Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1042 (2004) 61–68
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Study of short polystyrene monolith-fritted micro-liquid chromatography columns for analysis of neutral and basic compounds

C. Legido-Quigley [a,*], N.W. Smith [b]

[a] *Centre for Analytical Sciences, Imperial College, Exhibition Road, RSC1 London SW7 2AY, UK*
[b] *Microseparations Group, Department of Pharmacy, King's College, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK*

Received 11 August 2003; received in revised form 15 November 2003; accepted 7 May 2004

Abstract

This study details the effects of poly(styrene-divinylbenzene) (PS-DVB) frits in micro-HPLC columns for the separation of neutral and basic compounds. The procedure comprised the optimization of separations with only monolith or conventional fritted columns followed by method transference to short monolith-fritted columns. It was observed that a superior separation was achieved with the new columns compared to silica-fritted-packed columns once triethylamine (TEA) was added in small percentages. The separation of basic and neutral compounds was achieved in fast analysis times in the isocratic mode.

Keywords: Monoliths; Stationary phases, LC; Basic compounds

1. Introduction

Recently, very narrow HPLC-packed columns have become available in the market. Microbore columns are considered as columns with an internal diameter between 0.5 and 2 mm while capillary columns are regarded as having any internal diameter smaller than 0.5 mm [1]. The most important advantage of using microflows thorough capillary columns, is the higher sensitivity one can achieve. As the internal diameter of the column reduces the analysis is more sensitive [2]. Another important direct consequence of the small flow rates delivered at this mode is the excellent conditions for coupling a micro-HPLC to a mass spectrometer [3].

Monolithic capillaries have emerged as an alternative to traditional packed-bed columns for use in micro-HPLC and CEC. The driving force behind this research is the many advantages that they hold. They are easy to manufacture, the monolith being formed in situ, often via a one-step reaction process, and its properties such as porosity, surface area and functionality can be tailored. Another major advantage is that they eliminate the need for retaining frits, which can contribute to band broadening in HPLC and to bubble formation in CEC. Monoliths or continuous beds can be divided into two general categories:

The first type are silica-based monolithic columns, generally prepared using sol–gel technology. This technology can be applied to create a continuous sol–gel network throughout the column formed by the gelation of a sol solution within the capillary.

The second category is rigid polymer-based monolithic columns, and these include acrylamide-, methacrylate ester- and styrene-based polymers. The polymer network is generally formed inside the capillary by a step-wise chain polymerisation reaction [1].

At the beginning of the 1990s, Svec and co-workers [4–6] introduced these highly porous polymers as a monolithic separation medium for the reversed-phase HPLC separation of proteins. In the last decade, many groups have applied this technology for several applications in HPLC and CEC [7–11].

Poly(styrene-divinylbenzene) (PS-DVB) polymers are stable across a wide pH range (1–14), which cannot be endured by conventional LC packing materials. Due to its

* Corresponding author.
E-mail address: c.legidoquigley@ac.uk (C. Legido-Quigley).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.05.060

hydrophobic nature, the synthetic polymer in certain cases can be used directly as a reversed-phase stationary phase, without the need for further derivatisation. There are however two methods of introducing alkyl groups onto the surface of the polymer. Firstly, a monomer such as an alkylstyrene can be included in the polymerization mixture, however it must be soluble in the porogen, and precipitate when the polymer is formed. Secondly, the surface of the polymer can be alkylated after formation, by using for example, a strong Friedel–Crafts catalyst (alkyl halide in organic solvent).

In 2000, the group of Premstaller, Oberacher and Huber showed excellent applications of these polymer-based materials. The porogen in this case was a mixture of THF and decanol. This mixture would produce mesopores for the separation and macropores for the fast analysis of compounds. In their paper, the gradient elution ion pairing reversed-phase analysis of single and double stranded nucleic acids was achieved with efficiencies of 190 000 plates per meter [12,13]. Furthermore, the capillary columns were hyphenated to a mass spectrometer via electrospray ionization, enabling the mass recognition of the nucleic acids.

