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Separation of enantiomers on anion exchangers modified with heparin in liquid chromatography

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Abstract

The separation of enantiomers of chloroquine and other amine derivatives has been investigated by liquid chromatography using anion exchangers modified with heparin as the stationary phase. Modification and mobile phase conditions affected the separation of analytes. Separation of the chloroquine enantiomers could be achieved by using a buffer solution containing acetonitrile as the mobile phase.

Keywords: Enantiomer separation; Heparin-modified stationary phases; Chloroquine; Amines

1. Introduction

The retention behavior and selectivities of inorganic anions on anion exchangers can be remarkably altered after the electrostatic modification with anionic polysaccharides such as chondroitin sulfate, heparin and dextran sulfate [1–5]. These polysaccharides contain sulfate or carboxyl groups, which are possibly strongly adsorbed on the positively charged surface by ionic interactions. The ion-exchange capacity of the modified stationary phase could be changed depending on the number of ionic groups in the mucopolysaccharide skeleton and the amounts of the modifier. The negative charges of the mucopolysaccharides can repel anionic analytes and therefore decrease the retention of anions. It was observed that the retention of analyte anions on the modified stationary phase decreased with decreasing eluent concentration [1–5]. Such unusual retention behavior is due to the presence of both anion- and cation-exchange sites in the column. It was also demonstrated that both anions and cations were simultaneously separated on the modified stationary phase [2]. Ion-exchange properties were affected by the size of the modifier [4] as well as by the mobile phase employed for the separation [5].

As mucopolysaccharides are chiral, they can have a potential to provide enantioselectivity. Heparin has been employed as a chiral selector for enantioseparation in capillary electrophoresis [6–9], where racemic chloroquine, chlorpheniramine and 9-chlorodiltiazem enantiomers have been resolved. In LC chemically bonded heparin stationary phases have been developed for affinity chromatography of proteins and these stationary phases have been also applied to the resolution of chloroquine enantiomers [9]. Since the chiral recognition mechanism has not

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been elucidated yet, more work still needs to be done.

It is easy to introduce anionic polysaccharides into anion exchangers by electrostatic interaction [1–5]. This paper describes the chromatographic performance of heparin stationary phases electrostatically modified on anion exchangers and applies them for the enantiomeric separation in LC. The effects of modification and mobile phase conditions on the separation of enantiomers are examined.

2. Experimental

2.1. Apparatus

Microcolumn and conventional LC systems were used in this work. A microcolumn liquid chromatograph was comprised of an MF-2 Microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5 ml; Ito, Fuji, Japan), an ML-522 microvalve injector (Jasco, Tokyo, Japan) with an injection volume of 0.2 μl, a 100×0.32 mm I.D. microcolumn and a UV-970 UV detector (Jasco). The flow rate of the pump was kept at 4.2 μl min^{-1}.

The microcolumns were prepared in the laboratory from fused-silica tubing as reported previously [10]. The anion exchangers employed were TSK_{gel}IC-Anion SW and QAE-3SW (Tosoh, Tokyo, Japan), silica-based strongly basic anion exchangers. The former packing has an ion-exchange capacity of ca. 0.16 mequiv. ml^{-1} and a mean pore diameter of 14 nm, whereas the latter has an ion-exchange capacity of ca. 0.15 mequiv. ml^{-1} and a mean pore diameter of 23 nm.

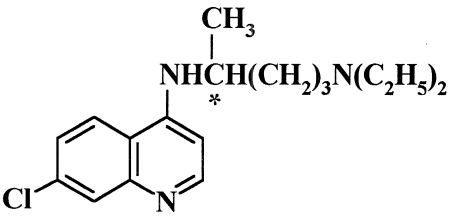
A conventional LC system was comprised from an 880-PU HPLC pump, a loop injector with an injection volume of 20 μl, a 50×4.6 mm anion exchange column (QAE-3SW), an UVIDEC-100 V UV detector (Jasco) and OR-990 optical rotation (OR) detector (Jasco). The detectors were coupled in tandem in this order. The data were handled by a Chromatopac C-R4A data processor (Shimadzu, Kyoto, Japan).

