# STUDIES ON IODINATED COMPOUNDS

## V. REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF IODIDE WITH CYCLODEXTRIN-CONTAINING MOBILE PHASES

MASAHIRO MIYASHITA and SABURO YAMASHITA*

*Department of Clinical Chemistry, Hoshi College of Pharmacy, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142 (Japan)*

(First received February 6th, 1989; revised manuscript received August 16th, 1989)

SUMMARY

$\alpha$-, $\beta$-, $\gamma$- and dimethyl-$\beta$-cyclodextrin (CD) were used as mobile phase additives in a reversed-phase high-performance liquid chromatographic determination of iodide ($I^-$). The retention of $I^-$ was decreased by the addition of CDs in mobile phases composed of hexyl-, heptyl- or octylammonium phosphate. The decrease in the retention was influenced by the molecular species and the concentration of CDs. Using 2 m$M$ heptylammonium phosphate (pH 4.0) containing 2 m$M$ $\beta$-CD as the mobile phase, determination of $I^-$ was performed with a UV detector at 226 nm. Good linearity of the calibration graphs and good reproducibility of repeated determinations were obtained for $I^-$ in the range 25 pmol (3.2 ng) to 12.5 nmol (1.6 $\mu$g).

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) has been widely used for the determination of iodide ($I^-$)[1–3]. Often alkylammonium salts are added to the mobile phase consisting of water–organic solvent mixtures, as ion-pairing reagents to increase the retention of $I^-$ on the non-polar stationary phase. The retention of $I^-$ is influenced and controlled by various mobile phase parameters such as molecular species and concentration of ion-pairing reagents, pH, ionic strength and content of organic modifiers[3–5].

Recently, host–guest interactions, using crown ethers (CEs) or cyclodextrins (CDs) as hosts, have been applied to the RP-HPLC separation of various solutes. Many compounds have been separated selectively on the basis of selective interactions of CEs or CDs with solutes. The host–guest interactions can also be utilized as a novel retention-controlling factor for the RP-HPLC separation of $I^-$. CEs, which are cyclic polyethers, are known to form complexes with metal ions and primary alkylammonium ions. We previously applied the CE, 18-crown-6, to the separation of $I^-$ and found that the retention of $I^-$ was increased by the addition of 18-crown-6 to mobile

phases composed of primary alkylammonium phosphates because of complex formation of CE with ion-pairing reagents[6].

CDs, which are homologous series of cyclic oligosaccharides with glucose units joined by $\alpha$-1,4-linkages, are known to form inclusion complexes with various guest compounds[7]. The inclusion phenomena have been applied to the separation of positional isomers of aromatic compound[8–10] and the resolution of racemic compounds into enantiomers[11–13] by using CDs as mobile phase additives in RP-HPLC. However, the use of CDs for the separation of $I^-$ has not previously been attempted.

This paper reports the application of CDs as mobile phase additives for the separation and determination of $I^-$. The aim of this study was to establish a novel RP-HPLC method for $I^-$ based on the inclusion interaction of CDs, controlling the retention behaviour.

EXPERIMENTAL

*Apparatus*

The HPLC equipment consisted of a Model 6000A pump, a U6K universal injector (Waters Assoc., Milford, MA, U.S.A.), a Model NS-310A variable-wavelength UV detector (Nippon Seimitsu Kagaku, Tokyo, Japan) and a Model VP-6511W pen recorder (Matsushita Communication, Yokohama, Japan). The HPLC column was a radial compression separation system with a Radial-Pak Nova-pak $C_{18}$ (4 $\mu$m) cartridge (100 mm × 5 mm I.D.) (Waters Assoc.). UV spectra were measured on a Model 100-50 double-beam spectrophotometer with a Model 200 recorder (Hitachi, Tokyo, Japan).

*Chemicals*

All reagents were of commercial guaranteed grade. Potassium iodide (KI) was obtained from Wako (Osaka, Japan) and CDs from Nakarai (Kyoto, Japan). Deionized, distilled water was used to prepare the mobile phases.

*Mobile phases*

Alkylammonium phosphate solutions were prepared by dissolving alkylamines in water and adjusting to the desired pH with phosphoric acid. After dissolving CDs in the alkylammonium phosphate solutions, all mobile phases were filtered through a 0.45-$\mu$m filter and degassed prior to use.

