Analytica Chimica Acta 1274 (2023) 341572

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Green strategies using solvent-free biodegradable membranes in microfluidic devices. Liquid phase microextraction and electromembrane extraction

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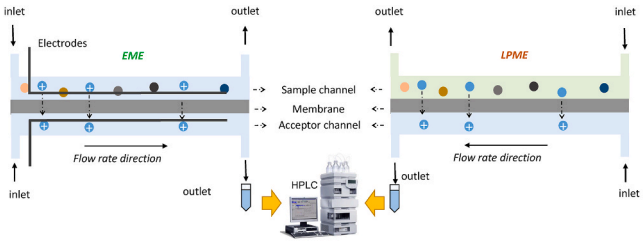
HIGHLIGHTS

- Solvent-free strategies in microfluidic devices have been proposed for the first time.
- Liquid phase microextraction and electromembrane extraction using agarose membranes have been compared in microchips.
- The microfluidic method has been successfully applied in biological samples with excellent clean-up.

GRAPHICAL ABSTRACT



The analytes (blue colour) are positively charged in both (sample and acceptor) solutions in EME. For LPME, the analytes (blue colour) are in their neutral form and positively charged in the sample and acceptor solution, respectively.

ARTICLE INFO

Handling Editor: Dr. L. Liang

Keywords:
Sample preparation
Microfluidic
Liquid phase microextraction
Electromembrane extraction
Solvent-free

ABSTRACT

In this work, a novel solvent-free microfluidic method based liquid phase microextraction has been proposed for the first time. A comprehensive study of liquid phase microextraction (LPME) and electromembrane extraction (EME) implemented in microfluidic formats has been carried out to investigate the efficiency of biodegradable membranes (such as agarose) without organic solvent to develop fully environmental microfluidic methods. For this study, non-polar and polar basic compounds (five) were selected as model analytes and different agarose membrane compositions were synthesized and tested with and without organic solvent (solvent-free). Under optimal experimental conditions, the extraction efficiencies obtained using solvent-free LPME-chip devices were similar to the ones obtained using solvent-free EME-chip devices at very low voltages (0.25 V), however, LPME microfluidic format was selected due to its simplicity. The proposed green microfluidic device was successfully applied in urine samples with recoveries between 80 and 93% for all analytes and relative standard deviation below 7% for all analytes. Results were compared with experiments previously conducted using conventional (polypropylene) membranes, observing that solvent-free microfluidic systems based on biodegradable solid support materials have proven to be an attractive alternative and offered the same advantages in terms of membrane stability allowing consecutive extractions compared to supported liquid membranes (SLM) microfluidic methods.

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https://doi.org/10.1016/j.aca.2023.341572
Received 17 March 2023; Received in revised form 22 May 2023; Accepted 28 June 2023
Available online 28 June 2023

1. Introduction

Liquid phase microextraction (LPME) and electromembrane extraction (EME) are two sample treatment techniques widely used in analytical chemistry in the last decades [1–4]. In these membrane-based techniques, the analytes are extracted from a donor phase to an acceptor phase through a supported liquid membrane (SLM), which is embedded in the pores of a solid support [5,6]. The LPME is carried out by passive diffusion while the EME is a technique that uses an electric potential to create a driving force for the analytes to pass through the membrane into the acceptor phase. Both LPME and EME, have shown promising results in various analytical applications, and they are efficient and reliable sample treatment techniques for the extraction and preconcentration of analytes from complex matrices [7]. In the last decade, the miniaturization of both techniques in microfluidic systems has offered several advantages such as low cost, simplicity, low sample volume required and high extraction efficiency [8–10]. These microfluidic systems, which are based on LPME or EME, utilize a solid support that acts as a membrane. The solid support, commonly made of polypropylene (PP) [11–14], separates the acceptor and donor channels. Furthermore, these systems have evolved to incorporate more sophisticated designs, enabling the simultaneous extraction of compounds with vastly different properties [15–17]. The membrane is usually impregnated with a toxic solvent although in recent years, the use of deep eutectic solvents (DESs) has proven to be an attractive alternative to replace these toxic solvents with green and natural ones in microfluidic devices based LPME [18, 19]. Microfluidic systems have been evolving in recent years, implementing also biodegradable membranes with the aim of achieving more environmentally friendly procedures [20,21]. Combining the advantages of microfluidic devices in sample treatment with eco membranes is of great importance. The eco membranes, such as agarose, offer sustainable and biodegradable alternatives, minimizing environmental impact. Studying these membranes with different analytes is necessary to assess their selectivity and efficiency, as these properties can vary depending on the specific analytes of interest. The use of agarose membranes has also been successfully used in traditional microextractions set-up and EME systems [22–25], offering advantages such as its easy fabrication, adjustability to get various dimensions and shapes, high inertness, and biodegradability. Recently, the development of new analytical methods based on solvent-free techniques has the potential to offer many benefits, including improved environmental sustainability, health and safety, cost-effectiveness, sensitivity, and selectivity [26–28]. Although one of the advantages of microfluidic systems is the use of very small amounts of organic solvent (3 μL) for LPME [29–32] and EME [33–38], we believe that studying new solvent-free microfluidic strategies will bring these devices closer to current green chemistry, offering new advantages to the current ones. In this work, new green (solvent-free) strategies were studied using biodegradable agarose membranes to achieve completely green microfluidic systems. A comprehensive study has been carried out for the microfluidic technique of LPME and EME, synthesizing new biodegradable membranes and investigating the effect of removing the organic solvent used to form the SLM. The model analytes selected for the study were basic polar and non-polar compounds and the results have been compared with previous microfluidic studies carried out by our group [17] which use polypropylene membranes. The logP and pK_a for the analytes were 0.42 and 9.67 for ATN, 1.75 and 9.67 for MTP, 5.04 and 9.68 for VRP, 4.43 and 10.47 for NRP, and 4.81 and 9.76 for AMP. For the first time, novel solvent-free microfluidic methods based LPME and EME will be studied. In addition, this study will discuss the competitiveness of the EME technique compared to LPME in solvent-free microfluidic systems, as well as the effectiveness of using biodegradable agarose membranes to achieve more environmentally friendly systems.

