Separation of purines and pyrimidines by normal-phase high-performance liquid chromatography using dimethyl sulfoxide in binary and ternary eluents

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Abstract

Chromatographic systems with a silica sorbent and mobile phases containing dimethyl sulfoxide have been studied. It has been established that the substitution of isopropanol by dimethyl sulfoxide in binary eluents results in a specific selectivity of the chromatographic system and shows an improvement of the peak shape for the solutes under study. When mobile phases consisting of hexane, isopropanol and dimethyl sulfoxide (solvents with a limited mutual solubility) are used, changes in retention characteristics and peak symmetry are caused by a transition from adsorption to partition sorption mechanism. The stationary liquid phase is generated dynamically in the pores of silica, even in the mobile phases not saturated with a polar component. If the phase ratio of the column reaches 0.1, partition dominates over adsorption and such mixed partition–adsorption (MPA) systems show very good peak symmetry for the solutes under study. The investigation has shown that dimethyl sulfoxide-containing MPA systems are applicable in analytical practice. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Mobile phase composition; Partition–adsorption systems; Purines; Pyrimidines; Dimethyl sulfoxide

1. Introduction

High-performance liquid chromatography (HPLC) has become a widely used chromatographic tool for the separation of complex organic mixtures. A majority of chromatographers practice their separations using reversed-phase (RP) systems. The RP mode has become a well established method for the separation of pyrimidine and purine derivatives [1–5]. Nevertheless, the RP mode is not always the best or only a choice for a given analytical task. Normal-phase (NP) separation is still of considerable importance [6]. A very efficient separation method of purines and pyrimidines on unmodified silica with dichloromethane–methanol–water mixtures as a mobile phase is known [7–9]. The use of RP separation in combination with NP can offer additional possibilities for quality control.

Unmodified silica is traditionally used in NP chromatography with a mobile phase of low or medium polarity. Polar organic substances require eluents of a high polarity in order to be eluted from unmodified silica. The lower aliphatic alcohols from methanol to propanol are most frequently used for this purpose as mobile phase components. Under these conditions, adsorption is usually the dominating retention mechanism, therefore the peak shapes of polar solutes are usually unsatisfactory. Aprotic

dipolar solvents were used as eluent additives for moderating silica surface [10–12]. Dimethyl sulfoxide (DMSO), a well known hydrogen bond acceptor which can form stable association complexes with compounds containing proton-donating functional groups, is capable of moderating the silica surface and increasing the selectivity for various functional classes. For instance, for separation of nitrogen heterocycles from hydroxyaromatic standards, the μPorasil column utilizing DMSO–carbon tetrachloride mobile phases yielded a superior separation due to the rapid elution of the nitrogen heterocycles and the stronger retention of sterically hindered hydroxyaromatic compounds [11]. A system consisting of a silica column and carbon tetrachloride containing DMSO has been found to offer an excellent repeatability [10]. The interactions of aprotic dipolar solvents with the silica surface might be reversible in spite of their highly polar nature.

Chromatograms on silica can be improved significantly if solvent-generated liquid–liquid systems are applied. According to this method [13–18], two or three immiscible solvents are equilibrated and, after the separation of layers, the less polar layer is used as a mobile phase. The polar component of this layer wets the silica surface better than the non-polar one, and covers it with a film of liquid that acts as a stationary phase in liquid–liquid partition chromatography. It has been shown [16,17] that cyclohexane saturated with DMSO generates a liquid stationary phase in pores of unmodified silica and such a system was found to be particularly useful for extremely fast and selective separations of polycyclic aromatic hydrocarbons. The partition mode offers two advantages, i.e., versatility and reproducibility [18]. But the extensive presaturation of the mobile phase with the stationary phase and precise thermostatting of the stock reservoir and column are the main drawbacks of the solvent-generated liquid–liquid systems [13].

Our previous investigations [19–22] showed that a stationary liquid phase is generated dynamically in the pores of silica, even in the mobile phases not saturated with the polar component. Such mixed partition–adsorption (MPA) systems are free of above-mentioned disadvantages of solvent-generated liquid–liquid systems [20].