*Procedure*

HPLC was performed at room temperature (*ca.* 20°C). The flow-rate of the mobile phase was 1.0 ml/min. The detection wavelength was set at 226 nm. The retention of $I^-$ was determined by injecting 5 $\mu$l of KI standard solution (1 · $10^{-4}$ $M$ in water). The capacity factor, $k'$, of $I^-$ was calculated using the equation $k' = (V - V_0)/V_0$, where $V$ is the retention volume of $I^-$ and $V_0$ is the hold-up volume of the column. The hold-up volume of the column was obtained from the retention volume of nitrate ion after injection of sodium nitrate using 60% acetonitrile as the mobile phase.

RESULTS AND DISCUSSION

*Effect of addition of CDs*

The capacity factor of $I^-$ was investigated by using three molecular species of 2 mM alkylammonium phosphate (pH 4.0) solutions containing 2 mM α-CD, β-CD, γ-CD, 2,6-di-O-methyl-β-CD (DM-β-CD) or no CDs as mobile phases. The results are given in Table I and the typical chromatograms are shown in Fig. 1.

TABLE I

EFFECT OF CD IN THE MOBILE PHASE ON CAPACITY FACTOR OF $I^-$

Mobile phase: 2 mM alkylammonium phosphate (pH 4.0) containing 2 mM CD.

| Ion-pairing reagent[a] | CD | | | | |
|---|---|---|---|---|---|
| | None | α-CD | β-CD | γ-CD | DM-β-CD |
| Hexylammonium | 5.10 | 3.33 | 3.33 | 3.90 | 0.96 |
| Heptylammonium | 12.18 | 6.68 | 7.82 | 7.94 | 2.05 |
| Oxtylammonium | 26.68 | 14.63 | 16.15 | 20.79 | 5.10 |

[a] Added as phosphate salts.

In all instances, the retention of $I^-$, which was influenced by the ion-pairing reagent used, was decreased by the addition of any CD to the mobile phase. The magnitude of the decrease in retention was dependent on the molecular species of CD; the decrease in retention was greater when a CD having a small condensation number of glucose units was used (γ-CD < β-CD < α-CD) or when a CD having methylated glucose units was used (β-CD < DM-β-CD).

*Retention mechanism of $I^-$*

It is interesting to consider the retention mechanism of $I^-$ with the CD-containing mobile phases in combination with the model of ion-pair chromatography. However, many different models of ion-pair chromatography have been proposed[14]. Here we adopt the dynamic ion-exchange model to explain the retention mechanism of $I^-$. According to this model, the retention of $I^-$ in mobile phase systems without CDs is governed mainly by the following two important equilibria: (1) sorption of ion-pair-
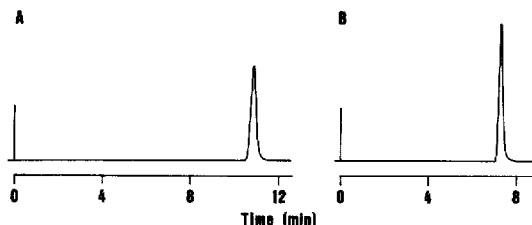


Fig. 1. High-performance liquid chromatograms of $I^-$. Mobile phase: (A) 2 mM heptylammonium phosphate (HAP, pH 4.0) and (B) 2 mM HAP (pH 4.0) containing 2 mM β-CD. For other conditions, see Experimental.

ing reagent (alkylammonium ion) and counter ion (phosphate ion) on the surface of a non-polar stationary phase and (2) ion exchange of $I^-$ with the counter ion. Moreover, when CDs are added to the mobile phase, interactions of the CDs with the mobile phase component, $I^-$ and the stationary phase, etc., further complicate the chromatographic equilibria. CDs, which are toroidal-shaped molecules having a relatively hydrophobic cavity, form inclusion complexes with various guest molecules in the cavity. Therefore, in the dynamic ion-exchange model, the retention of $I^-$ in CD-containing mobile phase systems will be considerably influenced by the formation of inclusion complexes between CDs and ion-pairing reagents or CDs and $I^-$.

