octanol, 1-nonanol, 1-decanol, 1-undecanol and 1-dodecanol were purchased from Merck (Darmstadt, Germany). All chemicals and reagents were of analytical grade.

Individual stock solutions of 500 mg L^{-1} of SAC, KTP, NAX, DIC and IBU were prepared in methanol and stored at 4ºC. Aqueous working solutions of NSAIDs were daily prepared by adequate dilutions from methanolic stock solutions using ultrapure water from Milli-Q Plus water purification system (Millipore, Billerica, MA, USA).

Tablets of IBU and SAC were obtained from Normon® and Bayer® laboratories, respectively.

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Porous polypropylene (PP) sheet membrane of 25 μm thickness, 55% porosity and 0.21 μm x 0.05 μm pores (Celgard 2500 micro-porous membrane) was purchase from Celgard (Charlotte, CN, USA). Porous polypropylene (PP) sheet membrane of 65 μm thickness, 30% porosity and 2.4 μm x 0.9 μm pores (Polypropylene membrane PP013001) was purchased from Sterlitech Corporation (Kent, WA, USA). Porous polypropylene (PP) sheet membrane of 100 μm thickness, 37% porosity and 0.84 μm x 0.5 μm pores (Accurel® PP 1E (R/P)) was purchased from Membrana (Wuppertal, Germany)

Platinum wire 0.25 mm 99.9% (metal basis) $\approx$ 1.05 g m^{-1} was purchased from Alfa Aesar® (Karlsruhe, Germany).

2.2 Electromembrane extraction set-up and procedure

EME was carried out as previously described by Aranda-Merino et al. [32] using a home-made device (Figure 1) consisting in a 2 mL screw-capped vial, with the base cut off, as acceptor phase compartment. A 10 mm × 10 mm piece of a polypropylene flat membrane was cut and placed into the vial cap that was screwed with an o-ring to prevent leaks. Then, the vial cap was immersed in the organic medium to impregnate the pores of the polypropylene support and the excess of solvent was removed using a medical wipe. Afterwards, the acceptor phase compartment was filled with 250 μL of an aqueous solution and immersed in 10 mL of aqueous donor solution containing the NSAIDs at a concentration of 1 mg L^{-1} each. Two platinum wires ending in a spiral shape were used as electrodes and placed in donor and acceptor solutions, respectively. The glass vial employed for housing the acceptor phase can be cleaned and reused as many times as needed.

NSAIDs were extracted from an aqueous donor solution (pH 5, not adjusted) to a pH 12 (10 mM NaOH) aqueous acceptor solution applying a potential difference of 80 V for 12 min with a laboratory DC power supply (Benchtop Instrument, Pennsylvania, USA) with programmable voltage within the range of 1-120 V. Donor solution was continuously stirred at 400 rpm and 1-nonanol modified with Aliquat®336 2.5% (w/v) was used as SLM. The system-current was monitored (every 0.5 s) using a digital multimeter connected in serial. Once the extraction was finished, the acceptor phase was collected with a Hamilton® Gastight® syringe and directly injected into the HPLC for analysis.

2.3 Chromatographic conditions

Analytes were determined using an Agilent Technologies 1100 series liquid chromatograph (Palo Alto, CA, USA) with a diode array (DAD) and a fluorescence detector connected in series mode.

NSAIDs were separated using a Hibar® 100–4.6 Purosphere® STAR RP-18e 3 µm particle size (100 mm × 4.6 mm i.d.) (VWR, Darmstadt, Germany) chromatographic column preceded by a guard column Kromasil® 100 Å pore size, C18, 5 µm particle size (15 mm × 4.6 mm i.d.) (Scharlab S.L., Barcelona, Spain). The column was thermostated at 25 °C.

Formic acid aqueous solution 0.1% (v/v) (component A) and acetonitrile (component B) were used as mobile phase at a flow rate of 0.8 mL min^{-1}, in gradient elution mode applying the following program: an initial composition of 55% component A and 45% component B was programmed in isocratic mode for 5 minutes and then linear elution gradient was programmed from 55% to 100% for component B for 5 min more. Finally, 2 min at 100% B isocratic elution was applied. The chromatographic separation was completed in 12 min. KTP and DIC were measured with DAD and SAC, NAX and IBU with FLD [32].

2.4 Calculations

Extraction efficiency was analysed according to the extraction recovery ($R(\%)$). This parameter is defined as the percentage of the moles number of target analytes extracted into the acceptor phase ($n_{f,a}$) respected to the moles number of the same analytes present in the donor solution at the beginning of the extraction procedure ($n_{i,d}$). The following equation (Eq. 1) was employed for recoveries calculations:

$$R\ (\%) = \frac{n_{f,a}}{n_{i,d}}\ x\ 100 = \frac{C_{f,a}\ x\ v_a}{C_{i,s}\ x\ v_d}\ x\ 100 \qquad (1)$$

where $C_{f,a}$ is the final analyte concentration in the acceptor phase, $C_{i,d}$ is the initial analyte concentration in the donor phase, v_a is the acceptor phase volume, and v_d is the donor phase volume.

