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## Note

### Use of free-flow electrophoresis for the purification of components separated by ion-pair chromatography

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(Received February 2nd, 1989)

After separation by ion-pair chromatography<sup>1–8</sup>, isolated components still contain the ion-pair reagent (*e.g.*, tetrabutylammonium salts for anionic and heptanesulphonic acid for cationic substances), and if further characterization of a component by spectroscopic methods is necessary the ion-pair reagent has to be removed. If the substance is a large molecule there is no problem as a separation by dialysis, ultracentrifugation or gel chromatography is possible. However, if the substance is a small molecule, as with dyestuffs, the separation could be difficult. There is a need for a simple method that can quickly separate ion-pair reagents from small molecules. Here we describe a suitable purification method using free-flow electrophoresis<sup>9–17</sup>.

#### PRINCIPLE

Flanked by the two electrolyte solutions with high ionic strength, the sample solution with low conductivity is admitted to the separation chamber (Fig. 1). Because of the stepwise field strength profile resulting from the different ionic strengths of the different solutions, sample ions migrate according to their charge at high speed to the boundaries formed by the sample solution and the electrolyte solutions. At these boundaries the sample ion will be slowed and thereby focused. With this simple separation principle, it is possible to separate anionic substances from cationic ion-pair reagents (*e.g.*, tetrabutylammonium) and cationic substances from anionic ion-pair reagents (*e.g.*, heptanesulphonic acid).

#### EXPERIMENTAL

All chemicals were purchased from Fluka (Buchs, Switzerland) and were of analytical-reagent grade. Free-flow electrophoresis was performed in an Elphor VaP 21 apparatus with a separation chamber of 100 × 250 × 0.8 mm (Bender and Hobein, Munich, F.R.G.). The separation method used was field-step electrophoresis<sup>14–17</sup>. The conductivity of the anodic and cathodic electrolytes was 5 mS cm<sup>-1</sup>, and that of the sample was 0.4 mS cm<sup>-1</sup>. Experiments were performed in ammonium acetate buffer at pH 7.0 under 320 V and 100 mA. The flow of the buffer in the separation chamber was 8 ml per fraction per hour. As electrode buffer a solution of

0.04 *M* disodium hydrogenphosphate and 0.03 *M* potassium dihydrogenphosphate was pumped through both electrode compartments. The detection of the purified dyestuff was done with an adapted UV-scanner at 280 nm.

Thin-layer chromatography with silica gel 60 plates (Merck, Darmstadt, F.R.G.) was used to check the purity of the fractions from free-flow electrophoresis. The mobile phase was *n*-propanol-formic acid (8:2). The ion-pair reagent was detected with  $\text{Cl}_2/\text{KI}$ -starch<sup>18</sup>.

## RESULTS

Free-flow electrophoresis is well suited to the preparative purification of components separated by ion-pair chromatography. Field-step electrophoresis allows a throughput of 50–100 mg h<sup>-1</sup>. Even short residence times of about 60 s are sufficient to achieve complete separation of dyestuffs from the ion-pair reagent (Fig. 2). Afterwards the separation components still contain ammonium acetate buffer from the

