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Stationary phase effects on the dynamic affinity of low-molecular-mass displacers

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Abstract

In this paper, the selectivity of a variety of cation-exchange stationary phases was investigated using a homologous series of displacer molecules based on pentaerythritol. These displacers were derived from pentaerythritol and contained either four trimethyl ammonium groups [pentaerythrityl-(trimethylammonium chloride)$_4$, PE(TMA)$_4$], benzene rings [pentaerythrityl-(benzyl dimethylammonium chloride)$_4$, PE(DMABzCl)$_4$], heptyl groups [pentaerythrityl-(heptyl dimethylammonium iodide)$_4$, PE(DMAHepI)$_4$] or cyclohexyl groups [pentaerythrityl-(cyclohexyl dimethylammonium iodide)$_4$, PE(DMACyI)$_4$]. This series enabled us to probe the secondary interactions that can play a role in the affinity of low-molecular-mass displacers for different stationary phases. The relative affinities of these displacers were examined using a displacer ranking plot based on the steric mass action (SMA) isotherm model. While hydrophobicity and aromaticity played important roles in generating the affinity to the hydrophilized polystyrene–divinylbenzene (Source 15S) and polymethacrylate-based (Toyopearl 650M) resins, these secondary interactions had a minimal impact on the selectivity in agarose resins coated with dextran (SP Sepharose XL), ''gel in a shell'' (S Ceramic HyperD F), and monolithic (Bio-Rad Uno S6) cation-exchange materials. Further, the results with a tentacular stationary phase (Fractogel EMD) suggest that the alkyl chains on PE(DMAHepI)$_4$ play an important role in increasing the affinity, possibly because of strong interactions between the alkyl moiety and the polymer matrix as well as between the charged groups and the polyelectrolyte tentacles. The results of this study provide insight into the design of high affinity, low-molecular-mass displacers for different cation-exchange stationary phase materials.

Keywords: Stationary phases, LC; Dynamic affinity; Cationic exchangers; Low-molecular-mass displacers; Pentaerythritol

1. Introduction

Ion-exchange displacement chromatography has been shown to be a promising technique for preparative protein separations [1–3]. In addition, displacement chromatography of proteins has been successfully carried out in hydroxyapatite [4–6] and hydrophobic interaction and reversed-phase chromatographic systems [7,8]. Various classes of displacers, such as polyelectrolytes [9–13], polysaccharides [14,15] and low-molecular-mass dendrimers [16], amino acids [17], and antibiotics [18] have been

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identified for ion-exchange systems. In fact, the ability to use low-molecular-mass displacers has attracted significant attention due to several distinct operational advantages [19]. However, the design of low-molecular-mass high-affinity displacers for the purification of highly retained biomolecules remains a challenge.

It has been shown that retention in ion-exchange systems is not purely based on electrostatic interactions [20–22]. Shukla et al. [23–25] have investigated the effect of displacer chemistry on dynamic affinity in cation-exchange systems. They showed that hydrophobic/aromatic interactions were important in affecting the affinity in certain resin systems. In addition, structural factors effecting displacer efficacy were studied for cation and anion-exchange systems where various displacers were screened for their efficacy using batch displacements and the results were evaluated using quantitative structure efficacy relationships (QSERs) [26,27]. In the present work, we extend the investigation of Shukla et al. to a wide variety of stationary-phase materials where we used four probe molecules to identify the dominant interactions contributing to displacer efficacy on each stationary phase. These materials include: agarose-based stationary phase with bound dextran (SP Sepharose XL), hydrophilized polystyrene–divinylbenzene (PS–DVB) stationary phase (Source 15S), polymethacrylate-based resin (ToyoPearl SP-650 M), ceramic coated polyacrylamide gel-filled resin (S Ceramic HyperD F), polymethacrylate-based resin containing tentacles (Fractogel EMD SO_3 650S) and a monolithic acrylamide-based stationary phase (Bio-Rad UNO S6). This investigation was carried out to provide an insight into the design of low-molecular-mass high-affinity displacers for different classes of stationary phase materials.

