



Equipment and preliminary results for orthogonal pressurized planar electrochromatography



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ABSTRACT

We report combination of overpressured layer chromatography (OPLC) and pressurized planar electrochromatography (PPEC) techniques into a single technique in which both OPLC and PPEC processes proceed simultaneously and orthogonally. The separation process with this new technique is performed in adsorbent layer of a chromatographic plate, which is equipped with special sealing margin on its whole periphery and closed under pressure in special chamber. We have named this separation technique as orthogonal pressurized planar electrochromatography (OPPEC). Examples of analytical and micropreparative (continuous) OPPEC separations are demonstrated.

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1. Introduction

Two-dimensional (2D) separations are usually performed in two consecutive stages. There are many papers on this subject in which different separation techniques (gas chromatography, liquid chromatography and electrophoresis) are involved [1–18]. Peak capacity of such separation is considerably higher than that of one-dimension. Principle of such separation is based on different separation selectivity in each, of two, separation systems involved. It is why various research groups are interested in development of this approach. The first two-dimensional separation was demonstrated for paper chromatography [19]. This approach has been developing for contemporary electrophoresis and chromatography techniques. However, combination of two different separation techniques into one separation process in which both involved modes proceed simultaneously seems to be especially interesting. The idea of combination of liquid chromatography and electrophoresis techniques in simultaneous two-dimensional separation process originated from the 1949 when Svensson and

Brattsten published the paper on continuous electrophoretic separation [20]. In their first apparatus they used glass powder as a bed in a vertical lucite frame. The sample mixture and buffer solutions were descendent flowing in capillaries formed between bed particles, and electric field was applied perpendicularly to the direction of flow of the buffer solution. Under such conditions sample mixture components, if their molecules formed ions, migrated in the directions different than that of the buffer solution. If separating system was continuously fed with sample solution then the mixture components formed continuous bands directed with different angles against the direction of buffer flow. The separated components were continuously collected at the bottom edge of the lucite frame. Various authors modified this device. The modifications were concerned with current stabilization and thermostatic regulation [21], application of filter paper sheets [22], improved electrode arrangement [23], 3 mm thick paper as the porous bed to which lower edge paper strips were attached [24,25]. Some progress in development of simultaneous two-dimensional separation with combined electrophoresis and chromatography was reported by Karlson [26]. In the device the mobile phase and sample solutions were delivered to separation system (stationary phase bed formed as a flat column between glass sheets) with special pumps. The mobile phase flow was driven by constant pressure of gas bladder formed above the stationary phase bed inlet. On

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opposite sides two metal channels supported the bed of the stationary phase. Potential was applied to the channels and in this way electric field was perpendicularly applied to direction of the mobile phase flow. Scott proposed more sophisticated equipment, which was named as continuous annular electrochromatography [27]. In this equipment rotating annulus chromatography column was subjected to electric field radially. The mobile phase was driven into movement axially by external pump. The separation system was continuously fed with sample mixture at the predetermined point of top part of the cylindrical column and separated solute zones were collected at different places of the bottom part of the column. Pseudo-two-dimensional separation technique with cylindrical stationary phase was also reported [28]. In the technique the mobile phase was driven into movement by electric field through cylindrical motionless column but sample entry position and sample collection position were rotated at the top and bottom of the column, respectively. Some papers have been published on continuous separation using electrophoresis micro devices [29–34]. In such devices a wide separation channel is continuously fed with carrier buffer and sample solution. Electrical field is usually applied at perpendicular angle to buffer flow. During separation sample components deflect from direction of the buffer flow, and can be collected as separated zones at the outlet of the wide chamber of a microdevice. This separation cannot be regarded as fully two-dimensional because only one separation mechanism in one direction is involved in the process. In more sophisticated devices perpendicular pH gradient of carrier buffer has been applied to the direction of its flow. Under such circumstances sample components can be separated with respect to their *pI* values. This approach has been developed for rectangular channels [35]. Šlais et al. reported the channel with increasing width and named the technique as divergent flow isoelectric focusing [36–38].

Real application of orthogonal simultaneous electrophoresis and chromatography in system with adsorbent layer of the chromatographic plate under ambient pressure was reported by Van Ooij [39]. The cover plate of the device was 2 mm apart from the adsorbent layer surface. So the separation system comprised three phases: gas, liquid and solid. Recently, the simplified device for this kind of separation, using three phase system, has been reported by Stevenson et al. [40]. The authors demonstrated successfully simultaneous two-dimensional separation of vitamins, amino acids and dyes.

