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Journal of Chromatography A, 1088 (2005) 57–66
JOURNAL OF CHROMATOGRAPHY A
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Comparative study of three teicoplanin-based chiral stationary phases using the linear free energy relationship model

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Available online 18 April 2005

Abstract

Teicoplanin (T) is a macrocyclic glycopeptide that is highly effective as a chiral selector for enantiomeric separations. In this study, we used three teicoplanin-based chiral stationary phases (CSPs) – native teicoplanin, teicoplanin aglycon (TAG) and recently synthesized methylated teicoplanin aglycon (MTAG). In order to examine the importance of various interaction types in the chiral recognition mechanism the three related CSPs were evaluated and compared using a linear free energy relationship (LFER). The capacity factors of 19 widely different solutes, with known solvation parameters, were determined on each of the columns under the same mobile phase conditions used for the chiral separations. The regression coefficients obtained revealed the magnitude of the contribution of individual interaction types to the retention on the compared columns under those specific experimental conditions. Statistically derived standardized regression coefficients were used to evaluate the contribution of individual molecular interactions within one stationary phase. It has been concluded that intermolecular interactions of the hydrophobic type significantly contribute to retention on all the CSPs studied here. Other retention increasing factors are n- and π-electron interactions and dipole–dipole or dipole-induced dipole ones, while hydrogen donating or accepting interactions are more predominant with the mobile phase than with the stationary phases. However, these types of interactions are not equally significant for all the CSPs studied.
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Keywords: LFER; HPLC; Chiral stationary phases; Macrocyclic glycopeptide; Teicoplanin; Teicoplanin aglycon; Methylated teicoplanin aglycon

1. Introduction

Macrocyclic glycopeptides are one of the fastest growing classes of chiral selectors nowadays. Chiral stationary phases (CSPs) based on macrocyclic glycopeptides have shown an excellent ability to separate various classes of racemic compounds (such as underivatized amino acids, acidic and also basic drugs) [1–3]. The structure of macrocyclic antibiotics possesses many functional groups (for example hydroxyl, amine, amide linkages, carboxylic acid, aromatic moieties and hydrophobic pockets) that offer different molecular interactions, including hydrophobic, ionic, hydrogen bonding, dipole–dipole, π–π and steric interactions.

A molecule of the glycopeptide teicoplanin (T) consists of an aglycon peptide "basket" with three attached carbohydrate moieties. The bulky saccharide moieties restrain access to hydrophobic "basket" that provides important interaction sites (Fig. 1A). On the other hand, the size and mobility of the saccharides allow steric repulsive interactions and their hydroxyl groups provide hydrogen binding sites. A modified form, teicoplanin aglycon (TAG) has the same aglycone "basket" but in comparison with teicoplanin it lacks the three carbohydrate units and an alkyl chain connected to one saccharide moiety (Fig. 1B). The steric effects of the carbohydrates disappear and so the aglycon becomes more accessible for some analytes. In addition, three new OH-groups are produced on the aglycone where the three saccharides are removed. Stronger interactions between exposed functional groups on the rim of the aglycon basket and some solutes can cause poor

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doi:10.1016/j.chroma.2005.03.105

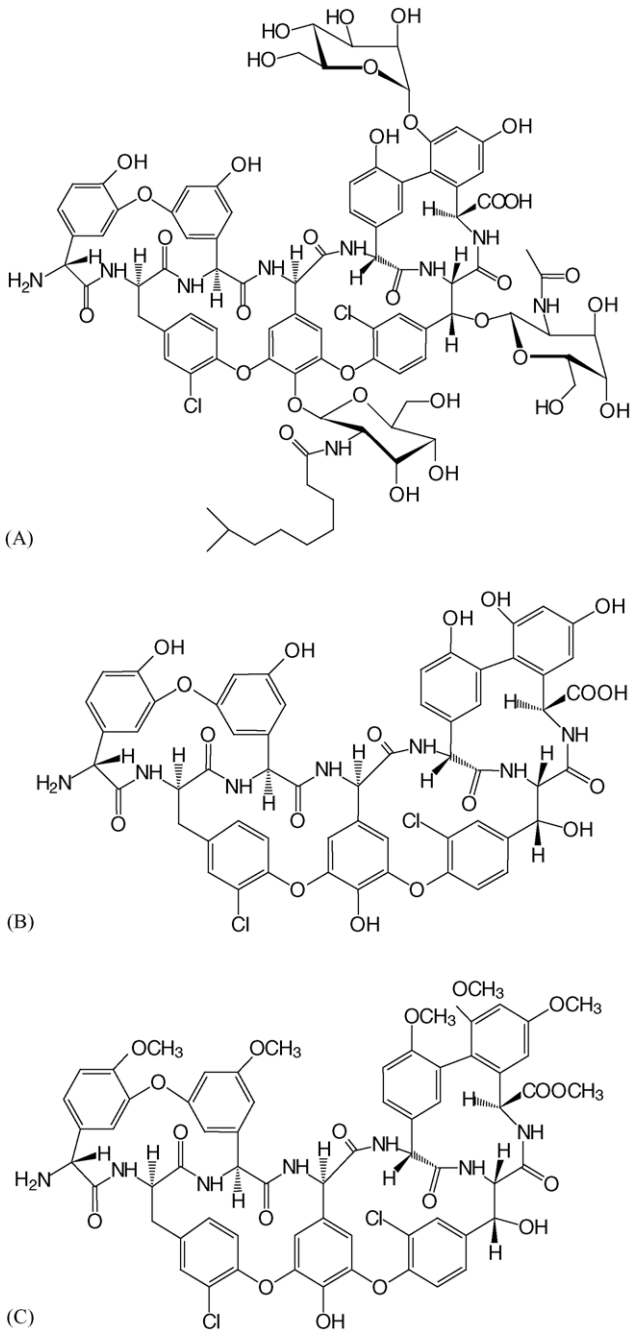

Fig. 1. Structures of the macrocyclic antibiotics: (A) teicoplanin; (B) teicoplanin aglycone and (C) proposed structure of methylated teicoplanin aglycon.

