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Performance of C18 Derivatized Silica Gels: A Structure-Performance Study

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Abstract: Significant performance differences in the separation of desamido-insulin from insulin and of amitriptyline from imipramine have been found for five, macroscopically similar, preparative C18 silica materials. Structure-performance relations have been used to understand this difference. Physical measurements on the unpacked silica gels as well as chromatographic measurements have been carried out. The latter measurements comprise: hydrophobicity, metal impurity, steric selectivity and silanol activity. The performance difference could not be explained by the difference in hydrophobicity, steric selectivity or metal impurity between the materials. Instead a correlation between the silanol ion exchange activity and the selectivity could be found. The highest performance was found under conditions where the silanol groups have a moderate activity. For the separations considered in this work, the silanol groups have a beneficial effect.

Keywords: Hydrophobicity, ion exchange, metal impurity, performance, silanol activity, steric selectivity

INTRODUCTION

Silica based gels with C18 derivatization are the most widely used materials for reversed-phase chromatography. These materials are used

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for the purification of various components, from small molecules to polypeptides. The main advantages of these materials with respect to polymer based materials is the higher mechanical stability as well as the fact that they are not swelling or shrinking during a chromatographic experiment (1). The main disadvantages are the pH instability and the presence of free silanol groups on the surface. Silica based C18 silica gels are made by grafting native silica with C18 aliphatic chains. The C18 chains are attached to the silanol groups present on the surface of the material. Due to steric hindrance this derivatization is never complete and some free silanol groups remain on the surface. In order to try to shield also the remaining silanol groups an end-capping is typically done. This consists of attaching to the remaining free silanol groups a small silane molecule (1–3).

Even with the endcapping some free silanol groups remain on the surface of the material. Different methods have been tried to reduce the silanol activity. For example cross linking of the alkyl-chains with different anchor groups can be applied or alkyl-bonding reagents with different functionalities can be used. However, no method is really able to completely deactivate the silanol groups. These groups can interact in different ways with an analyte (e.g. by hydrogen bonding or by ion exchange interaction) (2). The most important interaction is, however, ion exchange. When the silanol groups are active (deprotonated, i.e., at high pH) we have a mixed-mode kind of adsorption: hydrophobic interaction on the C18 chains and ion exchange on the deprotonated silanol groups (4). The mixed-mode adsorption can have a deleterious effect on the peak shape of some molecules and is therefore undesirable. However, for some applications (e.g. antidepressant separation) the hydrophobic interaction is not sufficient for a good resolution and the ion exchange contribution is in this case beneficial.

The activity of the silanol ion exchange sites is unique for each resin and, like all ion exchange interactions, is affected by pH. For pure silica at a pH below 3 the silanol groups are protonated and therefore inactive, at pH larger than 3, instead, the silanol groups are deprotonated and thus active (1). In commercial silica gels, the pH at which they become active, i.e., the acidity of the silanols, is dependent on different properties of the base silica. Metal impurities (especially iron and aluminium) for example typically strongly increase the acidity of the silanols (5).

The performance of a material depends on many structure parameters. Macroscopically similar materials (similar particle and pore characteristics, same derivatization) are typically showing very different performance in challenging chromatographic separations. For difficult separations, like for e.g. antidepressant separation or insulin purification, these differences can become very important. In order to understand

this difference in performance, the so-called structure-performance relationships can be a very useful tool.

Various authors proposed different tests for the structure characterization of reversed-phase materials. We can cite for example: the Engelhardt test (6,7), the Walters test (8), and the Tanaka test (9). These tests present alternative ways of measuring structure parameters like for example hydrophobicity, silanol activity and shape selectivity. Other authors focused their work in comparing the different test methods (10–15). Visky et al. investigated 24 structure parameters and showed that only four of them are relevant for the characterization of the materials (13). These parameters are hydrophobicity, silanol activity, metal impurity, and steric selectivity.

After a first physical characterization of the materials, the performance in the separation of two basic antidepressant drugs and of insulin from desamido-insulin is measured for five preparative C18 reversed-phase materials. The four structure parameters described previously are also measured for the materials investigated. Structure-performance relationships are then used to understand the difference in performance.

MATERIALS AND METHODS

Instrument

The chromatographic experiments are performed using an Agilent 1100 Series HPLC, equipped with a quaternary gradient pump (G1311A), an autosampler (G1329A), and a temperature controlled two column switch (G1316A). The detection is performed by a diode array detector (G1315A) and a refractive index detector (G1362A).

