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Dynamically generated stationary liquid phase systems with silica and ternary mobile phases containing ethylene glycol and formamide

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Abstract

Chromatographic systems with a silica sorbent and ternary mobile phases consisting of solvents with limited solubility have been studied. The investigation of mobile phases consisting of ethylene glycol, methanol and ethyl acetate has shown that a stationary liquid phase is generated dynamically in the pores of silica, even in the mobile phases that are not saturated with the polar component. The retention mechanism is mixed, and if the phase ratio reaches 0.1 partition dominates over adsorption. Such mixed partition–adsorption (MPA) systems show very good column efficiency and selectivity. Several types of ternary liquid systems were tested as mobile phases for MPA in HPLC of some purine and pyrimidine derivatives. The results demonstrated that such systems are applicable in analytical practice.

Keywords: Mobile phase composition; Mixed partition–adsorption systems; Stationary phases, LC; Purines; Pyrimidines; Ethylene glycol

1. Introduction

Normal-phase chromatography is not widely used for separation of highly polar solutes, mainly because of poor peak shape. In order to improve it, one can use better purified silicas [1] or polar chemically bonded phases [2]. Another approach is based on liquid–liquid chromatography systems [3,4], including dynamically generated ones [5–8].

Our previous papers [9,10] showed that chromatographic systems with unmodified silica and binary mobile phases consisting of ethyl acetate and the weakly soluble polar components ethylene glycol (EG) and formamide (FA) were useful in the chromatography of purine and pyrimidine derivatives. It was demonstrated that a liquid stationary phase consisting mainly of a polar solvent is deposited in the pores of silica during its equilibration with the mobile phase. Such dynamically generated stationary liquid phase systems showed very good column efficiency and peak symmetry for the studied polar solutes. We found that formation of a liquid stationary phase did not necessarily require saturation of the mobile phase, but was also possible with an unsaturated mobile phase.

The limited solubility of EG and FA in ethyl acetate restricts the elution strength of binary systems. It was shown [9] that addition of methanol to the mobile phase increased the elution strength. Therefore, some of the most polar solutes were

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tested in ternary mobile phases consisting of ethyl acetate (EA), EG and methanol (MeOH). It was found [9] that in order to maintain the good peak symmetry typical of the partition mode, the proportion of EG in the mobile phase should exceed the proportion of MeOH. However, this problem was not studied in detail. The addition of MeOH to the binary systems based on EA–EG and EA–FA does not only lead to changes in the elution strength of the mobile phase, but also increases the mutual solubility of the mobile phase components, due to the complete miscibility of MeOH with both solvents. Consequently, when the concentration of MeOH is considerable, the conditions for the generation of stationery liquid phase may become unfavourable.

Fig. 1 represents the triangular phase diagram for the ternary system ethylene glycol–methanol–ethyl acetate (EG–MeOH–EA). Points 6–9 below the equilibrium curve correspond to the composition of ternary systems which split into two co-existing liquid phases, one of which is presaturated with EG, but the other with EA. If the first of the coexisting phases is applied as a mobile phase, solvent-generated liquid–liquid systems with a retention partition mechanism are formed [5–8]. Points 2–5 above the equilibrium curve correspond to the compositions which are homogeneous and differ from one another by the degree of saturation with EG. 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 deposited liquid phase. It is not clear whether the compositions 2–5 are able to generate a sufficient amount of stationary liquid phase to produce good column efficiency and peak symmetry for the polar solutes.

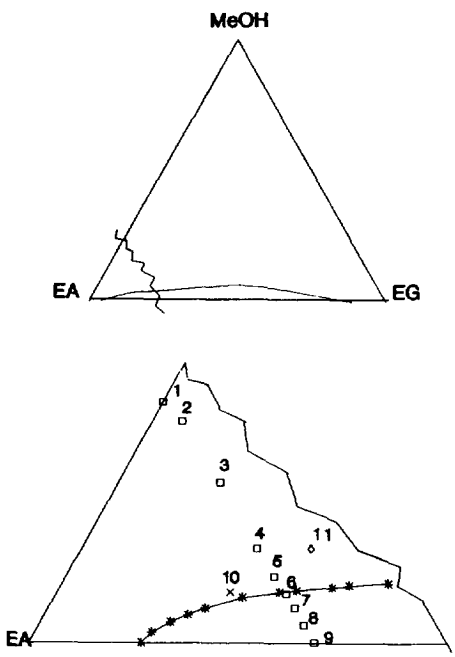

Fig. 1. Triangular phase diagram of the ternary system EG–MeOH–EA at ambient temperature.

