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Switching valve with internal micro precolumn for on-line sample enrichment in capillary zone electrophoresis

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ABSTRACT

The design of a switching valve containing a micro precolumn for on-line sample enrichment in capillary electrophoresis is described. Samples are loaded on to the precolumn with a micro liquid chromatographic pump, while desorption is performed by means of the electroosmotic flow. The influence of the precolumn on the capillary zone electrophoretic separation process was investigated. A zone-cutting procedure was introduced, by means of valve switching, to prevent excessive band broadening and shifting of the migration times. Using papaverine as a model compound, it was shown that sample volumes of up to 100 μl can be enriched on the precolumn. Calibration plots are linear over a concentration range of two orders of magnitude. The detection limit of papaverine is $5 \cdot 10^{-8}$ *M* (5 pmol injected; UV detection at 254 nm). The potential of the sample-enrichment valve for on-line coupling to micro liquid chromatography is discussed.

INTRODUCTION

Recent developments in micro separation systems have significantly improved their applicability. Especially in the field of capillary electrophoresis (CE), the combination of high separation efficiency, sensitive detection modes (*e.g.*, amperometric, laser-induced fluorescence, mass spectrometry) and special injection techniques provide low absolute detection limits [1]. Nevertheless, the analysis of complex samples still requires the development of sample treatment methods suitable for micro separations. Apart from the removal of components that can clog the analytical separation system, reduction of the large number of interfering compounds in complex samples is necessary. In addition, the limited injection volumes commonly used in CE (< 10 nl) restrict the detectability of analytes. In addition to off-line liquid–liquid extraction and precipitation methods, on-line coupled techniques such as liquid chromatography (LC)–capillary zone electrophoresis (CZE) [2] and isotachophoresis (ITP)–CZE [3] can be used for sample clean-up. Coupling of ITP to CZE has also been used to achieve analyte enrichment. Electrodesorption in combination with zone electrophoresis, isoelectric focusing and ITP can also be used for the desorption of protein ligates [4–7].

Recently, off-line solid-phase cartridges have been used to achieve trace enrichment in CZE [8]. In another study, capillaries with interactive walls, *i.e.*, coated capillaries which are frequently used in gas chromatography (GC), have been used for on-line preconcentration in CZE [9]. Using these techniques one order of magnitude analyte enrichment was achieved. However, so far the off-line techniques have not been very successful because they are time consuming and laborious, while the on-line techniques lack sample capacity or result in severe band broadening and, consequently, in loss of resolution. As the use of precolumns to increase sensitivity in GC is a well known technique, as an alternative to

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overcome the mentioned limitations, in this work a laboratory-made rotary-type switching valve was developed that contains a micro precolumn in order to obtain on-line sample enrichment in CZE. Sorption on to the precolumn is performed using an LC pump and desorption is achieved by using electroosmotic flow.

EXPERIMENTAL

Chemicals and solutions

Phosphoric acid, sodium dihydrogenphosphate, sodium hydroxide, concentrated hydrochloric acid, glacial acetic acid, tetrabutylammonium bromide, octanesulphonic acid and phenol were purchased from J. T. Baker (Deventer, Netherlands). Acetonitrile (HPLC grade) was obtained from Westburg (Leusden, Netherlands). Codeine, noscapine and papaverine hydrochloride were a gift from P. de Goede (Academic Hospital, Free University, Amsterdam, Netherlands); they were stored as concentrated 10 m*M* aqueous solutions in distilled, demineralized water at 5°C. All diluted samples (1 μM–0.1 m*M*) were prepared daily in demineralized, distilled water. Unless mentioned otherwise, electrophoresis was performed in an aqueous 7.5 m*M* phosphate buffer (pH 4.5) containing 25% (v/v) acetonitrile. In order to reduce gas bubble formation, sample solutions contained 5% (v/v) acetonitrile and were degassed with helium before use.