The goal of this work was to study the behaviour of some purine and pyrimidine derivatives on silica in mobile phases containing DMSO in adsorption (A) and MPA modes.

2. Experimental

The chromatographic measurements were performed on a Gilson Model 302 HPLC system, equipped with a spectrophotometer ($\lambda = 254$ nm). The columns of 150×4.6 mm were packed with unmodified silica Zorbax SIL, 5–6 μm (DuPont). Mixtures of DMSO and/or isopropanol (IPA) with ethyl acetate (EA), chloroform (CHCl$_3$) and hexane (HEX) were studied as mobile phases. All the solvents were purchased from commercial sources and were of analytical grade and used without any pretreatment. The flow-rate was 1.5 ml/min. The samples (25 μl, 0.1 mg/ml) were injected via a Rheodyne 7125 sampling valve.

The column was conditioned before each series of retention measurements. Conditioning included flushing with 50 ml of IPA–HEX (40:60) followed by the mobile phase under study. Usually 50 ml of the latter eluent was sufficient to obtain constant retention values.

The capacity factors of the solutes under study (k') were calculated according to the usual expression [23]. The system mobile phase volume was regarded to be equal to the benzene retention volume. The phase ratio of the column (V_s/V_m) was calculated according to Ref. [22]. The peak asymmetry was calculated by determination of the A/B ratio at 10% of the peak height [24].

Values of liquid–liquid equilibrium curve of the ternary system HEX–IPA–DMSO were determined by mutual titration (a microburet was used) of a 50 ml vigorously mixed binary system IPA–HEX (2.5:97.5; 5:95; 10:90 and so on) with DMSO at ambient temperature. If turbidity was observed, the solution was saturated.

