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Experimental study on the effect of the sample size on the band profile for a binary mixture showing no competitive interaction

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ABSTRACT

The effect of the sample size on the band profile of triphenylphosphine oxide and a piperidine derivative, for the pure components and for the binary mixture, were measured in reversed-phase chromatography. The experimental results showed reversal of the elution order with respect to the peak maximum. Deconvolution of the detector response for the mixture into individual component profiles was made by collecting fractions and subsequent analysis. The results show that the profile of the two components in the mixture is virtually the same as if they were injected alone. These results suggest that there is more than one type of adsorption site on the surface of the stationary phase and that each component interacts with a different site. Therefore, during the separation process there is hardly any competitive interaction between the two solutes.

INTRODUCTION

Single-component band profiles in overloaded elution chromatography can be predicted if the shape of the adsorption isotherm is accurately known [1,2]. This has been demonstrated experimentally in normal- and reversed-phase chromatography when the isotherm exhibits Langmuir shape [3–6]. Band profiles of binary mixtures can also be predicted when the competitive Langmuir isotherm is known [7,8]. This has been demonstrated experimentally with alcohols in reversed-phase chromatography [9], and with the separation of protected amino acid enantiomers on a bovine serum albumin stationary phase [10]. In the former case, a competitive Langmuir isotherm has been used empirically to fit the measured isotherm data. In the latter case a competitive biLangmuir isotherm has been employed in the light of a hypothesis of a two-site surface for interaction, one chiral and one non-chiral. Some solutes do not behave ideally and require more complex isotherm models to fit the empirical data [11,12]. For example, the separation of *cis*- and *trans*-androsterone at different

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Je vous présente, en cette occasion, mes meilleurs voeux de bonheur et succès.

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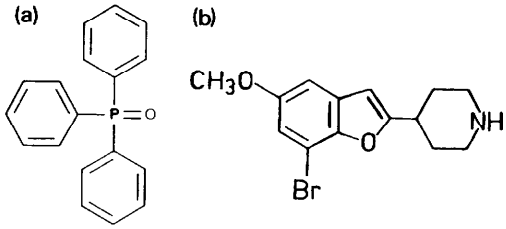

Fig. 1. Molecular structures of solutes used in this study: (a) triphenylphosphine oxide (PPO); (b) piperidine derivative (Pip).

sample sizes on a phosphate-buffer-modified silica could be predicted qualitatively by fitting the measured isotherm data to the LeVan-Vermeulen isotherm [13].

The prediction of non-traditional band shapes requires either the use of empirical models or an understanding of the basic mechanism of the separation, from which one can derive a model. In this paper, an unusual change of band shape with sample size, as seen in the detector response for a binary mixture, is described. A fundamental study is presented in order to understand the phenomenon that occurs. An interpretation of the results is made on the basis of binary and single-component band profile data.