Instrumentation

CZE was performed using a modified Brandenburg (Thornton Heath, UK) Alpha Series II high-voltage power supply which was operated in the constant-current mode (30 μA, voltage *ca.* 16 kV). All instruments were placed in a Plexiglas safety box. A 200 μm I.D. (340 μm O.D.) fused-silica capillary obtained from Polymicro Technologies (Phoenix, AZ, USA) with a total length of 60 cm was used for the electrophoretic separation. After installation of the capillary and after every ten experiments the capillary was washed with 0.1 *M* hydrochloric acid and 0.1 *M* potassium hydroxide in order to clean and activate the capillary wall. Glass vials containing platinum electrodes were used as electrode vessels. A fan was used to cool the outer wall of the electrophoresis capillary. Detection was performed at 250 nm with an Applied Biosystems (Ramsey, NJ, USA) Model 757 UV absorbance detector, modified for on-column capillary detection.

A laboratory-made six-port injection valve equipped with a 1.0-ml loop was used to load the sample. The sample was flushed on to the precolumn in the switching valve (see below) by means of an LDC/Milton Roy (Riviera Beach, FL, USA) Micrometric 5-ml syringe pump.

For the LC separation a slurry-packed 14 cm × 320 μm I.D. microcolumn containing 5-μm RoSil C_8 (RSL, Eke, Belgium), was used. An LKB (Bromma, Sweden) Model 2150 pump was used for mobile

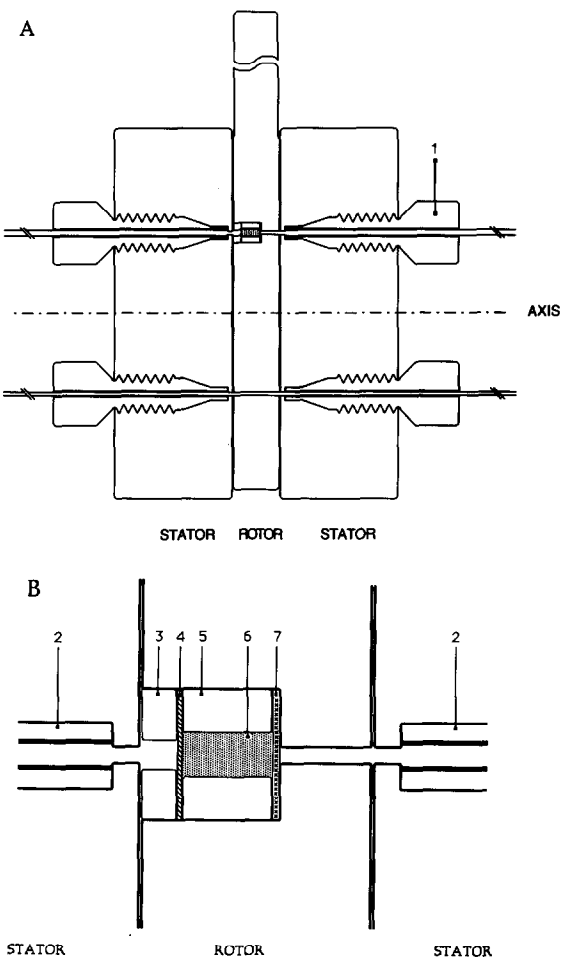

Fig. 1. (A) Cross-sectional view of the switching valve with (B) the precolumn. 1 = Finger-tight connections; 2 = capillary inlet/outlet; 3 = 1.5 mm × 0.3 mm I.D. PTFE tubing; 4 = metal screen; 5 = 1.5 mm × 0.5 mm I.D. PEEK tubing; 6 = PLRP-S packing material; 7 = PTFE screen.

phase delivery. Noscapine, codeine and papaverine were separated using methanol–water (40:60, v/v), containing 1% (v/v) glacial acetic acid, 10 m*M* tetrabutylammonium bromide and 2.5 m*M* octanesulphonic acid, as the mobile phase. The separation was performed at ambient temperature.

Switching valve with internal precolumn

The switching valve containing the micro precolumn was a rotary-type injection valve [10], modified for small sample volumes and containing a laboratory-made precolumn. Fig. 1A shows a cross-sectional view of the valve. The polyethylene rotor (6 mm thick) contains the precolumn and a 200 μm I.D. channel parallel to the precolumn. The precolumn (Fig. 1B) consisted of 1.5 mm × 500 μm I.D. (volume 0.2–0.3 μl) poly(ether ether ketone) (PEEK) tubing (Alltech, Zwijndrecht, Netherlands) slurry packed with 8-μm PLRP-S divinylbenzene–styrene polymer (Polymer Laboratories, Church Stretton, UK). The inlet of the column was closed with a metal screen (Metaalgaasweverij Dinxperlo, Dinxperlo, Netherlands) and the outlet with a PTFE screen (Alltech). The metal screen was needed for mechanical support during the sample loading procedure and to prevent clogging of the column inlet. A piece of 1.5 mm × 300 μm I.D. PTFE tubing was used to position the microcolumn in the rotor. The rotor was mounted between two poly(ethylene terephthalate) stators; it can be used at pressures up to 150 bar without leakage. Capillaries were connected to the valve with laboratory-made finger-tight connections.