2. Experimental

2.1. Chemicals and sample solutions

All reagents and chemicals were of analytical grade. Formic acid, hydrochloric acid, phosphoric acid, sodium hydroxide, methanol, 2-nitrophenyl octyl ether (NPOE), 1-octanol, dihexyl ether (DHE), tributyl phosphate (TBP), verapamil hydrochloride (VRP), amitriptyline hydrochloride (AMP), atenolol (ATN), metopropol (MTP), and nortriptyline (NRP) were purchased from Sigma Aldrich (Madrid, Spain). Agarose was purchased from Merk (Spain) and different flat agarose membranes were synthesized in the lab for its use as solid support. A flat polypropylene membrane from Celgard (Charlotte, NC, USA) with a 25 μm thickness, 55% porosity, and 0.21 × 0.05 μm pores was also used as solid support. Stocks solutions were prepared in methanol at 100 mg L^{-1} and daily working dilutions were prepared using ultrapure water from a Milli-Q Plus system.

2.2. Liquid chromatography

The separation was carried using an HPLC system consisted of a VWR-Hitachi (Barcelona, Spain) liquid chromatograph with a quaternary L-2130 pump. The injector was an autosampler L-2200. Separation was performed using a LiChroCART 75–4 Purosphere STAR RP-18e (3 μm, 75 mm × 4.0 mm i. d.) (VWR, Germany) proceeded by a guard column Kromasil1 100 Å, C18, (5 μm, 20 mm × 4.6 mm i. d.) (Scharlab S.L., Barcelona, Spain). The mobile phase consisted of 0.1% formic acid (pH 2.6) (component A) and methanol (component B) at a flow rate of 0.5 mL min^{-1}. The separation program selected was modified from our previous reported separation [17]. An initial elution gradient was programmed as follows: from 15% to 100% B for 18 min, and then this condition was kept for 3 min, followed by 5 min re-equilibration at 15% of B. The wavelengths used for DAD was 200 nm for VRP, NRP and AMP; and 280 nm for ATN and MTP. Chromatogram was completed in 20 min and retention times were 5.9, 12.8, 17, 19 and 19.8 min for ATN, MTP, VRP, NRP and AMP, respectively.

2.3. Fabrication and setup of the microfluidic device

For the comparative study of both microextraction techniques (LPME and EME), two different microfluidic devices were used. For LPME-based microfluidic experiments, a previously designed geometry [17] was used, and that device was modified to introduce the electrodes for EME-microfluidic experiments. For the fabrication of both devices, an Epilog Mini 24–30-W laser cutter was used (40% writing speed, 35% power, resolution of 1500, and frequency of 5000). Each device consisted of two symmetrical poly(methyl methacrylate) (PMMA) layers and each layer contained one channel of 23.0 × 3.0 × 0.13 mm (length × width × depth). Two inlets/outlet holes (1.5 mm od) were drilled to introduce the acceptor and sample solutions by using Teflon tubes. In addition, for the EME microfluidic device, two extra holes were drilled to each channel (acceptor and sample channel) to introduce the platinum electrodes along each channel to serve as electrodes. Fig. 1(A) and (B) shows a scheme of the LPME-chip device and EME-chip device, respectively. The flat membrane (PP or agarose) was located between the acceptor and sample channel, and it was impregnated with 3 μL with the organic solvent (if needed) to form the supported liquid membrane. No organic solvent was used for solvent-free experiments. Then, both devices (on-chip LPME and on-chip EME) were assembled by using six screws each. Before the extraction, sample and acceptor solutions were introduced in their respective syringes and both solutions were pumped into the microfluidic channels at 1 μL min^{-1}. A voltage was applied for EME-chip experiments during the extractions. The extractions were completed in 7 min, and the acceptor phase was collected by a micropipette and transferred to a micro-insert for its analysis by liquid chromatography.

