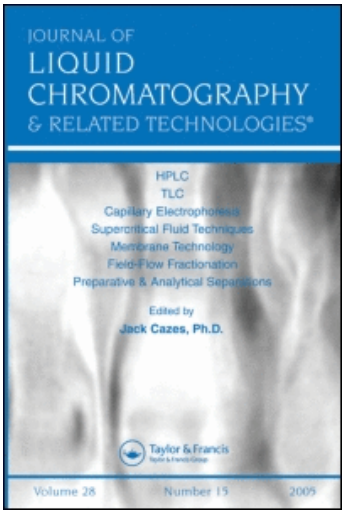

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Preparative Chiral Chromatography of *trans*-Stilbene Oxide Using Cellulose *tris*(Phenylcarbamate), Chiralcel® OC, as Stationary Phase

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ABSTRACT

Two approaches were studied to improve the productivity of preparative chiral separation. For a model case, *trans*-stilbene oxide as a racemate and cellulose *tris*(phenylcarbamate), "CHIRALCEL® OC," as a stationary phase were chosen. The productivity per unit CSP was improved as much as 52% by connecting two different sizes of columns (10 cm ID × 50 cm L and 5 cm ID × 50 cm L) in series. Further improvement may be possible by adjusting the eluent temperature several degrees lower than the jacket temperature of the columns. Preparative separation conditions regarding composition of mobile phase, separation temperature, loading amount of

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racemic substance, positions of fractions to be taken, and so on were also studied.

Key Words: Resolution; Chiral separation; High performance liquid chromatography; HPLC; Chiral stationary phase; Coating.

INTRODUCTION

It is essential to examine bioactivity of an enantiomer at the screening stage, where some quantity of such a highly optical pure enantiomer is required, especially for research and development of pharmaceuticals and agrochemicals.[1] The technical issues to be considered in a preparative separation using a single column will be: (1) preparation of columns; (2) optimization of preparative conditions; (3) automatic operation; (4) total feasibility study; (5) security of safety, etc. Among them, the selection of chiral stationary phase in relation with the above item 1, must be the most important issue from viewpoint of easy availability and chemical stability, as well as high chiral discrimination ability. Polysaccharide derivatives coated on silica-gel, in particular cellulose and amylose derivatives as chiral stationary phase, are most suitable since they have all of the abovementioned characteristics.[2–4] There is a comprehensive review of Francotte regarding preparative chiral chromatography.[5] However, optimal preparative conditions for a particular sample need to be investigated individually, and there are a couple of points which were not discussed in the review, such as making a gap between eluent temperature and jacket temperature of a column and connecting two columns, whose sizes are different. The purpose of this paper is to discuss these two interesting techniques, as well as the regular check points for a preparative separation to improve the productivity per unit CSP and solvent, using *trans*-stilbene oxide and cellulose *tris*(phenylcarbamate), "CHIRALCEL® OC" as a model case. We assumed that the price of the racemate is very cheap (in other words, productivity is more important than the recovery rate) and only the first eluted enantiomer is needed in this model.

EXPERIMENTAL

Chemicals

Hexane (HPLC grade) was purchased from Nakalai Tesque, Kyoto or Wako Pure Chemical, Osaka, 2-propanol from Nakalai Tesque. *Trans*-stilbene oxide in racemic form was purchased from Aldrich, Milwaukee and purified by recrystallization.

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Instrumentation

The automated preparative system, Model 590 (GL Sciences, Tokyo), as shown in Fig. 1 consisted of the following: main pump 4APUS-200 (maximum flow rate of 200 mL/min), sample pump PUS-60 (maximum flow rate of 60 mL/min), injection valve, UV detector Model 502T (230 nm) and fraction collector, and the system was controlled by a computer, NEC 9801. Column temperature was controlled by circulating thermo-controlled water in the column jacket. Two columns, whose sizes are 10 cm in internal diameter and 50 cm in length, and 5 cm in internal diameter and 50 cm in length, were involved in this study; either one 10 cm ID column or a pair of two columns in series was used. Both columns were packed with cellulose *tris*(phenylcarbamate), CHIRALCEL® OC, which is available from Daicel Chemical Industries, Ltd., Tokyo. The analytical and semi-preparative columns were also available from Daicel. The particle size of the stationary phase was 20 μm and the operation temperature was ambient, unless otherwise specified.