Columns

The ODS materials were obtained as powder and given for packing to HiChrom Ltd (UK). The packed column have 25 cm length and 0.46 cm I.D. Kromasil C18 was obtained from EKA Chemicals AB, Sweden; Daisogel SP ODS-BP from Daiso Co, Japan; YMC ODS-A was obtained from YMC, Japan; Chromatorex C18 SMB from Fuji Silysia, Japan and ZEOsphere C18 was kindly donated by Zeochem AG, Switzerland. All materials are C18 derivatized and fully endcapped. For the sake of simplicity the columns have been numerated as follows: Column 1: Kromasil C18, Column 2: YMC ODS A, Column 3: Daisogel SP ODS BP, Column 4: Chromatorex C18 SMB and Column 5: ZEOsphere C18.

Reagents and Buffers

Imipramine (imi), amitriptyline (ami), 2,2′-dipyridyl, phenol, *ortho*-terphenyl, triphenylene, amylbenzene, and benzylamine have been obtained from Sigma-Aldrich, Switzerland. Human insulin (ins) was purchased from Sigma-Aldrich. Desamido insulin (dins) was obtained from degradation of insulin in 0.01 M hydrochloric acid (Baker, Holland) at 40°C for six days (16). Potassium dihydrogen phosphate (KH_2PO_4) and ammonium dihydrogen phosphate ($(NH_4)H_2PO_4$) were obtained from Merck, Germany. Ammonium sulphate ($(NH_4)_2SO_4$) was purchased from Fluka, Switzerland. Methanol (MeOH) HPLC grade was obtained from Fisher scientific, UK, and Ethanol (EtOH) from Merck, Germany. All chemicals were "proanalysi" grade. HPLC grade water (H_2O) was obtained with a Millipore Synergy system equipped with a Simpac 2 filter. All buffer were filtered through a 0.45 μm filter and degassed for 15 minutes with a sonificator before use. The buffers used for the different chromatographic tests are summarized in Table 1.

All the experiments were performed at 25°C, the antidepressant separation was performed at a flow rate of 0.5 ml/min, all other experiments were done at 1 ml/min. The columns were equilibrated with 10 column volumes (CV) of the corresponding buffer before each experiment. After each experiment the columns were cleaned with pure methanol (5CV).

Physical Properties

The silica gels have first been characterized by measuring physical properties. The following properties have been measured for the unpacked silica gels: specific surface area, pore volume, pore size, particle

Table 1. Buffer used for the experiments (all concentrations are given in weight ratios)

Performed test	Buffer used
Metal impurity[13]	MeOH:H_2O (34:100)
Hydrophobicity[13]	MeOH:H_2O (371:100)
Silanol activity[13]	MeOH:H_2O:0.2 M KH_2PO_4 (34:90:10) pH = 2.7 to 6.3
Steric selectivity[13]	MeOH:H_2O (371:100)
Insulin purification[16]	EtOH:50 mM $(NH_4)_2SO_4$ (29.8:70.2) pH = 3.5/5
Antidepressants separation[18]	MeOH:25 mM $(NH_4)H_2PO_4$ (72:28) pH = 3 to 6.3

size distribution, and carbon content. Specific surface area, pore volume, and pore size have been determined by nitrogen adsorption (BET) after burning the C18 chains out (550°C in air). The particle size distribution has been measured with a Malvern Mastersizer. The carbon content has been determined by elemental analysis.

Performance Parameters

The selectivity factor and the resolution between two components have been chosen as the performance parameter. Two separations are considered. The first one is the separation of two tricyclic antidepressants: amitriptyline and imipramine. This separation is specially sensitive to ion exchange interactions since the antidepressants are basic molecules. The second separation is the separation of insulin from its main decomposition product: desamido-insulin. The latter separation is an important step in the insulin purification process.

The separation performance is first expressed in terms of the selectivity factor (α) between amitriptyline and imipramine and between insulin and desamido-insulin.

This is defined as:

$$\alpha_{i,j} = \frac{k'_i}{k'_j} \tag{1}$$

where $k'_{i,j}$ are the retention factors of the i-th and j-th components, respectively. The latter are defined as:

$$k'_i = \frac{t_{R,i} - t_{0,i}}{t_{0,i}} \tag{2}$$

where $t_{R,i}$ is the retention time, $t_{0,i}$ the retention time under non adsorption conditions for the i-th component. In case of asymmetrical peaks, the retention time has to be calculated from the first statistical moment of the elution peak.

Secondly, also the chromatographic resolution defined as:

$$R = \frac{2(t_{R,i} - t_{R,j})}{w_i + w_j} \tag{3}$$

is used. $w_{i,j}$ are the baseline width of the i-th and j-th components, respectively. Note that the resolution can be calculated only for baseline-separated peaks.