3. Results and discussion

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3.1 Preliminary studies about system stability

The assays were performed using a classical hollow fiber device (HF-EME) and a home-made device designed for flat membrane supports (FM-EME) at the same operational conditions described by Aranda-Merino et al. [32].

For HF-EME device, the presence of Aliquat®336 in the SLM (1-octanol) provoked an uncontrolled rise in the system current during the extraction, with average values higher than 2 mA. This fact caused an important increase in the electrolytic processes, generating an excess of H^+ and OH^- ions and therefore drastic pH changes in donor and acceptor solutions. Consequently, the system lost stability and the analytes did not extract. In order to reduce the electric current and stabilize the extraction procedure, some assays were conducted at lower voltages using the same amounts of carrier in the liquid membrane, but results did not show improvements so HF-EME device was discarded in this study.

Preliminary assays were also performed using the FM-EME device to check if the presence of the carrier in the organic solvent improved the extraction efficiency obtaining favourable results so FM-EME device was selected for subsequent studies.

Taking into account Aliquat®336 addition and carrier concentration raise the electrical conductivity of the SLM, some additional parameters were investigated and discussed to ensure the system stability. As reported in literature, EME procedures present some limitations that contribute to decrease the extraction yield. These drawbacks derived from several sources and are normally attributed to physicochemical processes, such us instability problems, high electrical resistance or double electrical layers formation around the SLM [33]. The additional parameters examined were the support thickness and porosity, the geometry of the electrodes and the organic solvent used as SLM.

3.1.1 Effect of the support thickness and porosity

The influence of the support thickness and porosity on the mass transfer was evaluated. Recently, some authors [34, 35] have described how electrokinetic migration of analytes in EME could be affected by the physical properties of the material employed to immobilize the organic solvent.

Three different flat polypropylene sheets were compared applying the optimal conditions described in Aranda-Merino et al. [32]: a) a 25 μm thick sheet with a pore size of 0.21 μm x 0.05 μm and 55% porosity, (b) a 65 μm thick sheet with a pore size of 2.4 μm x 0.9 μm and a porosity of 30% and (c) a 100 μm thick sheet with a pore size of 0.84 μm x 0.5 μm and a porosity of 37%. The sheets were characterized in terms of thickness and porosity by scanning electron microscopy (SEM) using a TENEO high-resolution scanning electron microscope (FEI, Spain). The scan images corresponding to each support are shown in Figure S1 (included in supplementary materials). The thickness was provided by the scanning electron microscope software, while the porosity percentage and the pore size were determined using the free imaging processing software FIJI-ImageJ.

Obtained results (Figure 2), show the extraction efficiency decreased when support thickness increase. 25 μm and 65 μm flat sheets gave similar results in terms of recoveries, being slightly higher the recoveries with 25 μm support. As can be seen, it is especially noticeable a decrease in extraction recoveries (4-10%) for the selected NSAIDs with the 100 μm support. A priori, it could be assumed that the differences in the recoveries could be due to the different porosities of the supports used, however, it seems that it is the thickness of the support what significantly influences the extraction performance, since the supports of 100 μm and 65 μm have very similar porosities and considerably different results were obtained. This behaviour could be explained considering that electrokinetic migration resistance is higher when thicker supports are employed, because the migration path and, consequently, the diffusion distance to the acceptor phase, rise and as a result, a significant decreased in the mass transfer is observed [33, 34].

3.1.2 Effect of the electrode configuration

Some authors have investigated the use of different shapes and geometries to enhance the analytes migration. Thus, Asl et al. [36], described the use of a cylindrical outer electrode around the hollow fiber, Moazami et al. [37] investigated how the electrode geometry affects to EME performance using three different shapes for the outer electrode, Asadi al. [38] introduced a rotating electrode in the acceptor phase to increase the mass

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transfer and more recently, Moazami [39] proposed the use of a round-headed platinum wire as inner electrode.

Therefore, to evaluate if electrode geometry affect to system stability and analytes migration, four different configurations were investigated (Figure 3), maintaining the platinum wire thickness at 0.25 mm.

As shown in Table 2, recoveries achieved for each analyte did not differ significantly (less than 5%). Previously studies, described above, reported an influence of electrode configurations in EME systems [36-39], however the results obtained in our study seemed to be independent on electrode configuration.

3.1.3 Linear alcohols as supported liquid membrane

The composition of the liquid membrane has an important role on mass transfer and selectivity in EME technique. Chemical properties of the SLM also determine, to a large extend, the electric current generated during the extraction process which is important in order to ensure the stability of the system [14]. Thus, selection of an adequate organic solvent is essential to achieve a selective, reproducible and efficient extraction procedure [13]. According to literature, the organic solvent used as SLM will depend on the nature of the target analytes and can be selected, to some extent, in terms of Kamlet and Taft solvatochromic parameters: α, β and π* [40, 41]. For the extraction of acidic compounds an adequate organic solvent should present high hydrogen bond acidity (α), low hydrogen bond basicity (β) and a moderate dipolarity-polarizability (π*) [15], being long-chain aliphatic alcohols the most suitable organic solvents. Aliphatic alcohols with more than eight carbons have been poorly studied, for this reason, in addition to 1-octanol, other homologues such as 1-nonanol, 1-decanol, 1-undecanol and 1-dodecanol were tested.

