Journal of Chromatography, 479 (1989) 377–386
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 683

USE OF ACHIRAL ION-PAIRING REAGENTS WITH CHIRAL STATIONARY PHASES

WILLIAM H. PIRKLE*, JEN-PING CHANG[a] and JOHN A. BURKE, III
School of Chemical Sciences, University of Illinois, Urbana, IL 61801 (U.S.A.)
(First received April 6th, 1989; revised manuscript received June 13th, 1989)

SUMMARY

The influence of the structure and concentration of achiral ion-pairing additives upon the retention and enantioselectivity of N-3,5-dinitrobenzoyl derivatives of α-amino acids and 2-aminophosphonic acids on a commercial (*R*)-N-(2-naphthyl)-alanine-derived chiral stationary phase has been examined. A mobile phase of methanol–aqueous phosphate buffer was used and the effect of methanol concentration was studied.

Increasing the concentration of the alkyltrimethylammonium ion-paring reagent enhances retention and enantioselectivity of N-3,5-dinitrobenzoyl derivatives of both α-aminocarboxylic acids and 2-aminophosphonic acids. An increase in the chain length of the alkyl portion of the ion-pairing reagent gives increased retention but no significant change in selectivity for the enantiomers. The concentration of the organic modifier in the mobile phase dramatically affects the retention of the enantiomers. A minimum in enantioselectivity occurs at about 60% methanol.

INTRODUCTION

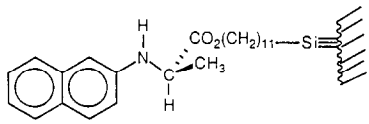
In most of the papers from this laboratory describing the development of bonded chiral stationary phases (CSPs) for the direct chromatographic separation of enantiomers[1–5], standard mobile phases of hexane and 2-propanol are used to facilitate the comparison of the various CSPs. These mobile phases usually afford near-maximum enantioselectivity but are not necessarily optimal for any given application, for other mobile phases can significantly affect band shapes, resolution factors, and peak dispersions. Reversed phase solvents can also be used although they almost always afford less enantioselectivity than does hexane-2-propanol and may, in the case of ionic analytes, lead to undesirably rapid elution. Addition of lipophilic ion pairing reagents to reverse mobile phases can increase the retention of ionic analytes and alter selectivity as is well known[6]. Pettersson and Schill[7] have used chiral ion-pairing agents in conjunction with achiral columns to separate enantiomers. Schill *et*

[a] On sabbatical leave from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 20031, China.

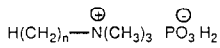
0021-9673/89/$03.50

al.[8] as well as Hermansson and Eriksson[9] have also studied the use of α-glycoprotein chiral stationary phases with aqueous mobile phases and indicate that charged and uncharged additives affect retention and, in many cases, afford significant improvement of chiral recognition of analyte enantiomers. The reason for the enhancement of chiral recognition is stated to be one enantiomer of the ion-pair "fits better in the chiral active site of the protein due to steric reasons"[9]. Owing to the complexity of the protein, this may or may not be an adequate explanation.

This paper reports the reversed-phase chromatographic behavior of enantiomers of the N-3,5-dinitrobenzoyl (DNB) derivatives of both α-amino acids and 2-aminophosphonic acids on a commercial (*R*)-N-(2-naphthyl)alanine-derived chiral stationary phase, **1**, using a mobile phase of methanol–aqueous phosphate buffer in conjunction with achiral ion-pairing agents of general type **2**. The CSP is well characterized structurally and mechanistically[10,11]



(R)-1



2 n = 5, 6, 7, 8, 12

EXPERIMENTAL

Apparatus

Chromatography at ambient temperature was performed using an Altex-100 A pump, an Altex 210 injector valve with a 20-μl loop, a Beckman Model 155 fixed-wavelength UV detector operating at 254 nm, and a Kipp & Zonen BD 41 chart recorder. A Regis (*R*)-N-(2-naphthyl)-alanine 250 × 4.6 mm I.D. column was used.