The eluents for MPA systems were prepared by slow addition of DMSO to the vigorously mixed IPA–HEX to ensure complete homogenization. The eluents for the solvent-generated liquid–liquid system were mixed vigorously during 6 h at a constant temperature to ensure a complete demixing. The conjugated polar phase was not removed from the
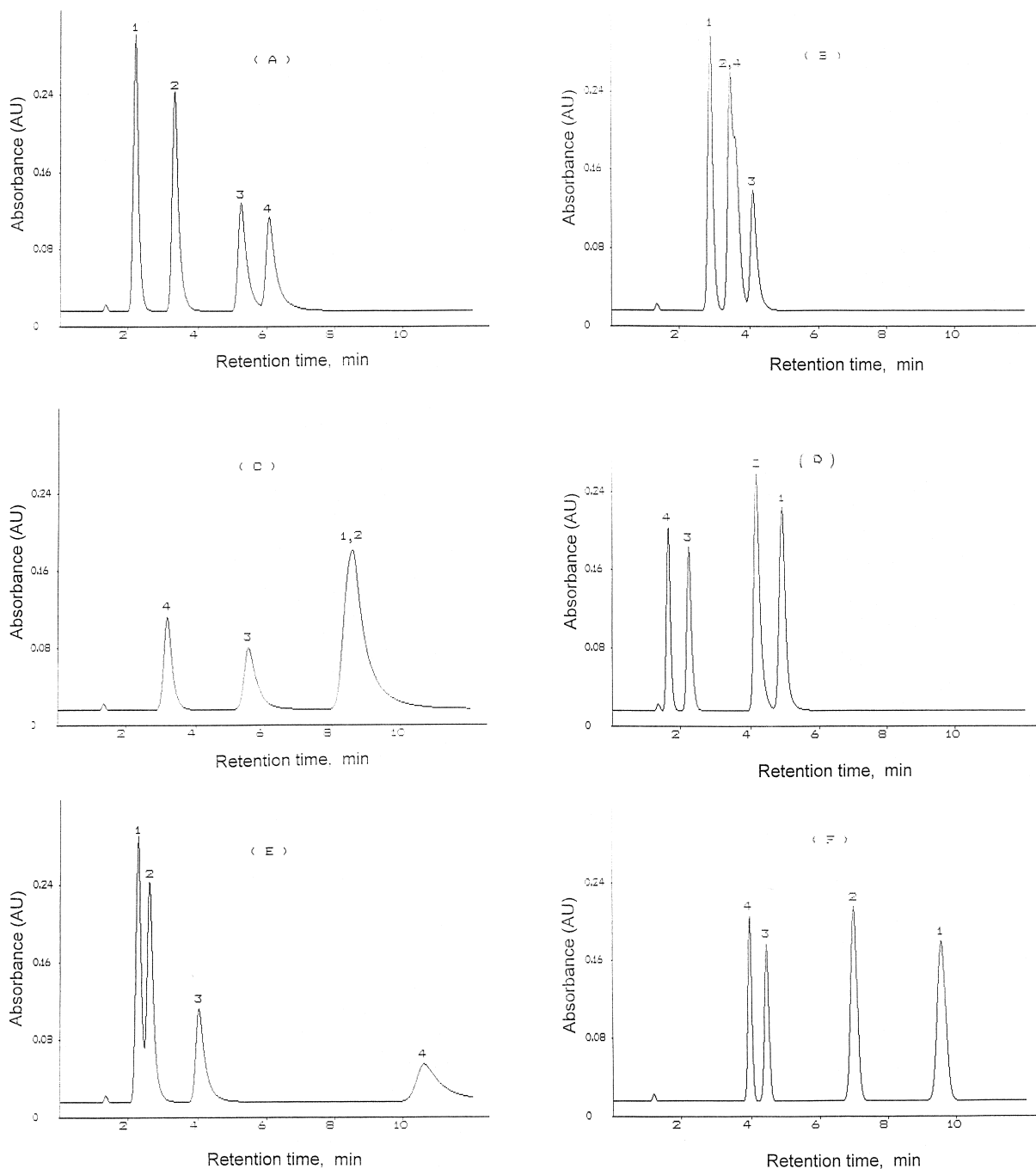
Fig. 1. Chromatograms of a test mixture of solutes: 5-fluorouracil (1); thymine (2); theophylline (3); caffeine (4). Mobile phases: 2% IPA in EA (A); 0.25% DMSO in EA (B); 5% IPA in CHCl$_3$ (C); 5% DMSO in CHCl$_3$ (D); 40% IPA in HEX (E); 6% DMSO and 14% IPA in HEX (F). Column, Zorbax SIL, 150×4.6 mm; flow-rate, 1.5 ml/min; detector, UV, 254 nm; ambient temperature; 0.32 AUFS. Injection volume, 25 μl; sample concentration in mobile phase, 0.1 mg/ml.

eluent container when pumping the apolar phase through the column.

3. Results and discussion

The results of the studies on the chromatographic behaviour of some purine and pyrimidine derivatives on silica in mobile phases consisting of IPA and/or DMSO in EA, CHCl$_3$ or HEX are presented in Fig. 1. It can be seen that all systems have a sufficient elution strength for the test solutes. At the same time, peak tailing is typical for adsorption systems. The peak shapes of the substances under study are not satisfactory in Fig. 1A, C and E (the peak asymmetry value is higher than 1.5), which is typical for NP chromatography of polar solutes on the Zorbax SIL columns [6]. The substitution of isopropanol by dimethyl sulfoxide in the adsorption systems (Fig. 1B and D) results in a specific selectivity and demonstrates a better peak symmetry for solutes under study (the peak asymmetry value is nearly 1.3). It can be seen that DMSO is a good eluent additive for moderating the silica surface. Nevertheless, the chromatogram in Fig. 1F indicates that the peak shape is much better (the peak asymmetry value is less than 1.2) when the mobile phase

consists of HEX, IPA and DMSO. Since such a ternary solution contains poorly mutually soluble components, dynamic generation of a stationary liquid phase on silica can be expected when such a solution is used as the mobile phase [19–22].