The experiments revealed that extraction recoveries decrease with chain length (Figure 4). 1-decanol, 1-undecanol and 1-dodecanol were less efficient as SLM since the results decreased in more than a 50% respect to 1-octanol. In addition, extractions with 1-undecanol and 1-dodecanol were barely reproducible. This behaviour might be attributed to their relatively high viscosity and a less affinity analyte-solvent, which results in a lower flow from donor to acceptor solution [17].

In accordance with the results depicted in figure 4, 1-octanol and 1-nonanol showed the better results. Some authors have reported that 1-nonanol improve the

system stability due to its lower water solubility [38]. Thereby, considering 1-nonanol average current was almost five times lower (300 µA 1-octanol vs 60 µA 1-nonanol) and the recoveries were quite close to 1-octanol, both solvents were selected for further optimization.

3.2 Modification of the supported liquid membrane using a cationic carrier. Influence of carrier concentration

Once optimized thickness and porosity of the support, electrode configuration and alcohol used as supported liquid membrane, the presence of a carrier dissolved into the SLM was studied. Considering the acidic nature of these analytes, a cationic carrier, Aliquat®336, was selected. To evaluate the influence of carrier concentration in EME performance, different amounts of Aliquat®336 were dissolved in 1-octanol and 1-nonanol.

For 1-octanol, carrier concentrations between 0.1-1% (w/v) were tested. The obtained results are shown in Figure 5. Compared to pure 1-octanol extraction efficiency improves at nearly all tested concentrations of Aliquat®336. Best results were obtained at carrier concentration of 0.25% (w/v) for SAC, NAX, DIC and IBU and 0.5% (w/v) for KTP and recoveries improved within 22% (DIC) and 60% (IBU) respect to the obtained with unmodified 1-octanol.

The use of carriers can increase the current generated when a difference of potential is applied. 1-nonanol allows higher percentages of Aliquat®336, ranging 0.5-7.5% (w/v) since it generated lower average currents than 1-octanol. As illustrated in Figure 6, all tested concentrations provided better extraction efficiency than the pure solvent. Recovery percentages increased for all compounds when carrier was used, and best results were achieved with 2.5% (w/v) Aliquat®336. Higher carrier concentration produced a decrease in extraction efficiency.

As can be seen in figure 7, the modification of 1-octanol and 1-nonanol with Aliquat®336 led to an improvement between 105% and 150% respect to 1-nonanol and between 56% and 96% respect to 1-octanol. These values show that the presence of a cationic carrier in the liquid membrane has a positive effect in the extraction of anionic compounds.

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Additionally, it can be also observed that, 1-nonanol pure solvent did not improved the extraction efficiency with respect to 1-octanol, however, the presence of Aliquat®336 in the SLM with 1-nonanol enhanced the extraction efficiency up to a56% and an approximately a 10% respecting 1-octanol with Aliquat®336.

Thereby, FM-EME set-up optimal conditions were finally established as follows: a donor phase pH 5 (pH value of 10 mL solution containing all NSAIDs at 1 µg mL^{-1}), pH 12 (NaOH 10 mM) for acceptor phase, applying a voltage of 80 volts for 12 minutes stirring at 400 rpm and 1-nonanol + 2.5% (w/v) Aliquat®336 as SLM. Under these conditions, average current recorded was 160 µA and recoveries for each analyte were 53% for SAC, 45% for KTP, 42% for NAX, 39% for DIC and 46% for IBU.

3.3 Validation of EME proposed procedure

Once optimal operational conditions were established, the proposed method was validated. Linearity, sensitivity, precision and accuracy were selected as quality parameters.

Linearity was evaluated using a ten-point (in triplicate) external calibration curve constructed using a least-square linear regression analysis. Standard mixtures of NSAIDs at concentrations within 0.05 and 1000 ng mL^{-1} were submitted to the proposed EME procedure. As can be seen, good linearity was obtained for all analytes with values ranging between 97.9% and 99.4% and regression coefficients $r^2 > 0.998$. Detection and quantification limits (LOD and LOQ) were calculated as the minimum concentration of analytes whose signal noise ratio was three and ten respectively [42]. LODs range within 0.02 ng mL^{-1} for NAX and 1.0 ng mL^{-1} for DIC and LOQs within 0.05 ng mL^{-1} for NAX and 3.0 ng mL^{-1} for DIC.

To determine repeatability and intermediate precision, aqueous standard solutions at three concentration levels (10, 100 and 500 ng mL^{-1}) were subjected, in triplicate, to the extraction procedure and measured in one single day and one day per week during two months, respectively. Relative standard deviation (% RSD) values were in the range 2-9% for repeatability and 5-10% for intermediate precision, respectively. Validation data are summarized in Table 3.

