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Preparative Liquid Chromatography and the *H/u* Curve*

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Abstract

H/u curves for columns of increasing internal diameter are shown to be useful for evaluating their LC performance. In particular, the *H/u* curve reveals whether the packing technique or the packing material is defective. *H/u* curves for very large internal diameter columns show deviations which result from friction heat and thermal expansion of the column tubing, creating a void at the column wall.

I. INTRODUCTION

In preparative high performance liquid chromatography (prep-LC) an important point is the evaluation of column performance and of column packing quality. We believe that the best way to do this is to run analytical size samples and to determine the plate number and *H/u* curves. If the values for these are up to standards, the system will also function optimally for larger scale preparative size samples.

H/u curves give peak dispersion against mobile phase velocity. They are often called "van Deemter" plots and are expressed mathematically by an equation in which an *A*, a *B*, and a *C* term are usually mentioned or discussed. Such *H/u* curves, through the derived *A*, *B*, and *C* terms, can teach us:

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Whether the packing procedure is correct
What is the quality of the packing material
Whether the chromatographic system is appropriate for the sample compounds

The *A*, *B*, and *C* terms of the van Deemter plot can be calculated mathematically or measured graphically as shown in Fig. 1. The *B* term is less important in practice and, in addition, the precision at the very low flow rates, where the *B* term can have an influence, is small. In earlier days of LC the *C* term was preponderant, and sometimes the other terms were both neglected. With the smaller particles of modern LC it has become apparent that the *A* term (the eddy diffusion or packing quality term) is the most important at optimum flow velocity. A practical example of how the *H/u* curve can help to evaluate column performance

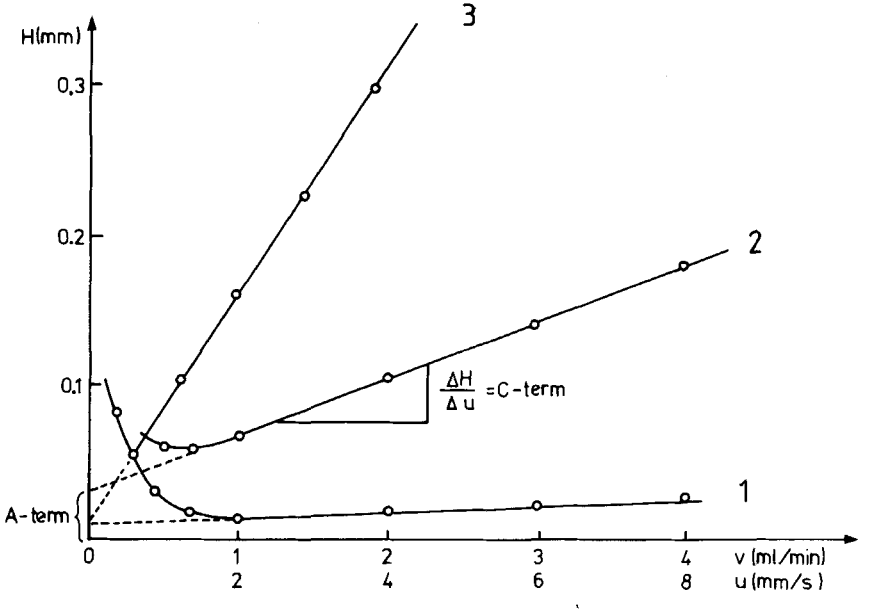

FIG. 1. Evaluation of van Deemter plot. Experimental conditions as follows. Curve 1: Column 25 × 0.46 cm filled with 5 μm RoSil-C18-D; mobile phase, acetonitrile/water, 75/25; sample, pyrene. Curve 2: Column 25 × 0.46 cm filled with experimental batch of 5 μm spherical octadecylated silica gel (pores too small); mobile phase, acetonitrile/water, 75/25; sample, pyrene. Curve 3: Column 25 × 0.46 cm filled with 5 μm RoSil-C18-D; mobile phase, water; sample, glucose.

and column quality is shown in Fig. 1. This presents the H/u curves for two columns (packed with two different 5 μm octadecylated silica gels) with two samples. Curves 1 and 2 are for pyrene (with acetonitrile/water, 75/25, as the mobile phase) and Curve 3 is for glucose (with water as the mobile phase).