2.2. Reagents

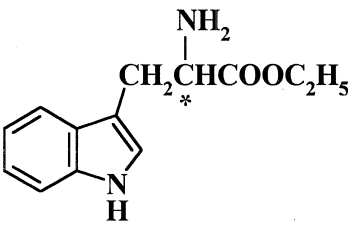
Bupivacaine was obtained from Sigma (St. Louis, MO, USA). Tryptophan ethyl ester was obtained from Tokyo Chemical Industry (Tokyo, Japan). Other reagents employed were of guaranteed reagent grade and were obtained from Nacalai Tesque (Kyoto, Japan). The reagents were used without any further treatment. Purified water was prepared by using a Milli-Q Plus system (Millipore, Molsheim, France). The mobile phases were prepared using the purified water. Phosphate buffers were prepared from potassium dihydrogen phosphate and dipotassium hydrogen phosphate, whereas acetate buffers were prepared from ammonium acetate and acetic acid. Fig. 1 illustrates the structures of the tested analytes.

2.3. Modification with heparin

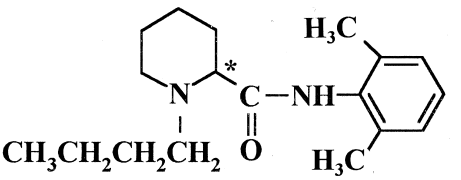
The anion exchange columns were first washed with water for 10 min and with 10 m*M* sodium



Chloroquine



Tryptophan ethyl ester



Bupivacaine

Fig. 1. Structures of tested analytes.

sulfate for 2 h. An aqueous solution of 0.1–1.0% (w/v) heparin was then passed through the column for 2 h, followed by washing with water for ca. 30 min.

3. Results and discussion

3.1. Effects of modification

Anion exchangers were modified with 0.1% (w/v), 0.5% (w/v) and 1% (w/v) heparin as described in the Experimental Section. The modified columns were employed for the separation of chloroquine enantiomers. It was found that the column modified with 1% heparin achieved baseline separation of the chloroquine enantiomers, whereas the columns modified with 0.1% (w/v) and 0.5% (w/v) heparin solution failed to resolve the enantiomers. Therefore, the concentration of heparin in the modification solution was kept at 1% (w/v) in the following experiments. In this paper, the stationary phase prepared by 1% (w/v) heparin solution is referred to 'high-load phase'.

3.2. Effect of buffer concentration and pH

The pH and the composition of mobile phases were found to be important for improvement of enantioselectivity. The effect of buffer concentration on the separation factor was investigated for chloroquine using 10–50 m*M* acetate buffer solution at pH 5.5 containing 30% acetonitrile. The results are described in Fig. 2. Better separation of enantiomers could be achieved by using mobile phases containing acetate buffer from 10 to 50 m*M*, where the separation factor (α) was about 1.20–1.24. The logarithm of the retention factors (log k_1 and log k_2) of chloroquine enantiomers decreased by increasing mobile phase concentration, where k_1 and k_2 are the retention factor of the first and the second eluted enantiomer, respectively. It is expected from the results in Fig. 2 that electrostatic interaction is involved in the retention of the chloroquine enantiomers.

The effect of buffer pH on the enantioselectivity was examined by using 30 m*M* buffer solutions (pH 4.5–7.0) containing 20% acetonitrile as the mobile phase. The effects of the buffer pH on the retention

