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Short communication

Off-line sample preparation by electrophoretic concentration using a micropipette and hydrogel

Alain Wuethrich, Paul R. Haddad, Joselito P. Quirino*

Australian Centre for Research on Separation Science, School of Physical Sciences-Chemistry, University of Tasmania, Tasmania 7001, Australia

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ABSTRACT

An off-line electrophoretic sample concentration technique for charged analytes in aqueous samples is presented. As a demonstration, nine anions including inorganic ions, a dye and benzenesulfonate derivatives were enriched from a 10 mL sample solution into 20 μL electrolyte inside a glass micropipette. A hydrogel was placed at one end of the micropipette while the other end was immersed in the sample. The electric field caused the movement and concentration of anions into the high conductivity electrolyte. The technique was applied to purified, drinking and river water and was optimised by changing applied voltage and voltage application time. The LODs after analysis by capillary electrophoresis was 1–19 ng/mL, 4–133 ng/mL and 18–80 ng/mL for purified, drinking and river water, respectively. The linear range was 0.002–0.048 to 0.1–2.4 μg/mL (R^2 of 0.993–0.999), 0.02–0.24 to 1.0–24 μg/mL (R^2 of 0.995–0.999) and 0.02–0.24 to 1.0–24 μg/mL (R^2 of 0.998–1.000), correspondingly. The intraday and interday repeatability (%RSD, $n = 6$) was ≤7.4% and 14.0%, respectively. The concentration factor was from one to two orders of magnitude. The technique was directly compatible with a liquid phase analytical technique, thus eliminated the additional steps (e.g., evaporation, elution and/or reconstitution) which are typically performed in sample preparation (e.g., liquid and solid phase extraction).

1. Introduction

In liquid–liquid extraction (LLE), the ideal case is to concentrate the target analytes from a large volume of sample into a much smaller volume of extraction phase [1–3]. However, for practical reasons LLE usually involves the use of an ample volume of extraction phase, with the sample and extraction phases being shaken, physically separated, and then the volume of the extraction phase is reduced by evaporation. The residue containing the extracted analytes is then usually reconstituted into a small volume of suitable solution prior to further processing.

Recently, innovative, environmentally friendly microscale techniques have emerged to reduce the amount of organic solvent used in LLE. Examples of such techniques include extraction into a solvent that is suspended in a drop (single-drop microextraction) [4–6], dispersed to increase the contact surface area (dispersive liquid–liquid microextraction) [7,8], or protected by a membrane such as a hollow fiber (hollow-fiber microextraction) [9–11]. Nevertheless, efficient extraction of polar or ionisable analytes that are more soluble in aqueous phases is still difficult. Thus, there has been increasing interest in the application of an electric field to enhance LLE of ionised and ionisable molecules from aqueous samples [12–16]. The electric field accelerates the transfer of charged molecules from the aqueous sample and then into the extraction phase [17]. In some cases, the aqueous sample and extraction phases were separated by a membrane or another liquid [18–24]. Selective enrichment of either cationic or anionic molecules can also be accomplished by manipulation of the polarity of the applied voltage. In addition, samples produced from these procedures do not normally require further processing and are often directly analysed.

In this communication, we propose an off-line electrophoretic sample concentration of charged analytes. We demonstrate the concentration of ionised analytes between two aqueous phases using an electric field, where the analytes from a low conductivity sample were concentrated to a high conductivity electrolyte. The electrolyte was immobilised inside a micropipette using a hydrogel situated at one end of the pipette. The enrichment effect was due to known principle of the Kohlrausch adjustment of concentrations when diluted components from a sample enter the region of more concentrated electrolyte [25–31]. The hydrogel was not used to separate the sample from the electrolyte, so the proposed approach did not use any physical barrier or membrane to separate the sample and acceptor phases, nor was any organic solvent

* Corresponding author. Tel.: +61 4 48122456.
E-mail address: jquirino@utas.edu.au (J.P. Quirino).

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required. A range of anionic compounds, including inorganic ions, a dye, and benzenesulfonate derivatives was effectively concentrated using a simple experimental set-up. Concentration factors were from one to two orders of magnitude and the approach was applied to fortified drinking and river water.

2. Experimental

2.1. Reagents and stock solutions

Purified water was obtained from a Milli-Q system (Millipore, MA, USA). All reagents (acetonitrile, acrylamide, *N*,*N*-dimethylacrylamide, ammonium acetate, phosphoric acid, sodium hydroxide, sodium borate and potassium persulfate) were obtained from Sigma–Aldrich (New South Wales, Australia) and used as delivered. Stock electrolyte solutions of sodium phosphate pH 2.4 and 1 mol/L ammonium acetate pH 8.3 were prepared in purified water. The pH of the stock solutions was adjusted when needed using 1 mol/L sodium hydroxide. The pH and conductivity of solutions were measured using a Bench-Top Meter (Sper Scientific, Australia). All stock solutions were sonicated and filtered using 0.45 μm filter prior to use. Drinking water was collected from a tap and river water from Derwent River (New Norfolk, Tasmania, Australia).

The analytes were also obtained from Sigma–Aldrich. Analyte stock solutions of 1 mg/mL each in purified water were prepared and stored at 5–8 °C when not in use. The analyte mixture consisted of potassium bromide, potassium bromate, potassium nitrate, 1,3,(6,7)-naphthalenetrisulfonic acid trisodium salt (7N), 2,6-naphthalenedisulfonic acid disodium salt (26N), 3-hydroxynaphthalene-2,7-disulfonic acid (2N), Orange G, and 4-vinylbenzenesulfonic acid (V).

