ELSEVIER

Journal of Chromatography A, 760 (1997) 235–244

JOURNAL OF CHROMATOGRAPHY A

# Optical resolution of new quinolone drugs by capillary electrophoresis with ligand-exchange and host–guest interactions

Takatomo Horimai*, Motomu Ohara, Mitsunori Ichinose

*Analytical Research Center, Production Technology Research Laboratories, Daiichi Pharmaceutical Co., Ltd, 1-16-13, Kitakasai Edogawa-ku, Tokyo 134, Japan*

Received 14 March 1996; revised 16 September 1996; accepted 17 September 1996

**Abstract**

A method for the optical resolution of new quinolone drugs (NQs) by capillary electrophoresis has been investigated. The NQs were adequately resolved using a γ-cyclodextrin (γ-CD)–Zn(II)–D-phenylalanine (D-Phe) solution as the running solution. The resolution depended on the components of the running solution and their concentrations. When L-Phe was used instead of D-Phe, inversion of the migration order of the enantiomers was observed. The resolution mechanism is considered to be due to a ligand-exchange interaction of the NQs with Zn(II) and D-Phe, in combination with a host–guest interaction of those with γ-CD.

*Keywords:* Enantiomer separation; Buffer composition; Pharmaceutical analysis; Quinolones; Ofloxacin

## 1. Introduction

In the pharmaceutical field, numerous drugs have been produced so far. Due to the high cost and the technical difficulties involved in their syntheses, optically active drugs usually have been provided as racemates. Since the report on thalidomide [1], however, it has been gradually recognized that some optically active substances (eutomers) are superior to their enantiomers (distomers) regarding their pharmacological, toxicological or pharmacodynamic effects [2]. Therefore, it is required that a new drug should be developed basically as a eutomer. For this purpose, excellent chiral resolution techniques are required.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been used as the techniques for assaying each enantiomer. In particular, HPLC has wide applicability to many drugs and a variety of chiral columns for HPLC are commercially available. These columns, however, have disadvantages: (1) They are relatively expensive, (2) their lifetimes are very short, and (3) the theoretical plate number is low in comparison with conventional reversed-phase columns.

In contrast, capillary electrophoresis (CE) has been regarded recently as an attractive resolution technique because of its high resolution efficiency, short analysis times and the requirement for small volumes of analytes and running solution additives, compared with HPLC. Several approaches for optical resolution by CE have been attempted. Some optical isomers are resolved using a running solution containing a chiral additive, such as natural or derivatized cyclodextrins (CDs) [3–10], metal chelating reagents [11–13], a crown ether [14], surfactants

*Corresponding author.


*PII* S0021-9673(96)00787-X

[15–18], polysaccharides [19] and proteins [20–23]. However, their applicability is often limited.

We have investigated a new optical resolution system for new quinolone drugs (NQs) by CE. NQs have bactericidal activities against a wide variety of bacteria. Some of them are optically active compounds whose enantiomers exhibit different pharmacological, toxicological or pharmacodynamic properties. Arai et al. [22] previously reported the optical resolution of ofloxacin (OFLX) by CE with bovine serum albumin (BSA) as a chiral selector [22]. Although this resolution mode is interesting, there is a lack of reproducibility for the migration behavior of the analytes because of the absorption of BSA on the inner wall of the capillary.

Therefore, we looked for a new system for the optical resolution of NQs by CE. Using a CD–metal(II)–amino acid solution as the running solution, the resolution of NQ enantiomers was achieved. The resolution mechanism is considered to be due to a ligand-exchange interaction, in combination with a host–guest interaction. In this study, optimization of the CE conditions was examined and the resolution mechanisms were also elucidated.

## 2. Experimental

### 2.1. Apparatus

CE studies were performed with a CE-800 system (JASCO, Tokyo, Japan) equipped a JASCO 875 UV detector. Untreated fused-silica capillaries [750 mm (effective length 500 mm)×50 μm I.D.] were purchased from JASCO. Electropherograms were recorded on a Shimazdu C-R4A Chromatopac (Shimazdu, Kyoto, Japan).

### 2.2. Reagents

NQs were obtained from Daiichi Pharmaceutical Research Institute (Tokyo, Japan). The chemical structures are shown in Fig. 1. These were dissolved in a 0.1 *M* aqueous sodium hydroxide solution at a concentration of 0.5 mg/ml. All chemicals used were of analytical-reagent grade. α-CD, β-CD, γ-CD, D-phenylalanine (D-Phe), L-phenylalanine (L-Phe), D-tyrosine (Tyr), D-serine (Ser), D-threonine (Thr), D-valine (Val), D-leucine (Leu), D-methionine (Met), D-aspartic acid (Asp), D-(−)-lysine monohydrochlor-