RESULTS AND DISCUSSION

Performance of the switching valve

In order to study the performance of the precolumn, the rotary type switching valve was coupled to an LC set-up (Fig. 2A). For this study the analytical column in the set-up was replaced with a 25 cm × 200 μm I.D. fused-silica capillary. Via the six-port valve, sample solution was flushed directly on to the micro precolumn in the switching valve by means of a micro LC pump (pump 1). An LC pump (pump 2) was used to pump the eluent through the switching valve, *i.e.*, the precolumn, via the capillary, to the UV detector.

Papaverine ($pK_a = 6.6$) was chosen as model compound. This analyte is hardly ionized in demineralized water so that trace enrichment on the polymer-packed precolumn will present no problems. To obtain information on the breakthrough characteristics of papaverine, 10^{-4} *M* sample solutions were loaded on to the micro precolumn (1.5 mm × 0.5 mm I.D.) until analyte breakthrough was observed. Breakthrough volumes larger than 1 ml were obtained when papaverine was dissolved in demineralized water. The addition of organic modifier to the sample solution resulted in lower breakthrough volumes: a breakthrough volume of 320 μl was obtained with a water–acetonitrile (95:5, v/v) sample solution, whereas almost immediate breakthrough (breakthrough volume 2 μl) occurred in the presence of 25% acetonitrile.

Because micro separation systems, and particularly CE, are sensitive to gas bubbles formed in the buffers used for the analytical separation, the addition of 5% of acetonitrile was found necessary in order to obtain efficient degassing. In all further experiments sample solutions in water–acetonitrile (95:5, v/v) were used. Furthermore, sample volumes loaded on the precolumn were 100 μl or less in order to prevent breakthrough.

Loading of the sample on to the precolumn involves three steps. First, the precolumn is washed with 10 μl of aqueous 7.5 m*M* phosphate buffer (pH 2.2)–acetonitrile (75:25, v/v) to remove contaminants from the precolumn. Next, the precolumn is conditioned with 10 μl of water–acetonitrile (95:5, v/v) at a flow-rate of 5 μl/min, after which the sample is loaded in the forward flush mode. Finally, the analyte is desorbed by the mobile phase and flushed to the detector via the fused-silica capillary. The sample is loaded on to the precolumn using a flow-rate of 5 μl/min. Using this flow-rate the peak width of the desorbed analyte is 30 s and the peak height is 0.56 absorbance units. If higher flow-rates (*e.g.*, 10 μl/min) are used the accuracy for small sample volumes will decrease [relative standard deviation (R.S.D.) > 10%] whereas for lower flow-rates the peak width increases and the peak height decreases.

The loading procedure was found to be linear over a range of 1–100 μl of sample solution loaded on to the precolumn. The plot of peak height (UV absorbance) *versus* sample volume was given by the equation $y = 0.40\ (\pm 0.01)x + 1.54\ (\pm 0.39)$ ($r^2 = 0.994$,

