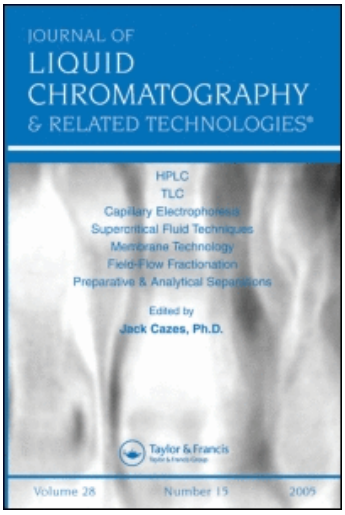

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PREPARATION OF MIXED C_{18}/C_1 HORIZONTALLY POLYMERIZED CHROMATOGRAPHIC PHASES

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ABSTRACT

The horizontal polymerization of trichlorosilanes on silica is discussed with regard to its use in chromatographic separations to reduce silanol activity and improve hydrolytic stability. It is shown that the use of C_1 spacers, which were previously demonstrated to provide very low silanol activity, are resistant to a pH 10 solution for at least 36 hr. It is also shown that the adsorptivity of water by silica gel is an important factor in achieving dense horizontal polymerization.

INTRODUCTION

Two imposing problems exist for presently available silica-based reversed phase chromatographic columns. First, unreacted surface silanols can deprotonate to form a negatively charged surface, causing strong adsorption of organic cations. This strong adsorption gives rise to peak tailing. Mobile phase additives, such as trifluoroacetic acid, amines, or phosphate ions, can reduce adsorption to these negatively charged sites on the surface.[1]

A better way to minimize such adsorption has been to use silica gel that has a high initial concentration of hydroxyl groups because the resulting hydrogen bonding among the silanols reduces their acidity.[2,3] Peak tailing is especially a problem in pharmaceutical applications because the pervasive amine functionality is positively charged at neutral pH. The second problem with available columns is that organosilane monolayers hydrolyze at pH extremes. Low pH, often needed to achieve efficient separations, causes degradation of commercial columns, which leads to added costs and down-time. The stability of silica-based columns is even worse at high pH, limiting typical applications to pH 8 and below.[4] At high pH, the silica substrate is attacked rather than the organosilane monolayer, ultimately resulting in bed collapse. Peak tailing is exacerbated by hydrolytic instability because hydrolysis exposes more surface silanols. There is a great demand for hydrolytically stable stationary phases having minimal silanol activity.

The majority of bonded chromatographic phases are "monomeric" phases, where a chlorodimethylorganosilane is covalently bonded to silica with 1:1 attachment of reagent to surface silanol. The straightforwardness of the reaction makes for a reproducible phase. However, most of the surface silanols remain unreacted, and some of these deprotonate to cause tailing. A technique called end-capping is often used to react some of the remaining silanols with a sterically smaller reagent, such as chlorotrimethylsilane. Endcapping reduces tailing of organic cations, but the improvement is temporary because the trimethylsiloxane groups hydrolyze readily. One variation has been devised to improve hydrolytic stability: larger sidegroups, such as isopropyl and isobutyl, replace the two methyl groups in the chlorodimethyloctadecylsilane reagent.[5,6]

Two factors might contribute to the higher stability. First, the bulky groups could sterically hinder attack at the siloxane bond. Second, the bulky groups could impede diffusion of the silicon moiety away from the site, allowing the siloxane bond to form again. These "sterically protected" phases are commercially available and are bonded to Zorbax-RX-sil, providing not only high stability but inherently lower silanol activity due to the lower acidity of Zorbax-RX-sil. As with any silica-based stationary phase, these cannot be used at excessively high pH.

If the use of silica could be avoided, high pH could be used routinely in liquid chromatography. This would allow separation of species that are only soluble or only separable at high pH, and would also open up a new area for HPLC: biotechnology. Presently, polymeric resins are used for purification in biotechnology because a base wash is required to remove irreversibly adsorbed proteins or cellular materials from the column. Copolymers of polystyrene and divinylbenzene provide hydrophobicity comparable to that of C_{18} monolayers, but offer virtually unlimited stability at high pH. However, polymeric resins are quite compressible, degrading

analytical separation efficiency and compromising resolution. A much less compressible stationary phase for use at high pH employs zirconia in place of silica.[7] Unlike silica, zirconia is not attacked by strong base. A thin polymer film, such as butadiene, provides a stable stationary phase covering the stable substrate. The one difficulty with zirconia is that its high surface charge gives rise to strong peak tailing of charged analytes such as proteins.[8]

There is not yet another substrate that satisfactorily replaces silica gel, and many research efforts are underway, using a variety of bonding schemes, to improve the performance of silica-based stationary phases. One alternative approach is the "polymeric" phase, where trichlorosilanes are mixed with a small amount of water and allowed to bond to the silica gel.[9] These polymeric phases are made of pure C_{18}, and have a bonding density of typically 5 $\mu mol/m^2$.