An alternative procedure for the formation of monolithic columns is to continuously bond liquid chromatography silica particles. The polymer solutions can be used to "glue" conventional LC particles. The first encapsulated studies for capillary chromatography were made in 1998 using sol–gel chemistry [14], and in the year 2000, the first methacrylate-based organic monolith was used to encapsulate conventional phases [15]. Here, the capillary was packed using the slurry pressure packing method to produce a standard micro column. The column was then filled with the chosen polymer solution to produce a continuously bonded bed. A review on encapsulated columns has been published recently [16]. An alternative to encapsulation is to dip the ends of the packed column into the polymer solution, thus creating polymer frits. The concept of monolithic frits for capillaries was patented by Merck, Germany in 2001 [17]. Several studies have been made with fritted columns and Oberacher and co-workers [18–21] studied polystyrene-based frits for the analysis of nucleic acids.

To our knowledge, this paper reports the first effort to use polystyrene-based monoliths as frits to immobilize a packed bed inside micro columns to analyze neutral and basic compounds. Recently, two studies of the behavior of basic compounds in silica-based commercially available monoliths have been reported for HPLC [22,23]. However, the analysis were performed at a maximum of pH 7 due to possible deterioration of the silica monolith at higher pH's. In this paper, we have studied the behavior of PS-DVB monoliths for the separation of neutral and basic compounds. In the first section, the behavior of the monolith was assessed with neutral and strongly basic compounds at all ranges of pH. The second section studies the possibilities of the monolith as a media for fritting micro columns and the advantages that might ensue when compared to silica-fritted columns. The last section deals with the manufacture of very short monolith-fritted columns and their applications for fast isocratic analysis.

2. Experimental

2.1. Chemicals and materials

The compounds used for analysis thiourea, dimethyl phthalate, anisole, naphthalene, uracil from Fluka, salmeterol and fluticasone propionate were provided by GlaxoSmithKline, desipramine (pK_a 10.6), nortriptyline (pK_a 9.7), imipramine (pK_a 9.5), amitriptyline (pK_a 9.4) and clomipramine (pK_a 9.4), sodium hydroxide, acetic acid 99% purity, monomer 3-(trimethoxysilyl)propyl methacrylate, tetrahydrofuran, styrene 99% purity, divinylbenzene 80% purity, decanol and azobisisobutyronytrile, isopropyl alcohol (IPA) and sodium silicate all purchased from Sigma–Aldrich. The XTerra^{TM} stationary phase was from Waters (USA) and it was donated by GSK (UK).

2.1.1. Solvents

Acetonitrile (190 far UV grade), water (HPCL grade), methanol and triethylamine (TEA) were purchased from Fisher Scientific. Acetic acid, ammonium formate and formic acid were purchased from Sigma–Aldrich.

2.2. Instrumentation

The micro-HPLC equipment used in this work comprised a Jasco (Japan) PU-1585 pump and an Applied Biosystems (USA) 757 detector for the isocratic mode analysis. Frit burner was purchased from Innovatech (UK).

2.3. Procedure

2.3.1. Optimization of monolith analysis

Monolith columns were fabricated by following a two-step procedure. First, the walls of 320 μm i.d. fused silica capillaries were silanized following the method published by Coufal et al. [24]. Next, the polymer mixture consisting of 2 μl tetrahydrofuran, 5 μl styrene, 5 μl divinylbenzene, 13 μl decanol and 10 mg/mL of initiator (0.25 mg azobisisobutyronytrile) was prepared [12]. The column was then filled with the mixture using a syringe and left to polymerise for 20 h at 60 °C.

2.3.2. Silica frits versus monolith frits

For the silica-fritted XTerra(tm) column, a 100 mm × 0.320 mm i.d. capillary was pretreated for the silanization of the capillary wall. Silica frits were necessary for these columns because it is not possible to fabricate a frit from the XTerra^{TM} phase. The silica frit was fabricated in the following manner. Pure silica was mixed with a small amount of aqueous sodium silicate solution (25% (v/v)) and IPA

to form a paste that was forced into one end of the capillary. Using a burner, the silica was fused and the resulting frit tested for resistance under pressure to 80,000 MPa. The column was then filled with the stationary phase using the slurry packing method to the desired length. Pure silica was packed at this stage in order to fabricate the second retaining frit.

When the pH study of neutral and basic compounds was performed this same column was washed by pumping dried methanol for 6 h and then dried under nitrogen for 1 h. After this, the column was dipped into the polymer solution to 0.2 cm distance from the frit and left to polymerize for 20 h at 60 °C. The silica frits were then cut off and the second round of analysis performed.