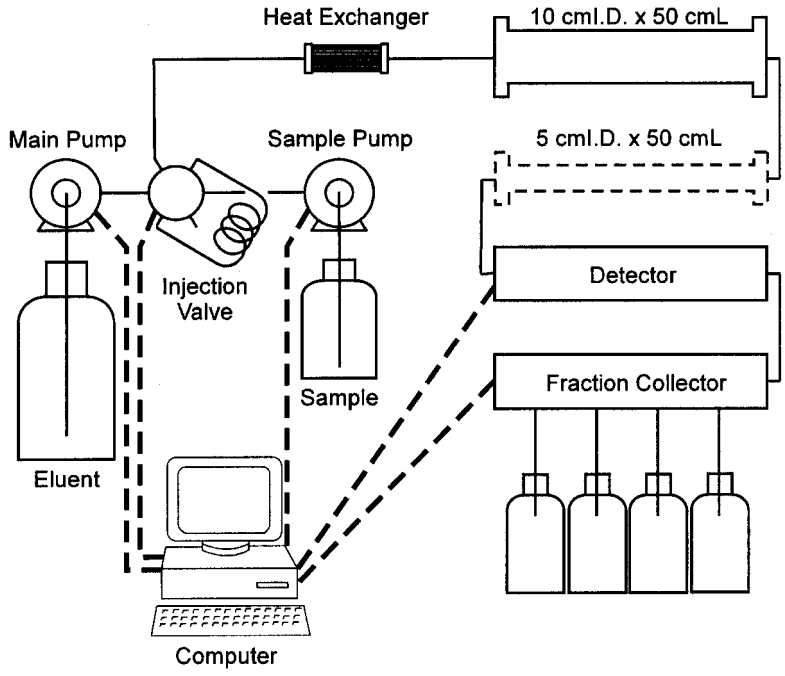

Figure 1. Flow diagram of preparative system.

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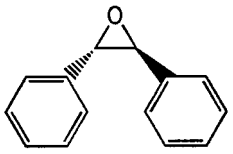
RESULTS AND DISCUSSION

Selection of Column as Stationary Phase

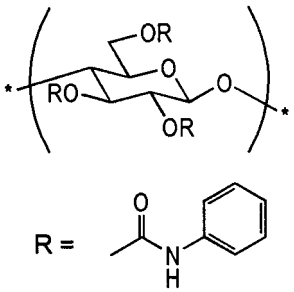
Optical separations of *trans*-stilbene oxide on cellulose-based chiral stationary phases have been reported.[2–4] Among them, CHIRALCEL® OC stationary phase was selected for the above-mentioned reasons, namely, easy availability, chemical stability, and high chiral discrimination ability, as well as low production cost for this preparative separation study. The structure of the solute and the chiral selector are shown in Fig. 2.

Comparison Between Analytical Column and Preparative Column

For comparison, two chromatograms of *trans*-stilbene oxide obtained with an analytical column (0.46 cm in diameter, 25 cm in length) packed with 10 μm particles of cellulose *tris*(phenylcarbamate), and a preparative column (10 cm ID × 50 cm L) packed with 20 μm particles were shown in Fig. 3, using the



Solute: *trans*-stilbene oxide (racemic)



Chiral selector: cellulose *tris*(phenylcarbamate)

Figure 2. Structures of solute and chiral selector.