Structure Parameters

The following structure parameters have been measured on the columns packed with the C18 derivatized silica gels: metal impurity, hydrophobicity, silanol activity, and steric selectivity. Several methods for the determination of these structure parameters are discussed in literature (6,8,9,12,13,18–20). For this work the following test methods have been selected. Metal impurity can be quantified using a metal chelating agent, i.e., 2,2′-dipyridyl (6,19,21,22). This molecule interacts strongly with the metals present on the surface of the material. Hydrophobicity can be measured from the retention factor of a very hydrophobic molecule like, i.e., amylbenzene (8,9,23,24). The silanol ion exchange capacity is evaluated from the selectivity factor between a basic (i.e., benzylamine) and a neutral compound (i.e., phenol) (9,25–27). Steric selectivity is determined using two aromatic hydrocarbons with similar physico-chemical properties, but a different molecular structure (i.e., *ortho*-terphenyl and triphenylene) (9,25,26,28).

RESULTS AND DISCUSSION

Physical Properties

The measured physical properties of the investigated materials are summarized in Table 2.

All the materials have specific surface areas between 324 and 349 m^2/g and pore size around 110 Å. The particle size is in the range of 15 μm, with the exception of YMC ODS A, which has a mean particle diameter of around 20 μm. YMC ODS A and Daisogel SP ODS BP have broader particle size distributions than the other materials. ZEOsphere

Table 2. Physical properties of the investigated silica gels.*: measured after burning the C18 chains out. Col. 1: Kromasil C18, Col. 2: YMC ODS A, Col. 3: Daisogel SP ODS BP, Col. 4: Chromatorex C18 SMB and Col. 5: ZEOsphere C18

Property	Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
Specific surface area* [m^2/g]	340	327	343	324	349
Pore volume* [mL/g]	0.93	0.94	1.00	0.89	0.95
Pore size* [Å]	109	114	117	110	109
Particle size, d(50) [μm]	15.4	19.4	16.6	14.1	15.4
Particle size, d(90)/d(10) [-]	1.54	1.74	1.71	1.51	1.61
Carbon content [%]	19.9	17.4	14.7	15.5	20.3

C18 and YMC ODS A have the highest carbon content, Chromatorex C18 SMB the lowest. The physical measurements show the macroscopic similarity between the materials investigated.

Performance Parameters

For the next experiments, the C18 derivatized silica gels were packed into chromatographic columns (see Section titled "Columns"). For the column performance evaluation two separations have been selected. A separation of two antidepressants and the separation of desamido-insulin from insulin. The antidepressants are basic molecules and therefore very sensitive to ion exchange interaction with the silanols. Retention factors, selectivities, and resolutions are measured for two pH values and summarized in Table 3.

Small changes in the selectivity factor can be seen for the materials investigated at constant pH, whereas changing the pH leads to significantly higher selectivites. The resolution is instead quite different even at constant pH. The materials were compared using always the same buffer composition, therefore without optimizing the buffer composition for each material. This leads to significant differences in retention factors. For example looking at Table 3 we can see that ZEOsphere C18 has smaller retention factors for the two antidepressants than the other materials. The consequence of shorter retention factors is that the separation performance is also smaller, since the time available for the separation is shorter. With a buffer optimization the retention factors could be increased and therefore, possibly, also the separation performance. On the other hand small retention factors have the advantage of leading to higher productivities, which is of course, also very important.

Table 3. Antidepressants retention factors, selectivity and resolution between imipramine and amitriptyline measured on all materials. Col. 1: Kromasil C18, Col. 2: YMC ODS A, Col. 3: Daisogel SP ODS BP, Col. 4: Chromatorex C18 SMB and Col. 5: ZEOsphere C18

	pH 5				pH 6.3			
Column	k'_{imi}	k'_{ami}	$\alpha_{ami,imi}$	$R_{ami,imi}$	k'_{imi}	k'_{ami}	$\alpha_{ami,imi}$	$R_{ami,imi}$
1	6.32	7.33	1.16	0.9	11.41	14.35	1.26	1.8
2	6.59	7.64	1.16	1.1	11.11	14.09	1.27	2.2
3	5.75	6.81	1.18	1.8	11.94	15.36	1.29	3.0
4	7.64	9.05	1.18	2.1	15.80	19.92	1.26	2.4
5	4.48	5.45	1.22	0.7	7.42	9.66	1.30	2.3

Table 4. Insulin and desamido insulin retention factors and selectivity between desamido-insulin and insulin measured at three pH values for all the materials. Col. 1: Kromasil C18, Col. 2: YMC ODS A, Col. 3: Daisogel SP ODS BP, Col. 4: Chromatorex C18 SMB and Col. 5: ZEOsphere C18

	pH 3.5			pH 5			pH 6.3		
Column	k'_{ins}	k'_{dins}	$\alpha_{dins,ins}$	k'_{ins}	k'_{dins}	$\alpha_{dins,ins}$	k'_{ins}	k'_{dins}	$\alpha_{dins,ins}$
1	4.57	5.93	1.30	4.04	5.89	1.46	5.36	11.19	2.09
2	7.55	9.92	1.31	6.50	9.00	1.38	8.82	17.07	1.93
3	9.57	12.64	1.32	9.18	12.82	1.40	15.75	29.66	1.88
4	10.27	12.20	1.19	9.13	13.30	1.46	14.00	27.11	1.94
5	3.39	4.13	1.22	3.04	4.13	1.36	3.74	6.37	1.70

The second separation considered involves desamido-insulin and insulin. Desamido-insulin is very similar to insulin and therefore this is a very challenging separation problem for chromatography. The separation has been done at three pH values. Retention factors and selectivities between desamido-insulin and insulin are summarized in Table 4. For this separation the resolution could not be calculated since there was no baseline separation between the two components.

