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Analytical capillary isotachophoresis of bis(2-ethylhexyl) hydrogenphosphate and 2-ethylhexyl dihydrogenphosphate

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ABSTRACT

Bis(2-ethylhexyl) hydrogenphosphate (H2DEHP) and 2-ethylhexyl dihydrogenphosphate (HDEHP) are widely used liquid cation exchangers. An analytical capillary anionic isotachophoretic method was developed for the determination of H2DEHP and HDEHP in methanolic and aqueous phosphate-buffered solutions. The detection limit of the method was 3 nmol for H2DEHP and 0.16 nmol for HDEHP. H2DEHP was determined in aqueous phases at concentrations down to 15 μmol/l when it was previously ion-pair extracted by use of tetrabutylammonium hydrogensulphate. The recovery and the intraassay relative standard deviation of the method including the ion-pair extraction procedure were 78% and 6.8%, respectively.

INTRODUCTION

Organic phosphates such as bis(2-ethylhexyl) hydrogenphosphate (H2DEHP) and 2-ethylhexyl dihydrogenphosphate (HDEHP) (Fig. 1) are widely used liquid ion exchangers for selective ion-pair extraction of inorganic cations such as zinc, uranium and trace rare-earth elements [1–4]. Also, H2DEHP has been shown to be remarkably selective with respect to organic cations such as various biogenic amines [5] including histamine [6], a potent mediator of allergy and hypersensitivity. The high selectivity of H2DEHP with respect to histamine is utilized in our laboratory for the selective extracorporeal removal of histamine from blood of patients with acute hepatic failure by means of hollow-fibre-supported liquid membranes (HFSLM) which contain H2DEHP.

In order to study quantitatively the stability of H2DEHP-containing HFSLM, an accurate and sensitive analytical method is required. Both the acidimetric and the photometric picrate method

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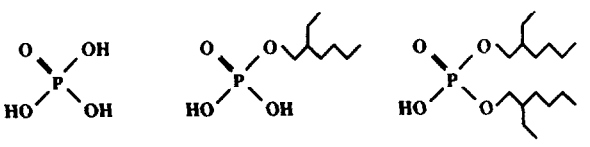

Fig. 1. Structures of inorganic phosphate (P_i), 2-ethylhexyl dihydrogenphosphate (HDEHP) and bis(2-ethylhexyl) hydrogenphosphate (H2DEHP).

[7] were found to be inapplicable for our purposes. Analytical capillary anionic isotachophoresis (ITP) has been shown to be an excellent analytical tool for the determination of inorganic and organic phosphates [8]. In this paper, an anionic ITP method for the determination of H2DEHP and HDEHP in organic and aqueous solution is described. For the sensitive determination of H2DEHP in aqueous buffered solutions, an ion-pair extraction procedure using the phase-transfer catalyst tetrabutylammonium hydrogensulphate (TBAHS) in chloroform was developed.

EXPERIMENTAL

Apparatus and materials

Anionic ITP analyses were performed on a Model 2127 Tachophor (LKB, Bromma, Sweden) using the conditions given in Table I. Isotachopherograms were recorded with an LKB Model 2120 line recorder at a chart speed of 0.5 mm/s. The terminator passed the detector at a potential of about 5 kV. H2DEHP and TBAHS were obtained from Serva (Heidelberg, Germany). A mixture of HDEHP and H2DEHP (45:55, w/w) was obtained from Merck–Schuchardt (Hohenbrunn, Germany). These and all other chemicals were of the highest quality available and used as received. Because of the low water solubility of H2DEHP and HDEHP, methanol was used to prepare stock solutions.