2. Theory

The steric mass action (SMA) formalism is a three-parameter model of ion-exchange designed specifically for representation of multicomponent protein–salt equilibrium in ion-exchange chromatography. The SMA formalism represents the adsorption process as a stoichiometric exchange of mobile phase protein and bound counter ions [28]:

$$C_i + \nu_i \bar{Q}_1 \Leftrightarrow Q_i + \nu_i C_1 \quad i = 2, \ldots, \mathrm{NC}$$

where C_i and Q_i refer to the concentration of protein in the mobile phase and on the stationary phase, respectively. C_1 refers to the concentration of salt in the mobile phase, $\bar{Q}_1$ refers to the concentration of bound salt available for exchange and ν is the characteristic charge (average number of sites that a molecule interacts with on the chromatographic surface, i.e. the effective charge of the displacer). The equilibrium constant of the exchange reaction between the solute and the salt counter-ions on the surface, K, may be written as

$$K_{1i} = \left(\frac{Q_i}{C_i}\right)\left(\frac{C_1}{\bar{Q}_1}\right)^{V_i} \quad i = 2, \ldots, \mathrm{NC} \tag{1}$$

In order to evaluate the efficacy of a displacer molecule, it is necessary to determine its dynamic affinity [28]. The dynamic affinity is defined as

$$\lambda = \left(\frac{K}{\Delta}\right)^{1/\nu} \tag{2}$$

where $\Delta = Q_d/C_d$ is the partitioning of the displacer and Q_d and C_d are the displacer concentrations in the stationary phase and mobile phase, respectively. The dynamic affinity is a measure of the ability of a solute to displace another solute at a specific displacement condition (determined by the value of Δ).

Taking the logarithm of both sides of Eq. (2) and rearranging it, the following relation can be written

$$\log \lambda = \frac{1}{\nu} \log K - \frac{1}{\nu} \log \Delta \tag{3}$$

By plotting $\log \lambda$ versus $\log \Delta$, the resulting displacer ranking plot [23] can be employed as a graphical tool for comparing the dynamic affinities of various displacer molecules over a variety of displacement operating conditions (determined by Δ). In other words, the higher the dynamic affinity line at a given Δ, the more efficacious a given molecule will be as a displacer. In this paper, the displacer ranking plot is used to study the relative affinities of a variety of high affinity, low-molecular-mass displacers for different classes of stationary phase materials.

In addition, to relate our discussion of solute affinity to solvent–polymer interactions, solubility parameters [29] were employed. Solubility parame-

ters can be calculated from the structural formula [29]:

$$\delta = \frac{\sum G}{M}\rho \quad (4)$$

where δ is the solubility parameter, G is the molar attraction constant, and ΣG is the sum for all the atoms and groupings in the molecule, ρ is the density and M is the molecular mass.

3. Experimental

3.1. Materials

Sulfopropyl strong cation-exchange columns, Source 15S (100×4.6 mm, 15 μm), SP Sepharose XL and its prototype (100×10 mm, 90 μm) were donated by Amersham Pharmacia Biotech (Uppsala, Sweden). Bulk stationary phase material, Fractogel EMD SO_3 650S (40 μm), S Ceramic HyperD F (50 μm), and ToyoPearl SP-650M (40–90 μm) were donated by EM Separations (Gibbstown, NJ, USA), BioSepra, Life Technologies (Rockville, MD, USA) and Tosoh Biosep (Montgomeryville, PA, USA), respectively. These stationary phases were slurry packed in 100×5 mm columns. A Bio-Rad UNO S6 cation-exchange column (53×12 mm) with strong cation-exchange sulfopropyl functional groups was donated by Bio-Rad (Hercules, CA, USA). Sodium monobasic and dibasic phosphate, and sodium chloride were purchased from Sigma (St. Louis, MO, USA). Pentaerythrityl-(trimethylammonium chloride)$_4$ [PE(TMA)$_4$], pentaerythrityl-(benzyl dimethylammonium chloride)$_4$ [PE(DMABzCl)$_4$], pentaerythrityl-(heptyl dimethylammonium iodide)$_4$ [PE(DMAHepI)$_4$] and pentaerythrityl-(cyclohexyl dimethylammonium iodide)$_4$ [PE(DMACyI)$_4$] were synthesized in Professor Moore's laboratory in the Department of Chemistry at Rennselaer Polytechnic Institute as described elsewhere [24,25]. The structures of these molecules are shown in Fig. 1.

