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Journal of Chromatography A, 678 (1994) 343–350

JOURNAL OF CHROMATOGRAPHY A

Migration behaviour of triiodinated X-ray contrast media containing diol groups as borate complexes in capillary electrophoresis

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First received 27 August 1993; revised manuscript received 26 May 1994

Abstract

The migration behaviour of several triiodinated X-ray contrast media containing diol groups was examined by capillary electrophoresis as borate complexes. A basic pH and a high concentration of borate buffer appeared to facilitate the complexation reaction and hence enhance the electrophoretic mobility and the resolution of solutes. Further, addition of organic modifiers to the background electrolyte was found to affect the resolution by decreasing the magnitude of the electroosmotic flow. A correlation between migration order and structure of the solutes is discussed in terms of degree of complex formation.

1. Introduction

Modern triiodinated X-ray contrast media (XCM) containing small diol groups have been developed, as the second generation of non-ionic contrast media, in succession to metrizamide (Amnipaque; Nycomed Imaging, Oslo, Norway). The compounds have high water solubility and chemical stability, low osmolality and significantly improved biological safety when compared with metrizamide [1].

Owing to their highly hydrophillic character (>50%, w/w), reversed-phase HPLC has been used routinely in the qualitative and quantitative analysis of these pharmaceuticals [2–5]. Recently, capillary electrophoresis (CE) has been developed as a potent and complementary tool to HPLC for analytical purposes [6,7]. One of the most common formats of CE is capillary zone electrophoresis (CZE), which primarily allows the separation of ionizable compounds. Interestingly, CZE has been extended to the analysis of certain non-ionizable compounds, through dynamic complexation [8]. This approach involves an in situ transformation of neutral solutes into negatively charged complexes, using borate buffer as a complexing additive in the background electrolyte. The concept has been applied in the separation of diol-containing compounds, including catechols [8], catecholamines [9], carbohydrates [10] and flavonoid-O-glycosides [11].

The objective of this work was to adopt the borate complexation concept to enable triiodinated XCM containing diol groups to be de-

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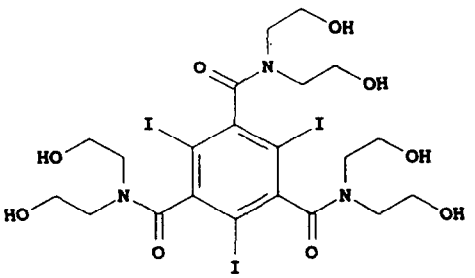
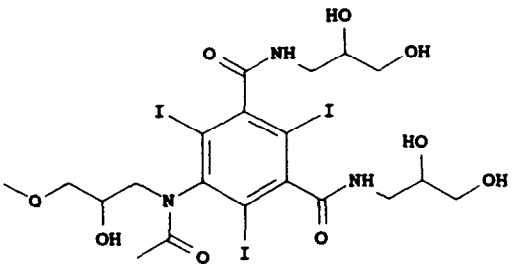
0021-9673/94/$07.00
SSDI 0021-9673(94)00502-Z

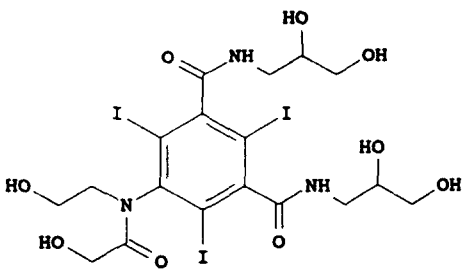
termined by CE. The compounds include iosemide, iopentol, ioversol, iopamidol and iohexol (Fig. 1). The emphasis was placed on studies of the effect of pH and concentration of borate buffer on the migration behaviour of solutes. Attempts to lower the electroosmotic flow and thereby affect the resolution was done by addition of organic modifiers. A correlation between migration order and structure of solutes was also elucidated.