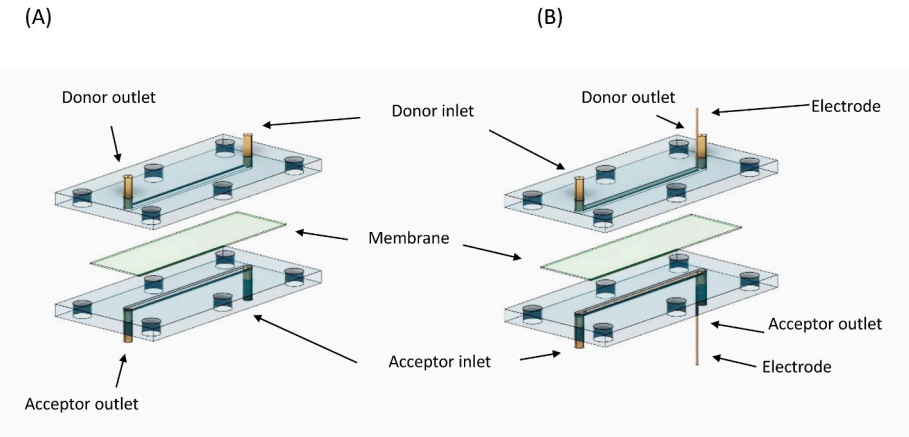

Fig. 1. Microfluidic device based (A) Liquid phase microextraction and (B) Electromembrane extraction.

2.4. Preparation of the agarose membranes

Agarose was obtained from Merck (Madrid, Spain) and it was added to 100 mL of deionized water and mixed. In this step, different weight of agarose (0.8 g, 0.6 g and 0.4 g) was tested to prepare different type of agarose films. Each different solution (containing different amount of agarose) was shacked and boiled at 100 °C to completely dissolve the agarose. Then, different aliquots of each warm solution (6 mL, 8 mL and 10 mL) was pipetted into a glass Petri dish (90 mm of inner diameter) and kept at room temperature for at least 30 min. Each Petri dish was dried in an oven at 50 °C for 24 h. Nine different agarose gel membranes were obtained. Each agarose film formed, was cut into a pieces of 25 mm length × 4 mm width and located between the acceptor and the donor channel for further use. All agarose films synthesized were tested as biodegradable solid support and were characterized by using Scanning Electron Microscope to check the thickness. Images were taken with a SEM operated at an accelerating voltage of 3 kV. Membranes were coated with approximately 10–20 nm Au/Pd. Thickness between 1.4 μm and 9 μm were obtained. Fig. 2 shows an image of the top (a) and the cross-section (b) by using Scanning Electron Microscope of a biodegradable agarose membrane (0.4 g).

2.5. Preparation of real samples

Human urine samples were collected prior consent. Non-diluted and 1:1 diluted urine sample from a 29-year-old female healthy volunteer were spiked with the model analytes at different concentration levels within the linear range (0.5, 0.8 and 1 mg L^{-1}). All samples were adjusted to pH 11 and filtered through Pall Nylaflo™ nylon membrane filter 0.45 μm and 32 mm diameter (Pall Corporation, Ann Arbor, MI, USA) prior to extraction. A flow rate of 1 μL min^{-1} was used to deliver the samples to the microfluidic device using a sample volume below 80 μL for at least 3 repetitive extractions.

3. Results and discussion

3.1. On-chip liquid phase microextraction

3.1.1. Study of the supported liquid membrane

In our previous studies, PP and agarose membranes were used for the extraction of compounds from different families with both acidic and basic functional groups [17,20,21], using traditional organic solvents as SLM, or even green natural solvents such as DES [19]. New studies have been carried out in this work with the aim of achieving a fully green microfluidic system and comparing the results with those previously

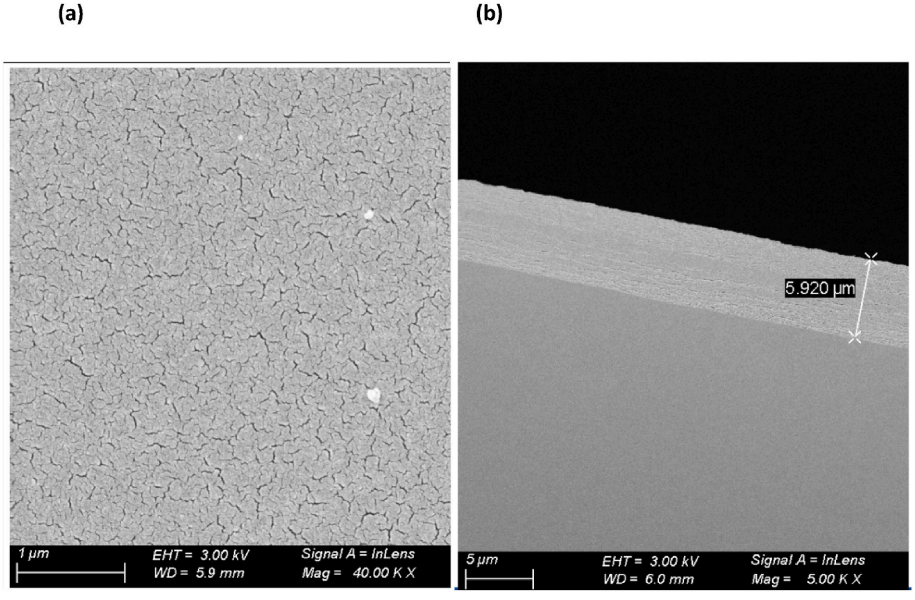

Fig. 2. Image of the membrane using SEM of the (a) top and (b) cross section.

studied by our group based on PP as a solid support.