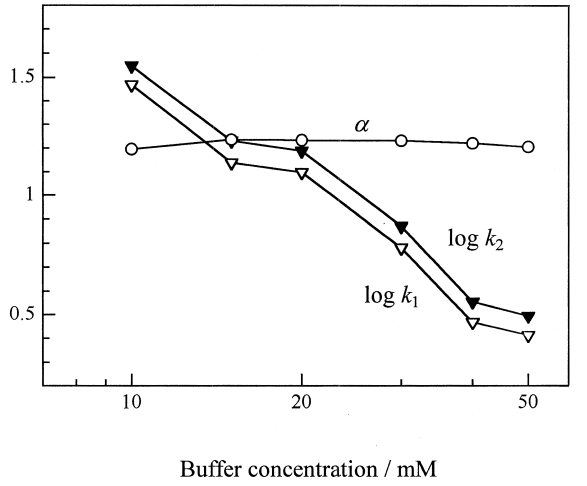


Fig. 2. Effect of buffer concentration on the retention behavior of chloroquine. Column: IC-Anion SW (high-load phase), 100×0.32 mm I.D. Mobile phase: acetate buffer (pH 5.5) containing 20% acetonitrile (v/v). Flow rate: 4.2 μl min^{-1}. Injection volume: 0.2 μl. Analyte: 0.1 m*M* chloroquine. Wavelength used for UV detection: 214 nm.

and the selectivity of the chloroquine enantiomers are shown in Fig. 3. It is seen that the retention and selectivity of the enantiomers are pH-dependent. Better selectivity for the chloroquine enantiomers was achieved at pH 5.5 to 6.0. It is expected that the pH is an important factor in improving the other enantiomers as well.

The modified stationary phase was stable when

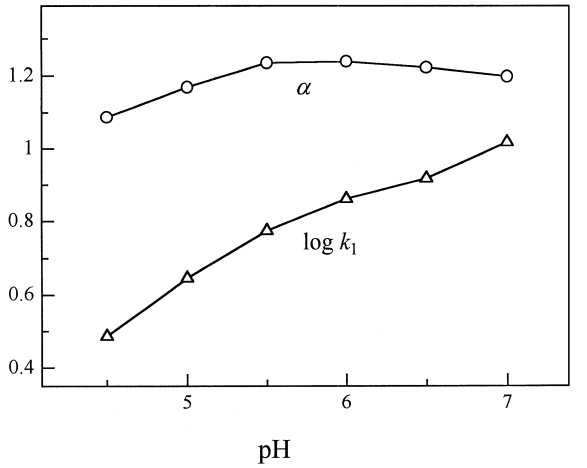


Fig. 3. Effect of pH on the retention behavior of chloroquine. Mobile phase: 30 m*M* acetate buffer containing 20% acetonitrile (v/v). Other operating conditions as in Fig. 2.

acetate or phosphate buffers were employed. However, sodium perchlorate or chlorides of alkaline-earth metals were contained in the eluent, heparin was gradually eluted out of the column. When the same procedures were employed for the preparation of the column, the column-to-column repeatability was satisfactory in terms of selectivity and resolution.

The data shown in Figs. 2 and 3 are obtained by using acetate buffers, where 1.1–1.24 of α are achieved, and the resolution (R_s) was at most 1.3. On the other hand, phosphate buffers resulted in better selectivity (1.2–1.4 of α) and better resolution (at most 1.6 of R_s) than acetate buffers. α and R_s achieved by the present method were better than those reported, e.g., $\alpha=1.2$ and $R_s=0.9$ [9].

3.3. Effects of acetonitrile concentration

Acetonitrile was used as the organic modifier. Fig. 4 describes the effect of acetonitrile concentration on enantioselectivity of chloroquine. The concentration of acetonitrile used in these experiments was 5–50% (v/v). The selectivity for chloroquine was optimum at 20% (v/v) of acetonitrile. The retention factors decreased by increasing acetonitrile concentration. The addition of organic modifier reduced retention of analytes due to the decrease in hydrophobic interaction, and the linear relationships between log k and the acetonitrile concentration were observed. The results in Fig. 4 suggest that hydrophobic interaction is involved in the retention of chloroquine enantiomers. In addition, acetonitrile as the organic modifier gave better peak shape than methanol. The column efficiency achieved by the present stationary phase is rather moderate, compared with those achieved in the common reversed-phase mode. This may be because the multimodal interactions such as hydrophobic and electrostatic interactions involved in the present stationary phase are possibly not fast and because helicity of heparin is expected to slow the diffusion of analytes.

