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Utility of small-particle silica in preparative chromatography

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ABSTRACT

Three columns filled with octadecylsilane-bonded spherical silica were studied to determine their behavior with different sample loads below and in the "overload" region. The stationary phases were as similar as possible, differing only in their particle sizes (8, 5 and 3 μm diameter). Optimum and non-optimum flow-rates, as determined by a plot of plate height *versus* flow-rate, were used with each particle size. Chromatograms were compared using peak width at half-height as a measure of preparative utility, after the method of Perry and Szczerba [*J. Chromatogr.*, 484 (1989) 267]. It was found that the three particle sizes became equivalent in peak width at a given sample load soon after entering the "overload" region. Although the larger particles gave a slightly wider range of linear peak width to sample load response, at no time did the larger particles offer a greater loading capacity than the smaller particles. Until overloaded, the larger particles gave less sample capacity than the smaller particles. The potential benefits of these findings as they influence throughput are discussed, and the terms "laboratory-scale" and "process-scale" preparative high-performance liquid chromatography are defined and discussed in the light of the results.

INTRODUCTION

In preparative high-performance liquid chromatography (HPLC), the desired purity is normally fixed; hence there is a minimum resolution which must be attained for the separation to be worthwhile. Once this needed resolution is attained, one must balance the sample capacity against the speed of the separation to maximize throughput within the limitations of the available hardware. Of course, the resolution is affected by both the speed and sample capacity of the separation, but purity is the one consideration which is set *a priori*, and also the prime factor when evaluating the utility of a separation.

The question of how best to achieve these goals is still subject to debate. In particular, the utility of small particle *versus* large particle stationary phases in preparative-HPLC remains a controversy. One school of thought holds that large (>20 μm) particles will give the most useful preparative separations owing to their low back-pressure, high capacity and low cost [1–3]. Implicit in this approach is the assumption that the separation is independent of the particle size if the column length is variable [4] over a wide range. Recently it has been shown theoretically that smaller particles allow higher production rates [5,6], even under conditions of column overload [7].

A method suggested by Perry and Szczerba [8] of comparing the preparative utility of small and large particle columns may shed more light on the advantages of small particles in preparative-HPLC. This method, termed the "equal-cut-point" approach, compared the peak widths at various loadings on columns of equal length containing C_{18} stationary phases of particle diameter 80, 40, 20 and 10 μm. For columns containing 20-, 40- and 80-μm materials there was a clear advantage for the column containing the smaller particles. Within the range of loadings studied, more sample could be loaded on the smaller particle column given equal peak widths.

Two findings of Perry and Szczerba's study are surprising: (1) although their results indicated a clear superiority for the smaller particle columns in

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the 20–80 μm range, here was no advantage of the 10- over the 20-μm material; no explanation was given for this finding; and (2) whereas theory predicts that peak width should be independent of stationary phase particle size for high (non-linear isotherm) loadings, this effect was not apparent in Perry and Szczerba's findings.

In this study we used the method of Perry and Szczerba to determine whether there is an advantage within packings based on particles of less than 10 μm diameter for use in preparative chromatography. Three 15-cm analytical columns were packed with reversed-phase material based on particles of 3, 5 and 8 μm diameter porous silica and peak widths were determined over a wide range of sample loads.

EXPERIMENTAL

Materials

Spherical silica used in the study was manufactured at Rainin Instrument (Berkeley, CA, USA) by a proprietary process. Physical data for the silicas are given in Table I. Particle sizes were determined using a Brinkmann (Westbury, NY, USA) Model 2010 particle size analyzer. Surface area and pore size were determined using the BET nitrogen sorption method on a Quantachrome (Syosset, NY, USA) Autosorb-1. Bonding of the C_{18} functionality was performed identically for the three silica sizes. Stainless-steel columns (150 × 4.6 mm I.D.) in the Dynamax format were packed using a high-pressure slurry packing apparatus.

Solvents were of HPLC grade from EM Science (Gibbstown, NJ, USA). Chromatography was isocratic and the mobile phase was methanol–water–acetic acid (80:20:1, v/v/v), degassed by sonication prior to use. Methyl salicylate was purchased from Humco Labs. (Texarkana, TX, USA) and used without further purification.