2.3.3. Fast micro-HPLC analysis with monolith-fritted short columns

Two capillaries, 5 and 3 cm long were packed following the same procedure as described in the previous section. The 50 mm × 0.320 mm column had two frits of 2 mm length while the 30 mm × 0.320 mm column had a 2 mm frit at one end and a 10 mm long frit at the other end. Both of these columns were used for the micro-LC experiments at flows up to 60 μL/min. The test mixture consisted of uracil and five strong basic compounds with pK_a's >9 and the same four neutral compounds used before. The analyses were all isocratic in order to assess column efficiency and the quickest possible separation without a gradient.

3. Results and discussion

3.1. Optimization of monolith analysis

When the columns filled with monolith were used for the analyses of the neutral or basic compounds two trends were observed. For neutral compounds, a Gaussian peak was observed at all pH's (pH 2–12). This result was expected, as the neutral compounds would only slightly change retention with the changing of the pH. A possible reason for this would be dependence between π-interactions in the benzene rings and mobile phase pH. This is difficult to asses, as shown in a study made by Davankov et al. [25], where the separation mechanisms of this polymer (in the hypercross-linked variant) in chromatography are not totally understood, concluding that the polymer is characterized by showing unusual selectivity under reversed-phase conditions.

The second trend was that the basic compounds were strongly retained at all the pH values independently of them being in the neutral or protonated form. The basic compounds were also thought to be experiencing irreversible adsorption. This implied that basic molecules in their neutral form did not interact in the same way as neutral compounds; performing electron-donating interactions at the same time as hydrophobic interactions. See Fig. 1, where a neutral retained compound (naphthalene) was compared to a strongly

3.3.1 Optimization of monolith analysis figures

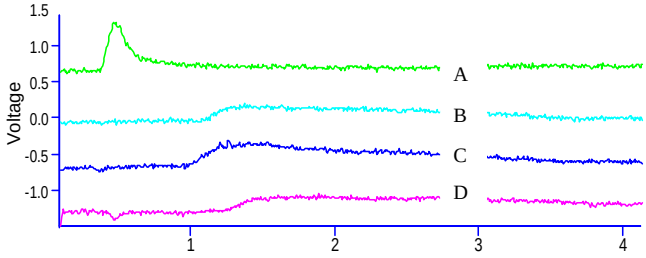


Fig. 1. Shows chromatograms of a neutral and a strongly basic compound on a PS-DVB monolith. All chromatograms: column: 50 mm × 0.320 μm. PS-DVB monolith only; flow: 40 μL/min; mobile phase: 50% water (pH adjusted) and 50% acetonitrile; detection: 210 nm. Temperature: 21 °C. Chromatogram A: naphthalene at pH 7 and mobile phase at pH 7; chromatogram B: nortriptyline at pH 10.8 and mobile phase at; chromatogram C: nortriptyline at pH 7 and mobile phase at pH 7; chromatogram D: nortriptyline at pH 3 and mobile phase at pH 7.

basic compound at different pH values. The same trend was found when the mobile phase was basic (pH 10.8) and acid (pH 3).

When a competing base was added to the mobile phase the results were very different (see Fig. 2). The basic compounds were strongly retained but a greatly improved peak shape was observed. Therefore, the sensitivity of the analysis was also improved, and it was also noted that TEA improved selectivity, as the separation of a neutral and the basic compound was achieved, whilst this did not occur without the additive. There are few possible reasons for this phenomenon. One possibility was of competition between the compounds and the additive. The basic cations strongly interacted with phenyl rings and could slightly interact to any unreacted alkenes. A cation-π bonding interaction could be feasible as the positively charged molecules were attracted to the negatively charged areas below and above the benzene rings or unreacted alkenes—therefore the triethylamine which was also protonated was competing for these sites with the basic compounds. Another possible explanation, although an