Chemicals

The ion-pair reagents: pentyl, hexyl, heptyl, octyl and dodecyl trimethylammonium phosphate were supplied by Regis. Analytes used in this study were available from prior studies. Methanol was purchased from Fischer. All other chemicals were reagent grade and used without further purification.

RESULTS AND DISCUSSION

From an initial investigation of the separation of the enantiomers of the DNB derivatives of 2-aminohexanoic acid and 2-aminobutylphosphonic acid using a mobile phase of methanol–0.01 *M* aqueous phosphate buffer (pH 6.86), is was found that the undesirably small capacity factors (k') of the analytes can be increased by addition of lipophilic quaternary ammonium ions to the mobile phase, Fig. 1. This was not

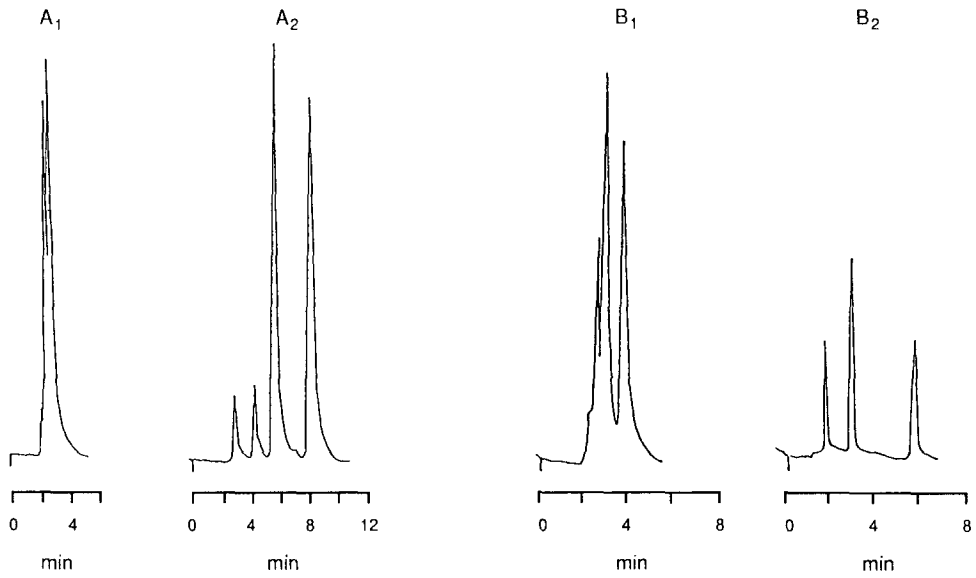
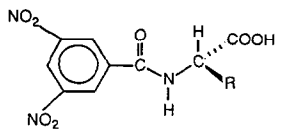
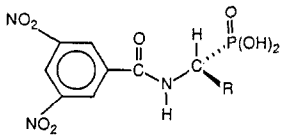


Fig. 1. Separations of the enantiomers of (A) N-(3,5-dinitrobenzoyl)-2-aminobutylphosphonic acid using methanol–0.01 *M* aqueous phosphate buffer (60:40, v/v) pH 6.86 and (B) N-(3,5)-dinitrobenzoyl)-2-amino-hexanoic acid using methanol–0.01 *M* aqueous phosphate buffer pH 6.86 (70:30, v/v). In chromatograms A_1 and B_1, no ion-pairing agent was present. In A_2 and B_2, 5 m*M* ion-pairing agent had been added to the mobile phase. These agents were hexyltrimethylammonium phosphate and heptyltrimethylammonium phosphate respectively. The flow-rate was 1 ml/min in each case.

unexpected, for the eleven carbon connecting arm used to link the chiral selector to the silica affords an appreciable lipophilic character to this CSP. Thus, retention occurs by both adsorption and partitioning processes. To better understand the lipophilic effects which contribute to retention, the relationships between retention, selectivity, structure and concentration of the pairing ions were investigated. From Fig. 2, it may be seen that an increase in the concentration of the dodecyltrimethylammonium reagent from 0.25 to 5 m*M* causes a significant increase in the capacity factors of both enantiomers of the DNB derivatives of leucine and 2-aminopentanoic acid until a limiting value is reached. More significantly, the separation factor, α, for both sets of enantiomers increases as the concentration of the ion-paring reagent is increased (Fig. 3). For the enantiomers of DNB-leucine, α is increased from 2.0 to 2.9 by the addition of 8 m*M* ion-pairing reagent. For all cases, the elution order of the analytes is that expected from prior studies of the chiral recognition mechanism used by this CSP[10]. For DNB α-amino acids of the type employed, **3**, the elution order from (*R*)-**1** is (*S*) before (*R*). For DNB-2-aminoalkyl phosphonic acids of type **4**, the order is (*R*) before (*S*) owing to the Cahn–Ingold–Prelog priority sequence. The presumption is that both types of analytes are resolved by the general mechanism previously described[10].