Accuracy was evaluated by recovery assays in blank urine samples collected from healthy volunteers. Fortified urine (10, 100 and 500 ng mL^{-1} final concentration) was microfiltered (0.22 µm), diluted with ultrapure water (1:100) and submitted to the EME

procedure described in section 2.2. Method recoveries were calculated as percentage of extracted compound, resulting values above 78% with relative standard deviation percentages less than 2.5% and no significant differences between the three concentrations spiked levels were found (Table 4).

Therefore, according to the obtained results for the different quality parameters evaluated, the suitability of the proposed EME procedure has been proved to human urine analysis. The results obtained show an improvement in sensitivity with respect to liquid membrane with pure solvent, especially significant in the case of KTP, NAX and DIC. Compared with the LOD and LOQ obtained using other supports such as HF, the results of this work improve for KTP and NAX [32]. On the other hand, the extraction recoveries are significantly higher than those previously reported for FM-EME and HF-EME of NSAIDs [29, 32]. Regarding the linear range, the proposed procedure represents a significant improvement as it increased up to one order of magnitude.

3.4 Application to human urine samples

Urine samples from patients under medical treatment were collected 2 hours after the ingestion of an oral dose of 500 mg tablet of acetyl salicylic acid (ASA) (Bayer®) and 8 hours after the ingestion of 600 mg tablet of IBU (Normon®), respectively and conveniently stored at 4ºC until analysis.

Samples were microfiltered (0.22 µm), diluted with ultrapure water (1:100) and subjected, in triplicate, to the proposed EME procedure before injection into the HPLC-DAD/FLD. Figure 8 shows the chromatograms corresponding to: (a) blank human urine, (b) human urine sample spiked with all NSAIDs at (100 ng mL^{-1}), (c) urine sample collected after 2 hours of the ingestion of 500 mg of ASA and (d) urine sample collected after 8 hours of the ingestion of 600 mg of IBU. As can be seen, all chromatograms have a good baseline as well as peaks with good resolution for all compounds. Furthermore, no interfering additional peaks corresponding to other possible compounds present in urine samples were observed, which reveal the excellent method selectivity and clean-up.

Once ingested, acetyl acetylsalicylic acid (ASA) is hydrolysed to salicylic acid (SAC), its main active metabolite. Salicylic acid is also pharmacologically active and has analgesic, anti-inflammatory and antipyretic effects. Aspirin is metabolized to SAC and other metabolites that are eliminated via kidneys, being mainly excreted in the

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urine. Approximately 75% is in the form of SAC, 15% is in the form of mono- and di-glucuronic conjugates, and the 10% remaining are free salicylates [43]. ASA is completely excreted between 4-6 hours after its intake. Concentration of SAC found in the analysed urine sample was 59 ± 2 μg mL^{-1}. This concentration is in accordance with metabolisation process of ASA taking into account sample was collected before total excretion.

On the other hand, IBU is metabolised in the liver and mainly eliminated via renal. Its elimination is considered complete after 24 hours. Approximately a 90% is excreted in the urine as metabolites or some of their complex forms and a 10% is excreted in its original in a period of 6-8 hours after the ingestion [44]. To ibuprofen analysis, urine samples were collected after complete excretion (8 hours) and an accordance concentration of 29 ± 1 μg mL^{-1} was found.

Therefore, obtained results indicate the proposed electrokinetic extraction procedure using 1-nonanol modified with 2.5% (w/v) Aliquat®336 as SLM in a static EME system that employs flat polypropylene sheets as support for the liquid membrane (FM-EME), can be successfully applied for the analysis of non-steroidal anti-inflammatory drugs in human urine samples.

4. Conclusions

The results obtained in this work indicate that the porosity of the support does not affects to a great extend to the extraction efficiency, however, its thickness significantly influences the extraction performance decreasing when support thickness increase. On the other hand, although previous studies have reported the influence of electrode configuration on EME systems, the results obtained in our study seem to be independent on electrode configuration.

Respecting the aliphatic alcohols as SLM, the experiments revealed that extraction recoveries decrease with chain length. 1-decanol, 1-undecanol and 1-dodecanol were less efficient and reproducible than 1-octanol and 1-nonanol, since their relatively high viscosity and low affinity for analyte, result in a lower flow from donor to acceptor solution.

Additionally, the presence of Aliquat®336 in the organic solvent allowed increasing the solubility of the NSAIDs and accordingly the mass transfer thanks to the

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carrier's ability to form ionic pairs with the analytes. Therefore, the use of 1-nonanol with 2.5% (w/v) of Aliquat®336 as modifier of SLM has demonstrated to obtain better sensibility and higher extraction recoveries respect to pure solvents. 1-nonanol pure solvent did not improved the extraction efficiency with respect to 1-octanol, however, the presence of Aliquat®336 in the SLM with 1-nonanol enhanced the extraction efficiency respecting 1-octanol with Aliquat®336. Finally, the proposed method has been successfully applied for the analysis of non-steroidal anti-inflammatory drugs in human urine samples.

Compliance with ethical standards

All urine samples were obtained from volunteers from whom informed consent was obtained.