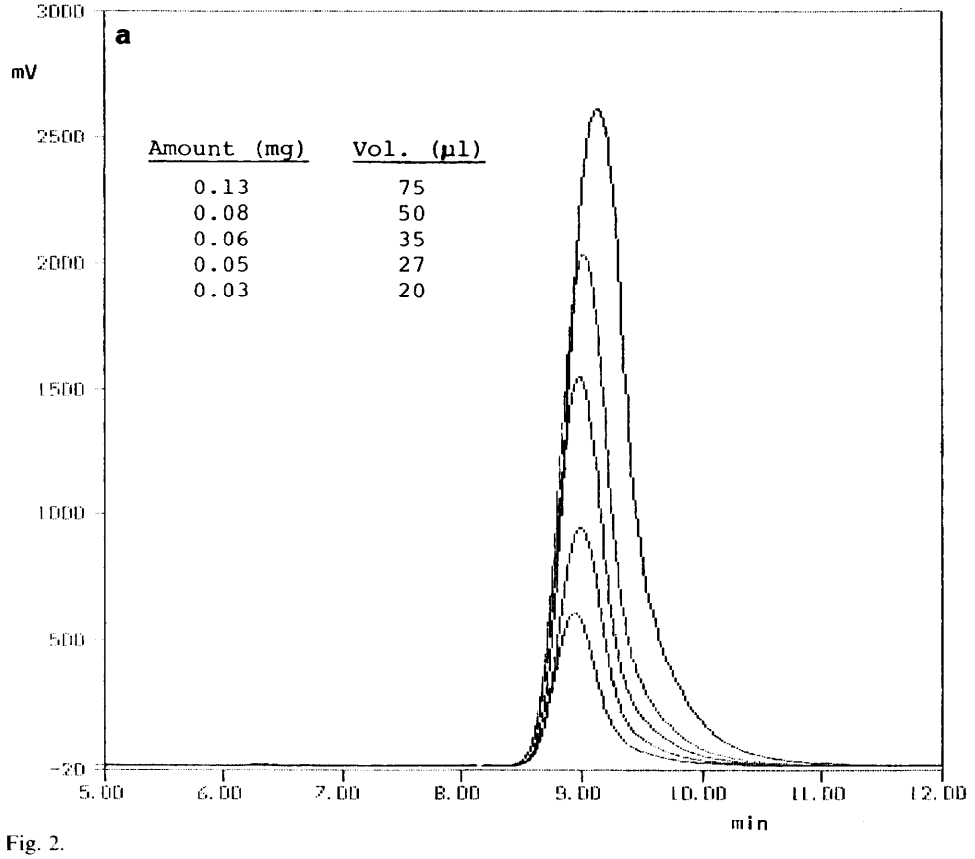

Fig. 2.

