ELSEVIER

Journal of Chromatography A, 762 (1997) 27–33

JOURNAL OF CHROMATOGRAPHY A

Relationship between stationary and mobile phase composition and its influence on retention factors of aromatic hydrocarbons in reversed-phase high-performance liquid chromatography

R. Nasuto*, L. Kwietniewski, J.K. Różyło

Faculty of Chemistry, M. Curie-Skłodovska University, 20-030 Lublin, Poland

Abstract

The effect of the composition of the mobile phase on the composition of the stationary phase and its influence on the retention factor, k', of polynuclear hydrocarbons has been determined. The decreasing relationship between log k' and the concentration of modifier (methanol) in mobile and stationary phases has been proved. It has been determined that the solubility of examined aromatic hydrocarbons in stationary phase is a reason for changes in the k'. This fact can be used in order to improve the effectiveness of separation of some mixtures.

Keywords: Stationary phases, LC; Mobile phase composition; Retention factors; Hydrocarbons; Polynuclear aromatic hydrocarbons

1. Introduction

In liquid chromatography, it is usually assumed that the chemical composition of the stationary phase does not change, irrespective of changes in the composition of the mobile phase [1–4]. According to this assumption, it is the type of stationary phase (of fixed physical characteristics) as well as the type of mobile phase (at the optimal temperature and flow-rate) that has a substantial effect on the distribution of chromatographed substances.

The assumption that the composition of the stationary phase does not vary (composition meaning the chemical composition of the surface layer of the column packing) with the changing composition of the mobile phase is incorrect and inconsistent with the rules of thermodynamics. Chromatographic systems with a liquid mobile phase and a fixed stationary phase should be treated as adsorption systems in which the composition of the stationary phase is the function of the composition of two or more mobile phases under conditions of dynamic equilibrium. Thus, the composition of the stationary phase that occurs during the flow of a mobile phase of a given concentration is determined by the real adsorption isotherm n_i^s vs. x_i^l (where n_i^s is the number of moles of the component that are adsorbed on the surface of the column packing and x_i^l is the fraction of this component in the mobile phase). It should be stressed that, to date, a definite theoretical or experimental method for determining the composition of the surface phase (n_i^s) as a function of the composition of the volume phase (mobile phase, x_i^l) has not been worked out. The real adsorption isotherm n_i^s (x_i^l) is determined on the basis of experimentally measured values, the so-called excess adsorption isotherm (n_i^σ vs. x_i^l) for the assumed model of the surface layer structure ("surface

*Corresponding author.

PII S0021-9673(96)00859-X

phase") [5–9]. Equations that exist in the scientific literature characterizing the change in the composition of the surface layer depending on the concentration of the volume phase, usually describe only a group of compounds and therefore they cannot be used as general equations. This explains why different k' values have been obtained for the same compound, especially if different models have been used for determining the stationary phase composition (under the same conditions).

The main aim of this paper is to show experimentally how the change in the composition of the stationary phase affects the mobile phase composition (methanol–water) as well as the influence of the changing composition of the stationary phase on the retention factor k' of aromatic hydrocarbons in reversed-phase high-performance liquid chromatography (RP-HPLC).

2. Experimental

2.1. Materials

Benzene, toluene, anthracene, phenanthrene, pyrene, chrysene, chlorodimethyloctadecylsilane and silica gel Si 100 (Merck; 10 μm particle size) were used in experiments.

2.2. Preparation of the Si 100 ODS

Silanization of the silica gel surface Si 100 (with a specific surface area of 235 $m^2\ g^{-1}$) was described in [10,11]. The process of silanization was driven by the catalyst, morpholine. The deposit of coal on the silanized surface of silica gel was 19.4% (w/w). This is related to a concentration of alkyl radicals on the silica gel surface equal to 4.01 μmol m^{-2}. The specific surface area of the silanized silica gel is 232 $m^2\ g^{-1}$. The specific surface area was measured on the basis of the thermodesorption isotherm of nitrogen. Therefore, it is quite feasible that the silica gel surface area was not changed following silanization.