The behaviour of the substances studied in chromatographic systems with silica and different mobile phases consisting of HEX, IPA and DMSO was investigated. Fig. 2 represents a triangular phase diagram for the ternary system HEX–IPA–DMSO. The mobile phase composition varied by changing the concentration of IPA and DMSO from 20 to 10% and 0 to 10%, respectively, while keeping HEX constant at 80%. A binary mobile phase (without DMSO, point 1 in Fig. 2) corresponds to the chromatographic system with an adsorption mechanism of retention. The compositions of the mobile phases with DMSO concentrations higher than 7% (points 6 and 7 below the equilibrium curve in Fig. 2) correspond to the ternary systems split into two co-existing liquid phases, one of which is presaturated with DMSO, but the other with HEX. The first of the coexisting phases is applied as a mobile phase, and a solvent-generated liquid–liquid system with a partition mechanism of retention is formed [7–12]. The compositions with less than 7% DMSO (points 2–5 above the equilibrium curve in Fig. 2) corre-
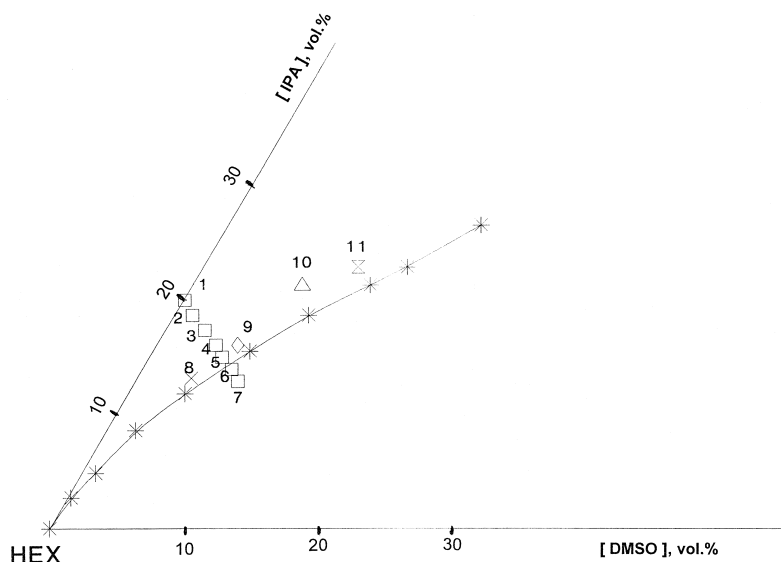

Fig. 2. Triangular phase diagram of ternary system HEX–IPA–DMSO at ambient temperature.

spond to the compositions which are homogenous and differ from the others by the degree of saturation with DMSO. The mechanism of sorption in such systems is mixed, involving adsorption on the silica surface and partition. The contribution of each process depends on the volume of the liquid phase deposited. The values of the calculated phase ratio (V_s/V_m) [22] are given in Fig. 3.

The relationship between the volume of DMSO in the mobile phase and the capacity factor (k') of the compounds under study is not linear (Fig. 4a). When the DMSO concentration is not more than 1%, a slight increase (solutes 1 and 2) and decrease (solute 3) in retention is observed; a sharp decrease in retention for solute 4 takes place. It can be seen that when the DMSO concentration is increased in the range of 1–6%, only a slight increase (solutes 1–3) or decrease (solute 4) is observed. A sharp increase in retention of all the solutes takes place when the amount of DMSO approaches its limit of solubility (more than 7%).
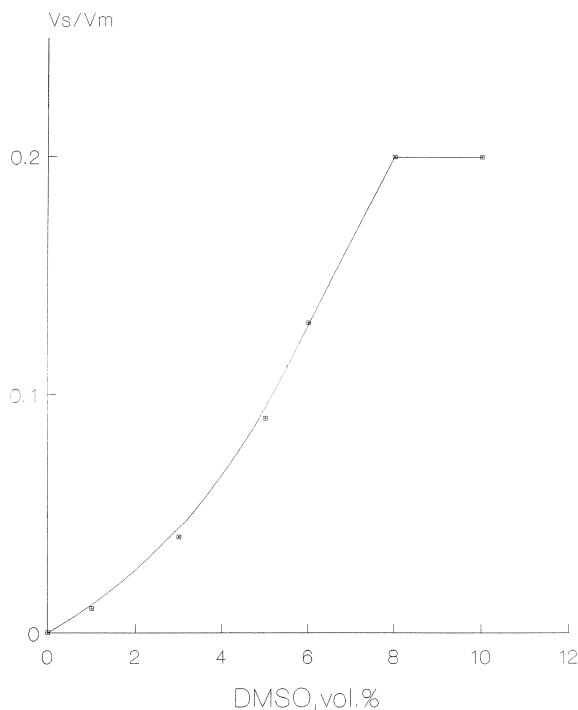