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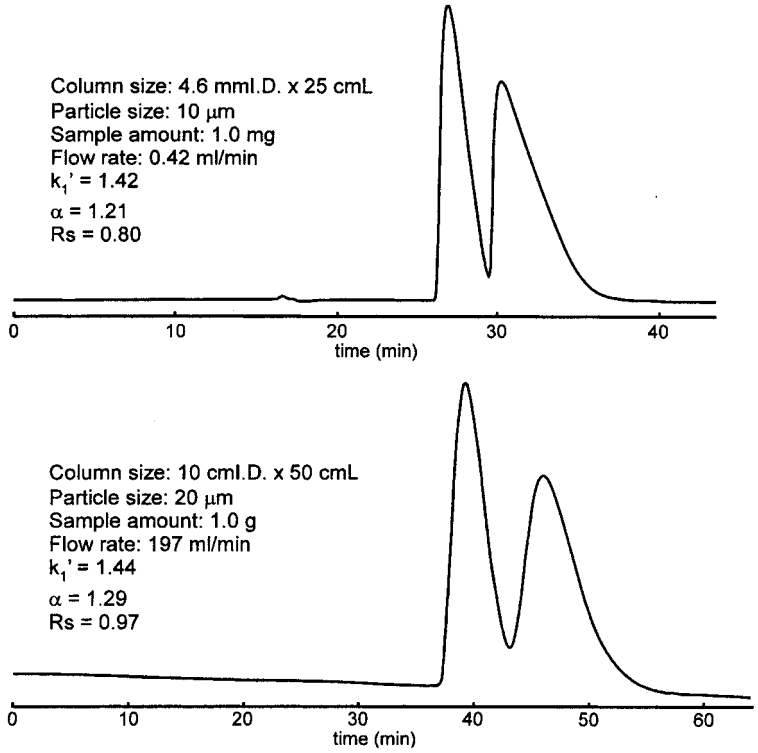

Figure 3. Comparison between analytical and preparative columns.

mixture of 90% of hexane and 10% of 2-propanol as eluent. These chromatograms were not optimized, but they were good enough to demonstrate their difference under a proportional condition. Almost the same capacity factor (k_1'), separation factor (α), and resolution (Rs) were observed on both the analytical size and the preparative size columns, when the same linear velocities of eluent, 3.3 cm/min, were used and the same loading amounts of *trans*-stilbene oxide per unit volume of the columns were injected. This result suggests that the preparative scale chromatogram can easily be predicted from the analytical scale chromatogram in the case of CHIRALCEL® OC.

Effect of Mobile Phases on Separation

Composition of mobile phase was optimized by using an analytical column with an overloaded condition. As described in the introduction, we

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assumed that only the first eluted enantiomer is needed in this model study. Therefore, it is not necessary to achieve a baseline separation as long as the first eluted enantiomer can be collected in high enantiomeric purity and reasonable recovery rate. As the ratio of 2-propanol to *n*-hexane is going down, the α value got lower by reducing the amount of 2-propanol, while the distance of the top of the peaks got wider and the valley between the peaks got deeper, which was preferred for the preparative purpose, as shown in Fig. 4.

