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Short Communication

Separation of phenylenediamine isomers by capillary zone electrophoresis

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ABSTRACT

A capillary zone electrophoretic method for the separation of positional isomers of phenylenediamine is presented. The phenylenediamines were baseline resolved using a fused-silica capillary. However, the analysis of samples containing both an excess of *p*-phenylenediamine and minor traces of the *meta* and *ortho* isomers, and also a high salt content, required a non-polar (DB-1) bonded phase in order to reduce the electroosmotic flow. The method can be used for purity testing of *p*-phenylene diamine and for the determination of impurities in hydrolysates of synthetic polymers.

INTRODUCTION

Phenylenediamines are widely used in the chemical industry, *e.g.*, in dye manufacture and as building blocks in synthetic polymers. The separation of the positional isomers of phenylenediamine by chromatographic methods is difficult. Liquid chromatographic methods [1–3] suffer from interferences from related compounds. Gas chromatographic methods are a good alternative from the resolution point of view, but they cannot be applied directly to aqueous samples. In addition, the cationic phenylenediamines have to be converted into their free bases, which are less stable.

Capillary zone electrophoresis (CZE), offering rapid and efficient separations of ionic compounds [4,5], has been successfully applied to the separation of positional isomers of aminobenzoic acid [6,7]. Employing minor differences in the pK_a values of *o*-, *m*- and *p*-phenylenediamine by selecting an appropriate pH of the buffer solution might be adequate for obtaining baseline resolution. However, the resolution of cationic analytes in CZE might be hindered by unintended interactions with the negatively charged surface of fused-silica capillaries. Apart from these interactions, the resolution of cationic analytes will also suffer from the similar directions of the electrophoretic migration and the electroosmotic flow. The resolution equation in CZE [4]:

$$R_s = 0.177\,(\mu_{ep1} - \mu_{ep2})\left[\frac{VL_d}{D(\mu_{epm} + \mu_{osm})L_t}\right]^{1/2} \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte, μ_{epm} the mean of the two electrophoretic mobilities, V the applied voltage, D the diffusion coefficient, L_d and L_t the length of the capillary to

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the detection window and the total length, respectively, and μ_{osm} the coefficient of electroosmotic flow, predicts that the maximum resolution will be obtained when the electroosmotic flow is low or, even better, in the opposite direction to the electrophoretic migration. Consequently, the separation of cationic analytes in unmodified fused-silica capillaries is theoretically less favourable. Several groups have studied modifications of fused-silica capillaries [8–18] and different types have recently been introduced commercially [19]. In this study, we used both unmodified fused-silica and DB-1 coated fused-silica capillaries for the separation of phenylenediamine isomers, traces of isomeric impurities and for the analysis of aqueous saline samples, this matrix simulating neutralized hydrochloric acid digests of synthetic polymers.

EXPERIMENTAL

Apparatus

An Applied Biosystems (San Jose, CA, USA) Model 270A capillary electrophoresis system [20] was used, equipped with a variable-wavelength UV absorbance detector, operated at 225 or 200 nm with a 0.5-s rise time. CZE was performed in 70 cm (50 cm from injection to detection) × 50 μm I.D. fused-silica capillaries (Applied Biosystems) and fused-silica capillaries coated with 0.05-μm DB-1 methylsilicone (J&W Scientific, Folsom, CA, USA). The voltage was +25 kV (constant-voltage mode) and the oven temperature was 30°C. Samples were introduced using a controlled vacuum system; the injection time was 0.5 or 1.0 s, which corresponds to volumes of *ca.* 1.5 and 3 nl, respectively. The coefficient of electroosmotic flow was calculated using the migration time of the system peak. The electrophoretic mobilities and the plate numbers were calculated using the equations in ref. 7. Data were recorded using a Nelson Analytical Model 4400 integration system.

Chemicals

Tris(hydroxymethyl)aminomethane (Tris) was obtained from Fluka (Buchs, Switzerland) and serine from Sigma (St. Louis, MO, USA). Phenylenediamine isomers were obtained from laboratory stock. All other chemicals were of analytical-reagent grade from J. T. Baker (Deventer, Netherlands). Distilled water was purified in a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Methods

The detection window in the DB-1 capillary was prepared by removing 4 mm of the polyimide outer film using a razor blade. Buffers were filtered through 0.45-μm Spartan 30/B membrane filters (Schleicher & Schuell, Dassel, Germany) prior to use. Stock solutions of the phenylenediamines ($1.0 \cdot 10^{-2}$ *M*) were prepared in water or saline solutions. Sample solutions were prepared by dilution of the stock solutions with the electrophoresis buffer.

