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Empirical relationship between chiral selectivity and mobile phase modifier properties

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Abstract

An empirical relationship was derived which relates properties of the mobile phase modifier to the chiral selectivity factor for a given analyte/chiral selector combination. Using carbon dioxide and heptane-based mobile phases, the effect of various mobile phase modifiers on Pirkle-type stationary phases may be accurately modeled using a two-parameter equation. Similar results are obtained using cellulosic stationary phases with carbon dioxide-based mobile phases. Modeling separations performed using heptane-based mobile phases with cellulosic stationary phases were not successful. The predictive ability of this modeling approach was demonstrated using novel modifiers and chiral analytes.

Keywords: Chiral selectivity; Mobile phase modifier; Chiral stationary phases, LC; Carbon dioxide

1. Introduction

In general practice, chiral method development is accomplished via a trial and error approach. Ideally, with molecular modeling it should be possible to predict an optimal mobile and stationary phase combination based on the structure of the chiral molecule. Lipkowitz's review of the molecular modeling of chiral interactions suggests that, while the ability to predict three-dimensional structures has progressed, chiral selectors and chiral analytes induce structural changes in one another as part of the recognition process [1]. Most models do not allow for mobile phase effects and the predictive success of modeling may be ultimately limited.

While true a priori predictions of chiral separations remain elusive, empirical relationships may prove useful in developing predictive models. Many researchers have noted a relationship between chiral selectivity and structural features of chiral analogs. Pirkle has extensively demonstrated the relationships between selectivity and π-basicity [2,3] and hydrogen bond donating ability [4] to chiral selectivity. Investigations by Roussel and Suteu showed that lipophilicity descriptors could be used to quantitatively assess the effects of various structural changes in the analyte to chiral selectivity [5].

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These relationships are useful when faced with the requirement to separate compounds similar to those previously encountered. They do not however address the potential control available through judicious mobile phase selection. There are innumerable reports noting the effect of different modifiers on chiral selectivity. Tambute and co-workers [6,7] examined the use of modifiers, varying in abilities to act as proton donors, proton acceptors and dipoles, and concluded that selectivity in their system depended on the steric hindrance of the alcohol modifier. Pirkle and Welch [8] also studied modifier effects on chiral selectivity and found that the influence of the mobile phase modifier was dependent upon the structure of the chiral analyte. In most literature examples of modifier effects, a single mobile phase modifier property is related to chiral selectivity whereas the selection process may involve many types of specific interactions.

An earlier study has shown that the effect of mobile phase modifiers on chiral selectivity for a variety of phenylalanine analogs could be rationalized based on simple molecular descriptors for the modifiers [9]. This procedure suggests that conditions for optimal chiral selectivity may be quantitatively predicted using a limited number of experimental data points obtained with judiciously chosen mobile phase modifiers. Using a particular chiral stationary phase, the choice of optimal mobile phase conditions may be selected by extrapolation of the empirical model results to other modifiers. This approach would rapidly determine the suitability of a particular chiral selector and the optimal conditions with which to separate the enantiomers, rather than resorting to trial and error.

In this work, the effect of mobile phase modifiers on chiral selectivity is explored for a wide range of chiral amino acid analogs. Separations are performed under normal phase and subcritical phase conditions using Pirkle-type and cellulosic chiral selectors. A variety of descriptors for the mobile phase modifiers are used to develop a correlation between the modifier's properties and the resulting chiral selectivity factors. The applicability of these simplified empirical relationships will be examined by determining the predictive ability of this approach using novel chiral compounds and mobile phase modifiers.

2. Experimental

2.1. Equipment – supercritical fluid chromatography (SFC)

The chromatographic system used in this study was a Gilson SF3 system (Gilson, Middleton, WI, USA). Carbon dioxide mobile phase was pumped with a Model 308 pump with a thermostated head. Modifiers (Table 1) containing 1% (v/v) trifluoroacetic acid were pumped with a Model 306 pump at 10% (v/v) of the total mobile phase. Mixing took place in a Model 811C dynamic mixer with a 1.5-ml mixing chamber. Fixed loop injections (10 μl) were made using a Model 231XL sampling injector. Column thermostating was accomplished using a Model 831 temperature regulator fitted with an externally driven cooling coil pumped with a chilled water–ethylene glycol mixture. Detection was accomplished at 210 and 254 nm using a Model 119 variable-wavelength UV detector with a 7-μl high-pressure flow cell. Column backpressure was maintained at 200 bar using a Model 821 pressure regulator.

