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Adsorption and partition mode in high-performance liquid chromatography of highly polar solutes on silica

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ABSTRACT

The investigation of chromatographic systems with silica and mobile phases consisting of ethyl acetate and weakly soluble polar components ethylene glycol, triethylene glycol and formamide has shown that mixed mechanism of sorption is possible. When the solubility of the polar component is good, as in case of methanol, the retentions observed are typical of adsorption chromatography. When ethylene glycol, which has limited solubility, is used as the mobile phase component, a liquid stationary phase consisting mainly of polar solvent is formed in the pores of silica. This leads to a mixed mechanism of retention, selectivity changes and improvement of the peak shape. The formation of a liquid stationary phase both from saturated and unsaturated solutions is observed.

INTRODUCTION

Reversed-phase chromatography has become the most popular method of separation and analysis of organic substances belonging to different classes and widely differing in their properties. Nevertheless, the reversed-phase mode is not always the best or only choice for a given analytical task. This decision is very often based simply on tradition or use in similar experiments. Therefore it is our opinion that the analytical potential of high-performance liquid chromatography (HPLC) on unmodified silica is often underestimated. One of the potential advantages of unmodified silica is its higher selectivity in terms of the structural differences of the chromatographed solutes. The use of normal-phase separations in combination with reversed-phase ones can offer additional possibilities in thorough process and quality control.

Very polar organic substances, many of them biologically important, require eluents of 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. Ethylene glycol (EG) and some other highly polar mono- and bifunctional additives have been mentioned as possible eluent constituents [1], some of them having even higher elution strength than methanol. It has been shown [2–9] that mobile phases saturated with polar solvents, *e.g.*, water [2,6,8,9], EG [2], dimethyl sulphoxide [3], triethylene glycol (TEG) [4], formamide [5] or ethanol [7], generate a liquid stationary phase in pores of unmodified silica. The

obtained liquid–liquid partition chromatographic systems have been shown to be useful in analytical chromatography. Nevertheless, solvents of this kind have not been used widely until now. For example, no EG-containing eluents are cited in a review of 700 drug analyses [10].

The high viscosity of many highly polar solvents is a distinct disadvantage. However, if their high elution strength is taken into account, relatively low
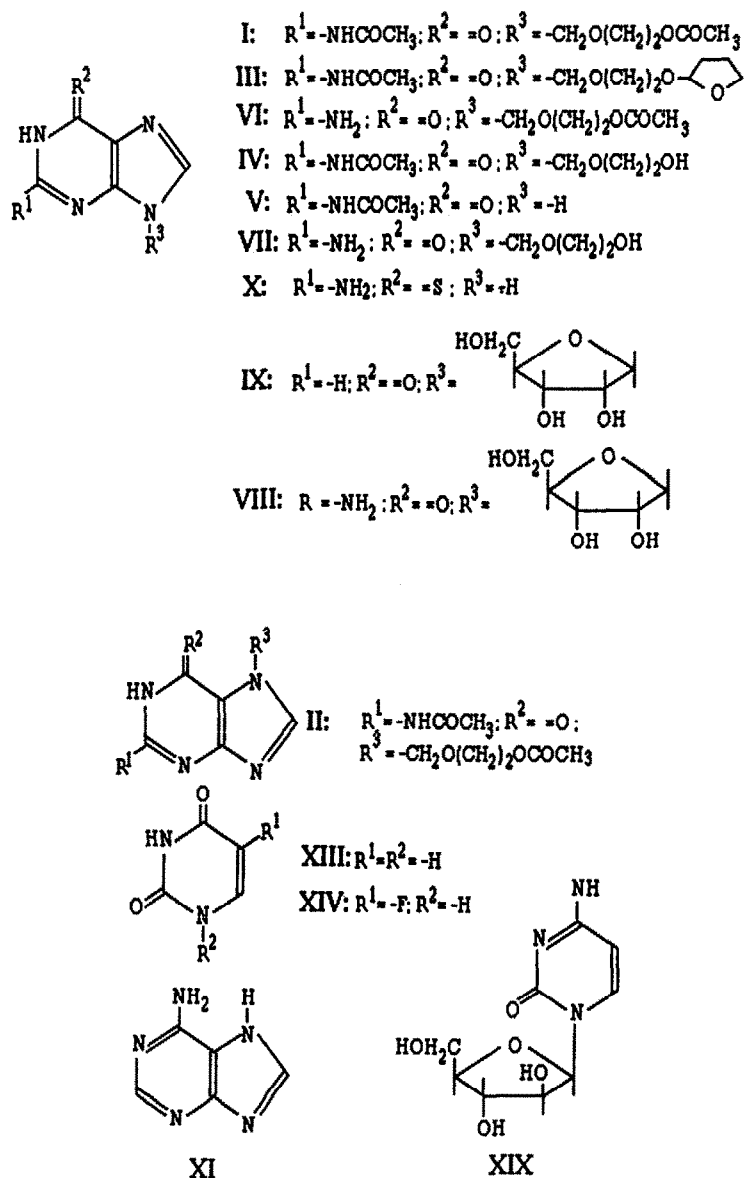

