Normal-phase high-performance liquid chromatography with highly purified porous silica microspheres ☆

J.J. Kirkland*, C.H. Dilks, Jr. and J.J. DeStefano

Rockland Technologies, Inc., 538 First State Boulevard, Newport, DE 19804 (USA)

ABSTRACT

Column reproducibility and peak shapes are improved for polar and basic solutes in normal-phase chromatography using new highly purified, low-acidity unmodified porous silica microspheres. The chromatographic characteristics of this new type B silica are compared with a conventional type A silica to determine the type and level of mobile phase modifiers needed for good results. The effect of sample type and sample loading on retention, column efficiency and peak shape also was measured. Critical normal-phase tests compared this new silica with several commercial silicas. The new silica shows special promise for developing rugged separation methods to analyze polar and basic compounds that traditionally are handled by reversed-phase chromatography. Normal-phase chromatography is well-suited for highly organic-soluble compounds and certain structures such as positional isomers.

INTRODUCTION

Normal-phase (NP) chromatography is a powerful complement to the more popular RP-HPLC method for separating non-ionic compounds. Recent surveys show that about one fifth of all HPLC separations now are performed by NP-HPLC [1]. Advantages for this method include the ability to operate with totally organic mobile phases of high solute solubility, which is especially important in preparative applications. NP-HPLC also shows extensive capability for selectivity changes by varying mobile phase constituents [2,3]. The ability to make band spacing changes with NP-HPLC usually exceeds that of the widely used reversed-phase method. NP-HPLC methods are especially useful for separating difficult mixtures containing positional isomers [3,4].

Current NP-HPLC applications commonly use columns containing polar bonded phases (e.g., cyano- diol- , etc.) [5]. Columns containing unmodified silica are less popular, because of problems in maintaining a constant surface activity for repeatable separations. Conversely, retention with bonded-phase NP-HPLC columns generally is more consistent, because of the insensitivity to small concentrations of water in the sample or the mobile phase [6]. Also, there are problems in obtaining unmodified chromatographic silica with reproducible separation characteristics, as seen in the present study. Therefore, the chromatographic reproducibility of bonded-phase columns for NP-HPLC generally is viewed as superior to columns of unmodified silica [7]. Finally, polar bonded-phase columns are less retentive in NP-HPLC than those with unmodified silica, often permitting the elution of highly

---

* Corresponding author.

polar compounds that are eluted with difficulty from the more retentive unmodified silica [6,8].

A common problem with unmodified silica columns with totally organic solvents is that polar compounds often show broad, tailing peaks, especially for basic components. This condition can exist even when the mobile phase contains basic additives designed to minimize this effect. Such undesirable characteristics complicate the design of rugged and reproducible quantitative methods. As a result, unmodified silica columns are not widely accepted for quantitative analyses.

On the other hand, basic compounds have been separated on unmodified silica with good band shapes using reversed-phase conditions where water was a major constituent of the mobile phase [9]. Biddlingmeyer *et al.* [10] reported that some basic drugs produced satisfactory peak shapes on silica gel using reversed-phase eluents. These and other studies (e.g., ref. 11) suggest that unmodified silica, when properly deactivated with water or other strongly sorbing polar compounds, can be successfully used for separating basic and other polar solutes with good peak shape and desirable properties for quantitation.

Unmodified silica columns present a special problem that must be solved for reproducible separations: the activity of the adsorbent surface must be maintained constant [12]. The most effective procedure to stabilize this surface activity is to control the level of water adsorbed to the silica surface. This often is accomplished by fixing the concentration (humidity) of water in the mobile phase [12], a procedure viewed as awkward by many users. So, polar, usually protic, mobile phase modifiers such as methanol or propanol typically are used instead of water to control surface activity. Use of protic organic modifiers generally is successful in NP-HPLC. But, misshapen or tailing peaks can occur as a result of highly non-linear and unusual adsorption isotherms that result from such systems [11,13]. The fundamental problem remains, however, that commercial unmodified chromatographic silica often exhibits an inhomogeneous adsorbing surface. This situation can result in broad, poorly shaped peaks when used for

separating many polar solutes, and especially compounds such as basic drugs.

All silica particles for HPLC are not alike. Different chromatographic silicas can vary greatly in their separation characteristics, primarily because of variations in the type and structure of silanol groups on the surface, as illustrated in Fig. 1. Silicas with a high population of un-bonded silanol groups generally are more acidic and "less friendly" in the separation of many polar and basic solutes. Geminal silanols are less acidic, and less of a problem for such solutes. Silicas with a high population of internally bonded or associated silanols also are less acidic, and "more friendly" for separating polar and basic compounds [14,15]. Contamination of the silica with certain metals such as iron and nickel can cause tailing peaks with compounds that form complexes with these contaminants. Finally, inclusion of other contaminants such as aluminum in the silica lattice activates and intensifies the acidity of silanol groups [16].

Previous studies have shown that chromatographic silicas can be arbitrarily classified into types to define their potential utility as supports in reversed-phase chromatography [14,15]. So-called type A silicas generally are more acidic and less purified. These materials apparently have less energy-homogeneous surfaces, and are more likely to exhibit tailing, misshapen peaks with more polar or basic solutes. Type B silicas are more highly purified and less acidic, and often are preferred for many RP-HPLC applications [17]. Some silicas, however, exhibit intermediate properties, as verified by data presented below, and by other sources [7,17].
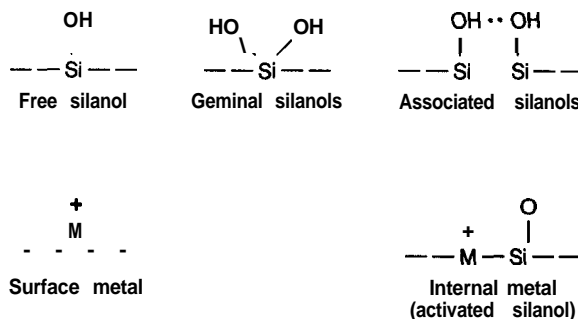

Fig. 1. The surface of silica supports for HPLC.

