Colloid Chemical Aspects of Slurry Packing Techniques in Microcolumn Liquid Chromatography

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The relationship between the coagulating properties of slurry suspensions, type of packing liquids, and final performance of slurry-packed microcolumns for liquid chromatography (LC) was investigated. The colloidal stability of stationary-phase suspensions was examined by means of sedimentation and \( \zeta \)-potential experiments. Expressions for the theoretical sedimentation rate of noncoagulated particle suspensions were derived for spherical particles with completely and incompletely filled pores and for irregularly shaped particles with incompletely filled pores. The performance of the slurry-packed microcolumns was predominantly determined by the selection of the packing liquid. It is shown that the packing liquid preferably has to be coagulating to obtain efficient and stable packed capillary LC columns. The selection of the slurry liquid on the column performance was of minor importance.

The relatively poor column efficiency and long-term stability of packed capillary columns have delayed the introduction and application of microcolumn liquid chromatography as an analytical technique. Possible reasons for this are the coagulating properties of slurry suspensions, containing alkyl-modified silica or poly(styrene–divinylbenzene) packing materials particles, and the physical/chemical properties of the applied packing liquids. Different packing techniques are proposed to overcome these problems.

Most of the packing procedures of columns in liquid chromatography (LC) are performed by using slurry packing techniques.\(^1\) As a result of the dispersion of the stationary-phase particles in a solvent, the attraction between the particles is drastically reduced, resulting in favorable packing conditions compared with dry packing methods.\(^2,3\) However, the type of slurry solvent strongly determines the stability of the slurry suspension. Bristow,\(^4\) and more recently Shelly et al.\(^5,6\) and Wang et al.,\(^7\) pointed out that the colloidal stability of packing material suspensions, that is, the coagulating behavior of stationary-phase particles in a solvent, can be critically important in slurry packing methods. These effects become even more critical in packed fused-silica capillary columns of 100–350-\( \mu \)m internal diameter (i.d.) because of the large value for the column length to column internal diameter ratio of such columns.\(^3\) In slurry packing methods, efforts are made to avoid coagulation of the stationary-phase particles. Currently applied slurry packing techniques are balanced and nonbalanced density methods, chemical and mechanical stabilization methods, and high- and low-slurry viscosity methods.\(^8\)

This paper reports a quantitative method describing the colloidal stability of porous, reversed-phase particles in apolar liquids, as based on the sedimentation rate of the particles. In addition, \( \zeta \)-potential measurements were performed to study slurry stabilities in more detail. The effect of the type of slurry and packing liquids on the resulting chromatographic performance (plate height, separation impedance, flow resistance parameter) is investigated extensively for nominally identical stationary phases. The results of this study were used to develop selection criteria for slurry packing techniques in microcolumn LC.

**THEORY**

Spherically Shaped Particles. Bristow\(^4\) pointed out that the theoretical sedimentation rate can be used to determine the colloidal stability of slurry suspensions. Suspensions in which the particle settling rate exceeded the calculated one were regarded as coagulated. The theoretical sedimentation rate of spherical stationary-phase particle suspensions can be determined with the Stokes equation but is only valid for solid particles at infinite dilution. At present, most stationary-phase particles are porous and infinite dilutions are impractical in column packing techniques. Both effects can be corrected for by taking the particle porosity, \( e _ p \), and a hindered settling function\(^9,10\) into account, which is represented by the term \((1 - \phi)^{- \kappa_2} \) multiplying a modified Stokes expression for the theoretical sedimentation rate of noncoagulated stationary-phase particles:

\[
\nu = \frac{(1 - \phi)^{- \kappa_2} d_p^2 (\phi_{\text{skel}} - \phi) (1 - \epsilon) g}{18 \eta}
\]

where \( \phi \) is the volume fraction of the particles, \( \kappa_2 \) is a particle/
solvent-dependent constant, \( q_{\text{dil}} \) and \( q_1 \) are the densities of the packing material and the liquid, respectively; \( \eta \) is the viscosity of the liquid, and \( g \) is the gravitational constant. \( \epsilon_i \) can be expressed as:

\[
\epsilon_i = \frac{V_{\text{pore}}}{V_{\text{hydro}}} = \frac{V_{\text{pore}}}{V_{\text{pore}} + 1/\rho_{\text{ske1}}} \tag{2}
\]