3.2. Apparatus

Linear gradients were run on a Pharmacia fast protein liquid chromatographic (FPLC) system consisting of two P-500 pumps and LCC-500 controller donated by Amersham Pharmacia Biotech. The column effluent was monitored using a Waters 484 UV–Vis absorbance detector (Waters, Milford, MA, USA) and the data were acquired using a QUICKLOG (Version 1.4) chromatography workstation (Strawberry Tree, Sunnyvale, CA, USA). Column effluent fractions during linear gradient runs were collected using a LKB 2212 Helirac fraction collector (LKB Bromma, Sweden).

3.3. Procedures

3.3.1. Determination of SMA parameters for displacers

The linear SMA parameters for displacers were obtained using retention times from linear gradient experiments. Because the displacers have relatively high affinity for these ion-exchange resins, it is more practical to obtain the data from gradient rather than isocratic experiments. Once the retention volumes were obtained, using at least two different gradient conditions, the values were substituted into the following equation to solve for the linear SMA parameters K and ν [24]:

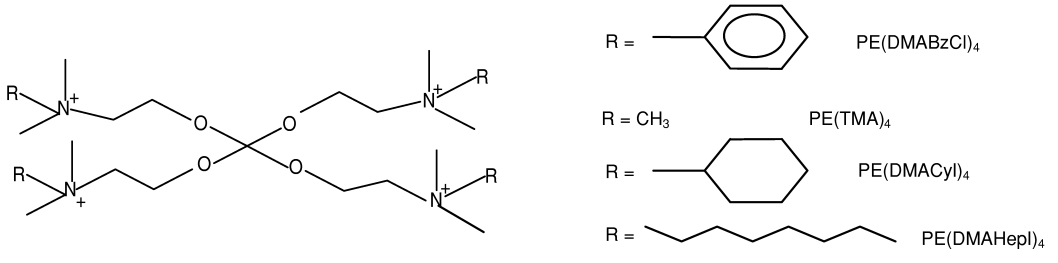

Fig. 1. Probe molecules used to screen the different stationary phase.

$$V_g = \left[\left(x_i^{\nu+1} + \frac{V_m K \epsilon \Lambda^{\nu} (\nu+1)(x_f - x_i)}{V_G} \right)^{\left(\frac{1}{\nu+1}\right)} - x_i \right] \frac{V_G}{(x_f - x_i)} \quad (5)$$

where V_g is the solute retention volume; x_i and x_f are the initial and final salt concentrations of the gradient, respectively; V_G is the total gradient volume; V_m is the dead volume, and ϵ is the total column porosity. Linear gradient experiments were carried out at different times between buffer A (20 m*M* phosphate, pH 6.0) and buffer B (20 m*M* phosphate, pH 6.0, containing 3 M NaCl). For these experiments, 25 μl of 2–3 m*M* displacer solutions were injected.

The ion capacities (Λ) of the stationary phases were determined using a titration method. Column volumes (10–20) of acetic acid at either pH 3.5 or 2.5 (depending on the stability of the stationary phase) were passed through the column. This treatment was followed with ten column volumes of deionized water. Additional column volumes (50–60) of 1 M KNO_3 were then passed through the column and the column effluent was collected. The column effluent was finally titrated against 0.01 M NaOH using phenolphthalein as an indicator. The ionic capacities of stationary phases employed in this work are presented in Table 1 (Note: the units are per stationary phase volume which does not include the volume in the pores or in the interstitial space of the column).