3.4. Separation of enantiomers

Figs. 5 and 6 demonstrate the enantiomeric separation of chloroquine under different operating conditions. Fig. 5 shows the enantiomeric separation of chloroquine on an IC-Anion-SW column modified with heparin using 30 m*M* acetate buffer (pH 5.5) containing 20% acetonitrile as the mobile phase. The separation factor of the analytes achieved was 1.24 under the conditions in Fig. 5.

Fig. 6 demonstrates the enantiomeric separation of

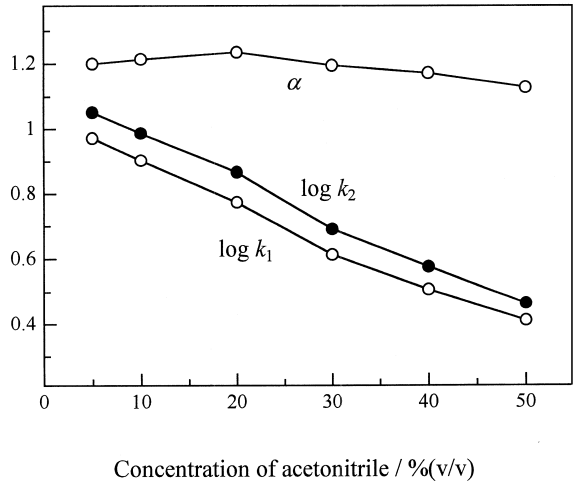


Fig. 4. Effect of acetonitrile concentration on the retention behavior of chloroquine. Mobile phase: 30 m*M* acetate buffer (pH 5.5) containing acetonitrile as indicated. Other operating conditions as in Fig. 2.

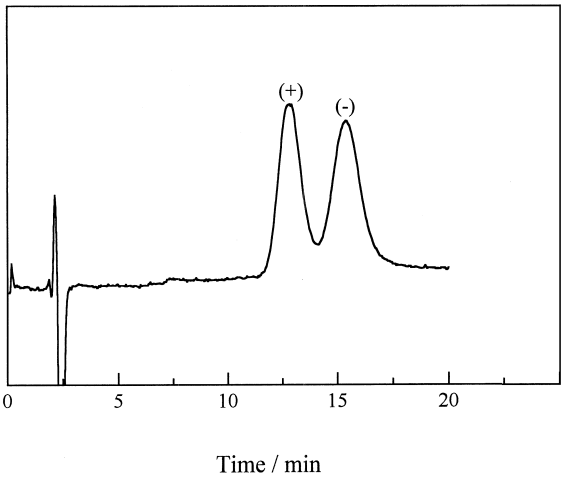


Fig. 5. Separation of chloroquine enantiomers on an IC-Anion-SW column modified with heparin. Mobile phase: 30 m*M* acetate buffer (pH 5.5) containing 20% acetonitrile. Other operating conditions as in Fig. 2.

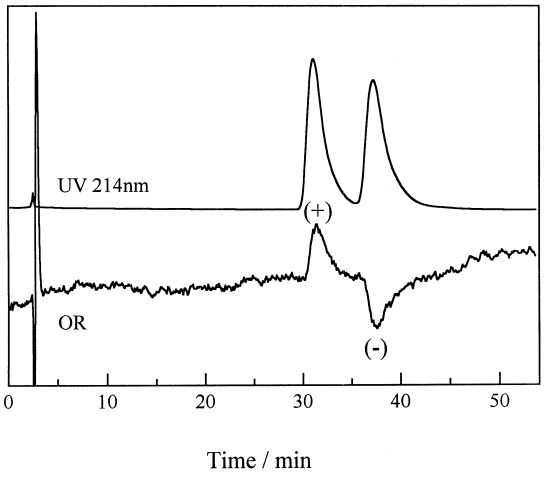


Fig. 6. Separation of chloroquine enantiomers on a QAE-3SW column modified with heparin. Column: QAE-3SW (high-load phase), 150×4.6 mm I.D. Mobile phase: 10 m*M* potassium phosphate (pH 5.0) containing 30% acetonitrile. Flow rate: 1.0 ml min^{-1}. Injection volume: 20 μl. Analyte: chloroquine. Wavelength used for UV detection: 214 nm.