2.3. Chromatographic measures

A liquid chromatograph, type 302, produced by the Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland, equipped with a 10-μl injection loop (Rheodyne, Berkeley, CA, USA) and a UV detector, set at 254 nm, was used. A refractive index (RI) detector was used to measure the ad-

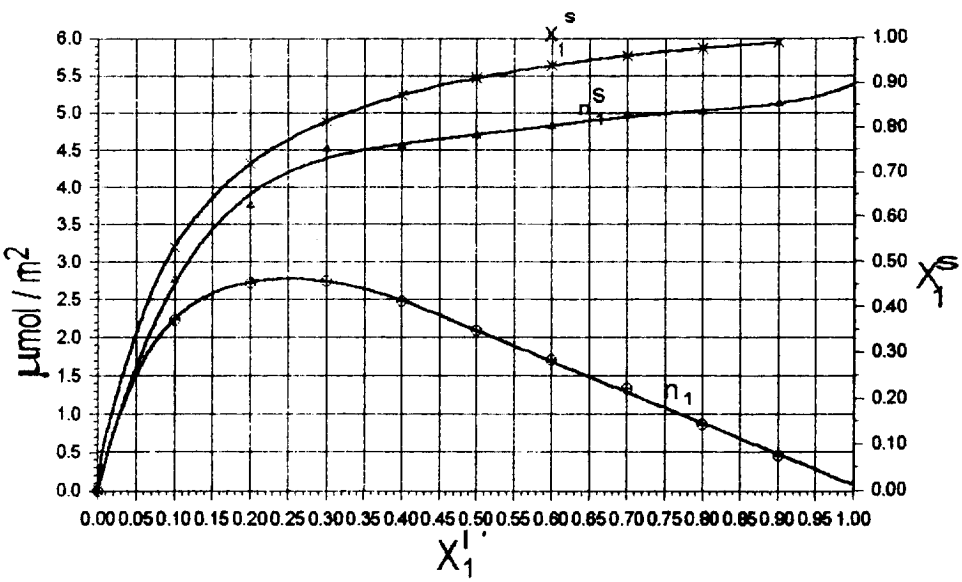

Fig. 1. Isotherms: real adsorption of methanol n_1^s vs. x_1^l; excess adsorption n_1 vs. x_1^l and its mol fraction in the surface phase x_1^s vs. x_1^l on silica gel Si 100 ODS.

sorption isotherm of methanol, which helped to get rid of the double peak effect. A stainless steel column (100×4 mm I.D.), the temperature of which was kept at 20±0.5°C was used. A methanol–water solution was used as the mobile phase at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 of the mol fraction of methanol in water. The flow-rate of the mobile phase was 0.5 cm^3 min^{-1}. Chromatographed substances were injected into the column as methanol solutions.

3. Results and discusion

Experimental determination of the excess adsorption isotherm of methanol of the methanol–water solutions (used as the mobile phase in chromatographic measurements) on silanized silica gel Si 100 ODS was necessary in order to solve the problem raised in this paper. This type of adsorbent was used to measure the retention of hydrocarbons that were examined by means of RP-HPLC. The excess adsorption isotherm of methanol (n_1^σ vs. x_1^l) was measured chromatographically [12–14] and is illustrated in Fig. 1, which also gives the real adsorption isotherm of methanol (n_1^s vs. x_1^l μmol m^{-2}), determined using the Everett equation [6], as well as the mol fraction of methanol in the surface phase (x_1^s vs. x_1^l).

Methanol adsorption isotherms will not be discussed in this paper as they are the subject of many publications dealing with the phenomenon of adsorption from liquid solutions on surfaces of adsorbents of different polarity [12,15,16]. We will discuss how adsorbed methanol (with its adsorption isotherm n_1^s vs. x_1^l) and the composition of the mobile phase influence the retention factor, k', of polynuclear aromatic hydrocarbons in RP-HPLC. As seen in Fig. 1, the concentration of methanol (x_1^s) in the surface phase is greater than in the volume phase (x_1^l), thus $x_1^s >> x_1^l$. Moreover, x_1^l approaches x_1^s as the concentration of methanol in the mobile phase