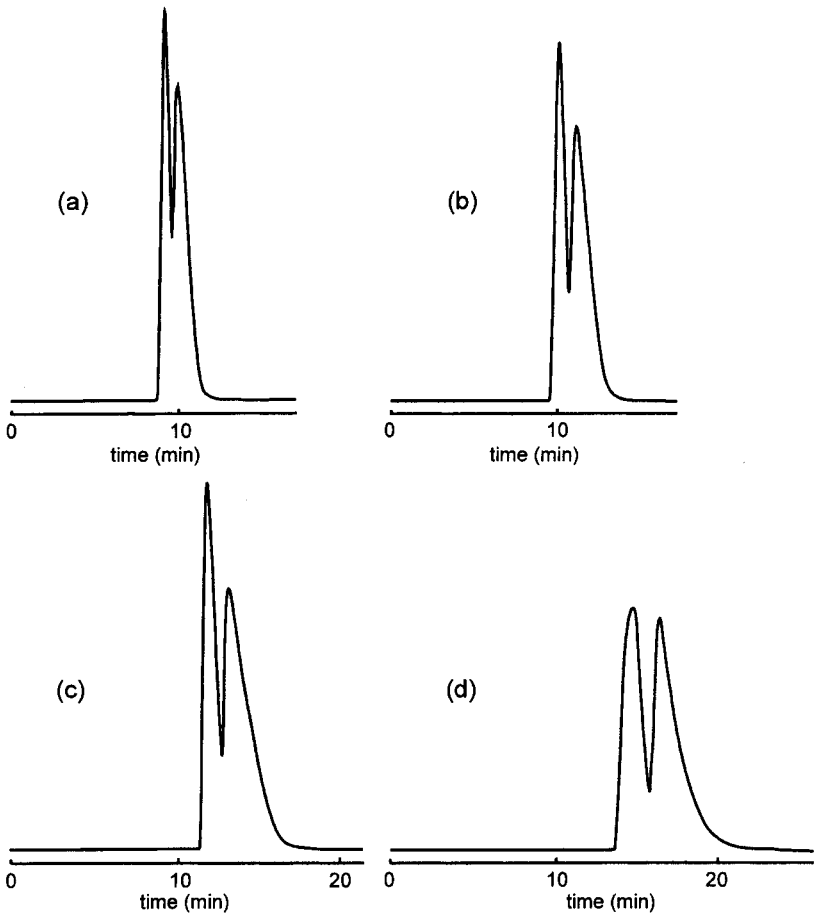

Figure 4. Effect of mobile phase composition. Column size: 0.46 cm ID × 25 cm L, particle size: 10 μm; sample amount: 2.0 mg; flow rate: 1.0 mL/min; temperature: r.t.; eluent: (a) hexane/2-propanol = 90/10; (b) hexane/2-propanol = 95/5; (c) hexane/2-propanol = 98/2; and (d) hexane/2-propanol = 99/1.

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On the other hand, the time between appearance and disappearance of the peaks is also one of the important factors affecting productivity of the enantiomer in the batchwise preparative separation. In the case of the ratio of 99% of *n*-hexane to 1% of 2-propanol, the peaks become considerably broader compared with those obtained with an eluent containing more 2-propanol, probably due to the considerably increased interaction of the analyte with the stationary phase. Consequently, this solvent ratio would not be suitable for the preparative separation of this sample.

Effect of the Operation Temperature on Separation

The operating temperature often affects chiral recognition ability more than a change of composition of the mobile phase as shown in Fig. 5. In general, the peak becomes sharper as the operation temperature gets higher, while the separation factor decreases. Since resolution of the peaks depend on both the plate number and the separation factor, it is difficult to predict which is better for optimum resolution, higher temperature or lower temperature. In the case of the separation of *trans*-stilbene oxide on CHIRALCEL® OC, higher temperature gave better separation between 5°C and 35°C. Moreover, not only the column temperature, but also the eluent temperature influences the separation, as Welsch et al. reported.[6] Therefore, the influence of the eluent temperature is investigated by holding the jacket temperature at 31.6°C with a 10 cm ID × 50 cm L column, as shown in Fig. 6. The eluent temperature was measured right before the column inlet by using a thermocouple. The left axis shows a resolution parameter, min/max, as indicated in Fig. 7, instead of the ordinary resolution parameter "Rs," because "Rs" is sometimes unable to be calculated under preparative conditions. The right axis shows the time between the rise of the first peak and the end of the second peak. The highest productivity can be achieved with the highest resolution parameter and the shortest injection interval. Judging from Fig. 6, the best eluent temperature is around 28.5°C, which is lower by about three degrees than the jacket temperature.

It is well known that moving speed of a solute at the center of a column is faster than that of near the wall.[7] On the other hand, the retention times become shorter when the column temperature gets higher, as shown in Fig. 5. Therefore, the moving speed of the solute would be the slowest in the center and the fastest near the wall if the eluent temperature is lower than the jacket temperature. It seems that this temperature difference, three degrees in this case, compensated the wall effect and resulted in the best separation. These results basically agree with Welsch's report, though the best temperature difference is much smaller than 14°, which was reported in their report by

