

Use of low volatility mobile phases in electroosmotic thin-layer chromatography

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Abstract

A variant of electroosmotic thin-layer chromatography is suggested with the use of low volatility compounds as mobile phases aimed at drastically decreasing the evaporation of the mobile phase and improving the reproducibility of the method. The linear movement velocity of zones of separated compounds is experimentally shown to increase 2–12-fold in electroosmotic chromatography (compared to similar values in traditional TLC). The separation efficiency is also considerably increased.

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1. Introduction

Thin-layer chromatography (TLC) discovered by Izmailov and Shraiber in 1938 [1,2] is one of the most simple, efficient, and economical methods for separation and analytical determination of chemical compounds and is presently widely used in analytical practice (see, for example, [3,4]).

Being developed during the last years mostly in the USA [5], the UK [6] and Poland [7,8] electroosmotic chromatography is one of the promising variants of planar chromatography allowing for overcoming the main drawback of traditional TLC—poor separation speed.

However, a number of researches in electroosmotic chromatography have discovered a new drawback of this method—heating of the sorption layer as result of passing electric current through the mobile phase even layer and consequently intensified evaporation of traditionally used volatile mobile phases with formation of dry regions on the TLC plate leading to termination of the electroosmotic chromatographic process. Therefore, further study and improvement of this promising method are expedient.

In this paper a new variant of electroosmotic TLC (EO-TLC) is suggested and studied in which low volatility mobile phases are used. The variant suggested here allows for eliminating the main drawback of traditional electroosmotic thin-layer chromatography—evaporation of the mobile phase. The new variant substantially increases the reproducibility of the method and extends its practical applications domain.

The use of low volatility mobile phases in traditional TLC has been first suggested in our earlier papers [9,10]. The use of such phases in TLC allows for realizing the following advantages of this method: (1) enhancement in experiment reproducibility (as result of elimination of the mobile phase evaporation); (2) simplification of the experiment (experiment can be conducted without a special chamber); (3) separation efficiency gain (as result of lower diffusion of chromatographic zones in a more viscous, as a rule, mobile phases).

As low volatility mobile phases for electroosmotic and, generally, planar chromatography we have first suggested dimethyl sulfoxide (DMSO), β,β' -oxydipropionitrile (ODPN), *N,N*-dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMAA). Some important physico-chemical properties of these compounds are presented in Table 1 [11,12]. Since compounds with the highest values of ε/η are most appropriate as mobile phases, among compounds in Table 1 primarily appropriate are acetonitrile

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Table 1

Some physico-chemical properties of dimethyl sulfoxide (DMSO), β,β' -oxydipropionitrile (ODPN), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMAA), acetonitrile and ethanol used as mobile phases in electroosmotic chromatography [11,12]

Compound	Boiling point (°C)	Viscosity, η (10^{-3} Pa s)	Dielectric permeability, ϵ	ϵ/η , rel.
DMSO	189	2.47	48.9	1.8
ODPN	270	9.35	66.6	0.65
DMF	152	7.96	36.7	0.41
DMAA	165	9.2	37.8	0.37
Acetonitrile	81.6	1.45	36.2	2.3
Ethanol	78.3	1.19	13.1	1.0

– among volatile compounds, and dimethyl sulfoxide – among low volatility compounds.

2. Experimental

Research was carried out on Merck 40 plates (Merck, Darmstadt, Germany) of size 10 cm \times 10 cm which have cut in pieces in the size 2.5 cm \times 10 cm. Preliminary a plate was washed out in ethanol–water (1:1, v/v) and then conditioned at 120 °C within 10 min. The plate has been used no more than in 24 h after air-conditioning. As mobile phases DMSO, ODPN, DMF, and DMAA were used, obtained from Lancaster Synthesis (Morecambe, UK). The equipment for practical realization of EO-TLC is shown in Fig. 1 a and b.