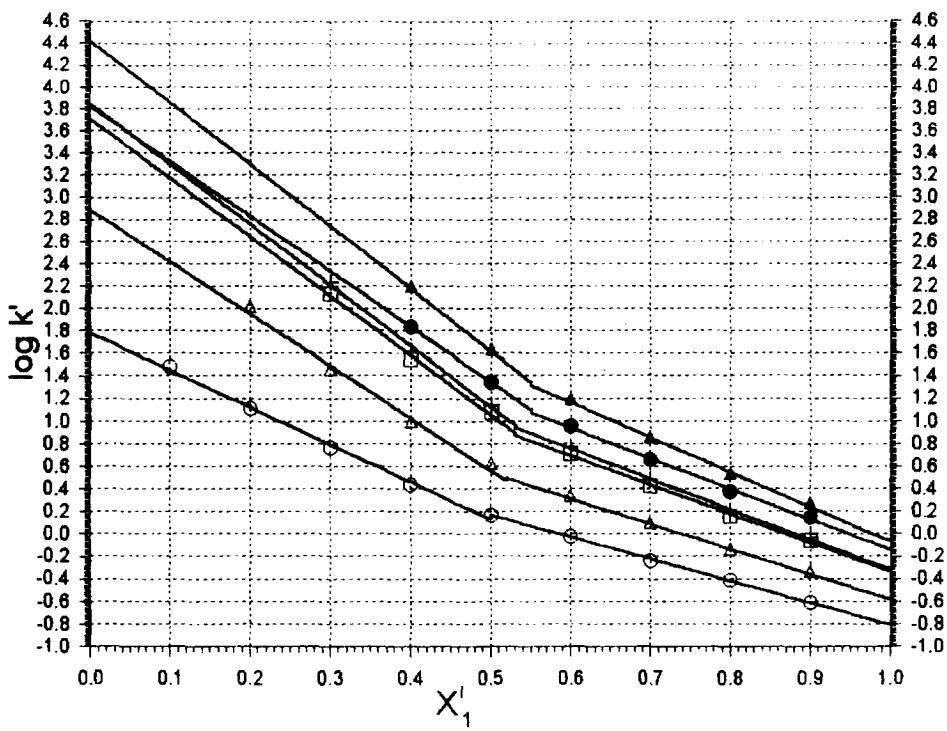

Fig. 2. Plots of the capacity factor log k' and the concentration of methanol in the liquid mobile phase x_1^l for aromatic hydrocarbons on silica gel Si 100 ODS: ○ = benzene, △ = naphthalene, + = anthracene, □ = phenanthrene, ● = pyrene, ▲ = chrysene.

increases. The stationary phase is made up of three components, radicals of octadecylsilane (ODS), adsorbed methanol and water, which can be described as follows: 4 μmol m^{-2} ODS + n_1^s (x_1^l) μmol m^{-2} adsorbed methanol + some number of mol of water (cannot be determined practically) at a given equilibrium concentration of methanol in the mobile phase (x_1^l). The chromatographic distribution of aromatic hydrocarbons occurs between the equilibrium mobile phase of methanol in water (which equals x_1^l) and the stationary phase of the composition described above. As the surface concentration of the bonded phase (ODS) is constant, its influence upon the value of k' is approximately constant, irrespective of the concentration of the mobile phase.

Therefore, k' depends only on changes in the concentration of methanol in water, both in the stationary and the mobile phase. Due to an indefinite amount of water in the stationary phase, changes in the value of k' of chromatographed hydrocarbons will be considered as functions of changes (1) in the methanol concentration in the mobile phase (x_1^l), (2) in the stationary phase (x_1^s), (3) in the number of mol of adsorbed methanol (n_1^s) on the surface packing unit and (4) in the number of atoms of carbon in the molecules of the aromatic hydrocarbons used.