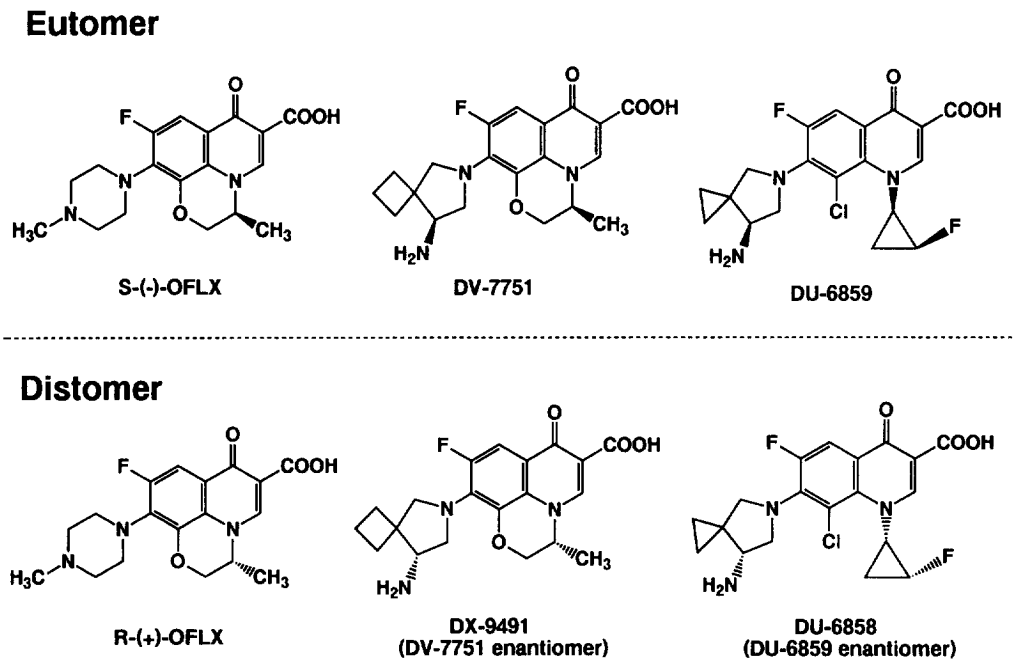


Fig. 1. Chemical structures and names of the investigated NQs.

ide (Lys) and D-proline (Pro) were purchased from Tokyo Kasei (Tokyo, Japan). D-Isoleucine (Ile) and D-arginine hydrochloride (Arg) were purchased from Sigma (St. Louis, MO, USA). Zinc(II) sulfate heptahydrate and copper(II) sulfate pentahydrate were purchased from Nacalai Tesque (Kyoto, Japan). Nickel(II) sulfate hexahydrate, iron(II) sulfate heptahydrate, ammonium acetate, phosphoric acid and sodium hydroxide were purchased from Kishida Chemical (Osaka, Japan). Running solutions were prepared by dissolving the additives in 10 m*M* ammonium acetate solution and adjusting the pH to 6.5 with a diluted aqueous ammonia or acetic acid solution. The running solutions were filtered through a 0.45-μm pore size membrane filter (Gelman Science, Tokyo, Japan) prior to use. Water was purified on a Milli RO-Milli Q system (Millipore Japan, Tokyo, Japan).

### 2.3. Procedure

All experiments were performed at ambient temperature. The applied voltage was constantly held at 10 kV. The analytes were monitored by the UV detector at 300 nm (OFLX and DV-7751 enantiomers) or at 295 nm (DU-6859 enantiomers). The sample solutions were injected by siphoning (20 cm height, 5 s). The capillary was pretreated with 0.1 *M* phosphoric acid, water and finally the running solution, under pressure, for 10 min prior to analysis.

### 2.4. Calculation

Resolution of each enantiomer of the NQ was characterized as $100\times\Delta t$ values, as introduced by Gassmann et al. [11] and Gozel et al. [12]:

$$100\,\Delta t = 2(t_2 - t_1)/(t_2 + t_1) \times 100 \quad (1)$$

where $t_1$ and $t_2$ are the migration times of the faster and slower migrating peaks, respectively. In Table 2, the resolution was also evaluated by measuring the $R_s$ values, as defined below:

$$R_s = 2(t_2 - t_1)/(W_1 + W_2) \quad (2)$$

where $W_1$ and $W_2$ are the peak bandwidths of the faster and lower migrating peaks, respectively.

The theoretical plate number of each of the faster peaks, $Nt_1$, is calculated as follows:

$$Nt_1 = 5.54(t_1/W_{1/2})^2 \quad (3)$$

where $W_{1/2}$ is the peak bandwidth at half of the height of the faster migrating peak.

## 3. Results

In preliminary experiments, the optical resolution of the NQs was examined by using running solutions containing some CDs, metal ions and amino acids. Among the reagents, the combined use of γ-CD, Zn(II) and D-Phe was found to be the most effective for resolving the NQ enantiomers. However, if running solutions lacking one of the components were used, no optical resolution was observed. Optimization of the CE conditions for the optical resolutions of the NQs was studied first.

### 3.1. Effect of CD on optical resolution

In the first stage of the investigation, the effects of CDs on the optical resolution of the NQs were evaluated using running solutions containing natural CDs [20 m*M* α, β or γ-CD, 10 m*M* $ZnSO_4$, 10 m*M* D-Phe–10 m*M* ammonium acetate (final pH of 6.5)]. We found that all of the NQ enantiomers were resolved only by using the γ-CD solution.