Also for this separation similar selectivity factors are found at constant pH for the different materials. For this separation, however, the differences between the materials are higher than for the antidepressant separation. A significant increase in selectivity can be seen for all materials going from pH 3.5 to pH 5. The increase is even larger when going from pH 5 to pH 6.3. Looking at the retention factors we can see that, also in this case, the high resolutions at pH 6.3 are achieved with very high retention factors. In this case, Kromasil C18 and ZEOsphere C18 show much shorter retention factors: compared to Chromatorex SMB C18, ZEOsphere C18 has more than a factor four smaller retention time for desamido-insulin (at pH 6.3). It is interesting to note that the materials showing the highest selectivity in the antidepressants separation are not the ones showing the highest performance in the insulin, desamido-insulin separation. Moreover the material ranking is also pH dependent. This result shows once more the very peculiar properties of different C18 derivatized stationary phases. For this work, the selectivity (and the resolution) between two components is selected as the only parameter describing the performance of the silica gels. It is clear that for a correct ranking of the materials more experiments, especially under overloaded conditions should be done. This is however outside the scope of this work.

Structure Parameters

Metal impurity, hydrophobicity, steric selectivity, and silanol activity are measured for all examined stationary phases. The measured parameters are summarized in Table 5.

Metal impurity is the parameter accounting for the presence of metals on the surface of the resin. This is expressed in terms of the retention factor $k'_{2,2'\text{-dipyridyl}}$. From the table, it can be seen that Chromatorex SMB C18 has the highest metal content, whereas ZEOsphere C18 the lowest. Metals accumulate on the surface of the materials during their use in a chromatographic equipment (19). The determination of the metal impurity was, therefore, done as the first experiment. However, some contamination from the hardware can not be excluded. The retention factor of 2,2′-dipyridyl is sensible only to the amount of metals present on the surface of the material and this can be quite different from the amount of metals present in the bulk of the resin. The metal ions in the bulk of the resin are strongly affecting the acidity of the silanol groups on the resin. The result of the 2,2′-dipyridyl test could in principle be related to the metal content measured by elemental analysis on the silica gels. This measurement is however very difficult to be applied to derivatized silica gels and was, therefore, not performed here.

The second parameter measured is hydrophobicity. This is the parameter accounting for the amount of accessible carbons on the surface and is measured with a very hydrophobic molecule, i.e., amylbenzene. The results are expressed by the retention factor of amylbenzene. Since all the materials are C18 derivatized, large differences are not expected. The Kromasil C18 and Chromatorex SMB C18 materials show, however, a slightly higher hydrophobicity. Looking at the carbon content reported in Table 2, one can see that ZEOsphere C18 has the highest carbon

Table 5. Structure parameters. Col. 1: Kromasil C18, Col. 2: YMC ODS A, Col. 3: Daisogel SP ODS BP, Col. 4: Chromatorex C18 SMB and Col. 5: ZEOsphere C18

Parameter	Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
$k'_{2,2'\text{-dipyridyl}}$	14.3	13.4	14.9	18.5	12.2
$k'_{\text{amylbenzene}}$	10.33	8.06	7.51	9.60	8.40
$\alpha_{\text{triphenylene,}ortho\text{-terphenyl}}$	1.70	1.45	1.34	1.35	1.80
$\alpha_{\text{benzylamine,phenol}}$, pH 2.7	1.64	1.67	1.71	1.73	1.68
$\alpha_{\text{benzylamine,phenol}}$, pH 3.5	1.68	1.69	1.77	1.77	1.73
$\alpha_{\text{benzylamine,phenol}}$, pH 5.0	1.72	1.75	1.80	1.82	1.77
$\alpha_{\text{benzylamine,phenol}}$, pH 6.3	2.12	2.15	1.96	2.09	1.83

content, but moderate hydrophobicity. On the other hand Chromatorex SMB C18 shows high hydrophobicity, but moderate carbon load. These differences can be explained by the fact that the carbon content represents the total amount of carbon atoms present on the material (from the C18 chains and the endcapping molecule, in all the pores of the material). The hydrophobicity, instead, is quantified by the retention factor of amylbenzene. This parameter is a function of the carbon molecules that can be accessed by amylbenzene, which is dependent also on the pore structure of the material.

