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Investigation of cross-linking chiral stationary phases within capillary columns

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ABSTRACT

Four diamide chiral stationary phases (CSPs), undecenoyl-L-valine-(*S*)-α-phenylethylamide (CSP-1), OV-225–L-valine-*tert.*-butylamide (CSP-2), XE-60–L-valine-*tert.*-butylamide (CSP-3) and polycyanoethyl vinyl siloxane 25% cyanoethyl–L-valine-*tert.*-butylamide, were cross-linked within both glass and fused-silica capillary columns with dibenzoyl peroxide (DBP) as the cross-linking reagent. The effects of the CSP skeleton on the degree of cross-linking and racemization (decrease in enantiomeric excess) of the stationary phase during cross-linking were investigated. The performance of the cross-linked columns in terms of enantioselectivity, thermal stability, acid–base characteristics and column efficiency were determined and compared. The results show that the CSP skeleton may have considerable effects on both cross-linking and racemization.

INTRODUCTION

Since the first paper on the direct separation of amino acid enantiomers by gas chromatography (GC) [1], the development of novel chiral stationary phases has attracted wide attention. In GC, the direct separation of enantiomers is more efficient, sensitive, accurate and faster than indirect separation. The performance of direct GC separation depends to a large extent on the performance of the CSPs or the chiral columns used. Usually cross-linked capillary columns have more stable films and can even be used in supercritical fluid chromatography (SFC) [2,3]. Great efforts have been made in preparing cross-linked chiral capillary columns in recent years. Several methods for the immobilization of CSPs within capillary columns have been suggested [4–7], but in the cross-linking of diamide CSPs racemization effects have been observed [6].

It was the purpose of this work to investigate the effects of CSP skeletons and cross-linking conditions on the performance of cross-linked chiral capillary columns in terms of the degree of cross-linking, decrease in enantiomeric excess (e.e.), etc.

EXPERIMENTAL

Chemicals and materials

CSP-1 was synthesized in our laboratory [8]. CSP-2, CSP-3 and CSP-4 were

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synthesized according to Frank *et al.* [9] and Saeed *et al.* [10] with some modifications [11]. OV-225, XE-60 and SE-54 were obtained from Chrompack, PEG 20M from Fluka, amino acids from Sigma, polycyanoethyl vinyl siloxane from Jiling Chemical Industry Co. and blank fused-silica capillary columns from Yongnian Optical Fibre Manufacturer.

Preparation of columns

Unless stated otherwise, the columns were statically coated with a 1% (w/v) dichloromethane solution of the stationary phase and a 6% (w/w, based on the stationary phase) solution of DBP. After static coating, the columns were conditioned under a carrier gas flow. Cross-linking was carried out with temperature programming from 40 to 190°C, the final temperature being maintained at 190°C for 2 h. The columns were then rinsed successively with pentane, dichloromethane and water. All the columns were tested in a GC RIA gas chromatograph equipped with a split injector and a flame ionization detector, before and after rinsing.

Determination of racemization

In order to determine the racemization of the stationary phase during cross-linking, the cross-linked columns were filled with 6 *M* hydrochloric acid, both ends were sealed and the stationary phases in the columns were hydrolysed at 110°C for 20 h [6]. After cooling the hydrolysates were collected, dried, derivatized and then analysed on a cross-linked CSP-4 column. Racemization was expressed as the decrease in e.e.:

$$D(\text{e.e}) = 1 - [A(\text{L}) - A(\text{D})]/[A(\text{L}) + A(\text{D})] = [2\,A(\text{D})]/[A(\text{L}) + A(\text{D})] \quad (1)$$

where D (e.e.) is the decrease in the enantiomeric excess and A(L) and A(D) are the peak areas of L- and D-Val, respectively, in the hydrolysates.

Derivatization

Amino acids were derivatized as N(O,S)-trifluoroacetyl isopropyl esters according to McKenzie and Tenaschuk [12].

RESULTS AND DISCUSSION

The structures and basic performances of the CSPs are listed in Table I.