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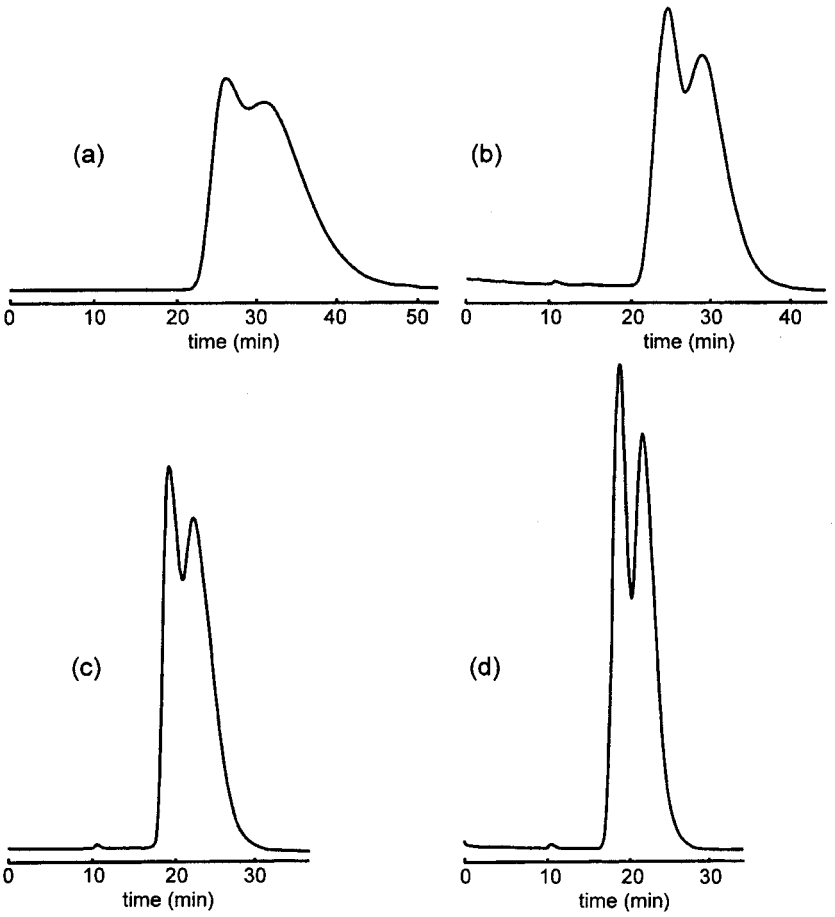

Figure 5. Effect of column temperature. Column size: 2 cm ID × 25 cm L; particle size: 20 μm; sample amount: 40 mg; flow rate: 6 mL/min; eluent: hexane/2-propanol = 98/2; temperature: (a) 5°C; (b) 15°C; (c) 25°C; and (d) 35°C.

using 2.2 cm ID × 25 cm L column.[6] Probably, this is mainly due to the difference of the heat capacity of the column hardware.

Effect of Sample Loading on Separation

Increasing the loading tends to make the value of min/max increased, and to make the time of the peak appearance gradually faster, as shown in Fig. 8.