A column well packed with a good packing material should give an A term of around 2 dp and, for 5 μm packing material, the C term should be very small. Curve 1 for Column 1 is in this category. If we now chromatograph glucose in water on this column (Curve 3), the intercept on the Y-axis is still about 10 μm or 2 dp (A term). The column is still well packed of course, but the chromatographic system (packing, solvent, and sample) are not well chosen. The C term is very large. The A term for Curve 2 (for pyrene on Column 2) is about 5–6 dp and the C term is also too high for a 5-μm material. Curve 2 shows that this particular packing material is defective (the pores are too small) and that the packing procedure is not really adapted to this material. This is the difficult point about LC column packing; it is not possible to know with certainty whether some other packing procedure could not produce a better result. We can only establish that a similar material with the same packing procedure does give good results.

Curve 1 shows good packing material, good packing procedures, and a well-adapted chromatographic system. Curve 3 shows good packing material, good packing procedures, but a badly chosen or not adapted chromatographic system (sometimes this is unavoidable). Curve 2 shows defective material and/or defective packing procedures.

Practically no information is available on peak dispersion versus mobile phase velocity in large i.d. prep-LC columns. The differences between prep- and analytical LC, and what conclusions can be drawn from the similarities or differences, is the subject matter of the present paper. Four parameters of prep-LC, which are usually changed compared to analytical LC, will be considered:

1. The increased column internal diameter (i.d.)
2. The increased particle size (dp)
3. The increased particle size spreading (dp 90/dp 10 ratio)
4. Changes in the derivatization chemistry of the packing materials

We will discuss the influence of these parameters on the plate number and the H/u curves, and evaluate the performance and quality of prep-LC systems.

II. EXPERIMENTAL

Instrumentation

Columns up to 22 mm i.d. were tested with a Varian 5000 Liquid Chromatograph, a Varichrom UV 50 detector, and a Valco 7000 psi loop injector (1/16″ connections). The equipment for larger i.d. columns consisted of a Knauer pneumatic LC pump, a Varichrom UV 50 detector (with flow splitter system), and a Valco loop injector (1/16″ as well as 1/8″ connections).

Columns, Packing Material

The stainless steel columns were all 25 cm long (unless mentioned in the text) with an internal diameter of 2.1, 4.6, 22, and 44 mm. Columns up to 22 mm i.d. were provided with Valco fittings and 1/32″ frits (2 μm pores). The 44 mm i.d. columns were provided with self-made flange-type fittings.

All packing materials were from Alltech-RSL, Eke, Belgium. 2, 3, 5, and 10 μm RoSil-C18-D are spherical octadecylated silica gels which are endcapped. 10, 20, and 30 μm RSil-C18-HL-D are irregular shaped silica gels, octadecylated to a high loading, and endcapped. Prep RSil-C18 is a preparative octadecylated silica gel with a mean particle size of 20 μm.

III. RESULTS AND DISCUSSION

1. Column Internal Diameter

In connection with the influence of column i.d., Guiochon (*1*) mentions that variation from 1 to 10 mm does not affect the shape of the *H/u* curve. Figure 2 shows the *H/u* curve for 10 μm RSil-C18-HL-D in 2.1, 4.6, 22, and 44 mm i.d. columns.

Columns from 2.1 to 22 mm i.d. give very similar *H/u* curves. Small differences in the *A* term occur. The resistance to mass transfer is roughly the same. For the 22 mm i.d. column, only a small increase of the *C* term is observed. The conclusions of Guiochon can therefore be extended to this diameter. For 44 mm (and consequently larger) i.d. columns, however, the curve is clearly different. These columns can be packed very efficiently ($h = 2$ to 2.5 at 1 mm/s linear flow velocity of the mobile phase). At higher flow rates ($u > 2$ mm/s), peak broadening and/or