Firstly, a comprehensive study was carried out on the supported liquid membrane using biodegradable as solid supports, such as agarose. First, an agarose membrane synthesized by our group (using 0.8 g of agarose) was tested with different organic solvents to compare the results with our previous studies in PP membranes. The organic solvents selected were those that had previously given good performance for the extraction of basic compounds of very different polarity in microfluidics [17]: DHE, 1-octanol, NPOE and 2:1 TBP:DHE, the latter being the optimum in our previous studies with PP membranes. The rest of the operational parameters were set as a guide to ensure that the analytes are neutral (donor phase) and positively charged (acceptor phase) for the extraction to take place. The experiments were carried out fixing 5 mM phosphate buffer (pH 1.5) as the acceptor phase, 5 mM phosphate buffer (pH 11) as the donor phase and 1 μL min^{-1} for both flow rate (donor and acceptor), following the conditions previously studied [17, 19]. Under these conditions, the compounds were extracted (in their neutral form) from the donor phase to the donor phase (positively charged) accordingly to their pK_a values [52]. Table 1 shows the extraction efficiencies obtained with different organic solvent tested (as SLM) impregnated in agarose membrane as solid support. As seen in Table 1, 2:1 TBP:DHE showed the best extraction efficiencies between 40 and 70% for all compounds compared to others SLMs. This SLM showed similar results when it was tested in PP membranes [17] under the same operational conditions. Therefore, biodegradable agarose membranes showed good effectiveness to replace PP membranes. Second, the thicknesses of the agarose membrane were optimized to achieve better mass transfer of the analytes through the membrane. The membranes were synthetized according to the described method in previous section 2.4, using the following combinations: (I) 0.4 g agarose/10 mL, (II) 0.4 g agarose/8 mL, (III) 0.4 g agarose/6 mL, (IV) 0.6 g agarose/10 mL, (V) 0.6 g agarose/8 mL, (VI) 0.6 g agarose/6 mL, (VII) 0.8 g agarose/10 mL, (VIII) 0.8 g agarose/8 mL, and (IX) 0.8 g agarose/6 mL. The thickness of the membranes varied between 1.4-6 μm, 4.6–7.2 μm and 5.5–8 μm for 0.4, 0.6 and 0.8 g of agarose respectively. Each agarose membrane was investigated with and without organic solvent as SLM. In Table 1S (supplementary information), the different membranes compositions are shown for (a) solvent-free and (b) 2:1 TBP:DHE as SLM. As seen, the membrane (II) and (III) showed better extraction efficiencies in general for all compounds, either with SLM (b) and as solvent-free (a). No significant differences were observed between II and III, although very thin membranes (II) were more brittle and broke more easily. A decrease in the extraction efficiency was observed between 10 and 33% in some cases under solvent-free conditions compared to PP membranes, except for AMP which showed an increase of 42% using membrane II. Based on the better efficiencies obtained for membrane II compared to the others solvent-free membranes, membrane (II) was selected for the rest of the experiments to develop a solvent-free microfluidic method. The selectivity and extraction efficiency could be affected by different parameters, such as thickness, chemical functionalization, amount others. The thickness of the agarose membrane affects the distance over

Table 1
Extraction efficiencies (RSD %) of the polar and non-polar basic using different organic solvents as the SLM in agarose membranes.

	Extraction efficiency % (RSD%, n = 4)				
Organic solvent	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
2:1 TBP-DHE	40.1 ± 2.6	62.6 ± 9.7	75.0 ± 2.3	69.4 ± 5.1	33.1 ± 3.5
DHE	42.0 ± 0.5	66.5 ± 0.8	74.5 ± 3.1	56.8 ± 10.2	26.1 ± 6.4
NPOE	35.8 ± 1.1	39.2 ± 4.7	44.7 ± 3.2	32.9 ± 8.3	0.8 ± 0.3
1-Octanol	38.9 ± 3.3	39.2 ± 7.2	59.6 ± 3.0	65.8 ± 2.5	11.2 ± 1.8

Table 2
Extraction efficiencies (RSD %) of the analytes using microfluidic based EME using different solvent-free agarose membranes.