Fig. 2 shows the effects of γ-CD concentrations on the optical resolution of the NQs. As an example, electropherograms of the optical resolution of OFLX are shown in Fig. 3. In the absence of γ-CD, no optical resolution of the NQs was observed. On the other hand, using running solutions containing γ-CD, the optical resolution of OFLX and DV-7751 enantiomers increased with increasing γ-CD concentration, up to 20 m*M*. Although the migration behavior of OFLX and DV-7751 enantiomers was similar, the optical resolution of the DU-6859 enantiomers was much better than was that of OFLX and DV-7751. Thus, 20 m*M* γ-CD was selected.

### 3.2. Effect of metal(II) on optical resolution

In the second stage of the investigation, the effects

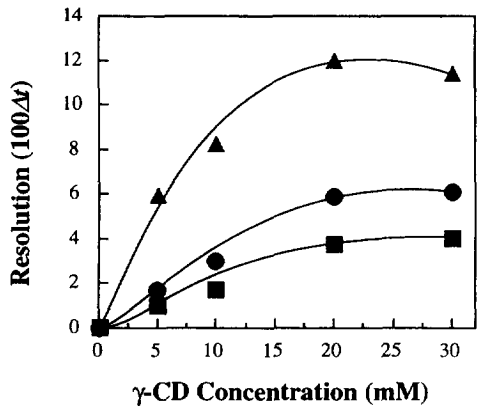


Fig. 2. Effect of the concentration of γ-CD on the optical resolution of NQs. Analytical conditions: capillary, 750 mm×50 μm I.D. (500 mm to the detector); running solution, 0 to 30 m*M* γ-CD, 10 m*M* $ZnSO_4$, 10 m*M* D-Phe–10 m*M* ammonium acetate (final pH=6.5); temperature, ambient; applied voltage, 10 kV; detection, UV at 300 nm for OFLX and DV-7751 enantiomers, and UV at 295 nm for DU-6859 enantiomers; injection, siphon method (20 cm, 5 s); sample, OFLX, 0.5 mg/ml; DV-7751 enantiomers, 0.5 mg/ml DV-7751–0.5 mg/ml DX-9491 (1:1, v/v); DU-6859 enantiomers, 0.5 mg/ml DU-6859–0.5 mg/ml DU-6858 (1:1, v/v). (■) OFLX, (●) DV-7751 enantiomers, (▲) DU-6859 enantiomers.

of metal on the optical resolution of the NQs were evaluated using running solutions containing some divalent metal ions (10 m*M* $FeSO_4$, $NiSO_4$, $CuSO_4$ or $ZnSO_4$), 20 m*M* γ-CD and 10 m*M* D-Phe in 10 m*M* ammonium acetate. The final pH of the running solutions was adjusted to 6.5 by adding diluted aqueous ammonia solution. Optical resolution of the NQs was observed using the running solutions containing Cu(II) or Zn(II). DU-6859 enantiomers were also resolved by the Ni(II) solution. No optical resolution of the NQs was observed with the Fe(II) solution. These results are very similar to those observed by ligand-exchange LC with a mobile phase containing a metal(II) ion, such as Cu(II) [24–28], Zn(II) [29,30] or Ni(II) [30].

Fig. 4 shows the effect of Zn(II) concentration on the optical resolution of the NQs, where the concentrations of γ-CD and D-Phe are fixed at 20 and 10 m*M*, respectively. As an example, electropherograms of DV-7751 enantiomers are shown in Fig. 5. No optical resolution of the NQs was observed without Zn(II) in the running solution. When using Zn(II)-containing solutions, optical resolution of DU-6859 enantiomers was only observed with Zn(II) concentrations of at least 1 m*M*. The other two enantiomers required Zn(II) concentrations of above 5 m*M*. The optical resolution of the NQs increased with increasing Zn(II) concentrations, up to 20 m*M*, with a migration order of distomer<eutomer. DU-6859 enantiomers were resolved much better than the other two enantiomers.

### *3.3. Effect of amino acids on optical resolution*

In the third stage of the investigation, the effects of ligands on the optical resolution of the NQs were evaluated using each running solution containing D-amino acids (see Table 1). All of the NQ enantiomers examined were resolved only when aromatic amino acids were used, except for DU-6859 enantiomers, which were also resolved using other amino acid such as Ser or Arg. No resolution of the NQs was observed using acidic amino acid solutions, although the reason for this was not clear. D-Phe was the most effective ligand for this system and was therefore selected as the ligand.

Fig. 6 shows the effects of the concentration of D-Phe on the optical resolution of the NQs, where the concentrations of γ-CD and Zn(II) are fixed at 20 and 10 m*M*, respectively. The resolution increased with increasing concentration of D-Phe, up to 10 m*M*. Thus, 10 m*M* D-Phe is optimal. Typical electropherograms of DU-6859 enantiomers are shown in Fig. 7.

As shown in Fig. 8, when DU-6859 enantiomers were resolved by the CE system with D-Phe, the eutomer (DU-6859) migrated more slowly than its distomer (DU-6858). Iversion of the migration order was observed when L-Phe was used. OFLX and DV-7751 enantiomers also showed the same behavior.