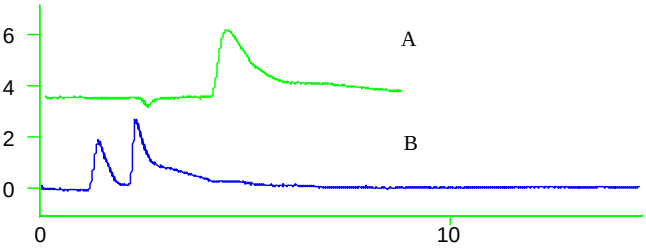


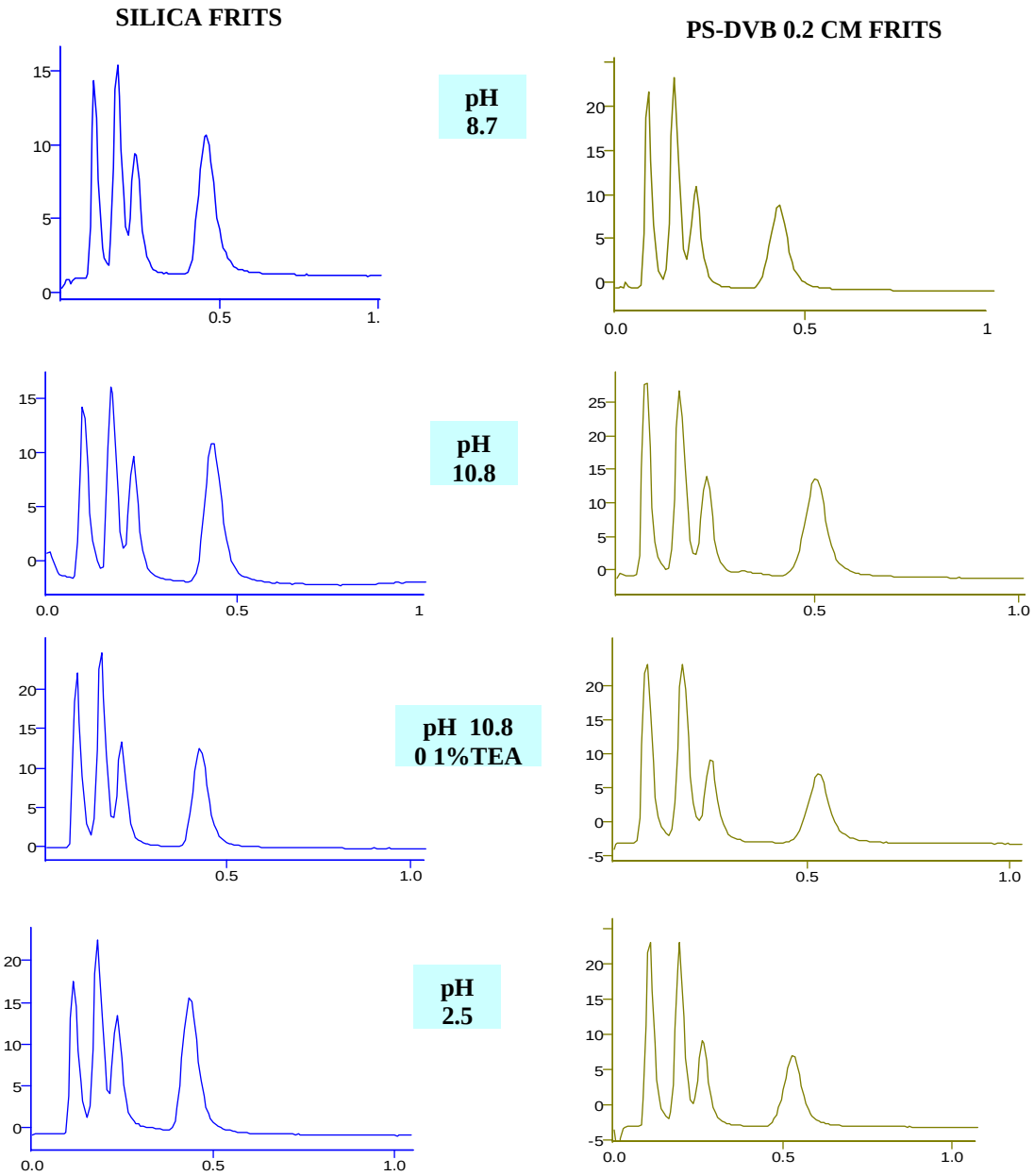
Fig. 2. Shows chromatograms of a neutral and a strongly basic compound on a PS-DVB monolith with the addition of 0.1% TEA. All chromatograms: column: 50 mm × 0.320 μm. PS-DVB monolith only; flow: 20 and 40 μL/min; mobile phase: 50% water (pH adjusted) and 50% acetonitrile; detection: 210 nm. Temperature: 21 °C. Chromatogram A: nortriptyline at pH 3 and mobile phase at pH 10.8; chromatogram B: naphthalene and nortriptyline at pH 10.8 and mobile phase at 10.8.

unlikely reaction, was that TEA was actually reacting with the monolith forming a stationary phase alkylated with ethyl groups and incorporating the positively charged nitrogen. This charge would repel the basic compounds and aid the chromatography.