3



4

The process by which the ion-pairing reagent increases retention is readily understood from prior work. The ion-pairing reagent interacts reversibly with the stationary phase to provide surface charge and with the analyte enantiomers to afford enantiomeric ion pairs. Both the so-called "ion pair" and "dynamic ion exchange" retention processes are presumably in operation[6]. Consequently, the curves which relate retention and enantioselectivity to the concentration of a given ion-pairing reagent are complex, for they are ordained by the values of the various equilibrium constants. Interestingly, plots of α *vs.* ion-pairing reagent concentration do not "flatten out" as do the plots of k' *vs.* reagent concentration. Both plots might have been expected to show similar curvature if the plots reflect only the extent of the pair formation in solution. Increasing the concentration of a given ion-pairing reagent will increase the surface charge of the stationary phase until the latter becomes saturated. Surface charge facilitates the retention of both enantiomers equally and does not itself contribute to chiral recognition. The retention component contributed by surface charge augments that afforded by the chiral recognition process, thus influencing the

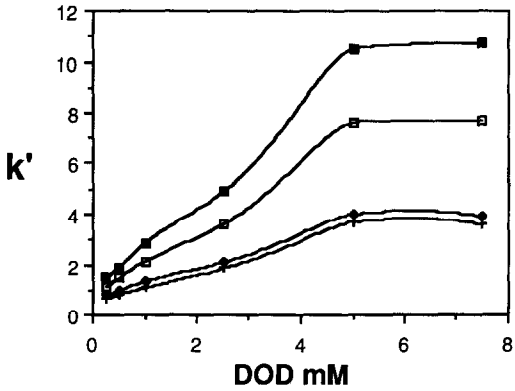


Fig. 2. Influence of the concentration of dodecyltrimethylammonium phosphate, DOD, in the methanol–aqueous phosphate buffer pH 6.86 (80:20, v/v) upon the retention of the enantiomers of the N-3,5-dinitrobenzoyl derivatives of leucine and 2-aminopentanoic acid. (+) (*S*)-DNB-2-aminopentanoic acid; (□) (*R*)-DNB-2-aminopentanoic acid; (◆) (*S*)-DNB-leucine; (■) (*R*)-DNB-leucine. The flow-rate was 1 ml/min. The curves for the two (*S*)-enantiomers are essentially coincident. The lower curve (+) has been displaced slightly for clarity.

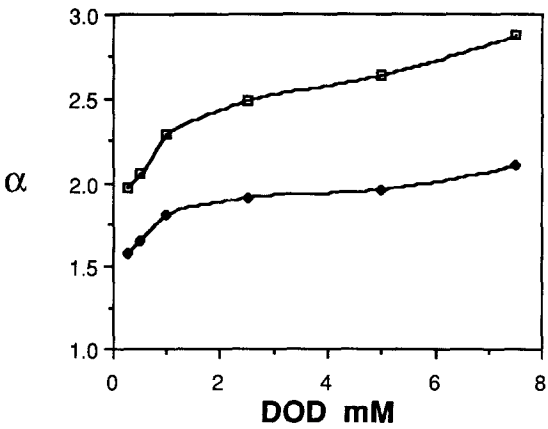


Fig. 3. Influence of the concentration of dodecyltrimethylammonium phosphate, DOD, in the mobile phase upon the separation factors for the enantiomers of DNB-leucine (□) and DNB-2-aminopentanoic acid (◆). Mobile phase and flow-rate were as in Fig. 2.

shapes of the plots of either k' or α *versus* ion-pairing reagent concentration. Additionally, one must consider the possibility that the ion-pairing reagent, by interacting with residual silanol groups on the silica[12], reduces the extent of non-enantioselective adsorption. That is, retention now more nearly occurs by only those processes which distinguish between the enantiomers. The reduction in retention through loss of the non-enantioselective processes might somewhat offset the increase in retention afforded by the higher concentration of ion-pairing reagent. Loss of non-enantioselective retention would also tend to increase enantioselectivity.

