Journal of Chromatography, 121 (1976) 13–22

CHROM. 9024

PREPARATION AND USE OF *IN SITU* CHEMICALLY BONDED SMALL-PARTICLE SILICA AS PACKINGS IN HIGH-PRESSURE LIQUID CHROMATOGRAPHY

R. K. GILPIN*, D. J. CAMILLO and C. A. JANICKI

Pharmacy Research Department, McNeil Laboratories, Camp Hill Road, Ft. Washington, Pa. 19034 (U.S.A.)

(Received November 24th, 1975)

SUMMARY

Previously we have demonstrated the feasibility of forming bonded phases on porous layer beads (Corasil II) by a completely *in situ* process. A logical extension of this *in situ* method has been its application to small-particle silica columns. Such columns have all the advantages of the bonded phases formed on porous layer beads with the added feature of much higher plate counts. In addition, when compared to commercially available small-particle bonded phase columns, the cost is reduced significantly. In this paper, both the initial column preparation procedure and the *in situ* technique applied to completely porous small-particle silica columns are presented. Columns were packed with a constant-pressure pump using a dibromoethane slurry procedure. After initial packing, all columns were conditioned with a solvent series of decreasing polarity and evaluated for performance using a test mixture of biphenyl ($k' \approx 0.5$–1.0) and nitrobenzene ($k' \approx 5$–10). After evaluation, suitable columns ($H = 0.008 \pm 0.002$ cm at a flow velocity of 0.55 cm/sec) were selected for *in situ* modification. By this procedure, differences due to variation in column packing were minimized. Subsequently, various modification procedures were investigated. As in the case of porous layer beads, control of pre-reaction surface hydration was found to be critical in determining the consistency of surfaces formed.

INTRODUCTION

In the past decade, bonded phases for chromatographic application have grown into an active and important area of research interest in the field of separation science. Initially, the greatest interest was in the development of bonded phases for gas chromatographic (GC) use. During the first three to five years of this period, a number of investigators[1–8] contributed to the establishment and acceptance of bonded phases as GC packings. More recently the emphasis in GC has turned to detailed

---

* To whom correspondence should be addressed.

studies of reaction mechanisms and solute interactions with the bonded phases[9–17], with the ultimate goal of optimizing bonded phase selectivity and reproducibility.

Perhaps even more significant than bonded phases as GC packings has been the development of permanently bonded phases for high-pressure liquid chromatographic (HPLC) use[6,18–27]. Recently there has been a great deal of interest in the use of completely porous small-particle silica materials (av. d_p 10 μm or less) and in the modification of these materials by chemically bonding various organic molecules to their surface[25,26].

The advantage of columns packed with these materials is that they provide a large number of theoretical plates in a short period of time. Thus, difficult separations may be achieved within minimal analysis times.

In most cases, both polar and non-polar siloxane phases are formed by the reaction of organosilanes with the silica surface via batch type processes. By this conventional means a great deal of care in the preparation is required to produce coatings that are reproducible and effective. If the amount of moisture is not precisely controlled, coatings may be formed which are either too thick or too thin or which are non-homogeneously distributed on the surface.

Previously, Gilpin *et al.*[23] have demonstrated the feasibility of forming bonded siloxane phases on porous layer beads (Corasil II) by a completely *in situ* process. This process was further modified[27] to provide a simple and efficient, yet more reproducible means of preparing bonded phases. A logical extension of this *in situ* method has been its application to columns packed with completely porous small-particle silica. Such columns have all the advantages of the bonded phases formed on porous layer beads with the added feature of much higher plate counts leading to improved separations with faster analysis times. In addition, when compared to commercially available small-particle bonded phase columns, the cost is reduced significantly.

In this paper both initial column preparation and *in situ* modification applied to completely porous small-particle silica are discussed. All columns have been packed using a dibromoethane slurry procedure, conditioned with a solvent series of decreasing polarity and evaluated for performance. After evaluation, suitable columns have been selected for *in situ* modification. By using this procedure, differences in column efficiencies due to variation in packing technique have been minimized. Subsequently, the *in situ* reaction procedure has been investigated in terms of overall column performance and reproducibility. HETP values obtained before (H_{before}) and after (H_{after}) chemical modification have been used to calculate $H_{\text{before}}/H_{\text{after}}$ ratios. Comparisons based on these ratios have provided a better means of evaluating the *in situ* reaction process.