Fig. 1. Solutes under study.

concentrations of such solvent should be sufficient to obtain reasonable capacity factors. Bifunctional solvents, being adsorbed onto silica, may generate a surface layer with polar groups in contact with both the mobile and stationary phases. This may lead to additional selectivity effects compared with monofunctional solvents.

The goal of this work was to investigate the sorption behaviour of polar solutes in normal-phase chromatography on silica with mobile phases containing ethyl acetate and several highly polar additives and to discuss the applicability of this approach in analytical chromatography.

EXPERIMENTAL

The substances under study were the derivatives of purine and pyrimidine shown in Fig. 1.

A DuPont Model 8800 HPLC system with spectrophotometric detector (λ = 254 nm) was used for retention measurements. A 250 × 4.6 mm I.D. column from DuPont was packed with Zorbax SIL. Samples (10–25 μl of 0.05–1 mg/ml solution in the mobile phase) were injected via a Rheodyne 7125 loop injector. The eluents consisted of ethyl acetate as the less polar solvent, and EG, TEG, formamide and methanol as polar modifiers. All solvents were of "pure" grade and were supplied by Fluka. Column temperature was 25°C.

Capacity factors were calculated according to a standard formula:

$$k' = \frac{t_R - t_0}{t_0} \tag{1}$$

where t_R and t_0 are retention times of solute under study and benzene, respectively.

RESULTS AND DISCUSSION

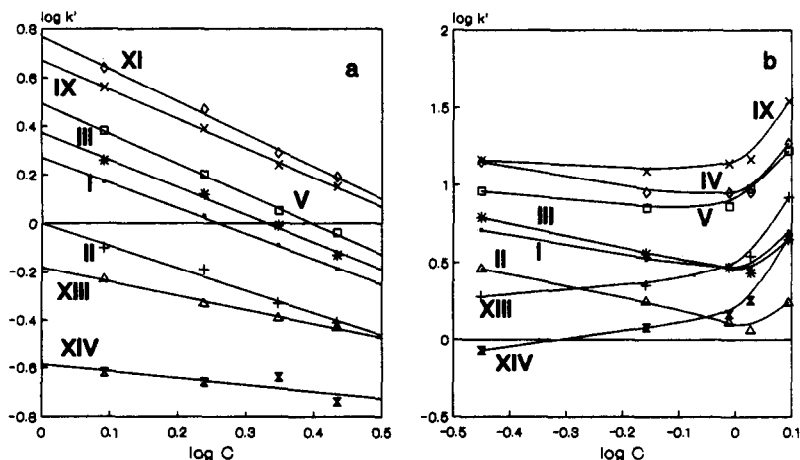Most of the substances under study are only slightly soluble in the hydrocarbons

Fig. 2. Relationship between the concentration of methanol (a) or ethylene glycol (b) and capacity factors.
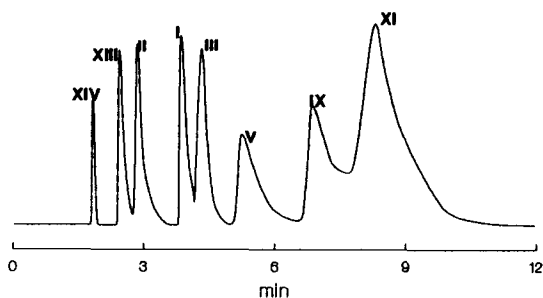
Fig. 3. Chromatogram of test solutes in the mobile phase, containing 5% methanol in ethyl acetate.

and chloroform that are generally used in normal-phase HPLC as less polar components of mobile phases. These solvents are immiscible with EG, and therefore ethyl acetate was chosen as a relatively non-polar solvent. It is completely miscible with methanol and dissolves up to 6.5% of EG at room temperature.

