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Comprehensive study of buffer systems and local pH effects in electromembrane extraction

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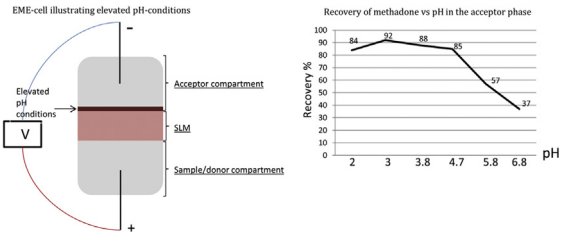
HIGHLIGHTS

- Buffers in EME provide stable pH, low current and high recoveries.
- Elevated pH conditions at the acceptor/SLM interface affect the extraction.
- Elevated pH conditions proved by visual inspection using the pH indicator phenolphthalein.
- Indicates why acceptor phase pH is more critical to EME than the donor phase.

GRAPHICAL ABSTRACT



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ABSTRACT

Different phosphate-, acetate- and formate buffers in the pH range 2.0–6.8 were tested for electromembrane extraction (EME) in a 96-well system. The five basic drugs haloperidol, loperamide, methadone, nortriptyline, and pethidine were selected as model analytes. The EME performance was tested with respect to extraction recovery, extraction current and pH-stability. The analytes were extracted from 200 μL buffer, through a 100 μm thick supported liquid membrane (SLM) of 2-nitrophenyl octyl ether (NPOE) immobilized in the pores of filters in a 96-well plate, and into 100 μL buffer acceptor phase. The extraction voltage was 50 V and the extraction time was 10 min. The acceptor phase was analyzed by HPLC-UV. The extraction current was ≤6 μA with all buffers, and pH was effectively stabilized during EME using buffers as donor (sample) and acceptor phase. For buffers with pH ≤ 4.8 as acceptor phase, the extraction recoveries were in the range 66–97% and with RSD <15%. With pH in the range 5.8–6.8 in the acceptor phase, the extraction recoveries decreased and were in the range 21–62%. This was attributed to elevated pH conditions in the acceptor/SLM interface. The presence of elevated pH conditions was visualized with phenolphthalein as pH sensitive color indicator. Increasing the buffer strength from 10 to 500 mM in an attempt to offset the elevated pH conditions gave no improvement, and elevated pH conditions remained. Elevated pH conditions in the acceptor/SLM interface were also observed when voltage was increased, and when NPOE was replaced with tributyl phosphate as SLM. The presence of

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elevated pH conditions close to the SLM in EME was discussed for the first time, and this information is highly important for future development of EME.

1. Introduction

Electromembrane extraction (EME) was introduced in 2006 [1] as a novel microextraction technique. EME was developed from hollow-fiber liquid-phase microextraction (LPME) [2], which in turn evolved based on the idea of solid-phase microextraction (SPME) [3], single-drop microextraction (SDME) [4], and supported liquid membrane extraction [5]. EME is performed in a three-phase system comprising two aqueous phases (donor (sample) and acceptor) separated from each other by an organic supported liquid membrane (SLM). The SLM is a thin film of an organic solvent which is immobilized in the pores in the wall of a porous hollow fiber. EME is based on electrokinetic migration of analytes in an electrical field sustained across the extraction system. This is in contrast to LPME which is based on diffusion of the analytes. EME provides faster extraction compared to LPME, while maintaining all the benefits of a microextraction system, such as (a) efficient sample clean-up, (b) high enrichment, (c) very low consumption of hazardous organic solvents, and (d) potential for automation and high-throughput. With an electrical field as the driving force for mass transfer, EME offers a unique possibility of tuning the extraction selectivity by the direction (polarity) and magnitude (voltage applied) of the electrical field [6]. For efficient EME, target analytes have to be charged. This means that for extraction of basic or acidic compounds, pH in the donor and acceptor phases has to be adjusted to a level where they are ionized. Up to date, pH adjustment has in most cases been accomplished using strong acids and bases (typically HCl and NaOH). In the area of pharmaceutical analysis, EME has been used to extract acidic drugs [7], basic drugs [8], and peptides [9] from different samples such as whole blood [10], plasma [11], urine [12], breast milk [13], and saliva [14]. Recent EME applications also include extraction of environmental pollutants [15], metal ions [16], and inorganic anions [17] related to environmental and food analysis applications.