TABLE I

PHYSICAL CHARACTERISTICS OF BASE SILICAS

Nominal particle size (μm)	Actual median particle size (μm)	Surface area (m^2/g)	Pore diameter (Å)
3	4.32	176	120
5	5.66	195	108
8	8.68	175	112

Equipment

Unless indicated otherwise, all equipment was obtained from Rainin Instrument. HPLC was performed using a Rainin HPX pump, a Rheodyne Model 7125 injector and a 20-μl sample loop. A Humonics Optiflow 1000 liquid flow meter was employed to ensure that consistent flow-rates were maintained. A Rainin UV-1 variable-wavelength detector was used in the extended range mode. A cell ratio of 7.5 was employed for extended range measurements. HPLC runs were controlled and data collected by Rainin Method Manager software used on an Apple Macintosh SE computer. A Knauer Model 87 variable-wavelength detector (10 mm flow cell) was used in one set of experiments.

Procedure

To prevent saturation of the extended range of the UV-1 detector, a wavelength (340 nm) was chosen such that the peak at maximum loading remained on-scale. This allowed detection over more than five orders of magnitude concentration range (0.005–140 g/l). The solute was dissolved in the mobile phase at eleven different concentrations, and the samples were run at three flow-rates, 0.4, 0.7 and 1.0 ml/min, on each of the three columns. When peak volume is plotted against sample load beyond overload, the three flow-rates give equivalent peak volumes (data not shown). The peak width in minutes at half-height of each peak was determined using Method Manager data reprocessing software.

RESULTS AND DISCUSSION

Fig. 1. shows the plate height (*H*) *vs.* flow-rate plots obtained for columns packed with the three different particle sizes. The asymmetries on each column were between 1.00 and 1.25 for toluene with methanol–water (65:35) as the mobile phase. The flow-rates for minimum plate height in columns of 4.6 mm I.D. were determined from these data to be 0.4 ml/min for the 8-μm, 0.7 ml/min for the 5-μm and 1.0 ml/min for the 3-μm particle sizes. Table II gives the approximate back-pressure of the three columns at each different flow-rate with methanol–water–acetic acid (80:20:1) as the mobile phase.

Figs. 2–4 depict the peak widths at half-height *versus* the column loads at the three different flow-

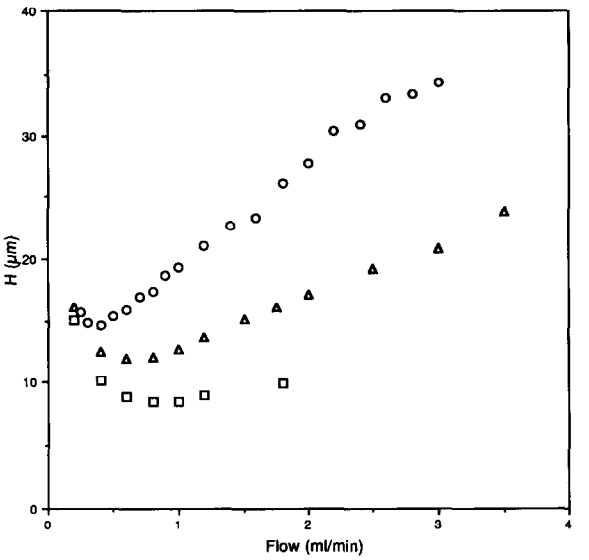

Fig. 1. Plot of plate height (H) *vs.* flow-rate for three particle sizes: ○ = 8; △ = 5; □ = 3 μm. All columns C_{18}, 150 × 4.6 mm I.D.; sample, toluene in methanol–water (65:35); mobile phase, methanol–water (65:35).