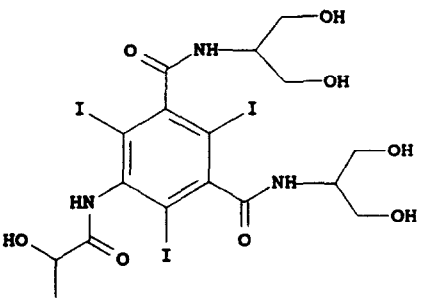
IOSEMIDE (1)
(MW = 849)



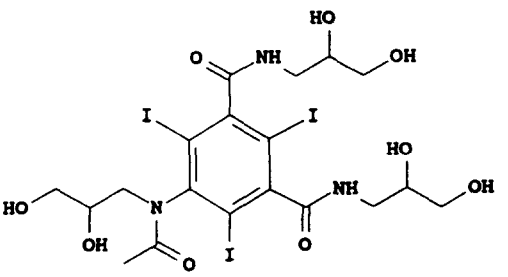
IOPENTOL (2)
(MW = 835)



IOVERSOL (3)
(MW = 807)



IOPAMIDOL (4)
(MW = 777)



IOHEXOL (5)
(MW = 821)

Fig. 1. Structures and molecular masses of the non-ionic XCM.

2. Experimental

2.1. Materials

Iohexol (Omnipaque) and iopentol (Imagopaque) were obtained from the laboratory stocks (Nycomed Imaging). Iopamidol (Iopamiro; Astra Tech, Sweden) and ioversol (Optiray, Mallinckrodt, St. Louis, MO, USA) were isolated from the commercially available pharmaceutical products. Iosemide was synthesized according to the method proposed by Gries et al. [12]. Borax (disodium tetraborate decahydrate), HPLC-grade methanol and 2-propanol were supplied by Merck (Darmstadt, Germany). Tris-(hydroxymethyl)aminomethane hydrochloride (Tris) and benzyl alcohol (as neutral marker) were purchased from Fluka (Buchs, Switzerland).

2.2. Buffers and sample preparation

Tris and borate buffer were prepared in Milli-Q-purified water with the pH adjusted with 0.1 *M* NaOH or 0.1 *M* HCl (Titrisol; Merck) and filtered through a 0.45-μm filter (Millipore, Bedford, MA, USA) before use. Approximately 0.1 mg/ml sample solutions were prepared in Milli-Q-purified water.

2.3. Apparatus

An Applied Biosystems (San Jose, CA, USA) Model 270A-HT capillary electrophoresis system was used. All experiments were performed in a 72 cm (50 cm from injection to detection) × 50 μm I.D. fused-silica capillary (Applied Biosystems).

The injection cycle was started with flushing of the capillary with 0.1 *M* NaOH for 2 min, followed by the applied buffer for 2 min. Samples were introduced at the anode (+) by hydrodynamic injection using a controlled vacuum system for 2 s and detected by UV measurement at 245 nm with a 0.5-s rise time. A constant voltage of 20 kV was applied and the oven temperature was maintained at 25°C. A Perkin-Elmer Model LCI-100 integrator was used to record the electropherograms. The attenuation was set at 16 mV and the chart speed at 1 cm/min.

3. Results and discussion

3.1. Evidence for borate complexation of the XCM

Initial experiments were performed to confirm the borate complexation of the XCM, using 50 m*M* Tris and 50 m*M* borate buffer, both at pH 9.2. Benzyl alcohol was used to measure the electroosmotic flow (EOF). Fig. 2 shows the electropherograms of the XCM obtained with these buffers.

As electrically neutral compounds, the XCM

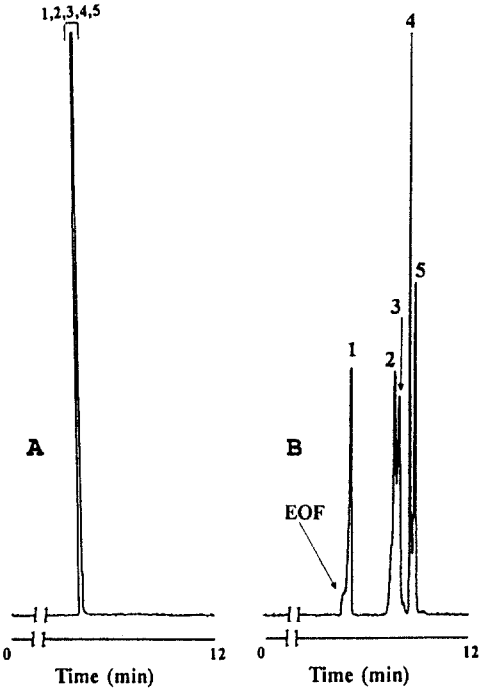


Fig. 2. Electropherograms of the XCM obtained with (A) 50 m*M* Tris and (B) 50 m*M* borate buffer, both at pH 9.2. Constant voltage, 20 kV; detection wavelength, 254 nm; temperature, 25°C. Peaks 1–5 as indicated in Fig. 1.