2.2. Equipment – high-performance liquid chromatography (HPLC)

Two different chromatographic systems were used in this study. The first system consisted of a Hewlett-Packard Model 1100 with a binary pumping system. Column thermostating was accomplished using the cooled column compartment. The second system was

Table 1
Solute solvatochromic parameters for mobile phase modifiers [10]

Modifier	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	V_X
Methanol	0.278	0.44	0.43	0.47	0.308
Ethanol	0.246	0.42	0.37	0.48	0.449
n-Propanol	0.236	0.42	0.37	0.48	0.590
Isopropanol	0.212	0.36	0.33	0.56	0.590
n-Butanol	0.224	0.42	0.37	0.48	0.731
Isobutanol	0.217	0.36	0.33	0.56	0.731
n-Pentanol	0.219	0.42	0.37	0.48	0.872
2-Methyl-2-butanol	0.194	0.30	0.31	0.60	0.872
4-Methyl-2-pentanol	0.167	0.33	0.33	0.56	1.013
2-Methoxyethanol	0.269	0.50	0.30	0.84	0.649
2-Ethoxyethanol	0.237	0.50	0.30	0.83	0.790

a Gilson SF3 system with the pressure regulation module bypassed. Mobile phase was pumped with a Model 308 pump. Fixed loop injections were made using a Model 231XL sampling injector. Column thermostating was accomplished using a Model 831 temperature regulator fitted with an externally driven cooling coil pumped with a chilled water–ethylene glycol mixture. Detection was accomplished at 210 and 254 nm in both systems. Mobile phases were premixed and degassed prior to use and contained 0.1% (v/v) trifluoroacetic acid.

2.3. Columns

α-Burke 2 columns (S configuration; 250×4.6 mm, 5 μm particles) were obtained from Regis Technologies (Morton Grove, IL, USA). Chiralpak AS columns (250×4.6 mm, 10 μm particles) were obtained from Chiral Technologies (Exton, PA, USA). Columns were conditioned with 10% (v/v) ethanol in carbon dioxide or heptane at a flow-rate of 1.0 ml/min (200 bar for SFC), 20°C for 4 h before initial use.

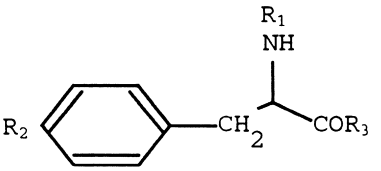
2.4. Chemicals

Carbon dioxide used in this study was Coleman grade obtained from MG Industries (Morrisville, PA, USA). Absolute ethanol was obtained from Quantum (Newark, NJ, USA). Heptane, methanol and isopropanol were from EM Science (Gibbstown, NJ, USA). *n*-Propanol, *n*-butanol and isobutanol were obtained from Burdick and Jackson (Muskegon, MI, USA). Trifluoroacetic acid, 2-methoxyethanol and 2-ethoxyethanol were from JT Baker (Phillipsburg, NJ, USA). *n*-Pentanol, 2-methyl-2-butanol, cyclohexanol and 4-methyl-2-pentanol were from Aldrich (Milwaukee, WI, USA). All modifiers were the highest grade obtainable.

Chiral analytes 1–17, 24 and 25 (Table 2) were obtained from Sigma (St. Louis, MO, USA), Bachem Bioscience (King of Prussia, PA, USA), Aldrich and Advanced ChemTech (Louisville, KY, USA) and were used without further purification. DMP 961 and 963 were synthesized within Dupont Pharmaceuticals Company.

Phenylalanine amide analogs 18–23 (Table 2) were prepared from *R*- and *S*-*N*-*tert*.-Boc-phenylalanine *N*-hydroxysuccinimide esters by reaction with the corresponding amine (or amine hydrochloride salt, with triethylamine) [11]. Example experimental procedure for *N*-*tert*.-Boc-D-phenylalanine *n*-propylamide: combine *N*-*tert*.-Boc-D-phenylalanine *N*-hydroxysuccinimide ester (0.36 g; 1 mmol), dichloromethane (5 ml) and *n*-propylamine (0.2 ml; 2.4 mmol) and age at 21°C. After 1 h, no *N*-*tert*.-Boc-D-phenylalanine *N*-hydroxysuccinimide ester was detectable by HPLC. Add 10 ml dichloromethane and 10 ml water and separate the organic

Table 2
Phenylalanine analog structure



Analog	R_1 [a]	R_2	R_3
1	Cbz	H	OH
2	Benzyl	H	OH
3	FMOC	H	OH
4	Acetyl	H	OH
5	Benzoyl	H	OH
6	Chloroacetyl	H	OH
7	Boc	H	OH
8	Boc	F	OH
9	Boc	Cl	OH
10	Boc	I	OH
11	Boc	NO_2	OH
12	Boc	OH	OH
13	Boc	OCH_3	OH
14	Boc	OCH_2CH_3	OH
15	Boc	$OCH_2C_6H_5$	OH
16	Boc	$CH_2C_6H_5$	OH
17	Boc	2,6-Dichlorobenzyl	OH
18	Boc	H	$NHCH_3$
19	Boc	H	$N(CH_3)_2$
20	Boc	H	NHC_2H_5
21	Boc	H	$N(C_2H_5)_2$
22	Boc	H	$NHCH(CH_3)_2$
23	Boc	H	$N(CH(CH_3)_2)_2$
24	Boc	Homophenylalanine	OH
25	Boc	Phenylglycine	OH

[a] Cbz is carbobenzyloxy, FMOC is fluorenylmethoxycarbonyl and Boc is *tert*.-butoxycarbonyl. Analytes 24 and 25 have one additional and one less methylene group between the phenyl ring and alpha carbon, respectively.

phase. Wash organic phase with additional 10 ml water and discard aqueous extract. Concentrate organic phase in vacuo to 0.31 g white solid in quantitative yield. Proton nuclear magnetic resonance (NMR) spectrum of product was consistent with literature values for chemical shifts. Analysis by chiral SFC showed enantiomeric excesses above 99%. All reagents were obtained from Aldrich and were reagent grade or better.