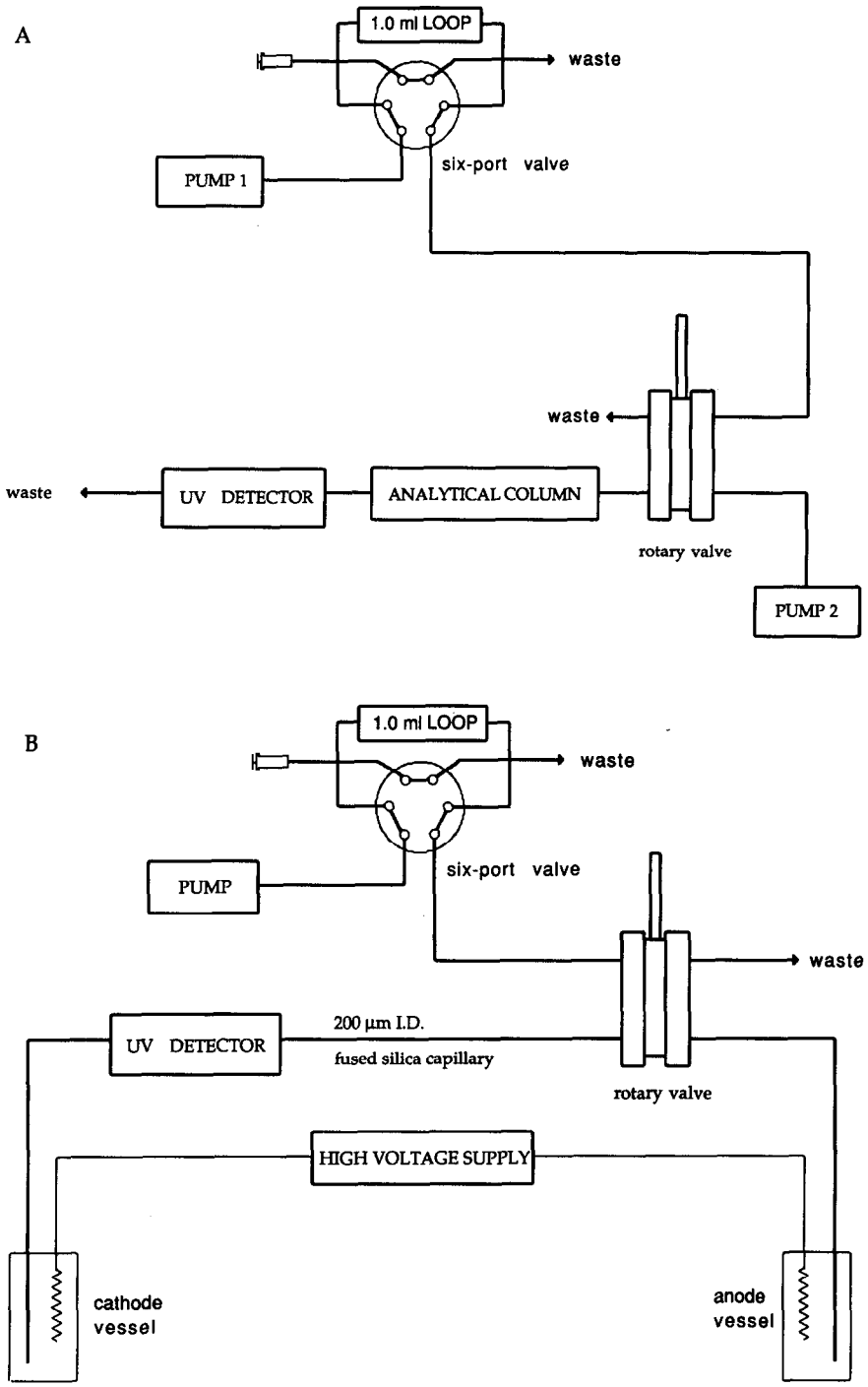

Fig. 2. Set-up of the switching valve coupled to micro separation systems. (A) Switching valve coupled to micro LC system. Pump 1 is used to flush the sample solution into the switching valve and pump 2 for eluent delivery. (B) Switching valve coupled to CZE system.

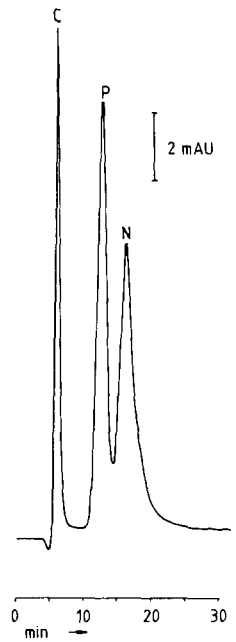

Fig. 3. LC–UV of a mixture of codeine (C) (10^{-5} *M*), noscapine (N) (10^{-5} *M*) and papaverine (P) (10^{-6} *M*) in water after enrichment of 100 μl of sample. The 1.5 mm × 500 μm I.D. precolumn was packed with 8-μm PLRP-S. UV detection at 250 nm. Eluent flow-rate, 3 μl/min.