where \( V_{\text{pore}} \) is the total pore volume per unit of mass and \( V_{\text{hydro}} \) the hydrodynamic particle volume per unit of mass. The application of \( \epsilon_i \) to calculate the effective density of a particle is only valid for immiscible substances, as is the case for packing material suspensions. \( \epsilon_i \) equals 0 if the particle is completely solid and theoretically equals 1 if it is completely porous, meaning no silica backbone is present anymore. As is shown later, eq 1 can only be applied for particles completely filled with slurry liquid: poly(styrene-divinylbenzene) (PS-DVB) particles. As a result of the possible inclusion of gas into the particle pores, eq 3 must be applied for alkyl-modified silicas to calculate the settling rate of noncoagulated porous particles:

\[
v = \frac{(1 - \phi)^{-q_{\text{dil}}^{1/2}(1 - \epsilon_i)+q_1(\epsilon_i - 1))g}{18\eta} \tag{3}
\]

where \( \epsilon_{\text{g}}/\epsilon_i \) is the fraction of filled particle pores. In this equation, we assumed that the density of the gas phase was negligible for the calculation the effective mass of the particles.

Irregularly Shaped Particles. Predicting the theoretical sedimentation rate is more complicated for irregularly shaped porous packing materials than for spherically shaped particles. The description of the shape of such particles and its influence on the free-falling behavior are complicating factors. The final settling velocity for irregularly shaped particles is usually determined by means of iterative calculating schemes. However, the theoretical sedimentation rate of noncoagulated particles can be calculated analytically by the combination of the equations that are applied in iterative calculation schemes, resulting in

\[
v = \frac{(1 - \phi)^{-q_{\text{dil}}^{1/2}(1 - \epsilon_i)+q_1(\epsilon_i - 1))g}{3\pi\eta} \tag{4}
\]

where \( \psi \) is an empirical shape factor, which is defined as the volume of the particle divided by the volume term obtained from the mean projected area diameter of the particle. \( d_A \) is the projected area diameter.

**EXPERIMENTAL SECTION**

Particle Characterization. The particle size distribution of the different stationary phases was measured with a Malvern 2600 particle sizer (Malvern Instruments, Worcestershire, UK). The skeleton density of the materials was determined with a gas (He). Volume densitometer (Stereopycnometer, Quanta-chrome Corp., Greenvale, NY). Pore volumes were assessed with an automatic multistep B.E.T. apparatus (Sorptomatic 1900, Carlo Erba Instruments, Milan, Italy). The projected area diameter of irregularly shaped particles was determined as described by Clift et al. Slurry densities were measured with a 10-nL capped liquid pycnometer, after sonication for 10 min and thermostating at 20 °C for 15 min in a water bath.

Particle Settling Device. A particle settling device was constructed from 130 × 7 mm i.d. glass tubes. The stationary-phase slurries were transferred into the tubes by means of a pipet. The tubes were closed by a rubber stopper to prevent solvent evaporation and placed in a homemade tube holder to ensure that they were held in a vertical position. The height of the slurry was measured from 5 min to 48 h after filling of the tubes. All sedimentation experiments were carried out twice.

⪽ Potential Measurements. The electrophototropic mobility of the packing material particles in the selected slurry liquids was measured by means of a Malvern Zetasizer 3 (Malvern Instruments) equipped with an AZ-4 cell for moderate conductivity measurements, i.e., electrophototropic experiments in solvents with a relatively high dielectric constant or an AZ-26 cell for low-conductivity measurements. The principles of detection and measurement with the AZ-26 cell are discussed by Arts et al. Column Preparation and Packing Equipment. Columns were constructed from 0.40 m × 320 μm i.d. uncoated fused silica tubing (Chrompack, Middelburg, The Netherlands). An EPM2000 glass filter frit (Whatman, Maidstone, UK) was cut out by the column. To hold the frit in position, a 0.080 m × 75 μm i.d., 280 μm outer diameter (o.d.) fused-silica capillary (LC Packings, Amsterdam, The Netherlands) was fixed into the large capillary using epoxy resin.

