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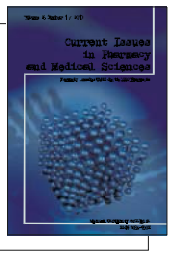

Effect of mobile phase buffer pH on separation selectivity of some isoquinoline alkaloids in reversed-phase systems of Pressurized Planar Electrochromatography and High-Performance Thin-Layer Chromatography

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ABSTRACT

Separation of some isoquinoline alkaloids (narcotine, chelidonine, dihydrocodeine, cinchonine, berberine, cinchonidine, papaverine, apomorphine) has been investigated with pressurized planar electrochromatography (PPEC) and high-performance thin-layer chromatography (HPTLC) in reversed-phase systems. The mobile phase consisted of acetonitrile and aqueous buffer (disodium phosphate and citric acid). The influence of the mobile phase buffer pH on migration distance (PPEC) and retardation factor (HPTLC) of the solutes has been investigated and compared. The results show different separation selectivity in both PPEC and HPTLC systems especially at pH range of buffer solution of the mobile phase that facilitates ionization of the solutes investigated.

Keywords: pressurized planar electrochromatography, PPEC, high-performance thin-layer chromatography, **HPTLC,** isoquinoline alkaloids

INTRODUCTION

Alkaloids are organic bases containing nitrogen in the heterocyclic ring, showing clear effect on the nervous system [1]. In view of different chemical structure, these compounds have been divided into a number of subgroups. Isoquinoline alkaloids belong to one of the subgroups. They are biogenetically related to phenylalanine or tyrosine. They can be isolated from plants belonging to the orders: Ranunculales, Papaverales, Geraniales, Rutales, Plumbuginales, Myrtiflorae and Rosales.

Thin Layer Chromatography (TLC) is very often applied as a tool for separation of alkaloids. Various alkaloids were determined by RP-TLC systems, with buffered aqueous eluents [10,11,13,15,16], ion-pair reagents [15,16, 21,22], or chiral selectors [4]. RP-TLC was used for determination of the lipophilicity of some alkaloids [23].

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Application of pressurized planar electrochromatography (PPEC) technique to separation of isoquinoline alkaloids in literature has not been reported so far.

PPEC is relatively new separation technique introduced by Nurok et al. [14]. In PPEC migration of the mobile phase against stationary phase is driven by electric field (electroosmotic effect). The advantages such as high performance, short separation time, and separation selectivity different than that of liquid chromatography (LC) make this technique very attractive for application in laboratory practice [17]. In addition, PPEC experiment proceeds in a closed system, so no vapor phase takes part in the separation process [2,3,5,6,7]. Because of the features mentioned, PPEC might also be highly suitable for the pharmaceutical and biomedical analysis [2,8,9,12, 19,20].

Based on the circumstances mentioned we have undertaken to perform investigations of migration of zones of some isoquinoline alkaloids (narcotine, chelidonine, dihydrocodeine, cinchonine, berberine, cinchonidine, papaverine, apomorphine) in PPEC systems and compare that with their retention in HPTLC ones.

MATERIALS AND METHODS

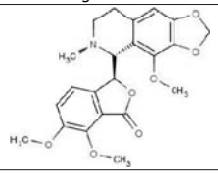
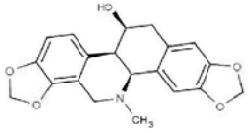
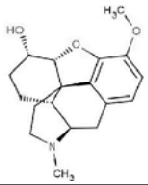
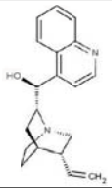
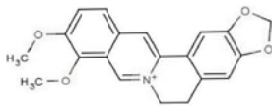
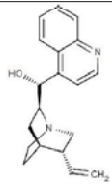
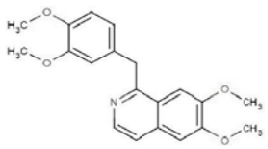
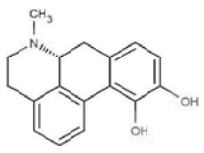
The mobile phase solution was prepared by mixing acetonitrile with appropriate buffer solutions. The organic modifier acetonitrile of the mobile phase was purchased from POCh (Gliwice, Poland).