The present study was designed to determine the characteristics of a new porous silica microsphere, Zorbax Rx-SIL, for use in NP-HPLC (sometimes called adsorption chromatography). We postulated that the high purity (>99.995%) and low surface acidity of this type B silica could minimize or eliminate some of the problems normally associated with conventional unmodified silicas for NP-HPLC. To define utility, we studied the type and level of mobile phase modifiers required for best operation. The effect of sample type on column efficiency and peak shape also was determined. The chromatographic properties of Zorbax Rx-SIL were compared to conventional chromatographic silicas, to define the best areas of potential utility for this new silica.

EXPERIMENTAL

Separations were performed with a Model 870 pump (DuPont, Wilmington, DE, USA) with the columns at ambient temperature. Detection was with a DuPont Model 860 UV detector at 254 nm. Sample injection was with a Model 7125 sample valve (Rheodyne, Cotati, CA, USA) with a 10-μl sample loop, unless otherwise noted. HPLC-grade solvents were obtained from Baker Chemicals (Phillipsburg, NJ, USA) and EM Science (Gibbstown, NJ, USA).

Dichloromethane was used as the principal organic mobile phase constituent in this study. Various water levels in dichloromethane mobile phases were established by appropriately blending dry solvent (v/v) with water-saturated solvent in the manner previously described [11]. "Dry" dichloromethane was prepared by treating with molecular sieve type 4A pellets (EM Science, Gibbstown, NJ, USA) overnight. "Wet" dichloromethane was prepared by equilibrating overnight with EM Science Grade 12 silica gel (29-200 mesh) containing 20% (w/w) of deionized water, as described in ref. 12. Mobile phases modified with protic solvents were prepared on a mass percent basis.

Highly purified dichloromethane is needed to obtain reproducible separations with unmodified silica. Some as-received HPLC-grade dichloromethane contains impurities that deleteriously change the surface of the silica. The same experience was found with dichloromethane that was allowed to stand in previously opened bottles for several days. Measurements with deionized water equilibrated with suspect dichloromethane showed a lower pH compared to satisfactory solvent. This result suggests the breakdown of dichloromethane to yield acidic impurities in the presence of light, air and water vapor. If contaminated with impurities from off-grade dichloromethane, columns usually can be regenerated by extensive purging with HPLC-grade methanol containing 10% deionized water.

All columns were equilibrated with at least thirty column volumes of a new mobile phase before taking data. (Because of the low viscosity of totally organic solvents, this equilibration can be performed rapidly at high flow-rates.) Duplicate measurements were made to ensure complete column equilibration.

Benzyl alcohol, benzanilide, acetoacetanilide, catechol and Cacetamidophenol were from Sigma (St. Louis, MO, USA). Caffeine was from Fisher Scientific (Fairlawn, NJ, USA). Quinoxaline and 3-pyridyl-acetonitrile were obtained from Aldrich (Milwaukee, WI, USA). Sulfanilamide was from U.S.P.C. (Rockville, MD, USA). PolyScience (Niles, IL, USA) supplied the herbicide mixture, and the plant growth hormones were from ICN Biochemicals (Cleveland, OH, USA). All test solutes were used as received.

Zorbax-SIL and Zorbax Rx-SIL columns were prepared internally. Similar columns are available from Mac-Mod Analytical (Chadds Ford, PA, USA). Zorbax-SIL columns contain a silica with a nominal surface area of 330 m^2/g. This silica arbitrarily was used as a model for type A silica during this study. As documented below, this silica is similar in characteristics to several of the type A silicas studied during this work. Zorbax Rx-SIL is a type B silica with a nominal surface area of 180 m^2/g. Typical analysis of impurities for this silica by inductively coupled plasma atomic-emission spectroscopy is: Na: 10 ppm (w/w); Ca: 4 ppm; K: <3 ppm; Al: <10 ppm; Mg: 4 ppm; Zn: 1 ppm; total: <35 ppm (no other impurities detected; 99.995% silica). All other silica columns were obtained either from Alltech, or directly from the manufacturer. All

columns tested were 15 x 0.46 cm I.D. unless otherwise noted. Particle sizes of the packings in all columns were nominally 5 μm. Chromatographic results were monitored with a Chrom-Perfect data station (Justice Innovations, Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Wafer-deactivation of silica

Previous studies have shown that deactivation of the unmodified silica surface is required for reproducible separations with good column efficiency and sample loadability [2,11,12]. Highly polar modifiers in the organic mobile phase bind to ("hot") sites of very high energy on the silica surface. The purpose of these modiiers is to compete for these "hot" sites more effectively than the solute molecules that are to be separated. The result is that the surface of the modified silica then is more energy-homogeneous -a more linear isotherm results. This condition provides for improved column efficiency, better peak asymmetry, and higher sample loadability.

Water previously has been found to be the best deactivating agent, even though maintaining the proper water level in immiscible mobile phase solvents often is deemed as inconvenient [2,4,5]. The level of water in the mobile phase needed to optimize performance depends on the type of silica used for the packing. Therefore, a study was made on the influence of water level modifier in the organic mobile phase on solute retention, column efficiency, peak asymmetry and sample loadability.

The effect of water content in dichloromethane mobile phase on the retention of benzanilide for a typical type A silica and the new type B silica is compared in Fig. 2. This plot shows that as the amount of water increases (measured as percent of water saturation for the organic solvent), retention *(k')* decreases as the silica surface is deactivated. The *k' versus* percent mobile phase water-saturation plot for the type A silica shows a steeper curvature at lower water concentrations. Conversely, the type B silica shows a flatter plot as the water concentration is decreased. These effects suggest better surface homogeneity for the type B silica. Simi-
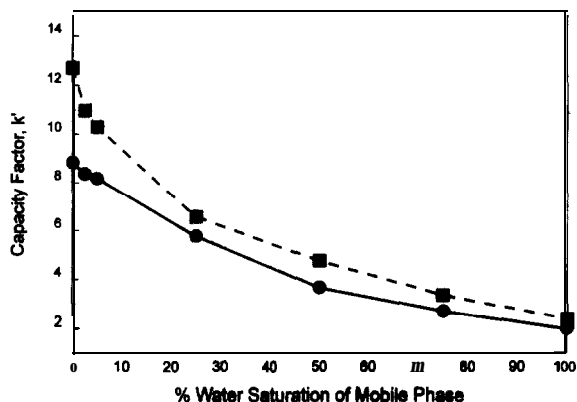

Fig. 2. Effect of mobile phase water content on retention for different silica types. Columns: 15 x 0.46 cm I.D.; mobile phase: dichloromethane; flow-rate: 1.0 ml/min; solute: 0.5 μg of benzanilide; temperature: ambient. ■ = Zorbax-SIL (type A); ● = Zorbax Rx-SIL (type B).

lar effects were found for phenol and benzyl alcohol as solutes. Absolute differences in retention for the two silicas is a function of differences in surface area. These and previous [11] results suggest that a 50% water-saturated mobile phase (1:1 v/v blend of "wet" and "dry" solvents) is a desirable compromise for water-deactivated systems with both silicas; retention is maintained and is less sensitive to small changes in water content.