The packing equipment consisted of a DSTV-122 air-driven fluid pump (Haskel Engineering, Burbank, CA) for constant pressure packing and a 0.19 × 0.0020 m i.d. stainless-steel slurry reservoir. The column was connected to the slurry vessel with a 0.2 mm i.d., 0.5 mm o.d. PVDF double cone (Merck, Darmstadt, Germany).

In this study, only reversed-phase particles were used. The packing material particles were suspended at a concentration of 100 kg·m⁻³ in a slurry liquid by sonication for 10 min. Afterward, the slurry was transferred to the slurry reservoir with a syringe. The columns were unless otherwise stated packed at a pressure of 500 bar during a 1-h period.

Chromatographic Instrumentation. The HPLC system consisted of a Model 100 DM syringe pump (Isco Inc., Lincoln, NE), a microUVIS20 absorbance detector (Carlo Erba Instruments), and a 60-nL CI4W injection valve (VICI-AG Valco Europe, Schenkon, Switzerland). To ensure that no stationary phase could enter the injection valve, a 0.2-μm nylon membrane (Alltech Associates Inc., Deerfield, IL) was positioned into the valve outlet to the column. UV detection was performed at 255 nm. A 900 Series interface and Nelson 5.0 software (Perkin Elmer Nelson, Cupertino, CA) were employed for data acquisition.

Column Evaluation. The reversed-phase columns were evaluated with a test mixture containing four components: uracil (dead volume marker), naphthalene, phenanthrene, and anthracene. The concentration of all components was approximately.
packing materials resulted in an overestimation of the theoretical Janssen Chmica (Beerse, Belgium), uracil, anthracene, naphtha-

The Netherlands). All chemicals were of p.a. grade. Water was

desedimentation rate compared to empirical determined settling
dp were from BioRad RSL nv. (Nazareth, Belgium).

Table 1. Median Particle Diameter, \( d_p \), Pore Volume, \( V_{pore} \), Skeleton Density, \( \rho_{skele} \), and Particle Porosity, \( \epsilon_p \), of Reversed-Phase Packing Materials

<table>
<thead>
<tr>
<th>stationary phase</th>
<th>( d_p ) (( \mu \text{m} ))</th>
<th>( V_{pore} ) (10(^{-3} \text{m}^3 \cdot \text{kg}^{-1} ))</th>
<th>( \rho_{skele} ) (10(^{3} \text{kg} \cdot \text{m}^{-3} ))</th>
<th>( \epsilon_p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>silica-based (spherical)</td>
<td>BioSil C18 HL 90-5 S</td>
<td>5.70</td>
<td>0.357</td>
<td>2.101</td>
</tr>
<tr>
<td></td>
<td>Nucleosil 100-5 C18</td>
<td>5.10</td>
<td>0.809</td>
<td>2.105</td>
</tr>
<tr>
<td></td>
<td>Spherisorb ODS-1</td>
<td>4.84</td>
<td>0.430</td>
<td>2.166</td>
</tr>
<tr>
<td></td>
<td>Zorbax ODS</td>
<td>4.40</td>
<td>0.358</td>
<td>1.987</td>
</tr>
<tr>
<td></td>
<td>Zorbax SB-C18</td>
<td>5.24</td>
<td>0.318</td>
<td>2.194</td>
</tr>
<tr>
<td>silica-based (irregular)</td>
<td>Polygosil 60-5 C18</td>
<td>6.39</td>
<td>0.658</td>
<td>2.077</td>
</tr>
<tr>
<td>poly styrene-based</td>
<td>RoGel RP</td>
<td>7.59</td>
<td>1.3</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* Averaged projected area diameter. \(^{1}\) For RoGel RP, the values for density and pore volume taken are as specified by the manufacturer.

