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Effect of the temperature and mobile phase composition on the retention behavior of nitroanilines on ligand-exchange stationary phase

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Abstract

This paper deals with the separation of isomers of nitroaniline by liquid chromatography using the ligand-exchange technique. The chromatographic separations were performed on the ligand-exchanger sporopollenin. The sporopollenin used as support of stationary phase was modified with carboxylated-ethylenediamine matrix and was loaded with cobalt(II) ions. Using the column packed with cobalt(II) loaded carboxylated diaminoethyl sporopollenin [Co(II)-CDAE-S], the retention behavior of 3- and 4-nitroanilines was investigated. The mobile phase used, was a mixture of 0.05 M NH_4OH in ethanol–water. The resolution was strongly affected by the presence of ammonium hydroxide in the mobile phase and a concentration of 0.05 M was shown to be necessary for the separation of analytes. To study the effects of temperature on the resolution, column runs were also performed at various temperatures (15–60 °C). With increasing temperature, a decreased interaction between the solutes and the ligand-exchanger was observed. Consequently, the best results were obtained using a mixture of 0.05 M NH_4OH in ethanol–water (10:90, v/v) as the mobile phase at a column temperature of 35 °C. Ligand-exchange chromatography on the Co(II)-CDAE-S could be a useful alternative method for the separation of nitroaniline.

Keywords: Ligand-exchange choromatography; Sporopollenin; Nitroaniline

1. Introduction

Nitroanilines are important pollutants in water because of their wide use in many industrial processes such as the manufacture of pharmaceuticals, dyes and synthetical colours [1]. Furthermore, they are of great environmental concern because of their high toxicity to living things [2]. For this reason, nitroanilines are chemical species that need monitoring, so the separation of their positional isomers is very important. In view of the importance of these compounds, a rapid and sensitive method of analysis is needed for their detection in the environment.

Nowadays, technique of ligand-exchange is preferable for the separation of neutral compounds in environmental samples due to its high selectivity. So far, the analysis of such neutral compounds has been widely performed by gas chromatography and high performance liquid chromatography. However, positional isomer separation is one of the most complicating areas of separation science because of their similar physical and chemical properties. Ligand-exchange chromatography, among other methods, has become more and more important for the separation of isomers. It utilizes the reversible formation of complex to separate neutral compounds which can coordinate the attached metal ions onto a solid support matrix [3,4]. Ligand-exchange technique has been employed for compounds coordinating with transition metal ions that form complexes of different stabilities [5]. Solvent molecules holding coordination sites on the matrix are exchanged with the ligands in sample. Briefly, ligand-exchange is based on the formation of metal complexes between the central metal and the analytes. Ligand-exchange is a process first described by Helfferich, and evaluated from the fundamental works of Walton and Stokes [6,7]. Since then, ligand-exchange technique has been widely applied to the separation of a large number of ligands such as proteins, amino acid, purin and pyrimidine bases [8–11] and numerous researches dealing with different applications of ligand-exchange have appeared [12–14].

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Packing materials for chromatographic separation of ligands have been continuously improved. In general, a solid phase support includes two aspects: the base support and the stationary phase that is chemically or physically immobilised onto a solid support and carries out the necessary functions. The base support plays a dominant role in the mechanical, chemical and thermal stability of packing materials. One of such materials is sporopollenin which possesses a high content of functional groups available for modification. This material may be successfully used at high temperatures, since it has a constant chemical structure that is stable under high temperature and in the presence of concentrated acids [15–18]. In this work, sporopollenin, whose surface has previously been chemically modified with a cobalt(II)-carboxylated-ethylenediamine matrix, was employed as stationary phase for the separation of nitroanilines. Ethylenediamine complexes possess a very stable structure, with a very minor dissociation tendency as well as having suitable functional groups for a ligand-exchange matrix [19–21]. In recent years, studies with ligands and modified sporopollenin have generated enhanced interest in the potential of sporopollenin as a support material; however there are very limited records on sporopollenin resin interaction with ligands. Up to date, there has been no investigation on the effect of temperature and mobile phases on the uptake of ligands by pretreated sporopollenin [22–24]. Adjusting the composition of the mobile phase and the temperature of column are the most widely used tools for controlling the separation of analytes in column chromatography. We therefore considered the effect of temperature and mobile phase on the separation of nitroanilines by Co(II)-CDAE-S resin. The aim of our study was to establish the most suitable conditions for the selective separation of 3- and 4-nitroaniline by Co(II)-CDAE-S.