## 4. Discussion

The optical resolution of the NQs depended on the components of the running solution and their concentrations. No resolution was observed when one of the running solution components was omitted. The resolution mechanism is thought to be due to the formation of the diastereomeric ternary complex by

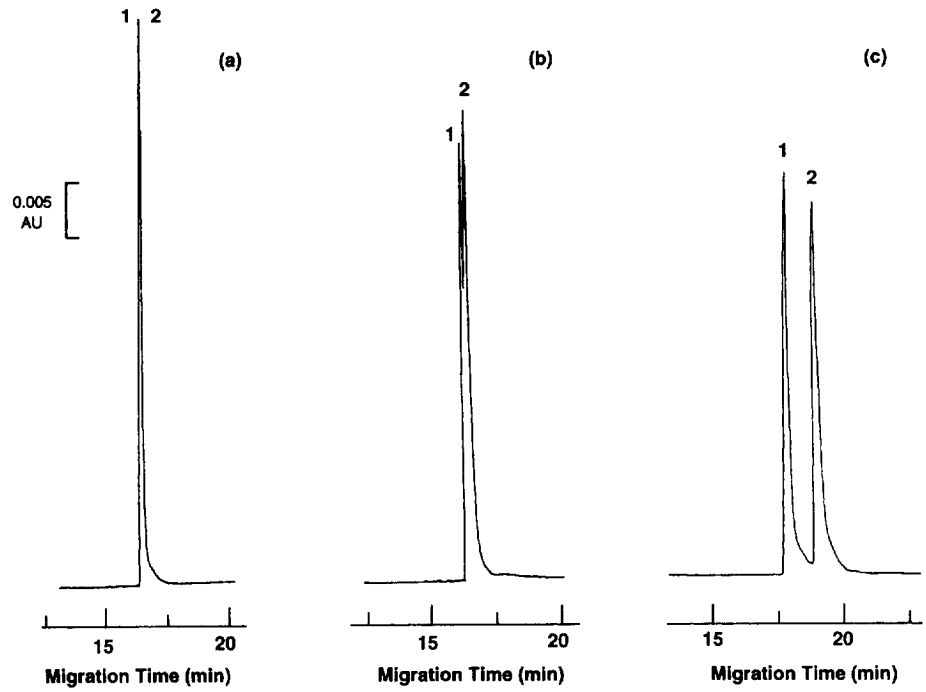


Fig. 3. Electropherograms of OFLX. Analytical conditions: running solution: (a) 0, (b) 5 and (c) 20 m*M* γ-CD, 10 m*M* $ZnSO_4$, 10 m*M* D-Phe–10 m*M* ammonium acetate (final pH = 6.5). Other conditions are as in Fig. 2. Peaks: 1 = *R*-(+)-OFLX; 2 = *S*-(−)-OFLX.

each of the enantiomers with Zn(II) and Phe (i.e., ligand-exchange interaction) in combination with the formation of an inclusion complex by each of the ternary complexes with γ-CD (i.e., host–guest interaction).

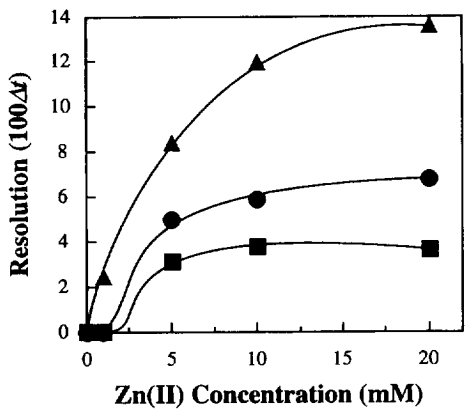


Fig. 4. Effect of Zn(II) concentration on the optical resolution of NQs. Analytical conditions: running solution: 0 to 20 m*M* $ZnSO_4$, 20 m*M* γ-CD, 10 m*M* D-Phe–10 m*M* ammonium acetate (final pH = 6.5). Other conditions and figure symbols are as in Fig. 2.

Since the NQs generally have a keto-carboxylic group in their structures, they have the ability to form complexes with various metal ions. Arai et al. [24] demonstrated the optical resolution of OFLX and some related substances by ligand-exchange HPLC with a Cu(II)–amino acid eluent system. They proposed a structure for the ternary complex of OFLX and the amino acid with Cu(II). In the present system, as well as the Cu(II)–amino acid system, the NQs form diastereomeric ternary complexes with D-Phe and Zn(II). Gassmann et al. [11] reported the optical resolution of dansylated (Dns) amino acids by CE using a Cu(II)–histidine running solution. They also demonstrated the optical resolution of Dns-amino acids using a Cu(II)–aspartame running solution [12]. Although the enantiomers of the NQs were expected to be resolved using the Zn(II)–D-Phe running solution, in accordance with their findings, resolution was not achieved. This suggests that a difference in stability exists between the ternary