The third parameter, the steric selectivity, quantifies the ability of a stationary phase to separate molecules based on their molecular structure, rather than on their physical or chemical properties (29). O*rtho*-terphenyl and triphenylene are very similar molecules, with the difference that one is twisted and the other planar. The steric selectivity is expressed in terms of the selectivity factor between triphenylene and *ortho*-terphenyl. The materials investigated show quite different steric selectivity. Kromasil C18 and ZEOsphere C18 show higher steric selectivities than the other materials. This parameter can, however, not be correlated directly to any of the measured physical properties of the silica gels. The chemistry of the octadecyl bonding molecule is surely affecting the steric selectivity. This effect could, however, not be investigated, since the chemical nature of the bonding molecules used by the different manufacturers are unknown.

The last, but probably the most important parameter is the silanol activity. Free silanol groups on the surface of the resin can interact in different ways with analytes, but the most important one is by ion exchange interaction (2). This interaction can be quantified with a basic component, which interacts with the negatively charged silanol groups, and a neutral one, which, instead, does not interact with the silanol groups and is used as a reference. Like all ion exchange interactions this is also pH dependent and therefore measurements at different pH values are done (see Table 5). In order to determine the ion exchange interaction, the following assumption is made: the pH affects only the silanol groups and not the charge of benzylamine. This assumption is reasonable since the pKa of benzylamine is 9.33 and therefore the molecule is always positively charged at the pH considered in these experiments. For all materials the silanol activity follows the expected trend, i.e., increases as the pH increases. The extent of increase and the absolute values are characteristic for each material. Kromasil C18, Daisogel SP-ODS-BP, and Chromatorex SMB C18 show a small activity increase from pH 2.7 to pH 5 and then a jump from pH 5 to pH 6.3. ZEOsphere C18 shows smaller and more constant activity. The silanol activity is strongly dependent on the purity of the starting silica material, on the preparation of the

material and on the derivatization technique used. The reasons behind the different silanol activities are, however, still not understood.

Correlation between the Structure and Performance Parameters

In this section we want to understand which structure parameter is at the origin of the different performance seen for the five materials in Section titled "Performance parameters". For this structure-performance relations are presented. The separation principle in reversed-phase chromatography is hydrophobic interaction. The first structure parameter to be investigated is therefore hydrophobicity. We assume that hydrophobicity is not affected by pH and therefore the hydrophobicity values listed in Table 5 are used for all pH values. The same assumption is also used for the steric selectivity and the metal impurity. In Figs. 1 the selectivity between amitriptyline and imipramine (a) and between desamido-insulin and insulin (b) are plotted as a function of hydrophobicity (expressed by $k'_{amylbenzene}$).

From the figures we can see that the selectivity is independent from the hydrophobicity measured for the investigated materials. It is expected that larger differences in hydrophobicity e.g. as obtained when comparing C18 with C8 or C4 materials, will instead have an influence on the selectivity.

The second structure parameter investigated is the steric selectivity. The selectivity for the separations investigated is plotted as a function of the steric selectivity (expressed by $\alpha_{triphenylene,ortho-terphenyl}$) in Figs 2(a) and (b). As it can be seen from the figures, the difference of steric selectivity between the materials is not affecting the selectivity. It can be concluded that the chemical and physical interactions between the molecules and the stationary phase are much more important than molecular structure effects. This is expected since the investigated molecules have very similar structures.

The third parameter is the metal impurity. Metal ions present on the surface of the materials can interact with solutes, for example via the formation of complexes. The selectivity is plotted as a function of the metal impurity (expressed by $k'_{2,2'-dipyridyl}$) in Figs. 3(a) and (b) for the two separations investigated. Also in this case almost constant selectivity is found for the different metal impurities.

The fourth parameter which can affect the selectivity is the silanol ion exchange interaction. Imipramine has a pKa of 9.5 and amitriptyline of 9.4 (18). These two molecules are clearly positively charged under the experimental conditions considered in this work and therefore can interact with the deprotonated silanol groups leading to retention