3.3.2. Displacer analysis

UV–Vis absorbance was used to monitor the column effluent during linear gradient analysis of PE(DMABzCl)$_4$. For the non-UV absorbing displacers (PE(TMA)$_4$, PE(DMAHepI)$_4$ and PE(DMACyI)$_4$) fractions of the column effluent were collected and the concentrations of these quaternary ammonium-containing compounds were determined by complexation with bromophenol blue followed by extraction of the complex as described elsewhere [30].

Table 1
Ionic capacity of the stationary phases

Stationary phase	Ionic capacity (mequiv./ml stationary phase)
SP Sepharose XL	917
SP Sepharose XL (prototype)	1023
Source 15S	660
UNOS6	760
ToyoPearl SP-650M	786
Fractogel EMD SO_3 650S	468
Ceramic HyperD F	741

4. Results and discussion

Shukla et al. [23–25] have previously investigated the affinity of a variety of dendritic, low-molecular-mass displacers. A homologous series of molecules based on pentaerythtritol were shown to be particularly efficacious in evaluating the behavior of different stationary phase materials. In the present work, we employ these displacers (Fig. 1) to study the effect of secondary interactions (e.g. hydrophobicity and aromaticity) on the selectivity of a wide variety of cation-exchange materials.

As seen in Fig. 1, the molecules in this series are based on the PE(TMA)$_4$ structure with the R group on the quaternary ammonium group consisting of either a benzyl [PE(DMABzCl)$_4$], a cyclohexyl [PE(DMACyI)$_4$], a heptyl [PE(DMAHepI)$_4$] or a methyl unit [PE(TMA)$_4$]. These displacers each have four charges but differ in their hydrophobicity and aromaticity. To classify these molecules according to their hydrophobicity, log *P* (octanol–water partition coefficient) calculations were performed using MOE software (Chemical Computing Group, Canada). To gain a better understanding of the structural components, additional descriptors such as the number of aromatic bonds and number of hydrogen-bond acceptor atoms were determined. In addition, to relate our results to the solubility parameter, the descriptor related to density was also included (Table 2). The calculation of solubility (cohesion) parameter is included in the Theory section. As seen in the table, PE(DMAHepI)$_4$ was the most hydrophobic (highest log *P* value) and PE(DMABzCl)$_4$ was the most aromatic (highest number of aromatic bonds) of all the molecules used.

The linear SMA parameters of the displacer molecules were determined on each stationary phase, as described in the Experimental section, and the

Table 2
Properties of the displacers

Displacer	M	F Charge[a]	Density	log P (o/w)[b]	b_{ar}[c]	a_{acc}[d]	ΣG[e]	δ
$PE(DMAHepI)_4$	1329.07	4	0.8754	16.019	0	4	14326	9.44
$PE(TMA)_4$	1008.47	4	1.0401	5.127	0	4	7870	8.12
$PE(DMABzCl)_4$	931.01	4	0.7022	9.887	24	4	13326	10.05
$PE(DMACyI)_4$	1337.05	4	0.8908	13.707	0	4	13626	9.08

[a] F charge, formal charge on the molecule.
[b] log P (o/w), octanol–water partition coefficient.
[c] b_{ar}, number of aromatic bonds.
[d] a_{acc}, number of hydrogen-bond acceptor atoms.
[e] ΣG values were calculated using Hoy's molar attraction constants [29,39].