EXPERIMENTAL

Equipment

All chromatographic experiments were performed on a Waters Assoc. Model 202 liquid chromatograph equipped with a 6000 p.s.i. pump and a 254-nm ultraviolet detector. Injections were made on column using Precision Sampling syringes. All column evaluations were carried out at ambient temperature (*ca.* 25°).

Columns

The micro-silica columns were slurry-packed by the following procedure: Micro-silica, LiChrosorb SI 60 (av. $d_p \approx 10\ \mu m$), from EM Labs. (Elmsford, N.Y., U.S.A.), was dried at 200° for 4 h. A slurry was prepared by placing 1.0 g of the dried silica in 10 ml of 1,2-dibromoethane and shaking the mixture for at least 1 h. Before use the 1,2-dibromoethane was purified through a column of SilicAR CC-7 silica from Mallinckrodt (St. Louis, Mo., U.S.A.). Columns were packed using a Haskel Model DST-126-32 liquid pump and a column-type slurry reservoir assembly. The slurry reservoir consisted of a 1.8 m × 1/4 in. O.D. (3 mm I.D.) stainless-steel column fitted with a 1/4-in. to 1/8-in. stainless-steel Swagelok reducing union on the column end and a 1/4-in. to 1/16-in. stainless-steel Swagelok reducing union on the pump side. A 25-cm column prepared from standard 1/8-in. O.D. stainless-steel tubing and a reducing union with a 2-μm frit was attached to the slurry reservoir. All tubing was cleaned with a non-polar and a polar solvent, rinsed with acetone and dried before packing. The 10-ml slurry of micro-silica was drawn into a 10-ml syringe and swept into the reservoir–column assembly. Approximately 6 ml of distilled water were added to the reservoir until solvent appeared from the downstream side of the column. The upstream side of the slurry reservoir was clamped shut, the 1/4-in. to 1/16-in. Swagelok reducing union was removed, and the reservoir attached to the Haskel pump. After opening the valve on the pump, 80 ml of *n*-hexane were pumped through the reservoir–column assembly, which was then removed from the Haskel pump and attached to a Waters Assoc. Model 6000 pump. One hundred milliliters of methanol were pumped through the reservoir–column system. After this initial packing the column was disconnected from the slurry reservoir, connected to the pump, and flushed with 100 ml of the following solvents: water, methanol, isopropanol, diethyl ether, 1,2-dichloroethane, and *n*-hexane. After conditioning, the columns were evaluated using a test mixture of biphenyl and nitrobenzene and a mobile phase of *n*-hexane.

Reaction procedure

After packing and evaluation a suitable column was attached to the Waters Assoc. Model 6000 pump, and 100 ml of water-saturated toluene pumped through at a rate of 5.0 ml/min, followed by various volumes of dry toluene at a rate of 5.0 ml/min. The column was disconnected from the pump, and a 2 ft. × 3/8 in. O.D. stainless-steel reservoir column filled with 30 ml of a 50% reaction solution of octadecyltrichlorosilane in dry toluene was connected between the pump and the column. The 50% octadecyltrichlorosilane reaction solution was chosen to insure an excess of silane monomer[23]. The reaction solution was pumped through the column at a rate of 1.0 ml/min, followed by 500 ml of dry toluene at the maximum attainable flow. This volume of dry toluene was needed in order to insure the elution of unreacted monomer from the column. The reaction reservoir was removed from the system, and 100 ml of water-saturated toluene, followed by 100 ml water–acetonitrile (5:95) were pumped through the reacted column.

Before evaluation all columns were conditioned with at least 300 ml of the mobile phase, acetonitrile–0.01 *M* ammonium carbonate (60:40).

Reagents

The toluene used was analytical reagent grade obtained from Mallinckrodt. The dry toluene was prepared by refluxing over calcium hydride (Fisher Scientific, Fair Lawn, N.J., U.S.A.) for at least 2 h and stored over calcium hydride. Acetonitrile and *n*-hexane (distilled in glass) were obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). The 0.01 *M* ammonium carbonate solution was prepared by adding 2.3 g of ammonium carbonate (Fisher Scientific) to a 2-l volumetric flask and diluting to volume with distilled water. Octadecyltrichlorosilane was obtained from Aldrich (Milwaukee, Wisc., U.S.A.) and was used in the condition received. The reaction solution was prepared by dissolving 50% by volume of the silane monomer in dry toluene.