Ion-pair extraction procedure

H2DEHP was ion-pair extracted from potassium phosphate-buffered aqueous solutions (10–100 mmol/l, pH 7.4) as follows: TBAHS (0.8 g in the main experiments) was added to a 45-ml sample of a potassium phosphate-buffered aqueous solution of H2DEHP. After shaking with 5 ml of chloroform for 5 min the layers were allowed to separate for 15 min and a 4.5-ml aliquot of the organic phase was transferred into a conical centrifuge tube. The solvent was completely evaporated under reduced pressure and the residue was dissolved in 100 μl of methanol. Up to 5 μl of this solution were injected into the Tachophor instrument.

Hollow-fibre experiments

A liquid membrane preparation that contained paraffin oil, *n*-dodecanol and H2DEHP (75:20:5, w/w/w) was supported on polysulphone hollow fibres (Fresenius, Bad Homburg, Germany) by a similar procedure to that described elsewhere [9]. A potassium phosphate-buffered (10 mmol/l, pH 7.4) solution of histamine (100 ml small scale; 5000 ml large scale) was circulated in the internal compartment and a potassium phosphate-buffered (100 mmol/l, pH 7.4) solution

TABLE I

ITP CONDITIONS FOR THE DETERMINATION OF BIS(2-ETHYLHEXYL) HYDROGENPHOSPHATE AND 2-ETHYLHEXYL DIHYDROGENPHOSPHATE

Tachophor	LKB, Model 2127
Capillary	Polytetrafluoroethylene, 20 cm × 0.5 mm I.D.
Leading electrolyte	10 mmol/l HCl, pH 3.3 (β-alanine) 0.25% (w/w) hydroxypropylmethylcellulose
Terminating electrolyte	10 mmol/l hexanoic acid
Detector	UV (254 nm) and conductivity
Driving current	50 μA
Solvent	Water
Sample volume	3–5 μl
Temperature	Ambient

(20 ml small scale; 1000 ml large scale) in the external compartment of the HFSLM reactor. Samples (20 or 45 ml) were taken from both compartments of the HFSLM reactor after 5 h and H2DEHP was ion-pair extracted and analysed by ITP as described above.

RESULTS AND DISCUSSION

Fig. 2 shows an isotachopherogram from the analysis of the commercially available mixture of H2DEHP and HDEHP. In Table II the reciprocal reference unit (RRU) values and the specific zone lengths of inorganic phosphate (P_i), H2DEHP and HDEHP are summarized. RRU values were calculated from the relative step heights of the conductivity signal relative to the terminating ion applying the following expression:

$$R_{rel} = (h_T - h_L)/(h_C - h_L)$$

where R_{rel} is the RRU value of a compound C, relative to that of the terminating ion; h_T, h_L and h_C are the heights of the conductivity signals for terminating, leading ion and compounds C, respectively.

As can be seen in Fig. 2, P_i, H2DEHP and HDEHP were completely separated from one another. These compounds appear as non-UV-absorbing zones resolved by UV-absorbing peaks from impurities. The UV-absorbing zone from

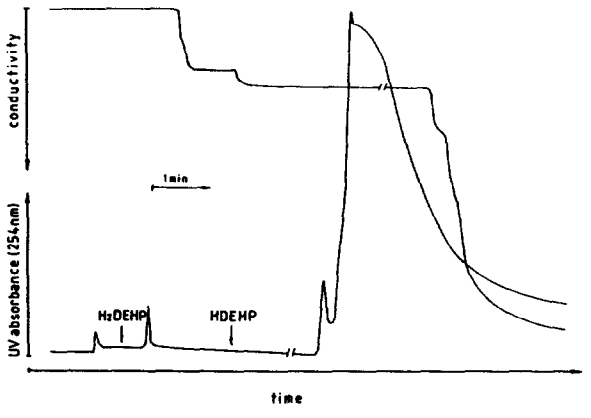

Fig. 2. Isotachopherogram from the analysis of 5 μl of a commercially available mixture of H2DEHP and HDEHP (55:45, w/w) in methanol (16 mg/ml). ITP conditions as described in Table I.