The effect of mobile phase water content on column efficiency is more striking for the two silica types, as shown in Fig. 3. The new type B
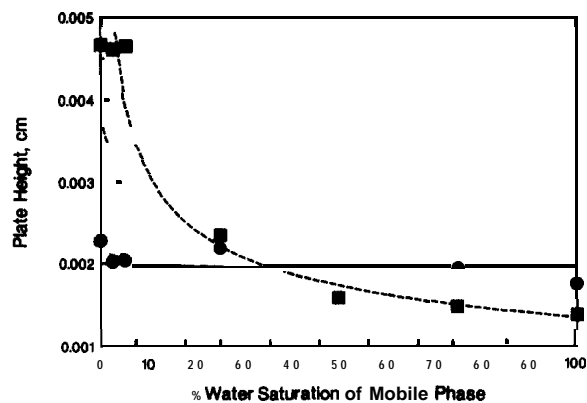

Fig. 3. Effect of mobile phase water content on column efficiency for different silica types. Conditions and symbols as in Fig. 1, except 38 μg of benzyl alcohol as solute.

silica shows little variation in the plate height of benzyl alcohol with varying water content in the mobile phase. On the other hand, the type A silica shows much poorer column efficiency (larger plate heights) at low water concentration. For dry dichloromethane the column plate height for the type A silica is more than twice that for the type B silica. Similar effects were found with benzanilide as the solute. These results suggest a non-linear adsorption isotherm for the type A silica at low water concentrations in the mobile phase. The new type B silica can be used with a wider range of water modifier concentration with little effect on separation resolution.

Peak asymmetries for benzanilide were strikingly different with varying amounts of water in the mobile phase for the two silicas, as illustrated in Fig. 4. The type A silica shows peak asymmetry values [11] in the 1.5-2.0 range with up to about 25% water saturation of the dichloromethane. As more water is added to dichloromethane, peak symmetry continuously improved. At 100% saturation, a nearly symmetrical peak was found. Conversely, with the type B silica, benzanilide showed a peak asymmetry value of cu. 1.4 with "dry" solvent, and an essentially symmetrical peak with all mobile phases with >10% water saturation. These data again suggest the superior surface homogeneity of the new type B silica.

The above conclusions are based on the premise that a heterogeneous silica surface leads to isotherm non-linearity and the related phenom-

ena of large plate heights and asymmetric, tailing peaks. These effects are discussed in detail in ref. 2, and the data of this study appear to support this concept. Since peak broadening and peak tailing also are influenced by sample size, small samples were used in this study to minimize this effect, as described below. It also should be noted that kinetic effects involving slow solute adsorption/desorption can affect plate heights and peak asymmetries. Additional studies with the two silica types are needed to measure possible differences in kinetic effects.

Sample loading effects on solute retention are similar for the two silica types with 50% water-saturated dichloromethane mobile phase, as shown in Fig. 5. Depending on the solute, up to 0.01-0.1 mg of sample/g of packing is allowed before a 10% change in k' occurs. Sample loadability is surface-area dependent, so the absolute solute loadability of Zorbax SIL is higher than that of Zorbax Rx-SIL. Similar results were found for phenol as the solute. It should be noted that 50% water-saturated dichloromethane mobile phase was arbitrarily selected because of the practicality of this system. Had mobile phase with less water been used, the differences between the two silicas probably would have been more striking, as suggested by the results in Fig. 2.
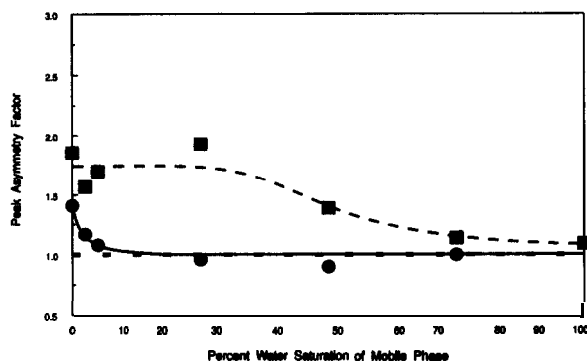
The effect of sample loading on column ef-

Fig. 4. Effect of mobile phase water content on peak asymmetry for different silica types. Conditions and symbols as in Fig. 1. -- (Peak asymmetry factor = 1) = symmetrical peak.
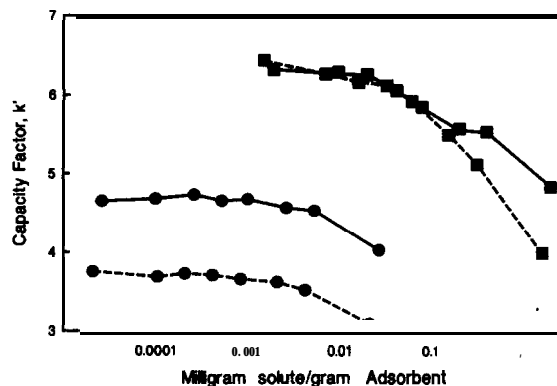