Fig. 3. Dependence of the column phase ratio on the concentration of DMSO in HEX–IPA–DMSO mobile phases (concentration of HEX constant at a value of 80%).
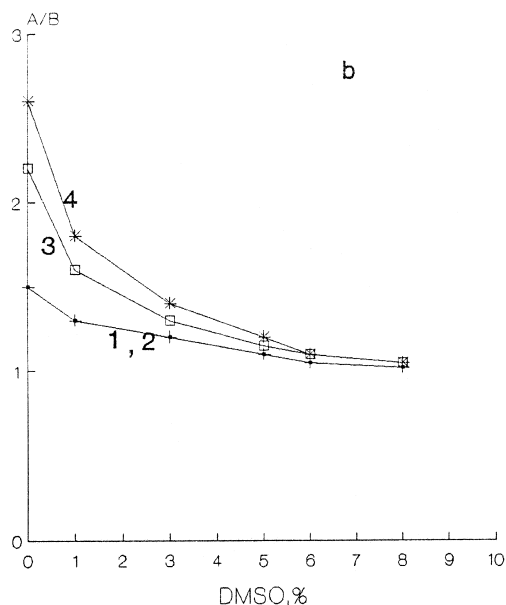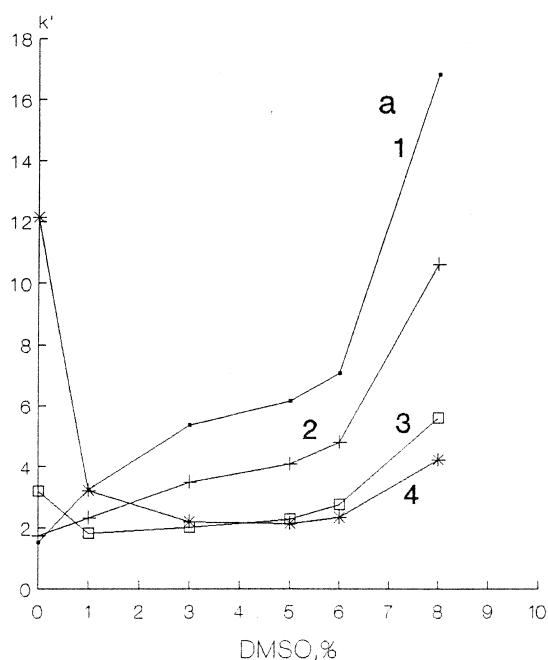