RESULTS AND DISCUSSION

Initial experiments were performed using an electrophoresis buffer of 40 m*M* Tris–acetate (pH 4.8). The excess of protonated Tris ions and the weakly acidic pH suppressed adsorption of the cationic phenylenediamines on the fused-silica wall, thus yielding good peak shapes without any tailing. The separation of a mixture consisting of $1.0 \cdot 10^{-4}$ *M* of *p*-phenylenediamine (PPD) and $5.0 \cdot 10^{-5}$ *M* of *m*-phenylenediamine (MPD) and *o*-phenylenediamine (OPD) is shown in Fig. 1. All isomers are easily baseline resolved within 6.5 min and non-ionic or anionic matrix components, if present, will show up at or later than 7.8 min (electroosmotic flow marker) and not interfere. The electrophoretic mobilities and the coefficient of electroosmotic flow were $0.282 \cdot 10^{-3}$, $0.137 \cdot 10^{-3}$, $0.085 \cdot 10^{-3}$ and $0.297 \cdot 10^{-3}$ $cm^2 V^{-1} s^{-1}$, respectively. The plate numbers ranged from 173 000 for PPD and MPD to 143 000 for OPD.

In order to test the feasibility for purity testing, an excess of PPD ($1.0 \cdot 10^{-2}$ *M*) was mixed with $1.0 \cdot 10^{-4}$ *M* of MPD and OPD. The electropherogram is shown in Fig. 2. It can be seen that, despite the 100-fold excess of PPD, the resolution has been maintained and the migration times are still the same. However, the response of MPD and OPD decreased significantly in comparison with Fig. 1. It can be concluded that the isomeric purity of PPD reagents can be determined in this way semiquantitatively only; the detection limit for the isomeric impurities will be of the order of 0.5%, relative to the main component.

Next, the feasibility of the determination of traces

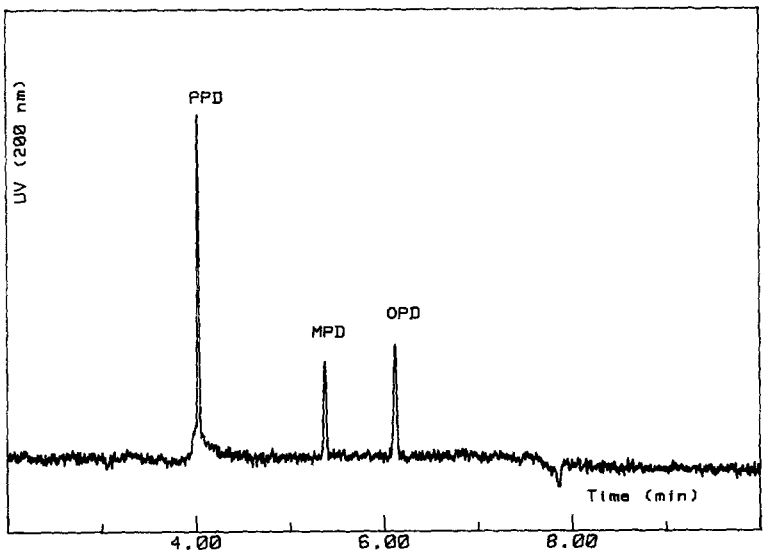

Fig. 1. CZE separation of positional isomers of phenylenediamine using an uncoated fused-silica capillary and 40 m*M* Tris–acetate buffer (pH 4.8). Constant voltage, +25 kV. Detection by UV absorbance at 200 nm. Injection, 1-s vacuum of 10^{-4} *M* PPD and $5 \cdot 10^{-5}$ *M* of both MPD and OPD. System peak (electroosmotic flow marker) at 7.8 min.

of OPD and MPD in an excess of PPD, dissolved in an aqueous saline solution, was investigated. The presence of 0.3 *M* sodium chloride in the sample turned out to be disastrous as OPD and MPD traces could not be detected owing to excessive band broadening. Nevertheless, the presence of high salt concentrations can also be exploited in a positive way [21,22]: the excess of sodium can act as a leading ion and provide a temporarily isotachophoretic stage with inherent peak focusing, provided that the appropriate terminating ion is present. Replacement of Tris– with serine–acetate (pH 4.5) should fulfil

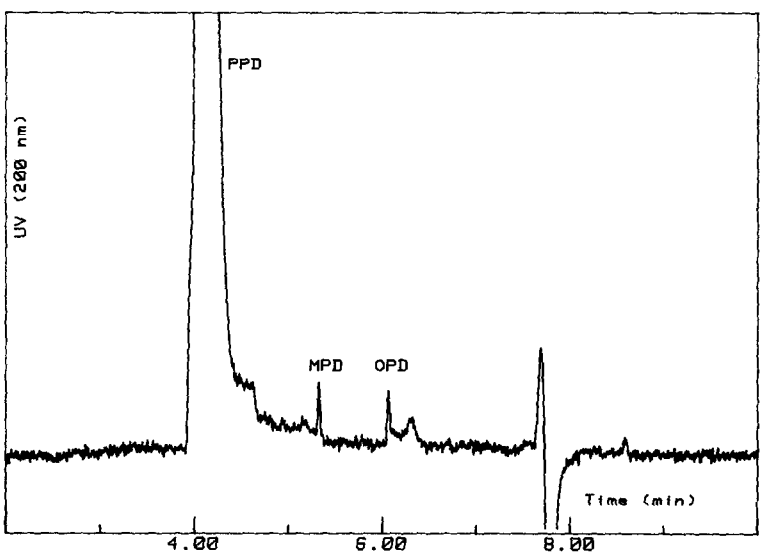

Fig. 2. CZE separation of an excess of PPD (10^{-2} *M*) and traces of MPD and OPD (10^{-4} *M* each). System peak (electroosmotic flow marker) at 7.8 min. Other conditions as in Fig. 1.