The formation of the inclusion complexes between part of the CDs and the ion-pairing reagents used in this study was suggested by the work of Miyajima *et al.*[15] They reported that $\alpha$-CD and $\beta$-CD form 1:1 inclusion complexes with hexyl-, heptyl- and octylammonium salts (chlorides) in aqueous solutions. When the CDs form complexes with alkylammonium (AA) phosphates in a mobile phase, the AA–CD complexes function as ion-pairing reagents. The hydrophobicity of the newly formed ion-pairing reagents is lower than that of the original reagents because the AAs are included in the CD cavity by van der Waals and hydrophobic interactions, whereas the outside of the CD molecules is hydrophilic. Hence the concentration of the ion-pairing reagents on the surface of a non-polar stationary phase is decreased when CD-containing mobile phases are used, and the retention of $I^-$ is decreased. The dependence of the retention of $I^-$ on the molecular species of CD is attributed to the differences in stability, which depend on the cavity size of the CDs, the alkyl chain length of AAs and the hydrophobicity of the AA–CD complexes formed. However, the decrease in the retention of $I^-$ observed on using $\gamma$-CD-containing mobile phases is not explained by the decrease in the hydrophobicity of the ion-pairing reagents, because $\gamma$-CD does not form inclusion complexes with hexyl-, heptyl- and octylammonium salts[15].

On the other hand, $\alpha$-, $\beta$- and $\gamma$-CDs also form inclusion complexes with $I^-$ in aqueous solutions[16]. When the CDs form complexes with $I^-$ in a mobile phase, the $I^-$–CD complexes function as anionic solutes. The ionic nature of the newly formed solutes is weaker than that of the original solutes, because the charge of $I^-$ is shielded by the complex formation with CDs. Therefore, the ionic interaction of the anionic solutes with cationic ion-pairing reagents on the stationary phase is decreased when CD-containing mobile phases are used, and the apparent retention of $I^-$ is decreased. The dependence of the retention of $I^-$ on the molecular species of CD is attributed to the differences in stability, which depend on the cavity size of CDs and the ionic nature of the $I^-$–CD complexes formed.

It has not been reported whether DM-$\beta$-CD forms inclusion complexes with alkylammonium salts and $I^-$. However, the marked decrease in the retention of $I^-$ observed when using DM-$\beta$-CD-containing mobile phases is noteworthy and requires a different retention mechanism. The methylation of CDs brings about changes in chemical and physical properties, *e.g.*, the hydrophobicity of methylated CDs is increased significantly and the adsorption of methylated CDs on the stationary phase is not negligible[17]. When DM-$\beta$-CD is adsorbed on the non-polar octadecyl stationary phase, the DM-$\beta$-CD–octadecyl complex functions as a stationary phase. The polarity of the newly formed stationary phase is greater than that of the original phase, because the polarity of the secondary phase, which is formed by DM-$\beta$-CD, is higher

than that of the primary phase, which is formed by octadecyl, in the stationary phase. Hence the concentration of the ion-pairing reagents on the surface of stationary phase is decreased when DM-$\beta$-CD-containing mobile phases are used and the retention of $I^-$ is then decreased.

In this manner, the interaction of CDs with ion-pairing reagents, $I^-$ and stationary phase may be demonstrated. As a result, we conclude that the retention of $I^-$ with CD-containing mobile phase would be decreased by the combined effect of these interactions of CDs.

*Effect of pH of mobile phase*

The dependence of the capacity factor of $I^-$ on the mobile phase pH was investigated by using 2 m$M$ HAP solution (pH 3.0–7.0) containing 2 m$M$ CD or without a CD as mobile phases. The pH of the HAP solution was not altered by the addition of CDs. Under these pH conditions, it is assumed from the p$K_a$ of heptylamine (10.65) that the heptylammonium added as an ion-pairing reagent exists in the fully dissociated form. The results ($k'$ profiles) are shown in Fig. 2 as a function of the mobile phase pH.

The maximum retention of $I^-$ was observed at pH 4.0 when a mobile phase without CDs was used, and similar results were also obtained when $\alpha$-, $\beta$- or $\gamma$-CD-containing mobile phases were used. Therefore, it is considered that the addition of these CDs to the mobile phase affects the magnitude of the retention of $I^-$, but does not affect the retention *vs.* pH profiles.

On the other hand, the retention of $I^-$ was almost independent of the mobile phase pH when a DM-$\beta$-CD-containing mobile phase was used.

*Effect of concentration of CDs*

The dependence of capacity factor of $I^-$ on the CD concentration in the mobile phase was investigated by using 2 m$M$ HAP (pH 4.0) containing 0–5 m$M$ CDs as mobile phases. The results are shown in Fig. 3 as a function of the CD concentration.

In all instances, the retention of $I^-$ decreased with increasing CD concentration. This behaviour may be caused by promotion of the formation of inclusion complexes between CDs and heptylammonium or CDs and $I^-$, and the adsorption of DM-$\beta$-CD on the stationary phase with increasing CD concentration.