The buffer solutions were prepared by mixing solutions of citric acid (Merck, Darmstadt, Germany) and disodium hydrogen phosphate (Standard, Lublin, Poland) or solutions of formic acid and aqueous ammonia (both Standard, Lublin, Poland). The other organic solvents (acetone and methanol) were received from POCh (Gliwice, Poland).

Silicone sealant solutions, Sarsil W and Sarsil H 50, were purchased from Zakłady Chemiczne Silikony Polskie (Nowa Sarzyna, Poland).

Standards of isoquinoline alkaloids were received from Sigma-Aldrich (St. Louis, Mo, USA). These solutions were prepared by dissolving 2 mg of investigated standards in 1 mL of acetone. Sample solutions were freshly prepared. Chemical structures and some chemical properties of investigated solutes are presented in Table 1.

Table 1. Chemical structures, the pK_A and log P values of investigated compounds

(1) NARCOTINE pK_A 6.44* log P 2.58*	**(2) CHELIDONINE** pK_A 5.73* log P 2.05*
	
(3) DIHYDROCODEINE pK_A 9.33* log P 1.55*	**(4) CINCHONINE** pK_{A1} 4.1 pK_{A2} 8.2** log P 2.7**
	
(5) BERBERINE pK_A4.69 *** log P 2.1**	**(6) CINCHONIDINE** pK_{A1} 4.0 pK_{A2} 8.2** log P 2.8**
	
(7) PAPAVERINE pK_A 6.03* log P 3.08*	**(8) APOMORPHINE** pK_{A1} 7.8 pK_{A2} 9.26 pK_{A3} 12.68* log P 2.87*
	

* http://www.chemicalize.org; **Moffat A.C., Osselton D. M., Widdop B., (2004). *Clarke's Analysis of Drugs and Poisons: in pharmaceuticals, body fluids, and post-mortem material.* 3rd. London: Pharmaceutical Press; *** Dzido T.H.: Modifications of retention of some alkaloids in the system silanized silica/methanol + water + di(2-ethylhexyl)orthophosphoric acid J. Chromatogr. 436, 257-266, 1988.

HPTLC mode was performed with 10x10 cm RP-18 WF254S HPTLC plates from Merck, (Darmstadt, Germany). The plates were washed before use by immersion in methanol for 1 min. After solvent evaporation the plates were activated in an oven at 105-110°C for 15 min. Sample solutions (0.7 μL) were applied on to the plate with aerosol applicator (Automatic TLC Sampler 4, Camag, Muttenz, Switzerland). Chromatograms were developed in the Horizontal DS-II-10×10 Chamber (Chromdes, Lublin, Poland) after 15 min saturation with the mobile phase vapor. The distance migration of the mobile phase was 45 mm (from the origin of sample application).

After the separation process, the plates were dried in air. The solute zones were registered under UV lamp with Visualizer and TLC SCANNER 4 (Camag, Muttenz, Switzerland).

PPEC experiments were performed with the device composed of PPEC chamber, high – voltage power supply (Consort, Turnhout, Belgium) and hydraulic press (Współpraca, Lublin, Poland). The cover in the PPEC device was pressed to the adsorbent layer of the chromatographic plate under pressure of 25 bar. Conceptual view of the equipment with longitudinal cross-section of the PPEC chamber was previously published [19].

Before PPEC experiments the adsorbent layer of the plates was immersed in methanol for 1 min and dried in air. Then the plates were activated in the oven (105°C) for 15 min. After each plate cooling, a margin 4 mm wide on its whole periphery was produced. The method used has been previously reported [18]. Subsequently the plates were placed in the oven at 105-110°C for 1 h to polymerize the sealant then left in a desiccator for use within 1 day.

RESULTS AND DISCUSSION

The relationships between retardation factor of investigated solutes and pH of buffer in the mobile phase of reversed phase HPTLC system is presented in Fig. 1. The mobile phase consists of 60% [v/v] acetonitrile in buffer solution at the pH range 3.0-8.0. Concentration of the buffer constituents in the mobile phase is in the range 1.00- 4.74 mM and 0.07-1.94 mM of disodium phosphate and citric acid, respectively.

Based on the data presented in Fig. 1, mild decrease of retention of investigated solutes with increase of buffer pH of the aqueous-organic mobile phase is observed. However, dihydrocodeine (3) stands for the exception in the buffer pH range 7.0-8.0.