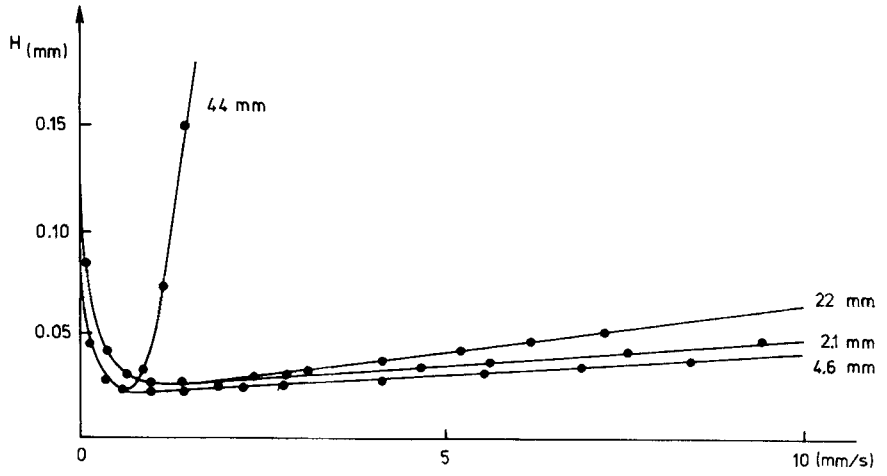

FIG. 2A. Influence of the column i.d. on the *H/u* curve. Experimental conditions: Columns stainless steel, 25 cm long, i.d. 2.1, 4.6, 22, and 44 mm, Valco end fittings (except for 44 mm i.d.); packing material, 10 μm RSil-C18-HL-D; mobile phase, acetonitrile/water, 75/25; sample, pyrene (k = 6), analytical sample size.

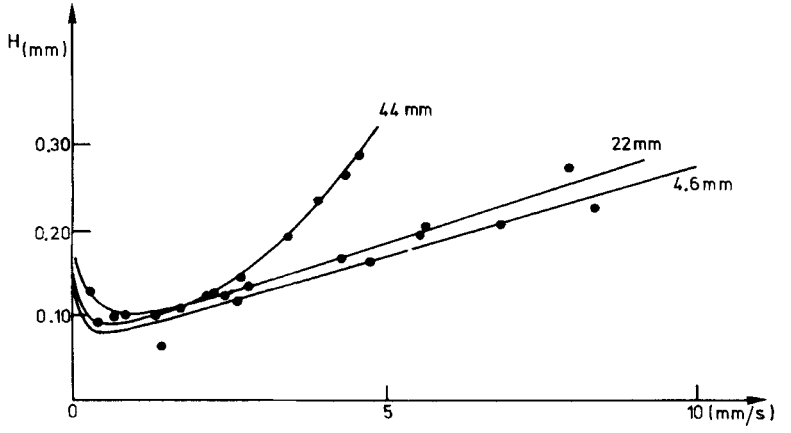

FIG. 2B. Influence of the column i.d. on the *H/u* curve. Experimental conditions: Same as Fig. 2A except for the packing material, 20 μm Prep RSil-C18.

doubling occurs. As a consequence, the C term increases very quickly. This is due to temperature effects. Similar effects can be obtained by heating the column wall or the mobile phase. The effect can be reduced—but not completely removed—by thermostatting and, even better, by isolating (*2*) the column.

At the higher flow rates friction heating becomes important. In the larger i.d. columns, heat dissipation becomes increasingly more difficult. This results in flow differences and even in local capacity ratio differences in the radial direction, as has been mentioned repeatedly in the literature (*3–6*). The deterioration of chromatographic results at higher flow rates has often been ascribed to these phenomena. We think, however, that the spectacular rise of the H/u curve for the 44-mm column is not only due to such effects; we think that the differences in thermal expansion coefficient between the packing material and the stainless steel mantle of the column is also responsible for the behavior of the H/u curve. While thermal expansion of silica gel, like that of quartz (0.53 μm/m/°C), is negligible, this is not the case for stainless steel (16 μm/m/°C). With a temperature increase of only 10°, for instance, in a column of 20 cm i.d., an opening of 16 μm starts to form at the column wall and the flow rate is consequently disturbed. This opening is not large, but sufficiently so to allow stationary phase particles to be transported downward. Gradually the opening at the top becomes larger and finally visible. The phenomenon is reversible for a few cycles with a freshly made column. Indeed, the curve in Fig. 2A can be reproduced, starting again at lower solvent speeds and with a cooled column. After a few of these cycles, column deterioration becomes irreversible. Considering the above, the effect should be less drastic in columns filled with coarser particles, having a lower back pressure, and, therefore, smaller thermal friction. This is shown in Fig. 2B for 20 μm particles in different i.d. columns. The same is true with the less viscous mobile phases of straight phase or adsorption chromatography. This assertion was repeatedly verified experimentally in the course of our H/u experiments.