Analysis of a polymeric C_{18} phase using quantitative ^{29}Si NMR dispelled the notion of "vertical polymerization": there is an average 1:1 attachment between reagent and surface silanol.[10] These still differ from the monomeric phases, despite their common 1:1 attachment, because there is frequent covalent bonding to nearest neighbors. The resulting nearest neighbor spacing is much closer than that for monomeric phases, offering unique shape selectivity that can be especially valuable for PAH analysis, distinguishing species based on nonplanarity.[11]

Polymeric phases have improved hydrolytic stability, presumably owing to the multiple bonding. The procedure for synthesis is likely to cause significant oligomerization before attachment to the surface, making the organization on the molecular scale unknown. These phases have been commercially available for years but are believed to be less reproducible than monomeric phases, possibly a consequence of oligomerization before bonding.

A newer innovation that also uses trifunctional reagents is made by reacting a trifunctional hydrosilane, such as $HSi(OCH_2CH_3)_3$, with silica gel to form a hydrosilane surface. The desired functional group is subsequently linked to theSi-H surface through a reagent having a terminal carbon-carbon double bond.[12] The functional group is attached by a direct Si-C bond, which is much more hydrolytically stable than the Si-O-Si bond formed in the conventional monomeric phases. The initial layer of Si-H groups forms a dense monolayer, thus protecting the silica substrate and lowering the silanol activity.[13]

The absence of sidegroups on the ligand allows the same denser bonding that is believed to give the polymeric phases their unique selectivity. One would expect these hydrosilane phases to be more reproducible than the polymeric phases because the olefin reaction involves no danger of oligomerization before bonding.

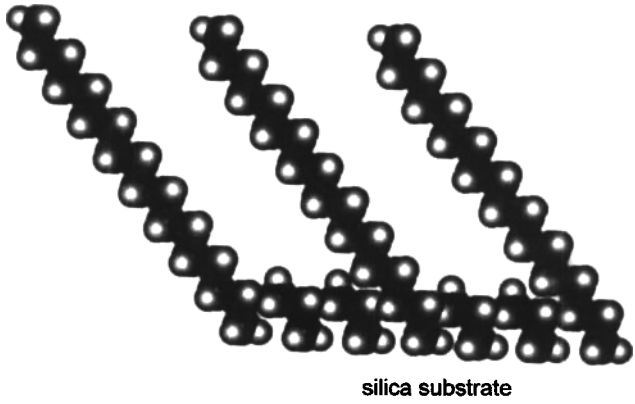

Figure 1. Space-filling model of a cross-section of the horizontally polymerized C_{18}/C_3 monolayer. The dense polymerization of trifunctional silanes in two-dimensions is designed to form a solid barrier immediately above the silica substrate, with little covalent attachment to the silica substrate. The mixing of C_{18} groups and short spacers gives a liquid-like density of functional groups to perform chromatographically as monomeric phases. The C_{18} chains are drawn in their all-trans conformations only for artistic convenience.

We have devised a bonding scheme for organosilanes on silica that also uses trifunctional silanes.[10,14,15] The bonding scheme is illustrated in Figure 1, where the process of "horizontal polymerization" , or "self-assembly" is used to make mixed monolayers. ^{29}Si NMR spectroscopy has revealed that the trifunctional reagents are bonded to one another, with only an occasional covalent bond to the surface.[10] The oriented copolymer covering the surface is intended to provide a steric barrier between the mobile phase and the silica substrate. This dense barrier would reduce surface charge by impeding the exchange of protons between the unreacted silanols and the mobile phase. The surface concentration of octadecyl (C_{18}) or other functional groups is adjustable by varying the reagent ratio.

