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COUPLING LIQUID CHROMATOGRAPHY WITH CZE BY MEANS OF A SOLENOID VALVE AND AN INJECTION DEVICE

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Abstract. The coupling of liquid chromatography with capillary zone electrophoresis in one hyphenated analytical system is described. A simple interface that transports the effluent from LC into CZE capillary is proposed. This interface consists of a solenoid valve and a home-made injection device and enables on-line analysis of the LC effluent by means of CZE in case of flow rates higher than 0.5 mL/min under computer control.

Key words: hyphenated methods, capillary zone electrophoresis, CZE injection.

INTRODUCTION

During the last two decades a number of hyphenated methods that utilize in one analytical system two or more principles of separation have been developed for the analysis and separation of complex mixtures. One of the variety of possibilities (GC–MS, GC–GC, CE–MS, etc.) is the combination of liquid chromatography (LC) with capillary zone electrophoresis (CZE). Pairing these techniques could result in good separation since, as was demonstrated by Steuer et al. [1], LC and CZE are highly orthogonal separation methods based on different properties of analysed species. The main "trick" in coupling LC with CZE is the automated transportation of the effluent sample from the LC column into the CZE capillary. The design of an LC–CZE interfacing system can be arranged in different ways depending on the LC column scale. For example, Jorgenson with co-workers used a six-port loop/valve for coupling the reversed-phase microbore HPLC to the CZE capillary [2] and a transverse flow gating interface for connecting microcolumn size exclusion chromatography to the capillary [3]. As a rule, the use of microseparation techniques for coupling

demands microscopic size interfacing arrangements, which are usually made with the aid of micromechanic and laser technologies. However, in case of conventional chromatographic columns with diameters of several or more millimetres, flow rates are relatively high and this enables to design and fabricate simple home-built interface devices using traditional labware.

This paper presents a simple computer controlled LC–CZE interface that consists of a solenoid valve and an injection device and that can be used at LC flow rates higher than 0.5 mL/min.

EXPERIMENTAL SECTION

A schematic diagram of the experimental set-up is shown in Fig. 1. The initial sample is injected manually (or under computer control) into the LC column by a valve/loop injector. The effluent from the LC column is directed into the solenoid valve 3, which has two output channels. One output channel, which is normally open (the valve is in off position), is connected to the monitoring UV detector and through this channel effluent goes to waste. The other, normally closed, channel is connected to the CZE injection device 1. When voltage (12 V dc) is applied to valve 3 (the valve is on), the normally open output becomes closed, while the normally closed output becomes open and the effluent from the

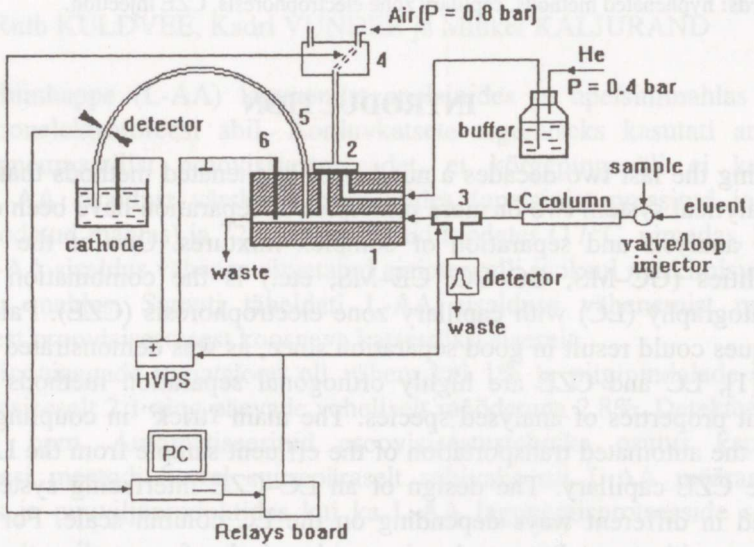

Fig. 1. Schematic diagram of experimental set-up of interfacing LC with CZE: 1, injection device; 2, membrane valve; 3, solenoid valve for LC effluent; 4, solenoid valve for air; 5, capillary; 6, electrode (grounded anode); HVPS, high voltage power supply.