The relationship between capacity factors and molar concentrations of EG and methanol in ethyl acetate is shown in Fig. 2. It is seen that ethyl acetate–methanol eluents have sufficient elution strength for most of the compounds. At the same time peak tailing is typical (Fig. 3), especially for strongly retained solutes. Solute behaviour, represented in Fig. 2a, is typical for normal-phase adsorption chromato-graphy. The relationship between log k' and the logarithm of molar concentration of methanol is almost linear. The system selectivity does not change very markedly with increase of polar solvent concentration. This relationship can be expressed by the linear equation:

$$\log k' = b - p \log C \tag{2}$$

where C is the molar concentration of polar solvent in the mobile phase. It is known that the silica surface in equilibrium with eluent may be covered by a layer of the most polar solvent (in this case, methanol). The displacement of these molecules by the solute molecule can occur in the act of sorption. The average number of released methanol molecules per solute molecule is equal to parameter p of eqn. 2. It may be concluded from Fig. 2a that p values in this data set lie between 0.5 and 1.3. These are typical values for normal-phase adsorption chromatography on silica [11]. It follows then that, notwithstanding the high polarity of the studied compounds, a one-point sorption scheme is prevailing, on average about one molecule of methanol being displaced from the surface upon the sorption of one solute molecule.

This is not the case when EG-containing mobile phases are used (Fig. 2b). The relationship between log k' and log C is not linear. When the concentration of EG is increased in the range 2–4%, only a slight decrease of retention is observed for most of the solutes. The slope is unusually low for adsorption chromatography on silica. A further increase of C leads to an increase in retention of some of the solutes. A similar relationship between the concentration of stronger eluent component and retention was described previously [7,8,12]. The observed effect results in selectivity changes and inversion of elution order for some pairs of solutes. Consequently, this parameter can
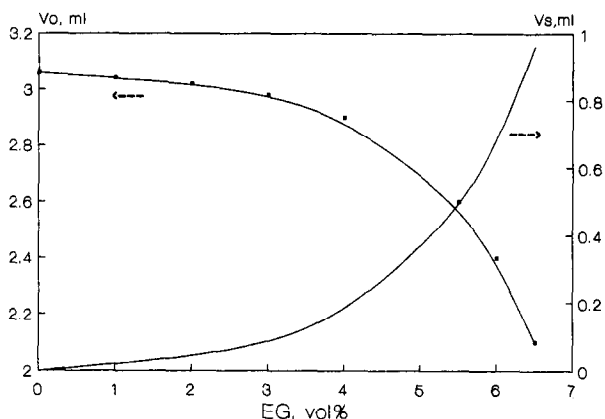
Fig. 4. Dependence of measured column dead volume (V_o) and calculated volume of liquid stationary phase. (V_s) on the concentration of ethylene glycol (EG) in the mobile phase.

be used as an efficient tool for selectivity variation. If the amount of EG used for mobile phase preparation exceeded the limit of miscibility C_1 (6.5%, v/v; log C = 0.062), a two-phase system was formed, and an ethyl acetate layer saturated with EG was used as eluent. Retention values observed with saturated eluents did not depend on the ratio of solvent volumes and were close to k' measured at C_1.