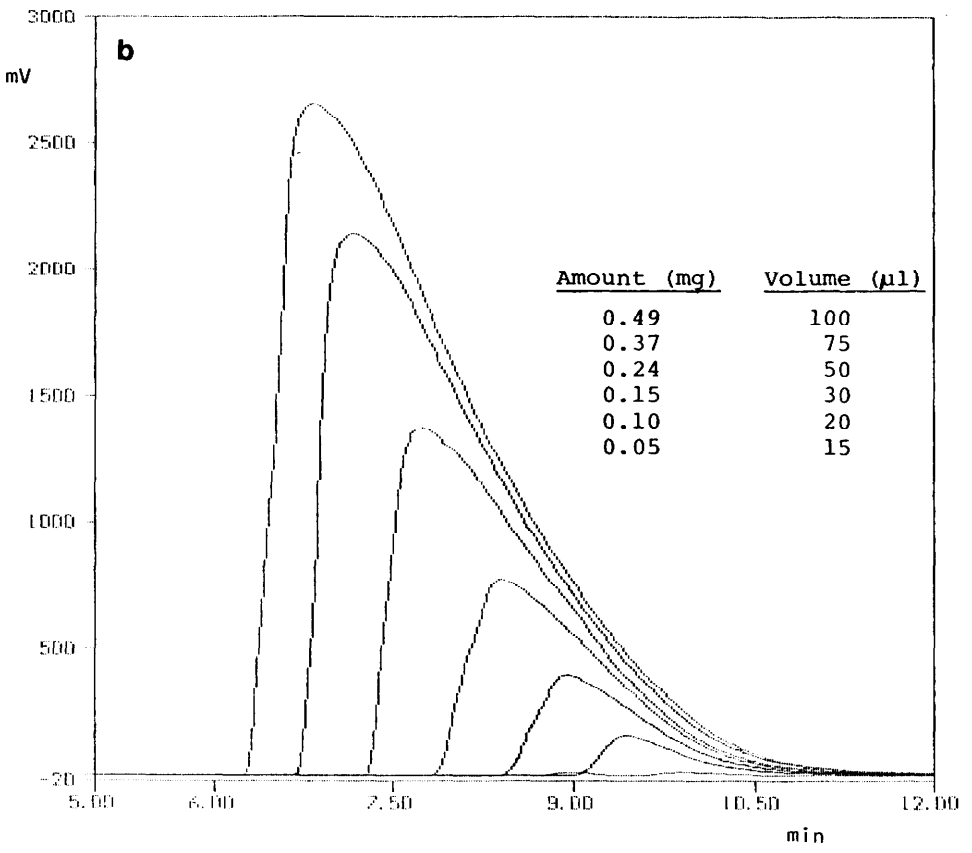

Fig. 2. Effect of the sample size on the pure components band profile. Column, 10-μm CN-Nucleosil, 25 × 0.46 cm I.D.; mobile phase, A–B (50:50) where A = acetonitrile–10 mM $(NH_4)_2SO_4$ (60:40) and B = 10 mM $(NH_4)_2SO_4$ in water; flow-rate, 1 ml/min; detection wavelength 230 nm. (a) Triphenylphosphine oxide; (b) piperidine derivative.

EXPERIMENTAL

Apparatus

Experiments were conducted on a modular high-performance liquid chromatograph consisting of a Kontron (Zurich, Switzerland) Model 425 low-pressure gradient controller and a Model 420 high-pressure pump, connected to the first Rheodyne (Cotati, CA, USA) Model 7010 valve of a Gilson (Middleton, WI, USA) Model 232/401 sample processor, a column, a Linear (Reno, NV, USA) Model 206 PHD rapid scanning detector. Then the outlet from the detector was connected to the second Rheodyne valve of the sample processor. The wires for control of the pump, the gradient former, the detector's output analog signal and the contact closures for the sample processor were connected to a Kontron multiport interface and to a Kontron adapted AT personal computer. Use of a Kontron data system Model 450-MT2 software, operating under MS-DOS version 3.3, allowed control of this modular system. The set-up and operation of the sample processor for this work were similar to that described previously [14].

Columns

Band profiles were determined on a 25 × 0.46 cm I.D. Nucleosil 10-μm CN column (Macherey-Nagel, Düren, Germany) packed in our laboratory. Analysis of the collected fractions was accomplished on a 25 × 0.46 cm I.D. CPS Hypersil, 5-μm CN column (Bischoff Chromatography, Wallisellen, Switzerland).

Chemicals

Triphenylphosphine oxide was purchased from Fluka (Buchs, Switzerland). The piperidine derivative (Ciba-Geigy, Basle, Switzerland) was obtained in our laboratory. The structures of the two solutes are shown in Fig. 1. Ammonium sulphate, (pro analysi) and Gradient-grade acetonitrile was purchased from Merck (Darmstadt, Germany). S.Q.S. HPLC-quality water was obtained from a Millipore system (Milford, MA, U.S.A.) All chemicals were used as received, without further purification.