There are two features that distinguish horizontally polymerized phases from the polymeric phase described earlier. First, the synthesis of the horizontally polymerized phase uses the water intrinsically adsorbed to silica gel, as illustrated in Figure 2. Consequently, no oligomerization occurs until the reagents have reached the surface. The use of a reproducible amount of adsorbed water provides a reproducible stationary phase. Second, a mixture of trifunctional silanes is used, providing a steric barrier with controllable coverage of the functional group .

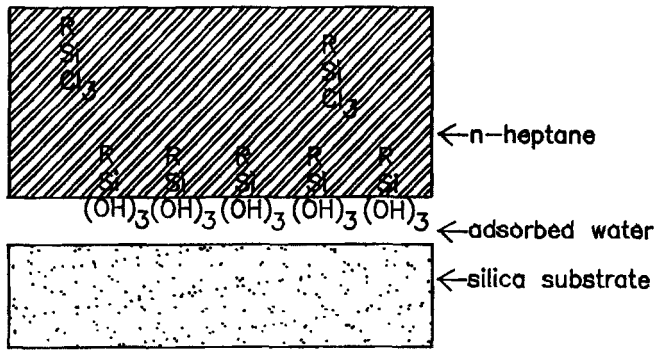

Figure 2. The oriented polymerization reaction. A monolayer of water adsorbed to the silica gel rapidly hydrolyzes the trichlorosilanes. The resulting trisilanols are adsorbed and oriented at the heptane/silica interface, and subsequently polymerize to form the dense monolayer.

The self-assembly of pure trichlorosilanes on silica surfaces has been studied by other groups with the goals of controlling the wetting properties of surfaces,[16,17] enhancing resolution in lithography,[18,19] electrochemical sensing,[20-22] molecular electronics,[23,24] preparing membrane mimetics[25] and biocompatible surfaces.[26] Our work represents the first use of the concept of applying the solid bonding density of self-assembly to make chromatographic stationary phases.

Previous Studies

Trifunctional silanes tend to be more susceptible to base hydrolysis than are monomeric silanes. This is because the silicon atom is a better electrophile when bonded to three oxygens compared to one, accelerating reaction with the bases, which are nucleophilic. This is the same reason that silica gel itself dissolves at high pH. Analogously, at low pH, trifunctional silanes are expected to be less susceptible to hydrolysis (per Si-O bond) because acids are electrophilic.

In stability studies of the C_{18}/C_3 horizontally polymerized phase, the retention time of benzo(a)pyrene was found to be constant at pH 2 over an observation period of one week.[15] This was not surprising given that the functional groups are the inherently acid-stable trifunctional silanes. However, the phase was also stable to a mobile phase at pH 10 over the observation period of 36 hours, again using benzo(a)pyrene as the probe.[15] The hydrolytic stability of a monomeric C_{18}, made on the same silica gel, Whatman Partisil, was poorer at both pH extremes.

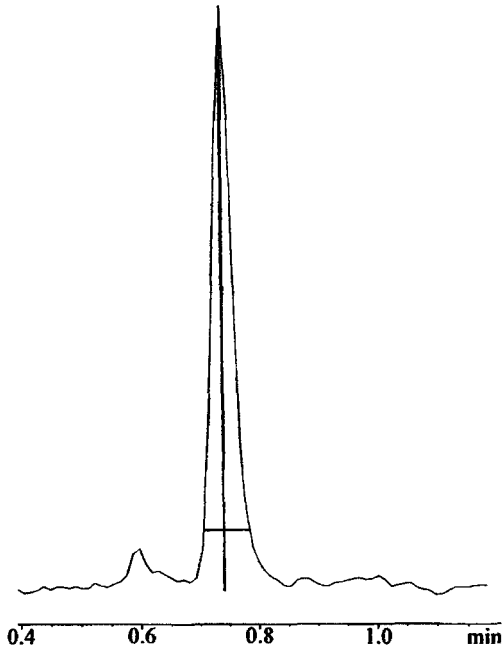

Figure 3. Chromatogram of aniline after 36 hr. exposure of stationary phase to pH 10 solution. The retention time is 0.75 min. and the asymmetry factor is only 1.2. A column length of 15 cm and flow rate of 2 mL/min. were used.