	Extraction efficiency % (RSD%, n = 3)				
Membrane (I)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	42.1 ± 0.9	47.7 ± 4.9	41.3 ± 0.8	42.7 ± 0.4	43.6 ± 0.7
0.25V	46.4 ± 1.1	52.3 ± 1.6	43.0 ± 0.5	50.4 ± 1.2	52.0 ± 1.4
0.5V	51.4 ± 2.1	54.7 ± 4.9	45.3 ± 1.6	50.0 ± 3.1	49.0 ± 4.2
1V	40.0 ± 2.3	37.2 ± 3.3	33.1 ± 0.8	38.5 ± 1.2	39.7 ± 0.7
Membrane (II)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	46.4 ± 9.1	43.0 ± 8.2	43.6 ± 0.8	44.4 ± 0.4	44.6 ± 0.7
0.25V	47.9 ± 3.4	53.5 ± 0.4	47.1 ± 0.8	44.9 ± 0.6	45.6 ± 2.1
0.5V	43.6 ± 1.3	55.8 ± 3.3	48.8 ± 1.6	47.4 ± 0.6	53.9 ± 2.8
1V	32.9 ± 4.2	39.5 ± 9.9	34.3 ± 2.5	38.9 ± 5.4	46.6 ± 6.2
Membrane (III)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	47.9 ± 1.4	50.0 ± 1.6	47.7 ± 1.6	52.6 ± 1.8	53.9 ± 1.4
0.25V	48.6 ± 0.8	51.2 ± 6.6	55.2 ± 2.5	53.4 ± 4.2	57.8 ± 2.8
0.5V	49.3 ± 3.1	51.2 ± 3.3	45.3 ± 0.4	48.7 ± 1.2	52.9 ± 2.8
1V	35.1 ± 2.9	51.2 ± 3.3	44.2 ± 1.6	47.4 ± 0.6	48.2 ± 4.2
Membrane (IV)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0,1V	26.6 ± 10.3	25.0 ± 1.1	26.6 ± 2.8	34.2 ± 3.5	32.1 ± 0.8
0,25V	29.8 ± 10.3	28.7 ± 8.8	28.6 ± 7.3	36.6 ± 8.4	33.2 ± 8.5
0,5V	33.9 ± 4.6	28.7 ± 1.8	29.9 ± 0.7	38.1 ± 2.1	36.4 ± 2.3
1V	17.1 ± 1.3	19.3 ± 2.5	19.3 ± 5.2	18.4 ± 2.0	32.4 ± 3.4
Membrane (V)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	33.1 ± 1.1	28.7 ± 1.8	26.6 ± 0.9	32.7 ± 2.8	30.4 ± 1.5
0.25V	32.3 ± 2.1	30.1 ± 7.1	31.8 ± 2.8	39.6 ± 4.2	37.5 ± 2.3
0.5V	28.2 ± 1.1	28.7 ± 1.8	28.6 ± 1.8	37.6 ± 2.8	36.4 ± 2.3
1V	18.5 ± 1.1	20.1 ± 1.8	16.9 ± 3.7	27.2 ± 3.5	29.3 ± 2.6
Membrane (VI)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	57.5 ± 3.5	44.9 ± 9.1	54.5 ± 3.7	39.7 ± 0.4	59.2 ± 2.3
0.25V	70.8 ± 5.9	59.0 ± 3.6	64.3 ± 0.9	45.6 ± 0.9	66.8 ± 0.8
0.5V	50.0 ± 4.1	51.3 ± 4.5	53.9 ± 1.9	43.8 ± 1.8	66.3 ± 2.5
1V	34.2 ± 1.2	38.5 ± 3.6	26 ± 5.5	27.2 ± 1.3	37.5 ± 2.3
Membrane (VII)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	41.4 ± 1.1	47.7 ± 1.6	41.3 ± 0.8	51.7 ± 0.6	52.9 ± 1.4
0.25V	43.6 ± 3.0	37.2 ± 3.3	30.2 ± 1.6	40.2 ± 4.8	37.3 ± 1.4
0.5V	42.1 ± 3.0	41.9 ± 3.3	39.5 ± 3.3	42.7 ± 0.5	48.0 ± 4.2
1V	31.4 ± 4.1	32.6 ± 3.3	29.7 ± 2.5	32.9 ± 1.8	37.3 ± 6.9

(*continued on next page*)

Table 2 (*continued*)

	Extraction efficiency % (RSD%, n = 3)				
Membrane (VIII)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	53.6 ± 5.1	51.2 ± 6.6	57.6 ± 4.1	57.7 ± 2.9	57.8 ± 4.2
0.25V	67.2 ± 17.8	67.2 ± 1.1	48.7 ± 0.9	65.1 ± 0.4	56.8 ± 1.9
0.5V	62.4 ± 3.7	45.4 ± 2.1	43.2 ± 0.5	61.4 ± 1.1	58.8 ± 0.3
1V	52.7 ± 1.2	54.0 ± 0.6	32.8 ± 2.3	49.5 ± 1.9	45.9 ± 17.8
Membrane (IX)	**ATN**	**MTP**	**VRP**	**NRP**	**AMP**
0.1V	42.1 ± 1.1	46.5 ± 3.3	42.4 ± 0.8	42.3 ± 0.6	41.7 ± 0.7
0.25V	54.3 ± 6.5	54.3 ± 2.7	39.4 ± 1.9	53.1 ± 0.4	43.3 ± 1.9
0.5V	56.4 ± 3.7	45.4 ± 2.2	43.2 ± 0.5	61.4 ± 1.1	49.3 ± 4.2
1V	48.1 ± 2.2	42.5 ± 2.4	28.5 ± 3.2	41.7 ± 3.2	49.8 ± 5.3