values are shown in Table 3. There are significant differences in the values of these parameters as the stationary phase and/or the displacer compound is changed. For example, the characteristic charges are consistently lower on the Hyper D resin, which indicates that, on average, the displacers are not interacting with as many charged sites on the weakly crosslinked polyelectrolyte gel phase inside these materials. This is counterintuitive since one would expect the ion-exchange sites to be more accessible in these systems. In addition, the characteristic charge on the Fractogel material is also low with the exception of $PE(DMAHepI)_4$ which had a significantly higher characteristic charge as well as equilibrium constant as compared to the other displacers. We believe that the elevated affinity is due to the ability of the alkyl side chains on the displacer to interact directly with the base poly(methylmethacrylate) material, as well as with the charged tentacles. Although the K values between different resins cannot be directly compared due to their dependence on the total ion capacity [28] it is still instructive to qualitatively compare these data. For example, a significant difference can be seen in the K values for the hydrophobic displacer $PE(DMAHepI)_4$. The K values for this displacer on the resins with a more hydrophobic backbone chemistry (e.g. Source 15S, ToyoPearl and Fractogel) were significantly higher than those on the more hydrophilic phases. These results confirm the importance of secondary interactions arising from both the resin backbone chemistry as well as the displacer. While ν is essentially constant for SP Sepharose XL, the value changes considerably for the prototype Sepharose XL material which has a higher dextran content. This observation indicates that subtle changes in selectivity may be effected by modifying the level of dextran in these systems. These results in Table 3 indicate that the mode of interaction and the strength of binding can be strongly affected by both displacer and stationary phase chemistry.

By evaluating the dynamic affinity (as defined in Eq. (1)) of these displacer probe molecules, we can now examine the relative affinities that would be expected under the nonlinear binding conditions found in displacement chromatography. Affinity

Table 3
Linear SMA parameters of the four probe molecules on different stationary phases

Stationary phase	$PE(TMA)_4$		$PE(DMAHepI)_4$		$PE(DMABzCl)_4$		$PE(DMACyI)_4$	
	ν	K	ν	K	ν	K	ν	K
SP Sepharose XL	3.17±0.08	2.18±0.76	3.2±0.06	2.32±1.16	3.18±0.04	4.62±0.67	3.3±0.06	2.08±0.81
SP Sepharose XL (prototype)	3.35±0.12	5.14±1.14	2.71±0.10	3.09±1.10	4.18±0.11	3.73±0.71	3.27±0.09	2.72±0.75
Source 15S	3.4±0.19	5.0±3.6	3.13±0.26	173±10.14	2.76±0.30	17.7±5.85	2.5±0.15	23±4.58
UNOS6	3.4±0.12	0.95±0.65	2.44±0.11	1.91±1.18	3.94±0.26	2.44±0.41	3.6±0.36	1.21±0.21
ToyoPearl SP-650M	3.5±0.08	12.5±6.25	2.44±0.43	99.8±10.78	3.46±0.18	65.2±6.77	3.68±0.26	21.9±5.1
Fractogel EMD SO_3 650S	2.58±0.16	21.4±9.8	3.84±0.14	501±62.45	2.55±0.09	25±10.14	2.82±0.53	20.3±11.07
Ceramic HyperD F	2.39±0.44	3.23±1.00	2.27±0.10	3.05±0.72	2.3±0.19	3.60±0.55	2.1±0.05	3.04±0.69

ranking plots are log–log plots and thus only significant changes in the dynamic affinities will be observed. Further, while the value of log *K* will affect the general location of the dynamic affinity lines, the characteristic charge affects the slope of these lines.

The Sepharose XL medium is composed of a rigid crosslinked agarose matrix with long, flexible dextran chains bearing charged groups. Agarose is an example of a polysaccharide stationary phase material which has a high degree of hydrophilicity [31]. It is an alternating copolymer of (1–3)-β-D-galactopyranose and (1–4)-3,6-anhydro-α-L-galactopyranose and is known to be very hydrophilic because of the presence of a high density of free OH groups [32]. The functional group (propane sulfonate) is added to the free hydroxyl sites, either directly or through spacer groups such as epichlorohydrin and 1,4-butanedioldiglycidyl ether [33]. Long dextran chains are coupled to the agarose backbone and this dextran greatly increases the exposure of the sulfopropyl charged groups. The prototype of this stationary phase (Sepharose XL prototype) differs from the commercially available resin only by the increased amount of dextran bound to the resin.