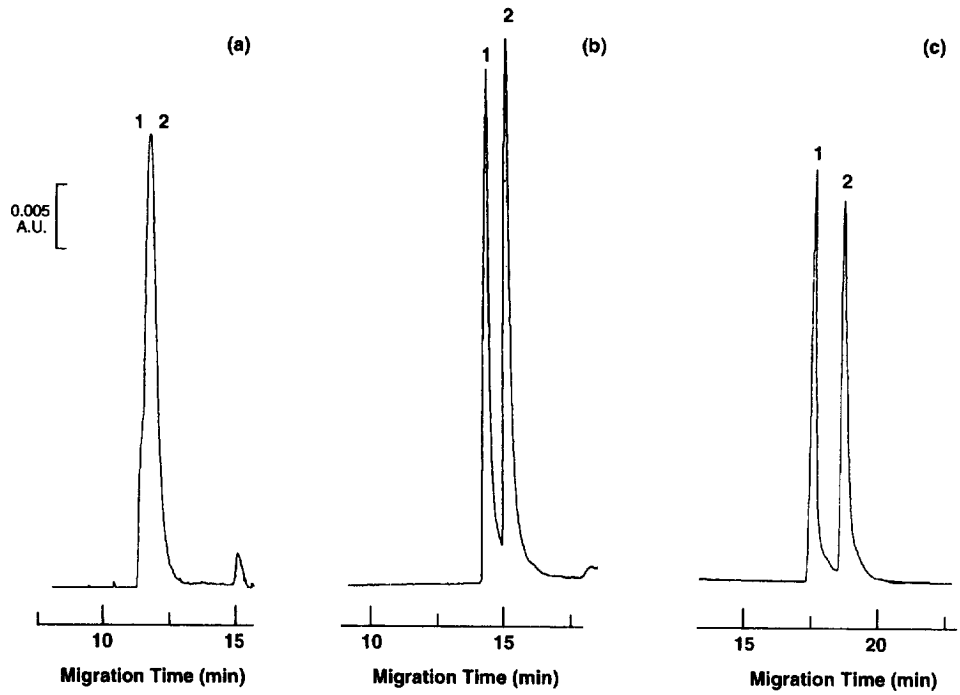


Fig. 5. Electropherograms of DV-7751 enantiomers. Analytical conditions: running solution: (a) 1, (b) 5 and (c) 10 m*M* $ZnSO_4$, 20 m*M* γ-CD, 10 m*M* D-Phe–10 m*M* ammonium acetate (final pH = 6.5). Other conditions are as in Fig. 2. Peaks: 1 = DX-9491; 2 = DV-7751.

complexes formed by each of the NQ enantiomers, which is difficult to achieve in the absence of γ-CD.

CDs are cyclic oligosaccharides consisting of α-(1,4)-linked D-glucopyranose units [31]. CDs, as well as crown ethers [34–37], are one of the well-known host molecules capable of forming an inclusion complex with a variety of substances, such as metal ions, positional, geometric, diastereomeric and/or optical isomers as the guest molecule, in their cavities [3–10,31–33]. Although we attempted to resolve the enantiomers of the NQs using each running solution containing CD (α, β, γ and dimethyl-β-CD solution), no optical resolution was observed. Optical resolution of the NQs were observed when using the Zn(II)–Phe running solution, only when γ-CD was present. The resolution depended on the size of the cavity and the concentration of the CD in the running solution. These suggest that γ-CD does not recognize the NQ enantiomers themselves, but diastereomeric ternary complexes of the NQs formed with metal(II) and amino acid.

The optical resolution of the NQs depended on the concentration of each of the components of the running solution, with an optimal concentration of each component being required for maximum resolution. Table 2 shows the $R_s$ values together with the $100 \times \Delta t$ values. Generally, in ligand-exchange HPLC [24,26–28] or CE [11–13], [metal] to [ligand] ratio is chosen as 0.5 in order to obtain adequate resolution. In the CE system, however, the best resolution was not necessarily achieved using a ratio of 0.5. The theoretical plate number of each of the faster-moving peaks, $Nt_1$, is also listed in Table 2. The $Nt_1$ gradually decreased with an increasing ratio of [Zn(II)] to [D-Phe].

Electroosmosis occurred towards the negative electrode under the experimental conditions. When methanol was used as a tracer for the electroosmosis in the 20 m*M* γ-CD–10 m*M* $ZnSO_4$–10 m*M* D-Phe solution, the electroosmotic flow, $\mu_{eo}$, was $1.62 \cdot 10^{-2}$ $mm^2$ $V^{-1}$ $s^{-1}$. The velocity of the NQs was faster than that of the electroosmosis. This suggest that the NQs, which are in zwitterionic form at pH

Table 1
Optical resolution ($100\Delta t$) of NQs with each D-amino acid

| | OFLX | DV 7751 enantiomers | DU-6859 enantiomers |
|---|---|---|---|
| *Aromatic* | | | |
| Phe | 3.74 | 5.86 | 12.0 |
| Tyr | 2.59 | 2.66 | 11.2 |
| *Hydrophilic* | | | |
| Ser | –[a] | – | 1.28 |
| Thr | – | – | 1.20 |
| Val | – | – | 1.15 |
| *Hydrophobic* | | | |
| Leu | – | – | not performed |
| Ile | – | – | 1.11 |
| Met | – | – | 1.01 |
| *Acidic* | | | |
| Asp | – | – | – |
| Glu | – | – | – |
| *Basic* | | | |
| Lys | – | – | – |
| Arg | – | – | 1.63 |
| *Imino acid* | | | |
| Pro | – | – | 1.07 |

Analytical conditions: running solution, 20 m*M* γ-CD, 10 m*M* $ZnSO_4$, 10 m*M* D-amino acid–10 m*M* ammonium acetate (final pH = 6.5). Other conditions are as in Fig. 2.
[a] No resolution.

6.5 and are almost at their p*I* values, form positively charged complexes in the running solution.