2.5. Sample preparation

Chiral analytes were dissolved in absolute ethanol to a final concentration of 1.0 mg/ml. When racemic mixtures were not available, equal proportions of D and L enantiomers were mixed to form synthetic racemic mixtures. Duplicate injections of racemic mixtures and the L enantiomers were made to determine the average selectivity factors and to confirm elution order.

2.6. Chromatographic conditions

Separations were performed using a flow-rate of 1.0 ml/min (with a backpressure of 200 bar for SFC) at 20°C. Selectivity factors for each of the chiral analytes were taken as the average of duplicate values.

2.7. Regression analysis

Multilinear regression analysis of the selectivity factor data was performed using Minitab (version 10extra, State College, PA, USA). All data were used for the regression analyses, but were inspected for systematic outliers. In this study chiral selectivity factor is fixed as:

$$\alpha = \frac{k'_{\mathrm{L}}}{k'_{\mathrm{D}}} \quad (1)$$

such that changes in elution order may yield α values of less than 1.0.

3. Results and discussion

A set of phenylalanine analogs, shown in Table 2, were chromatographed on an α-Burke 2 column using a carbon dioxide mobile phase modified with 10% (v/v) organic modifier containing 1% (v/v) trifluoroacetic acid. The resulting selectivity factors, obtained at 20°C with a flow-rate of 1.0 ml/min at 200 bar backpressure, are given in Table 3. Most of

Table 3
Selectivity factors for phenylalanine analogs on an α-Burke 2 column with carbon dioxide mobile phase

Analog	MeOH	EtOH	nPrOH	iPrOH	nBuOH	iBuOH	nPeOH	2-ME
1	0.922	0.875	0.863	0.830	0.853	0.858	0.870	0.853
2	0.944	0.902	0.886	0.862	0.878	0.880	0.894	0.876
3	0.890	0.815	0.789	0.737	0.775	0.786	0.810	0.781
4	0.855	0.746	0.709	0.656	0.692	0.683	0.724	0.710
5	0.945	0.903	0.887	0.862	0.878	0.883	0.892	0.876
6	0.949	0.899	0.882	0.847	0.873	0.873	0.890	0.872
7	0.915	0.864	0.847	0.823	0.844	0.851	0.862	0.840
8	0.906	0.850	0.853	0.808	0.841	0.833	0.846	0.794
9	0.897	0.842	0.825	0.800	0.819	0.822	0.837	0.822
10	0.905	0.858	0.848	0.816	0.841	0.834	0.849	0.842
11	0.952	0.929	0.932	0.913	0.936	0.921	0.924	0.922
12	0.825	0.770	0.749	0.730	0.714	0.743	0.767	0.736
13	0.810	0.723	0.700	0.661	0.685	0.692	0.707	0.697
14	0.806	0.714	0.688	0.647	0.675	0.677	0.697	0.688
15	0.876	0.816	0.796	0.768	0.785	0.782	0.797	0.800
16	0.953	0.940	0.943	0.938	0.943	0.937	0.931	0.927
17	0.923	0.880	0.864	0.842	0.854	0.854	0.863	0.870
24	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.962
25	1.042	1.080	1.093	1.107	1.100	1.101	1.087	1.081

the analogs eluted with the L enantiomer preceding the D enantiomer, thus resulting in selectivity factors less than 1.0 according to Eq. (3). This set of data show that the experimental selectivity factors for these compounds vary appreciably depending upon which organic modifier was used. Unfortunately, the R_3 phenylalanine analogs were not resolved using this column.

The selectivity factors for each phenylalanine analog were independently regressed against a wide variety of molecular descriptors for each modifier. Descriptors such as dipole moment [12], polarizability [13] and E_{30}^{T} [14] failed to show any consistent, statistically significant correlation with the experimentally determined selectivity factors ($p<0.05$). Specific molecular parameters, such as hydrogen bond donating, hydrogen bond accepting and dipolarity/polarizability *bulk* solvatochromic parameters [15] also failed to produce any consistent, statistically significant correlations.

A separate set of specific molecular parameters, the *solute* solvatochromic parameters for the individual organic modifiers did, however, result in a set of consistent, statistically significant correlations [10]. These parameters are primarily used for linear solvation energy relationship (LSER) modeling of achiral retention in gas chromatography (GC) [16,17], liquid chromatography (LC) [18,19] and SFC [20,21]. The *solute* solvatochromic parameters differ from bulk solvatochromic parameters in that *solute* parameters describe the molecules in an infinitely dilute state, where intermolecular interactions with other solute molecules are absent. Bulk parameters describe the molecules when they are capable of interacting with each other via hydrogen bonding, dipolar or other types of interactions. While it is not readily apparent why the *solute* solvatochromic parameters correlate with the selectivity factor data while the *bulk* solvent solvatochromic parameters do not, these descriptors are adequate for use in this empirical modeling approach. These parameters are available for an extremely wide range of organic molecules [10] and should allow for subsequent evaluation of a wide range of organic modifiers for chiral separations.