which analytes must travel through the membrane. A thicker membrane would result in a longer diffusion pathway for the analytes, potentially leading to slower extraction rates and decreased efficiency; and this can be relevant in microfluidic systems in which the amounts of fluid are very small. In this sense, smaller thicknesses are desirable. The thickness of the agarose membrane can also influence its mechanical stability. Thicker membranes are generally more robust and less prone to damage or tearing during the extraction process. This enhanced stability can ensure longer membrane lifespan and better overall performance. This was observed when using membrane (I) since the thickness was extremely small and consecutive extractions could not be carried out even at very low flows. In fact, the use of higher flow rates in microfluidics can determine different optimum thicknesses in the membrane, since higher flow rates in any of the phases could destabilize the membrane and could break if they are too thin. Furthermore, other physicochemical properties of the membrane can influence the extraction and stability phenomena and will be further studied to enhance the understanding of new applications.

3.1.2. Optimization of the acceptor and sample phase composition

The composition of the acceptor and the donor phase was optimized for solvent-free microfluidic extractions using agarose as solid support. Based on previous studies [17], phosphate buffer at different pHs were tested to keep system stability and avoid drift in the pH. The acceptor and donor flow rate were kept at 1 μL min^{-1}. During the extraction process, a variation in the pH was noted in the absence of a buffer solution, whereas the pH remained constant when a phosphate buffer was utilized. The stability of pH during extraction positively influenced the efficiency of the system. No significant differences were observed at different buffer concentrations (0 mM, 5 mM, 10 mM and 20 mM); however, the highest efficiencies (35–90% for all compounds) were achieved with a 5 mM concentration of phosphate buffer. First, donor pH composition was studied between pH 9–12, and 5 mM phosphate buffer (as acceptor phase) was fixed at pH 1.5 to keep the analytes positively charged. As seen in Fig. 3, the highest extraction efficiencies were obtained at pH 11. Second, the acceptor phase composition was tested within pH 1 and 3 by keeping 5 mM phosphate buffer (pH 11) as donor phase composition. The results are shown in Fig. 4 and the best efficiencies were observed between pH 1 and 1.5, depending on the compound, so a pH of 1.5 was set as a compromise for all compounds. 5 mM phosphate buffer at pH 11 and pH 1.5 were selected as donor and acceptor phase composition, respectively, for subsequent experiments. These experiments were tested in triplicate for donor and acceptor phase composition and an RSD between 1 and 6% was obtained for all analytes.

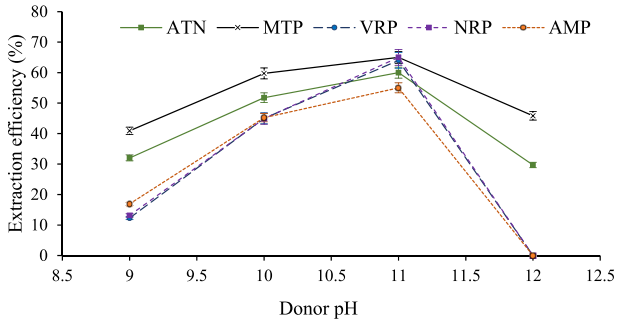

Fig. 3. Optimization of the donor composition using agarose membrane: 5 mM phosphate buffer (pH 1.5) as acceptor, flow rate: 1 μL min^{-1} (acceptor and sample).

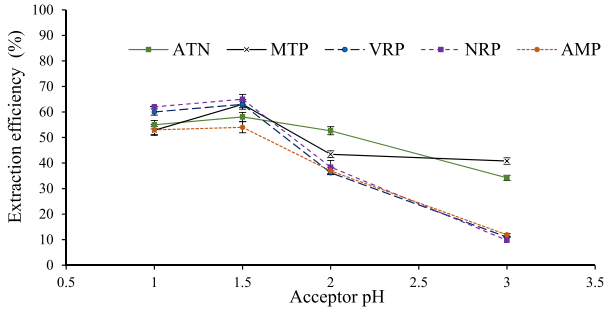

Fig. 4. Optimization of the acceptor composition using agarose membrane: 5 mM phosphate buffer (pH 11) as donor, flow rate: 1 μL min^{-1} (acceptor and sample).

3.1.3. Sample flow rate

The donor phase flow is another of the parameters to optimize in microfluidic systems. This study was carried out under optimal conditions previously optimized: 5 mM phosphate buffer (pH 1.5 for the acceptor phase and pH 11 for the donor phase), organic solvent-free and 1 μL min^{-1}. Previous studies have shown that the extraction efficiencies decrease when the donor or acceptor flow rate increases since the mass transfer is slower in passive diffusion. The residence time of the donor or acceptor phase in any of their respective channels leads to a trend of lower efficiencies, which depends on the type of compounds analysed. The donor flow rate was studied between 1 and 5 μL min^{-1}, observing the highest efficiencies at 1 μL min^{-1} as expected [17]. The efficiencies obtained were 58, 63, 53, 65 and 54% for ATN, MTP, VRP, NRP and AMP, respectively, at 1 μL min^{-1}. A clear decrease in the extraction efficiency was observed when increasing the donor flow rate since the residence time of the analyte decreased in the donor channel. Extraction efficiencies of 30, 50, 41, 33 and 29% for ATN, MTP, VRP, NRP and AMP, respectively, were obtained at 5 μL min^{-1}. Good relative standard deviation (<6%) were obtained within the studied range for triplicate experiments.