The complexation ratio of the CDs with heptylammonium or $I^-$ (CD:heptylammonium or CD:$I^-$) will be assumed to be 1:1[15,16]. However, other complexation
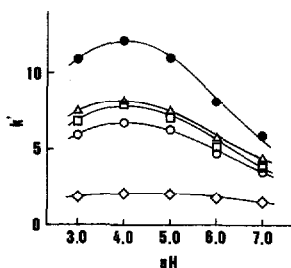


Fig. 2. Effect of mobile phase pH on the capacity factor of $I^-$. Mobile phase: 2 m$M$ HAP containing 2 m$M$ (○) $\alpha$-CD, (□) $\beta$-CD, (△) $\gamma$-CD or (◇) DM-$\beta$-CD or (●) no CDs.
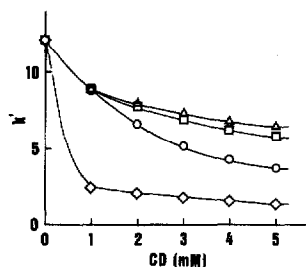
Fig. 3. Effect of CD concentration on the capacity factor of $I^-$. Mobile phase: 2 m$M$ HAP (pH 4.0) containing ($\bigcirc$) α-CD, ($\square$) β-CD, ($\triangle$) γ-CD or ($\diamond$) DM-β-CD.

ratios will not be negligible. In general, the relationship between solute retention and CD concentration in mobile phase gives an estimate of the complexation ratio of CD with the solute[18]. However, such an estimate was established on the basis that a CD interacts solely with the solute to form an inclusion complex in the chromatographic system. In our chromatographic system, it will be difficult to assume a definite ratio of complexation of the CDs with $I^-$, because multiple interactions of the CDs with $I^-$, the ion-pairing reagent or the stationary phase take place. Moreover, it is difficult to assume a ratio of complexation of the CDs with the ion-pairing reagent from the present results.

*Determination of $I^-$*

The determination of $I^-$ was performed with a UV detector using 2 m$M$ HAP (pH 4.0) containing 2 m$M$ β-CD as a mobile phase. The UV spectral characteristics of $I^-$ in the various solutions with or without CDs are given in Table II. There were almost no differences in the spectra, so the detection wavelength was set at 226 nm.

Calibration graphs for $I^-$ were prepared by injecting 10 μl of various concentrations of KI standard solutions at three detector sensitivities. The results are given in Table III.

The calibration graphs exhibit good linearity between amount of $I^-$ and peak height over the range of 25 pmol (3.2 ng) to 12.5 nmol (1.6 μg). The detection limit of $I^-$ was 0.5 pmol (64 pg) (signal-to-noise ratio = 3, 0.005 a.u.f.s.).

The reproducibility of peak heights was examined by repeated analyses ($n$ = 10) of 0.1, 1.0 and 10 nmol of $I^-$ at 0.01, 0.1 and 1.0 a.u.f.s., respectively. The relative

TABLE II

UV SPECTRAL CHARACTERISTICS OF $I^-$ IN VARIOUS SOLUTIONS

| Solution | $\lambda_{max}$ (nm) | Molar absorptivity, ε ($10^4$ l mol$^{-1}$ cm$^{-1}$) |
|---|---|---|
| Water | 226 | 1.40 |
| 2 m$M$ HAP (pH 4.0) | 226 | 1.42 |
| 2 m$M$ HAP (pH 4.0) containing 2 m$M$ α-CD | 226 | 1.40 |
| 2 m$M$ HAP (pH 4.0) containing 2 m$M$ β-CD | 226 | 1.38 |
| 2 m$M$ HAP (pH 4.0) containing 2 m$M$ γ-CD | 226 | 1.39 |
| 2 m$M$ HAP (pH 4.0) containing 2 m$M$ DM-β-CD | 226 | 1.37 |

TABLE III
REGRESSION LINES OF THE CALIBRATION GRAPHS FOR $I^-$

| Detector sensitivity (a.u.f.s.) | Amount of $I^-$ (nmol) | Regression line[a] | Correlation coefficient |
|---|---|---|---|
| 0.01 | 0.025– 0.125 | $y = 111x + 0.034$ | 0.9988 |
| 0.1 | 0.25 – 1.25 | $y = 11.8x - 0.018$ | 0.9996 |
| 1.0 | 2.5 –12.5 | $y = 1.06x + 0.310$ | 0.9999 |

[a] $y$ = Peak height (cm); $x$ = $I^-$ (nmol).

standard deviations of the peak heights were 1.43% (0.1 nmol), 1.35% (1.0 nmol) and 0.89% (10 nmol).