Behavior similar to this H/u curve of Fig. 2A for a 44-mm i.d. column can be observed for analytical columns and very small particles. This is shown in Fig. 3. Here, H/u curves for 2, 3, 5, and 10 μm spherical octadecylated silica gel packed in 4.6 mm i.d. columns are plotted. The sample is pyrene ($k' = 6$) and the mobile phase is acetronitrile/water 75/25. Under normal operating conditions ($v < 3$–4 mL/min or $u < 6$–8 mm/s), viscous heating does not contribute significantly to band broadening for conventional i.d. columns. However, with small particles, viscous solvents, and higher flow rates, deviations from the normal H/u curve are observed.

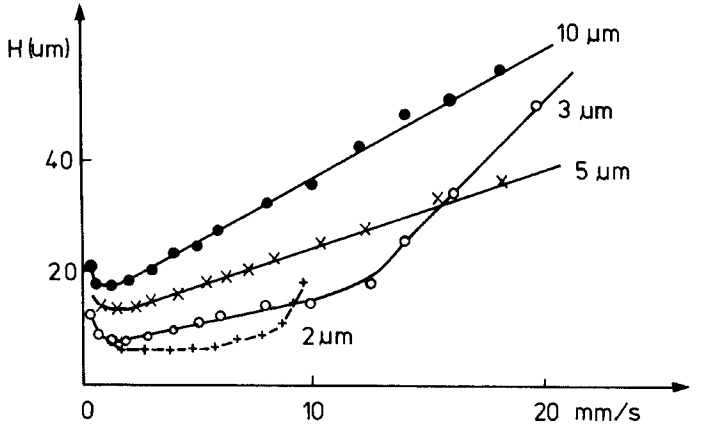

FIG. 3. *H/u* curves for small particles in analytical columns at high mobile phase velocity. Experimental conditions: Column, 10 × 4.6 mm filled with 2, 3, 5, and 10 µm RoSil-C18-D; mobile phase, acetonitrile/water, 75/25; sample, pyrene.

Whether the abnormal shapes of the *H/u* curves in Figs. 2 and 3 have the same origin is not clear. This particular point deserves further attention.

2. Increased Particle Size

The influence of increased particle size on the *H/u* curve is important for practical prep-LC. Curves for 10, 20, and 30 µm RSil-C18-HL-D are shown in Fig. 4. The sample is again pyrene, and the solvent system is acetonitrile/water (75/25). The columns were all 15 × 0.41 cm. The intercepts on the *Y*-axis or *A* terms are 0.8 dp for the 10-µm material, 1.8 dp for the 20-µm material, and 2.4 dp for the 30-µm material. This shows that it is more difficult to pack larger particles, at least with the applied slurry packing technique. One can never be sure that the packing technique used is the best for that particular material. This is an important point about packing LC columns.

What if another packing technique were to yield better results? Very often, in the past, this situation prevailed, especially at a time when a reduced plate height of about 5 was considered to be excellent. We are fairly confident—because we have tried and worked on so many packing procedures—that larger particles are more difficult to pack (as discussed above) but we cannot be certain. "More difficult" is then used here in the

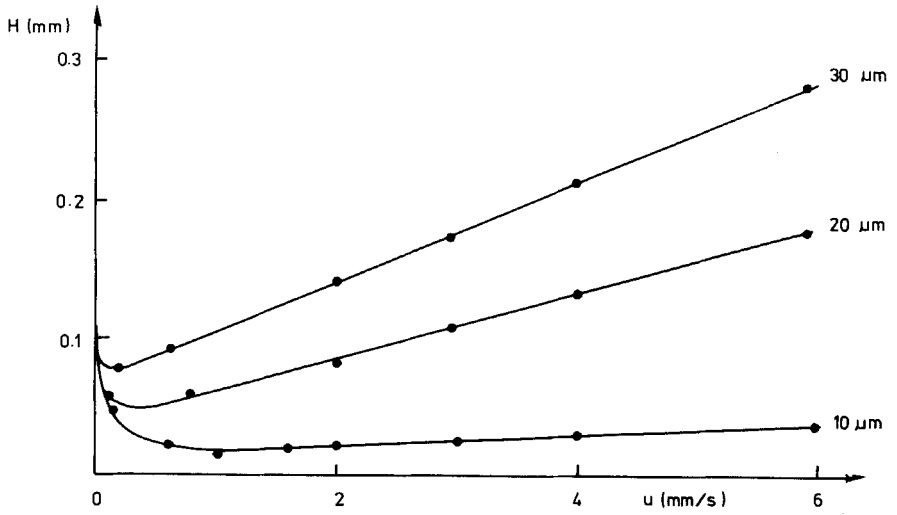

FIG. 4. Influence of the particle diameter on the H/u curve. Experimental conditions: 10, 20, and 30 µm RSil-C18-HL-D filled in a 25 × 0.46 cm column; sample and mobile phase, see Fig. 2A.