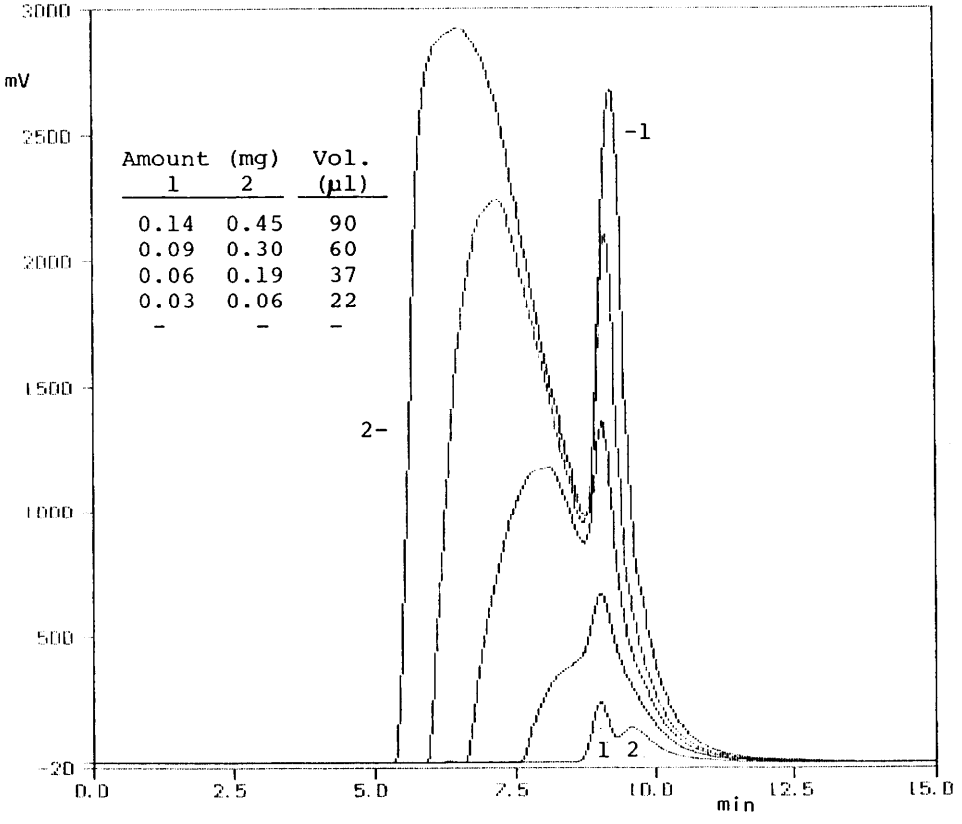

Fig. 3. Effect of the sample size on the binary mixture profile. Conditions as in Fig. 2. 1 = PPO; 2 = Pip.

RESULTS AND DISCUSSION

The effect of the sample size on the individual component band profiles of triphenylphosphine oxide (PPO) and a piperidine derivative (Pip) were measured (Fig. 2). The retention time of the peak maximum of the triphenylphospine oxide increases slightly with increasing sample size. This is indicative that the isotherm is slightly concave up. This is a moderate effect as no self-sharpening effect on the rear boundary is observed. The piperidine derivative takes on the typical profile of a compond with a Langmuir isotherm. As the sample size increases, the time of the peak maximum decreases, the front becomes self-sharpening, and the rear boundary tails to the value of its infinite dilution retention time.

When the two components are injected as a mixture (Fig. 3), at low concentration the PPO elutes first and the Pip elutes second with a separation factor, α, of 1.19. This has been confirmed by injection of the individual component in consecutive injections at low solute concentrations. As the sample size increases, however, it appears that the front of the second component passes the peak maximum of the first component. The roundness at the top of the band profile corresponding to the largest sample illustrated in Fig. 2b is due to detector saturation.