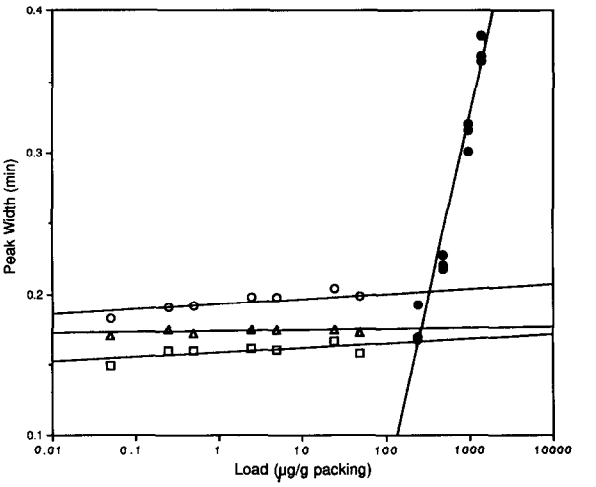

Fig. 2. Plot of peak width at half height *vs.* sample load at 0.4 ml/min flow-rate for three particle sizes: ○ = 8; △ = 5; □ = 3 μm; with (●) 3, 5 and 8 μm overloaded. All columns C_{18}, 150 × 4.6 mm I.D.; sample, methyl salicylate in methanol–water–acetic acid (80:20:1); mobile phase, methanol–water–acetic acid (80:20:1).

rates. The peak widths were determined using the Rainin UV-1 detector in the extended range mode of operation. This detector uses a unique flow cell which has a 9-mm flow path connected in series with a 1-mm flow path. In the analytical mode of operation the absorbance difference between the two flow paths is measured using dual-beam optics. The absorbance difference is equivalent to a flow path length of 8 mm. In the extended range mode of operation, when the absorbance limit is exceeded in the 9-mm flow path, the detector software automatically switches to single-beam operation and monitors the absorbance in the 1-mm flow path. The 1-mm absorbance response is scaled so that it becomes an extension of the 8-mm dual beam response. The result is an extension of the dynamic absorbance range of the detector by a factor of

TABLE II

BACK-PRESSURE FOR 150 × 4.6 mm I.D. C_{18} COLUMNS FOR DIFFERENT FLOW-RATES

Particle size (μm)	Back-pressure (p.s.i.)		
	0.4 ml/min	0.7 ml/min	1.0 ml/min
3	1250	2200	3100
5	650	1100	1600
8	250	450	650

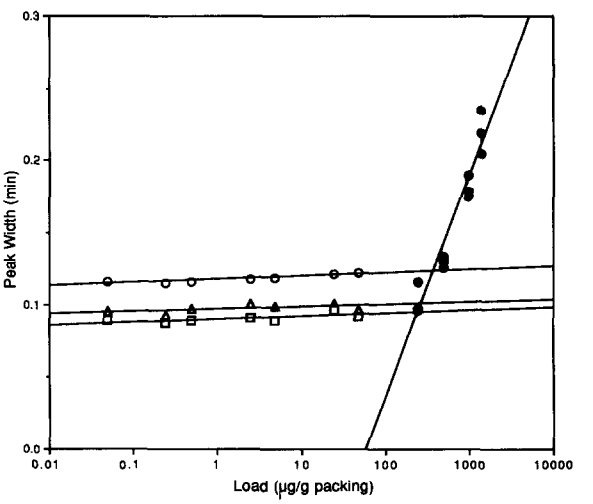

Fig. 3. Plot of peak width at half height *vs.* sample load at 0.7 ml/min flow-rate for three particle sizes: ○ = 8; △ = 5; □ = 3 μm; with (●) 3, 5 and 8 μm overloaded. All columns C_{18}, 150 × 4.6 mm I.D.; sample, methyl salicylate in methanol–water–acetic acid (80:20:1); mobile phase, methanol–water–acetic acid (80:20:1).

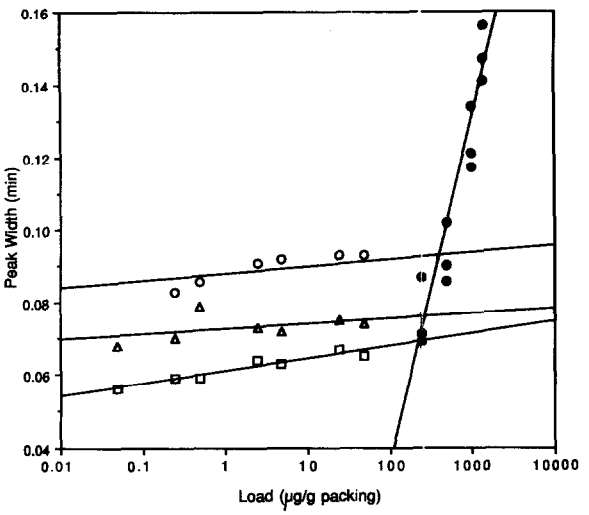

Fig. 4. Plot of peak width at half height *vs.* sample load at 1.0 ml/min flow-rate for three particle sizes: ○ = 8; △ = 5; □ = 3 µm; with (●) 3, 5 and 8 µm overloaded. All columns C_{18}, 150 × 4.6 mm I.D.; sample, methyl salicylate in methanol–water–acetic acid (80:20:1); mobile phase, methanol–water–acetic acid (80:20:1).