migrated with the same velocity as that of the EOF in CZE with the non-complexing Tris buffer. Interestingly, when borate buffer was applied, the migration pattern changed considerably. All compounds studied, except for iosemide, migrated more slowly than the EOF. This indicated that the compounds had to possess negative charges, presumably resulting from the complexation of borate with the diol groups of XCM.

According to the complexation mechanism postulated by Lorand and Edwards [13], the complexation involves a change of boric acid into borate anion on ionization and subsequent reaction with diols to yield a complex. As each XCM studied contains at least two diol moieties, the complexation of borate with XCM is considered to involve several equilibria, as illustrated in Fig. 3. In this figure, iohexol is assumed not to have any enantiomeric preference (*R* or *S*) for the borate complexation as the compound was eluted as a single peak under the conditions tested. Moreover, the complexation of the XCM with borate should be governed by the pH and concentration of the borate buffer. It was therefore of great interest to examine the effects of these parameters on the migration behaviour of the compounds.

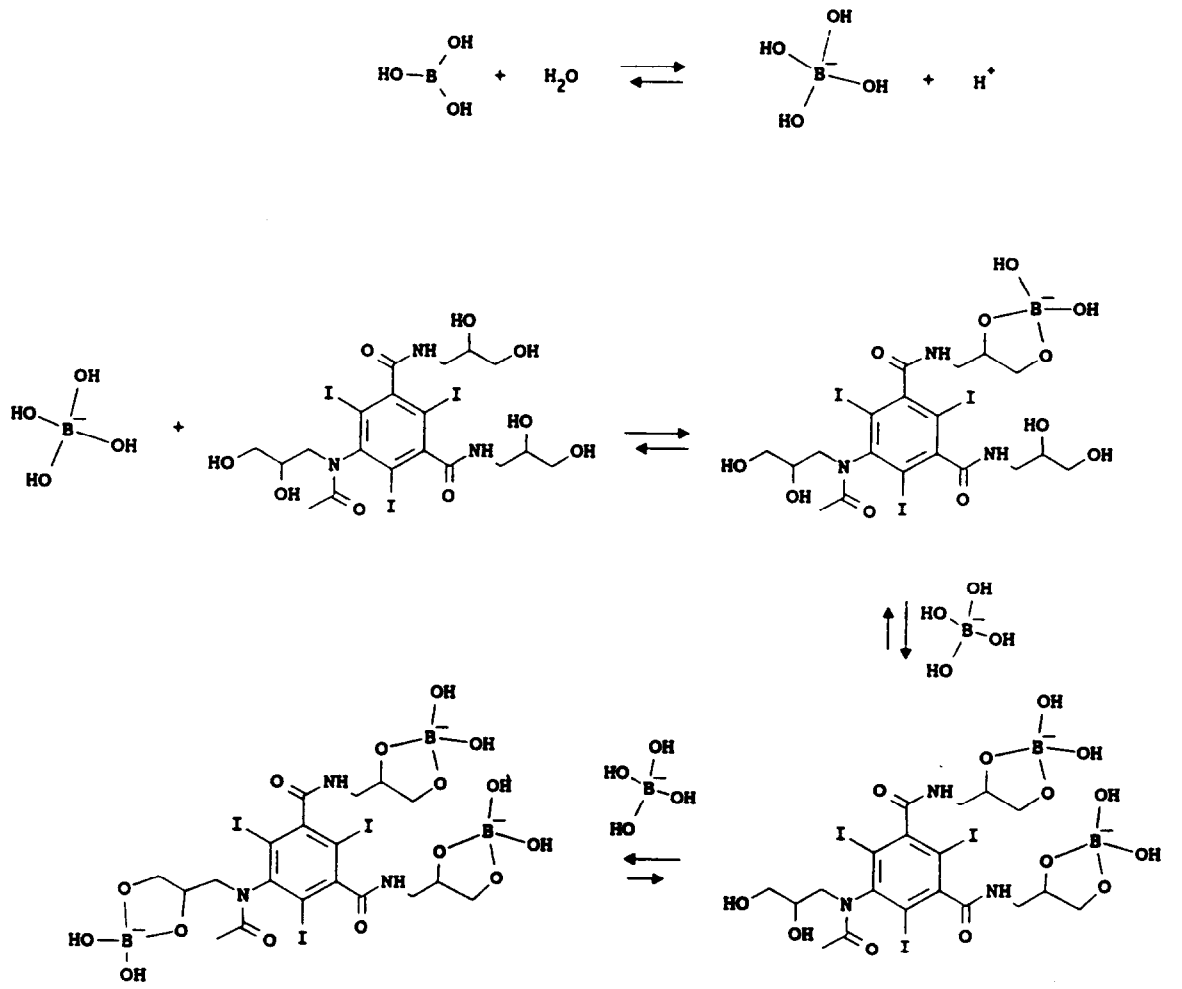


Fig. 3. Equilibria between boric acid, anionic borate and iohexol in aqueous medium.

3.2. Effect of pH

To verify the effect of buffer pH on the migration behaviour of the XCM, experiments were performed with 50 m*M* borate buffer. The results are shown in Fig. 4. The effective electrophoretic mobilities, μ(ep) in $cm^2\ V^{-1}\ s^{-1}$, were calculated using $\mu(ep) = L_{(d)}L_{(t)}/V[1/t_{eo)} - 1/t_{(m)}]$, where $L_{(t)}$ (cm) is the total length of the capillary, $L_{(d)}$ (cm) the distance between the inlet of the capillary and the detector, V (V) the applied voltage, $t_{(eo)}$ (s) the migration time of the neutral marker and $t_{(m)}$ (s) the migration time of the solute.