Acknowledgements

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Figure Captions

Figure 1. Experimental device for EME procedure using flat sheets as support for the liquid membrane.

Figure 2. Influence of the support thickness and porosity % RSD < 10%.

Figure 3. Schematic illustration of the different electrode configurations evaluated. (a.) Two platinum wires ending in spiral shape, (b.) two platinum wires ending in right angle, (c.) inner electrode in spiral shape and outer electrode in right angle, (d.) inner electrode in right angle and outer electrode in spiral shape.

Figure 4. Extraction recoveries obtained using long-chain aliphatic alcohol as SLM. % RSD < 10%.

Figure 5. Influence of the carrier concentration dissolved in 1-octanol. % RSD < 9%.

Figure 6. Influence of the carrier concentration dissolved in 1-nonanol. % RSD < 8%.

Figure 7. Recoveries at optimal conditions in pure solvents and solvents with Aliquat®336. % RSD < 8%.

Figure 8. Chromatograms corresponding to: (a) blank human urine, (b) human urine sample spiked with all NSAIDs at (100 ng mL^{-1}), (c) urine sample collected after 2 hours of the ingestion of 500 mg of ASA and (d) urine sample collected after 8 hours of the ingestion of 600 mg of IBU.

Figure 1

Figure 2

Figure 2

Figure 3

Figure 3

(a).   (b).   (c).   (d).