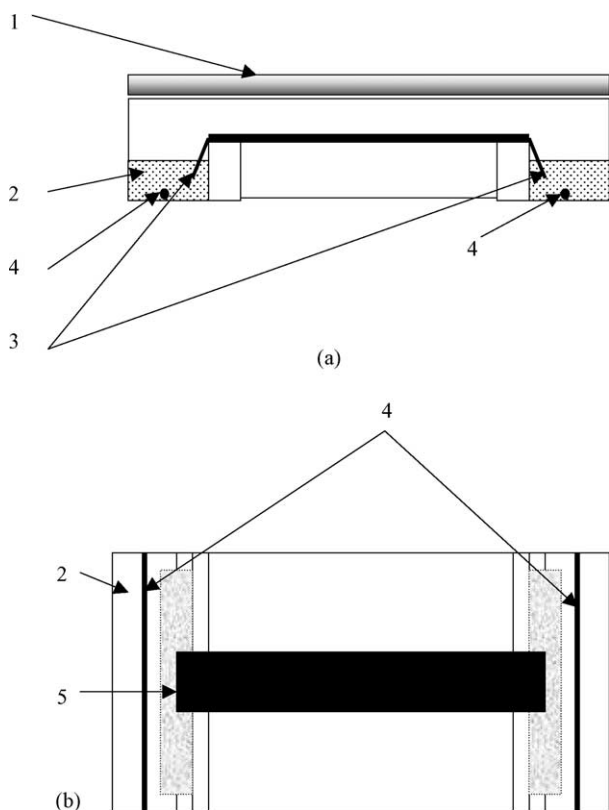


Fig. 1. The schematic image of the horizontal chamber for EO-TLC: (a) a side view and (b) the top view. 1=Demountable cover of the chamber; 2=mobile phase; 3=paper wick (filtering chromatographic paper); 4=electrodes; 5=TLC plate (sorbent layer was deposited to the bottom of the chamber).

Experiments for separation of dyes used as model compounds in EO-TLC were conducted in a horizontal chamber described more in detail in papers by Polish researchers (see [7,8]). The device used for carrying out of an electroosmotic planar chromatography consisted of two blocks—the high voltage rectifier (0–30 kV, the maximal current 1000 mA) and chamber for carrying out EO-TLC (Fig. 1).

The following dyes were mainly used as studied compounds: sudan II (orange color), crystal violet (violet color) and rhodamine 6G (pink color). Standard water and ethanol–water (1:1) solutions of dyes had concentration of 0.1%. Note that movement of dyes crystal violet and rhodamine 6G as ionic type compounds along the TLC plate may result simultaneously from two processes: first, electroosmotic movement of the mobile phase; and second, possible electrophoretic process for sorbates.

3. Results and discussion

As a comparison base, we first studied separation characteristics of dyes in traditional TLC with low volatility mobile phases. The experimental results obtained (averaged over three to six independent experiments) are presented in Table 2 (for DMSO mobile phase) and in Table 3 (for ODPN mobile phase).

From the results in these tables it follows, first, that the nature of the used low volatility mobile phase substantially influences the movement speed of separated compounds which can qualitatively be attributed to differences in viscosities of the mobile phases (for example, for ODPN the movement speed of sorbate zones is approximately two to five times lower than for DMSO and the viscosities ratio for these compounds is approx. 3.8); and second, addition of salt LiCl (only 0.01% LiCl) substantially influences the selectivity of separation with the addition of LiCl impairing the separation with DMSO and improving the separation with ODPN.

However, although the use of low volatility mobile phases noticeably improves the experiment reproducibility and allows for conducting separation without a chamber [9,10], the movement of these mobile phases along the sorption layer is usually considerably slower than of volatile mobile phases. Considering this, for the realization of advantages of low volatility mobile phases it appeared particularly appropriate to use EO-TLC characterized by substantially faster move-

Table 2

Mean linear movement speed (u) and *band broadening* characteristic (HETP) for dyes zones in traditional TLC with dimethyl sulfoxide (DMSO) and dimethyl sulfoxide (DMSO) with added 0.01% LiCl (DMSO + 0.01% LiCl) as mobile phases