Because the equilibrium constants for these two resins were of the same order of magnitude, the dynamic affinities of these probe molecules were similar. Thus, while there were subtle differences in the affinity plots for the different displacers in these two systems, their efficacies as displacers would be essentially the same on these two materials. These findings indicate that secondary interactions were not significant, and the efficacy of the displacers was purely a result of electrostatic interactions.

The Ceramic HyperD S material consists of functionalized poly(acrylamide)-based hydrogel polymerized within the large pores of a rigid ceramic bead. This type of sorbent is not only resistant to swelling or shrinking in response to ionic strength changes, like most of the silica and PS–DVB matrices, but also has a high binding capacity comparable to functionalized ''soft'' hydrogels [34].

In this case, the equilibrium constants for these displacers were of the same order of magnitude (Table 3). Therefore, the dynamic affinities of these probe molecules were very similar. Interestingly, the lower characteristic charges observed on this material resulted in steeper slopes of the dynamic affinity lines (figures not shown), which will make the affinity more sensitive to the concentration of displacer employed in the separation. While this is generally not desirable, it can sometimes be useful for creating selective displacements [35].

In contrast to the other stationary phases investigated in this study, the UNO S6 material consists of a continuous monolithic porous polymer. This continuous bed matrix is comprised of an acrylate type polymer that is formed directly in the chromatographic column [36]. Similarly to the previous stationary phases that were discussed, the equilibrium constants (Table 3) for these displacers were of the same order of magnitude and the dynamic affinities of these probe molecules were very similar. The lower characteristic charge of $PE(DMAHepI)_4$ relative to the other displacers resulted in a steeper slope for this particular dynamic affinity line (figures not shown). These results indicate that secondary interactions do not play a significant role in the affinity of displacers in this stationary phase material.

The Fractogel material [37] consists of linear polyelectrolyte chains which are coupled to the surface of a hydrophilic support material. These chains, or ''tentacles'', possess ionic sites and have been shown to reduce the contact between the solute and the support matrix, reducing the level of nonspecific interactions [31,38]. It has also been reported that in tentacle-type exchangers the flexibility of the charge arrangement allows additional electrostatic interactions [38]. The linear polyelectrolyte chains of *N*-trimethylaminomethyl acrylamide are introduced on the surface by radical grafting polymerization initiated by Ce^{+4} ions. The matrix used for the Fractogel-SO_3 sorbent is derived from polymerization of various methacrylate esters with vinyl alcohol emulsifiers [37]. The dynamic affinity plot for the Fractogel resin is shown in Fig. 2. There is a significant difference in the affinity of $PE(DMAHepI)_4$ relative to the other displacer molecules which have very similar affinity lines. As was shown in Table 3, the $PE(DMAHepI)_4$ had significantly higher values of both the equilibrium constant and the characteristic charge on the Fractogel material. The higher *K* value results in the generally higher position of the line relative to the

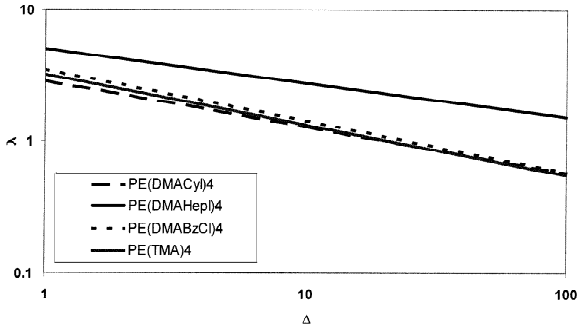

Fig. 2. Ranking of dynamic affinities of probe molecules on Fractogel column.