The relationship retention vs. pH of buffer in the mobile phase for some of the solutes (berberine, chelidonine, narcotine, papaverine) was also investigated by Petruczynik et al. [15]. The authors reported on retention increase with rise of buffer pH. Therefore, it is contrary to our findings. This discrepancy in retention change can be

concerned with different modifiers and their concentrations as well applied in the experiments. In the experiments reported by Petruczynik et al. and in the paper, 80% v/v methanol and 60% v/v acetonitrile were used, respectively. The explanation of the effect can be related to a change of the chromatographic system type. Very high modifier concentration in the mobile phase in system with silica based stationary phase of very low coverage density of non-polar ligands (0.5 μmol/m^2 for HPTLC RP 18W plates) can lead to transformation of the chromatographic system into normal-phase one from that of reversed-phase system, which usually comprises considerably lower concentration of the modifier in the mobile phase.

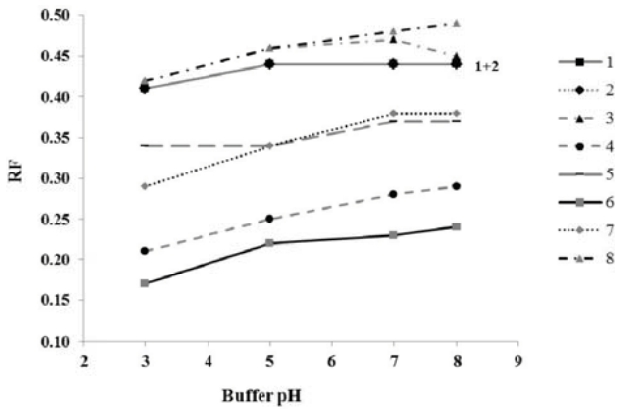


Fig. 1. Relationship R_F of solutes (isoquinoline alkaloids) vs. buffer pH of organic–aqueous mobile phase (60 % [v/v] acetonitrile + 40 % [v/v] aqueous buffer: citric acid and disodium phosphate) in system with RP18WF254 HPTLC plate (Merck). Solutes identification: narcotine (1), chelidonine (2), dihydrocodeine (3), cinchonine (4), berberine (5), cinchonidine (6), papaverine (7), apomorphine (8).

The effect of the retention decrease in the investigated buffer pH range may be explained by increase of ionization of silanol groups located on the stationary phase and change in ionization of the investigated substances, which are weak bases. The best separation selectivity of the investigated solutes was obtained when buffer pH was equal to 7.0.

In HPTLC system with buffer pH equal to 3.0, the order of retention increase of investigated compounds is as follows: apomorphine (8) + dihydrocodeine (3), narcotine (1) + chelidonine (2), berberine (5), papaverine (7), cinchonine (4), cinchonidine (6).

For other values buffer pH (3.0, 5.0, 7.0) the retention order of investigated substances is not changed with the exception of alkaloids such as berberine (5), $pK_A = 4.69$, and papaverine (7), $pK_A = 6.03$, the former is the weaker alkaloid base than the later. However, hydrophobic interactions of berberine with the stationary phase are then more favorable than that of papaverine.

The slight increase of retention of dihydrocodeine at high values of buffer pH in the mobile phase was probably concerned with its strongest basic property among the compounds investigated.

It should also be mentioned, that retention of narcotine (1) and chelidonine (2) was the same in the whole buffer pH range of the mobile phase. This behavior of these substances can be explained by their very similar physicochemical properties (similar chemical structure, pK_A values, and log P, Table 1).

In Fig. 2 the relationships migration distance of the solutes versus buffer pH of the mobile phase of PPEC system are presented.