sense that lower A term values are more readily obtainable with smaller particles. Also important in Fig. 4 is the difference in C term which is much larger for the larger particles ($C \approx dp^2$). While reasonably good efficiencies can be obtained at very low elution speeds with the larger sized packing materials, the efficiency drops rapidly when the solvent velocity is increased. This is important for practical prep-LC. Very often the particle size is increased for prep-LC. Since the pressure drop over the column is then so small, there is a tendency to increase the flow rate. These are conditions for which the column efficiency will be low. For some tasks this may be acceptable. However, reasonable efficiency with coarser particles is only attainable at very low solvent rates. At about 0.5 mm/s the efficiencies for the columns of Fig. 4 are roughly proportional to the particle sizes. The 30-µm material is 3 times less efficient than the 10-µm material. This is, however, a very low solvent rate. At the more usual flow rates for prep-LC, e.g., of 4 mm/s, the 30-µm material is about 10 times less efficient than the 10-µm material. This clearly shows the interest of small particle sizes for prep-LC purposes if a good efficiency is needed. The data for Fig. 4 are in accord with theory and were obtained for analytical conditions. For prep-LC it is most important to know the H/u relationship for loaded conditions. Up to now, no experimental data or theoretical models were published. As shown by Knox (*7*), it is still

valuable to use plate height and H/u curves for prep-LC, as long as we work in conditions where kinetics (lower loads, small injection volumes, and low concentrations) are important. In conditions where thermodynamics (high loads, large volumes, and high concentrations) are dominant, conclusions drawn from H/u curves should not be used anymore. Figure 5 shows the H/u relationship for analytical and loaded conditions for 10 and 20 μm particle size. For the analytical data, 1 μg/10 μL of a naphthalene solution was injected. For more loaded conditions, 100 μg/10 μL was applied. The asymmetry factor of the peaks increased from 1.53 to 1.56 for the 10-μm column and from 1.58 to 1.80 for the 20-μm column. Although it is not correct to compare plate numbers of peaks with different asymmetry factors, Figure 5 gives a general trend.

From this figure it can be deduced that the slope of the van Deemter plot or the C term increases only slightly for loaded conditions (from 1.2×10^{-2} to 1.87×10^{-2} s for 10 μm particles and from 2.08×10^{-3} to 3.56×10^{-3} s for 20 μm particles). The A term, however, is very much influenced. Theory does not predict that the A term or eddy diffusion should be influenced by changing the sample size. Our result is probably the consequence of a larger "starting zone" by injecting a sample size that locally, at the column top, swamps the available adsorption sites. So it is interesting to use smaller particles for high throughput and high efficiency at high mobile phase velocities. Most conclusions about H/u curves for analytical LC are still valid for loaded conditions.