RESULTS AND DISCUSSION

Column selection

Prior to *in situ* modification each of the slurry-packed micro-silica columns was conditioned with a solvent series of decreasing polarity[28]. This series consisted of water, methanol, isopropanol, diethyl ether, 1,2-dichloroethane, and *n*-hexane. After conditioning all columns were evaluated using a test mixture of biphenyl and nitrobenzene and a mobile phase of *n*-hexane. Five similar columns (based on capacity and efficiency data) were selected for initial optimization studies. Values of k' for both biphenyl and nitrobenzene obtained on these uncoated columns are listed in Table I. Shown in Table II are HETP values (H_{before}) obtained on these same columns for flow-rates of 0.33 and 0.55 cm/sec (0.9 ml/min and 1.5 ml/min). These values were calculated from plots of efficiency *vs.* linear velocity of the mobile phase over an operating range of 0.11–0.81 cm/sec (0.3–2.2 ml/min). In Fig. 1 a plot is shown of efficiency *vs.* linear flow for a typical uncoated micro-silica column. These data were obtained using nitrobenzene as the test compound and *n*-hexane as the mobile phase.

Columns with $H_{\text{before}} = 0.008 \pm 0.002$ cm over a linear velocity range of 0.33–0.55 cm/sec (0.9–1.5 ml/min) were selected for initial modification studies. Columns exhibiting these efficiencies were easily obtainable by the slurry-packing procedure used. Columns either more or less efficient were rejected for the optimization experiments.

By fully evaluating each column before modification and selecting similar columns based on capacity and efficiency data, differences in chromatographic behavior arising from variation in column-packing technique were minimized. Thus, a more controlled and consistent study of the bonding reaction was possible using the *in situ* process. This advantage is not true of batch type reaction procedures since the reacted material must be placed into the column following modification; thus, the uncertainty of the packing process itself adds to the uncertainty of the experiment.

Reaction studies

All columns were prepared using 30 ml of a 50% octadecyltrichlorosilane reaction solution. These reaction conditions were used to insure complete and reproducible surface coverage[23]. Octadecyltrichlorosilane was chosen as the monomer of choice since the alkyltrichlorosilanes of longer chain-length have been found to produce chromatographically preferable columns[25,27]. Columns prepared with alkyl-