Fig. 5. Effect of solute loading on retention for different water-deactivated silica types. Conditions as in Fig. 1, except 50% (v/v) water-saturated dichloromethane; varying amounts of (■) benxyl alcohol and (●) benzanilide solutes. Solid lines: Zorbax-SIL (type A); dashed lines: Zorbax Rx-SIL (type B).
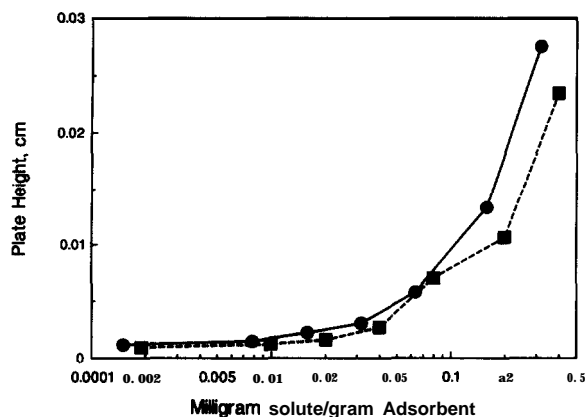
Fig. 6. Effect of solute loading on column efficiency for different water-deactivated silica types. Conditions as in Fig. 5, except benzyl alcohol as solute. ■ = Zorbax-SIL (type A); ● = Zorbax Rx-SIL (type B).
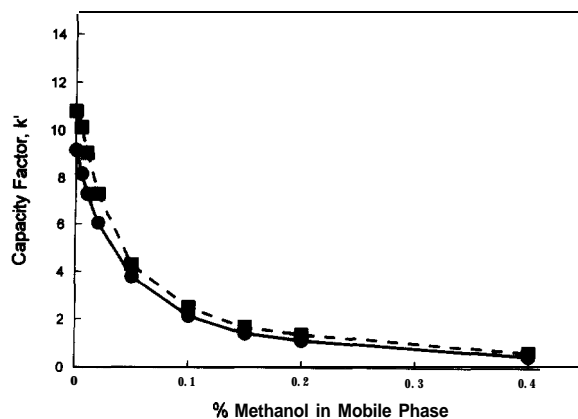

Fig. 7. Effect of methanol as modifier on retention for different silica types. Conditions and symbols as in Fig. 1, except methanol modifier; solute, 0.5 μg of benzanilide.

ficiency also is similar for the two silica types with water-saturated dichloromethane as the mobile phase, as shown in Fig. 6. Sample loadability is essentially equivalent for the two silicas, with small variations dependent on differences in packing surface area. In this system, 0.02-0.05 mg of sample was allowed before a significant change occurred in column efficiency. Again, differences between the two silicas were moderated by using 50% water-saturated mobile phase; lower concentrations of water would have accentuated differences, as indicated by the results in Fig. 3.

Deactivation of silica with protic modifiers

As mentioned above, many users consider the necessity to maintain a constant amount of water modifier in the organic mobile phase as experimentally awkward. Because of convenience, other highly-polar, organic-miscible modifiers such as methanol and propanol often are used in normal-phase separations to control and maintain the activity of the silica adsorbent. These polar organic compounds strongly bind to the silica surface, although they do not hold as tightly as water [2,18]. Because of the convenience of protic-solvent modifiers, a study was made with methanol and propanol to determine their effect on type A and B silicas.

Fig. 7 shows the effect of methanol concen-

tration in dichloromethane phase on the capacity factor, *k'*, of benzanilide. Similar results were found for the two silica types; both show sharply increasing *k' versus* percent methanol characteristics at low methanol concentrations. Small absolute *k'* differences are due to different packing surface areas. These results suggest that methanol-modified silica surfaces are less homogeneous than the water-modified silica of Fig. 2. Stated otherwise, water is a more effective deactivating agent than methanol, as previously noted [11,18]. The smaller change in *k'* for >0.1% methanol-modified dichloromethane suggests that at least 0.1% methanol should be used to ensure retention reproducibility for normal-phase separations.

But, the type A silica shows large changes in the plate height of benzanilide with different methanol-modifier concentrations, as illustrated in Fig. 8. Optimum plate height for the type A silica only is reached when 0.4% methanol modifier is used. In contrast, The new type B silica showed a constant plate height throughout the entire 0–0.4% methanol concentration range studied. These results again suggest the superior surface-energy homogeneity of the new type B silica.

Peak symmetries for benzanilide with 0.05% methanol modifier paralleled plate height results for the two silicas, as given in Fig. 9. Tailing peaks for the type A silica were eliminated only
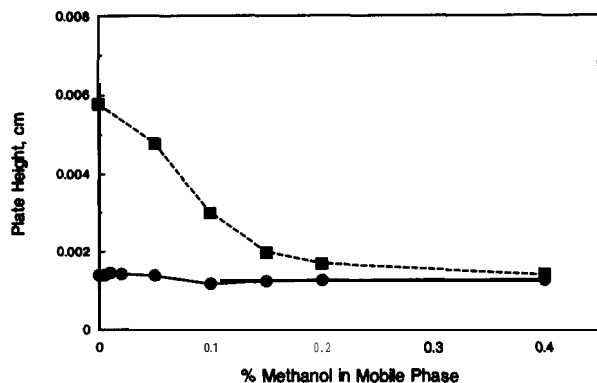
Fig. 8. Effect of methanol as modifier on column efficiency for different silica types. Conditions and symbols as in Fig. 7.
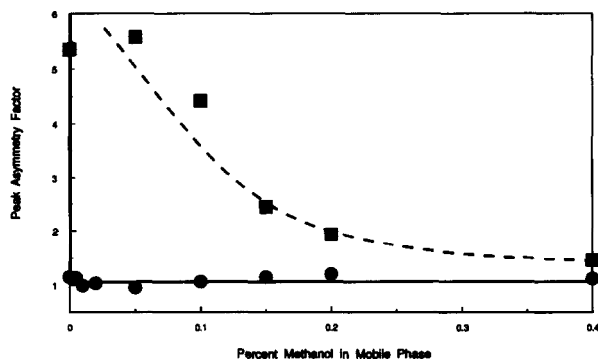

Fig. 9. Effect of methanol modifier on peak asymmetry for different silica types. Conditions and symbols as in Fig. 7.
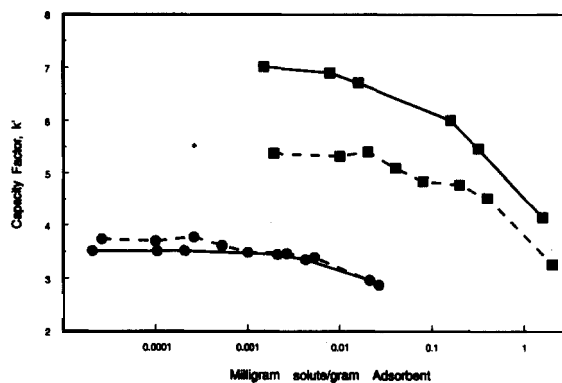

Fig. 10. Effect of solute loading on retention for different methanol-deactivated silica types. Columns as in Fig. 1; mobile phase: 0.05% methanol in dichloromethane; flow-rate: 1.0 ml/min; solutes as shown. ■ = Benzyl alcohol; ● = benxanilide; dashed lines: Zorbax-SIL (type A); solid lines: Zorbax Rx-SIL (type B).

when the methanol modifier content approached 0.4%. Similar results were found for benzyl alcohol as the solute. Phenol also showed larger asymmetry values for the type A silica, but less dramatic than for the other two solutes tested.

