Journal of Chromatography A, 1527 (2017) 1–9

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Full length article

On-chip pulsed electromembrane extraction as a new concept for analysis of biological fluids in a small device

Monireh Karami, Yadollah Yamini*, Yousef Abdossalami Asl, Maryam Rezazadeh

Department of Chemistry, Tarbiat Modares University, P. O. Box 14115-175, Tehran, Iran

ARTICLE INFO

Article history:
Received 23 August 2017
Received in revised form 19 October 2017
Accepted 20 October 2017
Available online 23 October 2017

Keywords:
On chip extraction
Pulsed electromembrane extraction
Codeine
Naloxone
Naltrexone

ABSTRACT

In the present work, an on-chip pulsed electromembrane extraction technique followed by HPLC-UV was developed for the analysis of codeine, naloxone and naltrexone as model analytes in biological fluids. The chip consisted of two channels for the introduction of the donor and acceptor phases. The channels were carved in two poly (methyl methacrylate) plates and a porous polypropylene membrane, which is impregnated by an organic solvent separating the two channels. Two platinum electrodes were mounted on the bottom of these channels and a pulsed electrical voltage was applied as an electrical driving force for the migration of ionized analytes from the sample solution through the porous sheet membrane into the acceptor phase. Using the pulsed voltage provided effective and reproducible extractions and could successfully overcome the disadvantages of applying constant voltages. Effective parameters of on-chip pulsed electromembrane extraction such as chemical composition of the organic solvent, applied voltage, pH of the donor and acceptor phases, flow rate and pulse duration were optimized using one-variable-at-a-time method. Under the optimized conditions, the model analytes were effectively extracted from different matrices and good linearity in the range of 10.0–500.0 $\mu g\,L^{-1}$ was achieved for calibration curves with coefficients of determinations (R^2) higher than 0.997. Extraction recoveries and%RSDs were obtained in the ranges of 28.6–32.9% and 2.15–3.8, respectively. Also, limits of detection were obtained in the ranges of 5–10 $\mu g\,L^{-1}$ and 2–5 $\mu g\,L^{-1}$ in plasma and urine samples, respectively.

1. Introduction

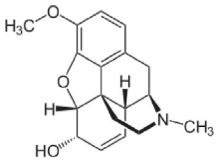
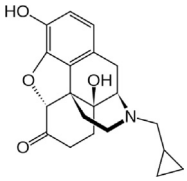
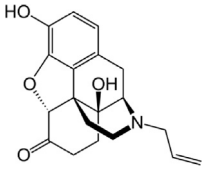
Sample preparation is a critical step in an analytical protocol and a measurement is as good as the sample preparation step which precedes it [1–3]. Liquid-liquid extraction (LLE) is one of the conventional techniques and has been used as a sample preparation method for several decades. It uses large volumes of organic solvents and the analyte. It is also time-consuming. Solid-phase extraction (SPE) is another approach to sample preparation. SPE can be automated and needs much less of an organic solvent than LLE; however, it entails complexities and additional cost [4].

Efforts to develop faster and greener extraction methods have resulted in the development of microextraction techniques including solid-phase microextraction (SPME) and liquid-phase microextraction (LPME). These methods have emerged to miniaturize conventional methods, to lead them to use smaller amounts of organic solvents and also to shorten analysis time. Liquid-phase microextraction emerged in 1990s [5,6]. As its name shows, in LPME, a few microliters of an organic solvent are used to extract analytes from aqueous samples. During the last decades, considerable amounts of attempts have been made to develop new methods of LPME thanks to their more efficient extraction of analytes of interest [7]. Electromembrane extraction (EME) is one of the LPME methods introduced by Pedersen-Bjergaard and Rasmussen in 2006 [8]. Certain advantages of this method are its being fast and great sample cleanup, which make it a suitable method for handling biological samples or any sample with a complex matrix. The base of this method lies in electrokinetic migration. In 2006, Pedersen-Bjergaard and coworkers showed that ionizable substances would be transported across a supported liquid membrane (SLM) by applying an electrical potential. Since 2006, a great deal of research has investigated the development of EME methods [9]. The electrical potential acts as a driving force and leads to the improvement of the extraction efficiency and decreases extraction time. As the extraction time increases, constant voltages, especially high voltages, can cause an increase in Joule heating through the SLM, which results in the instability of SLM during extraction so that the current level increases and electrolysis reactions occur in the acceptor and donor solutions. This drawback increases uncertainty of determination in EME and also decreases extraction efficiency [10–12]. To

* Corresponding author.
E-mail address: yyamini@modares.ac.ir (Y. Yamini).

https://doi.org/10.1016/j.chroma.2017.10.049
0021-9673/

Table 1
Chemical structures, pK_a and log $K_{O/W}$ of the model analytes.

