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# DRASTIC EFFECT OF CROWN-COMPLEXING CATION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY USING POLY(CROWN ETHER)-IMMOBILIZED SILICA

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A poly(crown ether)-immobilized silica behaves as a stationary phase for reversed-phase liquid chromatography as well as ion chromatography. The retention of several polar organic solutes in the reversed-phase liquid chromatography can be controlled easily by addition of alkali metal salts to the mobile phase, owing to electrostatic interaction between the solutes and the metal ion complexed by the crown ether moiety on the stationary phase.

Crown ether resins or immobilized crown ethers are attractive stationary phases for liquid chromatography of alkali and alkaline-earth metal ions and other ionic species.[1-3] This is true for poly(crown ether)-immobilized silicas where polymers incorporating pendant crown ether moiety are chemically bonded on silica gel.[4-6] The poly(crown ether) silicas possess hydrophobic moieties such as the polymer backbone and the aromatic ring besides the crown ether moiety. The modified silicas are, therefore, expected to interact hydrophobically with organic solutes as is the case in conventional stationary phases for reversed-phase liquid chromatography. In addition, the crown ether moiety on the modified silicas is, of course, able to complex alkali metal cations in mobile phases, which in turn brings about another interaction between certain organic solutes and the stationary phases, Here we report chromatographic property of a poly(crown ether)-immobilized silica on reversed-phase liquid chromatography which can be easily modified by using metal salt-containing mobile phases.

Poly(vinylbenzo-18-crown-8)-immobilized silica was employed here, which was prepared by the reaction of silica gel (10 μm, spherical) and 3-(methacryloyloxy)-

propyltrimethoxysilane, followed by radical copolymerization of the vinyl silica with 4'-vinylbenzo-18-crown-6,[7)] in a similar way to the previous poly(crown ether)-immobilized silicas.[4)] The residual silanol group was end-capped by treating with hexamethyldisilazane prior to the polymerization. Liquid chromatography (HPLC) of various disubstituted benzene derivatives and some other aromatic compounds[8)] was carried out using a column (4 mm i.d. X 15 cm length) packed with the poly(crown ether) silica and methanol/water(or a buffer) mixtures as the mobile phases. The chromatographic retention of the organic solutes was generally enhanced by increasing the water fraction in the mobile phase. This phenomenon is typical for conventional reversed-phase chromatography, suggesting that the chromatography on the poly(crown ether)-immobilized silica is substantially based on hydrophobic interaction between the solutes and the stationary phase. Some interaction like hydrogen bonding between the polar groups of the organic solutes and the crown ether moiety may also contribute to the chromatographic retention.

A most interesting thing in the reversed-phase liquid chromatography on the poly(crown ether)-immobilized silica is addition effect of alkali metal salts to the mobile phase. Remarkable changes were found in the chromatographic retention of some of the disubstituted benzene derivatives when some KCl was added to the mobile phase (methanol/buffer). For example, addition of a small amount of KCl to methanol/pH-5 buffer($CH_3CO_2H$-$CH_3CO_2Li$)(60/40) drastically increased retention times of nitrophenols, especially the *p*-isomer, on the poly(crown ether) silica (Fig. 1a). The similar retention behavior was observed in the case of amino- and nitro-benzoic acids. Such salt addition, however, caused very little effect on the retention times in the chromatography using polystyrene-immobilized silica which does not carry any crown ether moiety. This retention enhancement on the poly(crown ether) silica can be attributed to some electrostatic interaction. That is to say, stable complex formation of $K^+$ in the mobile phase with the benzo-18-crown-6 moiety generates positive charges on the stationary phase, thus inducing electrostatic attraction between the complexed $K^+$ and the negative charges or dipoles of the organic solutes. This electrostatic interaction is also supported by the fact that use of the higher-pH mobile phase, methanol/pH-8 buffer($NH_2CH_2CH_2OH$-HCl)(60/40), which promotes acid dissociation, retards the elution of nitrophenols much more significantly. Particularly, the *p*-nitrophenol could not be eluted out from the column even if the mobile phase includes $4 \times 10^{-3}$ mol dm$^{-3}$ KCl. Furthermore, there was hardly any retention enhancement of phenol and cresols which possess quite low acid