Using the *solute* solvatochromic parameters as descriptors for the mobile phase modifiers, only two of the descriptors showed any statistical significance. The modifier excess molar refraction term (R_2), a measure of excess electron density, and the modifier hydrogen bond donating term ($\Sigma\beta_2^H$) could be correlated with the natural logarithm of the selectivity factor for each analog using the following equation:

$$\ln \alpha = c + r_{eff}R_2 + b_{eff}\sum\beta_2^H \quad (2)$$

where c is the regression intercept. The fitted coefficients, r_{eff} and b_{eff} result from the fitting of the modifier data for each analog and should be different for each analog/stationary phase combination. The regression coefficients r_{eff} and b_{eff} and the correlation coefficients obtained for each of the phenylalanine analogs are given in Table 4. The overall fitting quality of Eq. (2) for these analogs under these separation conditions is shown in Fig. 1.

The predictive ability of this model was evaluated by using the coefficients for each analog, given in Table 4, along with the *solute* solvatochromic parameters for various other modifiers not used to build the regression model. The predicted selectivity factors are simply obtained by solving Eq. (2) using R_2 and $\Sigma\beta_2^H$ values from the literature for the modifier of

Table 4
Regression coefficients for phenylalanine analogs on an α-Burke 2 column with carbon dioxide mobile phase[a]

Analog	c	b_{eff}	r_{eff}	r
1	−0.31	−0.16	1.08	0.917
2	−0.27	−0.15	1.00	0.936
3	−0.54	−0.27	1.93	0.899
4	−0.87	−0.37	3.12	0.952
5	−0.27	−0.15	1.00	0.933
6	−0.31	−0.18	1.22	0.938
7	−0.32	−0.17	1.07	0.889
8	−0.26	−0.30	1.05	0.960
9	−0.39	−0.17	1.24	0.920
10	−0.36	−0.15	1.17	0.961
11	−0.14	−0.07	0.44	0.920
12	−0.51	−0.21	1.43	0.828
13	−0.74	−0.28	2.31	0.934
14	−0.79	−0.30	2.52	0.941
15	−0.51	−0.17	1.60	0.963
16	−0.08	−0.06	0.18	0.844
17	−0.35	−0.11	1.14	0.967
24	0.08	−0.10	−0.12	0.977
25	0.23	0.06	−0.76	0.942

[a] Regressions performed using methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, *n*-pentanol and 2-methoxyethanol as modifiers. r is the regression correlation coefficient.

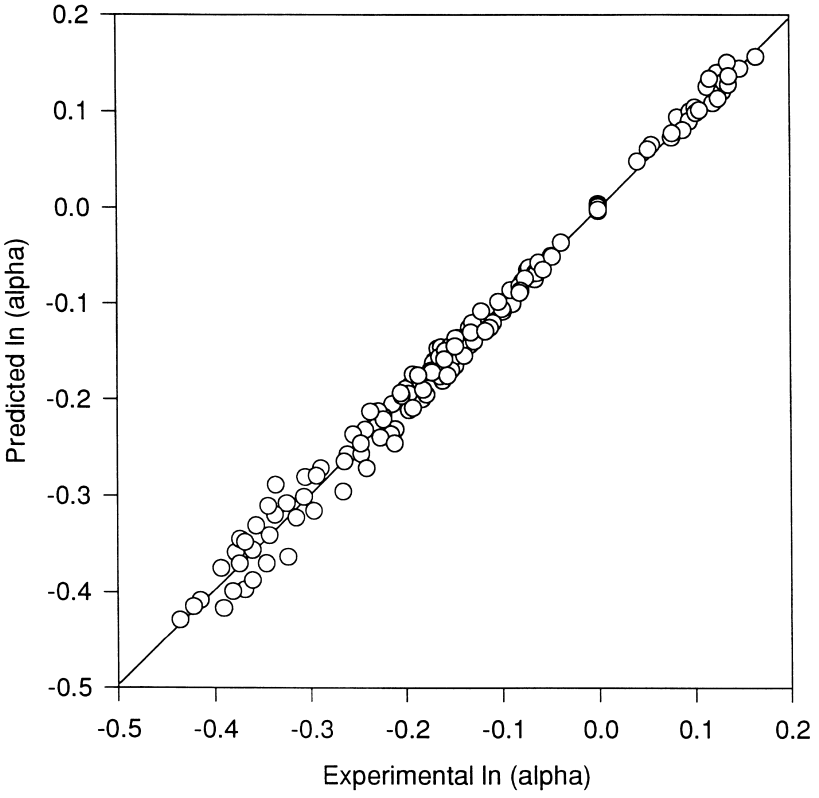


Fig. 1. Comparison of fitted versus experimental ln α values for phenylalanine analogs by SFC. Separations performed on an α-Burke 2 column using 10% (v/v) modifier containing 1% (v/v) trifluoroacetic acid. Flow-rate was 1.0 ml/min with a backpressure of 200 bar at 20°C. Modifiers used to generate regression data are methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, *n*-pentanol and 2-methoxyethanol.

interest. Table 5 shows the correlation between predicted and experimental selectivity factors using three other modifiers. In general, the predicted selectivity factors agreed well with those determined experimentally. In very few cases, such as when 4-methyl-2-pentanol was used to separate analog 25, the agreement was rather poor. Excluding this particular case, the overall error in the remaining 57 predictions averages 0.84% (excluding cases where the analyte elution was not observed). In each case, the correct elution order of the L and D enantiomers was predicted. This model also successfully predicted two cases for analog 24 where separation was not expected to occur ($\alpha = 0.997$ and 1.004 using 2-methyl-2-butanol and 4-methyl-2-pentanol, respectively).