The effect of sample loading on retention when using methanol as the mobile phase modifier for the two silicas is shown in Fig. 10. In this study, 0.05% methanol modifier was used in an attempt to accentuate possible differences between the two silica types. The data indicate similarities for the two silicas. In both systems, 0.01-0.1 mg of solute/g of packing is allowed before a 10% change in k' occurred. The somewhat higher loading capacity of the type A silica is attributed to the higher surface area.

Similar effects of sample loading on column plate heights were found for the two silicas, as

illustrated in Fig. 11. The new type B silica shows significantly smaller plate heights, as would be expected by the data in Fig. 8 with the 0.05% methanol modifier used. The results in Fig. 11 suggest that new type B silica is somewhat more sensitive to sample load than the type A silica. However, this difference could be due to the variation in the surface areas between the two silicas. Alternatively, the apparent higher sensitivity of the new type B silica to sample mass could be a result of the smaller peak volumes generated with this column, resulting in a
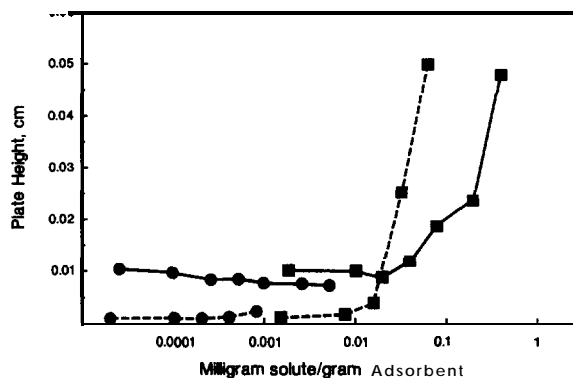

Fig. 11. Effect of solute loading on column efficiency for different methanol-deactivated silica types. Conditions as in Figure 10. ■ = Benzyl alcohol; ● = benzanilide; solid lines: Zorbax-SIL (type A); dashed lines: Zorbax Rx-SIL (type B).

higher level of overloading at the localized peak site on the packing.

Propanol also has been used as a mobile phase modifier in NP-HPLC with unmodified silicas. The effect of the amount of **2-propanol** modifier in dichloromethane on the **k'** of solutes for the two silicas was closely similar to that found for methanol. Both silicas showed **k' versus** percent modifier plots that were essentially the same shape as for methanol. However, large plate height differences were exhibited for the two silica types with **2-propanol** modifier, just as in the case for methanol in Fig. 8. The type A silica showed much larger plate heights, and more random variation in plate height as the concentration of **2-propanol** was changed.

Note, however, that use of modifying **protic** organic solvents with some unmodified silicas can result in misshapen peaks for certain solutes. Fig. 12 compares peaks shapes from three silicas, using 0.05% methanol as the mobile phase **mod-**

ifier. This low level of modifier concentration was used to accentuate the effect of unfavorable modifier composition. Note the unusual peak shapes for two type A-like silicas. Benzanilide produced a broad, tailing peak with **Zorbax-SIL** under these conditions. With the Spherisorb column, the elution order was reversed for **benz-anilide** and phenol, and the latter peak was broad and tailing (when chromatographed separately). Similar results were found with **2-prop-anol** as the modifier at low concentrations. Previous studies also have shown misshapen peaks for some solutes with an unmodified silica when methanol and propanol was used as mobile phase modifiers [5]. This condition apparently is the result of complex adsorption isotherms that occur with such systems at low **protic** solvent modifier concentrations [6]. These and other studies suggest that **protic** mobile phase modifiers should be used cautiously in NP-HPLC systems with unmodified silicas. For **dichlorome-thane** the level of **protic** modifiers probably should exceed 0.2% (v/v) for most silicas.

Comparison of commercial silicas

Eight commercial unmodified silicas were compared in terms of retention, selectivity, column efficiency and peak shape, using a mixture of polar and basic compounds as model solutes. Fig. 13 shows chromatograms of this test mixture for various columns. For this study, a favorable organic modifier concentration (2% methanol) was used in the dichloromethane mobile phase, to minimize potential misshapen-peak problems such as that discussed for Fig. 12. The **chroma-tograms** in Fig. 13 show large differences in the selectivity of the various silicas. Several peak inversions are noted for the same operating conditions. Peak shapes for the various test solutes sometimes were different, and for one column, sulfanilamide was very strongly retained with a very broad peak. Fig. 14 compares the retention (as capacity factor, **k')** of the various test solutes for these eight columns under the same operating conditions. Strong differences in the level of retention were found, suggesting different surface areas and/or different surface activities. Retention generally seems higher for the silicas that have type A qualities.
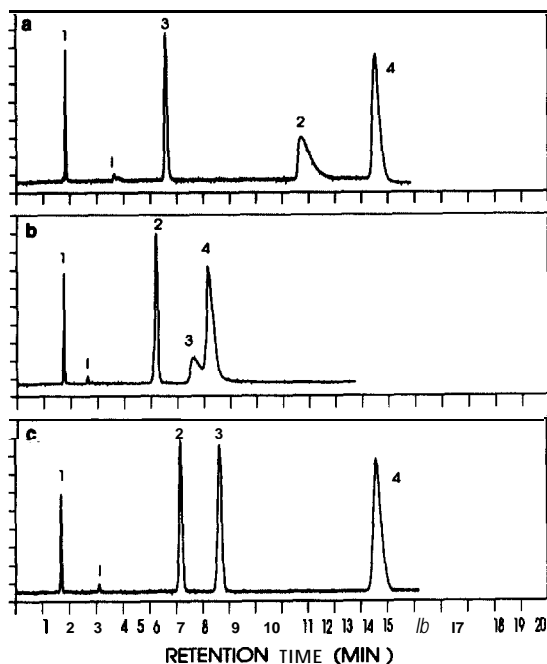