Inversion of the migration order of the enantiomers was observed by changing the chirality of the ligand in the present system. On investigating the optical resolution of the NQs by ligand-exchange HPLC with a Cu(II)–D- or L-Phe eluent, we also confirmed that the elution order of the enantiomers in the HPLC has similar tendencies in that in the present system: The order was distomer<eutomer, with D-Phe, and eutomer<distomer with L-Phe. In ligand-exchange HPLC, the elution order of enantiomers is basically dependent on both the stability of the ternary complexes and their retention on the stationary phase. As stated above, however, the differences in stability between the ternary complexes formed by each of the NQ enantiomers is difficult to determine in the metal(II)–amino acid solution. Therefore, it is considered that the elution order of NQ enantiomers in ligand-exchange HPLC depends largely on the retention of the ternary complexes on the stationary phase. The more stable complex is eluted last because of its stronger retention on the stationary phase, compared with that of the less stable complex. Changing the chirality of the ligand causes the NQs to form other diastereomeric ternary complexes, leading to a reversal in the elution order of the enantiomers. In contrast, the migration order of the NQ enantiomers in the present system is thought to depend on the stability of the inclusion complexes formed between the ternary complexes and γ-CD. Changing the chirality of the ligand changes the stability of the inclusion complexes by causing the formation of other diastereomeric ternary complexes, which leads to a reversal in the order of migration of the enantiomers. Each optical resolution ($100\Delta t$) of the NQs, with D- or L-Phe as a ligand, is listed in Table 3. The optical resolution by D-Phe was better than that by L-Phe. This is probably due to the chiral structure of γ-CD, the host molecule. In the present system, the ligand chirality is also responsible for determining the degree of optical resolution.

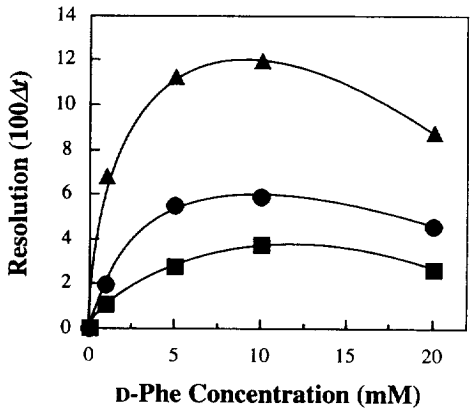


Fig. 6. Effect of D-Phe concentration on the optical resolution of NQs. Analytical conditions: running solution, 0 to 20 m*M* D-Phe, 20 m*M* γ-CD, 10 m*M* $ZnSO_4$–10 m*M* ammonium acetate (final pH = 6.5). Other conditions and figure symbols are as in Fig. 2.

The optical resolution of DU-6859 enantiomers is better than that of OFLX or DV-7751 enantiomers over a series of studies. DU-6859 enantiomers have a large group, such as a chiral monofluorocyclopropyl group, on their structure. In contrast, OFLX and DV-7751 enantiomers have a methyl group on their asymmetrical carbons, which are located on the oxazine ring. The former structures are relatively

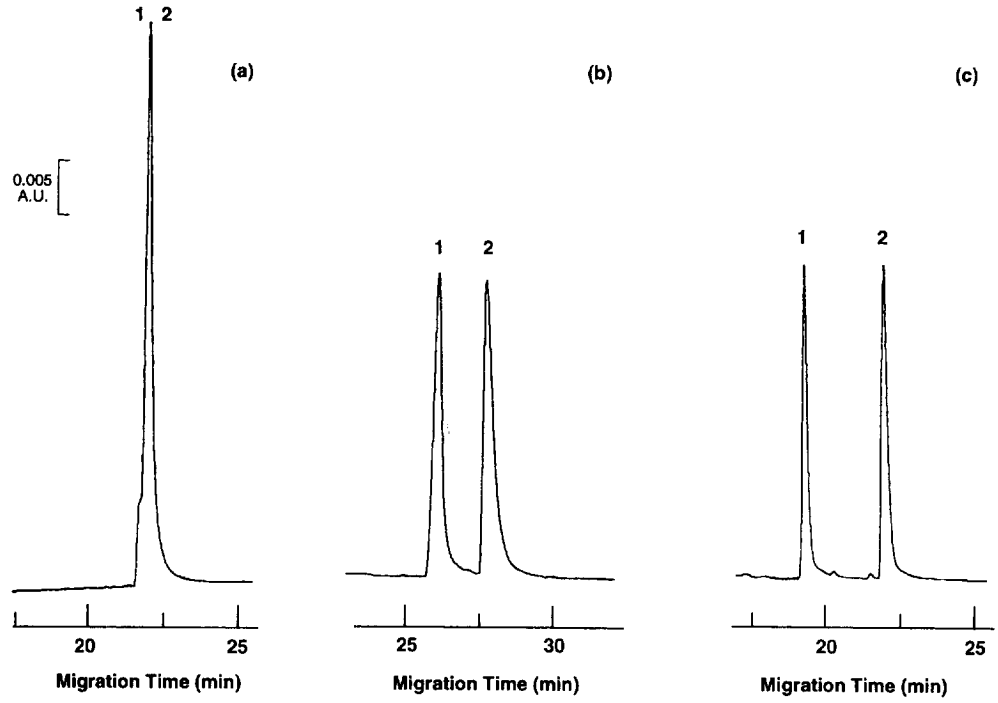


Fig. 7. Electropherograms of DU-6859 enantiomers. Analytical conditions: running solution: (a) 0, (b) 1 and (c) 10 m*M* D-Phe, 20 m*M* γ-CD, 10 m*M* $ZnSO_4$–10 m*M* ammonium acetate (final pH = 6.5). Other conditions are as in Fig. 2. Peaks: 1 = DU-6858; 2 = DU-6859.