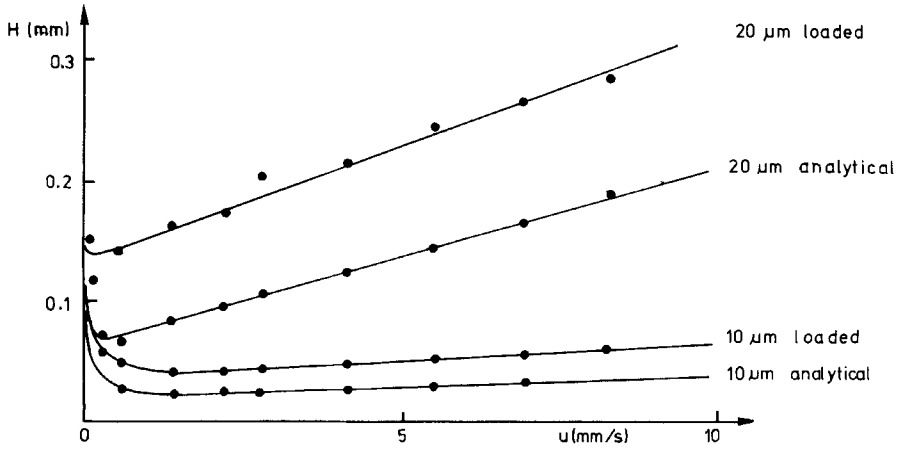

FIG. 5. H/u curves for analytical and loaded conditions. Experimental conditions: Column, 25 × 0.46 cm filled with 10 and 20 μm RSil-C18-HL-D; mobile phase, see Fig. 4; sample, naphthalene, 1 μg/10 μL for analytical run, 100 μg/10 μL for loaded conditions.

3. Increased Particle Size Spreading

The spread in the particle size of LC materials is a difficult point. For identical-sized particles or closely-sized particles it is relatively easy to evaluate or to determine the true particle size. Microscopic viewing would be easiest. Even then it would not be trivial with irregular-shaped particles (*8*). With Coulter Counter measurements the particle size when half the number or half the weight (dp 50) of the sample has been counted would be the same. The particle size deduced from chromatography data (back pressure and solvent viscosity) or effective particle size would also be the same. When the particles have some spread in size, all this is not true anymore. The larger the spread the more difficult it is to attribute a certain value to the mean particle size. With Coulter Counting, the dp 50 for size and weight is not the same anymore. Which dp 50 to choose? Estimation, in this situation, of the mean particle size under a microscope is also difficult. The chromatographic value is too much influenced by the fraction of the smaller particles to be of any use.

We have shown that the spread of the particle size is of minor importance for very small particles in analytical LC, if the ratio dp 90/dp 10 does not exceed 1.5 to 2.0 (*9*). This value appears to be surprisingly high. Still, efficiency is hardly affected by a large spread in particle size, but, as already stated, back pressure is higher. For larger particles as often used in prep-LC, the same situation is observed. There is no efficiency difference between closely sized 20 μm RSil-C18-HL-D (dp 90/dp 10 = 1.6) and Prep RSiL-C18-HL-D which also has a mean size of 20 μm but with a larger dp 90/dp 10 ratio (weight dp 90/dp 10 = 3.6). This ratio and the mean value were determined from both microscopic viewing and Coulter Counting. This is illustrated in Fig. 6. This shows that both materials pack well (same A term). This is also reflected in the same total porosities [ϕ_t (20 μm RSil-C18-HL-D) = 0.85 and ϕ_t (Prep RSil-C18-HL-D) = 0.87]. The back pressure for the Prep RSil-C18-HL-D (resistance factor ϕ = 1890) was twice as high as for the narrow sized 20 μm RSil-C18-HL-D (resistance factor ϕ = 980). Back-pressure differences are probably less important with these large particles. They are low anyhow. This is the reason why (the much cheaper) larger spreaded particles can and are used in prep-LC.

4. Changes in Derivatization Chemistry

The derivatization chemistry of packing materials for analytical sized LC columns has reached high levels of sophistication and often includes