The increase in enantioselectivity can be explained in another way. The CSP used for these studies has but one significant chiral recognition mechanism available to it for the analytes under study. One of the interactions involved is hydrogen bonding of the CSPs N–*H* to the oxygens of the C-terminal carboxyl group. This process is enantioselective, occuring more extensively for the more retained enantiomer, not just because it is more retained but because of the interactions involved. In methanol–water, the heavily solvated carboxylate ion must undergo some desolvation upon interaction with CSPs N–*H*. This enantioselective desolvation reduces the exothermicity of $\Delta\Delta H$ and, consequently, reduces the magnitude of the negative $\Delta\Delta G$ value. A structural change which reduces the initial extent of solvation of the C-terminal carboxyl (*e.g.* conversion from carboxylate to an ion pair or to an ester) will reduce the extent of enantioselective desolvation with a consequent increase in the separation factor, α, of the enantiomers. The other major interactions involved in the chiral recognition process, π–π bonding and hydrogen bonding of the DNBN*H* to the CSP's carbonyl oxygen, may also entail enantioselective desolvation. However, the extent of solvation (or desolvation) of these groups is not expected to be much influenced by the presence of absence of the ion pairing reagent. For example, the ion pairing reagent has little effect upon the enantioselectivity shown on CSP **1** by esters of DNB-amino acids.

Rather interestingly, the length of the alkyl group of the ion-pairing reagent affects retention strongly but has only a modest effect on enantioselectivity (Table I).

TABLE I

EFFECT OF ALKYL CHAIN LENGTH, *n*, OF THE 2 ALKYLTRIMETHYLAMMONIUM ION-PAIRING REAGENTS ON ENANTIOSELECTIVITY OF TYPE 3 AND TYPE 4 ANALYTES USING (*R*)-CSP 1.

Mobile phase: 5 m*M* pairing ion in methanol–0.01 *M* phosphate (70:30, v/v) (pH = 6.86); flow-rate: 1 ml/min.

R	α		
	n = 6	*n* = 8	*n* = 12
Type **3**: *α-amino acids*			
n-Butyl	3.09	2.96	2.92
n-Propyl	2.65	2.53	2.46
Ethyl	2.28	2.25	2.12
Methyl	2.08	1.98	1.93
Type **4**: *2-aminoalkylphosphonic acids*			
n-Octyl	2.79	2.69	2.67
n-Heptyl	2.48	2.54	2.57
n-Hexyl	2.30	2.51	2.46
n-Pentyl	2.25	2.38	2.37
n-Butyl	2.13	2.24	2.25
n-Propyl	1.97	2.12	2.10

The greater the length of the alkyl group, the more extensively these reagents partition into the stationary phase and the greater the surface charge of the latter. As mentioned previously, surface charge increases the retention of these anionic enantiomers equally. Indeed, plots of log *k'* *vs.* the number of methylene groups in the ion-pairing reagents alkyl substituent are linear, the lines for a pair of enantiomers being parallel. In Fig. 4A, the logarithms of the capacity factors noted for the more strongly retained enantiomers of three DNB-2 aminoalkylphosphonic acids are plotted against the number of carbons in the alkyl group of the pairing ion for three different 0.5 m*M* alkyltrimethylammonium ion-pairing reagents. The linear relationship between log *k'* and the number of carbons in the alkyl group of the paring ion is consistent with either the ion pairing or the dynamic retention mechanisms advocated for achiral ion pairing[6]. With each added methylene group in the alkyl portion of the 5 mM ion pairing reagent, the log of the capacity factor of each enantiomer increases by *ca.* 0.1 unit.