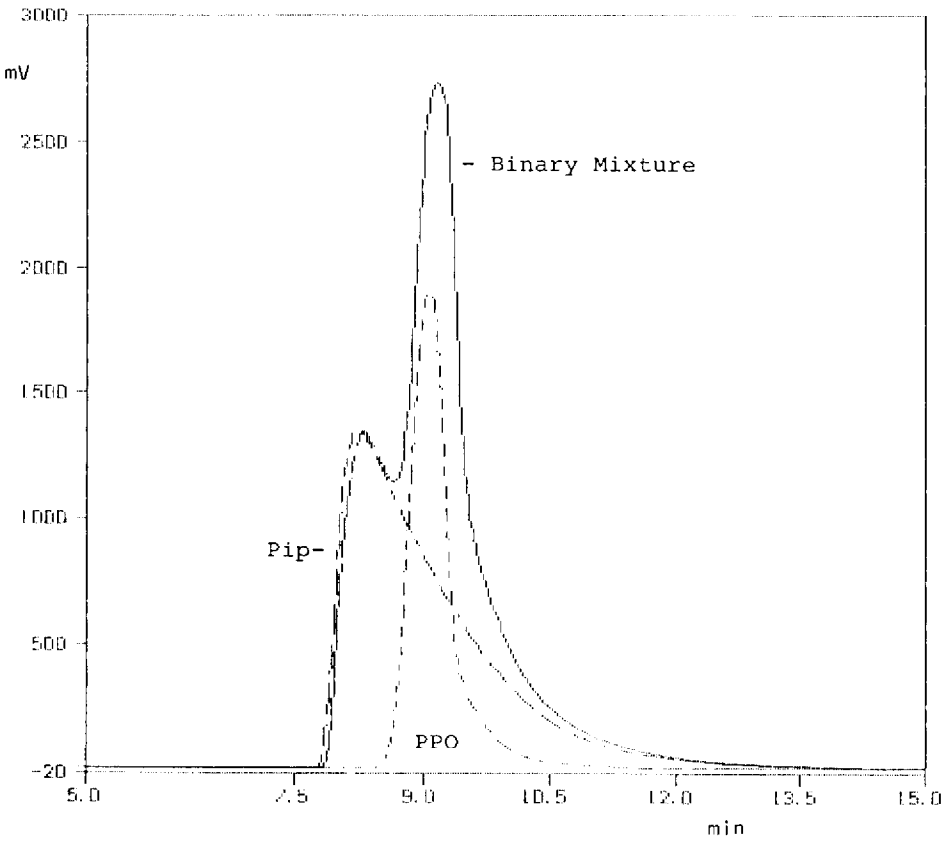

Fig. 4. Comparison on the single-component detector response profiles and that of the binary mixture at the same sample size. The conditions as in Fig. 2, except 50 μl injection (0.1 mg PPO and 0.25 mg Pip).

Fig. 4 illustrates an overlay of the individual detector response profiles of PPO and Pip compared with the total profile of the two component injected as a mixture. The amounts of PPO and Pip in the individual injections are the same as in the mixture. This figure suggests that the sum of the two individual profiles gives the total profile of the mixture; however, these data give no information about the individual profiles in the mixed zone.

One of the methods used to confirm the nature of the individual band profiles in the mixed zone is to collect fractions and develop a method for the quantitative analysis of each fraction [14]. Fig. 5a shows the detector response and Fig. 5b the result of the reanalysis of collected fractions. Several sets of data were taken with fraction collection and reanalysis. These experiments clearly show that the two data points at 8 min are outliers.

These results show that there is only a slight amount of competition and/or interaction between PPO and Pip. The simplest interpretation is that PPO and Pip interact with two independent sites for which the column saturation capacities are different. These results suggest that there are more sites available to PPO than to Pip, since the PPO remains practically linear in sample loading range where the Pip already exhibits a non-linear profile. Intermolecular interactions between the benzene