Separation Performance

The horizontally polymerized C_{18}/C_3 phase gave a chromatogram similar to that of a conventional C_{18} phase for a mixture of benzo(a)pyrene, hexanophenone and uracil; however, aniline was retained inordinately long.[15] The phase thus improved the hydrolytic stability but increased the silanol activity. This behavior indicates that the C_3 spacers did not form an adequately dense barrier over the silica substrate. A careful consideration of the steric interactions involved in creating an oriented, two-dimensional polymerization reveal that the C_3 spacers cannot be accommodated by the short Si-O-Si distances linking the reagents together. However, a network of C_1 groups is capable of forming a dense, two-dimensional methylsiloxane polymer.[27] Experimental measurements confirm this expectation. ^{29}Si NMR spectra revealed that a C_{18}/C_1 monolayer is comprised primarily of the alkylsiloxane groups covalently bonded to other reagent groups through all three oxygen atoms, with little attachment to the silica surface, while the C_{18}/C_3 monolayer is

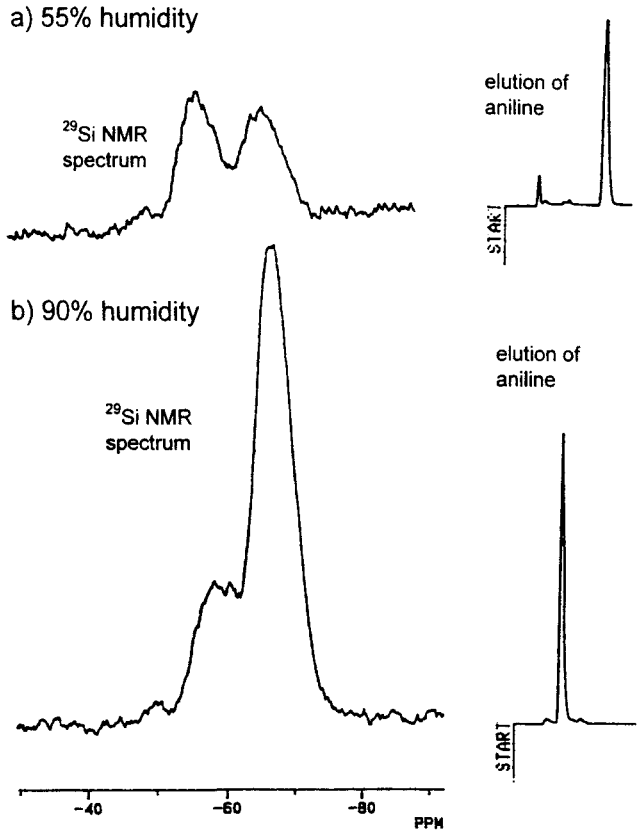

Figure 4. ^{29}Si NMR spectra and chromatograms of aniline for horizontally polymerized C_{18}/C_1 phase obtained upon pretreatment with a) 55% humidity and b) 90% humidity.

comprised primarily of the alkylsiloxane groups covalently bonded through only two oxygen atoms, with occasional attachment to the silica surface.[27] The NMR spectra thus reveal that the C_{18}/C_1 monolayer is a two-dimensional network while the C_{18}/C_3 monolayer is comprised of long chains packed close to one another. Since the reagents are bonded to one another, it would be possible for the small methyl groups to be oriented toward the surface.

Contact angle studies of C_1 monolayers on flat plates indicate that the methyl groups are oriented away from the surface: the contact angle is 78° for both the methylsiloxane monolayer on silica[27] and the methylthiol monolayer

on gold,[25] where the latter is known to have the methyl groups point away from the surface. The research thus shows that C_1 is a significantly better spacer group than C_3 for achieving maximum bonding density.

Finally, for the chromatographic test, aniline elutes early with little tailing, and a baseline separation of a mixture of cytochrome c genetic variants (bovine, equine, canine), was demonstrated using the C_{18}/C_1 phase.[27] These studies indicate exceptionally low silanol activity for the C_{18}/C_1 phase.

Stability Of The C_{18}/C_1 Phase

While the hydrolytic stability of the C_{18}/C_3 phase is excellent, the hydrolytic stability of the C_{18}/C_1 phase has not previously been tested. Hydrolytic stability of the C_{18}/C_1 phase is a concern because the shorter spacer group might be more prone to hydrolysis, particularly at defects, due to less steric hindrance. On the other hand, one might argue that the C_{18}/C_1 phase should be more stable due to its denser bonding. Over the course of our studies, we have not observed any unusual degradation of the C_{18}/C_1 phases.