TABLE I

STRUCTURES AND BASIC PERFORMANCES OF THE CHIRAL STATIONARY PHASES

CSP	Structure	M.p. (°C)	$[\alpha]_D^{20}$ in CH_2Cl_2	Maximum temperature limit (°C)
CSP-1	Undecenoyl–L-Val-(*S*)-α-NHCH(CH_3)C_6H_5	101–103	−36.0	180
CSP-2	OV-225–L-Val-NHC(CH_3)$_3$		− 7.3	190
CSP-3	XE-60–L-Val-NHC(CH_3)$_3$		−10.7	180
CSP-4	Polycyanoethyl vinyl siloxane–L-Val-NHC(CH_3)$_3$		− 8.8	180

TABLE II

EFFECTS OF DBP/CSP RATIO ON CROSS-LINKING AND RACEMIZATION

CSP	DBP (%)											
	1		2		6		12		20		100	
	d (%)[a]	*e* (%)[b]	*d* (%)	*e* (%)	*d* (%)	*e* (%)	*d* (%)	*e* (%)	*d* (%)	*e* (%)	*d* (%)	*e* (%)
CSP-2			40	20	65	26			62	30	60	38
CSP-3									12	3	23	7
CSP-4	39	2	43	2	68	2	60	4			55	10

[a] Degree of cross-linking (%).

[b] Decrease in e.e. (%) of the stationary phase.

CSP-1 is a stationary phase with vinyl groups, but its molecular weight is low (386) and it can only be co-cross-linked with polymeric molecules such as PEG 20M or SE-54 to obtain sufficient cross-linking. However, after adding polymeric molecules, the enantioselectivity of the CSP was decreased and the polarity changed [13].

The effects of the DBP/CSP ratio on the degree of cross-linking (%) and racemization of CSP-2, CSP-3 and CSP-4 are listed in Table II.

CSP-2 is a stationary phase with a polycyanopropyl siloxane skeleton (OV-225). A degree of cross-linking of 65% can be obtained, but with pronounced racemization (decrese in e.e. = 26%) (Table II).

CSP-3 and CSP-4 are stationary phases with a polycyanoethyl skeleton. Although the degree of cross-linking of CSP-3 is not satisfactory, the decrease in e.e. is only 7% even with 100% (w/w based on CSP-3) of DBP. CSP-4 also contains

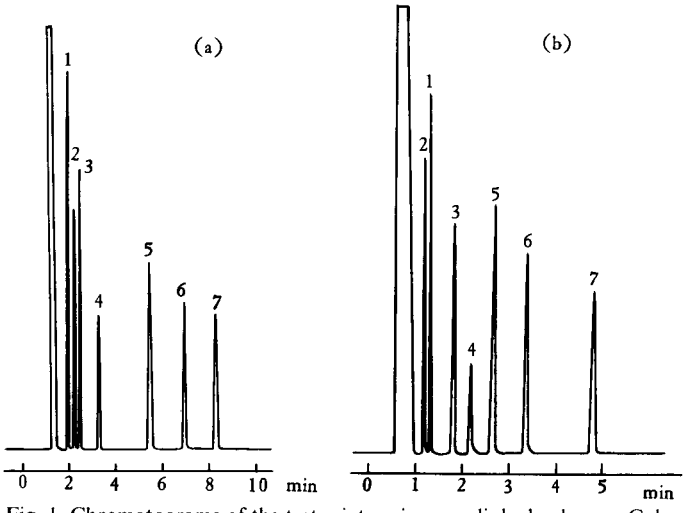

Fig. 1. Chromatograms of the test mixture in cross-linked columns. Columns (20 m × 0.25 mm I.D.): (a) cross-linked CSP-2 fused silica; (b) cross-linked CSP-4 fused silica. Temperature, 130°C; carrier gas, hydrogen. Peaks: 1 = dodecane; 2 = 2-octanone; 3 = tridecane; 4 = 1-octanol; 5 = naphthalene; 6 = 2,6-dimethylaniline; 7 = 2,6-dimethylphenol.

TABLE III

BASIC PERFORMANCE OF THE CROSS-LINKED COLUMNS

Stationary phase	d (%)[a]	e (%)[b]	CE (%)[c]	Acid/base[d]	Maximum temperature limit (°C)
CSP-1 + PEG 20M	50		75	1.02	190
CSP-1 + SE-54	50		75	1.04	190
CSP-2	65	26	80	1.02	210
CSP-3	12	3	55	1.01	
CSP-4	68	2	85	1.02	210

[a] Degree of cross-linking (%).
[b] Decrease in e.e. (%) of the stationary phase.
[c] Coating efficiency; sample, alanine; temperature, 120°C.
[d] Ratio of peak area per unit weight of 2,6-dimethylphenol to 2,6-dimethylaniline at 140°C.

vinylgroups, and gives good results with respect to both degree of cross-linking (68%) and racemization (decrease in e.e. = 2%) in comparison with the other CSPs studied. For CSP-2 and CSP-4, 6% DBP is optimum in preparing the cross-linked columns; a higher proportion did not increase the cross-linking but increased the racemization (Table II).