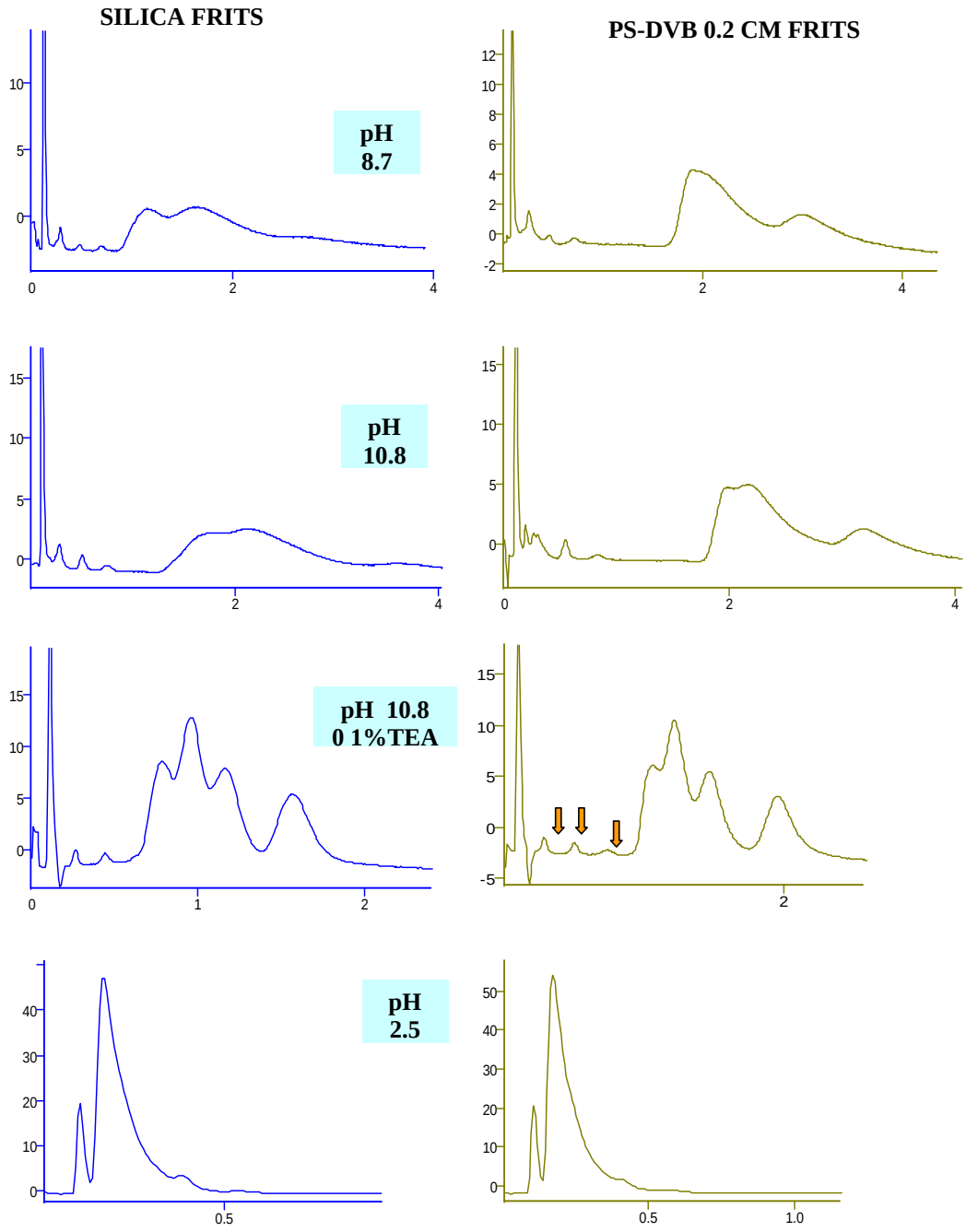
These results encouraged us to use this monolith to encapsulate a conventional phase (XTerra^{TM}) and then assess the extent of the monolith interactions with the test compounds.