The effect of other halogen ions on the determination of $I^-$ was examined by injecting solutions containing 1 nmol of $I^-$ with or without various amounts of other halogen ions. The results are given in Table IV. It was found that $I^-$ could be determined without any interference in the presence of up to a 100-fold excess of the other halogen ions. On the other hand, $I^-$ could not be determined quantitatively in the presence of a 1000-fold excess of the other halogen ions because the peak heights of $I^-$ were markedly lower, with peak broadening.

TABLE IV
EFFECT OF OTHER HALOGEN IONS ON THE DETERMINATION OF $I^-$

| Halogen ion (with $I^-$) | Added as | Amount added (nmol) | Recovery[a] (%) | Error (%) |
|---|---|---|---|---|
| None | — | — | 100.0 | 0.0 |
| $F^-$ | KF | 10 | 100.3 | +0.3 |
|  |  | 100 | 99.7 | −0.3 |
|  |  | 1000 | 53.2 | −46.8 |
| $Cl^-$ | KCl | 10 | 99.5 | −0.5 |
|  |  | 100 | 100.2 | +0.2 |
|  |  | 1000 | 47.8 | −52.2 |
| $Br^-$ | KBr | 10 | 100.4 | +0.4 |
|  |  | 100 | 99.4 | −0.6 |
|  |  | 1000 | 41.0 | −59.0 |

[a] Peak height of 1 nmol of $I^-$ = 100%. Values are averages of five determinations.

CONCLUSIONS

The retention characteristics and determination of $I^-$ by RP-HPLC were investigated using mobile phases containing CDs. It was found that the CDs decrease the retention of $I^-$ and the determination of $I^-$ is not affected by the presence of CDs in the mobile phase. The decreasing effect of the retention of $I^-$ may be the result of the formation of inclusion complexes between CDs and ion-pairing reagents or CDs and $I^-$, and by the adsorption of CDs on the stationary phase changing its original nature.

It is necessary to decrease the retention of I⁻ in ion-pair RP-HPLC to obtain a reasonable analysis time, as the retention is relatively high in conventional RP-HPLC. The present method represents a useful technique with a simple modification of the mobile phase in conventional RP-HPLC.

REFERENCES

1  W. J. Hurst, K. P. Snyder and R. A. Martin, Jr., *J. Liq. Chromatogr.*, 6 (1983) 2067.
2  M. Miyashita, K. Kaji, Y. Seyama and S. Yamashita, *Chem. Pharm. Bull.*, 32 (1984) 2430.
3  H. Nagashima and K. Nakamura, *J. Chromatogr.*, 324 (1985) 498.
4  N. E. Skelly, *Anal. Chem.*, 54 (1982) 712.
5  R. Vespalec, J. Neca and M. Vrchlabsky, *J. Chromatogr.*, 286 (1984) 171.
6  M. Miyashita and S. Yamashita, *J. Liq. Chromatogr.*, 9 (1986) 2143.
7  M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, 1978.
8  T. Sakai, Y. Ninuma, S. Yanagihara and K. Ushio, *J. Chromatogr.*, 276 (1983) 182.
9  D. Sybilska, J. Debowski, J. Jurczak and J. Zukowski, *J. Chromatogr.*, 286 (1984) 163.
10 J. Zukowski, D. Sybilska and J. Jurczak, *Anal Chem.*, 57 (1985) 2215.
11 J. Debowski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 237 (1982) 303.
12 J. Debowski, J. Jurczak and D. Sybilska, *J. Chromatogr.*, 282 (1983) 83.
13 Y. Nobuhara, S. Hirano and Y. Nakanishi, *J. Chromatogr.*, 258 (1983) 276.
14 W. R. Melander and C. Horvath, in M. T. W. Hearn (Editor), *Ion-Pair Chromatography*, Marcel Dekker, New York, 1985, Ch. 2, p. 27.
15 K. Miyajima, M. Ikuto and M. Nakagaki, *Chem. Pharm. Bull.*, 35 (1987) 389.
16 J. P. Diard, E. Saint-Aman and D. Serve, *J. Electroanal. Chem.*, 189 (1985) 113.
17 M. Tanaka, T. Miki and T. Shono, *J. Chromatogr.*, 330 (1985) 253.
18 D. W. Armstrong, F. Nome, L. A. Spino and T. D. Golden, *J. Am. Chem. Soc.*, 108 (1986) 1418.