The co-migration of the XCM with the EOF observed at pH 7 suggested that no complexation occurred at this pH. When the pH was made more basic, a structural preference for the formation of borate complexes was observed. The complexation of iosemide seemed to occur at pH 9.2, whereas the corresponding processes for the other XCM were observed at pH 8. Further raising the pH towards 10 led to a general increase in the electrophoretic mobility of the XCM. This can be explained in terms of an increase in the overall concentration of anionic borate that is active in the complex formation. Accordingly, the complex formation was facilitated at basic pH and the electrophoretic mobility of the solutes towards the anode was enhanced. Further, increasing the pH brought about an improved resolution of iopentol and ioversol, but at the expense of a loss of resolution of iopamidol and iohexol.

3.3. Effect of borate concentration

A plot of electrophoretic mobility of the XCM vs. borate buffer concentration at pH 9.2 is given in Fig. 5. As stated above, increasing borate concentration in the background electrolyte would favour the complex formation and result in more negative mobilities of the compounds.

Notably, high borate concentration appeared to improve the resolution of the four late-eluting XCM. The best separation of these four XCM was achieved with 75 m*M*. However, some small and unidentified peaks were also observed with this buffer.

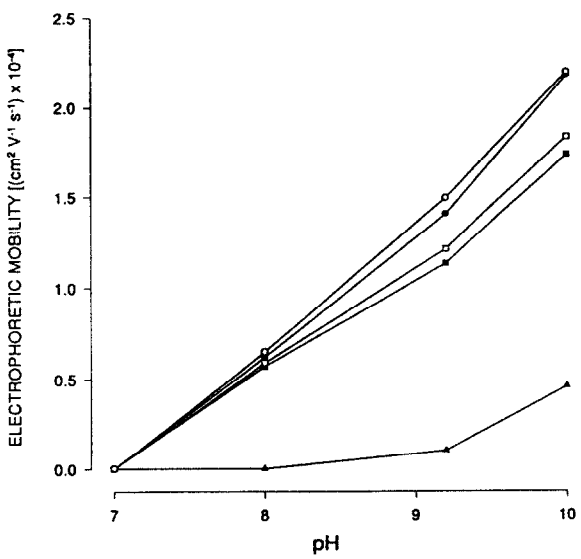


Fig. 4. Effect of buffer pH on the electrophoretic mobility of the XCM. Buffer concentration, 50 m*M* borate; other conditions as in Fig. 2. ○ = Iohexol; ● = iopamidol; □ = ioversol; ■ = iopentol; ▲ = iosemide.