The aim of the present work was:

1. To find the conditions under which the mobile phase consisting of EG, MeOH and EA is suitable for separation of the solutes under study [adenine (1), hypoxanthine (2), inosine (3), acyclovir (4), cytarabine (5) and guanosine (6) (numbers as in Fig. 2)].
2. To control retention and selectivity by changing the composition of the ternary mobile phase (e.g., replace EG with FA or EA with chloroform).

2. Experimental

The chromatographic measurements were performed using a DuPont Model 8800 HPLC system, equipped with a spectrophotometer (λ=254 or 280 nm). The columns (150×4.6 mm I.D.) were packed with unmodified silica Zorbax SIL, 5–6 μm (DuPont). Mixtures of EA and chloroform ($CHCl_3$) with MeOH, EG and FA were studied as the mobile phases. All the solvents were purchased from commercial sources, were of analytical grade and were used without any pretreatment. The flow-rate was 1.5 ml/min. The samples (10–25 μl, 0.025–0.1 mg/ml) were injected via a Rheodyne 7125 sampling valve.

The column was conditioned before each series of retention measurements. It included flushing with 50 ml MeOH and 50 ml EA or $CHCl_3$ followed by the mobile phase under study. Usually 350 ml of eluent was sufficient to obtain constant retention values.

The capacity factors of the solutes under study (k') and theoretical plate number (N) were calculated according to the usual expressions [11]. The system mobile phase volume was regarded to be equal to benzene retention volume. The peak width for col-

umn efficiency calculations was measured at half height. The phase ratio of the column (V_s/V_m) was calculated according to Ref. [10].

Values of the liquid–liquid equilibrium curve of the ternary system EG–MeOH–EA were determined by mutual titration of a binary MeOH–EA system with the EG at the ambient temperature.

3. Results and discussion

The chromatogram in Fig. 2 shows the behaviour of substances under study in typical condition for normal-phase adsorption chromatography on silica. According to Fig. 2, the mobile phase containing 16% (v/v) MeOH in EA possesses sufficient elution strength for test solutes. At the same time peak tailing is observed, which is typical for the adsorption chromatography of polar solutes on the Zorbax SIL column [1], and the system selectivity is poor.

The behaviour of the studied substances in chromatographic systems with silica and mobile phases consisting of EA, EG and MeOH was investigated (Fig. 3). The composition of the mobile phase was varied by changing the concentration of EG and MeOH from 0 to 16% and 16 to 0%, respectively, at the same time keeping the volume of ethyl acetate constant at value of 84%. The compositions of the mobile phases with the concentration of EG higher than 12.5% correspond to the one of the coexisting

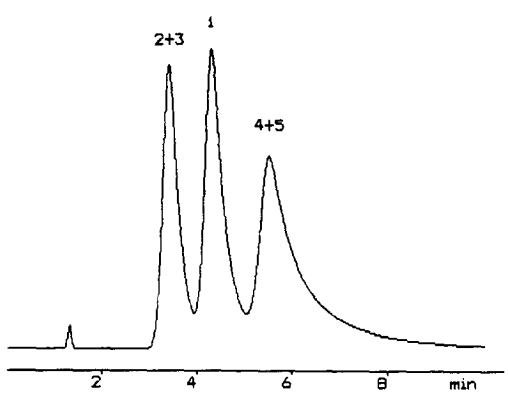

Fig. 2. Chromatogram of a test mixture of solutes: (1) adenine; (2) hypoxanthine; (3) inosine; (4) acyclovir; (5) cytarabine. Mobile phase, 16% MeOH in EA.

phases (saturated with EG). The use of such mobile phases leads to liquid–liquid systems with retention partition mechanism. The compositions with less than 12.5% EG correspond to the homogeneous systems. The use of such mobile phases leads to a chromatographic systems with a mixed retention mechanism.

Fig. 3a shows the relationship between the column efficiency (N) and the concentration of EG in the investigated mobile phases. As one can see the gradual replacement of MeOH with EG leads to an increase of the column efficiency in homogeneous mobile phases. In saturated mobile phases the column efficiency is high and unchanged.