3.2. Silica frits versus monolith frits

There are two possibilities when encapsulating conventional particles such as XTerra^{TM}; either "glue" the whole column or just making frits for the packing to hold in place. In our study, we opted for making frits as the monolith covering the whole bed would greatly affect the retention times by increasing the attractive interactions of both neutral and basic compounds.



Figs. 3 and 4. Analyses of four neutral compounds in Fig. 3 and four basic compounds in Fig. 4 on a 3 cm column before and after 0.2 cm monolithic frits were added. All chromatograms: column: 30 mm × 0.320 μm. Encapsulated 3.5 μm XTerra^{TM} phase; flow: 50 μL/min; detection: 210 nm; temperatures: 21 °C. All mobile phases: 50% water (pH adjusted) 50% acetonitrile.



Figs. 3 and 4. (*Continued*).

The first study performed consisted in manufacturing two 3 cm long columns from the same pressure-packed 10 cm capillary. One 3 cm column fritted with silica and the other fritted with 2 mm long monolith frits. The analyses were performed six times with each at pH 8.7, 10.8, 10.8 and 0.1% TEA and to finalise at pH 2.5. What was observed was that both columns performed good separation of neutral compounds at these pH's. These results can be observed in Fig. 3 were the difference between columns is negligible. When the same approach was used for the basic compounds the results were very different (see Fig. 4), the column did not separate the mixture at the same conditions, and only when TEA was used the compounds showed a Gaussian shape. When the monolithic frits in conjunction with the additive were used the bases were more retained and one-third impurity was detected in the chromatogram. From this study, it was decided that the 0.2 cm in total amount of monolith frits, was too small to have a relevant effect on the separation, although together with the additive there was potential for more selectivity.

When a very similar experiment was executed on a 10 cm long column, the results were more revealing. In Fig. 5, we

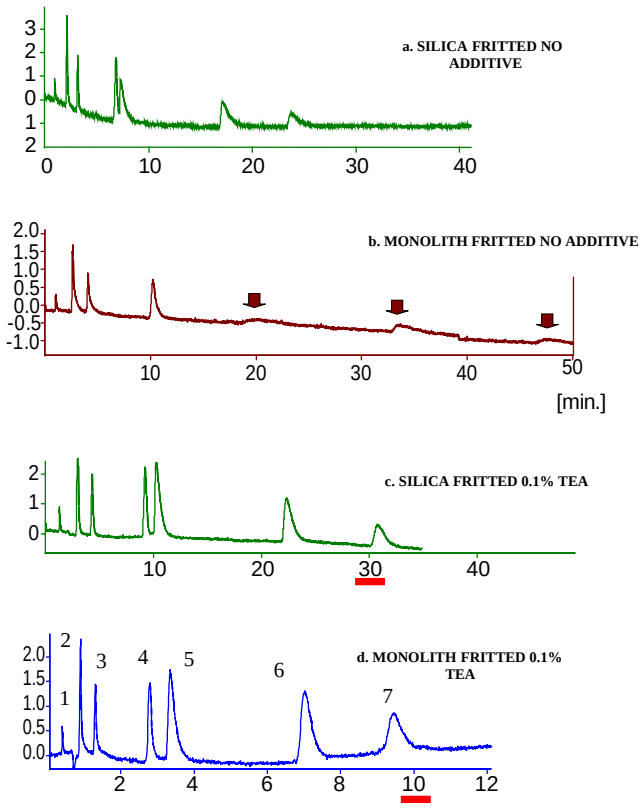


Fig. 5. Chromatograms show four neutral compounds and three strong bases eluting from the same XTerra™ column fritted with silica or PS-DVB 0.4 cm monolith. Basic compounds are strongly retained with the monolith frits, however when TEA is added greater resolution than with silica frits is obtained in one-third of the time. Column: 100 mm × 0.320 mm. Conventional 3.5 μm XTerra™ phase silica-fritted column that was later PS-DVB-fritted; flow chromatograms a, b, c: 20 and 40 μL/min for chromatogram d; mobile phase: 50% water 10 mM CAPSO (pH 12) and 50% acetonitrile; detection: 210 nm. Temperature: 21 °C. Peaks in order of elution: thiourea, dimethyl phthalate, anisole, naphthalene, nortriptyline, imipramine and amitriptyline.