eight without having to alter manually the length of the flow cell.

To confirm the accuracy of the extended range mode of operation of the UV-1 instrument, the measurements in Fig. 2 were also determined using a Knauer multi-wavelength detector. Loadings from 0.1 to 50 µg were determined at a wavelength of 250 nm and loadings from 100 to 2800 µg were determined at 350 nm. Both the UV-1 and the Knauer instruments (data not shown) gave similar peak widths.

Note that these graphs are depicted as straight lines, one for each particle size at concentrations below the level where column overload is apparent, and one line which includes the points for all three particle sizes above the concentration at which overload is obvious, as evidenced by an increase in peak width. The use of straight lines to express the data instead of a curve seems justified in the light of the expected high degree of linearity ($r^2 \geqslant 0.94$) exhibited by the data in the pre- and post-overload conditions. Note, however, that the lines are only for visualization purposes, and the data are actually two straight lines with an abrupt joining transition, which is no doubt a curve.

These graphs support the conclusion that the column with larger particles has a wider linear range in which the column is not overloaded [9]. More important, however, is the fact that the smaller particle column gives a narrower peak width for a given sample under many conditions. Only above the sample overload point of the larger particle column do the differing particle size columns become equivalent. Prior to this point, the smaller particle size column gives a narrower peak width than the larger particle size column even though the smaller particle column may be overloaded. This equivalence of differing particle sizes under overload conditions has been noted by other workers [6]. The difference in the onset of overload between columns of two particle sizes is indeed small (of the order of 125 µg per gram of packing between 3 and 8 µm particles), but at no point does the larger particle column give narrower peak widths. This behavior held for all three flow-rates tested.

These experiments support the proposal of Perry and Szczerba [8], in which an "equal cut point" approach is proposed as a more useful means of evaluating particle size with regard to utility in preparative HPLC. Perry and Szczerba's study encompassed larger particle sizes (10–80 µm) than presented here, and they used irregular silica, whereas we evaluated spherical silica. In addition, Perry and Szczerba used an injection solvent stronger than their mobile phase to allow the investigation of extremely high loadings [10]. Other workers [11] have noted the band broadening that can result from this practice. This could add disproportionately to peak width at high sample concentrations.

The need for the use of injection solvents stronger than the mobile phase reveals a problem which many researchers have commented upon, that of solubility. Particularly in reversed-phase chromatography, the sample solubility in the mobile phase may not be high enough to achieve overload. The solute used in our study, methyl salicylate, is infinitely soluble in 100% methanol, but only soluble to *ca.* 150 g/l in methanol–water–acetic acid (80:20:1). In this mobile phase, the capacity factor (k') of methyl salicylate is *ca.* 1.0. If a larger k' is needed, the solubility of the solute would be reduced even further, and mass overload conditions might not be achievable without larger injection volumes. For instance, if the mobile phase were

methanol–water–acetic acid (70:30:1), the sample loop would have to be doubled in size. At a composition of 60:40:1, the sample loop would need to be more than six times larger in order to deliver the same amount of sample. Clearly, this would cause increased band broadening.

In contrast to the system studied by Perry and Szczerba [8], the system studied in this work shows that the peak width does become independent of the particle size at high column loadings. We are unable to explain definitively their results in comparison with ours, but two factors seem likely to have contributed to the difference. First, Perry and Szczerba used an irregular silica which may have had a surface area greater than the 190 m^2/g of the spherical silica tested here. This would probably have caused a difference in the loading at which overload would have been initially apparent. Second, the higher concentrations of dibutyl phthalate (DBP) which Perry and Szczerba loaded utilized 100% methanol instead of the mobile phase as the injection solvent [10]. Solubility problems may well account for the difference in results, as DBP has a significantly lower solubility than the methyl salicylate used here, and a vastly higher solubility in methanol than in the mobile phase used by Perry and Szczerba. Our work only involved loadings at which the sample was soluble in the mobile phase, thus avoiding solubility-induced problems.