Fig. 4. Relationship between the DMSO concentration in the HEX–IPA–DMSO mobile phases (concentration of HEX constant at a value of 80%) and retention of the solutes under study k' (a) and peak asymmetry (b). Test solutes 1–4, Fig. 1.
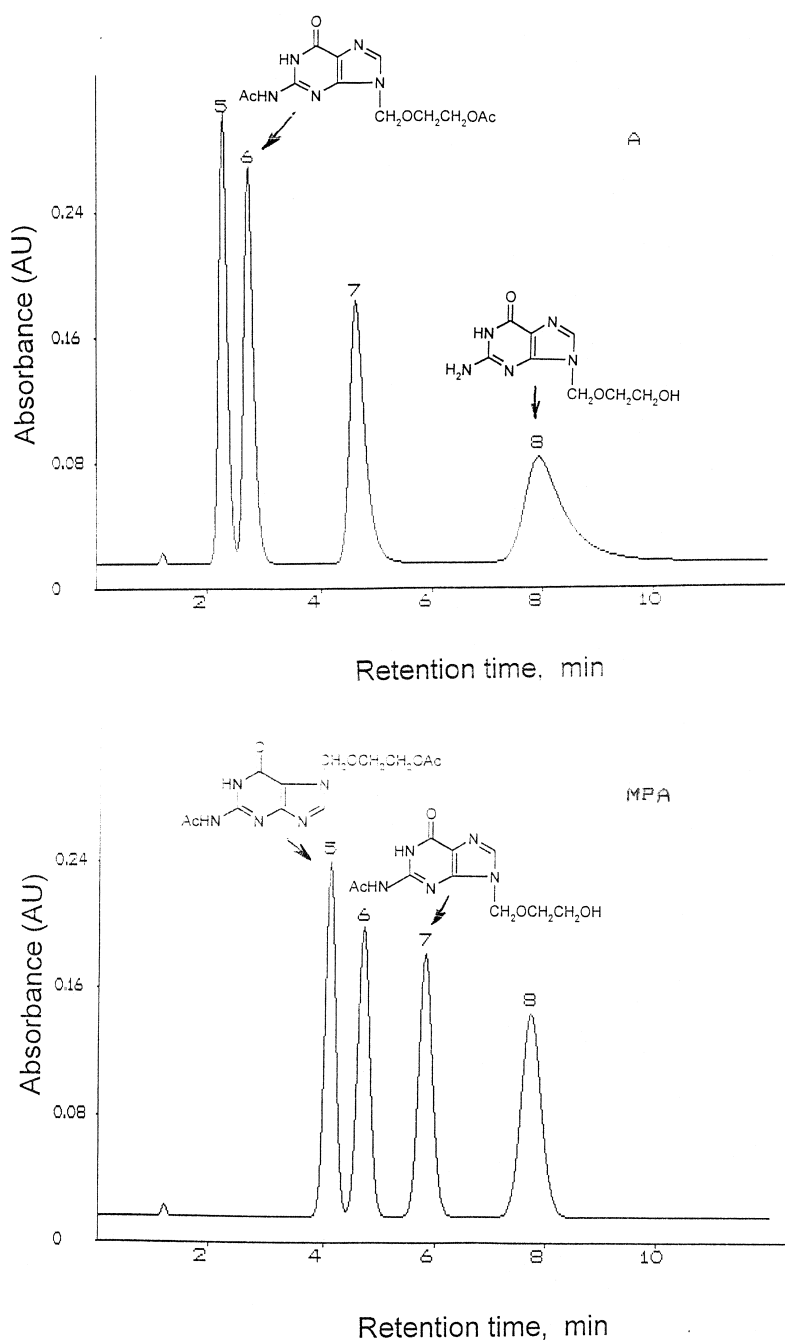
Fig. 5. Separation of the solutes studied (5–8) of the adsorption mode (A) and MPA mode (MPA). Mobile phase (A): 8% DMSO in EA; mobile phase (MPA): 13% DMSO and 21% IPA in HEX (composition corresponds to point 10 in Fig. 2); column, Zorbax SIL; 150×4.6 mm; flow-rate, 1.5 ml/min; detector, UV, 275 nm; ambient temperature; 0.32 AUFS. Injection volume 25 μl; sample concentration in mobile phase 0.1 mg/ml.

Fig. 4b represents the relationship between the DMSO concentration in the mobile phase and the peak symmetry of solutes under study. It is seen that when the phase ratio value is less than 0.05 the peak asymmetry is higher than 1.25 (Figs. 3 and 4b), and adsorption dominates under these circumstances. If the phase ratio value is higher than 0.1 the peak asymmetry is less then 1.1. Partition dominates over adsorption in such chromatographic systems and such mobile phases are good MPA systems. Fig. 5 demonstrates the advantage of the MPA mode over the adsorption mode in chromatographic systems with DMSO in the analysis of such polar solutes as acyclovir (8) and its possible impurities (5–7).