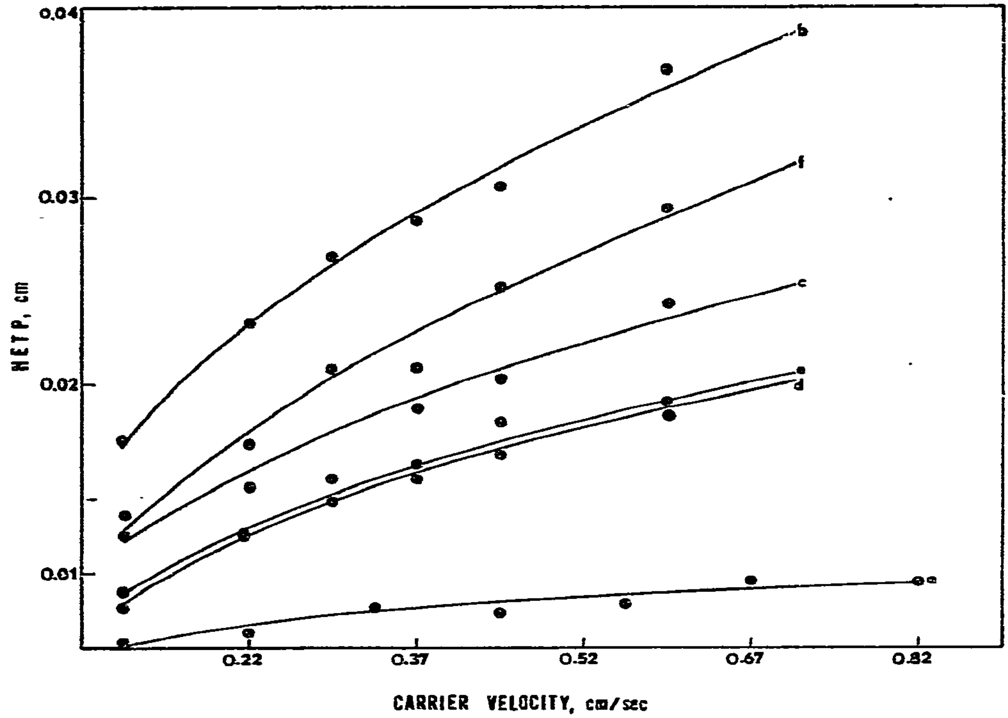

Fig. 1. Effect of mobile phase velocity on plate height for various unmodified and modified microsilica columns. (a) Typical unmodified column. (b–f) modified columns: (b) 50 ml, (c) 100 ml, (d) 200 ml, (e) 300 ml, and (f) 400 ml of dry toluene pre-reaction treatments.

trichlorosilane monomers of shorter chain-length such as ethyl- and hexyltrichlorosilane are less selective[25,27] with increased amounts of solute band spreading[27].

Previously it was demonstrated[27,29] that pre-reaction surface moisture was a critical factor in determining the bonded surface formed. In those cases where too little or too much physically adsorbed water was left on the silica surface prior to *in situ* modification, inefficient columns were obtained.

In Fig. 1 plots are shown of efficiency *vs.* linear velocity of the mobile phase for a series of octadecyltrichlorosilane columns prepared using different pre-reaction conditions. These data were obtained using 1,3,5-triphenylbenzene as a test solute and acetonitrile–0.01 *M* ammonium carbonate (60:40) as the mobile phase. The different pre-reaction treatments consisted of modifying the volume of dry toluene used to establish the amount of physically adsorbed surface moisture prior to modification.

In Fig. 2 a chromatogram is shown obtained from a test mixture of triphenylmethanol, triphenylmethane, triphenylsilane, and 1,3,5-triphenylbenzene on an octadecyltrichlorosilane *in situ* modified column. In this case, the column was rinsed with 100 ml of water-saturated toluene followed by 200 ml of dry toluene prior to the *in situ* bonding reaction.

In Table I capacity ratios (k') are tabulated for the four test solutes, triphenylmethanol, triphenylmethane, triphenylsilane and 1,3,5-triphenylbenzene, obtained on

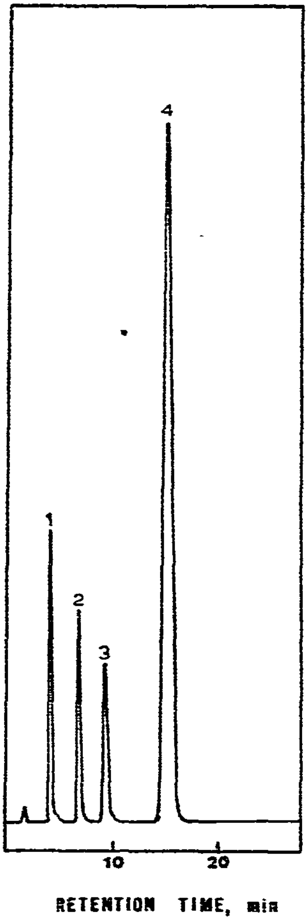

Fig. 2. Octadecyltrichlorosilane-modified micro-silica column. 200 ml of dry toluene pre-reaction treatment. Mobile phase, acetonitrile–0.01 *M* ammonium carbonate (60:40); flow-rate, 0.5 ml/min. 1 = Triphenylmethanol; 2 = triphenylmethane; 3 = triphenylsilane; 4 = 1,3,5-triphenylbenzene.

columns prepared using varying amounts of dry toluene to establish controlled surface moisture prior to the octadecyltrichlorosilane modification. These k' values were obtained over a flow range of 0.6–1.2 ml/min and are mean values of eight to twelve determinations. HETP values (H_{after}) for each of these columns are summarized in Table II. These data indicate that the amount of material bonded to the surface is decreased by a smaller and smaller percentage as a function of greater incremental volume changes of dry toluene used prior to modification. Arguments consistent with these data are that physically adsorbed water is removed to a decreasing degree as a function of increasing volumes of dry toluene used prior to the bonding procedure, resulting in smaller amounts of material bound to the surface. Eventually, conditions are established where removal of all physically adsorbed water has occurred or an equilibrium is attained prior to that point.