can observe the most representative results. All the analyses in this Figure were performed at pH 10.8 in order for the compounds to be sufficiently retained. When the silica-fritted column was used, Fig. 5a, the basic compounds showed peak tailing due to the residual silanol groups in the frits. The XTerra™ stationary phase used was manufactured via methyl group hybrid synthesis and a high percentage of the residual silanol groups are end capped. Therefore, the contribution to peak tailing from the stationary phase was minimal. When a small proportion of TEA (0.1%) was added to the mobile phase the peak shape improved and now the peaks 4 and 5 were totally resolved. All the compounds were eluted in 30 min (see Fig. 5c).

When the monolith-fritted column was used, the results were encouraging, as the 2 mm frits held the 100 mm × 0.32 mm long column at pressures as high as 40,000 MPa. As predicted from the previous section, the analyses of the basic compounds were poor. This shows the extent to which the monolith could affect the nature of the separation. Although the neutral compounds were more retained, the bases were strongly retained.

On the other hand, when 0.1% of TEA was added to the mobile phase a great improvement was observed (Fig. 5d). The peak shape was improved, the retention was decreased and the selectivity improved producing a superior column when compared with the silica-fritted version. Doubling the flow to 20 μL/min produced good results with a reproducible run time of approximately 10 min.

When the silica-fritted-column was compared with the monolith-fritted column for mechanical strength, it was noted that after many runs (>100) at backpressures of more than 30,000 MPa. the monolith frits could be flushed out. To solve this, it was decided to produce shorter columns and if necessary bigger frits up to one centimeter long so the monolith anchored the capillary better. On the other hand, it was also noted that the monolith-fritted columns performance would not deteriorate over time like the silica frits did. When several runs were performed at high pH the silica frits dissolved as expected. These results led us to believe that very short monolith-fritted columns could be fabricated for fast analyses of basic compounds.

3.3. *Fast micro-HPLC with monolith-fritted short columns*

The short columns were easy to make and had the advantage of using small amounts of stationary phase. Optimal conditions were studied by varying flow rate, concentration of acetonitrile, pH and concentration of TEA. Although the columns followed a conventional reversed-phase behavior, it was noted that the columns performance did not vary greatly when conditions were changed.

In Fig. 6, there is another example of the effect of the monolith-fitted column over the conventional analysis. The analyses were performed on a three centimeter long column all at 50 μL/min and the conditions were optimized for future

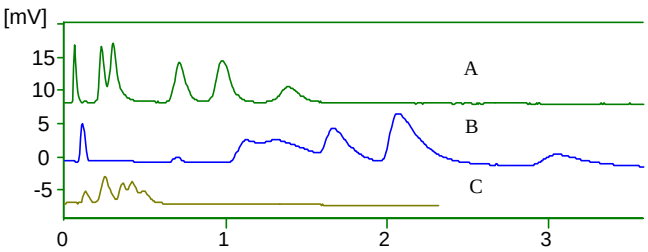


Fig. 6. Analyses of five basic compounds on a 3 cm column before and after 1 cm total monolithic frits were added. All chromatograms: column: 30 mm × 0.320 mm. Encapsulated 3.5 μm XTerra™ phase; flow: 50 μL/min; detection: 210 nm; temperatures: 21 °C. Chromatogram A: column with 1 cm PS-DVB frits, 50% water 20 mM ammonium formate (pH 9.7) 0.1% TEA and 50% acetonitrile 0.1% TEA; chromatogram B: column with 1 cm PS-DVB frits, 50% water 10 mM TRIS (pH 10) and 50% acetonitrile; chromatogram C: column with 0.2 cm silica frits; 50% water 20 mM ammonium formate (pH 9.7) and 50% acetonitrile.