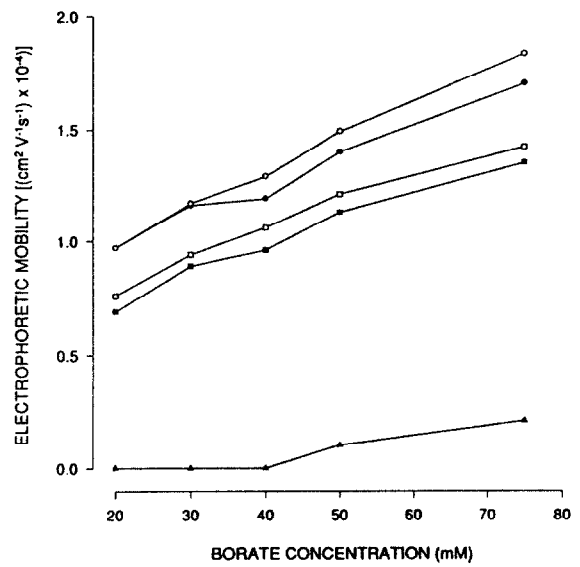


Fig. 5. Effect of borate concentration on the electrophoretic mobility of the XCM at pH 9.2. Other conditions as in Fig. 2. Symbols as in Fig. 4.

3.4. Effect of organic additives

So far, pH and borate concentration were observed to affect the resolution of the XCM, solely by increasing the differences in the electrophoretic mobilities. Alternatively, the resolution of solutes could be improved by decreasing the magnitude of the EOF according to Jorgenson and Lukacs [14]. A decrease in the EOF may be achieved by addition of an organic modifier to the background electrolyte [15].

The addition of up to 5% (v/v) of 2-propanol to 50 m*M* borate buffer (pH 9.2) was shown to lower the EOF, without having a significant effect on the resolution. A similar effect on the EOF was also observed when methanol was applied, but the resolution of iopamidol (**4**) and iohexol (**5**) was remarkably improved, particularly at 10% methanol (Fig. 6; for comparison, see Fig. 2B). Further increasing the concentration of methanol to 20% led to a greater resolution of iopamidol (**4**) and iohexol (**5**) at the cost of a slight loss of resolution of ioversol (**3**) and iopamidol (**4**).

3.5. Migration order and structure of solutes

When attempting to understand the migration order of the XCM observed in Fig. 2B, it is important to emphasize that the electrophoretic mobility of an ion in CE depends on its charge-to-size ratio. In the borate complexation mode, the magnitude of this ratio depends on the type and numbers of diol being complexed, as the complexation results in a slight increase in molecular mass and imparts negative charges to the molecule. Further, the borate complexation with the XCM is governed by equilibria between the uncomplexed and complexed forms. It is believed that the reaction equilibrium is probably achieved at high borate concentration. To confirm this, a solution of the XCM prepared in 50 m*M* borate (pH 10) was analysed by CE (50 m*M* borate buffer, pH 10, as background electrolyte) after 0, 4 and 24 h of continuous stirring. The results showed a constant migration time for the individual XCM at those time intervals. This indicated an instantaneous establishment of equilibria in the complex formation of the XCM