mass transfer. As the result improved selectivity but on the other hand reduced separation efficiencies of certain kinds of amino acids and their derivatives can be observed [4]. The separation efficiency could be improved by methylation of teicoplanin aglycon to block the hydrogen bonding groups. In the case of the recently prepared methylated teicoplanin aglycon (MTAG) the strong hydrogen bonding interactions can be thereby reduced (Fig. 1C). Methylation is realized using methyltriflate ($CH_3CF_3SO_3$) preferentially reacting with amines and alcohols, and diazomethane (CH_2N_2) modifying carboxylic acid and phenolic groups (see Section 2) [5].

One of the methods used for characterization and comparison of various reversed phase stationary phases is the linear free energy relationship (LFER) [6]. The LFER model has proved to be a useful tool for the analysis of solvation phenomena but it can be used also to characterize retention in various separation systems ranging from gas chromatography [7–10], high pressure liquid chromatography [11–15] to micellar capillary electrophoresis [16] and capillary electrochromatography [17]. Numerous reports were published on the application of LFER in comparative studies of stationary phase properties in HPLC in recent years [11–15,18]. Through LFER it is possible to gain insight into the molecular interactions that affect separations in a given chromatographic system and to elucidate differences in specific analyte-stationary phase interactions that are most important for retention on individual columns. The LFER method relates the phase transfer process of the analyte to the change of the Gibbs energy in the system [19]. The Gibbs energy related term can be separated into several molecular terms that are responsible for the individual interactions. The equation of LFER expresses then the relationship between the retention parameters determined for a representative series of analytes in a given separation system (e.g. retention factor) and the solute parameters (descriptors) [20]:

$$\log k = c + vV_x + a\sum \alpha_2^H + b\sum \beta_2^H + s\pi_2^H + rR_2 \quad (1)$$

The independent variables in Eq. (1) are solute descriptors as follows: V_x is the McGowan characteristic volume [21] in units of $cm^3\,mol^{-1}/100$, $\sum \alpha_2^H$ is the effective or overall hydrogen bond (HB) acidity [22], $\sum \beta_2^H$ is the effective or overall hydrogen bond basicity [22], π_2^H is the solute dipolarity/polarizability parameter [22] and R_2 is the excess molar refraction. The descriptors characterize properties of the solute molecule and account for the differences among them. The representative series of analytes should cover a wide range of interactions [23,24]. Therefore, solutes should be structurally diverse and the distribution of individual descriptors should be equal so that no interaction is preferred.

The regression coefficients in Eq. (1) reflect the different types of molecular interactions in a specific system, i.e., for the given LC column and mobile phase composition. Since Eq. (1) is applied to the distribution between the two phases in HPLC, the coefficients refer to differences in the properties between the stationary phase and mobile phase. The value v reflects the difference in hydrophobicity between the stationary and the mobile phases; a refers to the difference in hydrogen bond basicity between the stationary and the mobile phases; b is equal to the difference in the hydrogen-bond donating properties between the stationary and the mobile phases; s reflects difference in dipolarity/polarizability between the phases and r reflects the difference in propensity of the stationary and mobile phases to interact with solute n- and π-electron pairs. Therefore, various stationary phases

can be compared only in separation systems using the same mobile phase. The *c* intercept does not reflect any interaction. This coefficient involves various parameters affecting retention that are not expressed by regression coefficients [18].

All calculated regression coefficients are usually taken into account, also those that are not statistically significant. The model involving all regression coefficients is less precise and gives different results from those obtained by the optimal model handling just the statistically significant regression parameters.

A comparison of different stationary phases is possible by using the regression coefficients (if the columns that are compared are examined at the same temperature and mobile phase conditions). If the contributions of the various interactions within the scope of one stationary phase are studied, the use of regression coefficients is not accurate. The regression coefficients have different units, means and standard deviations in the solute equation. Statistically derived standardized coefficients equilibrate influences of the different units, their mean values are zero and the standard deviations are the same for all of them. The rigorous approach is thus, to use the ordinary regression coefficients to compare the different stationary phases and the standardized regression coefficients to analyze the various interactions within one stationary phase.

This work is focused on a study of the separation properties of teicoplanin-based chiral stationary phases. The aim of the study is to characterize and compare three chiral stationary phases: teicoplanin CSP, teicoplanin aglycon CSP and the recently synthesized methylated teicoplanin aglycon CSP using the linear free energy relationship. The results obtained by the different statistical approaches mentioned above are compared and discussed. The work is aimed at elucidating the molecular mechanism of retention (revealing the types of interactions responsible for the retention). Finally, the LFER parameters are correlated with results of enantioselective separations of certain analytes on the teicoplanin-based chiral stationary phases.