2. Experimental

2.1. Material

Sporopollenin (20 μm mesh, BDH Chemicals) which has been used as stationary phase was modified as ligand-exchanger using ethylenediamine, bromoacetic acid and $CoCl_2$ as we have previously reported [25]. Modification of sporopollenin and ligand-exchange of nitroanilines are illustrated in Fig. 1. Nitroanilines and all other chemicals were purchased from Merck Chemical Company and were of reagent grade. Deionized water was used in the preparation of the mobile phase.

2.2. Chromatographic runs

The ligand-exchange stationary phase was packed into a glass column by the conventional slurry packing method. The Co(II)-CDAE-S column was flushed with deionized water until the effluent was free of cobalt(II) ions and then equilibrated for 30 min with the mobile phase before analytes were injected. The chromatographic system used in the present study is a peristaltic pump (Alitea S2) and a UV–vis spectrophotometer (Shimadzu 160A) connected to the chromatographic column. The column temperature was controlled using a water jacket connected to a thermostated circulator within a deviation of ±0.1 °C and a heat exchanger was used for preheating the mobile phase before it reaches the column. Sample solutions were prepared by dissolving 1.0×10^{-2} mol of each analyte in 10 ml of mobile phase solution. The column was eluted with 0.05 M NH_4OH throughout the whole range (5–95% ethanol in water) of mobile phase, and elution behavior of analytes was investigated at various temperatures (15–60 °C) by means of their capacity factors and resolution. The capacity factor (k) was

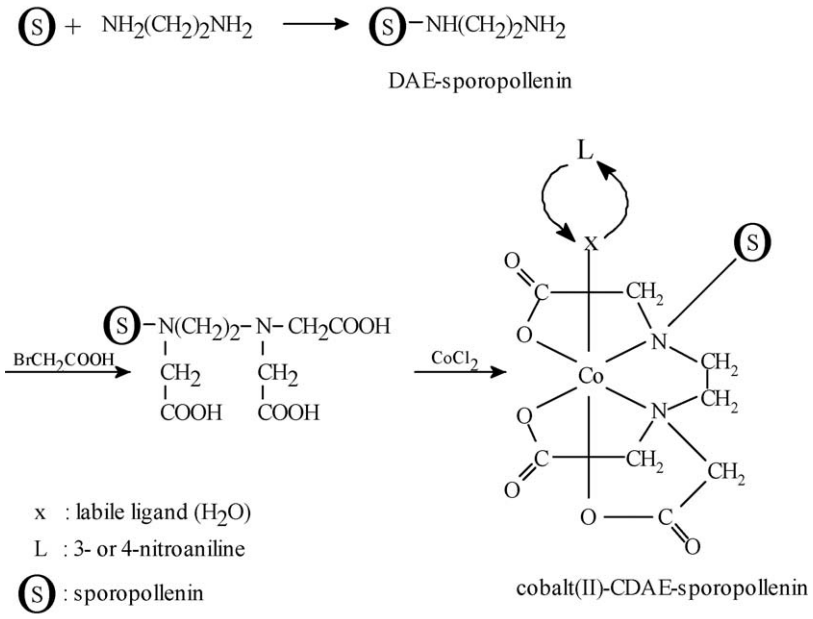

Fig. 1. Modification of sporopollenin as ligand-exchanger.

calculated using the following equation:

$$k = \frac{t_R - t_0}{t_0} \quad (1)$$

where t_R is the retention time of the analytes and t_0 is the retention time of a nonretained species. Resolution, R_s, for the two selected analytes at a given temperature was calculated using the following equation:

$$R_s = \frac{2(t_{R(2)} - t_{R(1)})}{W_{b(1)} + W_{b(2)}} \quad (2)$$

where $t_{R(1)}$ and $t_{R(2)}$ are retention times, $W_{b(1)}$ and $W_{b(2)}$ are peak widths at the baseline for analytes 1 and 2, respectively.