Fig. 12. Potential for misshapen peaks with protic modifiers for different silicas. Columns: 15 x 0.46 cm I.D.; mobile phase: 0.05% methanol in dichloromethane; flow-rate: 1.0 ml/min; solutes: 1 = 4 μg toluene, 2 = 0.5 μg benzanilide, 3 = 5 μg phenol, 4 = 38 μg benzyl alcohol. I = impurity. a = Zorbax-SIL, b = Spherisorb, c = Zorbax Rx-SIL.
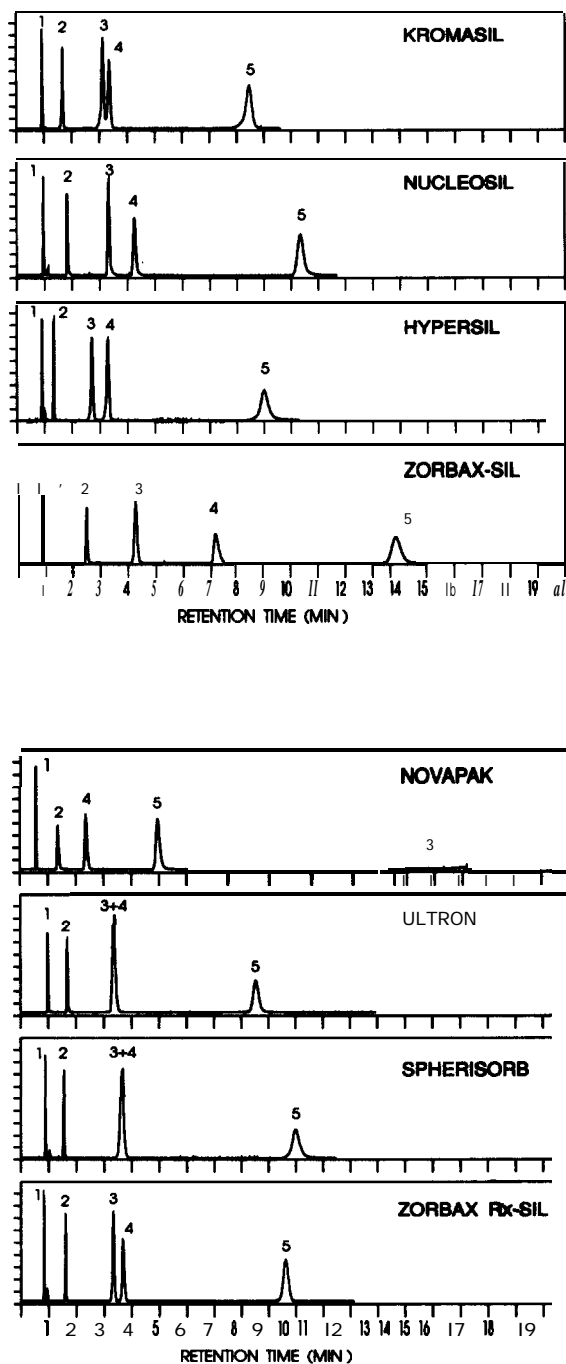
Fig. 13. Comparison of some commercial silicas: chromatograms. Columns as given in Experimental; mobile phase: 2.0% methanol in dichloromethane; flow-rate: 2.0 ml/min; solutes: 1 = 3 μg toluene, 2 = 1 μg quinoxaline, 3 = 0.25 μg sulfanilamide, 4 = 1 μg, caffeine, 5 = 0.5 μg 4-acetamidophenol.

Plate heights for various commercial unmodified silica columns are compared in Fig. 15. Efficiency generally appears highest for the silicas that resemble type B materials. Fig. 16 compares the peak asymmetry factors for each column. In this case, the difference in peak asymmetry values (positive or negative) from the theoretical Gaussian value of 1.0 is given for each silica column. Again, best peak shape is favored for type B-like silicas. Note, however, that column plate height and peak asymmetry comparisons are dependent on true packing particle sixes, and on column packing efficiency. Therefore, the comparisons in Figs. 15 and 16 should be viewed with care.

While the comparisons in Figs. 13-16 are informative, we believe that the test mixture used is not sufficiently critical to probe some of the important features of chromatographic silica. Accordingly, another test mixture was devised that shows more striking differences in the various silicas studied. Again, 2% methanol in dichloromethane was used as the mobile phase; no other modifier were used. In this test mixture, toluene is a neutral solute used to estimate t_0, and to define the effectiveness of the column packing method. Acetoacetanilide and catechol interact with heavy metals by different mechanisms to form complexes that can result in tailing or missing peaks. 3-Pyridyl-acetonitrile strongly sorbs to acidic sites, so this highly basic compound is a useful probe for the acidic nature of the silica.