$n = 6$, 10^{-6} *M* papaverine), where y and x are in milliabsorbance units and μl, respectively. As an example, Fig. 3 shows the reversed-phase ion-pair LC separation of a mixture of codeine, papaverine and noscapine with on-line trace enrichment.

Rotary switching valve coupled to CZE

The switching valve is made of synthetic material which allows the direct coupling to a high-voltage CZE system. The set-up for the CZE experiments is shown in Fig. 2B. The injection valve was placed in-line between two electrode vessels. The CZE separation was performed in a 60 cm × 200 μm I.D. fused-silica capillary. The high-voltage power supply was operated in the constant-current mode. In this mode temperature changes will have less influence on the electrophoretic separation than when using the constant-potential mode. In this study currents of up to 30 μA (electric field 180–250 V/cm) were used. The use of higher currents frequently caused the formation of gas bubbles, which led to breakdown of the electrophoretic separation process.

Instead of a hydrodynamic flow delivered by an LC pump, an electroosmotic (EO) flow, produced by means of the high voltage, was used for eluent delivery. If an electric field is applied over a fused-silica capillary having a negatively charged wall

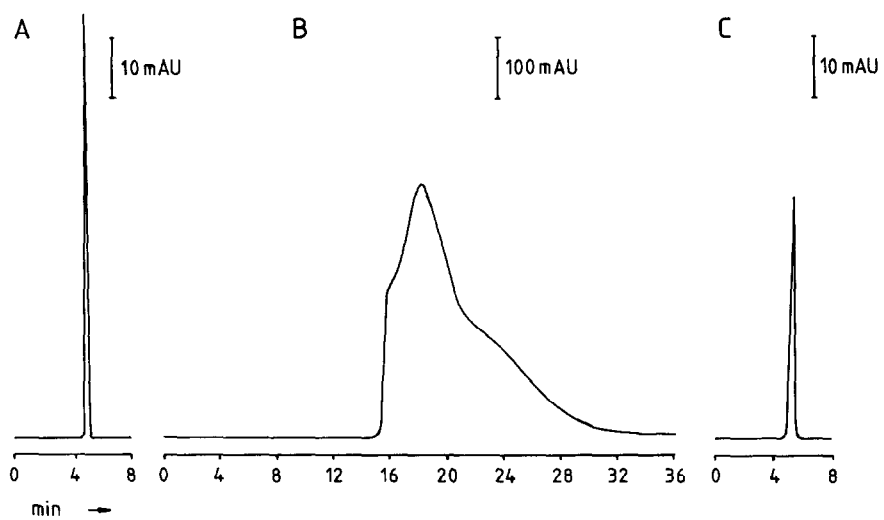

Fig. 4. (A) Electropherogram of 0.2 μl of papaverine (10^{-4} *M*) in water–acetonitrile (95:5, v/v) after direct injection. (B) Electropherogram of 5 μl of papaverine (10^{-4} *M*) in water–acetonitrile (95:5, v/v) with precolumn on-line during electrophoresis (current, 20 μA). (C) Electropherogram of 5 μl of papaverine (10^{-5} *M*) in water–acetonitrile (95:5, v/v) after electrodesorption procedure; desorption time, 45 s; current, 30 μA. For detailed conditions, see text.

surface, movement of the ion double layer will cause an EO flow. The EO flow-rate depends on parameters such as ionic strength, pH and viscosity of the buffer solutions and the electric field strength. In this study the EO flow and the analyte migration were both towards the negative electrode, which means that detection should take place at the cathode side of the capillary (Fig. 2B).

Sample enrichment and desorption. Enrichment of the papaverine-containing sample solution on the precolumn in the rotary switching valve was performed as described above. The precolumn was washed with *ca.* 10 μl of aqueous 7.5 m*M* phosphate buffer (pH 4.5)–acetonitrile (25:75, v/v) and conditioned with 10 μl of water–acetonitrile (95:5, v/v). Next, it was loaded with papaverine, dissolved in water–acetonitrile (95:5, v/v) at a flow-rate of 5 μl/min and washed with 0.2 μl of water–acetonitrile (95:5, v/v) to flush the valve dead volume. After washing, the precolumn was switched on-line with the CZE capillary. Next, a constant current of 30 μA was applied and the precolumn was desorbed using the EO flow. During desorption the analyte becomes charged due to the pH shift ($7 \rightarrow 4.5$) and electrophoresis will take place.