In Table II calculated ratios of HETP values for each column obtained before

TABLE I

VARIATION IN k' AS A FUNCTION OF COLUMN MODIFICATION

*Column modification**	*Unmodified***		*Modified****			
	k_1'	k_2'	k_1'	k_2'	k_3'	k_4'
50	0.55±0.02	5.6±0.14	2.7±0.18	8.1±0.44	13.6±0.82	22.9±1.2
100	0.66±0.05	6.3±0.27	2.3±0.08	5.5±0.15	8.5±0.25	15.4±0.43
200	0.56±0.07	5.9±0.49	2.2±0.08	4.4±0.15	6.4±0.21	10.9±0.38
300	0.55±0.05	5.8±0.33	1.9±0.13	4.0±0.25	6.0±0.34	10.5±0.54
400	0.64±0.05	6.5±0.33	1.7±0.06	3.5±0.12	5.2±0.18	9.2±0.31

* Milliliters of dry toluene used to establish controlled pre-reaction conditions.

** Mobile phase: *n*-hexane (test solutes: 1 = biphenyl, 2 = nitrobenzene).

*** Mobile phase: acetonitrile–0.01 *M* ammonium carbonate (60:40) (test solutes: 1 = triphenylmethanol, 2 = triphenylmethane, 3 = triphenylsilane, 4 = 1,3,5-triphenylbenzene).

TABLE II

VARIATION IN HETP AS A FUNCTION OF COLUMN MODIFICATION

Linear flow-velocity: A, 0.33 cm/sec; B, 0.55 cm/sec.

*Column**	H_{before} *(cm)***		H_{after} *(cm)****		H_{before}/H_{after}	
	A	*B*	*A*	*B*	*A*	*B*
50	0.0084	0.0097	0.028	0.035	0.30	0.28
100	0.0098	0.010	0.019	0.023	0.51	0.44
200	0.0080	0.0093	0.015	0.019	0.54	0.50
300	0.0071	0.0083	0.015	0.019	0.48	0.45
400	0.0084	0.0092	0.022	0.028	0.39	0.33

* Milliliters of dry toluene used to establish controlled pre-reaction conditions.

** Mobile phase: *n*-hexane (test solute: nitrobenzene).

*** Mobile phase: acetonitrile–0.01 *M* ammonium carbonate (60:40) (test solute: 1,3,5-triphenylbenzene).

and after the chemical modifications (H_{before}/H_{after}) are listed. These data are shown graphically in Fig. 3. Optimum columns were prepared using pre-reaction treatments of 100 ml of water-saturated toluene followed by volumes between 150 and 250 ml of dry toluene prior to chemical modification. These data are not unexpected and are in good agreement with recently reported data[29] for *in situ* modified Corasil II columns. In that work, the most efficient Corasil II columns were prepared using 50 ml of water-saturated toluene followed by 75 ml of dry toluene prior to chemical modification. Thus, removal of too little or too much physically adsorbed water results in production of inefficient columns. The optimum coating for this type of bonded phase is apparently formed at a point prior to the removal of all physically adsorbed water. Thus, these data would indicate that methods which use drying at 150° or greater are probably not the best means of forming similar bonded phase packings for liquid chromatographic use. Most workers agree that at about 150°, loss of physically adsorbed water has taken place and that above 150°, bound water loss occurs by condensation of adjacent hydroxyl groups[15,17,30,31].