It should be noted that the choice of 4-methyl-2-pentanol as a modifier was deliberate. Based on the regression coefficients in Table 4, the optimal mobile phase modifier would have a low excess molar refraction value and a high hydrogen bond donating value. Evaluation of a wide variety of alcohol modifier solute solvatochromic parameters in the literature and consideration of the physical properties of the potential candidates led to the selection of this relatively novel modifier.

The applicability of this modeling technique to other systems was explored by chromatographing the same series of phenylalanine analogs with the same set of organic modifiers under near critical conditions on a Chiralpak AS cellulosic chiral stationary phase. The resulting selectivity factors were regressed against the same modifier descriptors evaluated for fitting the data obtained using the α-Burke 2 column.

Table 5
Modeling separation factors using various modifiers on an α-Burke 2 column

Analyte	2-Methyl-2-butanol		2-Ethoxyethanol		4-Methyl-2-pentanol	
	α_{EXP}	α_{PRED}	α_{EXP}	α_{PRED}	α_{EXP}	α_{PRED}
1	0.809	0.822	0.818	0.830	0.801	0.803
2	0.842	0.847	0.843	0.854	eno	0.829
3	0.701	0.721	0.730	0.736	0.694	0.692
4	0.635	0.615	0.656	0.646	eno	0.573
5	0.835	0.847	0.843	0.854	eno	0.829
6	0.820	0.834	0.839	0.843	0.810	0.813
7	0.797	0.807	0.808	0.813	0.785	0.789
8	0.829	0.790	0.786	0.771	0.818	0.777
9	0.769	0.778	0.786	0.789	eno	0.757
10	0.800	0.800	0.806	0.813	0.787	0.780
11	0.918	0.908	0.903	0.910	0.903	0.900
12	1.000	0.699	0.695	0.708	eno	0.678
13	0.645	0.631	0.649	0.654	0.632	0.600
14	0.627	0.618	0.640	0.643	0.581	0.584
15	0.746	0.740	0.759	0.762	0.732	0.713
16	0.907	0.922	0.912	0.917	0.942	0.920
17	0.820	0.823	0.835	0.843	0.891	0.802
24	1.000	0.997	0.953	0.969	1.000	1.004
25	1.134	1.126	1.093	1.105	1.494	1.146

eno = Elution not observed.

Again, the only descriptors which showed any consistent statistically significant correlation were the *solute* solvatochromic parameters for the modifier. Coincidentally, the same two descriptors which fit this data set were the same two descriptors which fit the α-Burke 2 data set. The resulting regression coefficients and correlation coefficients obtained for each of the phenylalanine analogs are given in Table 6, in addition to those obtained for the R_3 analogs which are separated on the cellulosic phase.

It is evident from the correlation coefficients in Table 6 that the quality of the data fitting using Eq. (2) is not quite as good as was the case using the α-Burke 2 column. In some cases, Eq. (2) fits the experimentally determined selectivity factors rather poorly. Extrapolation of the model to a novel modifier, 2-methyl-2-butanol, resulted in average errors in predicted versus experimental selectivity factors of 3.85% (Table 7). While this stationary phase generally resulted in much higher selectivity factors than the Pirkle-type stationary phase, the complexity of the cellulosic selector proved problematic.

The cellulosic selector has multiple chiral recognition sites which play a role in the overall recognition process compared to the single chiral recognition site on the Pirkle-type chiral selector. This multiplicity of binding sites makes interpretation of recognition mechanisms significantly more complex [22]. In addition to the effect of the modifier on the analog-selector recognition process, the modifiers appeared to have a significant effect on other factors related to the recognition mechanism. The most significant is likely due to the differences in tertiary structure of the polymeric selector when different mobile phase modifiers are used. Other studies have shown that this tertiary structure is very important to the recognition process for these flexible selectors [23,24]. Despite this complexity, this modeling approach is quite good at predicting which mobile phase modifier would be most suited for enhancing the chiral selectivity based on the regression coefficients for each analog.