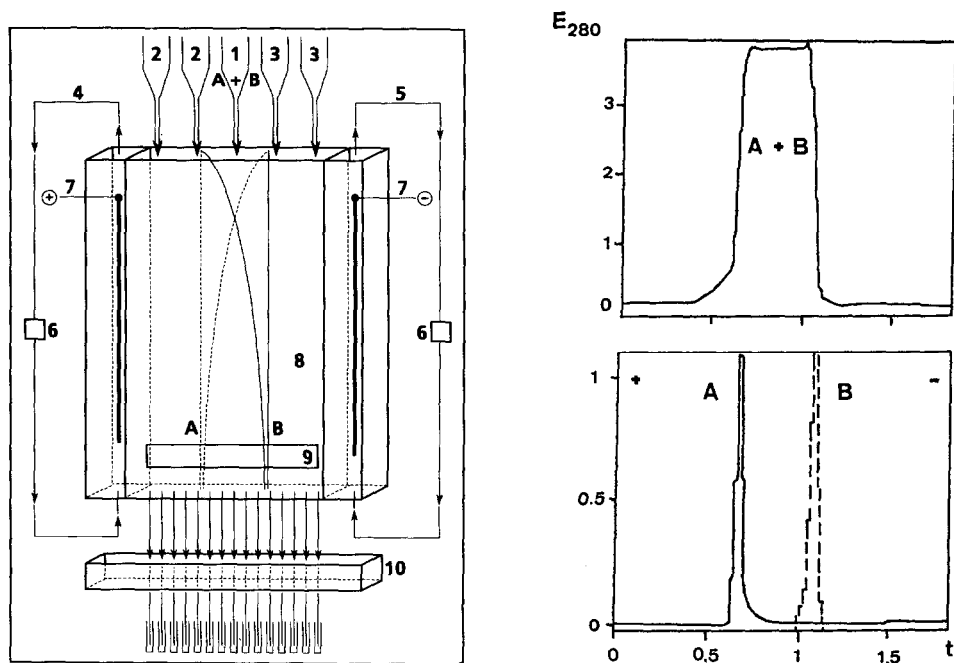


Fig. 1. Schematic diagram of the separation process. 1, Sample solution (mixture of dyestuff A with ion-pair reagent B); 2, anodic electrolyte; 3, cathodic electrolyte; 4, anodic electrode buffer; 5, cathodic electrode buffer; 6, electrode buffer pump; 7, electrodes (Pt); 8, separation chamber; 9, detection window; 10, fraction collector pump and fractions. Principle: the sample solution and the electrolytes are introduced at the top of the separation chamber consisting of two parallel plates. The electric field is applied perpendicular to the liquid flow. The charged molecules are deflected by the electrical field and are collected at the bottom through 90 outlet tubes.

Fig. 2. Separation of a dyestuff A<sup>-</sup> (fluorescein) from the ion-pair reagent B<sup>+</sup> (tetrabutylammonium). Above: the sample (mixture of 3 mg A and 30 mg B) is applied as a broad zone to the separation chamber. Below: separated zones A and B at the end of the separation chamber.

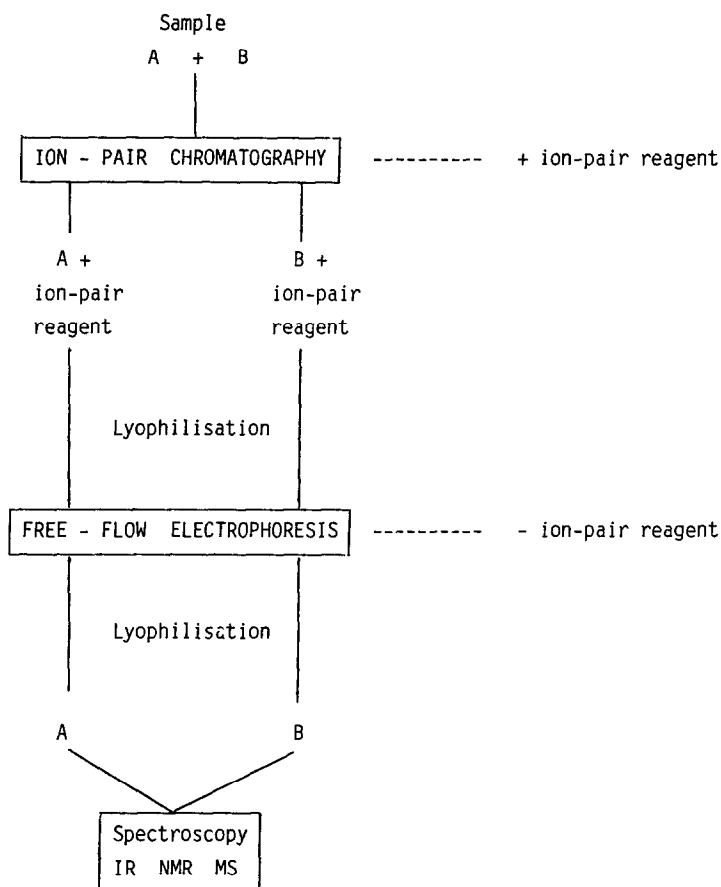


Fig. 3. Purification and isolation of dyestuffs by combination of ion-pair chromatography and free-flow electrophoresis.

electrophoresis, which can easily be removed by lyophilization. Substances purified in this way may be directly investigated using spectroscopic techniques (Fig. 3).

By means of free-flow electrophoresis, ion-pair chromatography can be used for the purification of components with similar molecular weight to the ion-pair reagent. This could be very helpful if the determination of the structures of dyestuffs and their impurities is necessary.

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