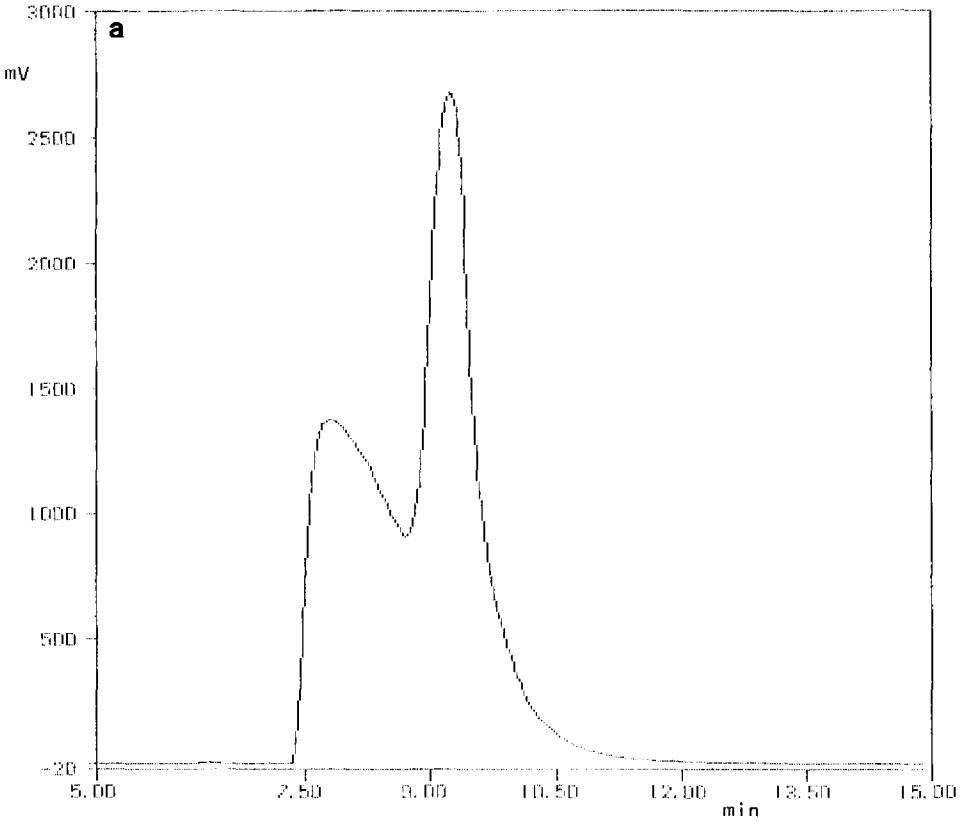

Fig. 5.

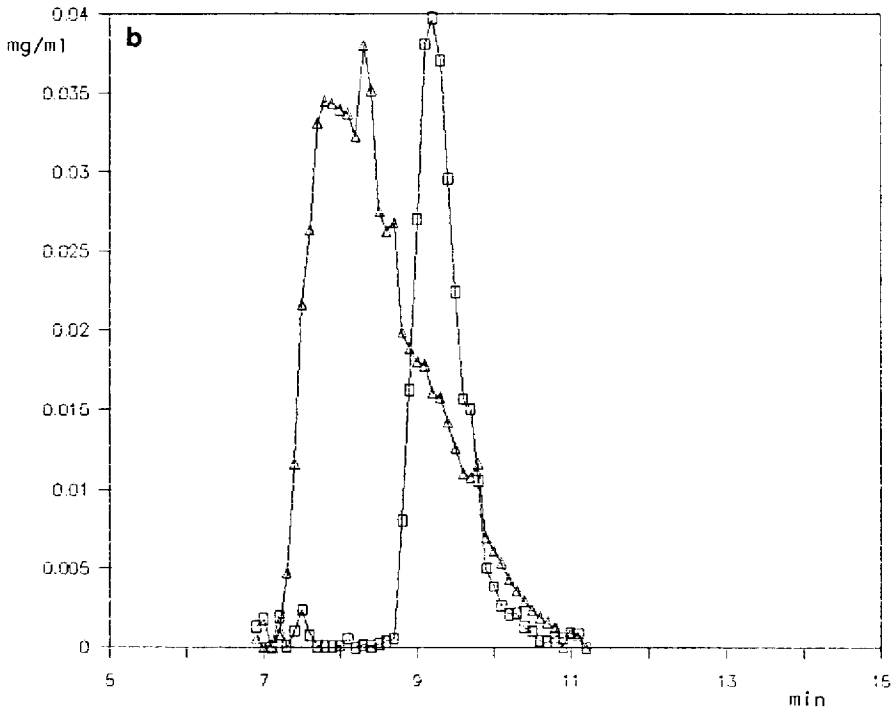

Fig. 5. Individual band profiles determined by fraction collection and reanalysis. Conditions as Fig. 2, except 50 μl injection (0.15 mg PPO and 0.31 mg Pip). (a) Detector Response; (b) individual profiles: ⊥ = PPO; ⌒ = Pip.

rings of the molecules of PPO could explain the slight increase in the retention time of the peak maximum, indicative of a convex isotherm. However, further experimental data on the surface properties of these would be required in order to draw a firm conclusion about the nature of the interactions [15].

The results in Fig. 5 also imply that the band profiles of the two-component mixture can be predicted quantitatively within a few per cent from only the single-component isotherm data. In the case of PPO the band profile at different sample sizes can be approximated with the estimation of coefficients for a linear isotherm or a slightly concave-up isotherm. The band profile of Pip can be predicted with the estimation of Langmuir isotherm coefficients. Isotherm data can be obtained in both cases directly from the band profiles [15–18] or by measurement of the single-component isotherm [19–21].