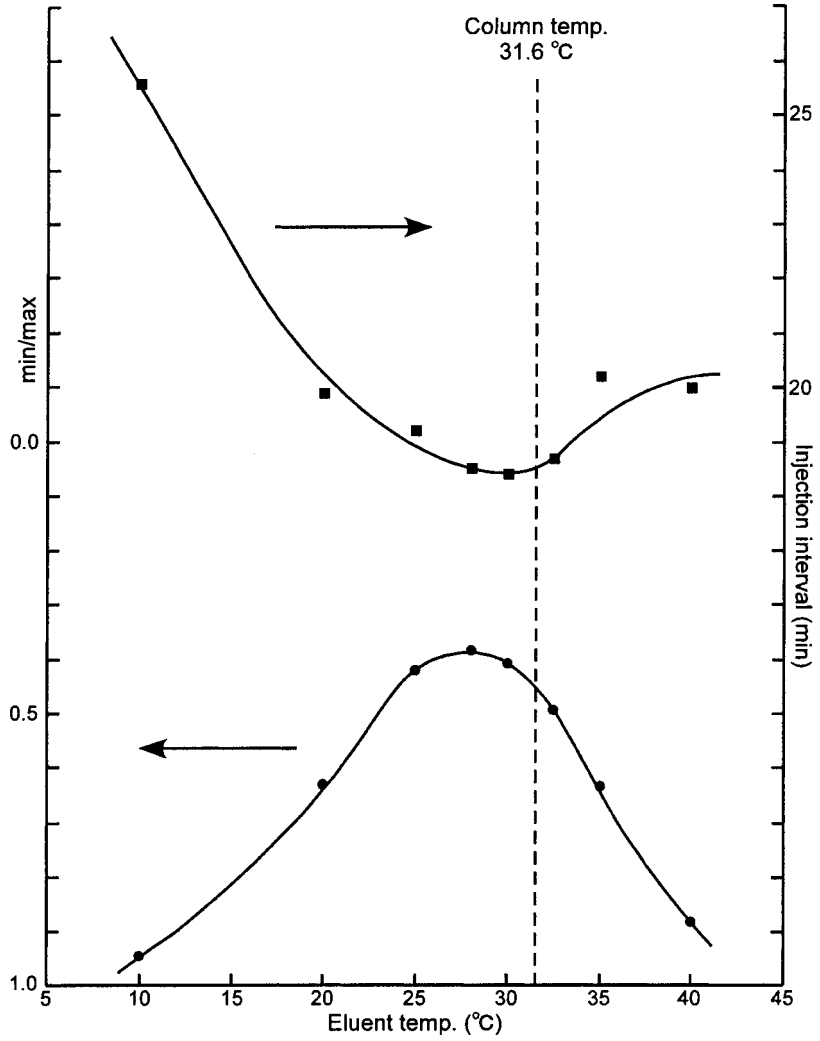

Figure 6. Relationship between column and eluent temperature. Column size: 10 cm ID × 50 cm L; eluent: hexane/2-propanol = 98/2; flow rate: 200 mL/min; sample amount: 4.0 g/inj.

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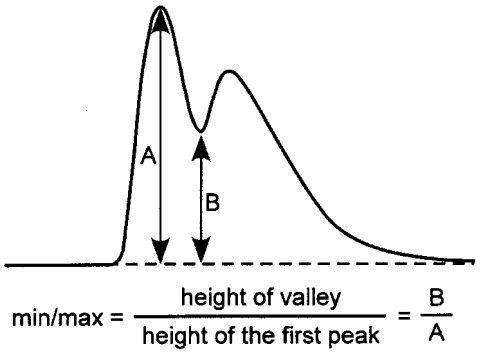

Figure 7. Resolution parameter for preparative column.

For a preparative separation, from our experiences, the tentative parameter of min/max will be around 0.5 when only the first elute enantiomer is collected.

Preparative Separation

Taking all the above-mentioned results into consideration, the preparative optical separation was conducted using the system explained in the Instrumentation section, as shown in Fig. 1. The loading amount of *trans*-stilbene oxide at one injection was 4.0 g dissolved in a mixed solvent of 98% of *n*-hexane and 2% of 2-propanol, and the flow rate was 200 mL/min. The enantiomeric purities measured by the analytical column for the fractions are shown in Fig. 9. As a result, the total enantiomeric purity of the fractions up to the third was 100% e.e. and the combined yield was 36.9%. This means about 74% (36.9 divided by 50.0) of (+)-form was recovered with 100% of optical purity. Furthermore, the total yield up to the 5th fraction reached 41.8% (corresponding to about 84% of total (+)-form), keeping 98.8% of the total enantiomeric purity. Supposing one collection cycle needs the time from the rise to the set of the two peaks, 67 injections (4.0 g/injection) per 24 hours will be possible and 98.9 g of (+)-form should be obtained at >99.9% e.e.