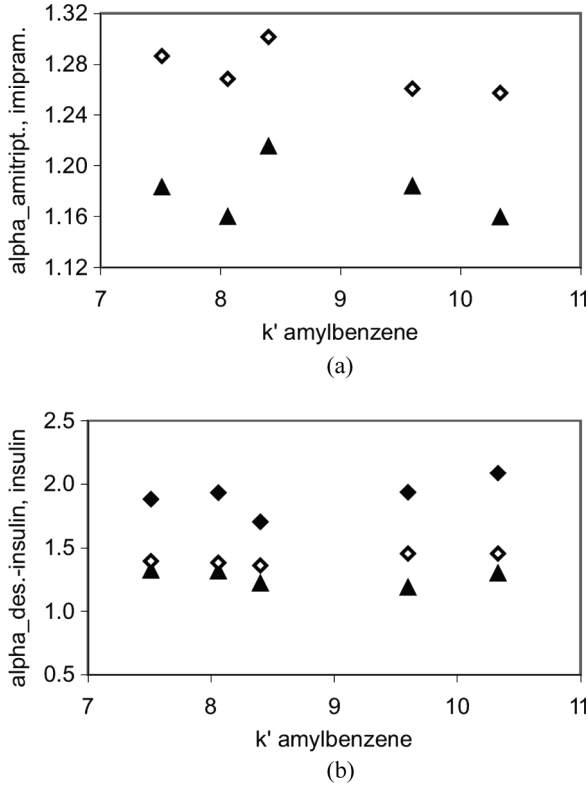

Figure 1. Selectivity (alpha) between amitriptyline (amitript.) and imipramine (imipram.) (a) and between desamido-insulin (des.-insulin) and insulin (b), plotted as a function of hydrophobicity expressed by $k'_{amylbenzene}$. The full triangles represent the experiments run at pH 3.3, the empty rhombus pH 5.0 and the full rhombus pH 6.3.

factors increasing with pH as shown in Table 3. Insulin has an isoelectric point of about 5.3 (30) and therefore it is also positively charged at pH 3.5 and 5. At pH 6.3 insulin should not be charged and therefore it should not interact with the silanol groups. The retention factors are, however, higher than at pH 5 (see Table 4), which is most likely due to stronger interaction with the silanol groups. It could be speculated that the insulin pI in the working buffer (containing ethanol) is higher than 5.3. The effect of ethanol on the pKa of different components is in fact reported in literature (31). The selectivity between amitriptyline and imipramine, measured at two pH values and the selectivity between

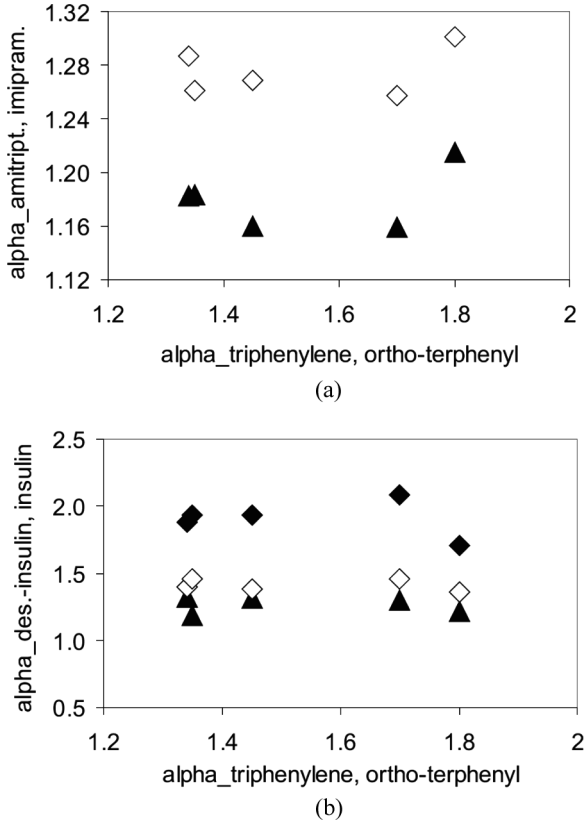

Figure 2. Selectivity (alpha) between amitriptyline (amitript.) and imipramine (imipram.) (a) and between desamido-insulin (des.-insulin) and insulin (b), plotted as a function of steric selectivity expressed by $\alpha_{\text{triphenylene},ortho-\text{terphenyl}}$. The full triangles represent the experiments run at pH 3.3, the empty rhombus pH 5.0 and the full rhombus pH 6.3.

desamido-insulin and insulin, measured at three pH values, are plotted as a function of the silanol ion exchange activity in Figs. 4(a) and (b), respectively.

In this case a clear dependence between the selectivity and the silanol activity, measured at different pH values, can be seen. In the case of the antidepressant separation (Fig. 4a), the selectivity is abruptly increasing at $\alpha_{\text{benzylamine,phenol}}$ of about 1.8 and then staying almost constant. In the case of desamido-insulin and insulin (Fig. 4b), the selectivity is more regularly increasing with $\alpha_{\text{benzylamine,phenol}}$. For both separations, the points with high selectivity are measured at high pH. In this case, for a