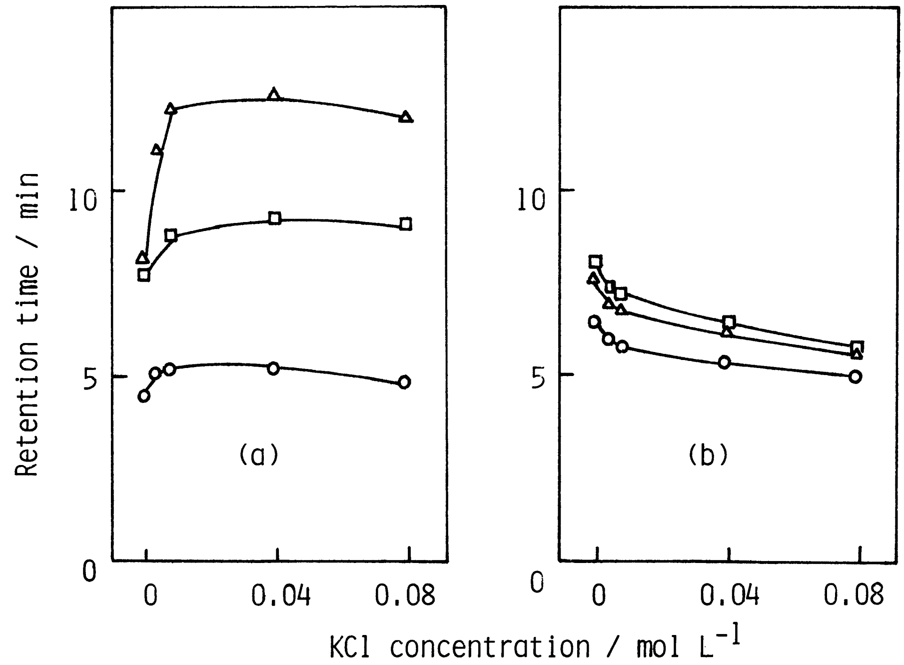

Fig. 1. Dependence of retention times for nitrophenols (a) and iodoanilines (b) on KCl concentration in mobile phase. ( △ ) *o*-isomer, ( □ ) *m*-isomer, ( ○ ) *p*-isomer; mobile phase: MeOH/$CH_3CO_2H$-$CH_3CO_2Li$ buffer (pH 5) (60/40 v/v); flow rate: 1 mL min$^{-1}$; sample size: 20 µL (2 x 10$^{-4}$ mol L$^{-1}$). (1 L = 1 dm$^3$)

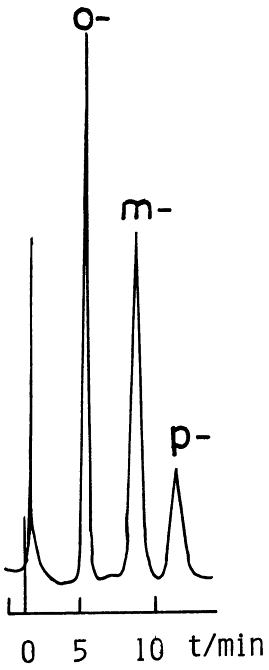

Fig. 2. Separation of nitrophenol isomers by reversed-phase liquid chromatography on poly(benzo-18-crown-6)-immobilized silica. mobile phase: MeOH/$CH_3CO_2H$-$CH_3CO_2Li$ buffer (pH 5) (60/40 v/v) containing 4 x 10$^{-3}$ mol L$^{-1}$ KCl; flow rate: 1 mL min$^{-1}$; sample size: 20 µL (2 x 10$^{-4}$ mol L$^{-1}$).

dissociation constants, even on the elution with the higher-pH mobile phase containing a large amount of KCl. In contrast, the chromatographic retention of disubstituted benzene derivatives carrying an amino group such as iodoanilines, nitroanilines, and toluidines was suppressed by adding KCl to the mobile phase, although the salt addition effect is not very drastic (Fig. 1b). This may be due to the electrostatic repulsion between the complexed $K^+$ and the anilinium ions or the depression of the weak interaction between the crown ether moiety and the amino groups.

A typical chromatogram shows excellent separation of three nitrophenol isomers, giving a good example for the improvement of the chromatographic separation on the poly(crown ether)-immobilized silica by using the KCl-containing mobile phase (Fig. 2). The *m*- and *p*-isomers were hard to separate by the elution with methanol/water(or a buffer)(60/40) not containing any KCl. Also, the higher water fraction of the mobile phases without KCl scarcely improve the separation of the two isomers.

Thus, the reversed-phase liquid chromatography using the poly(crown ether)-immobilized silica can be modulated markedly with some electrostatic interaction caused by the addition of crown-complexing cations to the mobile phase. This chromatography is promising for chromatographic separations of relatively polar organic compounds in reversed-phase layers.

References

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8) The organic solutes employed here were nitrophenols, cresols, iodoanilines, nitroanilines, toluidines, aminobenzoic acids, nitrobenzoic acids, phenol, benzene, and naphthalene.

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