Figure 4

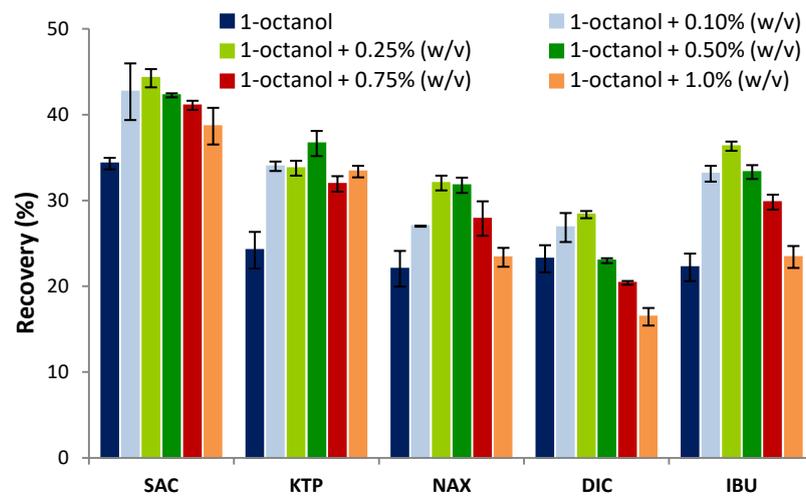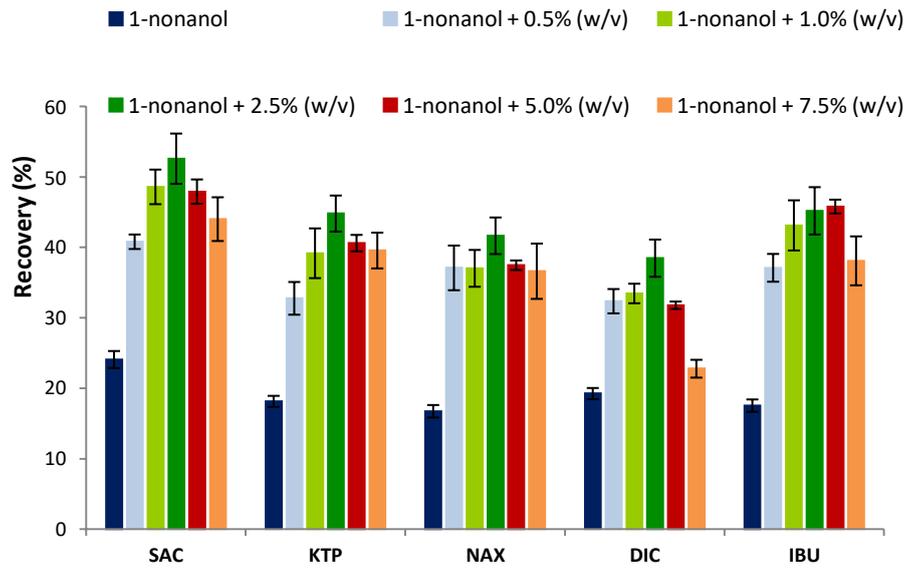Click here to access/download;Figure;Figure 4.pdf ±
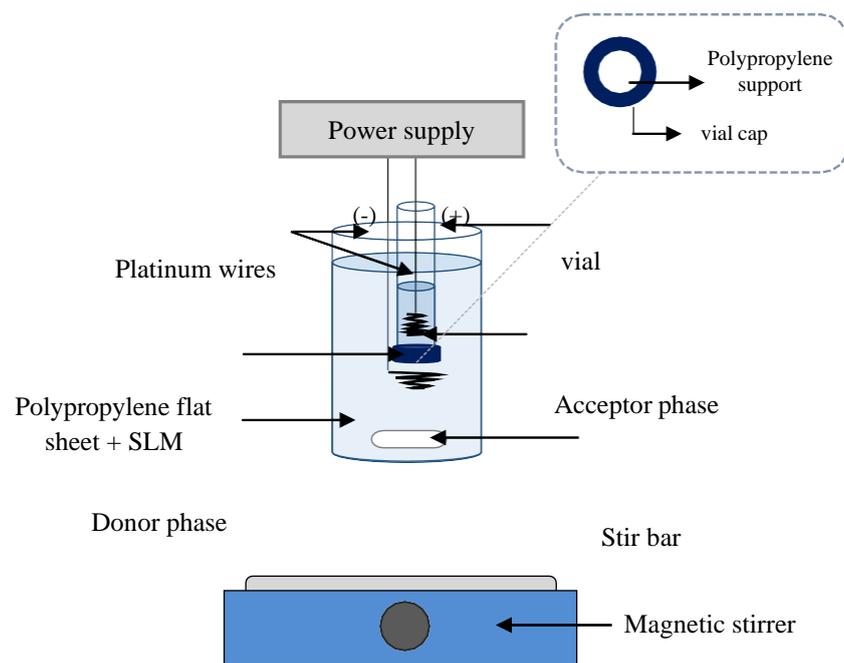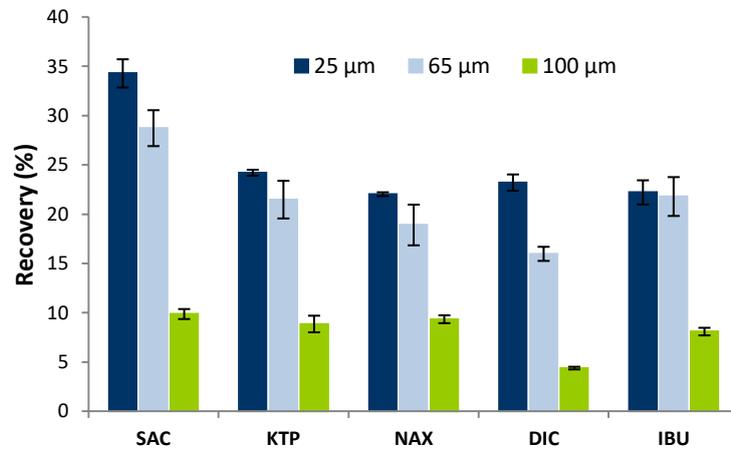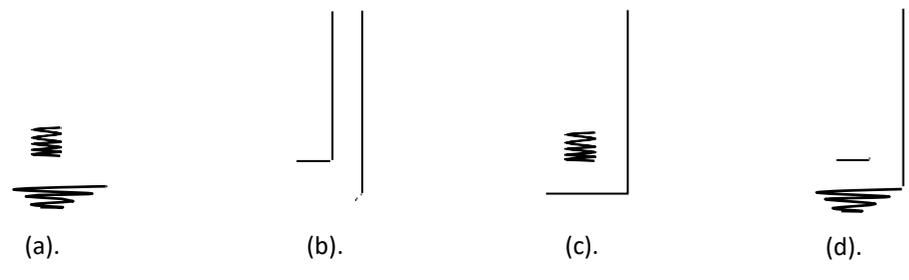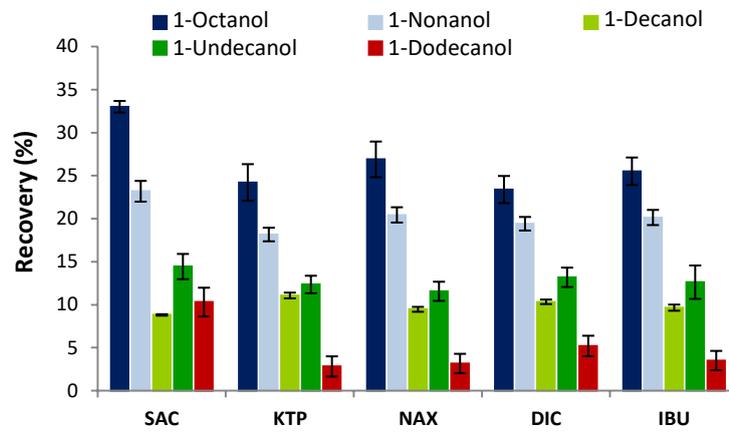
Figure 4