The relationship between the volume of EG in the mobile phase and capacity factor (k') of the compounds under study is presented in Fig. 3b. When the mobile phases are homogeneous only a slight increase in retention is observed. On the other hand, the sharp increase in retention of all solutes takes place if the saturated mobile phases are used.

Since the investigated homogeneous ternary solutions contain poorly soluble components, the dynamic generation of a stationary liquid phase on silica can be expected when such solutions are used as mobile phases [9,10]. Let us assume that under conditions of typical adsorption chromatography with an eluent strong enough (EA) benzene is not adsorbed and in this case its retention volume corresponds to the mobile phase volume V_m or total volume V_{mo} within column, apart from the silica. The formation of the dynamically generated stationary liquid phase with volume V_s that leads to a decrease in the mobile phase volume V_m and the total volume V_{mo}, in this case is $V_{mo}=V_m+V_s$. It is possible to estimate the volume of the stationary phase (V_s) as the difference between the total volume (V_{mo}) and the mobile phase volume (V_m): $V_s=V_{mo}-V_m$, where V_s is the volume of the dynamically generated stationary liquid phase; V_{mo} the retention volume of benzene in the typical adsorption mode; and V_m the retention volume of benzene in the dynamically modified system.

Fig. 3c represents the relationship between the EG concentration in the mobile phase and the column phase ratio (V_s/V_m) and mobile phase volume (V_m). The formation of the liquid stationary phase depends on the concentration of EG in the mobile