For a freshly prepared C_{18}/C_1 phase, we reported a short retention time and a low asymmetry value for the zone profile of aniline at neutral pH.[27] If this phase degraded, the exposed silica would partially deprotonate, retaining aniline more strongly and giving rise to tailing. We exposed this stationary phase to a solution of triethylamine in 85% acetonitrile/water at 30°C for 36 hours, the same harsh conditions and exposure time applied to the C_{18}/C_3 horizontally polymerized phase.

Figure 3 shows the chromatogram for aniline, where its zone maintains its short retention time and low asymmetry. These data alleviate concerns about the stability of methyl spacers.

Applicability to other Silica Gels

While impressively low silanol activity was demonstrated for the C_{18}/C_1 horizontally polymerized phase, this phase was made on Zorbax-300RX-sil, and it is not been established that horizontal polymerization works well with other silica gels. Initial experiments with a variety of other silica gels did not yield acceptable stationary phases. It is possible that these early problems were caused by different water adsorptivities of different silica gels.

The Zorbax-RX-sil had been exposed to 55% humidity for depositing the reagent water on the surface because this was the humidity reported to work well for self-assembled monolayers of C_{18} on silicon wafers.[29]

Recently it is has been shown that the density of self-assembled C_{18} monolayers on flat silica surfaces varies with the amount of reagent water available for the trichlorosilanes.[30,31] For chromatographic surfaces, we varied the amount of adsorbed water by varying the relative humidity to which the bare silica was exposed, then measured the ^{29}Si NMR spectra and the chromatographic behavior. The same conditions for cross-polarization were used as previously reported.[10]

The spectral intensities for the resonances due to the reagent silicon atoms can be compared to one another because their relaxation times are comparable.[10] Figure 4 shows the ^{29}Si NMR spectra of a C_{18}/C_1 phase on SMR22, a silica gel available from Davison, Inc., where a) 55% humidity was used, and b) 90% humidity was used.

The NMR spectrum in part a) shows weak cross-linking for the lower humidity: the predominant peak, at -58 ppm, owes to reagent silicon atoms having a terminal OH group. The spectrum in part b) reveals extensive cross-linking for the higher humidity: the predominant peak, at -68 ppm, owes to reagent silicon atoms bonded through all three oxygens to other silicon atoms. A fully cross-linked network would have a peak only at -68 ppm, so room still remains for improvement in the synthesis of horizontally polymerized monolayers. The stronger overall signal in the silicon NMR spectrum for case b also indicates a higher coverage of reagent silicon atoms, consistent with the denser monolayer.

The chromatograms, shown to the right of the NMR spectra, reflect these structural differences: for case b, aniline elutes earlier and with a much narrower peak. The humidity required for good horizontal polymerization on SMR22 thus differs markedly from that for Zorbax-RX: 55% vs. 90%. The likely reason Zorbax-RX is so adsorptive toward water is that it has a very high concentration of surface hydroxyl groups.[2]

The ability to vary surface water concentration to control the quality of horizontal polymerization suggests that horizontal polymerization can be used with virtually any silica gel, provided that the humidity is appropriately adjusted to obtain the appropriate water coverage. We are currently investigating the quantitative amount of water needed for different silicas to determine whether or not there is a requisite amount of water that is a constant.

Application to Capillary Electrophoresis

Strong adsorption due to surface silanol activity is also a major problem in capillary electrophoresis, particularly for proteins that are strongly positively charged. Horizontal polymerization has been shown to provide a surface coating that has virtually no charge, as indicated by the absence of detectable electro-osmotic flow and the elution of very surface-active proteins.[32] A mixture of allyl groups and C_1 groups were used in the horizontal polymerization, followed by copolymerization of the allyl groups with polyacrylamide to make the surface hydrophilic. This demonstrates the use of functional groups other than C_{18} in horizontal polymerization, as well as applicability of horizontal polymerization to another area of separation science.

Future Studies

Horizontal polymerization has thus far been demonstrated for C_{18} and allyl functional groups. The bonding scheme may find valuable application for separations requiring shorter alkyl chains, such as C_4, to minimize the denaturing of proteins. Other reactions of allyl groups for controlling the chemical functionality of the monolayer are also attractive, such as amino and other ion exchanging groups. Also, hydrophilic groups such as diols can be dispersed among the spacers to moderate the hydrophobicity of the surface. Finally, there is much more to be learn about the process of two-dimensional polymerization, and physical studies of this process may ultimately lead to chromatographic stationary phases stable toward very high pH.