For analytical method development or preparative chromatography, it is important to increase the separation factor between the two components. By modifying slightly the mobile phase and increasing the $(NH_4)_2SO_4$ concentration in water from 10 mM to 50 mM, the chromatogram in Fig. 6a was obtained. The addition of salt decreases the retention of Pip below that of PPO, and the retention time of PPO increases slightly. The capacity factors (k') are summarized in Table I. This result

suggests that PPO interacts according to the hydrophobic effect, suggestive of an adsorption mechanism, whereas Pip appears to undergo an ionic interaction on sites that are relatively few in number. Under these experimental conditions, because Pip elutes before PPO and because of the larger separation factor, the non-linear effects do not lead to an interference chromatogram.

It is also possible to change the retention properties of the components by using a column packing material from another manufacturer. Using the same mobile phase as in Fig. 6a, but on a 5-μm CN-Hypersil column, a good separation was also obtained. The chromatogram illustrating the band profiles at different sample sizes is shown in Fig. 6b. With this material as well, the profile of Pip exhibits strong non-linear behavior. Comparing Fig. 6b and 2b, the retention time of Pip is observed to be only slightly less than on the other column with the same mobile phase conditions. In contrast, the retention time of PPO drops significantly compared with the retention of Fig. 2. The capacity factors are summarized in Table I. This change in the elution order suggests that the density of sites for PPO is lower on this latter column than on the former. However, with a separation factor of over 7, quantitative analysis is possible. In fact, this column under these experimental conditions was used for the

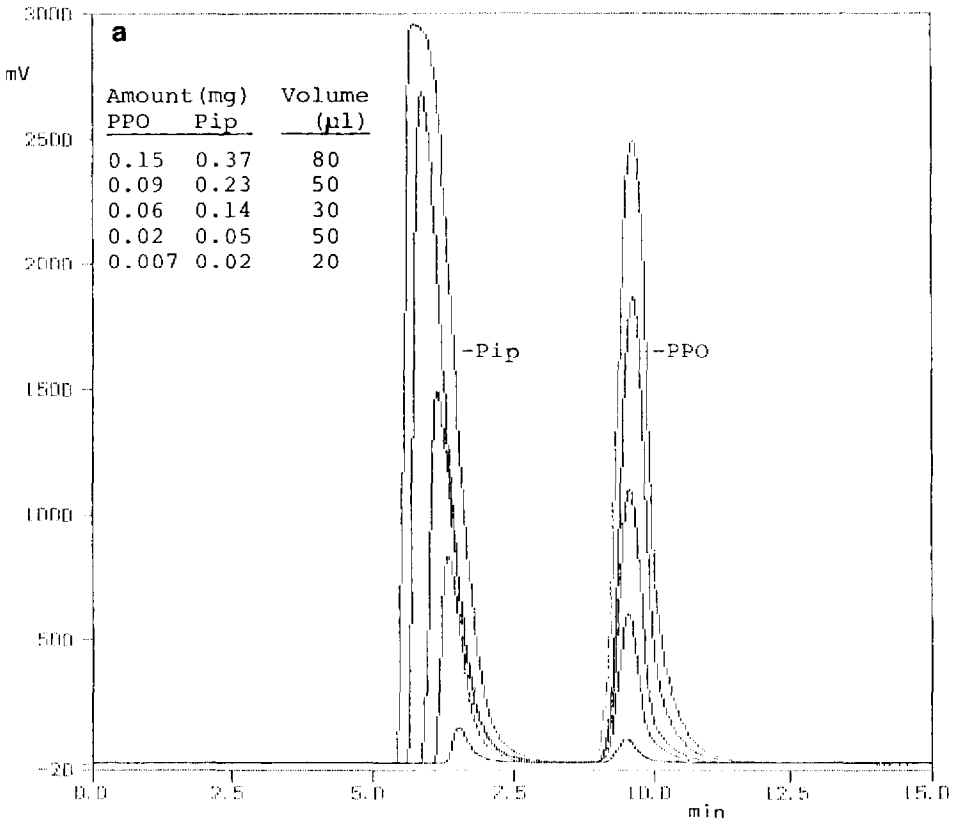

Fig. 6.