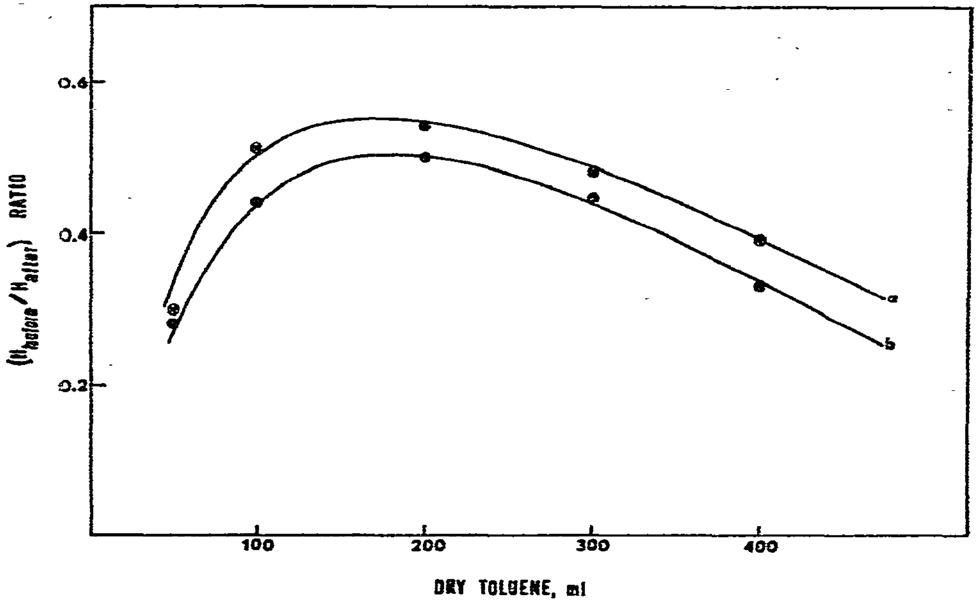

Fig. 3. H_{before}/H_{after} ratios as a function of pre-reaction treatment with dry toluene. Linear flow velocity: (a) 0.33 cm/sec, (b) 0.55 cm/sec.

Reproducibility

Reproducibility of the *in situ* process applied to completely porous small-particle silica columns has been examined. All columns were prepared using a pre-reaction treatment of 100 ml of water-saturated toluene followed by 250 ml of dry toluene. After pre-treatment, all columns were modified with 30 ml of a 50% reaction solution of octadecyltrichlorosilane. Before and after modification each column was evaluated chromatographically as previously discussed. A volume of 250 ml of dry toluene was chosen because: (a) this volume of dry toluene would produce effective surface coverage based on efficiency ratio data (H_{before}/H_{after}); (b) this volume would produce columns not previously examined in the optimization studies. k' values for each of the four test compounds on these octadecyl columns were calculated over a flow range of 0.6–1.2 ml/min using acetonitrile–0.01 *M* ammonium carbonate (60:40) as the mobile phase. Each mean k' value was calculated from eight to twelve determinations. In Table III relative average deviations of k' are listed for each of the test compounds on the five columns studied.

In Table IV variations in column efficiency are tabulated calculated from plots of HETP *vs.* linear velocity of the mobile phase at 0.33 and 0.55 cm/sec (0.9 and 1.5 ml/min). These data were obtained using nitrobenzene as the test solute and *n*-hexane as the mobile phase for the unmodified columns. After modification, 1,3,5-triphenylbenzene was used as the test compound and acetonitrile–0.01 *M* ammonium carbonate (60:40) as the mobile phase. As previously discussed, H_{before}/H_{after} ratios were calculated and also appear in Table IV. Previously, the performance of all columns were matched on the basis of similar values of efficiency. However, in this study, columns having varying values of efficiency were used. By comparing

TABLE III

REPRODUCIBILITY OF *in situ* PREPARED COLUMNS BASED ON k' DATA*

Test compound	*Mean k' values*	*Relative average deviation of k' (%)*
Triphenylmethanol	1.7	6.1
Triphenylmethane	3.9	8.1
Triphenylsilane	5.9	9.4
1,3,5-Triphenylbenzene	10.5	10.7

* Data obtained from five replicate columns (250 ml of dry toluene used to establish controlled pre-reaction conditions).

TABLE IV

REPRODUCIBILITY OF *in situ* PREPARED COLUMNS BASED ON HETP DATA*

Linear flow velocity: A, 0.33 cm/sec; B, 0.55 cm/sec.

*Column**	*H_{before} (cm)***		*H_{after} (cm)****		*H_{before}/H_{after}*	
	A	*B*	*A*	*B*	*A*	*B*
1	0.0099	0.011	0.019	0.014	0.51	0.47
2	0.011	0.013	0.021	0.024	0.52	0.53
3	0.011	0.013	0.021	0.024	0.52	0.51
4	0.011	0.014	0.020	0.025	0.53	0.55
5	0.008	0.009	0.014	0.018	0.57	0.51
				Mean values	0.53 ± 0.02	0.51 ± 0.03

* Data obtained from five replicate columns (250 ml of dry toluene used to establish controlled pre-reaction conditions).