The dependence of the values of log k' of aromatic hydrocarbons, obtained by RP-HPLC, as a function of the equilibrium concentration of the mobile phase, x_1^l, are presented in Fig. 2. The results show that there are two concentration intervals in which the values of log k' change rectilinearly with a change in velocity. In the first interval x_1^l=0.0–0.5 mol fraction, values of log k' vs. x_1^l decrease faster than in the interval of the mol fraction where x_1^l= 0.5–1. It should be stressed that the interval where log k' vs. x_1^l of the aromatic hydrocarbons examined decreases more steeply should be related to the interval, x_1^l, where the concentration of methanol in the stationary phase increases rapidly (Fig. 1). Decreasing values of log k', together with the increasing concentration of methanol in the mobile

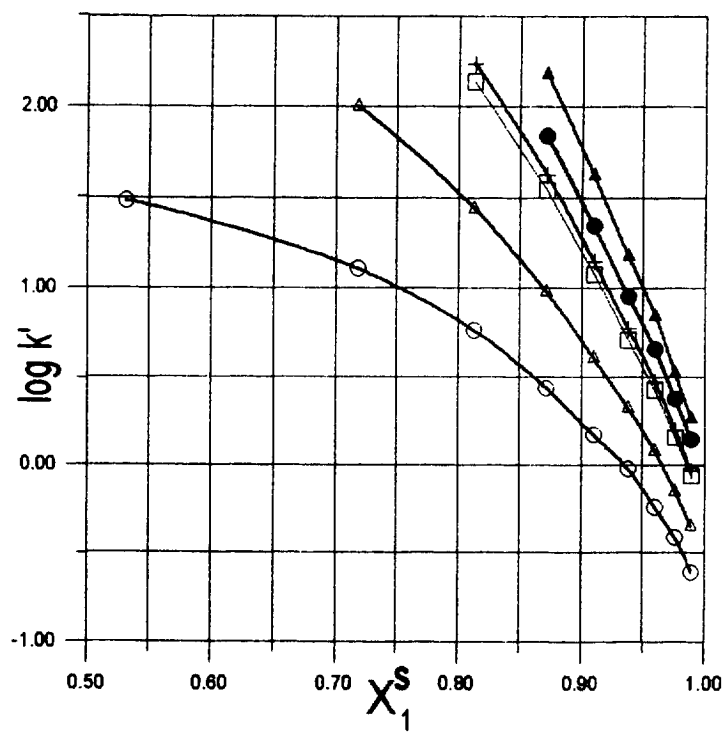

Fig. 3. Plots of log k' vs. x_1^s (x_1^s is the mol fraction of methanol in the stationary phase) for aromatic hydrocarbons obtained on silica gel Si 100 ODS: ○=benzene, △=naphthalene, +=anthracene, □=phenanthrene, ●=pyrene, and ▲=chrysene.

phase (and in the stationary phase) indicates that molecular interaction between the stationary phase and chromatographed hydrocarbons is decreasing. We may assume that it is mainly water, and not methanol, which is responsible for the log k' course, since, as can be seen in Fig. 1, the concentration of methanol in the stationary phase is greater than in the mobile phase (i.e., $x_1^s >> x_1^l$) over the entire concentration range of this phase. In the concentration interval of the mobile phase $x_1^l = 0.5–1.0$ of the mole fraction of methanol, the stationary phase composition undergoes smaller changes (which occur in the interval $x_1^l = 0.0–0.5$) which results in a slower decrease in the functions log k' vs. x_1^l. On the above basis, it can be claimed that in this interval, x_1^l, the mobile phase composition influences the change in log k' (x_1^l) to a greater extent (loss of water in this phase).

As seen in Fig. 2, decreasing log k' vs. x_1^l cross the concentration (x_1^l) axis at the values characteristic for a given hydrocarbon. In the point of crossing the function (log k') (x_1^l) values log $k' = 0$. A line equation can be used to describe such dependencies.

$$k'_{1,2} = k'_2 - ax_1^l \quad (2)$$

where $k'_{1,2}$ = retention factor of hydrocarbon, obtained using a two-component mobile phase for the methanol concentration interval $x_1^l = 0.0–0.5$; k'_2 = retention factor, when pure water was used as the mobile phase; x_1^l = mol fraction of the methanol in the mobile phase;and a = constant for a given hydrocarbon. Eq. (2) suggests that it is the methanol concentration in the mobile phase that is responsible for the decreasing function log $k'_{1,2}$ vs. x_1^l. However, as mentioned above, it is mainly water that is responsible for that kind of course; the loss of water in the mobile phase does not have to be equal to its loss from the strongly hydrophilic surface of the silica solid support (from the stationary phase). Thus, it is probable that the more water there is in the stationary phase compared with that in the mobile