3. Results and discussion

The column packing material used in this study, Co(II)-CDAE-S, is a sporopollenin, whose surface has previously been chemically modified with cobalt ion and a polydentating ligand, carboxylated-ethylenediamine. Sporopollenin is an extraordinary resistant biopolymer where the outer wall is composed of spores and pollen [15]. Ligand-exchange activity of the sporopollenin is due to the carboxylated ethylenediamine groups. Carboxylated ethylenediamine groups have a very stable structure with little possibility of dissociation; they are very suitable functional groups for a ligand-exchanger matrix [5]. The chelating ligand-exchanger resins are ion-exchange resins containing groups which are also able to complex with metal ions. Their adsorption mechanism is supposed to be through chelation instead of simple ion exchange, and as a consequence, they should be much more selective than ion-exchange resins [26]. In this work, *meta*- and *para*-positional isomers of nitroaniline which contain a little or no steric hindrance effect were chosen as the analytes to investigate the effects of substituents on the retention behavior of ligands.

This study was conducted in order to confirm the effect of column temperature and mobile phase composition on the retention of nitroanilines by Co(II)-CDAE-S. The mobile phase composition is the most critical variable in controlling the retention that provides information about the combined nature of the mobile phase and stationary phase. However, it is not always easy to predict the most suitable mobile phase composition to obtain optimum resolution of analytes, therefore, some preliminary experiments were carried out by changing the concentrations of ammonia and ethanol in the mobile phases in order to determine the effective composition of the mobile phase that would show tendency toward resolution in the range of 15–60 °C at various water–ethanol ratios (5–95%).

These preliminary experiments were carried out using a mini column of Co(II)-CDAE-S which was conditioned before the chromatographic runs. Under the conditions applied, the effective composition of mobile phase for the separation of analytes was found to be 10–15% ethanol. In this composition range, the most suitable concentration of ammonium hydroxide was found to be 0.05 M. Thus, the wide composition range of 5–95% ethanol was reduced to 10–15%. The chromatograms obtained from these experiments are shown in Fig. 2.

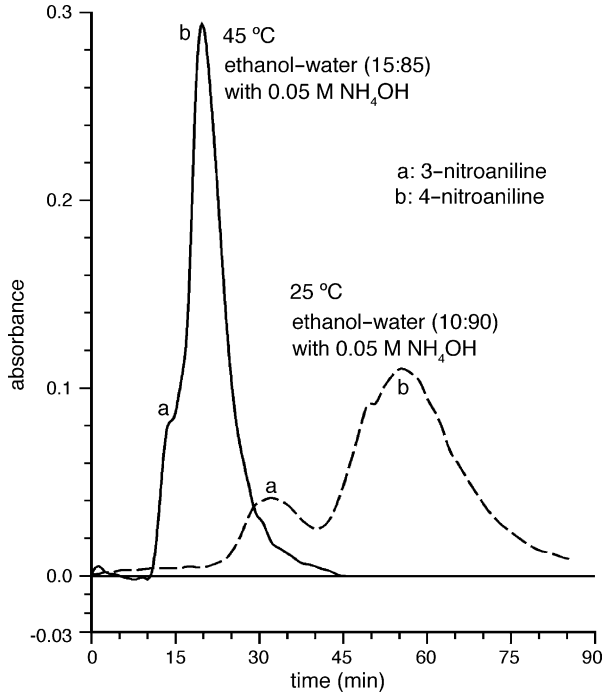

Fig. 2. Chromatograms for the resolution of nitroanilines on Co(II)-CDAE-S under the boundary conditions.