rates. Assuming that the silica particle pores would be completely filled with liquid after 10 min of sonication, \( \rho_{skele} \) should compare well with values for the density obtained with a liquid pycnometer. However, average values as obtained by stereopycnonometric experiments were \((2.0-2.2) \times 10^3 \text{kg} \cdot \text{m}^{-3}\), whereas with a liquid pycnometer, measurements values of \((1.5-1.9) \times 10^3 \text{kg} \cdot \text{m}^{-3}\) were found. These results indicate that the pores of the ODS particles are only partly filled with liquid. During sonication of the suspensions, gas might remain entrapped in the pores of the silica particles. Additional evidence for this assumption was found with liquid pycnometer experiments carried out at elevated pressures. Compression and decompression was used to remove the gas out of the pores. As an example, with Nucleosil 100-5 C18 in acetone, skeleton densities were found of 1.6055 \text{kg} \cdot \text{m}^{-3}, 1.6415 \text{kg} \cdot \text{m}^{-3}, \text{and} 1.6875 \text{kg} \cdot \text{m}^{-3} \text{at pressures of} 1.6, 1.2, \text{and} 1.0 \text{bar, respectively. As shown by the latter results, the inclusion of air is the most likely reason for the entrapping of air in the particle pores during sonication.}

The amount of entrapped air can be calculated by applying simple mass equations, knowing \( \rho_{skele} \) from a gas pycnometer, \( V_{pore} \), and the apparent skeleton density from a liquid pycnometer. Information about the filled pore fraction can be obtained by expressing the amount of entrapped air as a function of the particle porosity. All liquid pycnometer density measurements were duplicated. The repeatability of these experiments was found to be better than 2\%. In Table 2, \( \epsilon_p / \epsilon_{liq} \) is given for the different ODS particles in various liquids. Only 40–90% of the silica particle pores got filled with slurry liquid after sonication of the slurry during a 10 min period, as can be seen from the results in Table 2. With RoGel RP, the pores were completely filled with liquid in all cases.

Table 2. Fraction \( \epsilon_p / \epsilon_{liq} \) of the Pores Filled with Slurry Liquid after Sonication for 10 Minutes

<table>
<thead>
<tr>
<th>stationary phase</th>
<th>acetone</th>
<th>ACN</th>
<th>CCL(_4)</th>
<th>IPA</th>
<th>MeOH</th>
<th>THF</th>
</tr>
</thead>
<tbody>
<tr>
<td>silica-based</td>
<td>BioSil C18 HL 90-5 S</td>
<td>0.594</td>
<td>0.420</td>
<td>0.648</td>
<td>0.632</td>
<td>0.700</td>
</tr>
<tr>
<td></td>
<td>Nucleosil 100-5 C18</td>
<td>0.817</td>
<td>0.765</td>
<td>0.837</td>
<td>0.837</td>
<td>0.778</td>
</tr>
<tr>
<td></td>
<td>Spherisorb ODS-1</td>
<td>0.782</td>
<td>0.730</td>
<td>0.795</td>
<td>0.871</td>
<td>0.865</td>
</tr>
<tr>
<td></td>
<td>Zorbax ODS</td>
<td>0.528</td>
<td>0.530</td>
<td>0.777</td>
<td>0.666</td>
<td>0.721</td>
</tr>
<tr>
<td></td>
<td>Zorbax SB-C18</td>
<td>0.591</td>
<td>0.564</td>
<td>0.708</td>
<td>0.752</td>
<td>0.740</td>
</tr>
<tr>
<td>poly styrene-based</td>
<td>RoGel RP</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>BioSil RSL nv. (Nazareth, Belgium).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Characterization of the Particles. The particle diameter, skeleton density of the particles, and particle porosity must be known in order to determine the theoretical sedimentation rate of porous spherically shaped particles, according to eq 3. The results of the size distribution, nitrogen absorption, and stereopycnonometric measurements on six different octadecyl-bonded silicones (ODS) and a PS–DVB polymer material are given in Table 1. The median, \( d_{50} \), was taken as the average particle diameter, \( d_p \). The particle porosity is given in Table 1, which was calculated by use of eq 2.

Application of eq 1 to suspensions of noncoagulated silica-based packing materials resulted in an overestimation of the theoretical sedimentation rate compared to empirical determined settling

tions of stationary phases and liquids of varying densities and viscosities, normalized parameters were introduced.

normalized height $\Lambda$: $\Lambda = (h_{\text{clear}} - h_{\text{tot}})/h_{\text{tot}}$ (5)

where $h_{\text{clear}}$ and $h_{\text{tot}}$ are the heights of the clear top layer and of the total slurry, respectively.

normalized time $\tau$: $\tau = tv/h_{\text{tot}}$ (6)

where $t$ is time elapsed since the start of the sedimentation experiments and $v$ the theoretical sedimentation rate of noncoagulated particles according to equation 1, 3, or 4. For the hindered settling constant $K_s$, a value of $-5.4$ was used. A more detailed explanation about hindered settling is given elsewhere. In that paper, a detailed discussion is given of the coagulating behavior of ODS particles, as based on van der Waals interactions.