TABLE II

SPECIFIC ZONE LENGTHS AND RECIPROCAL REFERENCE UNIT (RRU) VALUES FOR INORGANIC PHOSPHATE (P_i), HDEHP AND H2DEHP USING THE CONDITIONS GIVEN IN TABLE I

Substance	Specific zone length (s/nmol)	RRU (mean ± S.D., $n = 6$)
P_i	5.37	6.24 ± 0.21
H2DEHP	0.29	4.60 ± 0.41
HDEHP	5.93	3.79 ± 0.13

an unknown compound that migrated behind HDEHP was always obtained when methanol was injected into the system. The specific zone lengths were obtained from the slopes of the calibration graphs obtained by injection of standard solutions of H2DEHP and HDEHP in methanol. The calibration graphs were linear in the range 5–50 mmol/l for H2DEHP and 0.3–3.0 mmol/l for HDEHP. From the calibration graphs the detection limit of the method was calculated to be 3 nmol for H2DEHP and 0.16 nmol for HDEHP. The precision of the method was determined by analysing monthly within a time of period of 6 months 100 nmol of H2DEHP. The mean specific zone length was 0.285 ± 0.008 (S.D.) s/nmol, and the interassay R.S.D. was 2.9%. The within-day ($n = 6$) R.S.D. was 1.8%.

Very low concentrations of H2EDHP and the presence of large amounts of phosphate in phosphate-buffered solutions used in HFSLM experiments make direct determination difficult and result in extremely long analysis times. These problems could be overcome by ion-pair extraction of H2DEHP from potassium phosphate-buffered aqueous solutions into chloroform by using TBAHS as ion-pairing agent. This procedure eliminates the interference by P_i (Fig. 3) as P_i is not extractable by TBAHS into chloroform. Also, the preconcentration effect significantly decreases the detection limit of the method. The recovery of H2DEHP and HDEHP from their phosphate-buffered solutions (250 and 15 μmol/l, respectively) was found to depend on the concentration of TBAHS in the buffer (Fig. 4). Sufficient recovery (75%) for H2DEHP was