** Mobile phase: *n*-hexane (test solute: nitrobenzene).

*** Mobile phase: acetonitrile–0.01 *M* ammonium carbonate (60:40) (test solute: 1,3,5-triphenylbenzene).

performance before and after bonding, the effect of the variation in column efficiency was minimized, as indicated by the consistency of the H_{before}/H_{after} ratios. The means and standard deviations for these ratios were 0.53 ± 0.02 and 0.51 ± 0.03 for 0.9 and 1.5 ml/min, respectively. The consistency of these data along with k' data demonstrates that *in situ* modified micro-silica columns can be prepared with a high degree of reproducibility and that comparisons based on H_{before}/H_{after} ratios are a more meaningful way of evaluating the overall reaction process.

REFERENCES

1 E. W. Abel, F. H. Pollard, P. C. Uden and G. Nickless, *J. Chromatogr.*, 22 (1966) 23.
2 C. J. Bossart, *ISA Trans.*, 7 (1968) 283.
3 F. Wolf and W. Heyer, *J. Chromatogr.*, 35 (1968) 489.
4 I. Halasz and I. Sebestian, *Angew. Chem.*, 8 (1969) 453.
5 W. A. Aue and C. R. Hastings, *J. Chromatogr.*, 42 (1969) 319.
6 J. J. Kirkland and J. J. DeStefano, *J. Chromatogr. Sci.*, 8 (1970) 309.
7 J. N. Little, W. A. Dark, P. W. Farlinger and K. J. Bombaugh, *J. Chromatogr. Sci.*, 8 (1970) 647.
8 A. H. Al-Taiar, J. R. Lindsay Smith and D. J. Waddington, *Anal. Chem.*, 42 (1970) 935.
9 J. B. Sorrell and R. Rowan, Jr., *Anal. Chem.*, 42 (1970) 1712.

10 R. Rowan, Jr. and J. B. Sorrell, *Anal. Chem.*, 42 (1970) 1716.
11 C. R. Hastings, W. A. Aue and J. M. Augl, *J. Chromatogr.*, 53 (1970) 487.
12 K. Unger and P. Ringe, *J. Chromatogr. Sci.*, 9 (1971) 463.
13 C. R. Hastings, W. A. Aue and F. N. Larsen, *J. Chromatogr.*, 60 (1971) 329.
14 K. Unger, *Angew. Chem., Int. Ed. Engl.*, 11 (1972) 267.
15 R. K. Gilpin, *Ph. D. Dissertation*, University of Arizona, Tucson, Ariz., 1973.
16 B. L. Karger and E. Sibley, *Anal. Chem.*, 45 (1973) 740.
17 R. K. Gilpin and M. F. Burke, *Anal. Chem.*, 45 (1973) 1383.
18 H. N. M. Stewart and S. G. Perry, *J. Chromatogr.*, 37 (1968) 97.
19 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1971) 207.
20 M. Novotný, S. L. Bektesh and K. Grohmann, *J. Chromatogr.*, 83 (1973) 25.
21 M. Novotný, S. L. Bektesh, K. B. Denson, K. Grohmann and W. Parr, *Anal. Chem.*, 45 (1973) 971.
22 E. Grushka and R. P. W. Scott, *Anal. Chem.*, 45 (1973) 1626.
23 R. K. Gilpin, J. A. Korpi and C. A. Janicki, *Anal. Chem.*, 46 (1974) 1314.
24 E. Grushka and E. J. Kikta, Jr., *Anal. Chem.*, 46 (1974) 1370.
25 R. E. Majors and M. J. Hopper, *J. Chromatogr. Sci.*, 12 (1974) 767.
26 R. S. Deelder, P. J. W. M. Claassen and P. J. H. Hendricks, *J. Chromatogr.*, 91 (1974) 201.
27 R. K. Gilpin, J. A. Korpi and C. A. Janicki, *Anal. Chem.*, 47 (1975) 1498.
28 L. R. Snyder, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, p. 125.
29 R. K. Gilpin, J. A. Korpi and C. A. Janicki, *Natl. Meet. Amer. Chem. Soc., 168th, Sept. 1974.*
30 R. K. Iler, *The Colloid Chemistry of Silicas and Silicates*, Cornell University Press, Ithaca, N.Y., 1955.
31 W. K. Lowen and E. C. Broge, *J. Phys. Chem.*, 65 (1961) 16.