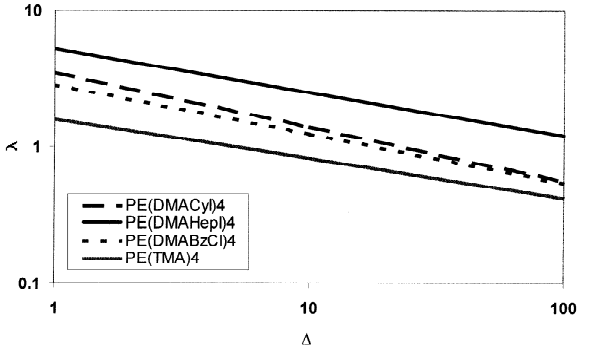

Fig. 3. Ranking of dynamic affinities of probe molecules on Source 15S column.

other displacers and the higher characteristic charge results in a lower slope which will enable this molecule to act as a high affinity displacer over a wide range of operating conditions.

The question then arises, why is this displacer so much better on this material? We believe that the elevated affinity is due to the ability of the alkyl side chains on the displacer to interact directly with the base poly(methylmethacrylate) material, as well as with the charged tentacles. Further, it is possible that the interaction of the long alkyl chains of the displacer with the base matrix makes the charges on the displacer more accessible for interaction with the stationary phase polyelectrolyte tentacles. This is a potentially significant result which is the subject of an ongoing investigation.

Toyopearl SP-650M ion-exchangers consist of a poly(methylmethacrylate) backbone bearing sulfopropyl charged groups. Fig. 4 shows the dynamic affinities of the probe molecules for this stationary phase. Because the equilibrium constants for the displacers were different on this material (Table 3), there is a noticeable difference in their dynamic affinity lines. The order of affinity is $PE(DMAHepI)_4 > PE(DMABzCl)_4 > PE(DMACyI)_4 > PE(TMA)_4$.

In order to examine this behavior in more detail, we have examined the solubility parameter [29] which can provide insight regarding the interaction of these displacers with the polymer base matrix. The traditional way of using the solubility parameter is to find a solvent that would solubilize a particular polymer. The smaller the difference between the values for solvent and polymer, the closer the solvent and polymer are in terms of their properties. For polymer–solvent mixtures, two materials with similar δ values gain sufficient energy on mutual dispersion to permit mixing [39]. The solubility parameters for these probe molecules were calculated using Eq. (4) and it was observed that the values, shown in Table 2, were always below the values for poly(methylmethacrylate) ($\delta = 18.0$) and polystyrene ($\delta = 18.5$). The highest value was obtained for $PE(DMABzCl)_4$ and the general order was $PE(DMABzCl)_4 > PE(DMAHepI)_4 > PE(DMACyI)_4 > PE(TMA)_4$. This suggests to us that more favorable interactions should take place between the $PE(DMABzCl)_4$ and the base matrices. However, $PE(DMAHepI)_4$ having the highest hydrophobicity, was observed to have the highest affinity for the poly(methylmethacrylate) and the PS–DVB based resins (discussed below). For the poly(methylmethacrylate) based resin (Toyopearl), the remaining molecules rank in the order predicted by the solubility parameter. From these results we can conclude that, while solubility calculations can provide useful information about the affinities in these systems, hydrophobicity must also be considered when interpreting these results. The benzyl containing displacer has a higher affinity for this material than the cyclohexane containing displacer as predicted by solubility parameter analysis. In this case, even though the hydrophobicity of the cyclohexane displacer is higher, it appears that aromaticity as well as hydrophobicity plays an important role. Polar inter-

actions would occur between the polar poly(methylmethacrylate) resin and polarizable aromatic ring containing displacers, thereby increasing the affinity. While these results are similar to those previously reported in our laboratory for the Waters poly(methylmethacrylate) material, there are some differences. In particular, for the Waters material, the highest affinity displacer was the $PE(DMABzCI)_4$, indicating that aromaticity may play an even more important role in the poly(methylmethacrylate) resin.