The device for orthogonal simultaneous electrophoresis/electrochromatography and chromatography separation with adsorbent layer covered with plastic cover under pressure has been described by Vanmiddlesworth's in his PhD thesis (J.G. Dorsey as the Professor directing dissertation) [41]. So the separation system comprised two phases: liquid and solid (adsorbent). In the device the chromatographic plate was placed face-down. Sample solutions were injected of-line or on-line into the adsorbent layer using microsyringe. In the latter mode the sample injection was performed through special hole in the adsorbent layer cover. The electrodes, platinum wire 0.51 mm in diameter, were located in two opposite troughs beneath surface of the adsorbent layer. The troughs were filled with sea sand ensuring that hydrodynamic flow of the mobile phase was uniform through whole adsorbent layer. It should be emphasized that in this mode overpressured layer chromatography (OPLC) was applied in the one separation direction and pressurized planar electrochromatography/electrophoresis (PPEC) in the second orthogonal direction.

The OPLC technique was worked out by Hungarian scientists [42]. It is very well known and established in scientific literature [43–45]. In 2004 Nurok et al. introduced PPEC for the first time [46]. This technique is an analogue to capillary electrochromatography (CEC) because of mobile phase movement is driven by electric field against an adsorbent layer. PPEC is characterized by some

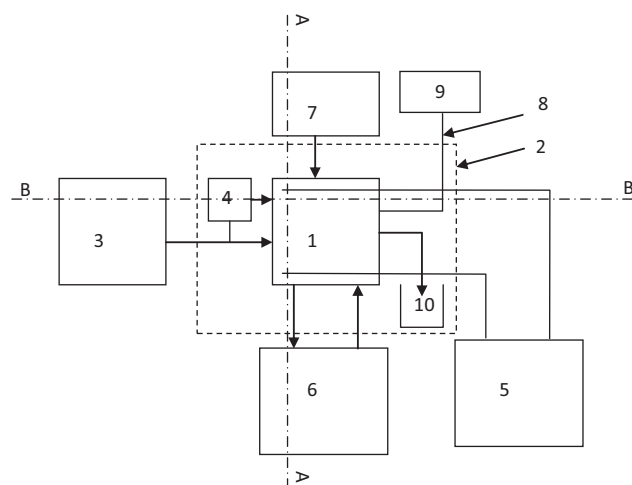


Fig. 1. Conceptual view of the device for orthogonal pressurized planar electrochromatography (OPPEC): 1 – OPPEC chamber, 2 – security cabinet, 3 – syringe pump, 4 – microinjection valve, 5 – DC high voltage power supply, 6 – thermostat, 7 – hydraulic pump, 8 – thermocouple, 9 – temperature digital display, 10 – waste reservoir.

advantages especially in comparison to thin layer chromatography/high performance thin layer chromatography (TLC/HPTLC): high performance, short separation time and different separation selectivity [47–56]. The latter is especially valid in relation to liquid chromatography and electrophoresis systems and can be attractive to application to 2D separation. Our group has reported few examples of 2D separation with PPEC and HPTLC (2D PPEC/HPTLC) [17,18]. In this approach both techniques were consecutively applied. However, the real challenge of further development of PPEC seems to be involved in its combination with OPLC into single 2D separation technique in which both liquid chromatography and electrophoresis/electrochromatography are simultaneously and orthogonally proceeded. In the paper we present preliminary results for analytical and micropreparative/continuous separations with this technique using new device and we have named the technique as orthogonal pressurized planar electrochromatography (OPPEC).

2. Experimental

2.1. Materials used

RP-18 W 10 cm × 20 cm HPTLC plates were from Merck, Darmstadt, Germany these were cut into 10 cm × 10 cm pieces. The mobile phase solution was prepared by mixing acetonitrile (POCH, Gliwice, Poland) with bidistilled water and buffer solution. The buffer solution of pH 3.0 was prepared by mixing solution of citric acid, analytical grade (POCH), 0.1 M and solution of disodium hydrogen phosphate, analytical grade (POCH), 0.2 M. The sample solutions were prepared in a mixture of acetonitrile in water. The mixtures of test solutes in the mobile phase solution were composed of food dyes (all from Institute of Dyes and Organic Products, Zgierz, Poland).

2.2. Orthogonal pressurized planar electrochromatography (OPPEC) device

Conceptual view of the OPPEC device for 10 cm × 10 cm chromatographic plates is presented in Fig. 1. It comprises OPPEC chamber (1) placed in the security cabinet (2), syringe pump (3), microinjection valve (4), or additional pump for feeding the adsorbent layer with a sample solution, DC high voltage power supply

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