bulky compared with the latter structures. Therefore, γ-CD effectively recognizes the structural differences of the ternary complexes formed by the bulkier NQs, which leads to the better optical resolution of the DU-6859 enantiomers. While comparing the optical resolution of OFLX with that of DV-7751 enantiomers, the latter is better than the former. This may be due to a steric effect at the $C_{10}$ position.

## 5. Conclusion

We developed an optical resolution system for NQs by CE using a γ-CD–Zn(II)–Phe running solution. The present investigation leads to the following conclusions.

1. The resolution depends on the components of the running solution and their concentrations. No resolution is observed in the absence of one of the components of the running solution.
2. The resolution depends on the size of the cavity of CD: The resolution is only observed using the γ-CD running solution.
3. Apart from using a Zn(II) running solution, resolution is also observed using a Cu(II)- or Ni(II) running solution.
4. Aromatic amino acids such as Phe or Tyr are the most effective ligands for this resolution system.
5. Reversal of the migration order of the enantiomers is observed by changing the chirality of the ligand.
6. The resolution is considered to proceed as follows. (1) Formation of the diastereomeric ternary complex by each of the enantiomers with Zn(II) and Phe (ligand-exchange interaction) and (2) formation of the inclusion complex by each of the ternary complexes with γ-CD (host–guest interaction).

The mixed resolution mode may be useful for substances other than the NQs. In the future, it is expected that a test to determine the purity of NQs

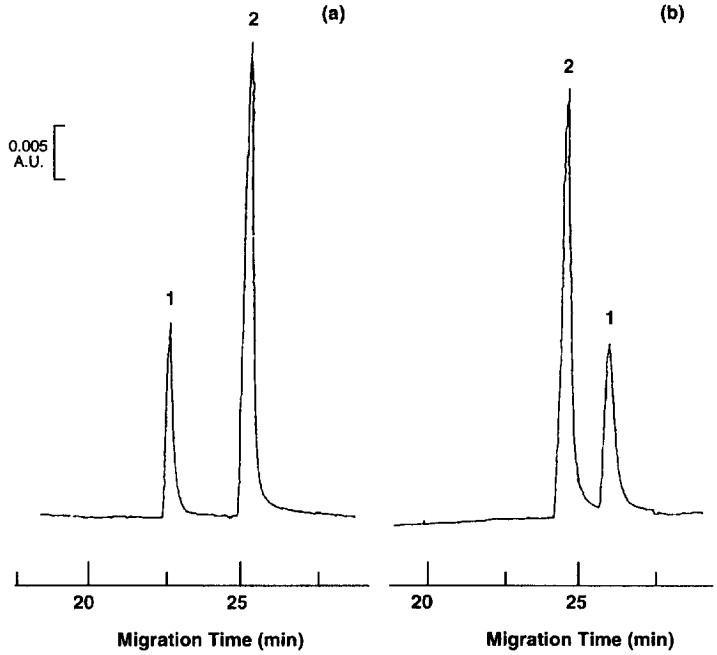


Fig. 8. Electropherograms of the optical resolution of DU-6859 enantiomers with (a) D-Phe and (b) L-Phe as the ligand. Analytical conditions: running solution: 20 m*M* γ-CD, 10 m*M* $ZnSO_4$, 10 m*M* Phe–10 m*M* ammonium acetate (final pH = 6.5), sample: 0.5 mg/ml DU-6859–0.5 mg/ml DU-6858 (7:3, v/v). Other conditions are as in Fig. 2. Peak identifications are as in Fig. 7.

Table 2
Effects of [D-Phe]–[Zn(II)] on the optical resolution ($100\Delta t$ and $R_s$) and theoretical plate number ($Nt_1$) of NQs

| | [D-Phe]–[Zn(II)] | $100\Delta t$ | $R_s$ | $Nt_1$ |
|---|---|---|---|---|
| OFLX | 5:10 | 2.75 | 1.46 | 56 000 |
| | 10:10 | 3.74 | 2.10 | 42 000 |
| | 20:10 | 2.58 | 1.08 | 37 000 |
| DV-7751 | 5:10 | 5.47 | 1.99 | 21 000 |
| enantiomers | 10:10 | 5.86 | 2.15 | 24 000 |
| | 20:10 | 4.54 | 1.25 | 14 000 |
| DU-6859 | 5:10 | 11.2 | 6.33 | 58 000 |
| enantiomers | 10:10 | 12.0 | 6.19 | 50 000 |
| | 20:10 | 8.74 | 4.74 | 36 000 |

Analytical conditions are as in Fig. 6.