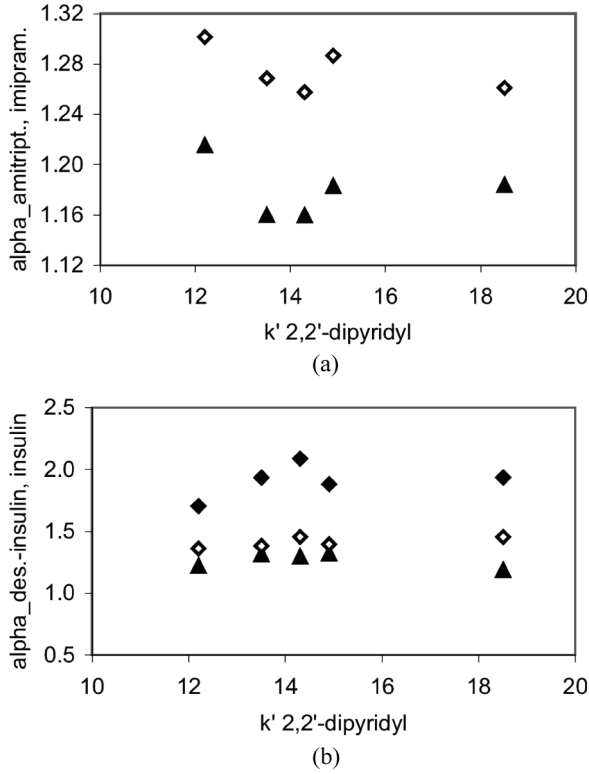

Figure 3. Selectivity (alpha) between amitriptyline (amitript.) and imipramine (imipram.) (a) and between desamido-insulin (des.-insulin) and insulin (b), plotted as a function of metal impurity expressed by $k'_{2,2'\text{-dipyridyl}}$. The full triangles represent the experiments run at pH 3.3, the empty rhombus pH 5.0 and the full rhombus pH 6.3.

good resolution between the investigated compounds the ion exchange contribution given by the silanol groups is necessary.

Very high silanol activities are, however, leading to strong tailing as can be seen in Fig. 5(a), where the chromatograms of the antidepressants at pH 6.3 are shown for the five materials investigated. Columns 1, 2, and 4 have higher silanol activities than Columns 5 and 3 (refer to Table 5). In Fig. 5(b), the chromatograms of the insulin, desamido-insulin separation at pH 3.5 are shown. Strong tailing is negatively affecting the chromatographic purification performance since it increases the time needed for the separation and thus decreases the productivity. This effect can be seen by calculating the chromatographic

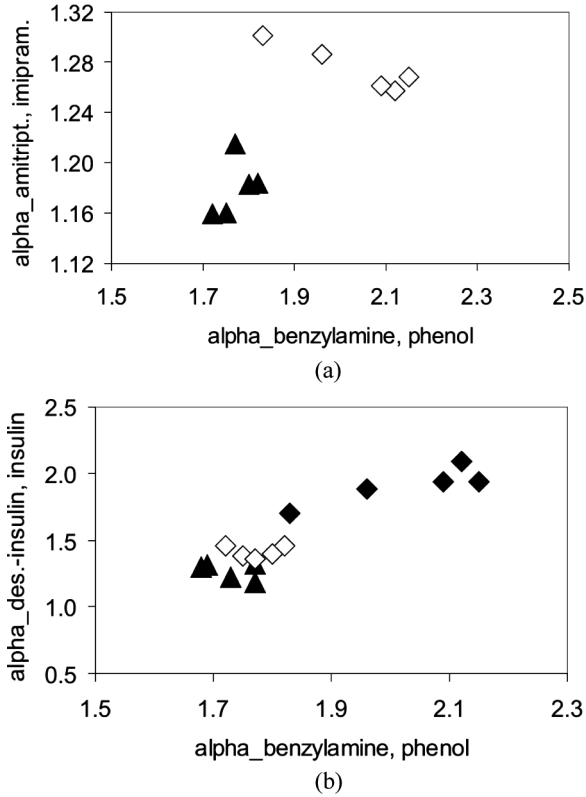

Figure 4. Selectivity (alpha) between amitriptyline (amitript.) and imipramine (imipram.) (a) and between desamido-insulin (des.-insulin) and insulin (b), plotted as a function of the silanol ion exchange activity expressed by $\alpha_{benzylamine,phenol}$. The full triangles represent the experiments run at pH 3.3, the empty rhombus pH 5.0 and the full rhombus pH 6.3.

resolution between the two investigated components. Since this calculation needs baseline separation between the peaks, the resolution could be calculated only for the antidepressant separation. This is shown in Fig. 6.

As it can be seen the points at very high silanol activity (large $\alpha_{benzylamine,phenol}$) correspond to a lower resolution value. Looking at the picture it seems that there is an optimal moderate silanol activity for this separation. The existence of an optimum silanol activity has, in fact, already been reported in literature (32). It is assumed that the tailing is due to thermodynamic factors only (two sites adsorption), and mass transport limitations differences between the materials due to