When papaverine was desorbed in the forward-flush mode, significant band broadening occurred (compare Fig. 4A and B). This band broadening is probably caused by a combination of several effects; apart from the slow desorption, which was also observed with packed capillaries in other studies [4–7], the relatively large precolumn volume, the dead volume of the injection valve and the disturbance of the EO flow profile caused by the back-pressure of the precolumn are important factors. In addition, the migration time was considerably higher, *i.e.*, 13 min, in the presence (Fig. 4B) than in the absence (Fig. 4A) of the precolumn. The increase in migration time is the result of a *ca.* 70% decrease in the EO flow that occurs on switching the precolumn on-line with the electrophoresis capillary. The EO flow will decrease because of the mechanical resistance of the precolumn and, as the inner diameter of the precolumn (500 μm) is significantly larger than that of the electrophoresis capillary (200 μm), the electric field strength will be smaller. An increase in migration time was also observed in experiments with phenol ($pK_a = 10$), instead of papaverine used as test compound. Under the test conditions phenol is neutral and will therefore have a migration velocity equal to the EO flow. For currents of both 25 and 35 μA it was found that the EO flow-rate decreased by 60–75%, *i.e.*, from 27 and 35 mm/min to 7 and 14 mm/min, respectively, when the precolumn was switched on-line.

TABLE I

DEPENDENCE OF ANALYTE RECOVERY ON ELECTRODESORPTION TIME

Conditions: loading of 5 μl of 10^{-5} *M* papaverine solution; 1.5 mm × 0.5 mm I.D. precolumn; current, 30 μA; $n = 2$, R.S.D. < 5%.

Electrodesorption time (s)	Peak height (milliabsorbance units)	$w_{0.5}$[a] (s)
30	13	< 30
40	34	30
45	44	30
60	42	50

[a] Peak width at half-height.

To prevent excessive band broadening and increased migration times, desorption of the sample was carried out in the backflush mode and a zone-cutting procedure by means of valve switching was used. After loading of the sample on the precolumn the rotor was switched and backflush desorption of the analyte was performed by applying the current (30 μA) for a limited period of time (30–60 s). After (part of) the analyte had been transferred into the capillary, the power supply was switched off and the rotor was switched back to its initial (load) position. Finally, the high voltage was switched on again and electrophoresis was continued (current 30 μA). For the time used for electrodesorption in the backflush mode, a compromise between sensitivity and separation efficiency had to be found. Large desorption times will cause an increase in the amount of analyte transferred into the separation capillary, leading to an increased peak height but, unfortunately, also an increased peak width (*cf.*, Table I). Using an electrodesorption time of 45 s (current 30 μA), the band broadening was dramatically reduced compared with results obtained with forward flush desorption (compare Fig. 4C and B). Further, the migration time now was approximately the same as that obtained with direct injection of the sample (Fig. 4A).

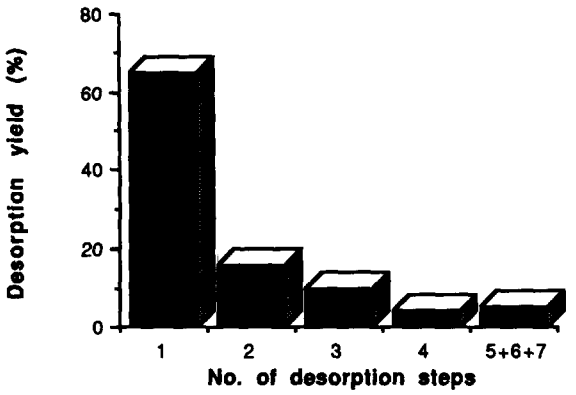

Fig. 5. Yield of the electrodesorption of papaverine for seven repeated desorption steps by means of valve switching, after a single sample loading. Backflush time, 45 s; current, 30 μA. For detailed conditions, see text.

To determine the amount of analyte desorbed during the 45-s desorption step, the valve switching procedure was repeated seven times (*i.e.*, 7 × 45 s with a current of 30 μA) after a single loading of 10 μl of a papaverine solution, without flushing of the precolumn in between. After the seventh cycle desorption of papaverine was complete. Fig. 5 shows the yield of papaverine after each desorption step, expressed as a percentage of the total yield. The yield after the first injection was 65%. In all further experiments an electrodesorption time of 45 s was used. However, it will be obvious that the desorption time used is optimized for papaverine, and that it should be modified when other analytes are to be determined.