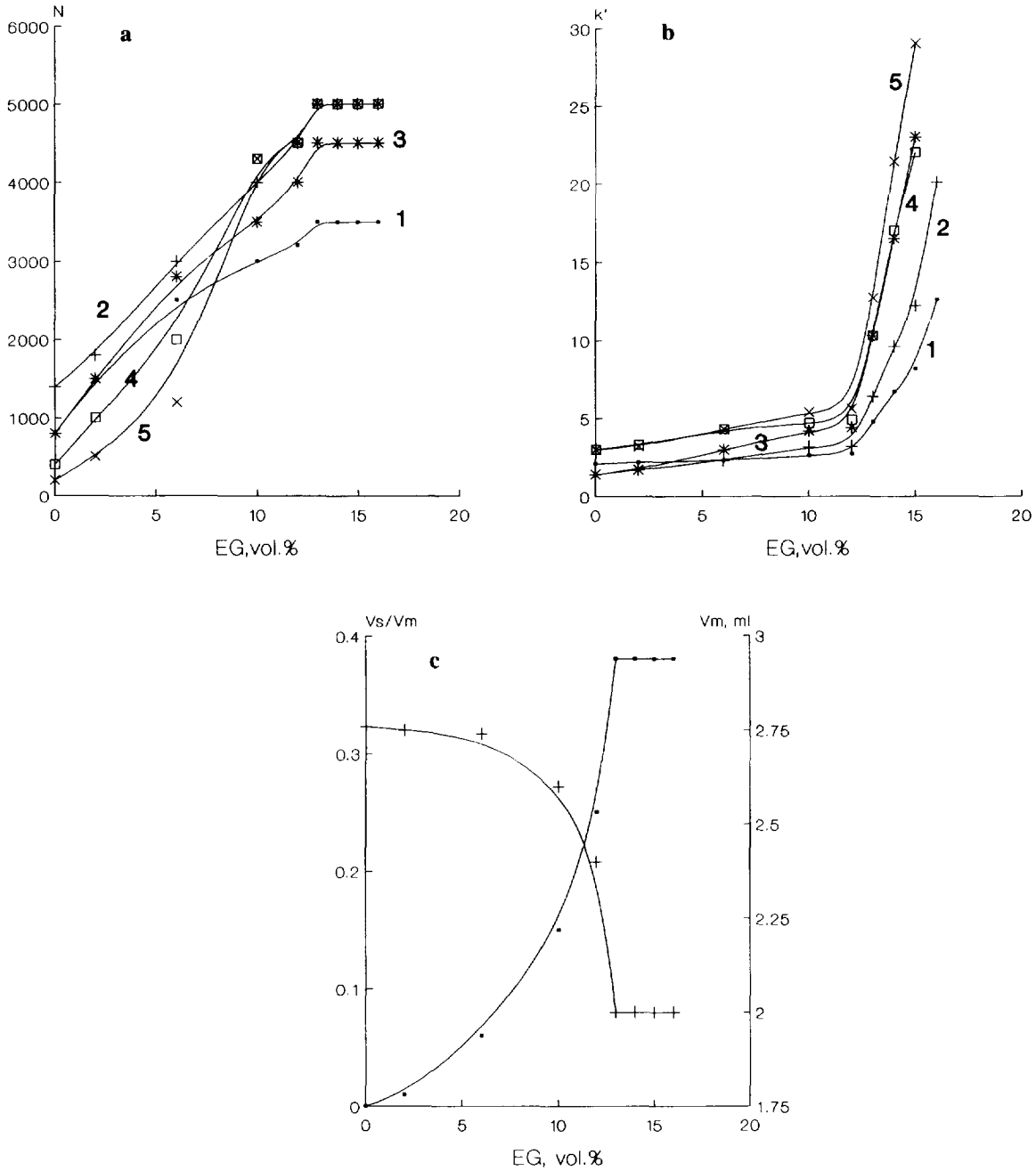

Fig. 3. Effect of the EG concentration in the ternary mobile phase EG–MeOH–EA on theoretical plate number N (a), retention of the solutes under study k' (b), column phase ratio V_s/V_m and mobile phase volume V_m (c).

phase. It follows from Fig. 3c that an amount of the stationary liquid phase is generated in all cases when a homogeneous mobile phase is used. If the concentration of EG is less than 2% the column phase ratio increases slowly and its value is less than 0.02. Adsorption dominates under these circumstances

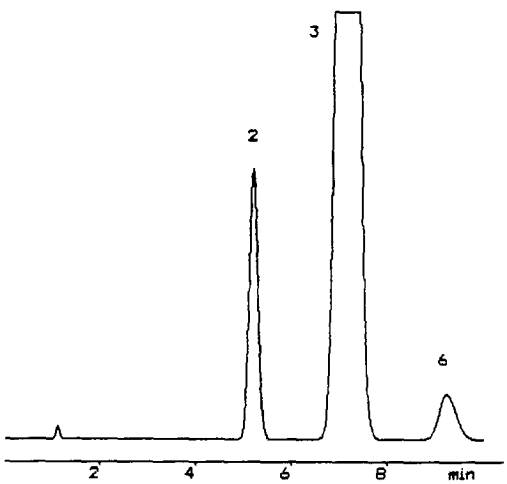

Fig. 4. Separation of inosine (3), hypoxanthine (2) and guanosine (6). Mobile phase, EA–EG–MeOH (84:12:4).

[10]. The column efficiency and the system selectivity for such systems are poor (Fig. 3a,b). If the column phase ratio is between 0.02 and 0.1 (EG concentration 3–8%), the column efficiency and the system selectivity are better than in the adsorption mode, but worse than in the partition mode (saturated mobile phases with more than 12.5% EG). If the column phase ratio is higher than 0.1 (EG concentration 8–12.5%) the column efficiency is good and the system selectivity is better than in the case of partition systems. Conditionally, we call such systems as mixed partition–adsorption (MPA) considering that a retention partition mechanism prevails over adsorption. It may be concluded from Fig. 3 that good separation conditions for the test solutes (1–5) could be obtained in MPA systems. The compositions of the investigated mobile phases in Fig. 3 correspond to points 1–9 in Fig. 1. One can see that for realization of the MPA mode one must choose the compositions which are located not very far from the equilibrium curve (i.e., not further than point 4).

The findings demonstrated that MPA systems are applicable in normal-phase chromatography of purine derivatives. The chromatogram in Fig. 4 represents the separation of inosine (3) and its typical impurities hypoxanthine (2) and guanosine (6) [12]. According to Fig. 4 the selectivity and peak shape is much better in the MPA system than in adsorption system (Fig. 2).

The selectivity of MPA systems obtained on different columns is shown in Table 1. The columns (see Table 2) have been used in the laboratory for routine analysis for at least one year in traditional organic solvents, that included also acids (No. 5) and bases (No. 6). It can be concluded from Table 1 and Table 2 that the volume of the dynamically-generated stationary phase is larger and retention is stronger on the silicas with larger surface areas (smaller pores). Also, the surface activity is important, which is obviously seen from the comparison of data on Silasorb columns (see Table 1, Nos. 3–6). Nevertheless, the selectivity obtained in the MPA mode does not differ much and, therefore, such MPA systems are applicable in analytical practice.