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Figure 8

Figure 8

Table 1

Click here to access/download;Table;Table 1.docx ☒
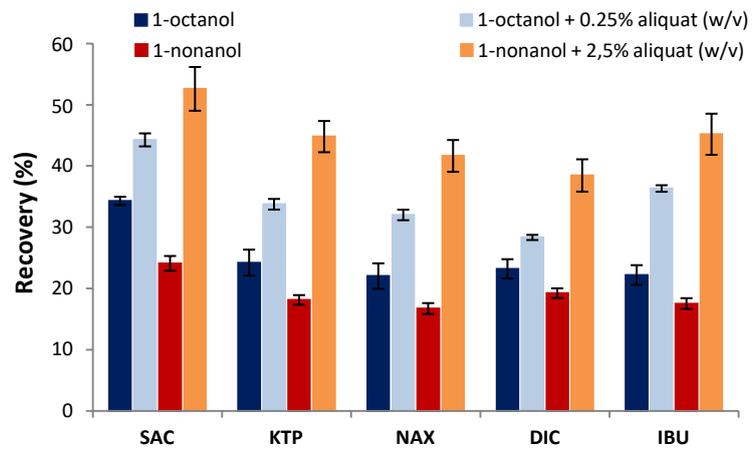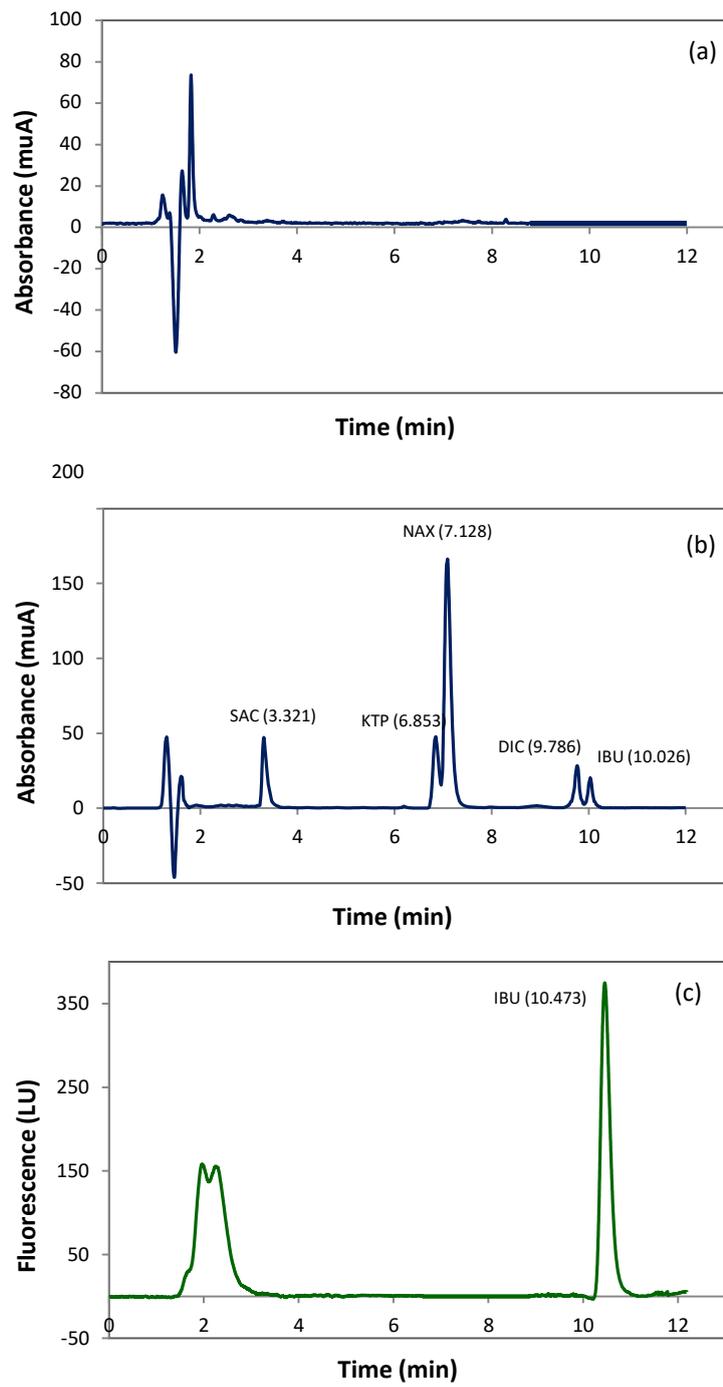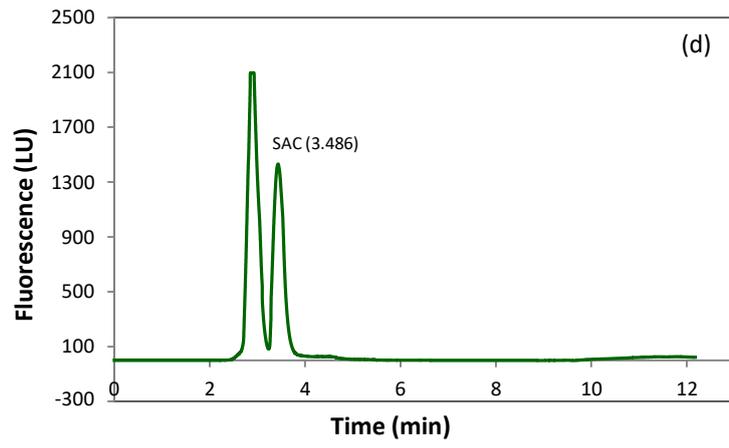
Table 1. Chemical structures and properties of the five selected NSAIDs