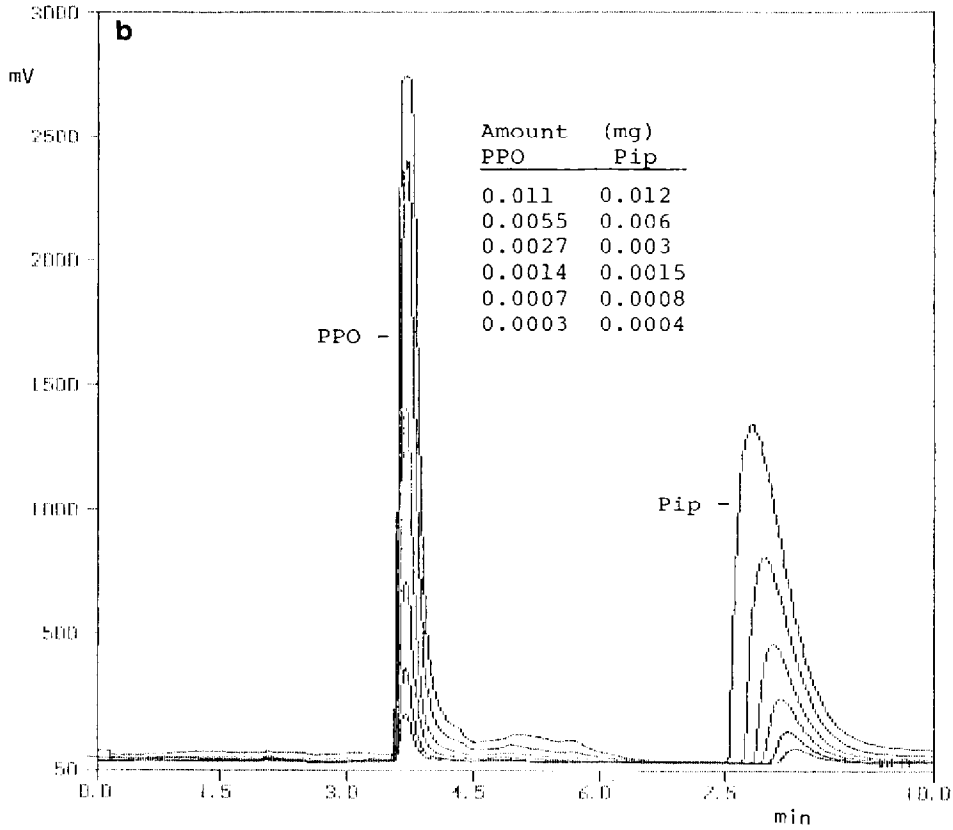

Fig. 6. Illustration of an improved method: (a) conditions as Fig. 1, except 50 mM $(NH_4)_2SO_4$ in solutions A and B; (b) new column, 5-μm CN-Hypersil, 25 × 0.46 cm I.D.; flow-rate, 1 ml/min; 50 mM $(NH_4)_2SO_4$ in A and B; detection wavelength 208 nm.

TABLE I

COMPARISON OF THE INFINITE DILUTION CAPACITY FACTORS

	k'_{PPO}	k'_{Pip}	α
Nucleosil 10 mM salt	2.03	2.41	1.19
Nucleosil 50 mM salt	1.18	2.17	1.84
Nucleosil 10 mM salt	1.88	6.64	3.53
Hypersil 50 mM salt	0.24	1.78	7.42

analysis of the fractions for Fig. 5b. Lastly, by decreasing the salt concentration from 50 mM to 10 mM, the capacity factor of both components is increased, but the separation factor is decreased (Table I).

CONCLUSIONS

The experimental chromatograms illustrate a situation in which two solutes in overloaded conditions behave as if there is virtually no interaction. This suggests that the solutes are absorbed on different sites, thus there is practically no competition. Band profiles under these conditions can be predicted quantitatively using only single-component isotherms.

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