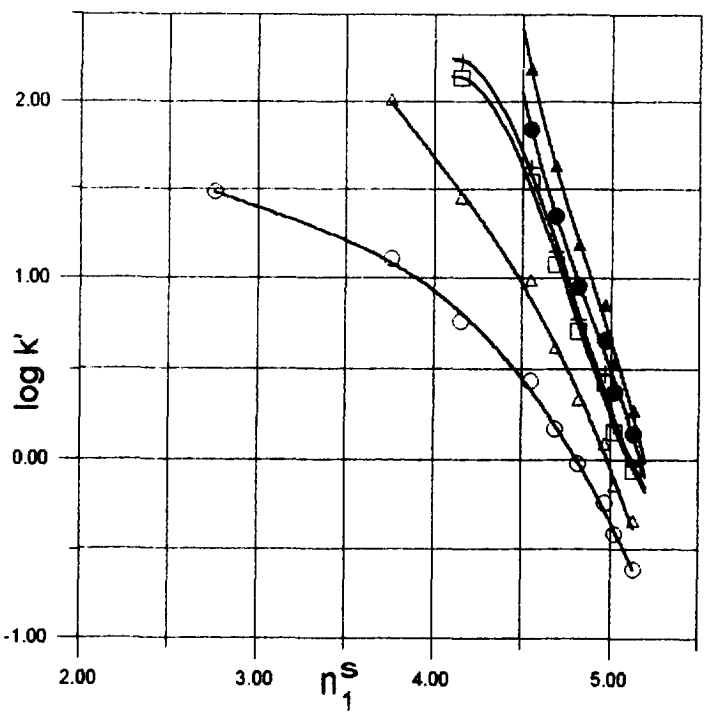

Fig. 4. Plots of log k' vs. n_1^s (n_1^s is the number of mol of methanol in the stationary phase) of aromatic hydrocarbons obtained on packing Si 100 ODS: ○ = benzene, △ = naphthalene, + = anthracene, □ = phenanthrene, ● = pyrene, ▲ = chrysene.

phase, causes a decrease in the solubility of chromatographed hydrocarbons in this phase. This occurs despite the fact that the content of hydrophobic hydrocarbon chains is greater in the stationary phase than in the mobile phase.

The influence of methanol in the stationary phase (x_1^s upon the change in the k' value of polynuclear aromatic hydrocarbons) is presented in Fig. 3. As has been mentioned previously, the methanol concentration in the stationary phase exceeds its concentration in the equilibrium mobile phase ($x_1^s >> x_1^l$). It somehow gives an explanation to the fact that the functions log k' vs. x_1^s run in the interval of considerably greater values x_1^s (see Figs. 2 and 3). In Fig. 3, it is seen that these functions are steeper for hydrocarbons with larger numbers of carbon atoms in a molecule (and less solubility in water). Decrease course of those functions is similar to the increase of adsorption of methanol on the stationary phase vs. its concentration in the mobile phase (Fig. 3)

Fig. 4 presents the dependence of log k' on n_1^s (n_1^s is the number of μmol of methanol adsorbed on a 1 m^2 surface of the column packing Si 100 ODS). It is seen that the functions decrease in a non-linear way with the quantity of methanol adsorbed on the surface of Si 100 ODS. The course of the above functions is similar to the function log k' vs. x_1^s (Fig. 3).