3.2. On-chip electromembrane extraction

3.2.1. Study of the operational parameters

In EME, the extraction of the analyte from the donor phase to the acceptor phase is carried out by electromigration when a potential difference is applied between both phases. Fig. 1B shows a scheme of the

on-chip EME device, which includes an electrode in each of the channels (donor and acceptor). Preliminary experiments were performed by setting the donor and acceptor phases at 5 mM phosphate buffer (pH 2) to ensure positively charged basic analytes in both phases. Initially, different organic solvents were tested: NPOE, 1-octanol and the mixture 2:1 TPB:DHE. Our previous experience using on-chip electromembrane extraction for the extraction of parabens [37] showed that these systems offered good current intensity at low voltages, so a voltage of 5 V was initially set. The acceptor and donor flow rate were set at 1 μL min^{-1} and the extraction time was 7 min. Surprisingly, high currents (greater than 100 mA) were observed, leading to electrolysis, and an unstable system with high relative standard deviations (>30%). The following experiments were carried out in order to develop a solvent-free microfluidic method based electromembrane extraction. For the study, different agarose membranes (I, II, III, IV, V, VI, VII, VIII and IX) within a voltage range from 0.1 to 1.0 V were tested. Voltages higher than 1.0 V showed higher current intensity and a more unstable system, while current intensities between 0.005 and 0.19 μA were observed between 0.1 and 1.0 V. Table 2 shows the extraction efficiencies obtained for each agarose film at different applied voltages. As seen in Table 2, the extraction efficiencies were between 41-60%, 30–65% and 46–58% for 0.8 g, 0.6 g and 0.4 g of agarose, respectively. The best extractions were obtained between 0.25 and 0.5 V regardless of the thickness of the membrane, offering current intensities around 0.02 μA and 0.03 μA for 0.25 V and 0.5 V, respectively. The membrane (VIII) showed slightly higher efficiencies (between 45.6 and 70.8%) for all compounds. Extraction efficiencies obtained by on-chip EME were comparable to those obtained by on-chip LPME for the full range of polar and nonpolar basic compounds and no significant differences were observed between both solvent-free microfluidic methods. However, the LPME on-chip system was simpler since it did not require applied voltage and a power supply. Therefore, the LPME procedure was selected for the development of a green solvent-free microfluidic method, also using agarose membranes as solid support.

4. Evaluation of analytical performance

The microfluidic method was evaluated using on-chip LPME. The device selected was the one illustrated in Fig. 1A. The evaluation of the analytical performance was based on international guidelines [39]. A calibration curve was constructed at seven different concentrations for each analyte, using a least-square linear regression analysis. Table 3 summarizes the calibration parameters of the method: linear range, detection limits, quantitation limits and extraction efficiencies in standard solutions. As seen in Table 3, different linear ranges were obtained for the analytes, observing a linear relationship with r^2 values over 0.9993 for all compounds. Method detection limits were 0.12 μg mL^{-1}, 0.11 μg mL^{-1}, 0.11 μg mL^{-1}, 0.13 μg mL^{-1} and 0.15 μg mL^{-1} for ATN, MTP, VRP, NRP and AMP, respectively. Under optimal operational conditions, the extraction efficiencies were within 53–65% for all compounds (polar and non-polar basic compounds). The reproducibility and intraday repeatability of the microfluidic method was tested by replacing the agarose membranes in the same device and other different device at different days, obtaining a relative standard deviation below 7% and 5%, respectively.

Table 3
Method detection limit (LOD), method quantitation limit (LOQ) and extraction efficiencies at optimal conditions.

	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	R^2	Linear Range (μg mL^{-1})	EE[a]
ATN	0.12	0.40	0.9993	0.40–5	58 (3)
MTP	0.11	0.37	0.9995	0.37–5	63 (2)
VRP	0.11	0.37	0.9993	0.37–5	53 (2)
NRP	0.13	0.43	0.9997	0.43–5	65 (4)
AMP	0.15	0.50	0.9996	0.50–5	54 (2)

[a] % Extraction efficiency (%RSD, n = 3).