In order to estimate the flexibility of HEX–IPA–DMSO MPA systems the concentration of components in the mobile phases was varied. When the concentration of polar components in ternary mobile phases increases a decrease in retention is observed, which is typical for NP chromatography. It is demonstrated that the selectivity does not change very markedly with alterations in concentration and proportion of polar components (Fig. 6a and b; compositions of the mobile phase correspond to points 5 and 9, respectively, in Fig. 2). If considerable selectivity alterations are necessary the best approach is to use another MPA system (Fig. 6c and d).
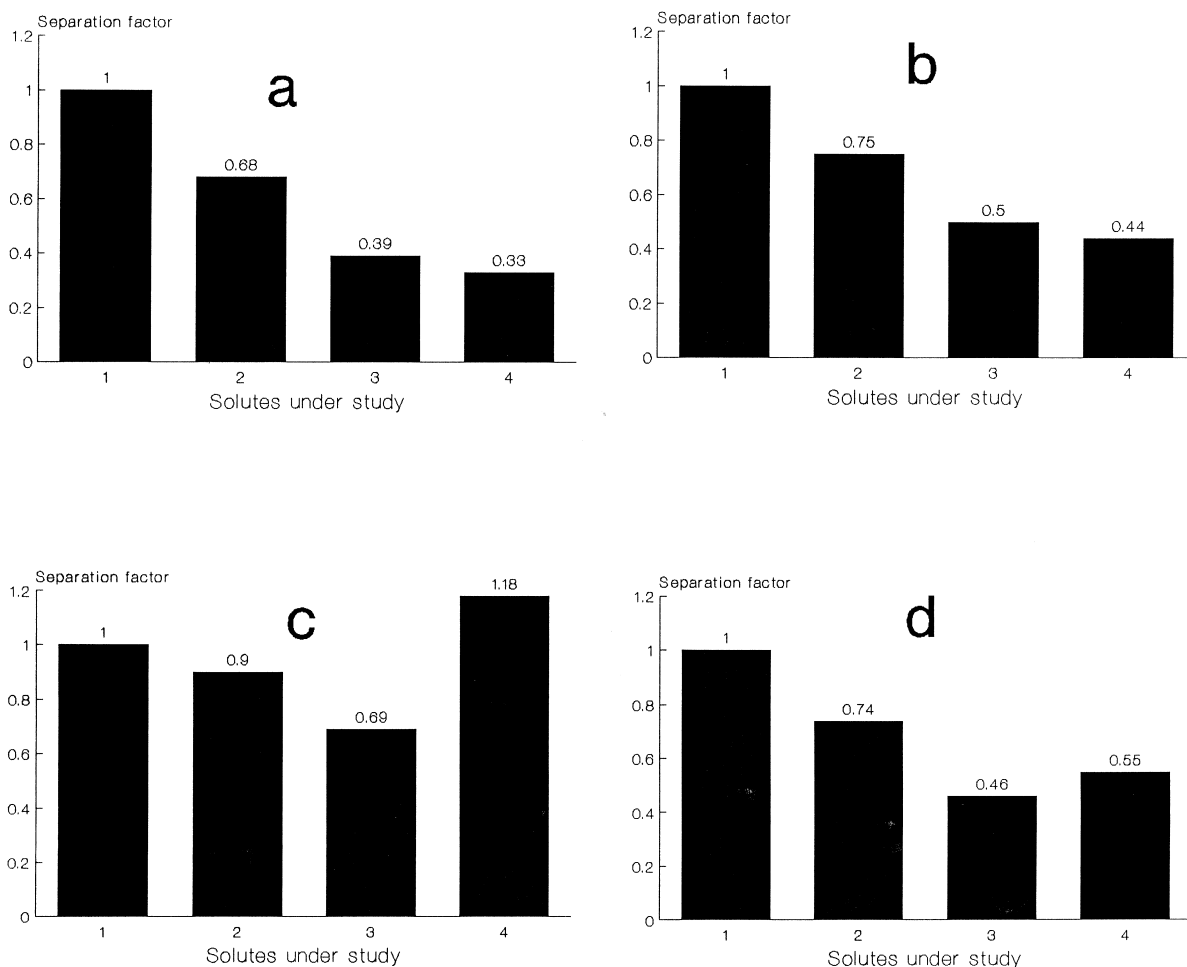

Fig. 6. Separation factor α for solutes 1–4 (Fig. 1) in MPA systems. Mobile phases: (a) HEX–IPA–DMSO (80:14:6); (b) HEX–IPA–DMSO (72:18:10); (c) HEX–IPA–ethylene glycol (87:10:3); (d) HEX–IPA–formamide (57:38:5).