2. Experimental

2.1. Equipment and chromatographic conditions

All measurements were performed on a Delta Chrom SDS 030 liquid chromatograph (Watrex, Prague, Czech Republic) consisting of a SDS 030 pump, Rheodyne 7125 injector with 20 μl loop and an UV–vis detector. Clarity 2.1 software was used for process control and data handling. The flow rate was set to 0.6 ml/min, the temperature was $22 \pm 1\,^{\circ}C$. Compounds were detected at 254 and 214 nm. The dead time was determined using the system peak.

2.2. Columns

Three teicoplanin-based chiral stationary phases were compared. The teicoplanin column (250 mm × 4.6 mm, particle size 5 μm, pore size 8 nm) and teicoplanin aglycon column (250 mm × 4.6 mm, particle size 5 μm, pore size 8 nm) were manufactured in ASTEC (Whippany, NJ, USA). The sorbent of methylated teicoplanin aglycon column (150 mm × 4.6 mm, particle size 5 μm, pore size 12 nm) was synthesized in the Department of Chemistry of the State University of Iowa (Iowa, USA) and the column was packed by ASTEC. See the structures of the chiral selectors in Fig. 1.

2.3. Chemicals and solutions

Methanol (MeOH) for HPLC was purchased from Sigma-Aldrich (Sigma-Aldrich, Prague, CR). Triethylamine (purity >99.5%) (TEA) and glacial acetic acid (purity >99%) were products of Fluka (Fluka, Prague, CR). Water was prepared with a Milli-Q water purification system (Millipore, Milford, MA, USA).

The test solutes for LFER were of analytical grade purity and were obtained from Sigma–Aldrich (Sigma–Aldrich, St. Louis, USA). List of the 19 solutes and their corresponding descriptors pertaining to Eq. (1) are shown in Table 1.

The racemic analytes: tyrosine, *N-tert*-butyloxycarbonyl tyrosine (*t*-BOC-tyrosine), 2-(2-chlorophenoxy) propionic acid, 2-(3-chlorophenoxy) propionic acid and 2-(4-chlorophenoxy) propionic acid for chiral separations evaluated in this study, all of p.a. purity, were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA).

The 1% triethylamine acetate buffer (TEAA) was prepared by titration of 1% (by volume) aqueous solution of TEA with acetic acid to pH 4.20. Mobile phase was prepared by mixing 20 volume parts of methanol and 80 volume parts of the buffer.

Table 1
Test solutes and their solvation parameters

Solut	V_x	$\sum \alpha_2^H$	$\sum \beta_2^H$	π_2^H	R_2
Phenol	0.775	0.60	0.30	0.89	0.81
Benzamide	0.973	0.49	0.67	1.50	0.99
2-Naphthol	0.144	0.61	0.40	1.08	1.52
Resorcinol	0.834	1.10	0.58	1.00	0.98
Benzophenone	1.481	0.00	0.50	1.50	1.45
Hydroquinone	0.834	1.16	0.60	1.00	1.00
o-Cresol	0.916	0.52	0.31	0.86	0.84
Benzonitrile	0.871	0.00	0.33	1.11	0.74
m-Cresol	0.916	0.57	0.34	0.88	0.82
Benzaldehyde	0.873	0.00	0.39	1.00	0.82
Benzyl alcohol	0.916	0.33	0.56	0.87	0.80
Toluene	0.857	0.00	0.14	0.52	0.60
o-Toluidine	0.957	0.23	0.45	0.92	0.97
Benzene	0.716	0.00	0.14	0.52	0.61
Naphthalene	1.085	0.00	0.20	0.92	1.34
Catechol	0.834	0.85	0.52	1.07	0.97
Dibenzothiophene	1.379	0.00	0.18	1.31	1.96
Nitrobenzene	0.891	0.00	0.28	1.11	0.87
Ethylbenzene	0.998	0.00	0.15	0.51	0.61

Note: The correspondent descriptors were obtained from the literature [24]. McGowan characteristic volume was calculated from atom and bond contributions according to the ref. [21].

Stock solutions of solid samples were prepared in concentration 1 mg/ml and stock solutions of liquid samples in concentration 20 μl/ml using methanol as a solvent. These stock solutions were afterwards diluted to obtain roughly equivalent detection signals of all the test compounds.

2.4. Data collection and processing

All retention times of the test solutes were measured in triplicate using the same methanol–1% TEAA buffer, pH 4.20 (20/80, v/v) mobile phase. The retention factors were calculated from the peak maxima. The average standard deviation of all the measurements of the retention factor (k) was less than 1.5%.

The regression coefficients in the LFER equation Eq. (1) were obtained from a series of measurements of the retention data of the set of 19 structurally different test solutes with known descriptors (see Table 1). At least three to four solutes should be used for each regression coefficient [18]. The resulting values were calculated for each separation system by multiple linear regression analysis of log k against the solute descriptors using NCSS software (NCSS, Kaysville, UT, USA). Complete model utilizing all regression coefficients, as well as, the optimal model handling just the statistically significant regression parameters' values were used. The optimal model is statistically derived through the use of the algorithm "All Possible Regressions" that fits all regressions involving one regressor, two regressors, three regressors, etc. Once the procedure finishes, the optimal model is determined [25].