While EME has been studied for 10 years and close to 250 EME papers have been published (Scopus), there are still fundamental questions and challenges that remain unanswered. One very important challenge is the occurrence of electrolysis in the donor and acceptor phase upon application of the electrical field. Thus, pH may decrease at the anode (Equation (1)) and may increase at the cathode (Equation (2)):

$$H_2O\ (l) \rightarrow \tfrac{1}{2}\ O_2\ (g) + 2H^+\ (aq) + 2e^-\ (\text{anode}) \quad (1)$$

$$2H^+\ (aq) + 2e^- \rightarrow H_2\ (g)\ (\text{cathode}) \quad (2)$$

Changes in pH have been reported in several EME papers, and due to the small volumes involved the changes may be significant [18,19]. The level of electrolysis is determined by the extraction current, which is carried by mass transfer of analyte and background ions across the SLM, and by the extraction time. The extraction current in turn, is mainly determined by the applied voltage and by the chemical composition of the SLM. Therefore the level of electrolysis can normally be controlled by careful selection of experimental conditions. As a consequence of electrolysis, EME will always be associated with pH changes (especially the acceptor phase), even under low extraction current conditions (<50 μA) [20]. In most papers published up to date, dilute solutions of HCl and NaOH have been used as donor and acceptor phase. In some of those papers pH changes have most probably been relatively small, whereas in others major pH changes may have challenged the extraction performance. Nevertheless, for future development of EME, focus should be directed towards stabilizing pH. This is considered important in order to enhance the reliability and robustness of EME procedures, and also for the general acceptance and implementation of the concept. Analytical scientists will prefer operating an extraction system under constant pH conditions, as long as it is obvious that performance is pH dependent.

In a few EME papers, special attention has been directed towards stabilizing pH in the acceptor phase. In one recent paper, the basic drug substances procaine, nortriptyline, and papaverine were extracted using 500 mM formic acid as acceptor phase [21]. Even after 40–80 min of EME, pH in the acceptor phase was still within 0.2 units from its origin, and due to the buffer capacity analyte molecules were not back-extracted into the SLM. While 10 mM HCl may be prone to pH shift, 100 mM HCl also provided stable pH in the acceptor phase, and after 80 min of operation the shift in pH was only 0.1 [21]. Successful use of very strong solutions of mineral acid was reported in another paper related to μ-EME. In this case pH changes up to eight units were observed using 1–10 mM HCl as acceptor phase, whereas pH stabilized when 100 mM HCl was used [19]. In the μ-EME system, the volume of acceptor phase was only 1.5 μL. However, electrolytically induced pH changes up to 8.5 pH units were also observed in experiments performed in a traditional EME system based on a hollow fiber, where the volume of acceptor phase was 20 μL [18]. In a paper by Huang et al., acetate buffers were tested as acceptor phase for selective EME of peptides based on their isoelectric point [22]. In that work, 75 mM acetate buffer pH 5.2 was stable within 0.1 pH units during EME.

The use of different buffer systems in EME applications is not new and has already been investigated by different research groups. However, the challenge lies in choosing the right one for the application in terms of recovery, pH stability and extraction current. In the first part of this work we investigated different buffer systems for use in the pH range 2.0–6.8. During testing with different buffers, we discovered a layer of elevated pH in the acceptor boundary layer in close contact with the SLM. Although several publications have discussed on formation of electrical double layers at the SLM surfaces [23–27], we are to the best of our knowledge the first group to address the effect of pH difference between the SLM and the bulk acceptor phase. . Because this layer of elevated pH has major impact on the performance and mass transfer, it was investigated systematically for the first time in the second part of this paper. The layer of elevated pH may better explain data already published in the literature, and knowledge about it is very important for future EME work.

2. Experimental

2.1. Chemicals and materials

All chemicals were of high analytical grade (>95%) Haloperidol, loperamide, methadone, nortriptyline, pethidine and ammonium molybdate were all purchased from Sigma Aldrich (St Louis,