Fig. 17 shows chromatograms of this definitive test mixture that were obtained on eight commercial silica columns. Kromasil and Nucleosil gave similar results in this test; all four compounds eluted, with catechol tailing badly for both silicas and with reduced peak areas. Kromasil exhibited peak fronting for 3-pyridyl-acetonitrile; there also is the hint of an unresolved impurity in the beginning of the acetoacetanilide peak. Acetoacetanilide tailed somewhat with Nucleosil. Catechol was totally missing from the Hypersil chromatogram, with the other test solutes eluting normally. Zorbax-SIL failed to elute 3-pyridyl-acetonitrile, but the other solutes eluted normally, with overlapping of two compounds. Novapak gave results similar to
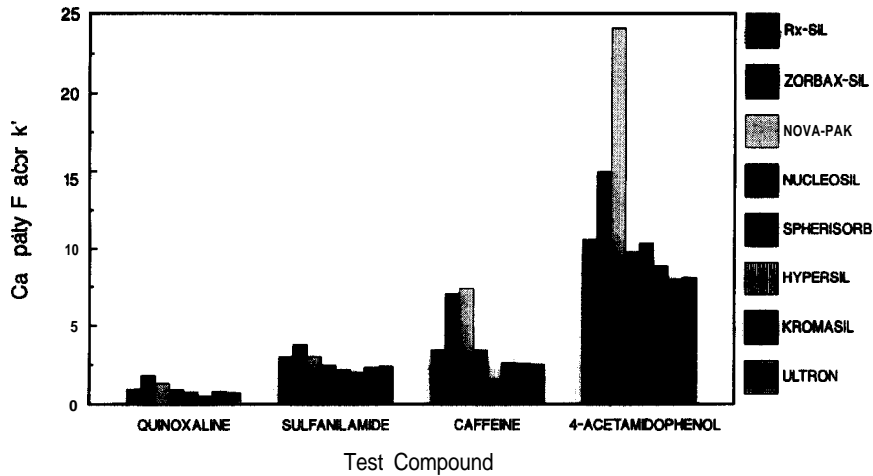
Fig. 14. Comparison of some commercial silicas: retention. Conditions as in Fig. 13.
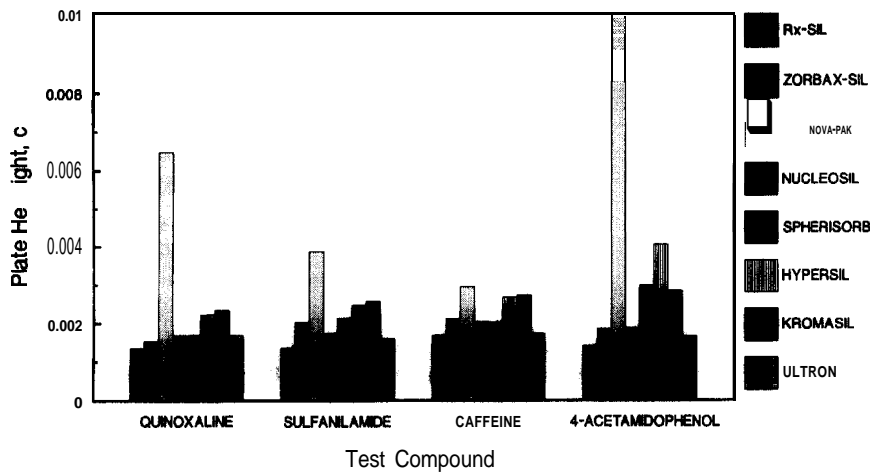

Fig. 15. Comparison of some commercial silicas: column efficiency. Conditions as in Fig. 13.
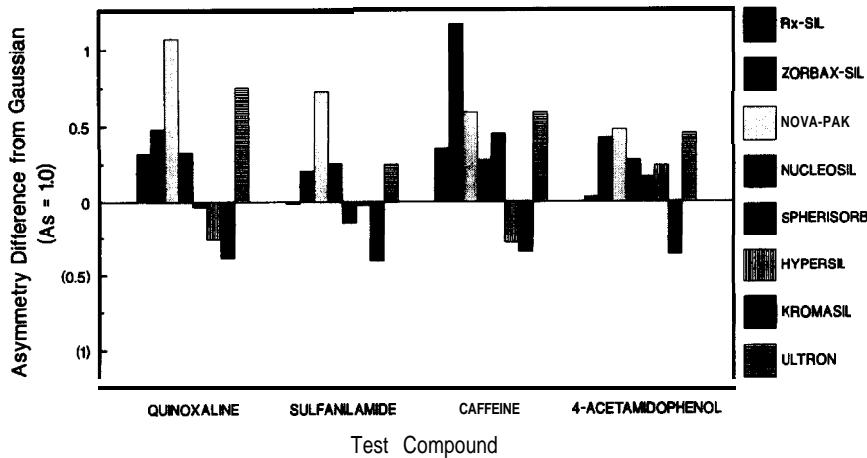

Fig. 16. Comparison of some commercial silicas: peak asymmetry. Conditions as in Fig. 13.
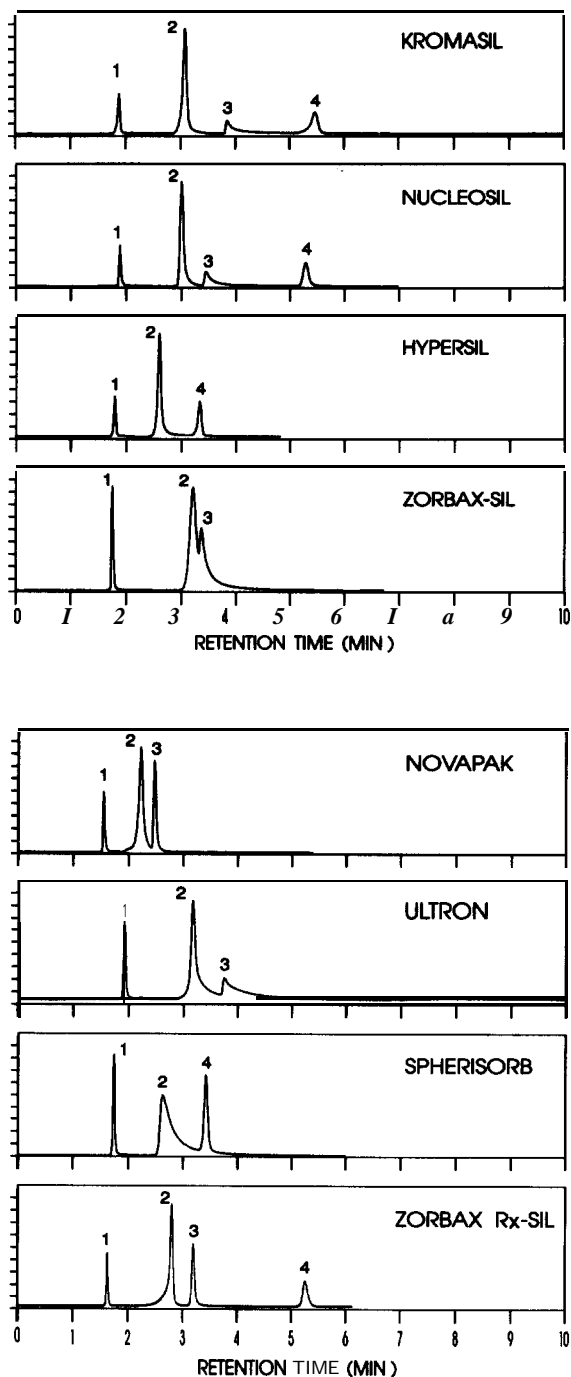
Fig. 17. Comparison of some commercial silicas: chromatograms. Columns as given in Experimental; mobile phase: 2.0% methanol in dichloromethane; flow-rate: 1.0 ml/min; sample volume: 1.0 μl; solutes: 1 = 1 μg toluene; 2 = 0.04 μg acetoacetanilide; 3 = 1 μg catechol; 4 = 0.1 μg 3-pyridyl-acetonitrile.