3. Results and discussion

Enantioseparations of several diverse chiral solutes were performed using various mobile phase compositions on the teicoplanin, teicoplanin aglycon and methylated teicoplanin aglycon CSPs. Subsequently, the mobile phase composed of methanol and 1% TEAA, pH 4.20 (20/80, v/v) was selected for the following study, since considerable differences in enantioseparations on the individual CSPs were observed under these conditions. Different retentions and enantioresolutions (see Table 2) are caused by diverse types and magnitudes of the analyte-stationary phase interactions. Therefore, the linear free energy relationship method applied to this particular separation system is an attempt to elucidate the specific interactions responsible for retention and enantioresolution.

Since interactions between the analytes and the chiral selectors should be dominant, it was necessary to eliminate the unfavourable silanophilic activity. Thus, the silanophilic interactions were reduced by using a buffer solution with triethylamine [26].

3.1. Chiral separation using the teicoplanin-based CSPs

In order to evaluate the effect of the variations in the analyte structure on enantioselectivity, two sets of analytes were chosen: (1) tyrosine versus *t*-BOC-tyrosine and (2) three structural isomers of chlorophenoxypropionic acid. The chromatographic results are summarized in Table 2. Fig. 2 shows the different separation behaviours of tyrosine and its N-blocked analogue. While tyrosine can be enantioresolved, with different R-values, on all three chiral stationary phases, blocking of the amino group of the amino acid with a non-polar substituent eliminates the separation on the TAG and MTAG CSPs under these experimental conditions.

Table 2
The separation parameters of the chiral solutes using T, TAG and MTAG CSPs; $k(S)$, retention factor of (S) enantiomer; $k(R)$, retention factor of (R) enantiomer; R, resolution

Solute/chiral stationary phase		T	TAG	MTAG
(*R*,*S*)-Tyrosine	$k(S)$	0.27	0.71	0.70
	$k(R)$	0.37	1.4	1.35
	R	1.45	3.81	2.75
(*R*,*S*)-*t*-BOC-Tyrosine	$k(S)$	0.89	2.89	3.27
	$k(R)$	1.16	2.89	3.27
	R	2.05	0.00	0.00
(*R*,*S*)-2-(2-Cl Phenoxy) propionic acid	k	0.75	2.75	2.65
		1.04	4.05	3.84
	R	1.07	1.87	1.41
(*R*,*S*)-2-(3-Cl Phenoxy) propionic acid	k	0.89	3.4	3.23
		1.05	4.65	4.32
	R	0.57	1.48	1.09
(*R*,*S*)-2-(4-Cl Phenoxy) propionic acid	k	0.85	3.34	3.11
		1.26	4.63	5.37
	R	1.43	1.73	1.82

Note: The *S*-forms of tyrosine and *t*-BOC-tyrosine elute first which is in agreement with the literature [1].

The isomers of the chlorophenoxy-propionic acid were almost baseline separated on all three CSPs (see Table 2). The different resolution values of these isomers on these columns reflect the importance of the steric compatibility between the derivatives and the CSPs. They also show that different special interactions are involved in the retention mechanism on these columns, which are the subject of the following LFER study.

The relatively broad peak width of the chiral compounds was attributed to heterogeneous sorption kinetics [27]. This result flows from the investigation of the adsorption isotherm at concentration ranges where non-linear effect appears [27,28]. The peak asymmetry, especially of the second eluted enantiomer, may indicate interactions with additional (at least two others) chiral adsorption sites in the stationary phase contributing to retention.

3.2. The LFER model

The unique advantage of the LFER approach is in its ability to independently quantify the contributions of individual