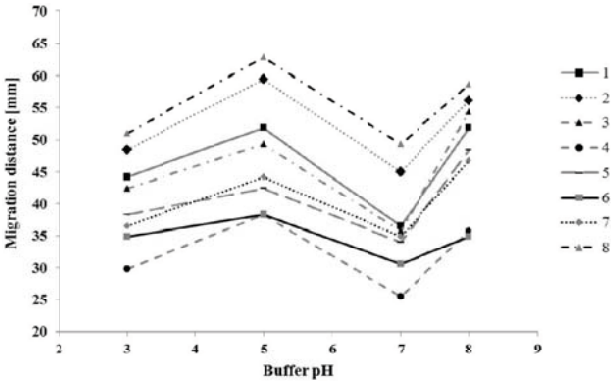


Fig. 2. Migration distance of investigated solutes vs. buffer pH of the organic–aqueous mobile phase (60 % [v/v] acetonitrile + 40 % [v/v] aqueous buffer: citric acid and disodium phosphate) in system with RP-18 WF254 HPTLC plate (Merck), PPEC, potential 1.00 kV, experiment time 10 min. The solute legend as in Fig. 1.

The relationships show a maximum at buffer pH 5.0 and a minimum at pH 7.0. The course of the curves is quite different from that for the corresponding thin-layer chromatography system. In such systems, PPEC with silanized silica based stationary phase, electroosmotic flow of the mobile phase rises with increase of buffer pH of the mobile phase [5].

Positively charged molecules of investigated solutes migrate toward the negative electrode – cathode. The direction of migration of zones of investigated compounds is consistent with the direction of electroosmotic flow of the mobile phase. Therefore at the initial range of buffer pH (3-5) in the mobile phase an increase of migration distance of investigated solutes is observed. However, at higher values of buffer pH (5-7), the ionization of weak bases, such as investigated isoquinoline alkaloids, is reduced, thus the contribution of the electrophoretic effect in solute migration is diminished. In this way at the higher range of pH values the plots of migration distance of these solutes vs. pH of buffer solution in the mobile phase show minimum.

The largest difference in migration distances of investigated substances at buffer pH equal to 3.0 and 5.0 are observed.

In PPEC system At buffer pH equal to 3.0, the order of increase in the migration distance of solutes is as follows: cinchonine (4), cinchonidine (6), papaverine (7), berberine (5), dihydrocodeine (3), narcotine (1), chelidonine (2), apomorphine (8).

However, in system of buffer pH 5.0 in the mobile phase, increase in migration distances of the investigated compounds is as follows: cinchonine (4) + cinchonidine (6), berberine (5), papaverine (7), dihydrocodeine (3), narcotine (1), chelidonine (2), papaverine (7), apomorphine (8).

Based on the correlation graphs migration distance of the solutes (in PPEC system) vs. their retardation factor (in HPTLC system) , considerable changes of separation selectivity between PPEC and HPTLC systems can be observed (Figs 3-6).

The order of increase of the values of determination coefficient, R^2, assigned to the specific values of buffer pH

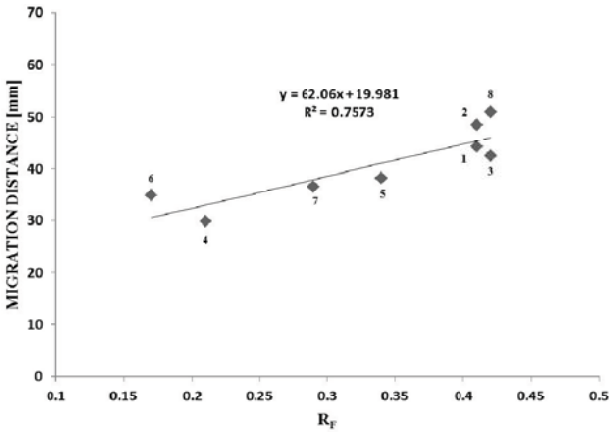


Fig. 3. Migration distance of investigated solutes vs. buffer pH of the organic–aqueous mobile phase (60 % [v/v] acetonitrile + 40 % [v/v] aqueous buffer of pH 3.0: 1.94 mM citric acid and 1.00 mM disodium phosphate) in system with RP-18 WF254 HPTLC plate (Merck). The solute legend as in Fig. 1.