Zorbax-SIL; the basic probe did not elute, but the other solutes eluted normally. Ultron also did not elute the basic probe, and catechol was reduced in size with tailing. Spherisorb completely captured catechol, with tailing of **aceto**-acetanilide. **Zorbax** Rx-SIL eluted all test solutes normally, again with evidence of a small impurity at the beginning of the acetoacetanilide peak.

The results of the studies summarized in Figs. 13-17 generally agree with the listing given in ref. 11 regarding silica supports best suited for the reversed-phase chromatography of basic compounds. Highly polar and basic compounds are most favorably chromatographed with **less**-acidic, highly purified type B silicas. In the present study of the characteristics of some unmodified silicas for NP-HPLC, results suggest that the order of desirable type B qualities are: Zorbax Rx-SIL > Nucleosil > Kromasil ≈ **Hy**-persil > Spherisorb > **Zorbax-SIL** > Ultron > Novapak. Differences between successive listings may not be significant in all applications, since the chromatographic effects may be compound dependent.

Chromatographic reproducibility

A prime consideration in the use of unmodified silicas for NP-HPLC is separation reproducibility with various lots of materials over long time periods. Constant characteristics of the silica is required for repeatable separations and rugged analytical methods. Fig. 18 shows the variation found in manufactured lots of Zorbax
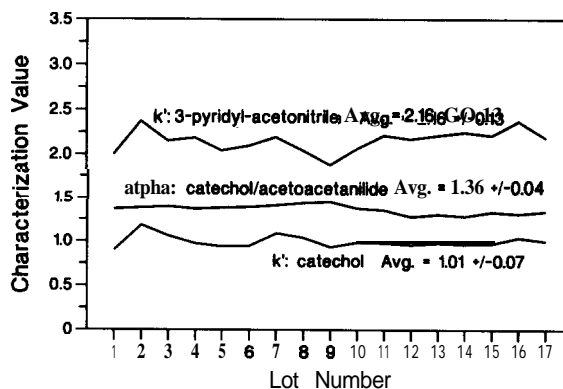

Fig. 18. Chromatographic reproducibility of Zorbax Rx-SIL silica. Conditions as for Fig. 17.

Rx-SIL over a two-year period. Small standard deviations were found in k' values for the highly sensitive compounds used in the test system described for Fig. 17. Slight k' variations are due to small changes in the surface areas of the various lots. More importantly, the α values for the sensitive catechol /acetoacetanilide pair show small variations over this two-year span, suggesting the potential for reproducible separations and rugged analytical methods with this silica.

CONCLUSIONS

In this study, large differences in retention and band spacing in normal-phase chromatography were found for eight different commercial unmodified silicas. The silicas studied show gross variations in separation resolution and analytical reproducibility. Highly purified, less-acidic type B silicas (e.g., Zorbax Rx-SIL) generally give superior results and better reproducibility in NP-HPLC. Still, good performance in some applications is available from some more acidic, less-pure silicas.

These studies reaffirm that deactivation with water usually gives the best chromatographic results with all silica types. The surfaces of water-deactivated silicas appear most homogeneous, producing more efficient columns and superior peak shapes. Still, methanol and 2-propanol are effective and more convenient deactivating agents when used at appropriate levels (e.g., >0.2% in dichloromethane). In some systems, however, these modifiers may cause misshapen peaks for some solutes, particularly when used at low concentrations.

REFERENCES

1 R.E. Majors, *LC-GC*, 9 (1991) 686.
2 L.R. Snyder, *Principles of Adsorption Chromatography,* Marcel Dekker, New York, 1968, Ch. 4.
3 L.-A. Trueddson and B. E. F. Smith, *J. Chromatogr., 214 (1981) 291.*
4 B. Stancher and F. Zonta, *J. Chromatogr., 234 (1982) 244.*
5 L.R. Snyder, J.L. Glajch and J.J. Kirkland, *Practical HPLC Method Development,* Wiley, New York, 1988, Ch. 4.
6 E.L. Weiser, A.W. Salotta, A.M. Flach and L.R. Snyder, *J. Chromatogr., 303 (1984) 840.*
7 L.R. Snyder, J.L. Glajch and J.J. Kirkland, *Practical HPLC Method Development,* Wiley, New York, 1988, Ch. 3.
8 A.W. Salloto, E.I. Weiser, K.P. Caffey, R.L. Corty, S.C. Racine and L.R. Snyder, *J. Chromatogr., 498* (1990) 55.
9 R.J. Flanagan and I. Jane, *J. Chromatogr., 323 (1985) 173.*
10 B.A. Bidlingmeyer, J. Korpi and J. Little, *Chromatographia, 2 (1982) 83.*
11 J.J. Kirkland, *J. Chromatogr., 83 (1973) 149.*
12 L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography,* Wiley, New York, 1979, Ch. 9.
13 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.,* 461 (1989) 19.
14 J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega and J.J. Kirkland, *J. Chromatogr., 352 (1986) 275.*
15 J. Köhler and J.J. Kirkland, *J. Chromatogr., 385 (1987) 125.*
16 R.K. Iler, *The Chemistry of Silica,* Wiley, New York, 1979, p. 711.
17 M.A. Stadalius, J.S. Berus and L.R. Snyder, *LC-CC,* 5 (1987) 495.
18 L.R. Snyder, in J.J. Kirkland (Editor), *Modern Practice of Liquid Chromatography,* Wiley-Interscience, New York, 1971, Ch. 4.