An example of a graph of normalized height of a sediment vs normalized time for two different slurry liquids at three different slurry concentrations is shown in Figure 1a,b. The solid lines in this figure represent the normalized theoretical sedimentation rate for noncoagulated suspensions. As can be seen, Nucleosil 100-5 C18 in acetone clearly shows noncoagulated behavior. The theoretical and experimental sedimentation curves are in good agreement. However, the same stationary phase, Figure 1b, coagulates in CCl4.

This approach for particle sedimentation provides information about the extent to which the particles are coagulated, whereas the sedimentation quotient as introduced by Shelly and Edkins provides only relative information. Furthermore, the sedimentation rate is more sensitive toward coagulation than the sedimentation quotient, especially for low suspension concentrations. All investigated silica-based stationary phases were noncoagulated in acetone, ACN, IPA, and THF. Exceptions were BioSil C18 HL 90-5 S, which was coagulated in ACN, and Nucleosil 100-5 C18, which was coagulated in IPA. Coagulation of all silica materials took place in ACN/H2O 70:30 (v/v), CCl4, and MeOH. Here, Spherisorb ODS-1 and Zorbax SB-C18 were exceptions because they did not coagulate in MeOH and only moderately coagulated in ACN/H2O 70:30 (v/v). RoGel RP coagulated in CCl4, IPA, and MeOH.

A comparison of the theoretical rates of sedimentation of irregularly shaped particles with the experimental settling rate can be performed in a similar way. To demonstrate this, Polygosil 60-5 C18 was employed for sedimentation experiments in acetone. The shape factor, $\psi$, was estimated to be 0.26. The slurry concentration was 100 kg·m$^{-3}$. The results are shown in Figure 2. The open circles represent normalized times if a ±5% deviation in $\epsilon_i$ (the most critical sedimentation parameter) was assumed. Again, the theoretical and experimental settling rate are in fairly good agreement for irregularly shaped particles.

$\zeta$-Potential Measurements. $\zeta$-potentials of the particles were measured to determine whether electrostatic or steric effects play a decisive role in slurry stabilization. A measure for the degree of coagulation is the ratio $\alpha$ of the initial slope of the experimentally determined and the theoretically calculated sedimentation rates, this is $\alpha = (\Delta A_{\text{exp}}/\Delta x_{\text{exp}})/(\Delta A_{\text{theor}}/\Delta x_{\text{theor}})$. $\alpha = 1$ for stable suspensions, and $\alpha > 1$ for coagulated suspensions.

In Figure 3, $\alpha$ is given for four types of packing material particles in MeOH as a function of the $\zeta$-potential. With all suspensions that are stable according to the sedimentation experiments, $\zeta$-potentials in the order of $-40$ to $-100$ mV were found. Electrostatic repulsion forces are therefore believed to be predominant in the stabilizing effects of these kinds of particles. Because of the geometry of the measuring cell and refractive index differences, it was impossible to determine the $\zeta$-potential of unstable and CCl4 suspensions, respectively.

Chromatographic Results. A number of solvents were selected as slurry and packing liquids in the packing procedure for the microcolumns, based on the results of the sedimentation experiments. A representative example of the results obtained is given in Table 3, where the average chromatographic performances for capillary columns packed with Nucleosil 100-5 C18 as the stationary phase are summarized. Of each type of column at least three were prepared and tested. In all LC experiments the mobile-phase velocity was 4 $\mu$L·min$^{-1}$, a value close to the optimum

Figure 1. Normalized height of the sediment, $\Lambda$, as a function of the normalized time, $\tau$, for Nucleosil 100-5 C18 in (a, top) acetone or CCl4 (b, bottom) as the dispersion liquid. Slurry concentrations: (C) 50, (O) 100, and (Δ) 150 kg·m$^{-3}$. The solid line represents the theoretical rate of sedimentation for noncoagulated stationary phase particles.
Table 3. Column Performance of 320-μm-i.d. Capillary Columns Packed with Nucleosil 100-5 C18, Using Different Slurry and Packing Liquids