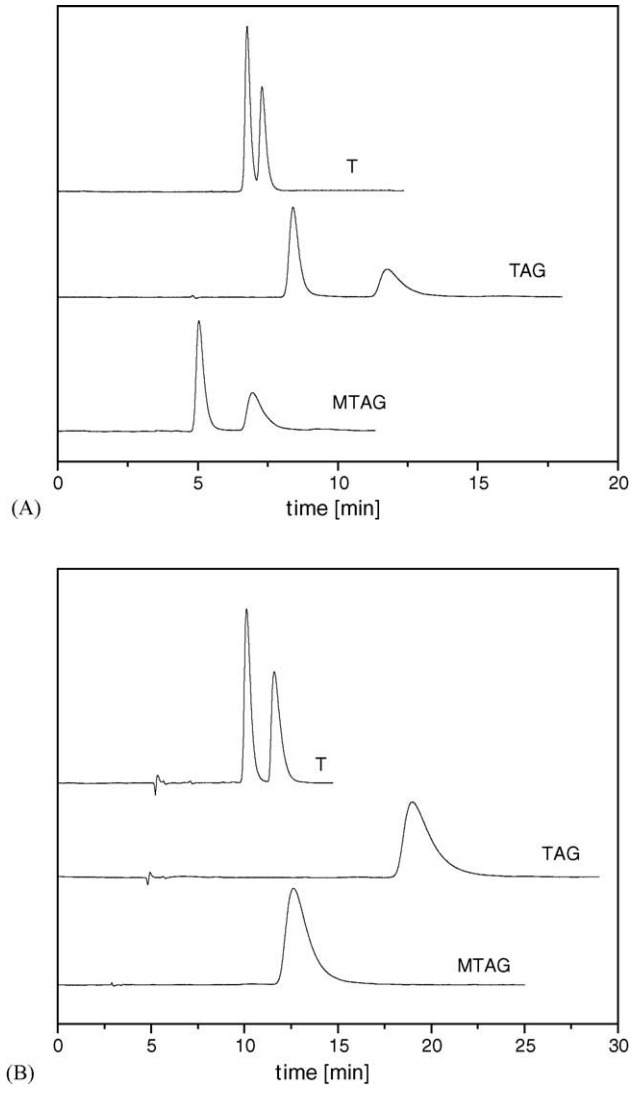

Fig. 2. Chromatograms of (A) tyrosin and (B) *t*-BOC-tyrosine on the teicoplanin-based CSPs. Conditions: mobile phase methanol–1%TEAA, pH 4.20 (20:80, v/v); 0.6 ml/min; UV detection at 254 nm; temperature 22 °C.

types of molecular interactions to retention. As has been described in Section 1, the differences in certain types of interactions of analytes between the stationary phase and the mobile phase are characterized by the regression coefficients of Eq. (1).

The regression coefficients of the complete LFER calculated for the three columns investigated are summarized in Table 3. Plots of the experimental $\log k$ against calculated $\log k$ values for all three stationary phases show no serious outliers thereby indicating that the LFER model strongly correlates with experimental results. The equations of the linear regressions were as follows: $\log k_{calc} = -0.01 + 0.93 \log k_{exp}$, $R = 0.97$, standard deviation (SD) = 0.09 for T CSP; $\log k_{calc} = 0.03 + 0.94 \log k_{exp}$, $R = 0.97$, SD = 0.11 for TAG CSP and $\log k_{calc} = 0.03 + 0.96 \log k_{exp}$, $R = 0.98$, SD = 0.10 for MTAG CSP. The regression coefficients obtained from the optimal LFER are shown in Table 4. The *p*-values in

Table 3
Regression coefficients of the complete LFER equation (± 95% confidence interval) and statistical parameters of individual equations

	v	(95% CI)	*p*	*a*	(95% CI)	*p*	*b*	(95% CI)	*p*	*s*	(95% CI)	*p*	*r*	(95% CI)	*p*	*c*	(95% CI)	*p*	*R*
T	0.91	(±0.65)	0.010	−0.39	(±0.26)	−0.007	−0.55	(±0.71)	0.120	0.35	(±0.41)	0.091	0.02	(±0.39)	0.909	−1.03	(±0.39)	0.000	0.966
TAG	1.05	(±0.78)	0.012	−0.57	(±0.32)	0.002	−0.09	(±0.86)	0.829	−0.07	(±0.50)	0.777	0.42	(±0.47)	0.072	−0.57	(±0.47)	0.022	0.970
MTAG	1.16	(±0.70)	0.004	−0.32	(±0.29)	0.034	−1.07	(±0.77)	0.010	0.40	(±0.45)	0.077	0.22	(±0.42)	0.272	−0.67	(±0.43)	0.005	0.977

Note: *R* is the correlation coefficient, *p* is statistical *p*-value.

Table 4
Regression coefficients of the optimal LFER equation (± 95% confidence interval) and statistical parameters of individual equations

	v	(95% CI)	*p*	*a*	(95% CI)	*p*	*b*	(95% CI)	*p*	*s*	(95% CI)	*p*	*r*	(95% CI)	*p*	*c*	(95% CI)	*p*	*R*
T	0.93	(±0.40)	0.000	−0.38	(±0.20)	0.001	−0.57	(±0.57)	0.051	0.36	(±0.41)	0.036	X			−1.04	(±0.30)	0.000	0.966
TAG	0.98	(±0.69)	0.009	−0.61	(±0.19)	0.000	X			X			0.42	(±0.36)	0.025	−0.57	(±0.43)	0.013	0.970
MTAG	1.44	(±0.45)	0.000	−0.22	(±0.22)	0.053	−1.29	(±0.65)	0.001	0.53	(±0.38)	0.010	X			−0.80	(±0.35)	0.000	0.975

Note: *R* is the correlation coefficient, *p* is statistical *p*-value. Symbol **X** means insignificant interaction.

Tables 3 and 4 represent the significance of the individual coefficients. Comparing the *p*-values of the complete model (Table 3) and the optimal model (Table 4), we can see, that *p*-values are lower in the case of the optimal model. Thus, the regression coefficients are more significant for the optimal model [25]. The data in Table 4 are more reliable because only statistically significant coefficients were included in the LFER model. Obviously, including statistically insignificant coefficients decreases the precision of the other regression coefficients.

The standardized coefficients of the optimal model are depicted separately for each stationary phase in Fig. 3A–C. As was described in Section 1 these coefficients should be used for comparison of various types of interactions within one stationary phase.

3.3. Comparison of the teicoplanin-based chiral stationary phases using LFER

A term-by-term analysis of the LFER results yields a quantitative measure of the contributions of the individual solute-stationary phase interactions to retention. A positive coefficient value reflects that the given molecular interaction is stronger in the stationary phase and so it increases retention of solutes. A negative coefficient reflects stronger interaction of the solutes with the mobile phase. See Table 4 for the regression coefficients used to compare the interactions among the different chiral stationary phases and the standardized coefficients in Fig. 3 to compare the various interactions on the same CSP.