2.2. Hydrogel preparation

Hydrogels were prepared directly in 3 mL capacity polypropylene syringes without plunger where the narrower end was sealed with parafilm. The polymer mixture was made by mixing 55 wt% acrylamide (monomer), 99% *N*,*N*-dimethylacrylamide (co-monomer), 0.5 mol/L ammonium acetate at pH 8.3, purified water, and 5 wt% potassium persulfate (initiator). The ratio of monomer, co-monomer and initiator was 15:1:1.5. The mixture was heated at 60 °C for 10 min [32].

2.3. Electrophoretic sample concentration

The set-up for electrophoretic concentration consisted of a high voltage power supply (Matsusada, Japan) capable of providing adjustable voltages of 0–30 kV (0.1 kV increments), 20 μL micropipettes with a length of 6.4 cm and an inner diameter of 0.3 mm (Microcaps, Drummond Scientific Company, USA), 3 mL disposable plastic syringes (Terumo, Philippines), and 20 mL capacity scintillation vials (Sigma–Aldrich). A hydrogel was prepared in the syringe (see Section 2.2) and the micropipette was filled with 50 mmol/L of ammonium acetate pH 8.3, which acted as electrolyte. The sample solution was stirred during electrophoretic concentration at 600 rpm.

2.4. Analysis of standards, samples and concentrates

Capillary electrophoresis (CE) was used to analyse the standards, samples and concentrates. CE was performed on a Beckman MDQ system (Fullerton, CA, USA). Fused-silica capillaries (60 cm long, 50 cm to the detection window) were obtained from Molex (Phoenix, AZ, USA). The separation electrolyte and voltage was 150 mmol/L sodium phosphate at pH 3 and −20 kV, respectively.

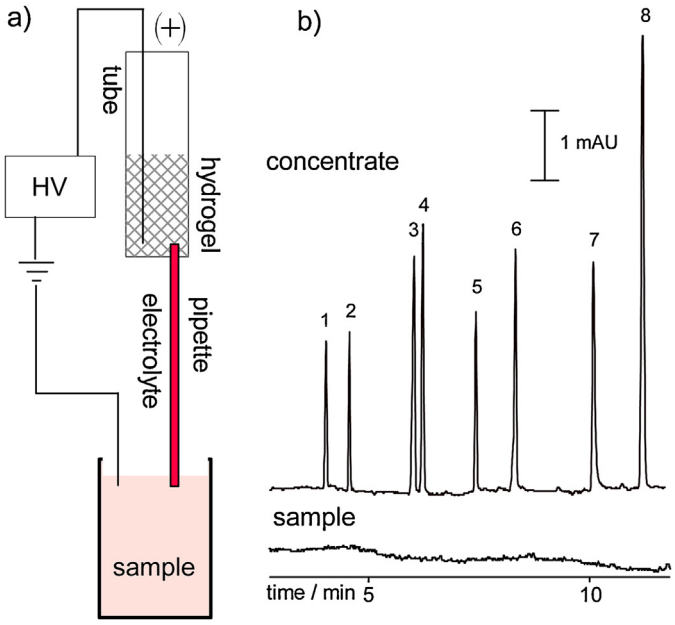

Fig. 1. (a) Scheme for off-line electrophoretic sample concentration using a micropipette and hydrogel. (b) Electropherogram of sample (bottom) and electrolyte after sample concentration (top). For (b), the sample solution was 0.5–12 μg/mL of bromide (peak 1), nitrate (2), bromate (3), 7N (4), 26N (5), 2N (6), Orange G (7), V (8) in purified water. Electrolyte was 50 mmol/L ammonium acetate at pH 8.3. Electrophoretic concentration was performed at 1.3 kV for 20 min. CE conditions are in Section 2.

The capillary was thermostated at 20 °C, the detection was at 200 nm, and the sample injection was by pressure at 50 mbar for 5 s.

3. Results and discussion

3.1. Scheme

The strategy is shown in Fig. 1(a) for anionic target analytes. The experimental set-up is shown in Supplementary material Fig. S1. An acrylamide-based hydrogel was prepared in a cylindrical plastic tube with open ends (in this case, the tube used was a plastic syringe with the plunger removed). A micropipette was filled with an electrolyte that acted as the concentration or acceptor phase. The micropipette was partially inserted into the bottom of the hydrogel and a platinum wire was attached to the top of the hydrogel. The hydrogel prevented the electrolyte from flowing out of the pipette and also supported the electrical current when voltage was applied. The other end of the micropipette and another platinum wire were then dipped into the aqueous sample. The platinum wires were connected to the voltage power supply and grounded. Voltage was applied with positive polarity at the hydrogel end, and this produced an electric field which caused the entry and electrophoretic concentration of anions from the aqueous sample to the aqueous electrolyte inside the micropipette. The net flow inside the pipette during voltage application was zero because of the hydrogel [32]. The entire electrolyte was then manually transferred and analysed using CE.

3.2. Proof of concept

Fig. 1 also shows an electropherogram (b) of the electrolyte (50 mmol/L ammonium acetate at pH 8.3) after electrophoretic concentration of 10 mL sample at 1.3 kV for 20 min. An electropherogram of the sample that contained three inorganic anions,