Table 3
Optical resolution ($100\Delta t$) of NQs with each phenylalanine

| | OFLX | DV-7751 enantiomers | DU-6859 enantiomers |
|---|---|---|---|
| D-Phe | 3.74 | 5.86 | 12.0 |
| L-Phe | 2.08 | 3.45 | 6.06 |

Analytical conditions: running solution: 20 m*M* γ-CD, 10 m*M* $ZnSO_4$, 10 m*M* Phe–10 m*M* ammonium acetate (final pH = 6.5). Other conditions are as in Fig. 2.

will be available and that a pharmacokinetic study will be carried out.

## References

[1] G. Blaschke, H.P. Kraft, K. Fickentscher and F. Köhler, Arzneim-Forsch., 29 (1979) 1640.
[2] S. Hara, K. Koga and K. Shudo (Editors), Molecular Chirality, Kagakudojin, Kyoto, 1993.
[3] A. Guttman, A. Paulus, A. Cohen, N. Grinberg and B.L. Karger, J. Chromatogr., 448 (1988) 41.
[4] H. Nishi, T. Fukuyama and S. Terabe, J. Chromatogr., 553 (1991) 503.
[5] J. Snopek, H. Soini, M. Novotny, E.S. Keulemansova and I. Jelinek, J Chromatogr., 559 (1991) 215.
[6] E. Francotte, S. Cherkaoui and M. Faupel, Chirality, 5 (1993) 516.
[7] C. Quang and G. Khaledi, Anal. Chem., 65 (1993) 3354.
[8] M. Heuermann and G. Blaschke, J. Chromatogr., 648 (1993) 267.
[9] H. Nishi, Y. Kokusenya, T. Miyamoto and T. Sato, J. Chromatogr. A, 659 (1994) 449.
[10] A. Sano, K. Watanabe and H. Nakamura, Anal. Sci., 11 (1995) 667.

[11] E. Gassmann, J.E. Kuo and R.N. Zare, Science, 230 (1985) 813.
[12] P. Gozel, E. Gassmann, H. Michelsen and R.N. Zare, Anal. Chem., 59 (1987) 44.
[13] C. Desiderio, Z. Aturki and S. Fanali, Electrophoresis, 15 (1994) 864.
[14] R. Kuhn, C. Steinmetz, T. Bereuter, P. Haas and F. Erni, J. Chromatogr. A, 666 (1994) 367.
[15] A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, Anal. Chem., 61 (1989) 1984.
[16] S. Terabe, M. Shibata and Y. Miyashita, J. Chromatogr., 480 (1989) 403.
[17] K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, J. Chromatogr., 559 (1991) 209.
[18] D.C. Tickle, G.N. Okafo, P. Camilleri, R.F.D. Jones and A.J. Kirby, Anal. Chem., 66 (1994) 4121.
[19] H. Nishi, K. Nakamura, H. Nakai, T. Sato and S. Terabe, Electrophoresis, 15 (1994) 1335.
[20] S. Busch, J.C. Kraak and H. Poppe, Anal. Chem., 64 (1992) 3024.
[21] L. Valtcheva, J. Mohammad, G. Pettersson and S. Hierten, J. Chromatogr., 638 (1993) 263.
[22] T. Arai, M. Ichinose, H. Kuroda, N. Nimura and T. Kinoshita, Anal. Biochem., 217 (1994) 7.
[23] Y. Ishihama, Y. Oda, N. Asakawa, Y. Yoshida and T. Sato, J. Chromatogr. A, 666 (1994) 193.
[24] T. Arai, H. Koike, K. Hirata and H. Oizumi, J. Chromatogr., 448 (1988) 439.
[25] V.A. Davankov and Yu.A. Zolotarev, J. Chromatogr., 155 (1978) 285.
[26] S. Lam, F. Chow and A. Karmen, J. Chromatogr., 199 (1980) 295.
[27] S. Lam and A. Karmen, J. Chromatogr., 239 (1980) 451.
[28] N. Nimura, A. Toyama, Y. Kasahara and T. Kinoshita, J. Chromatogr., 239 (1982) 671.
[29] J. LePage, W. Lindner, G. Davis, D. Seitz and B.L. Karger, Anal. Chem., 51 (1979) 433.
[30] W. Linder, J. LePage, G. Davis, D. Seitz and B.L. Karger, J. Chromatogr., 185 (1979) 323.
[31] M.L. Bender and M. Komiyama, Cyclodextrin Chemistry, Springer-Verlag, New York, 1978.
[32] Y. Matsui, T. Kurita, M. Yagi, T. Okayama, K. Mochida and Y. Date, Bull. Chem. Soc. Japan, 48 (1975) 2187.
[33] K. Fujimura, S. Suzuki, K. Hayashi and S. Masuda, Anal. Chem., 62 (1990) 2198.
[34] L.R. Sousa, D.H. Hoffman, L. Kaplan and D.J. Cram, J. Am. Chem. Soc., 96 (1974) 7100.
[35] G. Dotsevi, Y. Sogah and D.J. Cram, J. Am. Chem. Soc., 98 (1976) 3038.
[36] E.P. Kyba, J.M. Timko, L.J. Kaplan, F. Jong, G.W. Gokel and D.J. Cram, J. Am. Chem. Soc., 100 (1978) 4555.
[37] L.R. Sousa, G.D.Y. Sogah, D.H. Hoffman and D.J. Cram, J. Am. Chem. Soc., 100 (1978) 4569.