As mentioned, increasing the length of the alkyl substituent of the ion-pairing reagent does have a modest effect upon enantioselectivity. Increasing the length of the alkyl group from hexyl to dodecyl produces a slight drop in enantioselectivity for the DNB α-amino acids and a slight gain for the DNB 2-aminophosphonic acids. The origin of these small effects is unclear. The size of these effects can be judged from Fig. 4B and from the data in Table I. This situation is not unlike that which one obtains for the reversed-phase separation of the enantiomers of several DNB-α-amino acid esters. There is little change in α as the length of the alkoxy group of the ester is changed (Table II). It is evident that whatever lipophilic interactions may be undergone by the alkyl portion of the ion-pairing reagent (or the alkoxyl of the ester) with the CSP, these have but a slight influence on chiral recognition. Curvature noted in a

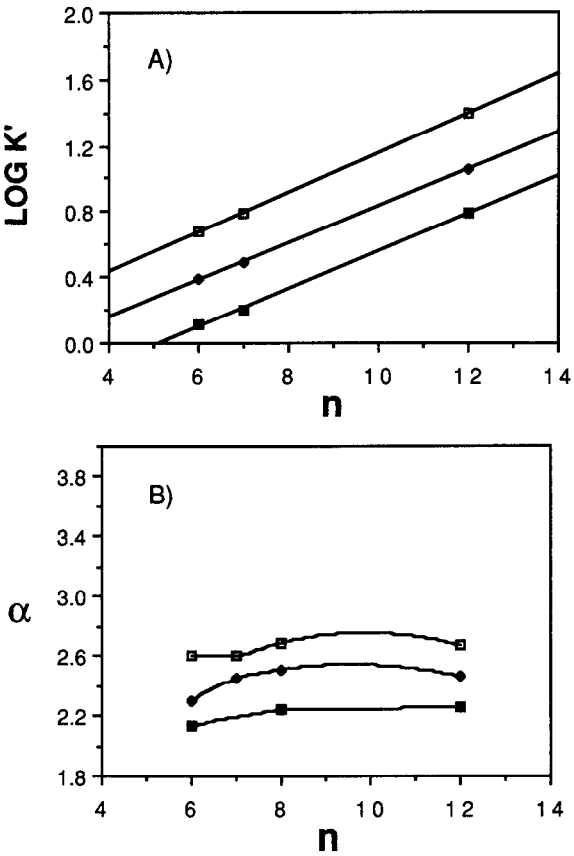


Fig. 4. (A) The dependence of log k' for the (*S*)-enantiomers of several DNB-2-aminoalkylphosphonic acids on the number, n, of methylene groups in the alkyl portion of the type **2** ion pairing reagents. (□) DNB-2-aminooctylphosphonic acid; (◆) DNB-2-aminohexylphosphonic acid; (■) DNB-2-aminobutylphosphonic acid. The mobile phase was 5 m*M* pairing ion in methanol–0.01 *M* phosphate buffer pH 6.86 (70:30, v/v). (B) The dependence of the separation factors for the enantiomers of several DNB-2-aminoalkylphosphonic acids on the number, n, of methylene groups in the alkyl portion of the type **2** ion pairing reagents. (□) DNB-2-aminooctylphosphonic acid; (◆) DNB-2-aminohexylphosphonic acid; (■) DNB-2-aminobutylphosphonic acid. The mobile phase was 5 m*M* pairing ion in methanol–0.01 *M* phosphate buffer pH 6.86 (70:30, v/v).

TABLE II

EFFECT OF THE LENGTH OF THE ALKOXYL GROUP OF ESTERS OF TYPE **3** α-AMINO-ACIDS ON ENANTIOSELECTIVITY, α, USING THE (*R*)-N-(2-NAPHTHYL)ALANINE CHIRAL STATIONARY PHASE

Mobile phase: methanol–water (90:10, v/v); flow-rate: 1 ml/min.