this requirement. The mobility of serine is very low as the pH of the buffer is relatively close to the isoelectric point of serine. The serine–acetate buffer showed an increased separation speed and an increase in the electroosmotic flow ($\mu_{osm} = 0.686 \cdot 10^{-3}$ cm^2 V^{-1} s^{-1}) owing to the decreased ionic strength and the lower tendency of serine to interact with the capillary wall. The positional isomers were still baseline separated but the resolution and peak capacity decreased because of the increase in the electroosmotic flow (*cf.*, eqn. 1); moreover, an excess of PPD in a saline matrix overlapped with traces of MPD and OPD. Decreasing the electroosmotic flow while maintaining the new buffer ion would be attractive. Therefore, a hydrophobically (DB-1) modified fused-silica capillary was investigated.

With the modified DB-1 capillary, the electroosmotic flow decreased towards $0.424 \cdot 10^{-3}$ cm^2 V^{-1} s^{-1} (system peak at 5.5 min) using serine–acetate. When the samples were dissolved in a 0.3 *M* sodium chloride sample matrix, the intended focusing occurred: the peak width decreased from 4.0 to 2.0 s while the migration time increased by 1 min (the plate number of OPD increased from 86 000 to 445 000). The electropherograms obtained with a real sample are shown in Fig. 3: (1) the electropherogram of $1.0 \cdot 10^{-2}$ *M* PPD in 0.3 *M* NaCl, where the two arrows indicate traces of isomeric impurities; (2) the same sample spiked with $1.0 \cdot 10^{-4}$ *M* MPD, where the arrow indicates the OPD impurity in the original sample, and (3) the sample spiked with $1.0 \cdot 10^{-4}$ *M* of both OPD and MPD. Note that the system peak has shifted from 5.5 towards 6.5 min because of the focusing step. Consequently, the system peak can no longer be used as an electroosmotic flow marker. A disadvantage of isotachophoretic-like sample introduction techniques is the decreased reproducibility of migration times, which actually become matrix dependent. Consequently, identification and quantification via standard addition techniques is recommended. The isomeric impurities in our PPD reagent were thus found to represent 0.1% of MPD and OPD. The detection limit of OPD relative to an excess of PPD will be lower than 0.05%, which is adequate both for purity testing of PPD reagents and for the detection of impurities in hydrolysates of synthetic polymers.

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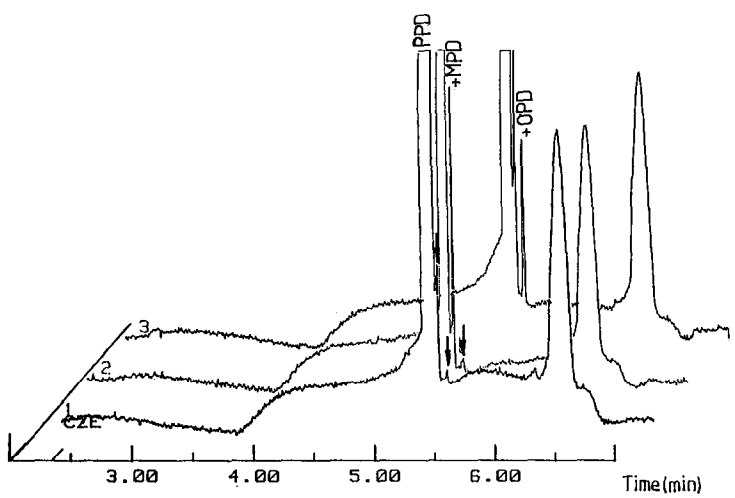

Fig. 3. Electropherograms of (1) 10^{-2} *M* PPD in 0.3 *M* sodium chloride, (2) spiked with 10^{-4} *M* MPD and (3) spiked with 10^{-4} *M* MPD and 10^{-4} *M* OPD. Conditions: DB-1 coated fused-silica capillary and 0.6 *M* serine–acetate buffer (pH 4.5). Injection, 1-s vacuum of a $2 \cdot 10^{-2}$ *M* PPD solution in 0.6 *M* NaCl, diluted 1:1 with buffer, followed by 10-s vacuum injection of the buffer. Detection by UV absorbance at 225 nm. System peak at 6.5 min.

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