The on-line enrichment procedure for CZE was investigated for sample volumes of up to 100 μl, using water–acetonitrile (95:5, v/v) containing 10^{-5} *M* papaverine. The plot of UV absorbance *versus* sample volume in Fig. 6A shows satisfactory linearity ($r^2 = 0.992$). A linear calibration graph was also obtained when analysing 1-μl samples containing 10^{-5}–10^{-3} *M* papaverine ($r^2 = 0.998$) (Fig. 6B). The R.S.D. for four consecutive injections (5 μl of sample, 10^{-5} *M* papaverine) was 6%. With the described enrichment–desorption and CZE–UV procedure a limit of detection of $5 \cdot 10^{-8}$ *M* papaverine (signal-to-noise ratio = 3; UV detection at 254 nm) was achieved; using a 100-μl sample an absolute limit of detection of 5 pmol can be obtained.

CONCLUSIONS

The rotary switching valve with an internal precolumn has promising features for on-line analyte enrichment in capillary separation systems. The high pressure resistance (up to 150 bar) of the valve allows coupling to micro LC, as demonstrated by the analysis of the opiate mixture. As the valve is constructed from synthetic material it can be coupled to CE systems, which is an advantage over micro precolumn-containing switching valves described previously [11]. In comparison with published sample treatment methods for CZE, the main advantage of the present system is that it allows (i) on-line sample pretreatment and, thus, automation, (ii) the next injection to be carried out while the CZE separation is still running and (iii) selective sample pretreatment by using suitable stationary phases. In addition, the system is easy to operate.

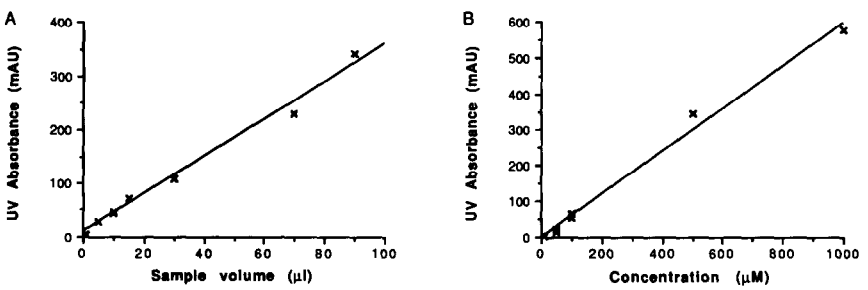

Fig. 6. (A) Plot of peak height (milliabsorbance units) *versus* loaded sample volume (μl) (10^{-6} *M* papaverine). The equation is $y = 3.5\ (\pm 0.1)x + 9.7\ (\pm 5.7)$, where x represents the sample volume and y the UV absorption signal. (B) Plot of peak height (milliabsorbance units) *versus* papaverine concentration (μ*M*) in 1 μl of a water–acetonitrile (95:5, v/v) sample solution. The equation is $y = 6.0\ (\pm 0.2)x + 2.3\ (\pm 8.5)$, where x represents the sample concentration and y the UV absorption signal.

Keeping the precolumn on-line with the separation capillary during the electrophoretic run caused excessive band broadening and increased migration times. The introduction of a rapid zone cutting–desorption procedure essentially solved this problem. As regards the analytical performance of the set-up, sample enrichment was linear over a large range of volumes (1–100 μl). This corresponds to an increase in the sensitivity of 2–3 orders of magnitude compared with a 0.2-μl direct injection. Furthermore, enrichment was linear over a concentration range of at least two orders of magnitude.

The present switching valve can also be coupled to other micro separation systems. For example, coupling to ITP has the advantage that zone sharpening will occur during the ITP run. This zone sharpening effect, which depends on the concentration and mobility of analytes and leading electrolyte, will suppress the zone broadening owing to slow analyte desorption. This means that ITP can be performed with the precolumn in-line, that is, quantitative desorption of the analyte from the precolumn can now be achieved.

Further investigations are to be made in order to study the influence of real samples on the recovery, selectivity and linearity of the desorption procedure.

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