In order to estimate the flexibility of MPA mode we varied the concentration of polar components in ternary mobile phases, as well as replaced EG and EA with FA and $CHCl_3$, respectively. The results

Table 1
Reproducibility of retention (k') and selectivity (α) of the MPA system EA–EG–MeOH (84:12:4)

Column		Retention (k')			Separation factor α	
No.[a]	Phase ratio	Hypoxanthine (2)	Inosine (3)	Guanosine (6)	(k'_3/k'_2)	(k'_6/k'_2)
1	0.13	3.78	5.56	7.89	1.47	1.42
2	0.24	5.25	8.21	11.99	1.56	1.46
3	0.30	7.98	12.07	17.60	1.51	1.46
4	0.30	7.72	11.65	16.76	1.51	1.44
5	0.30	8.20	12.48	18.07	1.52	1.45
6	0.18	4.32	6.38	9.04	1.48	1.42

Flow-rate, 2 ml/min; UV, 254 nm.
[a]See Table 2.

Table 2
Specification of silica columns

Column No.[a]	Packing material[b]				
	Trade name	Particle size (μm)	Pore size (Å)	Surface area (m^2/g)	Pore volume (ml/g)
1	Supelcosil LC-SI	5	100	170	0.6
2	Zorbax SIL	5–6	70	350	0.8
3	Silasorb SPH 600	5	60	600	1.0
4	Silasorb SPH 600	5	60	600	1.0
5	Silasorb SPH 600	5	60	600	1.0
6	Silasorb SPH 600	5	60	600	1.0

[a]Dimensions, 250×4.6 mm; columns No. 1 and 2 were obtained from the manufacturer; columns No. 3–6 were prepared internally.
[b]Particle shape, spherical.

given in Table 3 show that when the concentration of polar components in all ternary mobile phases is increased a decrease of retention is observed for all test solutes, which is typical for normal-phase chromatography. When the concentration of polar solvents is the same (see Table 3, systems 2 and 3) the proportion of EG and MeOH does not influence retention very much. It is seen that selectivity does not change very markedly with changing in polar solvents proportion and concentration in one ternary system (see Fig. 5a–d). The obtained results suggest that system selectivity can be varied by replacing EG and EA with FA and $CHCl_3$. It is seen that chloroform possesses higher elution strength than ethyl acetate (see Table 3, systems 2 and 8) which is unusual for normal-phase chromatography [11]. A substitution of EG by FA (see Fig. 5a–d and Fig. 5e; Fig. 5f–g and Fig. 5h) leads to considerable selectivity alterations. However, the substitution of ethyl acetate by chloroform (see Fig. 5g–d and Fig. 5f–g; Fig. 5e and Fig. 5h) causes only minor selectivity changes. A substitution of EG by FA, as well as substitution of EA by $CHCl_3$ leads to inversion of the elution order for the inosine/acyclovir (3/4) pair.

Acknowledgments

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Table 3
Retention of the solutes studied (1–5) in MPA systems

No.	Mobile phase	Composition (v/v)	Concentration of polar components (v/v)	Retention (k') of the solutes studied				
				1	2	3	4	5
1[a]	EA–EG–MeOH	87:10:3	13	4.71	5.40	5.40	11.0	13.0
2[b]		84:12:4	16	2.72	3.20	4.40	4.91	5.60
3[c]		84:10:6	16	2.65	3.20	4.20	4.70	5.40
4[d]		81:13:6	19	2.19	2.61	3.60	4.08	4.38
5	EA–FA–MeOH	85:13:2	15	3.27	8.00	31.3	19.8	31.1
6		83.5:15:1.5	16.5	2.63	5.42	15.1	11.3	15.1
7	$CHCl_3$–EG–MeOH	88:10:2	12	4.23	4.91	6.46	5.51	8.91
8		84:12:4	16	2.08	2.21	2.75	2.60	4.05
9	$CHCl_3$–FA–MeOH	80:10:10	20	2.00	3.11	7.10	4.70	10.0

[a]Point 10 in Fig. 1.
[b]Point 5 in Fig. 1.
[c]Point 4 in Fig. 1.
[d]Point 11 in Fig. 1.