The utility of this model for the Pirkle-type chiral stationary phase was examined under normal phase conditions. Table 8 shows that under these conditions, the fits to Eq. (2) are fairly good. The quality of the fits, as measured by the regression correlation coefficients, is comparable to those obtained using carbon dioxide-based mobile phases. As was the case with the carbon dioxide-based mobile phases, the R_3

Table 6
Regression coefficients for phenylalanine analogs on a Chiralpak AS column with carbon dioxide mobile phase[a]

Analog	c	b_{eff}	r_{eff}	r
1	−2.69	1.59	5.23	0.982
2	−0.79	0.65	2.20	0.926
3	0.20	0.09	−0.65	0.394
4	−0.21	−1.14	2.98	0.847
5	−0.78	0.66	2.14	0.919
6	0.44	0.03	−0.65	0.217
7	−1.17	0.71	3.31	0.954
8	−1.76	0.74	5.14	0.978
9	−1.42	0.51	4.34	0.966
10	−1.09	0.42	3.37	0.950
11	−1.36	1.50	2.38	0.989
12	−0.01	0.17	0.33	0.835
13	0.15	0.74	−0.82	0.935
14	−0.19	1.11	−0.30	0.961
15	0.31	0.47	−0.82	0.766
16	−0.55	0.58	1.05	0.912
17	−0.58	0.35	2.12	0.959
18	−1.32	−0.59	3.98	0.987
19	−0.66	−0.98	2.47	0.972
20	−1.36	−0.66	4.23	0.983
21	−2.59	−1.76	8.76	0.915
22	−1.36	−0.65	3.92	0.992
23	−2.95	−1.10	10.2	0.864
24	−1.98	1.10	4.82	0.975
25	−1.99	0.44	5.34	0.980

[a] Regressions performed using methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol, *n*-pentanol and 2-methoxyethanol as modifiers. r is the regression correlation coefficient.

analogs were not resolved. The predictive ability of the model was again evaluated using a novel modifier with an average error of 2.33% (Table 7).

When Eq. (2) is applied to selectivity factor data obtained using a cellulosic chiral stationary phase with the phenylalanine analogs under normal phase conditions, the model becomes very inaccurate. Table 9 shows that the quality of the fits, as measured by the regression correlation coefficients, is rather poor. Analysis of the data shows that the scatter in the fitted data is random and is not due to data from one or two aberrant modifiers. The predictive ability of the model in this application also suffers as a result of poor modeling. Table 7 shows that the average error in predicted versus experimental selectivity factor using 2-methyl-2-butanol is 7.11%. In some cases, the relative elution order is not accurately predicted.

While it is not entirely clear why the model is unsuccessful under these conditions, the source of the problem may be surmised. Cellulosic chiral stationary phases are known to change their tertiary configuration depending upon the swelling ability of the solvent [25,26]. This tertiary structure has been implicated as an important factor in determining the overall chiral selectivity for various chiral analytes. With heptane-based mobile phases, the modifier may play a more important role in determining the equilibrium tertiary configuration than under SFC conditions. This dramatic change in the chiral recognition process would prove difficult for an empirical model such as this to accurately predict. Carbon dioxide-based mobile phases may attenuate the effect of differential swelling by various modifiers by more fully swelling the polymeric stationary phase. Carbon dioxide is known to swell many polymers, to extremely large extents in some cases. With this variable removed, the chiral recognition process may be more straightforward with respect to modifier effects.

A more interesting test of the predictive ability of this modeling scheme was performed by evaluating the optimal conditions for the separation of DMP 961 and DMP 963, investigational new drugs for the treatment of human immunodeficiency virus (HIV), shown in Fig. 2. These molecules are structurally dissimilar to the probe analytes used in this study. These compounds were chromatographed using a Chiralpak AS column with four conventional modifiers (methanol, ethanol, *n*-propanol and isopropanol) and 25% (v/v) carbon dioxide at 20°C with a backpressure of 200 bar. When the selectivity data were regressed against various descriptors for the modifiers, only the *solute* dipolarity/polarizability (π^*) and McGowan's characteristic volume (V_X) [27] terms showed any statistical significance. The resulting equation describes the observed selectivity factors very well ($r = 0.979$ for DMP 961 and $r = 0.991$ for DMP 963):

$$\ln \alpha = c + s_{eff}\pi^* + v_{eff}V_X \quad (3)$$

Table 10 shows the predicted and experimental selectivity factors for two additional modifiers which are expected to improve the separation, based on the coefficients for Eq. (3) and the solute solvatochromic descriptors for the modifiers from Table 1. The

Table 7
Modeling separation factors using 2-methyl-2-butanol as a mobile phase modifier

Analyte	Chiralpak AS/SFC		α-Burke 2/NPLC		Chiralpak AS/NPLC	
	α_{EXP}	α_{PRED}	α_{EXP}	α_{PRED}	α_{EXP}	α_{PRED}
1	1.000	0.486	0.869	0.849	0.833	0.313
2	1.000	1.027	0.863	0.848	1.000	0.982
3	1.322	1.136	0.776	0.754	1.000	1.424
4	1.000	0.729	0.752	0.652	1.000	0.950
5	1.000	1.032	0.862	0.845	1.000	0.963
6	1.000	1.394	0.859	0.872	0.886	0.677
7			0.855	0.818	1.000	0.621
8			0.801	0.772	0.693	0.508
9			0.775	0.751	0.833	0.590
10			0.794	0.767	1.000	0.735
11			0.898	0.878	1.000	0.733
12			0.663	0.586	1.139	0.883
13			0.669	0.598	2.020	1.534
14			0.639	0.570	1.661	1.353
15			0.685	0.678	1.566	1.468
16			0.922	0.886	1.906	1.017
17			0.844	0.781	1.244	0.917
18	0.466	0.406			0.173	0.312
19	0.430	0.464			0.223	0.290
20	0.484	0.392			0.143	0.223
21	0.102	0.143			0.123	0.095
22	0.447	0.372			0.128	0.199
23	0.203	0.196			0.118	0.232
24			1.000	n/a	1.000	0.614
25			1.197	1.227	0.609	0.259