R	α		
	Ethoxyl	*Butoxyl*	*Octoxyl*
n-Propyl	2.27	2.13	2.09
n-Hexyl	2.35	2.31	2.32
n-Decyl	2.50	2.48	2.48
n-Tetradecyl	2.48	2.47	2.50

α *vs.* n plots stems largely (Fig. 4B) from the behavior of the least retained enantiomers which elute slightly "too late" when the shorter chain ion-pairing reagents are used. "Too late" means that these log k'_1 values fall slightly above an otherwise linear log k'_1 *vs.* n plot. The origin of this effect is unknown.

The major inference drawn from the data in Table I and Fig. 4A and B is that the length of the alkyl portion of these ion-pairing reagents has little effect upon the association constants for either ion-pair formation or dynamic ion exchange. Hence, the length of the alkyl group has little effect upon α but does affect retention in the usual manner.

Effect of methanol concentration

If retention and enantioselectivity are influenced by ion-pair formation and by enantioselective desolvation, one certainly expects that the concentration of methanol

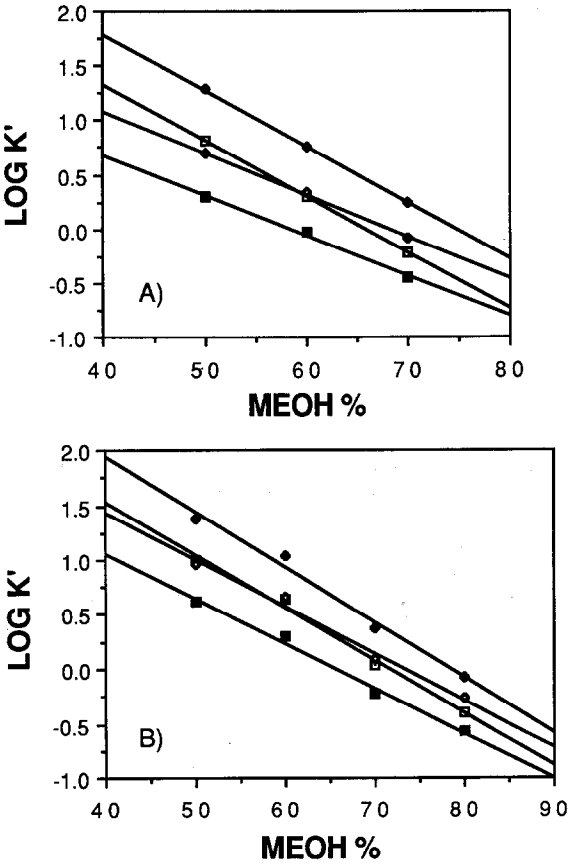


Fig. 5. The effect of the methanol content of the mobile phase on the retention of DNB-α-amino acids and DNB-2-aminoalkylphosphonic acids. A: (■) (*S*)-DNB-2-aminobutanoic acid; (◇) (*R*)-DNB-2-aminobutanoic acid; (□) (*S*)-DNB-2-aminohexanoic acid; (◆) (*R*)-DNB-2-aminohexanoic acid. B: (■) (*R*)-DNB-2-aminobutanoic acid; (◇) (*S*)-DNB-2-aminobutanoic acid; (□) (*R*)-DNB-2-aminohexylphosphonic acid; (◆) (*S*)-DNB-2-aminohexylphosphonic acid. In all instances, mobile phase was as indicated in Fig. 1 but containing 5 m*M* hexyltrimethylammonium phosphate.

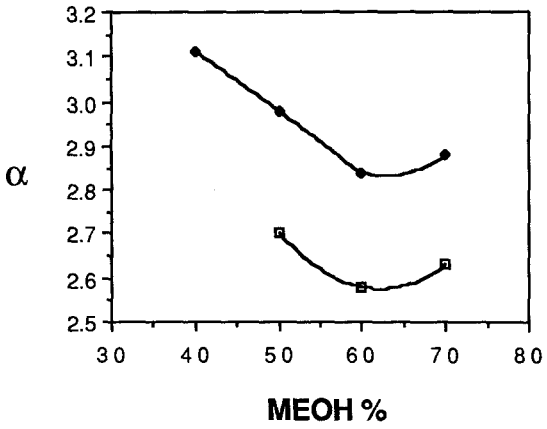


Fig. 6. Effect of the composition of the methanol–aqueous buffer mobile phase on the enantioselectivity of DNB-α-amino acids. (◆) 2-aminohexanoic acid; (□) 2-aminopentanoic acid. The mobile phases were 5 m*M* in hexyltrimethylammonium phosphate.