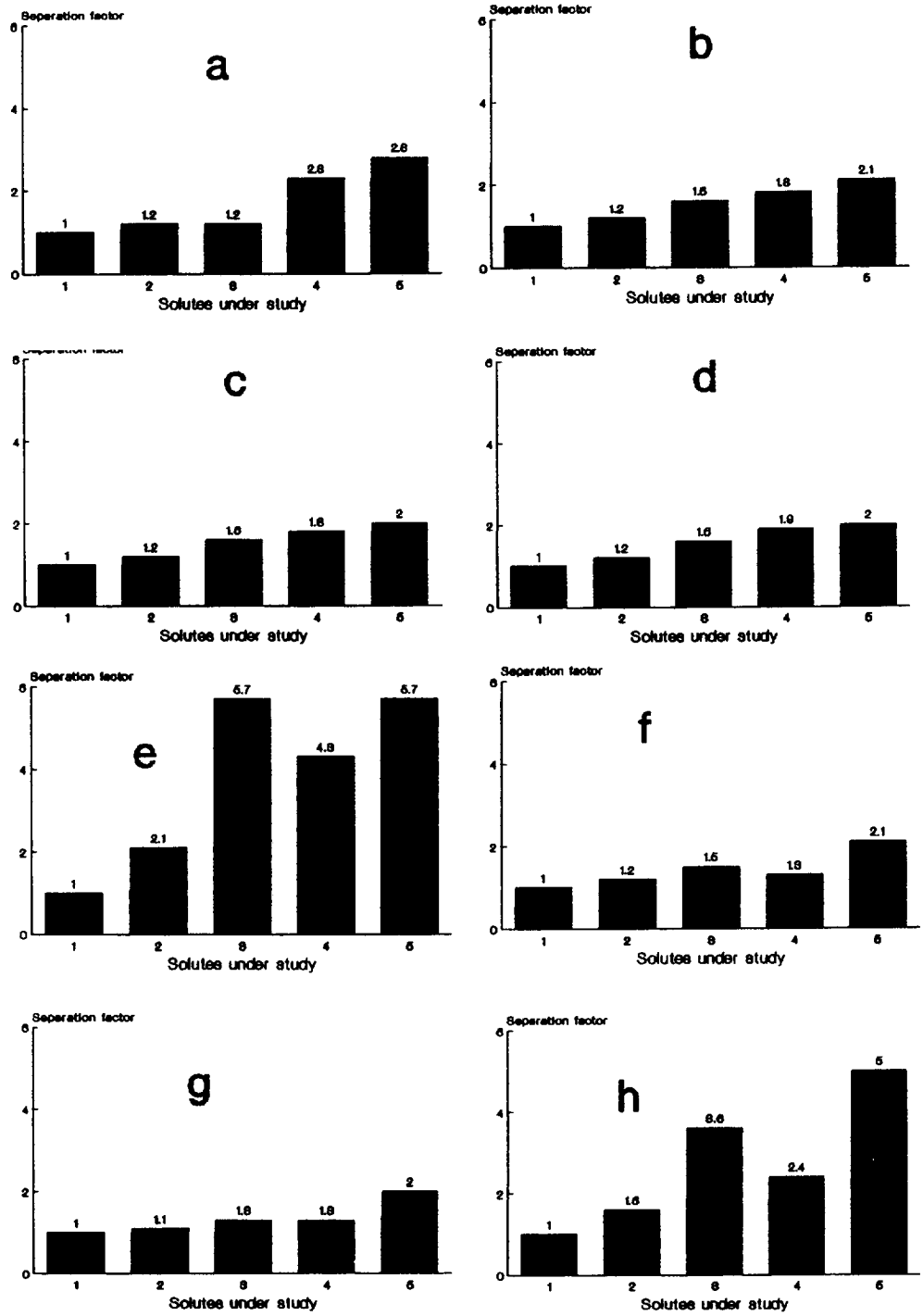

Fig. 5. Separation factor α for solutes 1–5 in MPA systems (a–h). Mobile phase composition (see Table 3): (a) No. 1; (b) No. 2; (c) No. 3; (d) No. 4; (e) No. 6; (f) No. 7; (g) No. 8; (h) No. 9.

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