Name	Chemical structure	Abbreviation	pK_a	log $K_{O/W}$
		Codeine	Code	1.34	9.19
		Naltrexone	Nalt	1.36	8.88
		Naloxone	Nalo	1.62	7.84

overcome the drawback, pulsed electromembrane extraction was developed in 2012 by Yamini's research group [13]. They used a pulsed voltage by means of a simple and inexpensive electronic device, which created pulsed voltages in combination with common DC constant power supplies and could successfully increase the extraction efficiency by applying a high voltage without making significant instability in the SLM. Despite all the improvements in EME methods, they still needed large amounts of sample solutions, which was not practical in the case of some samples. The first effort to downscale the EME method was done by Petersen et al. in 2010 in a microfluidic device [14]. After that, several reports tried to miniaturize EME on chip-based devices [15–17]. In all of the previous works, constant voltages were used as well. In the case of on-chip EME, electrodes are fixed on the bottom of the chip so that the distance between them remains unchanged during the extraction so that the repeatability of the method improves [16]. Furthermore, the short distance between electrodes in this system gives rise to an increase in the strength of the electric field between the electrodes compared with conventional EME. Thus, the drawbacks already explained can be very serious.

In the present study, an on-chip pulsed electromembrane method was developed to overcome the drawbacks of the conventional on-chip EME setups. The aim of this work is to use pulsed EME on a chip device to first overcome the disadvantages of using constant voltage and then to exploit the advantages of miniaturized systems. It is expected that the proposed method can improve extractability by increasing stability and using higher applied voltages.

2. Experimental

2.1. Chemicals and materials

Naltrexone (Nalt), naloxone (Nalo) and codeine (Code) were sincerely gifted by Temad Co. (Tehran, Iran). The chemical structures and properties of the drugs are shown in Table 1. 2-Nitrophenyl octyl ether (NPOE), tris-(2-ethylhexyl) phosphate (TEHP), and di-(2-ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs, Switzerland). 1-Octanol, 1-undecanol, methanol, HCl, and NaOH were obtained from Merck (Darmstadt, Germany). Acetonitrile was purchased from Caldon (Ontario, Canada). All chemicals used were of analytical grade. The Accurel PP 1E polypropylene membrane sheet with a wall thickness of 100 μm and a pore size of 0.1 μm was bought from Membrana (Wuppertal, Germany). Ultrapure water was prepared by a Young Lin aquaMAx purification system 370 series (Seoul, Korea).

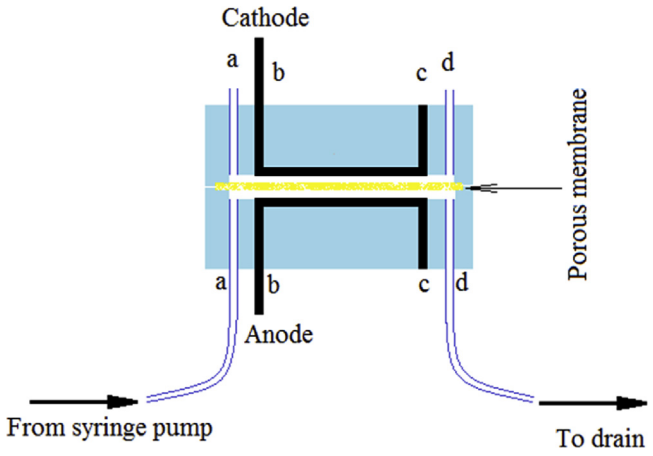


Fig. 1. A schematic of the designed chip for PEME.

2.2. Solutions

Stock solutions of the drugs at 1 mg mL^{-1} as well as a standard solution of the mixture of the drugs containing 50 μg mL^{-1} of each drug were prepared in methanol and stored at 4 °C. Aqueous working solutions were daily prepared by diluting the standard solution at 0.5 μg mL^{-1} in aqueous solutions of HCl at the intended concentration.

2.3. Real samples

Human urine samples were collected from a healthy volunteer and used on the day of collection. The sampling procedure was fulfilled according to the guidelines of research ethics. Human urine was filtered through a 0.45 μm pore size cellulose acetate filter from Millipore (Madrid, Spain) and then diluted 1:4 with ultra-pure water.

Frozen drug-free human plasma samples (blood group $^{+}$B) were obtained from the Iranian Blood Transfusion Organization (Tehran, Iran) and stored at −4 °C. The samples were allowed to thaw at room temperature, shaken before extraction, and diluted 1:15 as well.

2.4. HPLC conditions

The chromatographic separations were performed on an Agilent 1260 Series HPLC system (Waldbronn, Germany). The system consisted of a quaternary pump, a degasser and a UV–vis detector. Chromatographic data were recorded and analyzed using ChemStation for LC system software (version B.04.03). The separations were accomplished on an ODS-3 column (250 mm × 4.6 mm) with a 5-μm particle size from MZ-Analysentechnik (Mainz, Germany). A mixture of 25 mM phosphate buffer (pH 8) and acetonitrile (60:40) was used as the mobile phase via an isocratic elution at a flow rate of 1.0 mL min^{-1}. The wavelength of the detector was set at 210 nm.

2.5. Chip fabrication and pulsed electromembrane extraction

The designed chip device used in pulsed electromembrane extraction (PEME) is schematically demonstrated in Fig. 1. The chip