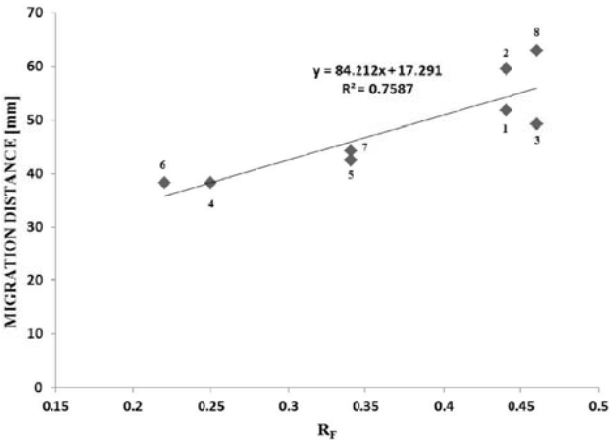


Fig. 4. Migration distance of investigated solutes vs. buffer pH of the organic–aqueous mobile phase (60 % [v/v] acetonitrile + 40 % [v/v] aqueous buffer of pH 5.0: 1.19 mM citric acid and 2.51 mM disodium phosphate) in system with RP-18 WF254 HPTLC plate (Merck). The solute legend as in Fig. 1.

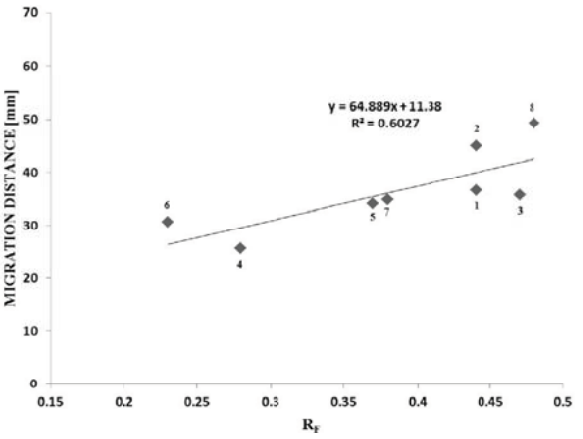


Fig. 5. Migration distance of investigated solutes vs. buffer pH of the organic–aqueous mobile phase (60 % [v/v] acetonitrile + 40 % [v/v] aqueous buffer of pH 7.0: 0.32 mM citric acid and 4.21 mM disodium phosphate) in system with RP-18 WF254 HPTLC plate (Merck). The solute legend as in Fig. 1.

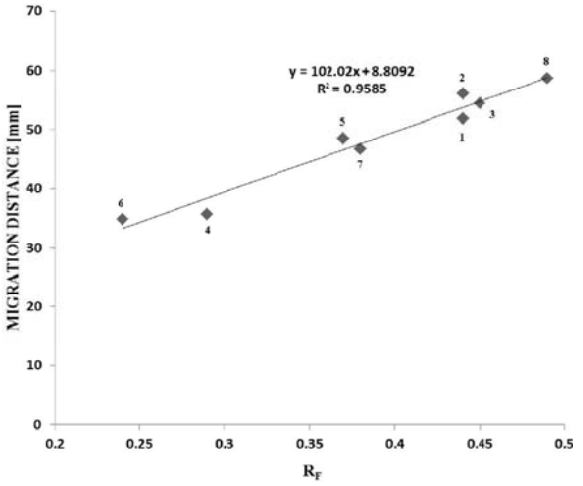


Fig. 6. Migration distance of investigated solutes vs. buffer pH of the organic–aqueous mobile phase (60 % [v/v] acetonitrile + 40 % [v/v] aqueous buffer of pH 8.0: 0.07 mM citric acid and 4.74 mM disodium phosphate) in system with RP-18 WF254 HPTLC plate (Merck). The solute legend as in Fig. 1.

is as follows: 0.6027 (pH 7.0) .7573 (pH 3.0) .7587 (pH 5.0) .9585 (pH 8.0). The maximum value of R^2 is obtained for buffer pH 8.0 (Fig. 6), what indicates similar separation selectivity in both PPEC and HPTLC systems. It is reasonable because pH value of buffer in the mobile phase is the highest one among the values applied to investigations. Under such circumstances, the solutes undergo the lowest ionization and then in PPEC system participation of solute electrophoresis in its migration mechanism is minimally involved. On the other hand, in the PPEC systems with buffers of lower pH values than 8.0 the solutes undergo stronger ionization and electrophoretic effect is then more shared in their migration mechanizm. So lower values of determination coefficient are then observed. Combination of such systems, i.e. PPEC and HPTLC, in two-dimensional separation process can be very advantageous for separation efficiency of complicated sample mixtures.

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