<table>
<thead>
<tr>
<th>slurry liquid</th>
<th>packing liquid</th>
<th>h</th>
<th>Φ</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetone</td>
<td>acetone</td>
<td>2.08</td>
<td>587</td>
<td>2530</td>
</tr>
<tr>
<td>MeOH</td>
<td>MeOH</td>
<td>4.25</td>
<td>493</td>
<td>11100</td>
</tr>
<tr>
<td>THF</td>
<td>THF</td>
<td>7.48</td>
<td>1150</td>
<td>68000</td>
</tr>
<tr>
<td>acetone</td>
<td>THF</td>
<td>2.35</td>
<td>639</td>
<td>3740</td>
</tr>
<tr>
<td>THF</td>
<td>MeOH</td>
<td>2.68</td>
<td>701</td>
<td>5320</td>
</tr>
<tr>
<td>acetone</td>
<td>ACN/H2O</td>
<td>2.43</td>
<td>608</td>
<td>3630</td>
</tr>
<tr>
<td>ACN/H2O</td>
<td>ACN/H2O</td>
<td>2.42</td>
<td>571</td>
<td>3340</td>
</tr>
<tr>
<td>MeOH</td>
<td>ACN/H2O</td>
<td>2.25</td>
<td>609</td>
<td>3100</td>
</tr>
<tr>
<td>THF</td>
<td>ACN/H2O</td>
<td>2.34</td>
<td>849</td>
<td>4600</td>
</tr>
</tbody>
</table>

* Columns were tested with an eluent composition ACN/H2O 70:30 (v/v). The eluent velocity was 4 μL/min. The test compound was anthracene (k ~ 4). 1 ACN/H2O 70:30 (v/v).

Changing the packing procedure using the same slurry and packing liquid into a system consisting of noncoagulating or moderately coagulating slurry liquids and coagulating packing liquids resulted in good quality capillary columns, as can be seen in Table 3. These results indicate that, to obtain well-packed capillary fused-silica columns, the type of slurry liquid is of minor importance compared with the type of packing liquid. No consolidation was observed for columns packed with ACN/H2O 70:30 (v/v). In all other cases, consolidation of the chromatographic bed was moderate.

Results similar to the Nucleosil 100-5 C18 microcolumns were obtained for columns packed with BioSil C18 HL 90-5 S as the stationary phase. In the latter experiments, the slurry liquid, ACN, was used constantly and the packing liquids were varied. In Figure 4, the obtained Knox h−ν curves are depicted for the applied slurry solvents for the BioSil C18 HL 90-5 S stationary phase. From the results in Figure 4, it can be seen that good columns were obtained with the coagulating packing liquids, ACN, ACN/H2O 70:30 (v/v), and MeOH. The noncoagulating packing liquids, IPA and THF, yielded columns of lower separation efficiency. In these experiments, acetone was an exception too. Although acetone showed noncoagulating properties, it yielded very good results when applied as the packing liquid. As yet, no reasonable explanation for this has been found.

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Figure 2. Normalized height of the sediment, Λ, vs the normalized time, τ, for Polygosil 60-5 C18 in acetone as the dispersion liquid: (•) εi = 0.577 and (○) εi ±5%. The solid line represents the theoretical rate of sedimentation for noncoagulated stationary-phase particles, using εi = 0.577.

Figure 3. Degree of flocculation, α, vs the ζ-potential for (○) Spherisorb ODS-1, (●) Zorbax SB-C18, and (□) Nucleosil 100-5 C18, and (●) RoGel RP packing materials in MeOH as the dispersion liquid. The concentration of the slurry from which α was derived was 100 kg/m³.

Figure 4. Knox h−ν plots for fused-silica capillary columns packed with BioSil C18 HL 90-5 S. CCl₄ was used as the slurry liquid. Packing liquids: (○) acetone, (●) ACN, (□) ACN/H₂O 70:30 (v/v), (△) IPA, (□) MeOH, and (●) THF. Test conditions as in Table 3.