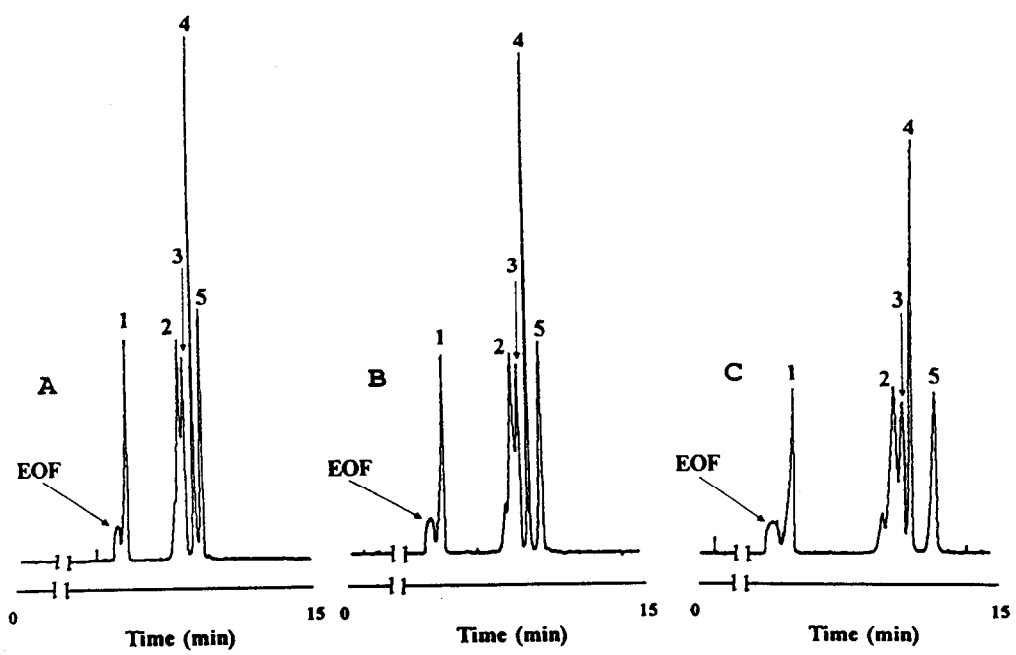


Fig. 6. Electropherograms of the XCM obtained with addition of (A) 5, (B) 10 and (C) 20% (v/v) methanol to 50 m*M* borate buffers at pH 9.2. Other conditions as in Fig. 2. Peaks 1–5 as indicated in Fig. 1.

at high borate concentration. Throughout the study, the borate complexation of iosemide was observed to take place only if the borate concentration was high enough. The resultant complex would have an eight-membered ring structure. Owing to the ring size and strain generally existing in such a ring structure [16], the complexation reaction is considered to be unfavorable. As a result, it is most probable that only a 1:1 iosemide–borate complex was formed at equilibrium. The iosemide–borate complex would therefore have the lowest charge density among the XCM and migrate fastest through the capillary, as observed in this study.

In contrast, the other XCM possess either 1,2-diol or 1,3-diol groups, all of which could react smoothly with borate [17–19], to form almost strainless five- or six-membered rings, respectively. As there are two such diol moieties in iopentol, ioversol and iopamidol, 1:2 solute–borate complexes were expected to be formed at high borate concentration. These complexes have the same charge density and the migration order is dependent on their masses, i.e. iopentol (**2**) > ioversol (**3**) > iopamidol (**4**). It should be noted that ioversol contains a third diol group, namely $N(COCH_2OH)(CH_2CH_2OH)$, in addition to the two 1,2-diol groups. An eight-membered ring, similar to that of iosemide, could in principle be formed on complexation. Unlike the diol of iosemide, which formed a borate complex at high borate concentration, the corresponding reaction of that of ioversol was not observed. If it took place, ioversol would have an overall charge density similar to that of iohexol and migrate more slowly than iohexol because of its lower mass. Under all conditions tested, ioversol was observed to migrate faster than iohexol, indicating that ioversol must have a lower charge density than iohexol. In other words, ioversol has only two 1,2-diol groups which are active in the borate complexation.

Lastly, iohexol, with its three 1,2-diol moietities, would possess the highest charge density of the XCM studied on complexation. It would therefore migrate the slowest through the capillary in spite of clearly being a larger solute than ioversol (**3**) or iopamidol (**4**).

4. Conclusions

The results demonstrate that CE is a useful technique for the rapid and efficient separation of non-ionic triiodinated XCM containing diol groups as borate complexes. Further, CE gives only a single peak for the individual XCM whereas multiple peaks are usually observed in HPLC, owing to the restricted rotation in amides [2]. This makes CE more attractive for both qualitative and quantitative analysis of these pharmaceuticals.

Acknowledgements

The author thanks Kebo Lab (Oslo, Norway) for lending the CE instrument and Professor A. Rogstad for critically reading the manuscript.

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