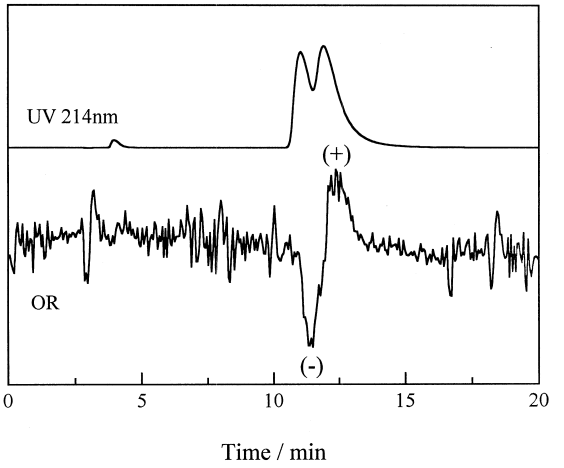


Fig. 7. Separation of tryptophan ethyl ester enantiomers on a QAE-3SW column modified with heparin. Mobile phase: 10 m*M* potassium phosphate (pH 5.0) containing 10% acetonitrile. Analyte: tryptophan ethyl ester. Other operating conditions as in Fig. 6.

chloroquine by using a phosphate buffer as the mobile phase on a QAE-3SW conventional size column (high-load phase). In Fig. 6 the UV and OR detectors were coupled in tandem. The retention, selectivity and resolution of chloroquine decreased as the ionic strength of mobile phase increased. This behavior is also consistent with an ion-exchange type mechanism. The OR detection of chloroquine enantiomers shows that the (+)-enantiomer was eluted first. This means that the (−)-enantiomer exhibited stronger binding to heparin than the (+)-enantiomer. Hydrophobic interaction as well as ionic interaction possibly contribute to the enantiorecognition.

Fig. 7 demonstrates the separation of tryptophan ethyl ester on the QAE-3SW column modified with heparin using phosphate buffer as the mobile phase. In case of enantiomeric separation of tryptophan ethyl ester, the (−)-enantiomer was eluted first, and the separation factor was 1.09.

Fig. 8 shows the enantiomeric separation of bupivacaine on the QAE-3SW column modified with heparin using phosphate buffer as the mobile phase. The enantiomers of bupivacaine could be partially separated and the separation factor was 1.07, which can be recognized for the OR detection.

Other amine derivatives such as primaquine, tryptophanamide, chlorpheniramine maleate, quinacrine, promethazine, chlorcyclizine could not be resolved under these experimental conditions. Operating conditions are being optimized to achieve enantiomeric separation of other analytes.

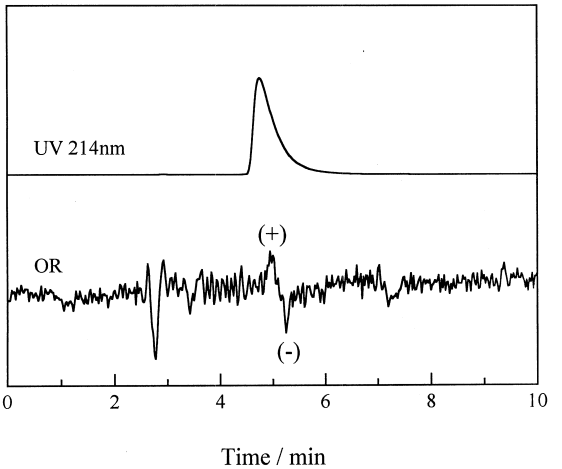


Fig. 8. Separation of bupivacaine enantiomers on a QAE-3SW column modified with heparin. Mobile phase: 20 m*M* acetate buffer (pH 6.0). Analyte: bupivacaine. Other operating conditions as in Fig. 6.

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