Further experiments were conducted as shown in Fig. 10 connecting the two columns in series to improve the productivity per unit CSP and solvent. It is rather usual to use a longer and/or wider column, or connecting the same size of the columns to increase the loading amount per injection. Instead, we connected two different sized columns, that is, 10 cm ID × 50 cm L and 5 cm

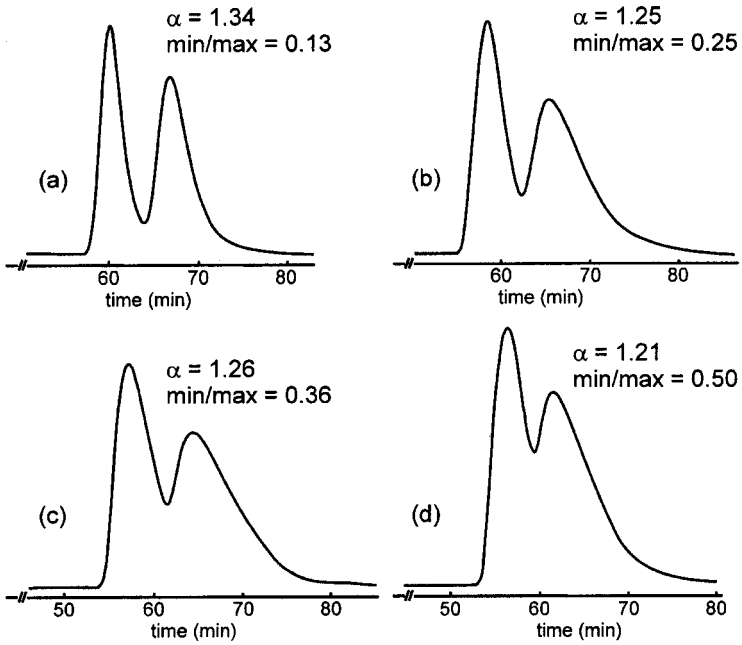

Figure 8. Effect of sample loading. Column size: 5 cm ID × 50 cm L; particle size: 20 μm; flow rate: 30 mL/min; eluent: hexane/2-propanol = 98/2, temperature: r.t.; sample amount: (a) 0.06 g; (b) 0.4 g; (c) 0.7 g; and (d) 1.0 g.

ID × 50 cm L columns in this order. We thought the solute was so diffused as not to saturate the narrower column when it reaches the second column. In addition, it is not necessary to sacrifice too much retention time, because the linear velocity of the narrower column is four times faster than the first column. As a result, the loading amount can be increased from 4.0 to 8.25 g per injection, which is more than a two-fold increase, by adding 25% of the stationary phase to serve as the additional narrower column without reducing the resolution. The flow rate needs to be decreased from 200 to 170 mL/min, but not to exceed the pressure limit of the narrower column, as shown in Fig. 10. Although, the retention time and the injection interval become longer because of the lower flow rate and the longer column length in total, it is still possible to obtain 163 g of the enantiomer (36.5% as the total yield) with 99.9% e.e. of the total enantiomeric purity within 24 hours, which is 65% higher than the result from the single column, and the productivity per kg—stationary phase is increased by 52%.

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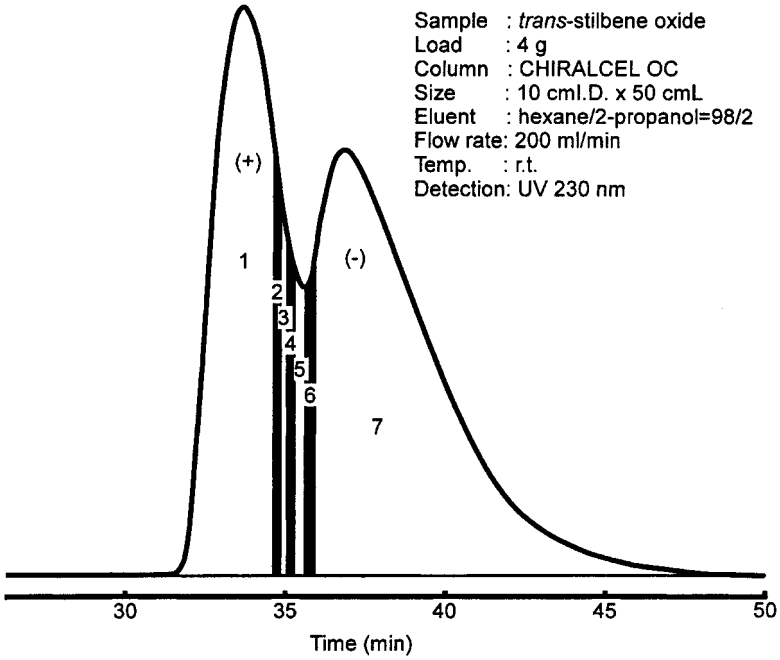