Fig. 1 shows the chromatograms of a mixture of apolar, polar, acidic and basic compounds with known composition by the cross-linked columns. The peaks in the chromatograms are almost symmetric.

TABLE IV

α-VALUES OF AMINO ACID ENANTIOMERS ON THE CROSS-LINKED COLUMNS

Amino acid	CSP-1 + PEG 20M		CSP-2		CSP-4	
	α	T (°C)	α	T (°C)	α	T (°C)
Ala	1.030	100	1.089	100	1.133	100
Val	1.042	100	1.069	100	1.096	100
Thr	1.026	100	1.048	110	1.088	100
a-Ile	1.046	100	1.053	120	1.114	100
Ile	1.044	100	1.047	120	1.100	100
Leu	1.050	100	1.107	100	1.187	100
Pro					1.013	100
Ser	1.020	100	1.034	130	1.062	120
Asp	1.009	130	1.026	130	1.034	130
Met	1.031	130	1.039	150	1.079	140
Phe	1.030	130	1.033	150	1.067	140
Glu	1.023	130	1.034	150	1.069	140
Orn			1.029	190	1.037	190
Tyr			1.023	190	1.028	190
Lys			1.026	190	1.033	190
Trp			1.021	190	1.028	190
n-Val	1.038	110	1.078	120	1.148	100
n-Leu	1.038	110	1.066	120	1.154	100

TABLE V

THERMAL STABILITY OF WALL-COATED AND CROSS-LINKED CSP-4 COLUMNS

Sample: alanine. Test temperature: 130°C.

Wall-coated			Cross-linked		
Temperature (°C)	Time (h)	α	Temperature (°C)	Time (h)	α
190	4	1.087	190	2	1.085
190	14	1.087	200	12	1.083
190	24	1.086	200	22	1.081
190	44	1.082	200	42	1.080

The basic performances of the cross-linked capillary columns are listed in Table III. It can be seen that CSP-4 gives the best results with respect to both degree of cross-linking and racemization among the four CSPs studied.

The separation factors (α-values) of amino acid enantiomers on the cross-linked columns are listed in Table IV. Table V shows the thermal stablity of both wall-coated and cross-linked CSP-4 columns. The cross-linked column can be used up to at least 200°C without noticeable bleeding and racemization.

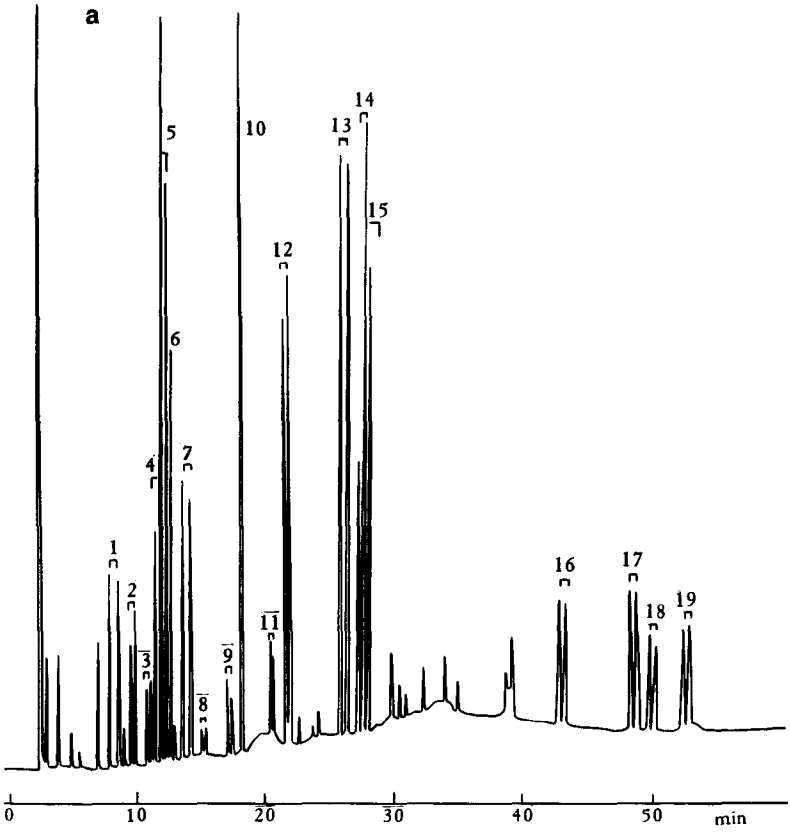

Fig. 2.