The major contribution toward retention for all the three CSPs is hydrophobicity. The *v* parameter is absolutely dominant in the case of MTAG. This highest *v*-value, compared to the T CSP and the TAG CSP, reflects the strongest dispersion interaction of solutes with the MTAG stationary phase. This increase in the dispersion interactions (relative to the teicoplanin and TAG CSPs) is related to methylation of carboxylic acid and phenolic groups of TAG.

The hydrophobicity of the teicoplanin-based stationary phases decreases in the following sequence: MTAG > TAG > T. Although teicoplanin has a hydrophobic alkyl chain connected to one saccharide moiety its hydrophobicity calculated from LFER is lower than the hydrophobicity of the teicoplanin aglycon that lacks this hydrophobic chain. This result can be explained by the hydrophilic nature of the saccharides that are present only in the teicoplanin molecule. Moreover, the hydrophobic chain of teicoplanin may not be exposed to the hydrophilic (water rich) mobile phase but it should be rather shielded in the cavity (and thus not available for hydrophobic interaction with solutes).

The next significant contribution to retention on the TAG CSP is the n- and π-electron interactions expressed by the positive value of coefficient *r*. The n- and π-electron containing groups (aromatic rings, carbonyl groups) are most accessible on TAG. The *r* coefficients are insignificant for T and for MTAG CSPs (Table 4 and Fig. 3). This means

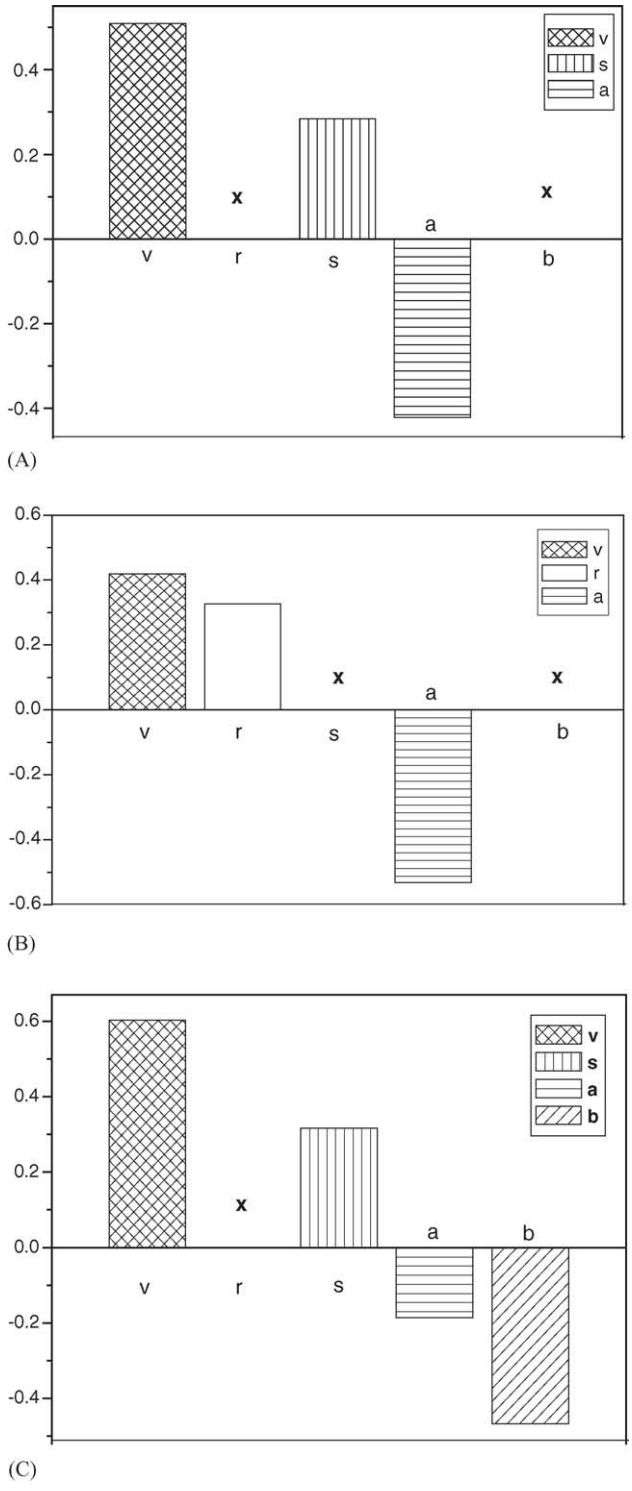

Fig. 3. Comparison of the standardized coefficients for (A) teicoplanin (B) teicoplanin aglycon (C) methylated teicoplanin aglycon chiral stationary phases in methanol–1%TEAA, pH 4.20 (20:80, v/v). The regression coefficient values were obtained from the optimal LFER equation. Symbol **x** means insignificant interaction. Standard deviation is the same for all coefficients and is equal to (A) 0.10, (B) 0.13 and (C) 0.10.

that the n- and π-electron interactions of the solutes are the same in the stationary phase and in the mobile phase. It is important to mention here that in the reversed phase mode the surface of the stationary phase is modified by sorption of the mobile phase. The extent of the sorption depends on both the stationary phase and the mobile phase composition. (If an achiral apolar octadecyl silane stationary phase is used, then mainly the organic modifier, MeOH, is adsorbed on the surface. The situation is slightly different with MA based CSPs, which are more polar, and so greater portion of the polar component of the mobile phase will be sorbed on it.) The higher *r* coefficient on the TAG CSP may be related to the proposition that it is more poorly activated by the mobile phase.