predicted selectivity factors agree fairly well with the experimental selectivity factors, despite the fact that the LSER parameters for these two additional modifiers lie outside the range of parameters used to develop the regression coefficients. The predicted selectivity factors are systematically larger than the observed selectivity factors, but effectively indicate which polar modifiers would be most suitable for optimizing the separation. The improvement in the chiral selectivity by using a model-predicted modifier versus a conventional mobile phase modifier is shown in Fig. 3 for DMP 963. Further gains in selectivity may be achieved by choosing other modifiers with desirable solute solvatochromic descriptors. However, the modifier's physical and chemical characteristics, such as stationary phase and detector compatibility, may restrict the total number of useful modifiers. While the degree of chiral selectivity in Fig. 3 is excessive for routine analytical scale separations, it has important implications for preparative and process scale separations.

The range of chiral compounds and chiral stationary phases which may be modeled using this approach remains to be determined. It is evident from the data presented here that some systems, particularly systems utilizing a carbon dioxide-based mobile phases, are more suited towards this type of modeling approach than heptane-based mobile phases. The basis for this, as well as possible mechanistic interpretations of the model regression coefficients also remain to be determined in future work.

4. Conclusions

An empirical model was developed which correlates the experimental selectivity factors for a variety of amino acid analogs with descriptors of the mobile phase modifier. With carbon dioxide-based mobile phases, the model fits data obtained using a Pirkle-

Table 8
Regression coefficients for phenylalanine analogs on an α-Burke 2 column with heptane mobile phase[a]

Analog	c	b_{eff}	r_{eff}	r
1	−0.58	0.08	1.90	0.949
2	−0.68	0.18	2.10	0.967
3	−1.28	0.35	4.06	0.965
4	−3.26	1.10	11.2	0.887
5	−0.69	0.18	2.13	0.966
6	−0.65	0.13	2.24	0.963
7	−1.04	0.36	3.21	0.957
8	−1.32	0.46	4.05	0.957
9	−1.41	0.46	4.37	0.954
10	−1.25	0.37	3.93	0.961
11	−0.72	0.34	1.99	0.911
12	−3.20	1.54	8.98	0.914
13	−2.46	0.78	7.62	0.972
14	−2.55	0.80	7.77	0.938
15	−1.77	0.54	5.45	0.939
16	−0.54	0.23	1.45	0.928
17	−1.25	0.45	3.78	0.925
25	1.04	−0.29	−3.41	0.921

[a] Regressions performed using ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol and *n*-pentanol as modifiers. r is the regression correlation coefficient.

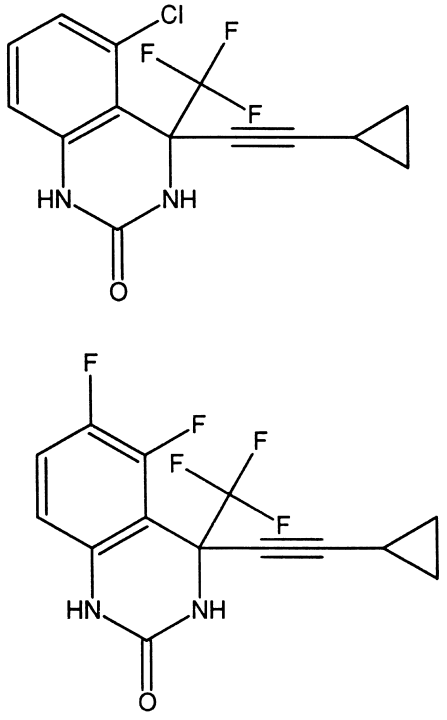


Fig. 2. Structures of DMP 961 (top) and DMP 963 (bottom).

Table 9
Regression coefficients for phenylalanine analogs on a Chiralpak AS column with heptane mobile phase[a]

Analog	c	b_{eff}	r_{eff}	r
1	−1.78	−1.28	7.15	0.533
2	1.20	−1.38	−2.01	0.733
3	2.58	−1.25	−7.61	0.592
4	−4.37	3.35	11.9	0.825
5	−0.26	−0.14	1.58	0.399
6	4.01	−4.18	−9.75	0.938
7	−3.13	1.06	10.4	0.688
8	−3.87	0.99	13.4	0.769
9	−2.26	−0.03	9.02	0.760
10	−1.93	0.48	6.88	0.493
11	0.29	−1.50	1.54	0.675
12	−2.17	0.88	7.82	0.820
13	0.10	0.65	−0.32	0.336
14	−0.93	1.31	2.30	0.471
15	−4.09	4.43	9.36	0.639
16	0.14	0.08	−0.88	0.399
17	−0.59	0.14	2.16	0.469
18	−3.90	0.45	12.7	0.978
19	−3.89	0.12	13.3	0.845
20	−3.73	−0.62	13.4	0.989
21	−6.46	−0.72	23.4	0.869
22	−3.41	−1.21	13.0	0.981
23	−9.94	5.24	27.5	0.679
24	−0.48	−1.16	3.55	0.504
25	−2.47	−2.63	13.9	0.868