It is seen that a sharp increase in retention of all solutes takes place when the amount of EG approaches its limit of solubility. The increase of EG concentration in the mobile phase leads to a decrease in t_0 values. It is most pronounced near C_1 (Fig. 4) but undoubtedly takes place over the whole range of C. The observed effects can be explained by deposition of EG-rich bulk liquid in the pores of silica. It acts as additional amount of stationary phase. The obtained results show that formation of a liquid stationary phase does not necessarily require saturation of the mobile phase, but is possible with unsaturated solutions as well. Probably the deposition of bulk liquid is stimulated by the adsorption forces in the pores of silica. The volume of the stationary phase formed may be estimated from the decrease of t_0. The data in Fig. 4 show its dependence on EG concentration. It follows that retention with a mixed mechanism takes place over a wide range of EG concentrations. The phase ratio for the system with a saturated mobile phase is estimated to be about 0.5. The role of the partition process depends on the concentration of polar solvent and on the chemical structure of the solutes. It is most important at high C and for relatively less polar solutes. It is probably dominant for substances XIII and XIV that were only slightly retained by adsorption even at low EG concentration.

A correlation was found between k' and partition coefficients measured by the shake-flask method. It is further evidence of a partition mechanism of separation. It was concluded from comparison of the partition coefficients and capacity factors that the phase ratio of the system is about 0.6. This value is in a good agreement with the value obtained from decrease of t_0.

The elution strength of binary ethyl acetate–EG systems is not sufficient for some of the most polar solutes. Therefore a series of ternary ethyl acetate–EG–
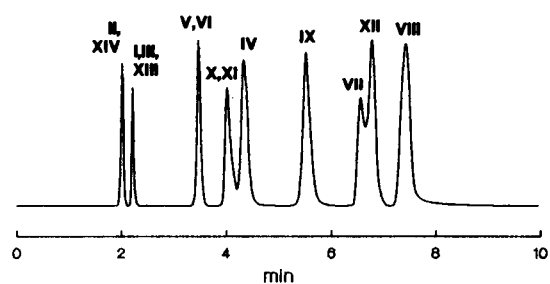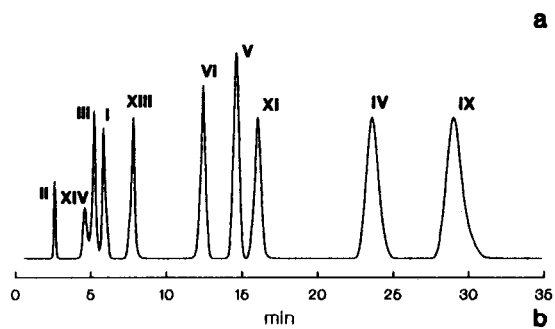
Fig. 5. Chromatograms of test solutes in (a) ethylene glycol-saturated mobile phase and (b) ternary solvent consisting of methanol–ethylene glycol–ethyl acetate (6:10:84).
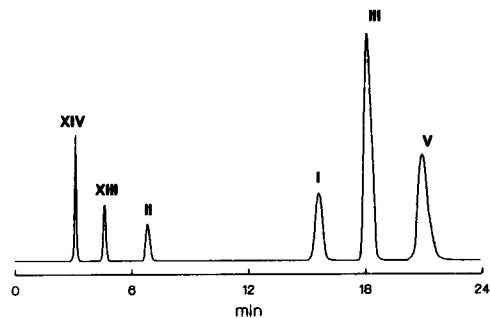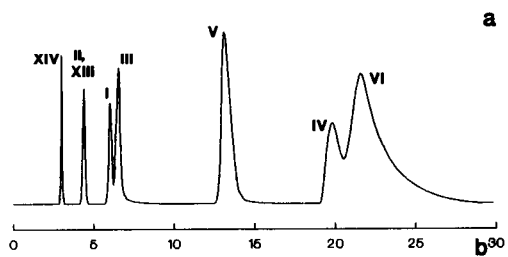

Fig. 6. Chromatograms of test solutes in the mobile phases consisting of ethyl acetate with addition of 4% triethylene glycol (a) and 1% formamide (b).

methanol mixtures was tested. Fig. 5b shows one of the chromatograms thus obtained. All the solutes under study are eluted at reasonable k' values. The experiments show 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 methanol.

Similar results were obtained with TEG and formamide as mobile phase constituents (Fig. 6). It should be emphasized here that in these cases the concentration of the polar solvents was far from saturation. Therefore the properties of such systems should differ from those of partition systems reported earlier [13].

The chromatograms in Figs. 5 and 6 show that peak shape is much better in the systems with a mixed or partition mechanism than in adsorption system ethyl acetate–methanol (Fig. 3). Each of the solvents produced systems with different selectivity of separation.

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