The other retention increasing factors are dipole–dipole and dipole-induced dipole interactions that are significant for the MTAG and T CSPs (viz. Fig. 3 and Table 4). The high polarizibility of these stationary phases is also influenced by the high polarity mobile phase that is adsorbed on the stationary phase surface. The lowest value of the coefficient *s* on TAG is then in accord with the above-mentioned lower sorption of the mobile phase on this CSP.

The other interactions involved in the model, i.e. hydrogen bond donor and acceptor interactions lower the retention. This is due to the fact that water present in the mobile phase is a very strong hydrogen bond acid and methanol is both a hydrogen bond acid and base. The negative a coefficients (see Table 4 and Fig. 3) indicate that this type of interaction of solutes is preferred in the mobile phase. The sorption of the mobile phase on the stationary phase increases its basicity. Again, the highest absolute value of the *a* coefficient, on the TAG stationary phase, apparently reflects the lowest solvation effect of the mobile phase there. As can be seen in Fig. 3 the *b* coefficient is significant only on the MTAG column. Methylation of the carboxylic acid group and phenolic groups contributes to the smallest acidity of the MTAG stationary phase.

Fig. 4 shows the contributions of the individual molecular interactions to retention (sum of 100%) on each of the studied CSPs. The absolute values of the standardized regression coefficients of the optimal model (in %) are used for this purpose. These results show that T and MTAG CSPs offer quite similar interaction possibilities with the exception of acidity and basicity. However, the ratio of the various interaction types on these two stationary phases is not equal. By contrast the interactions responsible for retention on the TAG CSP are completely different.

Generally, comparing the teicoplanin-based CSPs to achiral RP-HPLC systems (for example ref. [15]) the following differences can be found: achiral RP-HPLC systems provide markedly higher *v* coefficient values than the teicoplanin-based CSPs. On the contrary, a much greater diversity of intermolecular interactions contribute to retention on these chiral stationary phases compared to the achiral ones. Significantly different hydrogen bond acidity, basicity, and dipolar interactions can be observed on these CSPs. Numerous in-