In order to investigate the influence of temperature on the separation process, two series of experiments at each of 25, 35 and 45 °C, were carried out using the above mobile phase compositions. The first series of experiments was carried out using a mixture of 15:85 ethanol–water. These conditions caused only a small increase in column efficiency as is shown in Fig. 3, whereas

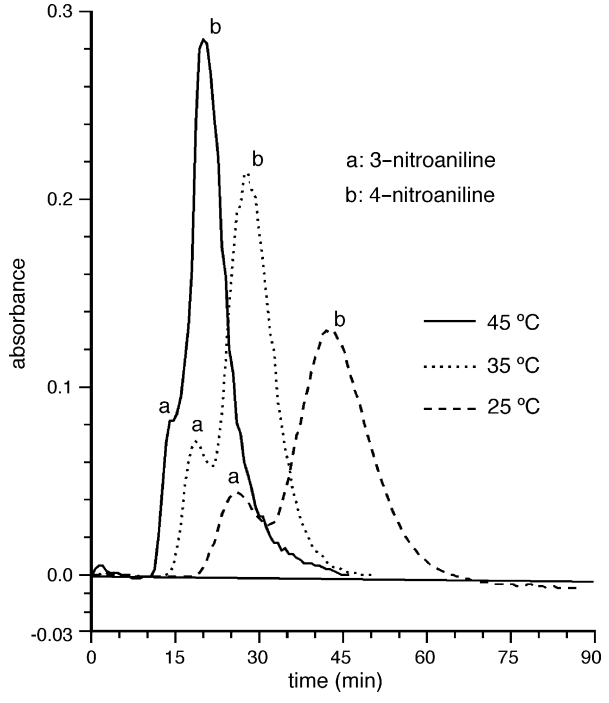

Fig. 3. Chromatograms of the nitroanilines on Co(II)-CDAE-S with 0.05 M of NH_4OH in ethanol–water (15:85) at various temperature.

Table 1
Data matrix for chromatogram of analytes on the Co(II)-CDAE-S[a]

Mobile phase	Temperature (°C)	k_1 (3-nitroaniline)	k_2 (4-nitroaniline)	α	R_s
Ethanol:water (15:85)[b] (Fig. 3)	25	14.60	24.60	1.68	0.72
	35	10.20	15.80	1.55	0.67
	45	7.60	11.00	1.45	0.52
Ethanol:water (10:90)[b] (Fig. 4)	25	18.60	32.40	1.74	0.87
	35	14.20	22.60	1.59	0.82
	45	8.00	12.40	1.55	0.58

[a] Column size: 4 mm × 50 mm.
[b] With 0.5 M ammonia.

in the second experimental series which was carried out using a mixture of 10:90 ethanol–water, a significant increase in column efficiency was observed (Fig. 4). The chromatographic parameters at the three temperatures are given in Table 1. Although chromatographic runs obtained at 25 °C had high capacity factors (k), the resolution was still not satisfactory because the band broadening in the column was inevitable at low temperatures. This can be attributed to slower mass-transfer between the mobile and the stationary phases at low temperatures. In this case, experimental conditions which band broadening occurs more slowly than band separation should be determined in order to obtain a satisfactory separation. A chromatographic separation is optimized by varying experimental conditions until the analytes of a mixture are separated completely with a minimum expenditure of time. Relative migration rates are controllable by the adjustment of experimental variables, thus permitting improvement in separations. Optimization experiments are aimed at either (i) reducing band broadening or (ii) altering relative migration rates of the analytes.