LC column flows into the injection device 1. The CZE injection gadget 1, proposed by Kaljurand et al. [4], was made from a Plexiglas block (2.5 × 2.5 × 3.5 cm) into which L-shaped and T-shaped channels with i.d. 1 mm were drilled. One end of the L-shaped channel was connected to the CZE buffer vessel that was kept under excess pressure of helium (to avoid air bubbles) and the other end served as a buffer input hole into the membrane valve 2. The T-shaped channel had one outlet arm (to the waste) and two inlet arms. The outlet arm in which a grounded electrode and the inlet end of the capillary were placed was used as an input CZE reservoir, as its volume was big enough compared with the capillary size. Through one inlet arm of the T-shaped channel the LC column effluent could fill the CZE input reservoir (channel) and through the other inlet arm the buffer could flow into the CZE input reservoir when valve 2 was open.

The principle of operation of the membrane valve is illustrated in Fig. 2. The actuating element in this design is the thin flexible PTFE membrane. When air pressure higher than the buffer pressure is applied (Fig. 2b), the Teflon membrane will be pressed against the Plexiglas block wall and close the holes that connect the L-shaped channel with the T-shaped channel. When the air pressure is released (Fig. 2a), the buffer will press the membrane off the wall and rapidly fill the CZE input reservoir pushing out the liquid from there.

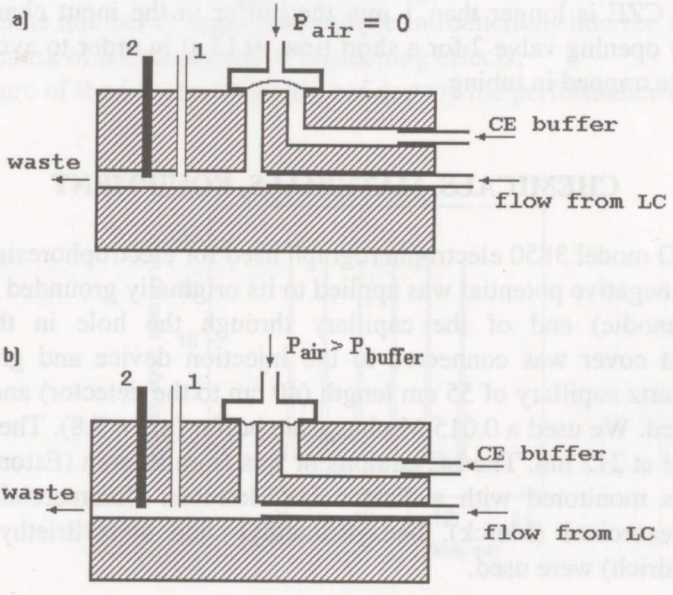

Fig. 2. Principle of the operation of the injection device. (a) Air pressure is released and buffer can flow into the CZE inlet reservoir (channel); (b) Air pressure is applied and the membrane valve is closed. When LC effluent flows into the injection device it fills the inlet channel replacing the buffer there, otherwise the buffer is in the CZE inlet reservoir. 1, capillary; 2, electrode.

Releasing the air pressure only for a short time period (0.15–0.2 s) enables to replace the liquid in the input reservoir with buffer very quickly without bubbling and without an interruption of the electric current if high voltage between the electrodes is applied. Applying/releasing the air pressure was controlled by switching on/off the pneumatic solenoid valve 4, operating at 24 V dc.

Computer control of the set-up was performed via an input/output interface board ADC-16 (Keithley) and an external home-made relays board. The relays board supplied appropriate voltage to valves 3 and 4 and switched on/off the high voltage power supply (HVPS). The software to control LC–CZE instrumentation was written in Borland C++ and the program allowed entering the predetermined time of switching on valve 3 after the injection in the LC column was done and the time period valve 3 was on. At the moment when valve 3 is switched on the high voltage is disabled (i.e. electrophoresis is stopped), and the LC effluent passes the inlet end of the capillary transporting sample material into the CZE input reservoir. Then at the moment when valve 3 is switched to the off position, HV becomes possible and electrokinetic injection of the sample begins. After the predetermined injection time has elapsed the pulse of buffer through valve 2 rapidly flushes out the sample to waste and fills the input channel without switching off the HV – the CZE analysis starts. The written program allows automatic repetition of such introductions of LC effluent into CZE in the course of an LC run. If the interval between the introductions of sample into CZE is longer than 1 min the buffer in the input channel will be refreshed by opening valve 2 for a short time (0.15 s) in order to avoid diffusion of the sample trapped in tubing.