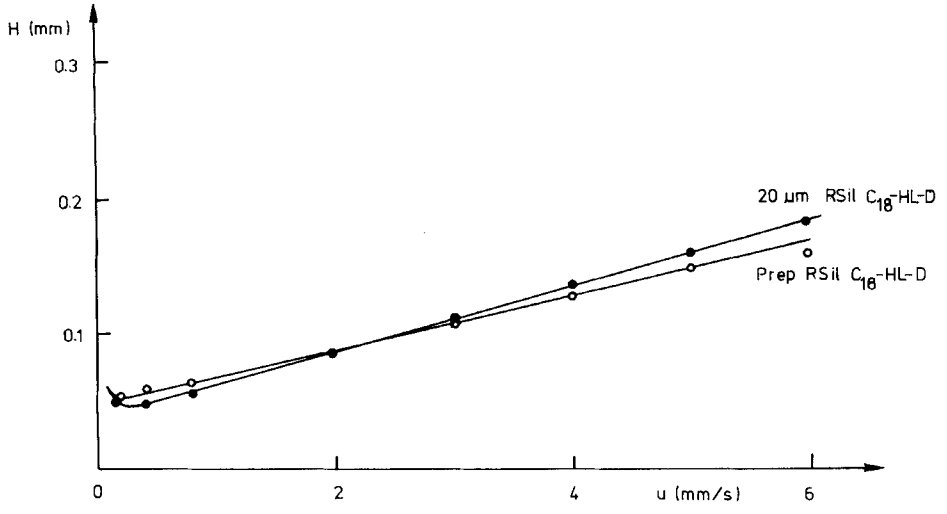

FIG. 6. Influence of the particle size distribution on the H/u curve. Experimental conditions: Column, 25 × 0.46 cm filled with 20 µm RSil-C18-HL-D (dp 90/dp 10 = 1.6) and Prep RSil-C18-HL-D (dp 90/dp 10 = 3.6); sample and mobile phase, see Fig. 2.

many steps and sometimes uses very expensive chemicals. This is not always possible for prep-LC packings, where very large amounts of these materials may be necessary. Simplified procedures are, or can be, followed and therefore require evaluation.

Figure 7 shows great differences in efficiency for the same silica gel, derivatized in only slightly different ways. Both materials pack reasonably well; they produce nearly the same A term. There is, however, a clear difference in the C term. This figure illustrates that for coarser particles ($C \approx dp^2$) the chromatographic system (sample, mobile phase, stationary phase) has to be well optimized in order to reduce resistance to mass transfer. If we had measured the efficiency at just one solvent speed of the columns of Fig. 7, we could not have concluded that the packing procedure was good and that the derivatization procedure was at fault in this case. Curve 3 in Fig. 7 shows the data for the packing material produced by the good derivatization procedure but filled with a bad packing procedure. If we had measured the plate number again at one mobile phase velocity, we could have concluded that the material was at fault, while the H/u curve shows that it is the packing technique. This illustrates the interest of determining H/u curves.

Finally, the reader interested in a more detailed discussion of prep-LC in general is referred to our recent book (*10*).

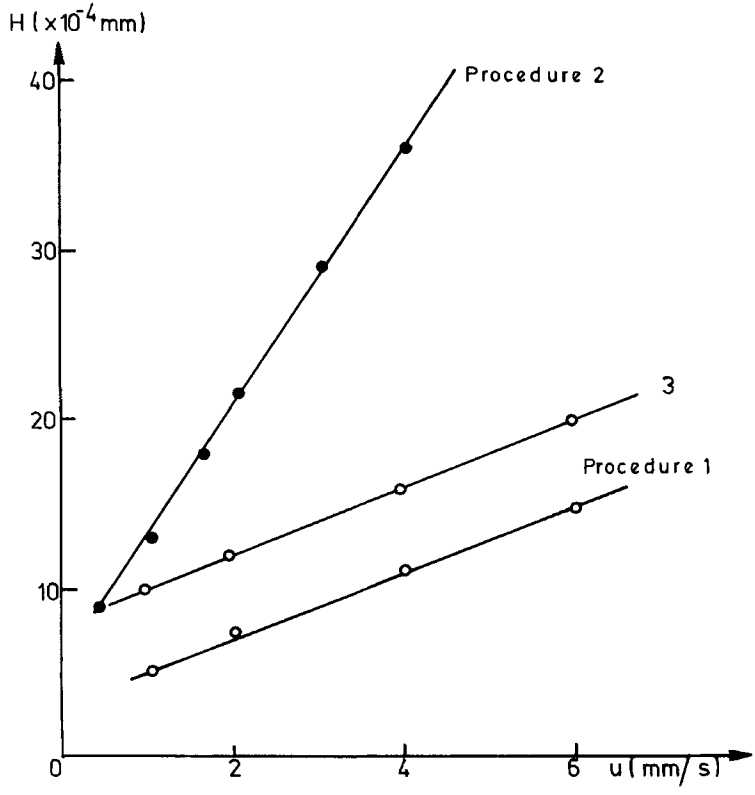

FIG. 7. *H/u* curves for different derivatization procedures. Curves 1 and 2: Same silica gel, same alkylsilane, practically identical loading percentage. Curve 3: Same packing material as for Curve 1 but bad packing procedure.

IV. CONCLUSION

We have shown that it is of interest to determine the *H/u* curve of (prep)-LC columns. In practice, this is much easier than usually thought. While many curves of the figures shown were obtained by measuring a large number of points, some were derived with only five to six measurements. As the curves prove, this is usually enough.

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