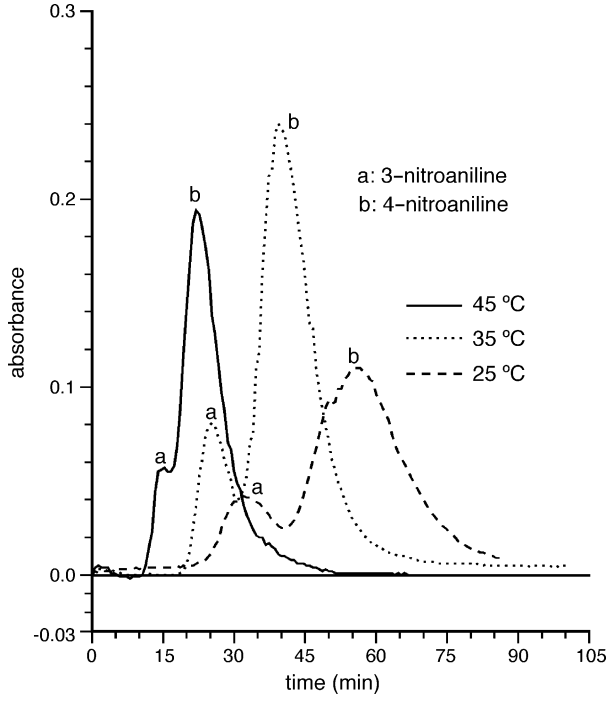

Fig. 4. Chromatograms of the nitroanilines on Co(II)-CDAE-S with 0.05 M of NH_4OH in ethanol–water (10:90) at various temperature.

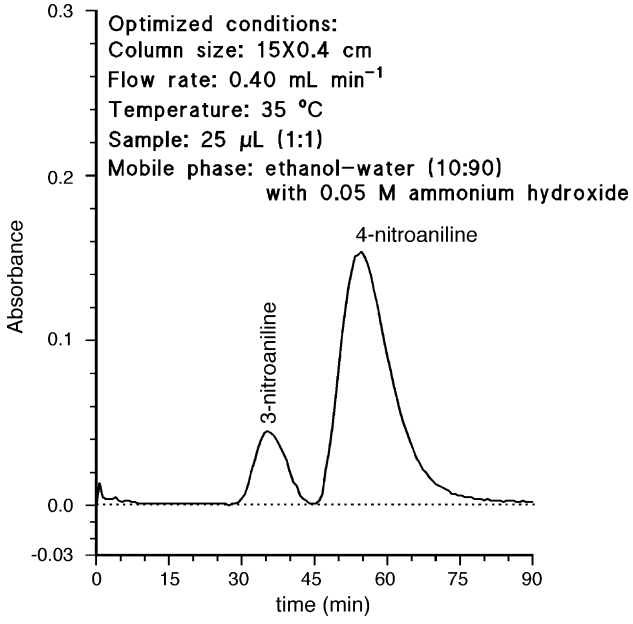

Fig. 5. Chromatogram for the nitroanilines on Co(II)-CDAE-S.

The chromatogram obtained at 25 °C has clearly revealed long retention times and broad tailing peaks whereas the resolution of the analytes at 35 °C led step by step to a better separation. As in all types of chromatography, resolution is dependent on the column length as well as the flow rate. Therefore, in order to achieve a better separation, experiments were performed with a mobile phase flow rate fixed at 0.40 mL min^{-1} and the band broadening problem that lowers the efficiency of the column was solved by changing the column length. Thus both positional isomers of nitroaniline were eluted and a baseline resolution was achieved within a reasonable time under the chromatographic conditions chosen as given in Fig. 5. Retention of analytes by ligand-exchangers is a complex phenomenon that involves several kinds of interaction; in ligand-exchange, the primary effect is the metal–ligand coordination, however this is not the only effect and thus detailed studies will be needed to fully confirm this concept.

4. Conclusion

On the basis of this study, it appears clearly that the resolution of nitroanilines on the Co(II)-CDAE-S was very much dependent on the content of the mobile phase. As the

concentration of the ethanol was decreased, the separation of 3- and 4-nitroaniline improved remarkably. However, the experimental results indicated that the solute–solvent interactions involved in the retention process could not be a dominant factor for controlling the retention time. The retention of nitroaniline on the Co(II)-CDAE-S was mainly governed by the formation of metal–ligand complexes between the analytes and the ligand-exchange matrix. The difference in the stability of these complexes is dependent on the interactions between the ligands and the metal ions of the packing material. On the other hand, at an elevated temperature, the adsorption of molecules on the surface and the viscosity of mobile phases decreased, while the solubility of analytes increased. Consequently, the retention time of the nitroanilines depended greatly on temperature; with increasing temperature, a decreased interaction between the solutes and the stationary phases was noticed and at temperatures above 45 °C, no resolution was observed. The optimum temperature for the separation of analytes by resin was found to be 35 °C.

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