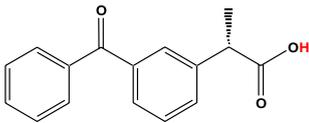| Chemical Structure	Chemical properties[a]
Salicylic acid (SAC)	IUPAC name: 2-Hydroxybenzoic acid Molecular formula: $C_7H_6O_3$ MW: 138,12 g/mol pK_a: 3,01 log P: 2,011
Ketoprofen (KTP)	IUPAC name: (RS)2-(3-benzoylphenyl)-propionic acid Molecular formula: $C_{16}H_{14}O_3$ MW: 254,281 g/mol pK_a: 4,23 log P: 2,911
Naproxen (NAX)	IUPAC name: (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid Molecular formula: $C_{13}H_{18}O_2$ MW: 206,26 g/mol pK_a: 4,84 log P: 2,876
Diclofenac (DIC)	IUPAC name: 2-(2-(2,6-diclorophenylamino)phenyl) acetic acid Molecular formula: $C_{14}H_{11}NCl_2O_2$ MW: 296,148 g/mol pK_a: 4,18 log P: 4,548
Ibuprofen (IBU)	IUPAC name: (RS)-2-(4-(2-methylpropyl)phenyl) propanoic acid Molecular formula: $C_{14}H_{14}O_3$ PM: 230,26 g/mol pK_a: 4,41 log P: 3,50
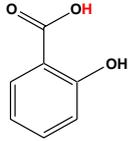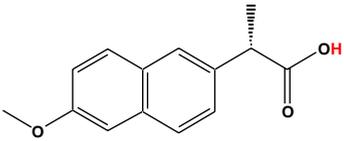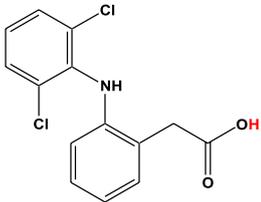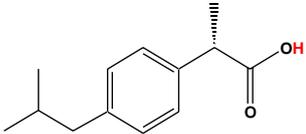
[a]Data collected from http://www.chemspider.com/ and https://pubchem.ncbi.nlm.nih.gov/

Table 2

Table 2. Recoveries obtained with different electrode configurations.

Config.	SAC[b]	KTP[c]	NAX[b]	DIC[c]	IBU[b]
			Recoveries (%)[a]		
(a).	34.3 ± 0.673	24.2 ± 2.13	22.3 ± 2.07	23.2 ± 1.58	22.3 ± 1.61
(b).	30.7 ± 1.61	20.2 ± 1.27	20.2 ± 1.21	19.0 ± 0.87	19.5 ± 1.80
(c).	29.1 ± 1.76	19.7 ± 0.826	19.5 ± 1.76	20.7 ± 1.74	18.9 ± 1.56
(d).	31.5 ± 1.72	20.5 ± 1.41	20.5 ± 1.63	21.5 ± 0.922	21.4 ± 0,962

[a]Average of three determinations ± standard deviation. %RSD < 9%

[b]FLD detection

[c]DAD detection

Table 3

Table 3. Method validation results: linear range, linearity, LOD, LOQ and EME recovery.

AINES	Linear range (ng mL⁻¹)	Linearity		LOD (ng mL⁻¹)	LOQ (ng mL⁻¹)	EME Recovery (%)
		(r²)	(%)			
SAC[b]	1.5-1000	0.9990	99	0.50	1.5	53
KTP[c]	1.5-1000	0.9988	98	0.50	1.5	45
NAX[b]	0.05-1000	0.9996	99	0.02	0.05	42
DIC[c]	3.0-1000	0.9983	98	1.0	3.0	39
IBU[b]	2.0-1000	0.9995	99	0.60	2.0	45

[a]Average of three determinations. RSD < 2.5%.
[b]FLD detection
[c]DAD detection

Table 4

Table 4. Recovery percentage in fortified urine samples.

Spiked level (ng·mL^{-1})	Recovery (%)[a]				
	SAC[b]	KTP[c]	NAX[b]	DIC[c]	IBU[b]
10	80.8 ± 5.9	94.5 ± 4.9	84.5 ± 7.4	98.8 ± 7.5	82.5 ± 6.3
100	77.7 ± 6.0	91.9 ± 4.7	80.1 ± 5.2	95.7 ± 7.7	87.7 ± 5.7
500	78.5 ± 5.6	90.4 ± 5.9	82.5 ± 6.5	84.7 ± 6.3	87.1 ± 5.4

[a]Average of three determinations ± standard deviation.

[b]FLD detection

[c]DAD detection

Conflict of interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

N. Aranda-Merino: Investigation, Methodology, Validation, **C. Román-Hidalgo**: Investigation, Validation, **J.L. Pérez-Bernal**: Conceptualization, Formal Analysis, **M. Callejón-Mochón**: Supervision, Visualization, **M. Villar-Navarro:** Formal analysis, Writing-original draft, Writing-review and Editing, **R. Fernández-Torres**: Conceptualization, Methodology, Writing-review and Editing.

Figure S1

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Supplementary Material
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