Fig. 5 illustrates the dependence log k' on n_c when a constant amount of methanol is adsorbed on the Si 100 ODS. These functions, both for phenanthrene and anthracene, prove that it is the size of the molecules of the aromatic hydrocarbons that influence, to a great extent, the retention factor k'. As seen in Fig. 5, log k' of phenanthrene and anthracene (which have fourteen carbon atoms each) do not

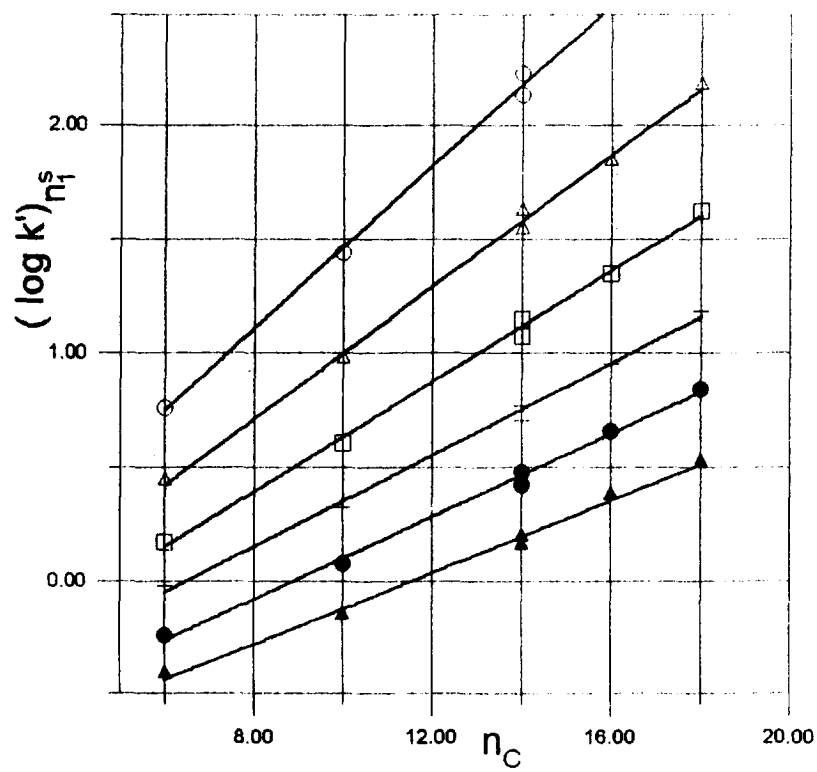

Fig. 5. Plots of log k' vs. number of carbon atoms (n_c) at constant surface concentrations of methanol in the stationary phase (n_1^s): ○=4.15, △=4.55, □=4.69, +=4.82, ●=4.97 and ▲=5.02 μmol m^{-2}.

differ practically from one another despite their differences in structure.

References

[1] G. Marko-Warga and D. Barcelo, Chromatographia, 34 (1992) 146.
[2] N. Sadlej-Sosnowska and I. Sledzińska, J. Chromatogr., 595 53 (1992).
[3] J. Puncocharova, L. Vodicka and J. Kriz, J. Chromatogr., 267 (1983) 222.
[4] A.M. Stalcup, D.E. Martire and S.A. Wise, J. Chromatogr., 442 (1988) 1.
[5] D.H. Everett, Trans. Faraday Soc., 60 (1964) 1803.
[6] D.H. Everett, Trans. Faraday Soc., 61 (1965) 2478.
[7] A.W. Kisielew and Ł.F. Pawłowa, Nieftiechimia, 2 (1962) 861.
[8] G. Schay, Acta Chim. Acad. Sci. Hung., 19 (1956) 281.
[9] G. Schay and L.Gy. Nagy, Acta Chim. Hung., 50 (1961) 207.
[10] B. Buszewski, Z. Suprynowicz, R. Lodkowski and R. Nasuto, Chem. Anal., 26 (1981) 685.
[11] Z. Suprynowicz, B. Buszewski, A. Waksmundzki and J. Gawdzik, Chem. Anal., 23 (1978) 325.
[12] G.A. Berendsen, L. De Galan, J. Liq. Chromatogr., 1 (1978) 561.
[13] E.H. Slaats, W. Markowski, J. Fekete and H. Poppe, J. Chromatogr., 207 (1981) 299.
[14] J.F.K. Huber and R.G. Gerritse, J. Chromatogr., 58 (1971) 137.
[15] A.W.J. De Jong, J.C. Kraak, H. Poppe and F. Nooitgedacht, J. Chromatogr., 193 (1980) 181.
[16] D. Fiat, M. Folman and U. Garbatski, J. Phys. Chem., 65 (1961) 2018.