5. Real samples analysis

Urine samples were collected from one healthy adult female volunteer. The solvent-free agarose membranes were tested in real urine samples and the results were compared with those obtained in our previous report using PP membranes with organic solvent. For these experiments, urine samples were spiked at three different levels (containing all compounds) at 0.5, 0.8 and 1 μg mL^{-1}. Each sample was pH adjusted prior its submission to the microfluidic device. Non-diluted and one dilution factor (1:1 v/v, using Mili-Qwater) were studied for the spiking recovery assay. Table 4 shows the spiking recoveries in human urine samples, by using the solvent-free microfluidic method proposed. The spiking recoveries were between 80 and 93% for all compounds with no sample dilution. Slightly higher recoveries were obtained with 1:1 sample dilution sample. RSD were below 5% for all compounds. Fig. 5 represents a (A) spiked urine sample (B) a blank urine sample at 0.8 μg mL^{-1}. Solvent-free biodegradable membranes implemented in microfluidic devices showed an excellent clean-up for sample treatment. The stability of the membranes was also studied, observing a good repeatability for more than 3 consecutive extractions. Repeatability and intraday repeatability in human samples were studied in triplicate at 0.5 mg L^{-1}, observing a relative standard deviation below 5% in both cases.

Compared to traditional liquid-liquid extraction methods, microfluidic systems offer several advantages, including significant volume reduction, simplify sample treatment steps, and the potential for membrane reuse. Additionally, the use of agarose membranes in these systems adds an eco-friendly aspect due to their biodegradability and sustainable nature.

6. Conclusions

In this work, solvent-free microextractions using biodegradable membranes in microfluidic devices for LPME and EME have been proposed for the first time. The complete elimination of the organic solvent and the competitiveness of LPME compared to EME make microfluidic systems powerful tools in sample treatment. The study was carried out for the simultaneous extraction of polar and non-polar basic compounds as model analytes. Different solvent-free strategies were studied and implemented in liquid phase microextraction and electromembrane extraction in microfluidic formats. Similar extraction efficiencies were obtained for on-chip LPME (54–65%) compared to on-chip EME (45–70%), noting that in this case, electromigration did not accelerate

Table 4
Recoveries (average of three determinations ± standard deviation) from spiked non-diluted and 1:1 diluted urine samples.

Samples	Spiking recovery ± SD (%) (n = 3)					
	TYR	ATN	MTP	VRP	NRP	AMP
Urine 0.5 μg mL^{-1} (non-diluted)	80 ± 2	91 ± 4	83 ± 2	84 ± 3	90 ± 3	84 ± 3
Urine 0.5 μg mL^{-1} (1:1 dilution)	79 ± 4	89 ± 1	85 ± 3	88 ± 2	87 ± 2	83 ± 2
Urine 0.8 μg mL^{-1} (non-diluted)	88 ± 2	90 ± 2	84 ± 3	86 ± 1	89 ± 1	89 ± 3
Urine 0.8 μg mL^{-1} (1:1 dilution)	85 ± 2	91 ± 1	86 ± 2	85 ± 2	90 ± 2	87 ± 3
Urine 1 μg mL^{-1} (1:1 dilution)	84 ± 3	87 ± 2	84 ± 5	86 ± 3	88 ± 1	86 ± 1
Urine 1 μg mL^{-1} (non-diluted)	86 ± 2	87 ± 3	84 ± 2	87 ± 2	88 ± 2	83 ± 1

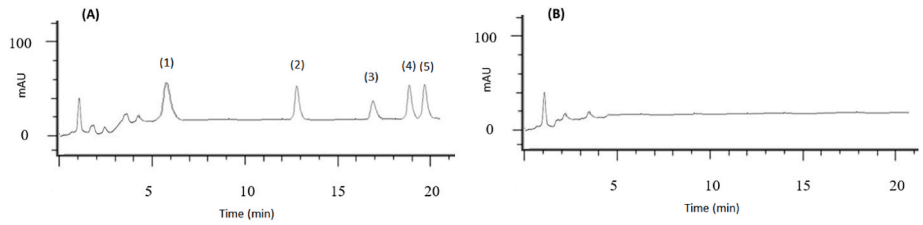

Fig. 5. Chromatogram of (A) a spiked human urine at 0.8 μg mL^{-1} and (B) a blank human urine sample, both, after submitted to the microfluidic device: (1) ATN, (2) MTP, (3) VRP, (4) NRP, (5) AMP.

mass transfer and did not improve extraction efficiency at voltages below 0.5 V. A comprehensive study carried out with and without supported liquid membrane demonstrated that solvent-free based agarose membranes can be considered an alternative for PP and toxic organic solvent replacement. The organic-solvent-free microfluidic device was successfully applied in human urine sample, observing high spiking recoveries (>80%) and provided an excellent clen-up. Agarose has demonstrated to be a versatile, easy-to-use, and stable material that is well-suited for use in microfluidic devices. We believe that this study provides new insights and attempts in the development of green sample preparation approaches. Physicochemical studies will be further explored to enhance the understanding of new applications utilizing eco membranes, encompassing real samples and diverse analyte types.

Ethics declarations

The sample was provided voluntarily and with informed consent.

CRediT authorship contribution statement

Alejandro Martín: Formal analysis, Investigation, Validation. **Elia Santigosa:** Data curation, Visualization. **María Ramos-Payán:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

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.

Data availability

The data can be shared if the manucript once the manuscript is accepted

Acknowledgements

This work was supported by the project PID2021-123073NB-C22 from the Spanish Ministry of Science and innovation (MCIN).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2023.341572.

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