in the mobile phase should affect these parameters. Higher methanol concentration reduces retention by increasing the solubility of the lipophilic analyte in the mobile phase. Plots of the logs of the capacity factors of the enantiomers of several DNB-α-amino acids and DNB-2-aminoalkyl-phosphonic acids as a function of methanol concentration are shown in Fig. 5A and B. These plots are depicted as linear and of comparable slope. In actuality, the data points are slightly sinusoidal of the depicted lines. Consequently, methanol concentration also influences enantioselectivity (Fig. 6), a minimum being noted at *ca.* 60% methanol. The occurence of a minimum might have been anticipated, for some mobile phase composition will afford maximum solvation where a maximum degree of enantioselective desolvation will occur upon interaction with the CSP and a minimum value of α will be observed. Superimposed upon this is the effect of mobile phase composition on the extent of ion-pair formation. The recent report by Katz et al.[13] that methanol–water (60:40, v/v) solutions contain the maximum concentration of methanol–water associate is worth mentioning, for the minimum of α noted at this composition may not be coincidental. However, no minimum in retention occurs in 60% methanol. In this regard, these systems differ from several of those reported in ref. 11.

CONCLUSION

Achiral ion-pairing reagents have no unusual influence upon the chromatographic behavior of the enantiomers of DNB-amino acids on the N-(2-naphthyl)-alanine CSP. Their use does increase retention and enantioselectivity in an explainable manner but leads to no lipophilic interactions which contribute significantly to chiral recognition. This is not to say that there are no lipophilic interactions whatsoever, but the effect of these interactions upon chiral recognition (*e.g.*, the curvature of the α *versus n* plots in Fig. 4B) are small and their origin is obscure. The bulk of the increased enantioselectivity which is observed is attributed to a reduction in the extent of solvation of the analytes carboxylate anion group upon ion-pair formation and a

consequent reduction in the enthalpic component of the enantioselective desolvation which occurs upon adsorption onto the CSP. Similar reductions in the extent of enantioselective desolvation may partially account for the effects of ion-pairing reagents on the enantioselectivity noted using protein CSPs[8,9].

ACKNOWLEDGEMENT

This work was supported by a grant from the National Science Foundation.

REFERENCES

1 W. H. Pirkle and D. W. House, *J. Org. Chem.*, 44 (1979) 2169.
2 W. H. Pirkle, J. M. Finn and J. L. Schreiner, *J. Org. Chem.*, 103 (1981) 3964.
3 W. H. Pirkle, T. C. Pochapsky, G. S. Mahler, D. E. Corey, D. S. Reno and D. M. Alessi, *J. Org. Chem.*, 51 (1980) 4991.
4 W. H. Pirkle and T. J. Sowin, *J. Chromatogr.*, 396 (1987) 83–92.
5 W. H. Pirkle and J. E. McCune, *J. Chromatogr.*, 441 (1988) 311–322.
6 W. R. Melander and C. Horvath, in M. T. W. Hearn (Editor), *Ion-Pair Chromatography* (*Chromatographic Science Series,* Vol. 31), Marcel Dekker, New York, 1985.
7 C. Pettersson and G. Schill, *J. Liq. Chromatogr.*, 9 (1986) 291.
8 G. Schill, I. W. Wainer and S. A. Barkham, *J. Liq. Chromatogr.*, 9 (1986) 641.
9 J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.*, 9 (1986) 621.
10 W. H. Pirkle and T. C. Pochapsky, *J. Am. Chem. Soc.*, 109 (1987) 5975.
11 W. H. Pirkle, J. A. Burke, III and S. Wilson, *J. Am. Chem. Soc.*, in press.
12 G. B. Cox and R. W. Stout, *J. Chromatogr.*, 384 (1987) 315–336.
13 E. D. Katz, C. H. Lochmüller and R. P. W. Scott, *Anal. Chem.*, 61 (1989) 349–355.