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velocity of the mobile phase for each case. From the first three rows in this table it can be seen that if the same solvent were used as the slurry and the packing liquid, low efficient columns were obtained in terms of h, Φ, and E irrespective of the coagulating properties of the suspensions. However, acetone was a clear exception in this respect. Similar results were found for other stationary phases, as shown later. For all investigated stationary phases, the lowest efficiencies were obtained for THF/THF as slurry liquid and packing liquid. Moreover, during the conditioning step of these columns, a consolidation of the chromatographic bed was observed of about 20–30 mm over a total column length of 0.40 m. An explanation of this effect is given in the next section, where attention is paid to the contradiction between h and Φ for columns packed with THF as both slurry and packing liquid.
Application of the results of the experiments described above to the other investigated reversed-phase packing materials showed similar results. In summary, going from a noncoagulating slurry/packing liquid system, IPA/IPA, to a noncoagulating/coagulating system, acetone/MeOH, for Spherisorb ODS-1, h-values decreased from 8.3 to 3.9. For Zorbax SB-C18, h was 19.1 in a THF/THF system; this value decreased to 4.7 for a system using acetone as the slurry liquid and MeOH as the packing liquid. For Zorbax ODS, the h-value was 3.5 for a noncoagulating/coagulating system THF/(ACN/H2O 70:30 (v/v)). These results of reduced plate heights are moderate compared to the results obtained for Nucleosil 100-5 C18 and BioSil C18 HL 90-5 S. However, in the latter experiments, the particles coagulated moderately in the applied packing liquids. Application of more coagulating packing liquids might increase the performance of these columns. For RoGel RP an h-value of 6.4 was observed when ACN was applied as the slurry and packing liquid. For a noncoagulating/coagulating system, THF/(ACN/H2O 70:30 (v/v)), h was reduced to 4.4. Similar results were reported recently by Cortes and Pfeiffer21 for another PS–DVB material tested in the size exclusion chromatography mode for incompletely permeating compounds.

Chromatographic Bed Structures. The most plausible explanation for the extreme consolidation phenomena, observed in the experiments for columns packed with THF as the slurry and the packing liquid, is that the electrostatic repulsion forces between the particles during the packing process could not be overcome by the applied packing pressure. Alternatively, the particles were pushed away from each other by the same kind of electrostatic forces, after disconnecting the column from the packing equipment, both resulting in a very loosely packed bed. During conditioning of the THF/THF packed capillary columns, the loosely packed stationary-phase particles coagulate and reorganization of the chromatographic bed takes place. This consolidation process is random and results in a very disordered bed, as indicated by the reduced plate height and flow resistance parameter of the Nucleosil 100-5 C18 THF/THF packed columns. These ideas are supported by scanning electron microscope pictures of cross sections of packed capillary columns, of which examples are given in Figure 5a,b. To obtain correct information about the structure of the chromatographic bed, the particles were embedded in a low-viscosity resin22 prior to the cutting of the column. The main demands for such a resin are a low viscosity in its monomeric state and a negligible difference in density between the monomeric and polymeric state.

Figure 5a shows the cross section of a chromatographic bed of a Nucleosil 100-5 C18 packed column, using MeOH as the slurry and packing liquid, respectively. This column showed a relative low h of 2.7 and a moderate back pressure, ΔP, of 94.3 bar over a column length of 0.412 m, which is in agreement with the observed bed structure, i.e., a homogeneous tightly packed bed. In Figure 5b, a packed bed cross section is shown for a Zorbax SB-C18 packed column, using THF as the slurry and the packing liquid, respectively. This column showed a very high h of 21.9 and a ΔP of 165.8 bar over a column length of 0.445 m. The reason for the low performance of the column is obvious. As can be seen from Figure 5b, the bed structure is very disordered and even channels are present. However, examination of other slices of cross sections of this column also showed tightly packed particles, explaining the very high back pressure of the column.

Reproducibility Studies. BioSil C18 HL 90-5 S was also used to test the reproducibility of packing procedures using the same slurry and packing liquids as described in the section Chromatographic Results. Each packing procedure was repeated 5 times in order to gain statistical information about the repeatability in h, E, and Φ at the optimum mobile-phase velocity of these procedures. The results are given in Table 4. The coagulating packing liquids ACN, ACN/H2O 70:30 (v/v), and MeOH generally showed a better repeatability for h than the noncoagulating packing liquids acetone, IPA, and THF. The repeatability obtained with acetone as the packing liquid was, however, only slightly higher. Far the best results were obtained with ACN/H2O 70:30 (v/v) as the packing liquid. h, E, and the repeatability were significantly better compared to the other liquids. It is noteworthy that BioSil C18 HL 90-5 S particles in ACN/H2O 70:30 (v/v) were more coagulated compared to the other packing liquids.