Fraction	Enantiomer ratio (+) / (-)	Total yield (%)	Total enantiomeric purity (% e.e.)
1	100 / 0	↓	↓
2	100 / 0		
3	100 / 0	36.9	100 (+)
4	98.4 / 1.6	39.5	99.8 (+)
5	91.1 / 8.9	41.8	98.8 (+)
6	73.4 / 26.6		
7	12.1 / 87.9		

Figure 9. Fractionating point, optical purity, and yield.

It is also worth saying that a chiral separation is normally a binary separation and it is very effective to make overlapping injections to achieve the maximum productivity. Needless to say, it is essential to pre-purify the racemate as chemically clean as possible for the overlapping injections, because the injection interval becomes longer if there are any peaks of impurities before or after the peaks of the enantiomers.

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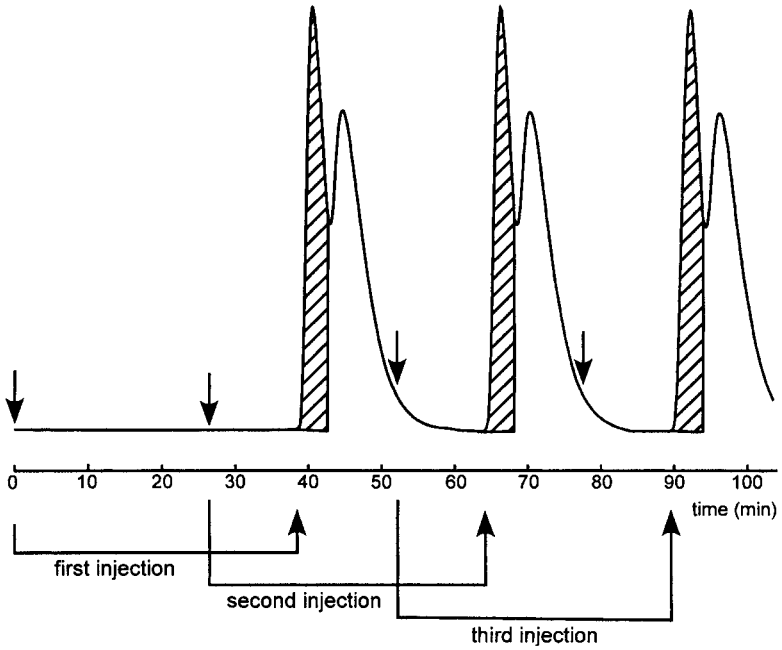

Figure 10. Preparative separation in continuous mode. Column size: 10 cm ID × 50 cm L + 5 cm ID × 50 cm L; eluent: hexane/2-propanol = 98/2; flow rate: 170 mL/min; sample amount: 8.25 g/inj.; injection cycle: 55 times/day; processed racemate: 454 g/day; collected enantiomer: 166 g/day; enantiomeric yield: 73.0%; enantiomeric purity: 99.9%.

CONCLUSION

In the preparative separation, it is important to optimize the chromatographic conditions, considering the balance between optical purity, yield, and time required. A chiral stationary phase needs to be carefully chosen from the viewpoint of separation ability, chemical resistance, durability, etc., because it has the greatest influence on the productivity. On the other hand, several experiments will be necessary to determine the proper preparative conditions, such as, effects of composition of mobile phase, flow rate of eluent, temperature, loading amount per injection, position to be fractionated, etc. We have conducted a model study using *trans*-stilbene oxide and CHIRALCEL® OC, and found that the productivity per given amount of stationary phase can be increased by connecting two columns of different dimensions in series. It may be possible to improve this productivity further by optimizing the eluent

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temperature as we described in the section of temperature effect. Unfortunately, the 5 cm ID column, which we have used this time, was not equipped with a column jacket and we could not confirm this point. The combination approach will be tried in our future study.

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