Duration of experiment (min)	Sorbate	DMSO		DMSO + 0.01% LiCl	
		u (mm/min)	HETP (μm)	u (mm/min)	HETP (μm)
5	Rhodamine 6G	0.51	190	0.50	220
	Crystal violet	0.20	180	0.50	120
10	Rhodamine 6G	0.50	110	0.40	310
	Crystal violet	0.20	130	0.40	210

Table 3

Mean linear movement speed (u) and *band broadening* characteristic (HETP) for dyes zones in traditional TLC with β,β' -oxydipropionitrile (ODPN) and β,β' -oxydipropionitrile (ODPN) with added 0.01% LiCl (ODPN + 0.01% LiCl) as mobile phases

Duration of experiment (min)	Sorbate	ODPN		ODPN + 0.01% LiCl	
		u (mm/min)	HETP (μm)	u (mm/min)	HETP (μm)
5	Rhodamine 6G	0.10	300	0.50	210
	Crystal violet	0.10	120	0.50	180
10	Rhodamine 6G	0.10	240	0.50	160
	Crystal violet	0.10	200	0.20	110

ment of the mobile phase (compared to the traditional variant of TLC).

Tables 4 and 5 show experimental data for dye zones movement speeds and diffusion in separations using electroosmotic chromatography with low volatility compounds as mobile phases (DMSO and ODPN).

As is seen from the data in Tables 4 and 5, zone movement speeds of the studied dyes are substantially higher in electroosmotic chromatography. Increases in movement speeds of the dyes chromatographic zones (compared to speeds in traditional TLC) are shown in Table 6. As is seen from this table, linear movement speed of the chromatographed compounds zone along the adsorption layer in electroosmotic chromatography is increased 2–12-fold (3–12-fold in 75% cases) compared to speed of the same compounds in traditional TLC. The separation duration is usually decreased by the same rate.

It is also important to note that in changing from traditional to EO-TLC the separation efficiency is also considerably increased which is indicated by a considerable decrease in the height equivalent to a theoretical plate (HETP). For example,

if DMSO is used as the mobile phase the change from traditional to electroosmotic TLC gives a 4–6-fold decrease in HETP. Similar effect is observed when ODPN is used as the mobile phase.

The decrease in HETP signifies also a noticeable enhancement in the resolution of the method due to change from traditional to electroosmotic TLC.

Therefore, when low volatility compounds are used as the mobile phase the change from traditional TLC to EO-TLC brings about substantial improvements in basic chromatographic characteristics. Movement speeds of chromatographic zones are increased 4.5–7.0-fold with simultaneous 4–6-fold decrease in HETP.

We investigated also *N,N*-dimethylformamide and *N,N*-dimethylacetamide (see Table 1) as low volatility mobile phases in TLC and EO-TLC (see Table 7). As can be seen from this table, the main chromatographic characteristics processes with DMF and DMAA qualitatively are analogous those, that we had investigated before (see Table 6).

It is important to note that the use of low volatility mobile phases allowed for stabilizing the chromatographic pro-

Table 4

Mean linear movement speed (u) and *band broadening* characteristic (HETP) for dyes zones in electroosmotic TLC with dimethyl sulfoxide (DMSO) and dimethyl sulfoxide (DMSO) with added 0.01% LiCl (DMSO + 0.01% LiCl) as mobile phases

Duration of experiment (min)	Sorbate	DMSO		DMSO + 0.01% LiCl	
		u (mm/min)	HETP (μm)	u (mm/min)	HETP (μm)
5	Sudan II	2.7	50	2.8	39
	Rhodamine 6G	1.2	20	1.4	25
	Crystal violet	1.6	25	2.2	25
10	Sudan II	2.2	43	2.4	30
	Rhodamine 6G	1.0	17	1.5	16
	Crystal violet	1.5	25	1.8	10

Conditions of a series of experiments of 5 min duration: voltage 1 kV, current 210 μA (DMSO) and 380 μA (DMSO + 0.01% LiCl); for a series of experiments of 10 min duration—voltage 1 kV, current 220 μA (DMSO) and 390 μA (DMSO + 0.01% LiCl).