CONCLUSIONS

The theoretical free settling rate of noncoagulated spherical and irregularly shaped reverse-phase packing materials in aqueous/organic solvents can be determined by means of a modified Stokes equation that accounts for possible inclusion of

Table 4. Average Values (n = 5) of the Reduced Plate Height, \( h \), Separation Impedance, \( E \), and Flow Resistance Factor, \( \Phi \), with Relative Standard Deviation (%), in Parentheses) for 320-\( \mu \)m-i.d. Capillary Columns Packed with BioSil C18 HL 90-5 S

<table>
<thead>
<tr>
<th>Packing Liquid</th>
<th>( h ) (%</th>
<th>( E ) (%</th>
<th>( \Phi ) (%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>2.37 (16.3)</td>
<td>3980 (32)</td>
<td>698 (12.7)</td>
</tr>
<tr>
<td>ACN</td>
<td>2.41 (14.0)</td>
<td>3570 (31)</td>
<td>600 (5.8)</td>
</tr>
<tr>
<td>ACN/H( _2 )O 70:30 (v/v)</td>
<td>2.11 (2.2)</td>
<td>2650 (10)</td>
<td>595 (10.7)</td>
</tr>
<tr>
<td>IPA</td>
<td>2.79 (24.2)</td>
<td>5180 (25)</td>
<td>629 (4.7)</td>
</tr>
<tr>
<td>MeOH</td>
<td>2.32 (15.8)</td>
<td>3240 (31)</td>
<td>597 (9.3)</td>
</tr>
<tr>
<td>THF</td>
<td>4.05 (66.0)</td>
<td>19300 (12)</td>
<td>832 (20.5)</td>
</tr>
</tbody>
</table>

*\( \text{CCl}_4 \) was the slurry liquid; columns were tested with an eluent composition ACN/H\( _2 \)O 70:30 (v/v); the test compound was anthracene (\( k \sim 4 \)).

In the pores of the stationary-phase particles. The degree of particle coagulation in slurry and packing liquids can be derived by comparison of the experimentially determined sedimentation rate of the particles with the theoretically calculated sedimentation rate of noncoagulated particles. No general rules for the coagulating behavior of ODS particles are derived. However, THF and acetone have noncoagulating properties for all investigated ODS stationary phases. The results of the stability experiments of reversed-phase particle suspensions, measured by sedimentation, were in good agreement with the \( \zeta \)-potential measurements.

The chromatographic performance of a packed bed in a microcolumn is strongly influenced by the nature of the packing liquid rather than by the slurry liquid. A packing liquid must have coagulating properties to obtain good results in terms of reduced plate height and flow resistance parameter. From the results of this study, the slurry liquid obviously is of minor importance for the final performance of packed capillary columns. The applied pressures are most likely large enough to overcome the attraction forces between the particles during the packing process, even in the case of the use of coagulating slurry liquids. No general rules exist for the selection of a proper slurry liquid. Finally, it can be concluded that for each stationary phase the individual packing solvent and packing condition has to be optimized to achieve satisfactory chromatographic performance. However, the results from this research provide adequate tools for the proper selection of packing liquid to be used in packing techniques for capillary LC columns.

ACKNOWLEDGMENT

The authors acknowledge E. M. van Oers (Eindhoven University of Technology, Department of Inorganic Chemistry, Eindhoven, The Netherlands), A. Spoelstra (Eindhoven University of Technology, Department of Polymer Technology, Eindhoven, The Netherlands), and M. E. H. Vanderbroeck for their technical assistance. We also thank A. B. J. M. Dams (Rockland Technologies, Inc., Nuenen, The Netherlands) and C. Dewaele (BioRad RSL nv., Nazareth, Belgium) for their generous gifts of the Zorbax SB-C18 and BioSil C18 HL 90-5 S stationary phases, respectively.

Received for review December 8, 1994. Accepted April 5, 1995.*


AC941187D