Table 1

Reproducibility of selectivity coefficient (α) in the MPA system with HEX–IPA–DMSO (72:18:10) mobile phase

No.	Column: trade name (dimensions, mm)	Packing material		Phase ratio (V_s/V_m)	Retention (k')		Selectivity coefficient, α (2/3)
		Specific surface area (m^2/g)	Specific pore volume (ml/g)		Thymine (2)	Theophylline (3)	
1	LiChrospher Si-60 (125×4.0)	700	0.85	0.21	3.80	2.50	1.52
2	Silasorb 600 (250×4.0)	600	1.00	0.21	3.11	2.10	1.48
3	LiChrosorb Si-60 (250×4.0)	500	0.70	0.19	2.89	1.90	1.52
4	Zorbax SIL (150×4.6)	350	0.80	0.20	3.00	2.00	1.50
5	Supelcosil LC-SI (250×4.6)	170	0.60	0.18	2.56	1.69	1.51
6	Nova Pak Silica (150×3.9)	120	0.30	0.13	1.66	1.09	1.52

The selectivity of the MPA system (composition of the mobile phase corresponds to point 9 in Fig. 2) obtained on different columns is shown in Table 1. It can be concluded that the volume of the dynamically-generated stationary phase is larger and retention is stronger on the silicas with larger surface area and pore volume. But the selectivity obtained in the MPA mode does not differ much on different silica columns.

The following two series of experiments (Table 2) were performed to assess the degree of reversibility of column modification (composition of the mobile phase corresponds to point 8 in Fig. 2). In one series of experiments (set 1 in Table 2), the column was washed as described in Experimental, then equilibrated with the mobile phase studied. Such washing–equilibration cycles were repeated six times in 2 days. The standard deviation of the obtained retention times was used as a measure of reproducibility [20]. After each equilibration, the whole set of retention times was measured. In the second set of experiments (set 2 in Table 2), equilibration of the column and measurement of the retention were repeated six times during 2 weeks followed by several separations performed with other solvents (e.g., mobile phases in Fig. 1). The system was washed again and equilibrated with the initial mobile phase. It is seen that the reproducibility of retention time is satisfactory. The results (Tables 1 and 2) show that the MPA systems with DMSO are applicable in analytical practice.

4. Conclusions

Dimethyl sulfoxide is an appropriate eluent additive for moderating the silica surface in adsorption chromatography. The substitution of IPA by DMSO in binary mobile phase results in improvement of the peak shape and specific selectivity for some purine and pyrimidine derivatives. DMSO is immiscible with HEX, and, therefore, changes in retention characteristics and peak symmetry in HEX–IPA–DMSO systems are induced by transition from adsorption to partition mechanism of sorption. A stationary liquid phase is generated dynamically, and if the phase ratio exceeds 0.1 partition dominates over adsorption. For such HEX–IPA–DMSO MPA systems a good selectivity and peak symmetry of solutes under study are typical. MPA systems are an alternative approach to the separation of polar solutes on silica. The studies on HEX–IPA–DMSO mobile phases enlarge the number of MPA systems applicable in analytical practice.

Table 2

Reproducibility of retention times in system with HEX–IPA–DMSO (76:16:8) mobile phase

Solutes under study[a]	Retention time, $t_R \pm SD$ (min) ($n=6$)	
	Set 1	Set 2
1	7.31±0.05	7.32±0.11
2	5.47±0.06	5.56±0.13
3	3.83±0.04	3.86±0.08
4	3.37±0.04	3.39±0.06

[a] See Fig. 1.

Acknowledgements

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