Many discussions on preparative HPLC have overlooked the problem of sample solubility, but it is significant nonetheless and, as previously noted, it is especially troublesome in reversed-phase chromatography. Unlike analytical chromatography where the constraints on the mobile phase are limited only to maximizing resolution, preparative chromatography has the added complication of demanding maximum solubility of the sample in the mobile phase. When working under overload conditions, where the peak width is independent of particle size, the larger particle stationary phase would be the most suitable owing to its low back-pressure and consequent high flow-rates. However, when sample solubility makes overload impossible, the use of small particles will allow the use of mobile phases that maximize sample solubility. In reversed-phase chromatography, solubility is normally aided by increasing the proportion of organic solvent in the mobile phase. However, this will cause a decrease in k', which will decrease the resolution if all other factors are held equal. If a smaller particle stationary phase is used, this decrease can be compensated for by the higher plate count which smaller particles normally give:

$$R_s = 0.25N^{1/2}(\alpha - 1)/[k'/(k' + 1)] \quad (1)$$

where R_s is the resolution, N the plate number and α the separation factor. This has the added benefit of decreasing the run time per separation, thereby increasing the throughput. Halving the k' would require squaring the number of plates to maintain the same resolution, so there are practical limits to this approach.

Alternatively, if more than sufficient resolution can be attained at a given k', the flow-rate may be increased. Although a decrease in plate number will occur at any flow-rate greater than the optimum, the throughput will increase. The flexibility that small particle size preparative systems offer has already been utilized by some workers to effect difficult separations [12]. Hardware limitations impose the greatest restriction on the extent to which these techniques can be utilized.

Clearly, the use of small particles in preparative HPLC offers increased throughput under certain conditions. However, the conditions that warrant their use need to be defined more clearly. Although several workers have commented on the different types of preparative HPLC [4,13], there has been no standardized categorization of the conditions under which preparative chromatography is conducted. The needs and resources of industrical "process-scale" chromatography should be distinguished from those of "laboratory-scale" methods.

Process-scale chromatography involves situations where equipment is dedicated to a specific separation and the production rate (throughput/cost) is the dominant consideration. Normally the equipment is large, with columns ranging from 5 cm to hundreds of centimeters in diameter. Often the length of the column is tailored to the desired separation. Process chromatography usually also requires dedicated areas for the equipment and for solvent storage.

This is in contrast to laboratory-scale work, where the length of the column is limited to those which can be purchased, and the range of flow-rates and column diameters is constrained by the limita-

tions of available HPLC pumps. Perry and Szczerba [8] have previously set forth these conditions as those likely to confront the laboratory-scale preparative chromatographer. Usually these systems are not dedicated to a single separation in order to produce a single product but are used to prepare many different samples for other purposes such as spectroscopic analysis or field testing. A lengthy study to determine the ideal column type and solvent system to increase α values and maximize solubility may not be justified. Laboratory-scale work is often done on the standard laboratory bench and probably is limited to columns of 1–5 cm I.D. The versatility which small particles offer to the preparative chromatographer would seem to be most valuable for laboratory-scale preparative work.

There is definitely some overlap between these two types of preparative chromatography and, as hardware capabilities are improved, the division between the two will probably increase to larger column diameters. The availability of more powerful pumps would allow the use of larger diameter columns or standard size columns (25 cm or less in length) with smaller particles on the laboratory scale.

CONCLUSIONS

The use of small particles in laboratory-scale preparative chromatography is seen to be most advantageous on several grounds. A large number of plates makes a separation easier and allows the freedom to manipulate the mobile phase and flow-rate to obtain the highest throughput while guaranteeing purity for all but the most difficult separations. These advantages are most apparent in reversed-phase separations, where small particles give smaller peak widths than would larger particles at almost all loadings. When solubility considerations are also taken into account, it is clear that the use of small particles in laboratory-scale preparative HPLC has benefits that improvements in hardware capabilities will increase.

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