



Influence of some operation variables on continuous separation process of orthogonal pressurized planar electrochromatography



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ABSTRACT

Orthogonal pressurized planar electrochromatography (OPPEC) is the new separation technique, in which two simultaneous and orthogonal processes of electrochromatography/electrophoresis and chromatography proceed under external pressure. It means in the OPPEC separation system a mobile phase solution hydrodynamically flows against adsorbent layer of the chromatographic plate and electric field is orthogonally applied to it. This technique can be applied to analytical and preparative separations. In the paper we report influence of some operating variables on continuous preparative OPPEC separation of test dyes in system with HPTLC RP-18W plates from Merck and methanol–buffer mobile phase. The following operation variables are discussed: external pressure exerted on the adsorbent layer, mobile phase flow velocity, and the ratio of flow velocities of the mobile phase to the sample solution, voltage applied to electrodes, modifier concentration in the mobile phase, concentration and pH of buffer.

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1. Introduction

In our last paper we had presented the principle of action, construction of equipment and preliminary results with a new separation technique named orthogonal pressurized planar electrochromatography (OPPEC) [1]. OPPEC is the technique in which two-dimensional (2D) separation process can be performed in adsorbent layer of a chromatographic plate. The characteristic feature of this technique is orthogonal and simultaneous combination of both liquid chromatography and electrophoresis/electrochromatography into single separation system under pressure. It means in OPPEC system the mobile phase hydrodynamically flows against adsorbent layer of the chromatographic plate in one determined direction and electric field is applied orthogonally to it. In the OPPEC technique the adsorbent layer stands for a kind of flat column that is placed on a carrier plate and covered with Teflon foil, which is pressed to the this adsorbent layer under external pressure higher than inlet pressure of the mobile phase. The equipment described can operate with

removable chromatographic plate. This feature of the equipment, in contemporary stage of its development, is advantageous due to flexibility of the technique—can be applied to analytical and continuous micro-preparative separations. In analytical OPPEC separated sample zones (peaks/spots), if solutes undergo ionization, can be spread in the whole region of the adsorbent layer what leads to considerable enhancement of peak capacity of the separation system in comparison to that of one dimensional separation. When preparative mode is applied then the solutes can be separated in adsorbent layer of the chromatographic plate in form of bands shifted under different angles from the direction of the hydrodynamic flow of the mobile phase if they undergo ionization. If solute molecules are neutral then they do not show deviation from direction of the mobile phase flow, and their bands can be separated according to frontal chromatography principle. Solute of ionic form obey this rule too if their bands are deviated at the same angle from the direction of hydrodynamic flow. It should be underlined that in preparative variant of OPPEC technique the separated solute can be collected continuously at the outlet of the separation system and/or stayed as bands on the adsorbent layer when separation is finished.

Special pattern of sealing margin on whole periphery of the adsorbent layer is the key solution for principle of action of the OPPEC equipment, because it enables to locate the electrodes in electrode compartments above the adsorbent layer and then the

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mobile phase uniformly flows throughout the adsorbent layer during separation process and in addition the chromatographic plate can be easily replaced with another one if required [1].

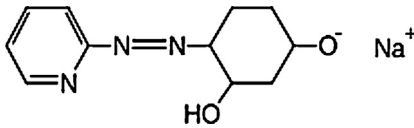
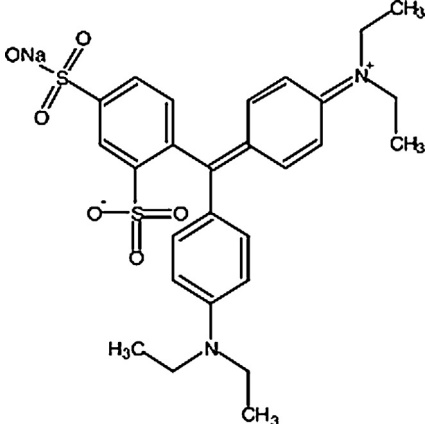
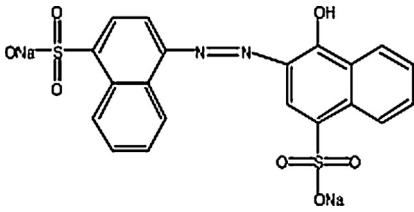
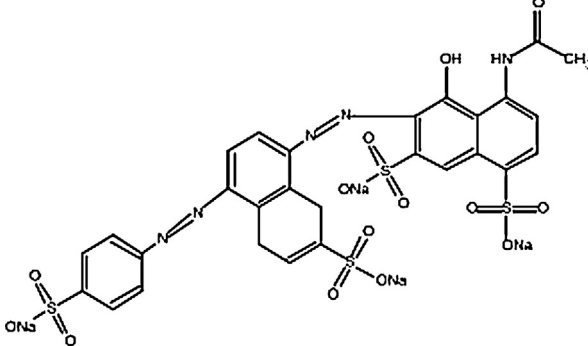
Principle of orthogonal and simultaneous 2D separation originates from 1949 [2]. This approach was also developed for flat column [3], cylindrical column under pressure [4], and adsorbent layer on chromatographic plate under ambient conditions, in system with three phases (gas, liquid and solid) [5,6]. Some experiments with simultaneous and orthogonal chromatography and electrophoresis for analytical separations were presented in doctorate thesis of Vanmiddlesworth under supervision of Prof. Dorsey [7], however, the results have not been published in scientific journal yet. Nevertheless, we discussed somewhat deeply the achievements by others in our last paper in this regard [1]. Additionally, it should be mentioned that the first idea of continuous separation, analogous to that of OPPEC technique, was published in 1952 [8]. Moreover, it should also be underlined that knowledge

regarding influence of different operation variables on separation with OPPEC is scarce so far.

As it was mentioned above our OPPEC equipment can operate both in analytical and continuous preparative modes, however, in the case of the former mode our present equipment suffers from the effect of too large dead volume concerned with injection of sample solution. We hope that in one of our next papers we demonstrate a solution of this obstacle too. The latter mode is very attractive for application to laboratory practice because an OPPEC separation system can be continuously fed with a mixture solution and separated mixture components can be continuously collected at the outlet of this system as well.

In present paper we show and discuss our next results regarding OPPEC separation of a test dye mixture, which was continuously supplied to the adsorbent layer of reversed-phase OPPEC system under influence of the following operating variables: flow velocity ratio of the mobile phase and the sample mixture solutions,

Table 1
Chemical structure of the dyes investigated and their *pI* values.

Name of dye	Chemical structure	<i>pI</i> values ^a
4-(2-pyridylazo)resorcinol monosodium salt (PAR)		3.88
Patent blue (PB)		1.67
Azorubine (AZ)		-
Brilliant black (BB)		2.33

^a *pI* values calculated using MarvinSketch software [12].

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