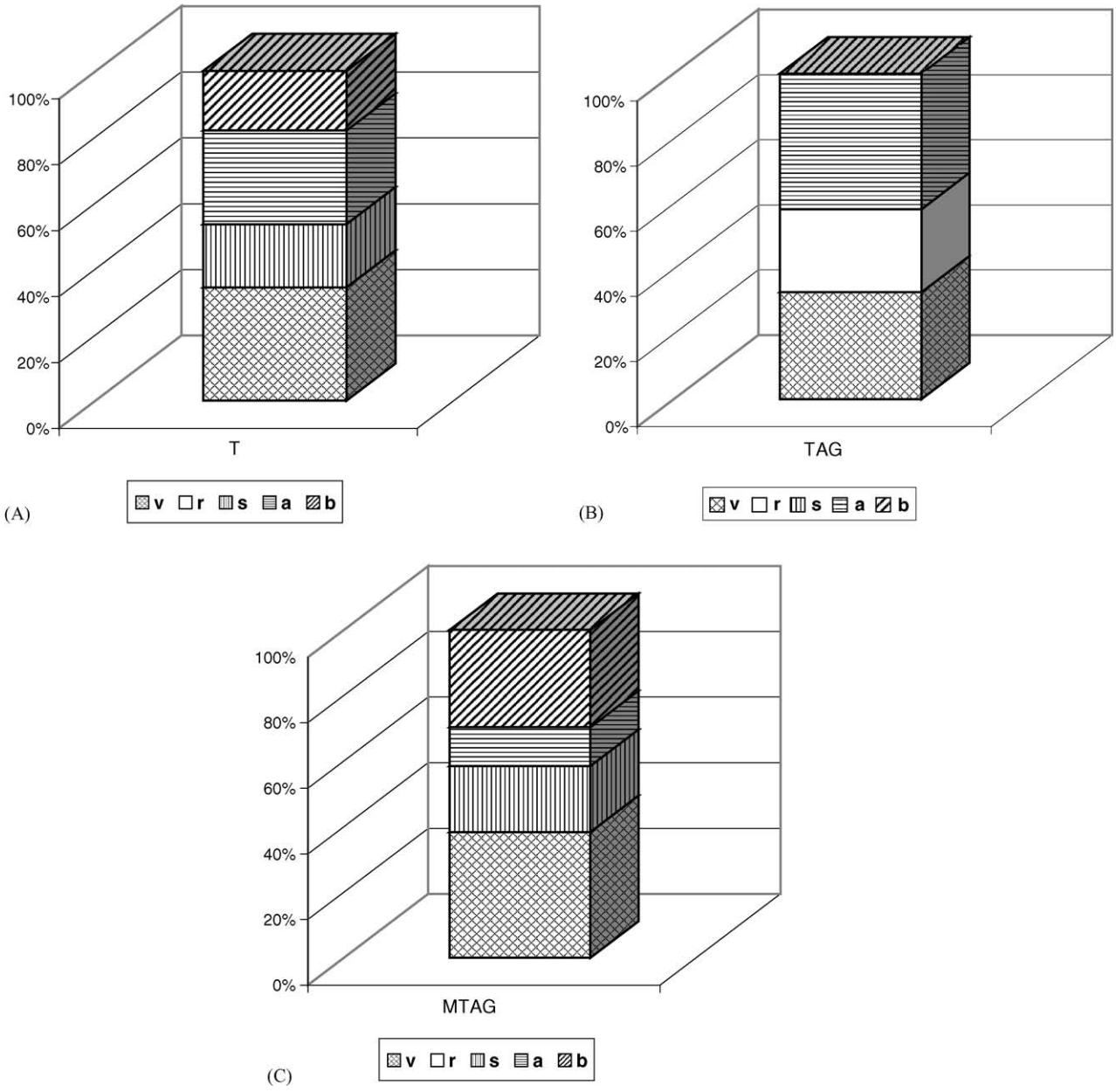

Fig. 4. The comparison of the ratio of various interactions for the (A) teicoplanin, (B) teicoplanin aglycon and (C) methylated teicoplanin aglycon chiral stationary phases. The ratio are determined using standardized coefficient of the optimal model.

teractions affecting the retention are important for stereoselective discrimination but on the other hand they can cause decreases in the separation efficiency.

3.4. The utilization and limitation of LFER results for chiral separations

As the LFER model is based on the Gibbs energy relation it characterizes the whole separation system under the given experimental conditions. The influence of the mobile phase on a separation is often studied and described in the literature because it is a fundamental parameter. The regression coefficients are calculated then for various mobile phase compositions. The relation of the changes of regression coefficients obtained in this way enables the choice and optimization of the separation system. Here we investigated the extent of interactions under just one mobile phase composition because the work was aimed at the evaluation of the differences of the three CSPs under the given condition that was used for chiral separations.

The LFER is a general method for characterization of separation systems. Of course, there are certain limitations of the LFER approach. For example ion exchange or Lewis acid/base interactions are not included in the model [29]. Another imprecision can originate from the descriptor V_x that combines dispersion and cohesivity [30].

The application of LFER to chiral separations is not explicit. It must be noted that for enantioresolution is important that there is a difference in the interaction energies of the individual enantiomers. Nevertheless, it is possible to utilize the regression coefficients for estimation of retention and possibly chiral separation. While prediction of retention of solutes is straight forward for prediction of enantioselective separation the structure of the analyte, mainly the functionalities and bulky groups surrounding the stereogenic centre, must be taken into account if related to the LFER results describing the separation system. As was discussed previously, hydrophobic interactions markedly contribute to retention on the T, TAG and MTAG CSPs in the reversed phase separation mode. Therefore, the increase of retention of *t*-BOC-tyrosine with a bulky hydrophobic group clearly follows the order of v values on the compared CSPs, MTAG > TAG > T (see Table 2). However, for chiral recognition at least two additional simultaneous interactions are necessary. (These interactions can be both attractive and repulsive, also inclusion or exclusion can be assumed a type of complex interaction.)

Let us consider now for example the phenoxypropionic acids. The π- and n-electron interactions characterized by the r coefficient value contribute to the great retention on the TAG stationary phase, while they are insignificant on the T and MTAG CSP. This result reflects the accessibility of the aromatic rings in TAG. The electron interactions can contribute then to the greatest chiral resolution of the structural isomers of the chlorophenoxypropionic acid on the TAG CSP (Table 2). The influence of the polarity/polarizibility coefficient, in addition to the v coefficient, can explain the second highest retention and resolution values of these isomers on the MTAG column.

The role of hydrogen bonding basicity (regression coefficient a) and acidity (regression coefficient b) in the interaction mechanism cannot be overlooked. The contribution of this type of interaction to chiral recognition depends, of course, on the structure of the analyte to be separated. Due to significant values of the regression coefficients a on all the teicoplanin-based chiral stationary phases their ability to separate acidic enantiomers is obvious. The highest absolute a-value on the TAG CSP will favour enantioresolution of compounds possessing an acidic group in a suitable position to the chiral centre on this CSP. The highest absolute value of the hydrogen bonding acidity obtained on MTAG can also help in the separation of analytes with basic functionalities.

The results of the enantioseparations on these teicoplanin-based CSPs surely depend on other factors like the size of solute with respect to the size of the chiral selector's cavity or on the compatibility of steric configuration of chiral centres in the aglycon basket and the structure of the analyte. The perfect fit of an analyte to the cavity is required for successful enantioseparation in the reversed phase mode. The size of the chiral selector cavity could be probably added as a new parameter to the LFER equation (or it can be considered separately) to extend the applicability of this equation for stereoselective interaction. This possibility is a subject for further detailed study.

Despite the limitations of LFER, it is a complex method that is useful for comparison of different separation systems including chiral stationary phases.

Acknowledgements

The work was financially supported by the Grant Agency of the Czech Republic, grant no. 203/03/0161 and by the Ministry of Education, Youth and Physical Training of the Czech Republic, research project MSM J 13/98: 1131000001. Also support of this work by the National Institutes of Health, NIH RO1 GM53825-08, is gratefully acknowledged. The authors want to thank Dr. Jarmila Zocová for her advice on the statistical procedures.

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