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REFERENCES

1. J. Paesen, P. Claeys, E. Roets, J. Hoogmartens, J.Chromatogr., **630**, 117 (1993).

2. J. Köhler, D. B. Chase, R. D. Farlee, A. J. Vega, J. J. Kirkland, J. Chromatogr., **352** 275 (1986).

3. J. Köhler, J. J. Kirkland, J. Chromatogr., **385**, 125 (1987).

4. J. J. Kirkland, M. A. van Straten, H. A. Claessens, J. Chromatogr., **691**, 3-19 (1995).

5. J. J. Kirkland, J. L. Glajch, R. D. Farlee, Anal. Chem., **69,** 2 (1989).

6. J. J. Kirkland, C. H. Dilts, J. E. Henderson, LC•GC, **11**, 290 (1993).

7. J. Nawrocki, C. J. Dunlap, P. W. Carr, J. A. Blackwell, Biotech. Prog. ,**10**, 561-573 (1994).

8. L. F. Sun. P. W. Carr, Anal. Chem., **67**, 3717-3721 (1995).

9. L. C. Sander, S. A. Wise, Anal. Chem., **56**, 504 (1984).

10. H .O. Fatunmbi, M. D. Bruch, M. J. Wirth, Anal. Chem., **65**, 2048 (1993).

11. L. C. Sander, S. A. Wise, J. Chromatogr. **316**, 163 (1984).

12. J. E. Sandoval, J. J. Pesek, Anal. Chem., **63**, 2634-2642 (1991).

13. C. -H. Chu, E. Jonsson, M. Auvinen, J. J. Pesek, J. E. Sandoval, Anal. Chem., **65**, 808-818 (1993).

14. H. O. Fatunmbi, M. J. Wirth, Anal.Chem., **64**, 2783 (1992).

15. H. O. Fatunmbi, M. J. Wirth, Anal.Chem., **65**, 822 (1993).

16. N. L. Abbott, C. B. Gorman, G. M. Whitesides, Langmuir, **11**, 16 (1995).

17. P. E. Laibinis, G. W. Whitesides, D. L. Allara, Y.-T. Tao. A. N. Parikh, R. G. Nuzzo, J. Am. Chem. Soc., **113**, 7152 (1991).

18. C. R. K. Marrian, F. K. Perkins, S. L. Brandow, T. S.Koloski, E. A. Dobisz, Appl. Phys. Lett., **64**, 390 (1994).

19. C. B. Ross, L. Sun, R. M. Crooks, Langmuir, **9**, 632 (1993).

20. F. Malem. D. Mandler, Anal. Chem., **65**, 37 (1993).

21. D. E. Weisshaar, B. D. Lamp, M. D. Porter, J. Am.Chem. Soc., **114**, 5860 (1992).

22. R. J. Willicut, R. L. McCarley, Langmuir, **11**, 296 (1995).

23. C. B. Gorman, H. A. Biebuyck, G. W. Whitesides, Chem. Mat., **7**, 526 (1995).

24. X. O. Zhang, X. -Z. You, S. -H. Ma, Y. Wei, J. Mat. Chem., **5**, 643 (1995).

25. Y. Maeda, H. Yamamotot, H. Kitano, J. Phys. Chem., **99**, 4837 (1995).

26. R. S. Petember, M. Matsuzawa, P. Liesi, Syn. Met., **71**, 1997 (1995).

27. R. W. P. Fairbank, Y. Xiang, M. J. Wirth, Anal. Chem. **67**, 3879 (1995).

28. C. D. Bain, E. B. Troughton, Y -T. Tao, J. Evall, G. M. Whitesides, R. G. Nuzzo, J. Am. Chem. Soc., **111**, 321 (1989).

29. S. R. Wasserman, Y -T. Tao, G. M. Whitesides, Langmuir, **5**, 1074 (1989).

30. J. G. Terlingen, J. Feijen, A. S. Hoffman, J. Colloid Int. Sci., **155**, 55-65 (1993).

31. D. H. Flinn, D. A. Guzonas, R. -H. Yoon, Colloids Surf., **87**, 163-176 (1994).

32. M. Huang, E. Dubrovcakova, M. Novotny, H. O. Fatunmbi, M. J. Wirth, J. Micro. Sep., **6**, 571 (1994).

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