Table 5

Mean linear movement speed (u) and *band broadening* characteristic (HETP) for dyes zones in electroosmotic TLC with β,β' -oxydipropionitrile (ODPN) and β,β' -oxydipropionitrile (ODPN) with added 0.01% LiCl (ODPN + 0.01% LiCl) as mobile phases

Duration of experiment (min)	Sorbate	ODPN		ODPN + 0.01% LiCl	
		u (mm/min)	HETP (μm)	u (mm/min)	HETP (μm)
5	Sudan II	6.3	23	6.1	10
	Rhodamine 6G	1.2	34	1.2	21
	Crystal violet	1.0	31	0.90	29
10	Sudan II	6.2	12	6.5	10
	Rhodamine 6G	0.9	44	1.0	27
	Crystal violet	0.7	24	0.9	21

Conditions of a series of experiments of 5 min duration: voltage 5 kV, current 105 μA (ODPN) and 117 μA (ODPN + 0.01% LiCl); conditions of a series of experiments of 10 min duration—voltage 5 kV, current 105 μA (ODPN) and 110 μA (ODPN + 0.01% LiCl).

Table 6

Relative increase in the linear movement speed of dyes $\rho = u_{\text{eo}}/u_{\text{tr}}$ (u_{eo} is the linear speed of the dye zone in EO-TLC, u_{tr} is the linear speed of the dye zone speed in traditional TLC) with low volatility mobile phases DMSO and ODPN

Duration of experiment (min)	Sorbate	Characteristics of linear speed increasing, ρ			
		DMSO	DMSO + 0.01% LiCl	ODPN	ODPN + 0.01% LiCl
5	Rhodamine 6G	2.4	2.8	12	2.4
	Crystal violet	8.0	4.5	10	3.0
10	Rhodamine 6G	2.0	3.8	9.0	2.0
	Crystal violet	7.5	4.5	7.0	4.5

cess. Such undesirable effects as sharp changes in the electric current and voltage, termination of the chromatographic process as result of evaporation of the mobile phase from the TLC plate were not observed in our experiments with mobile phases with low volatility. Therefore, the results obtained indicate perspectives for further development of this new variant of electroosmotic TLC with low volatility mobile phases.

The aim if this work was demonstrated the peculiarities a new EO-TLC variant. The main goal of chromatography is resolution [13]:

$$R_s = \frac{\sqrt{N} \alpha - 1}{4} \frac{k}{\alpha} \frac{1}{k - 1} \quad (1)$$

where N is the number of theoretical plates, α is the separation factor, and k is the retention factor of the second component.

Thus resolution (R_s) in chromatography is a function of three factors: selectivity (S), efficiency (E), and capacity (C) [14]:

$$R_s = SEC \quad (2)$$

Table 7

Linear speed (u) and *band broadening* characteristic (HETP) for dyes zones in traditional TLC and electroosmotic TLC using as mobile phases N,N -dimethylacetamide (DMAA) and N,N -dimethylformamide (DMF)

Variant of chromatography	Sorbate	DMAA			DMF		
		u (mm/min)	$u(\text{EO-TLC})/u(\text{TLC})$	HETP (μm)	u (mm/min)	$u(\text{EO-TLC})/u(\text{TLC})$	HETP (μm)
TLC	Sudan II	5.2	1.0	11	4.7	1.0	13
	Crystal violet	0.6	1.0	170	0.6	1.0	170
EO-TLC	Sudan II	8.0	1.5	20	9.0	1.9	17
	Crystal violet	7.1	11.8	17	8.5	14.1	14

Conditions of experiment: duration of chromatographic process, 5 min; voltage, 2 kV.

where

$$S = \frac{\alpha - 1}{\alpha} \quad (3)$$

$$E = \frac{\sqrt{N}}{4} \quad (4)$$

$$C = \frac{k}{k + 1} \quad (5)$$

In EO-TLC it is believed, that S and C are the same as in traditional TLC, but E is attenuated most. Consequently, as a rule

$$R_s(\text{EO-TLC}) > R_s(\text{TLC}) \quad (6)$$

As is shown in Eq. (6), resolution in EO-TLC must be not worse than in TLC. Consequently, there is a good reason to use EO-TLC for analytical purposes.

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