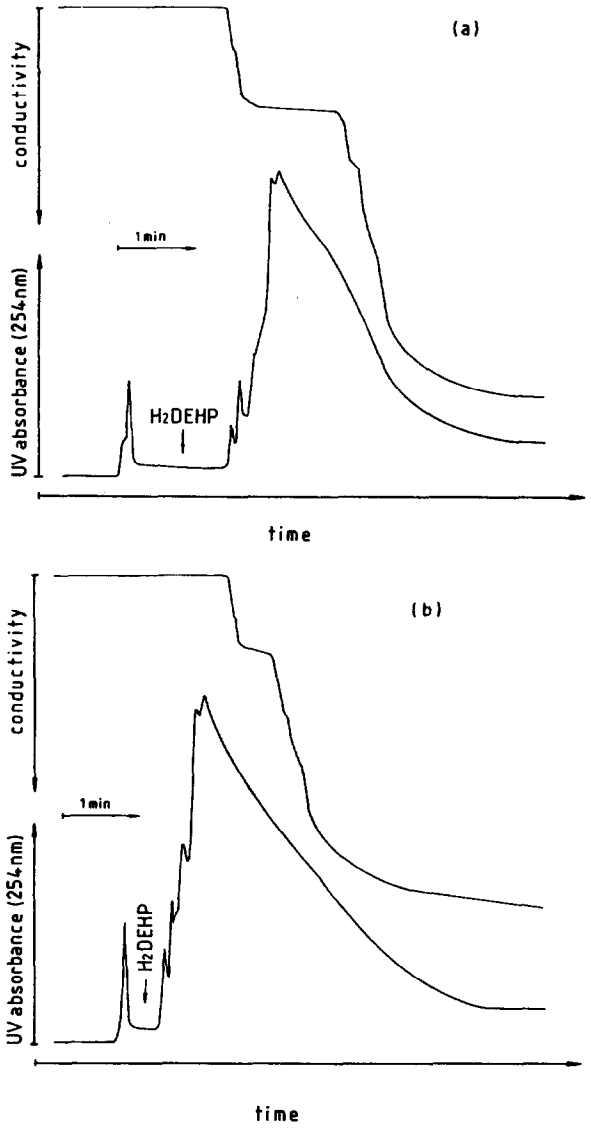

Fig. 3. Isotachopherograms from the separate analysis of H2DEHP following ion-pair extraction with TBAHS (a) from a standard solution (200 μmol/l) in phosphate-buffered solution and (b) from the internal compartment solution of a small-scale HFSLM experiment.

achieved by using 0.8 g of TBAHS in a 45-ml sample volume (final concentration 50 mmol/l). When H2DEHP (24–240 μmol/l) was ion-pair extracted using 50 mmol/l TBAHS, a straight line was observed with the regression equation $y = 0.777x - 3.702$, $r > 0.997$, where y is the measured and x the initial concentration of

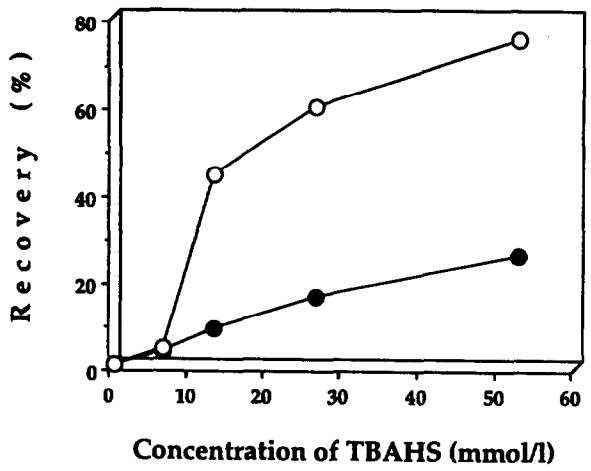

Fig. 4. Effect of the concentration of TBAHS on the recovery of (○) H2DEHP and (●) HDEHP (15 and 250 μmol/l, respectively) from potassium phosphate-buffer solutions (10 mmol/l, pH 7.4). The ion-pair extraction procedure is described under Experimental.

H2DEHP in the buffer. The slope of this line gives the recovery of the ion-pair extraction for H2DEHP, which amounted to 77.7%. Under these conditions, up to a 300-fold preconcentration of H2DEHP was achieved. The ion-pair extraction enables H2DEHP to be determined in aqueous solutions at concentrations down to 15 μmol/l. The intra-assay R.S.D. of the overall procedure was determined by analysing six samples of H2DEHP in buffer (100 μmol/l) and was found to be 6.8%.

In HFSLM experiments on both small and large scales, no H2DEHP was detectable in the external compartment of the reactor after 5 h. At this time the concentrations of H2DEHP in the internal compartment of the reactor were 55 ± 10 μmol/l for the small-scale and 180 ± 17 μmol/l for the large-scale experiments (mean ± S.D., $n = 3$). These values correspond to about 10% of the initial amount of H2DEHP in the HFSLM in both experiments. The release of H2DEHP from the HFSLM may be due to shear forces rather than physical solubility. Preliminary results show that the stability of the liquid membrane mainly depends on the composition of the liquid membrane, especially on its *n*-dodecanol content, and on other experimental conditions such as pores size and membrane

thickness of the hollow fibres and flow-rate through the hollow fibres.

The ITP method described here is currently used in our laboratory for stability studies on HFSLM in order to find the optimum composition of the liquid membrane and the experimental conditions for *in vivo* application. This ITP method should also be equally suitable in other fields in which H2DEHP, HDEHP and other related organic phosphates are involved, *e.g.*, in determining formation/dissociation constants of ion pairs between H2DEHP and inorganic or organic cations in two-phase systems [5].

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