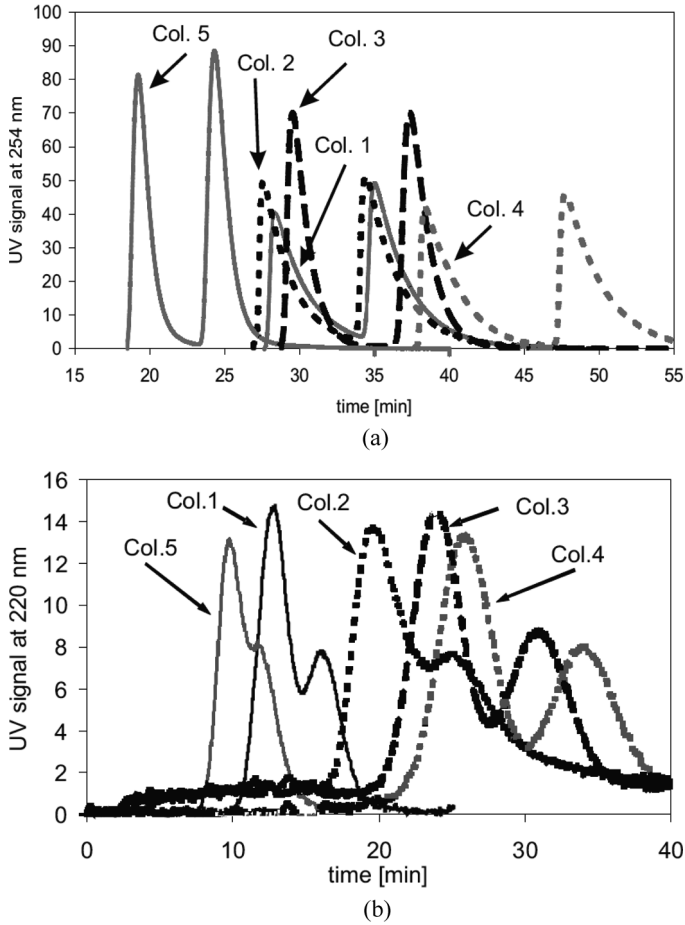

Figure 5. Chromatograms of the antidepressant separation at pH 6.3 (a) and the insulin, desamido-insulin separation at pH 3.5 (b) run on all columns. The first eluting peaks are imipramine and insulin, respectively. Col. 1: Kromasil C18, Col. 2: YMC ODS A, Col. 3: Daisogel SP ODS BP, Col. 4: Chromatorex C18 SMB and Col. 6: ZEOsphere C18.

different pore diameters and particle sizes are not considered. Looking at Table 2 it can be seen that the materials have very similar pore diameters and particle sizes with the exception of YMC ODS A, which shows higher mean particle size. For this material a slightly lower mass transport can be expected. This is, however, not changing the trend seen in Fig. 6.

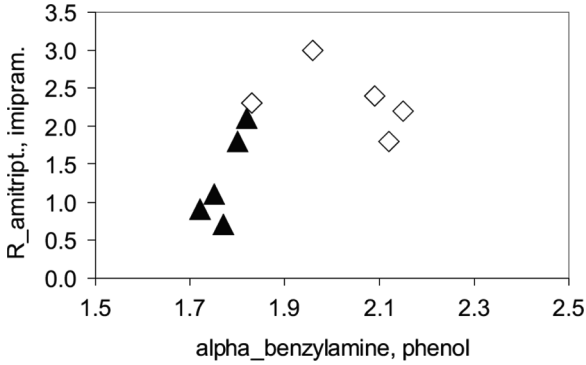

Figure 6. Resolution (R) between amitriptyline (amitript.) and imipramine (imipram.), plotted as a function of the silanol ion exchange activity expressed by $\alpha_{benzylamine,phenol}$. The empty rhombus represent the experiments at pH 5.0 and the full rhombus pH 6.3.

CONCLUSIONS

Five macroscopically similar, commercially available, C18 derivatized preparative reversed-phase stationary phases have been investigated. Physical properties of the silica gels have been measured with standard methods. The performance (selectivity factors and resolution) in the separation of desamido-insulin from insulin and from amitriptyline from imipramine has been found to be significantly different for the materials investigated. In order to explain this difference, structure-performance relations have been developed. For this, structure parameters have been measured in packed column. Hydrophobicity, metal impurity, steric selectivity, and silanol activity, have been determined. Some of these parameters exhibited large differences for the different materials. The different performance found for the materials investigated at different pH could not be related to the differences in hydrophobicity, steric selectivity, or in metal impurity between the materials. Instead it was found that the performance is related to the silanol ion exchange activity measured at different pH values. The highest selectivities were found at high pH, where the silanols are very active. Measurement on the resolution showed, however, that too high silanol activity is leading to large tailing and thus to a decrease in the performance. For the two separations investigated the ion-exchange contribution of the silanols is beneficial, since apparently the C18 chains alone are not able to resolve these compounds. It looks like that there is an optimal silanol activity for the antidepressants separation.

The trend in column manufacturing of removing the silanols from the material surface is, at least for the separations considered in this work, heading in the wrong direction. The best would be to be able to control the silanol activity and tune it properly for the different separations.

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