Source 15S is a hydrophilized PS–DVB resin used for protein chromatography. Fig. 3 shows the dynamic affinities of the probe molecules for the Source 15S stationary phase. Because the equilibrium constant (Table 3) for $PE(DMAHepI)_4$ is significantly higher than the other displacers, the dynamic affinity line for this displacer lies above the others. While the dynamic affinities of $PE(DMABzCI)_4$ and $PE(DMACyI)_4$ are similar, the affinity of $PE(TMA)_4$ was the lowest of the set. These results indicate that the hydrophobicity of the displacer is very important in determining the dynamic affinity on this resin material. These results are in accord with previously obtained results for Poros HS50 resin [25] which is also a hydrophilized PS–DVB stationary phase material. When the solubility parameter analysis was carried out, it was seen that it did not predict the order of efficacy. Instead, the efficacy followed the order of hydrophobicity: $PE(DMAHepI)_4 > PE(DMACyI)_4 > PE(DMABzCl)_4 > PE(TMA)_4$. Thus, for the Source15S stationary phase, hydrophobicity is the dominant factor in increasing the efficacy of these displacers.

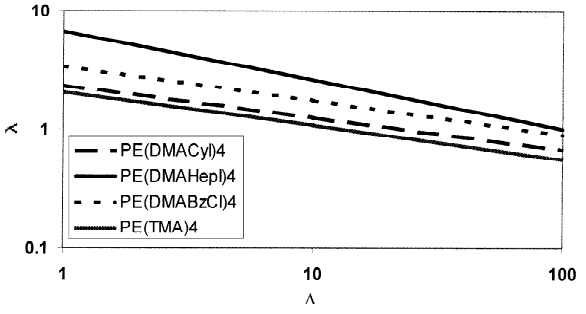

Fig. 4. Ranking of dynamic affinities of probe molecules on ToyoPearl column.

5. Conclusions

In this paper, the selectivities of different stationary phases were investigated using four $PE(TMA)_4$ based displacer probe molecules. The results indicate that the mode of interaction and the strength of binding of displacers can be strongly affected by both displacer and stationary phase chemistry. The SMA parameters were examined on various stationary phase materials and the results indicated that there are significant differences in the values of these parameters as the stationary phase and/or the displacer compounds are changed. By evaluating the dynamic affinity of these displacer probe molecules, we examined the relative affinities under the nonlinear binding conditions found in displacement chromatography. The results indicated that on some of the resin materials there was a significant effect of displacer chemistry on the dynamic affinity. For example, in the Fractogel material, there was a significant difference in the affinity of $PE(DMAHepI)_4$ relative to the other displacer molecules. This effect was attributed to the higher K value for this displacer resulting in a higher dynamic affinity line. Further, the higher characteristic charge of this compound resulted in a lower slope which will enable this molecule to act as a high affinity displacer over widely varied operating conditions on this resin. We believe that these results indicate that the alkyl side chains on the displacer may interact directly with the base poly(methylmethacrylate) material, as well as with the charged tentacles. Results also indicated that the hydrophobicity of the displacer can play a significant role in the dynamic affinity on hydrophilized polystyrene–divinylbenzene materials. In addition, for a poly(methylmethacrylate) resin, it appears that both hydrophobicity and aromaticity play an important role in the affinity. On the other hand, for the more hydrophilic resins examined in this study (Sepharose XL, Ceramic HyperD S, and UNO S6), the dynamic affinity plots did not indicate significant effects of displacer chemistry (e.g. hydrophobicity and aromaticity) on the efficacy of these displacers. The results of this study provide useful qualitative ''rules of thumb'' for the design of high-affinity low-molecular-mass displacers for different classes of stationary phase materials.

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