CHEMICALS, MATERIALS, EQUIPMENT

The ISCO model 3850 electropherograph used for electrophoresis was rebuilt so that high negative potential was applied to its originally grounded cathode and the inlet (anodic) end of the capillary through the hole in the capillary compartment cover was connected to the injection device and grounded. An uncoated quartz capillary of 55 cm length (40 cm to the detector) and 75 μm i.d. was employed. We used a 0.015 M phosphate buffer (pH = 7.8). The CZE signal was detected at 212 nm. The LC equipment was from Inkrom (Estonia). The LC effluent was monitored with a Kratos UV detector. Commercially available chemicals resorcinol (Merck), benzyltrimethyl- and benzyltriethylammonium chloride (Aldrich) were used.

RESULTS AND DISCUSSION

In order to test the set-up performance we pumped aqueous solution of resorcinol through the LC column (filled with anion exchanger) and introduced effluent sample into the CZE capillary six times (once per minute). As illustrated in Fig. 3, the injection device has a good reproducibility and preserves high efficiency of capillary electrophoresis.

To demonstrate the work of the proposed interface a sample containing two benzyltrialkylammonium salts (benzyltrimethylammonium chloride and benzyltriethylammonium chloride) was injected into the same LC column. Since cations could not be separated in the anion exchanger column, they all quickly passed the column and appeared together in the detector as a system peak. However, taking samples from various points of the system peak profile and their analysis with CZE clearly showed that at least two kinds of species were present in the system peak (Fig. 4).

Therefore we can conclude that our relatively simple interface consisting of a solenoid valve and an injection device may be used for the coupling of several types of liquid chromatography (ion chromatography, HPLC, etc.) with CZE. As in the application of any technique, some precautions should be kept in mind:

– the liquid flow rate through the LC column should be relatively high in order to fill the CZE inlet channel within reasonable time, since the HV is disabled and this contributes to peak broadening;

– a moderate number of sequential sample introductions into the capillary can be made because of accumulation of broadening effects;

– the nature of the LC eluent should not destroy the performance of CZE.

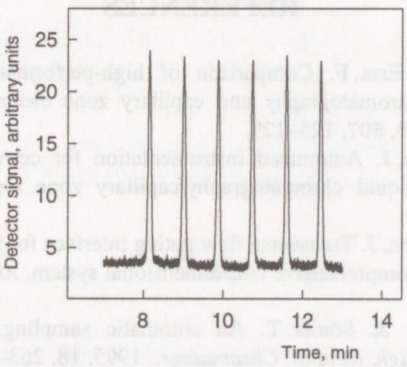

Fig. 3. Electropherogram of six sequential injections of resorcinol solution performed with the injection device.

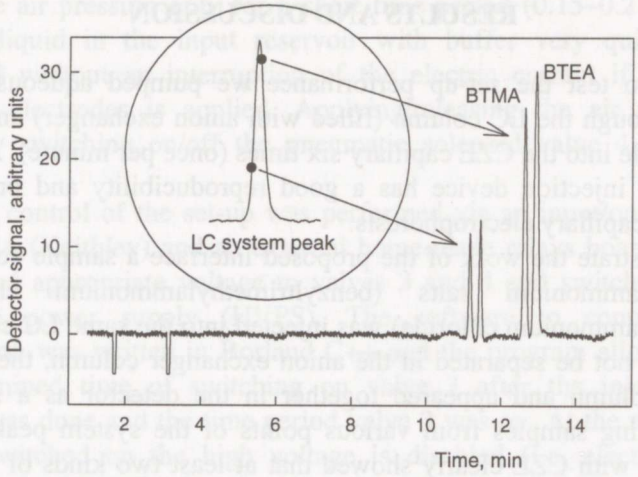

Fig. 4. Electropherogram of samples taken from the LC system peak (BTMA and BTEA are benzyltrimethyl- and benzyltriethylammonium chloride respectively). Negative peaks are a result of current interruption while the sample is flowing into the injection device.

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VEDELIKUKROMATOGRAAFI ÜHENDAMINE TSOONKAPILLAARELEKTROFOREESI SÜSTEEMIGA ELEKTROMAGNETILISE KLAPI JA SISESTAMISSEADME ABIL

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Kahe lahutusmeetodi – vedelikukromatograafia (LC) ja tsoonkapillaarelektroforeesi (CZE) – ühte analüütilisse süsteemi ühendamiseks on ehitatud arvutiga juhitav liiteseade, mis võimaldab automaatselt transportida LC kolonnist väljuva proovi CZE kapillaari. Konstrueeritud liidese kaks olulisemat elementi on väike elektromagnetiline klapp ja CZE sisestamisseade. On näidatud, et LC kolonnist väljuvaid lahutamata ühendeid võib edasi lahutada kapillaarelektroforeesi abil.