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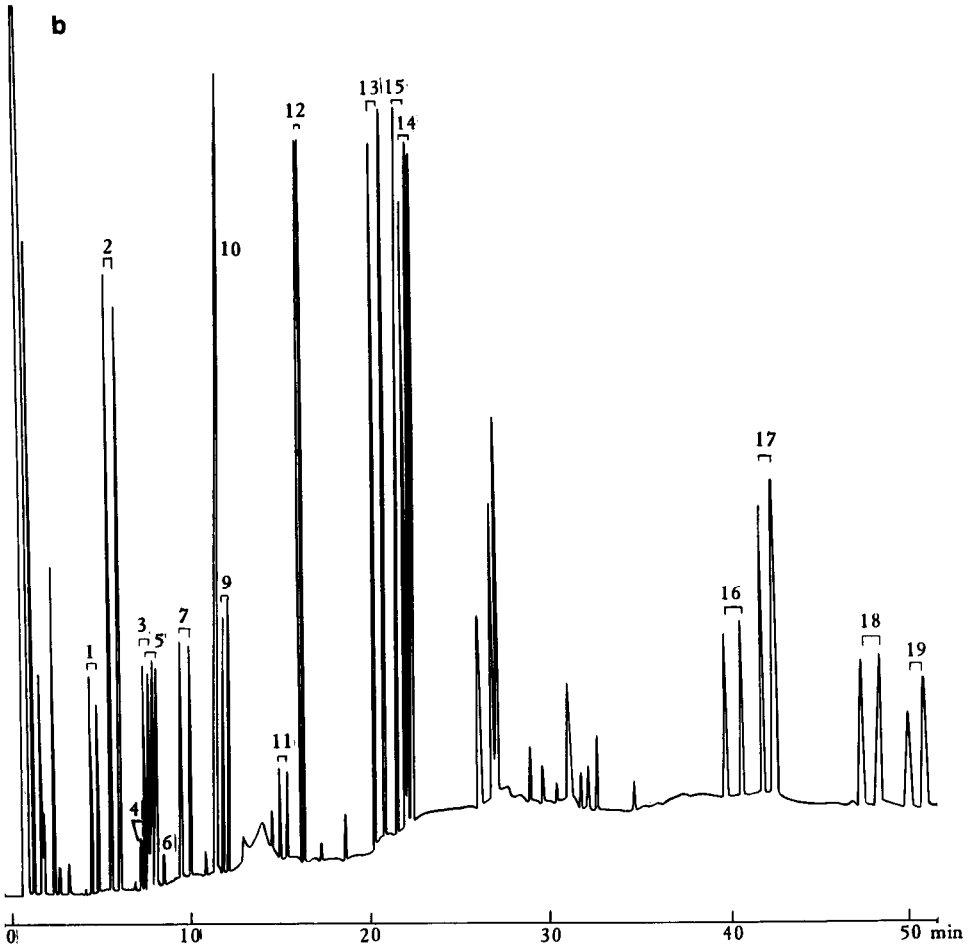

Fig. 2. Enantiomeric separation of amino acids by GC in cross-linked columns. Columns (20 m × 0.25 mm I.D.): (a) cross-linked CSP-2 fused silica; (b) cross-linked CSP-4 fused silica. Temperature, 100°C (6 min), programmed at 4°C/min to 190°C; carrier gas, hydrogen. Peaks: 1 = Ala; 2 = Val; 3 = Thr; 4 = a-Ile; 5 = Ile; 6 = Gly; 7 = Leu; 8 = *n*-Leu; 9 = Ser; 10 = Pro; 11 = Cys; 12 = Asp; 13 = Met; 14 = Glu; 15 = Phe; 16 = Orn; 17 = Tyr; 18 = Lys; 19 = Trp (D-enantiomers eluted first).

TABLE VI

REPRODUCIBILITY OF CROSS-LINKED CSP-4 COLUMNS

Column	α^a	k'(L)^a	d (%)	e (%)	CE (%)^a	Acid/base^b
1	1.133	4.59	68	2	85	1.02
2	1.134	4.64	69	2	87	1.02
3	1.133	4.65	69	2	82	1.02
4	1.133	4.79	71	2	80	1.03

[a] α, k'(L) (capacity factor of L-alanine) and CE were tested at 100°C. Sample: alanine.
[b] See Table III.