[a] Regressions performed using ethanol, *n*-propanol, isopropanol, *n*-butanol, isobutanol and *n*-pentanol as modifiers. r is the regression correlation coefficient.

type chiral stationary phase quite well, but is somewhat less accurate modeling data obtained using a cellulosic stationary phase. Predicted relative elution order among enantiomers and selectivity factors was quite good for Pirkle-type chiral stationary phases, but resulted in poorer predictions using cellulosic stationary phases. Using heptane-based mobile phases, the model fit data obtained using a Pirkle-type chiral stationary phase with precision nearly equal to that obtained using carbon dioxide-based mobile phases. Relative elution order of the enantiomers and relative effectiveness of a variety of additional modifiers were correctly predicted. When cellulosic chiral stationary phases were used under normal phase conditions, the model was far less accurate. Under these circumstances, the mobile

Table 10
Modeling separation factors for DMP 963 and DMP 961

Modifier	DMP 961		DMP 963	
	α_{EXP}	α_{PRED}	α_{EXP}	α_{PRED}
Modifiers used to build original model				
Methanol	1.29		2.01	
Ethanol	1.44		2.57	
n-Propanol	1.89		3.60	
Isopropanol	3.37		6.30	
Modifiers suggested by the model				
Isobutanol	3.34	3.28	6.84	6.65
2-Methyl-2-butanol	6.37	6.28	13.78	13.25

phase's effect on the tertiary structure of the cellulosic stationary phase is surmised to be the root cause of the failure. Empirical models, such as those developed in this study, allow prediction of selectivity factors for alcohol modifiers beyond those used to develop the models.

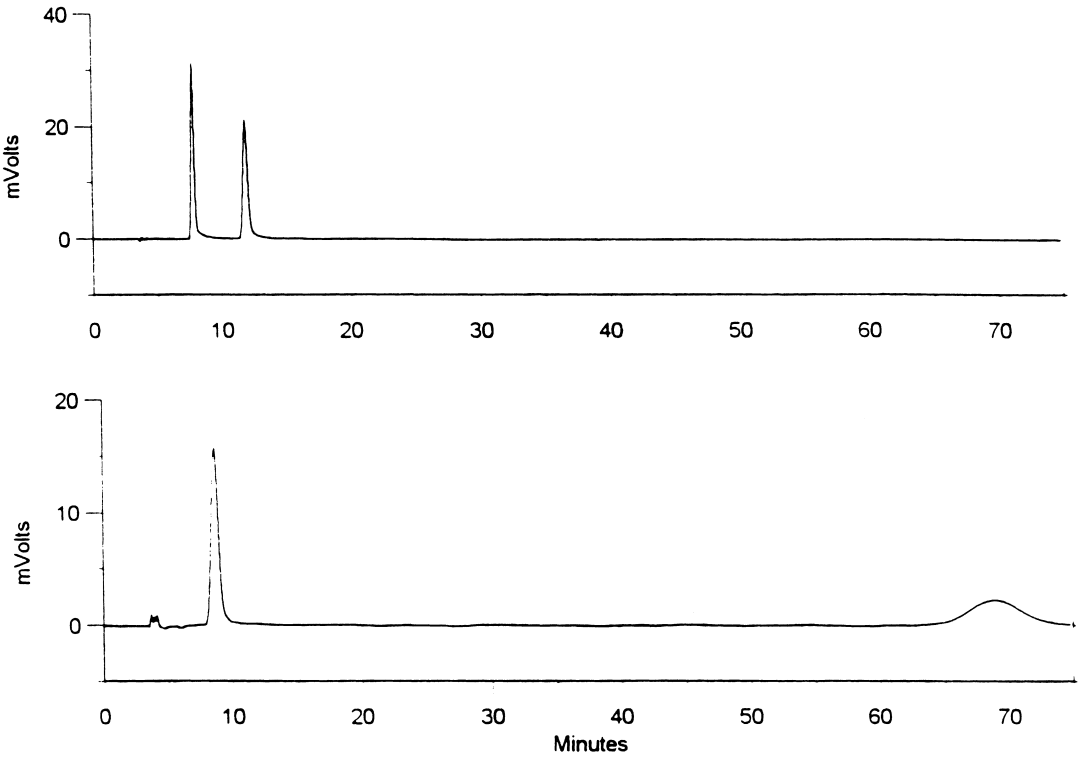


Fig. 3. Separation of DMP 963 using various modifiers: (a) methanol, and (b) 2-methyl-2-butanol. Separations performed on a Chiralpak AS column (250×4.6 mm) with carbon dioxide–modifier (75:25) with a flow-rate of 1.0 ml/min at 20°C with a backpressure of 200 bar.

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