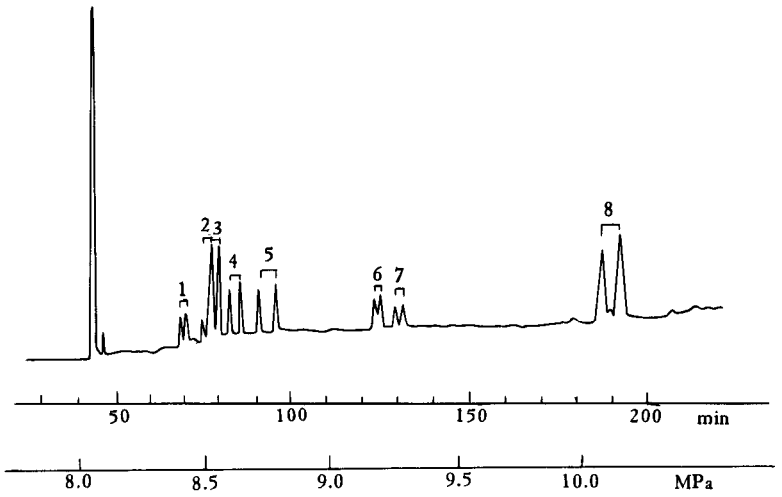

Fig. 3. Enantiomeric separation of amino acids by SFC in a cross-linked CSP-2 column. Column, 20 m × 0.10 mm I.D., glass; mobile phase, carbon dioxide, 8.0 MPa (60 min) increased at 0.05 MPa/min; column temperature, 60°C; detector, flame ionization (250°C). Peaks: 1 = Val; 2 = a-Ile; 3 = Ile; 4 = *n*-Val; 5 = Leu; 6 = Ser; 7 = Asp; 8 = Met (D-enantiomers eluted first).

Good reproducibility of the preparation of the cross-linked chiral capillary columns was obtained. Table VI gives the data for four cross-linked CSP-4 columns with 6% DBP.

Fig. 2 shows chromatograms for the separation of amino acid enantiomers with the cross-linked columns. Fig. 3 shows the chromatogram of some amino acid enantiomers obtained with a cross-linked CSP-2 column using SFC.

CONCLUSION

The CSP skeleton may have a large effect on both the degree of cross-linking and racemization with DBP as the cross-linking reagent. CSP-4 has both a polycyanoethyl siloxane skeleton and vinyl groups and gives the best results in terms of degree of cross-linking and enantioselectivity (α-values) among the four CSPs studied.

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REFERENCES

1 E. Gil-Av, B. Feibush and R. Charles-Sigler, *Tetrahedron Lett.*, (1966) 1009.
2 K. Grob and G. Grob, *J. Chromatogr.*, 213 (1981) 211.
3 J. Bradshaw, S. K. Aggarwal, C. A. Rouse, B. J. Tarbet, K. E. Markides and M. L. Lee, *J. Chromatogr.*, 405 (1987) 169.

4 W. Roder, F.-J. Ruffing, G. Schomburg and W. H. Pirkle, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 655.
5 P. Macaudière, M. Caude, R. Rosset and A. Tambuté, *J. Chromatogr. Sci.*, 27 (1989) 383.
6 G. Lai, G. Nicholson and E. Bayer, *Chromatographia*, 26 (1988) 229.
7 G. Schomburg, I. Benecke and G. Severin, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 391.
8 L. Zhou, Z. Zhang and X. Lou, in *Proceedings of the 3rd China–Japan Joint Symposium on Analytical Chemistry, Hefei, China, 1988*, Academia Sinica and the Japan Society for Analytical Chemistry, p. 231.
9 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr. Sci.*, 15 (1977) 174.
10 T. Saeed, P. Sandra and M. Verzele, *J. Chromatogr.*, 186 (1979) 611.
11 S. Zhang, G. Wang, T. Zhang and L. Zhou, *Huaxie Sheji*, 7 (1985) 197.
12 S. L. McKenzie and D. Tenaschuk, *J. Chromatogr.*, 173 (1979) 53.
13 X. Lou and L. Zhou, in *Proceedings of the 3rd Chinese National Symposium on Capillary Chromatography, Lanzhou, China, 1988*, Chinese Chemical Society, p. 42.