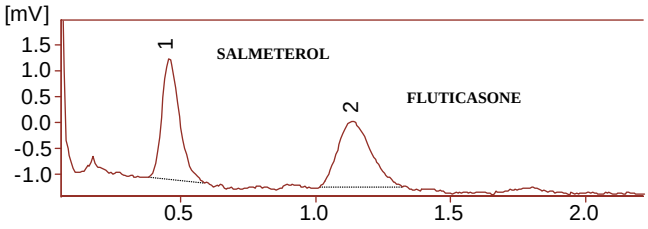


Fig. 7. The figure shows a PS-DVB-fritted column performing an analysis of a weak base and a neutral compound with. Column: 50 mm × 0.320 mm. Encapsulated 3.5 μm XTerra™ phase with 1 cm PS-DVB frits; flow: 30 μL/min; mobile phase: 50% acetonitrile, 50% H_2O with 0.1% formic acid; detection: 210 nm; temperature: 21 °C.

hyphenation to a mass spectrometer following the conditions used in a previous study [3].

In chromatogram 6A, the silica-fritted column showed little retention and a flow rate of 50 μL/min the backpressure was only 5800 MPa. At this flow, the separation could not be achieved with 50% organic in the mobile phase. In our opinion, the column did not have enough stationary phase. After this, the column was monolith fritted to 1 cm total length. When the column was one-third filled with monolith the backpressure increased to 15,200 MPa and the loadability improved to a mean value of 107%. It was believed that the increased surface area inside the column was responsible for these two observations. Chromatograms 6B and C show the effect from an analysis with no additive to an analysis with 0.1% of TEA in a 3 cm column long column when one-third of it has been monolith filled.

A study was performed for the separation of salmeterol and fluticasone propionate. Being salmeterol a weak base and fluticasone a neutral drug, it was observed that the analysis was possible at a mobile phase consisting of water and organic and a 0.1% of formic acid. These are conditions widely used for MS and further applications like the direct injection of plasma are currently being studied (Fig. 7).

Fig. 8 shows a useful application of these short columns, the isocratic separation of the five strong basic compounds on a monolith-fritted column. The three last bases showed

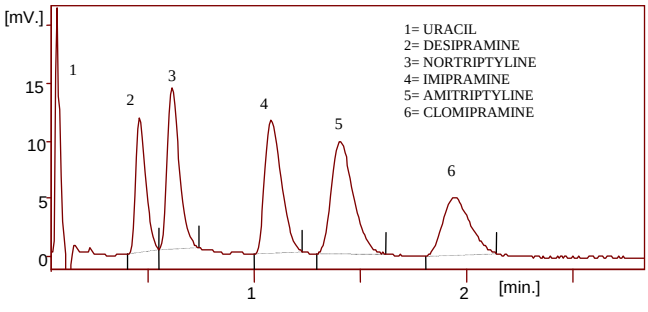


Fig. 8. The PS-DVB fritted columns performed a more selective analysis than those fritted with silica frits. Column: 50 mm × 0.320 mm. Encapsulated 3.5 μm XTerra™ phase with PS-DVB frits; flow: 50 μL/min; mobile phase: 50% water 10 mM CAPSO (pH 10.8) and 50% acetonitrile 0.1% TEA; detection: 210 nm; temperature: 21 °C.

efficiencies in the order of $N = 20.000$ plates per meter in times shorter than 2 min and the peaks show acceptable asymmetry values calculated at half height ($A_s = 1.533$ for the last eluting compound clomipramine). These results are encouraging as in a recent study of silica monoliths the asymmetry of nortriptyline at pH 7 was as high as $A_s = 5.43$, in our case by combining an organic monolith with a conventional phase showed an asymmetry value of $A_s = 1.77$ at pH 12. This separation is both faster and with better resolution than those achieved by conventional columns of the same length, internal diameter and stationary phase.

4. Conclusions

It was proved in this study that frits formed with PS-DVB monolith phases were not suitable for the analysis of basic compounds unless a competitive base like TEA was added. In that case, the decreased retention together with the improved peak shape made an excellent media to analyze basic compounds in the fast mode. The fast separations were achieved in conditions such as those desired for hyphenation to a mass spectromer leaving room for further improvement by using fast gradients at high temperature. The practical advantages seen over a stationary phase for basic compounds were improved mechanical strength, improved loadability, improved longevity and improved resolution permitting faster overall separations.

Acknowledgements

This work was carried out thanks to a CASE award grant provided by GlaxoSmithKline (GSK) in the UK. The authors also thank the Royal Academy of Engineering for the travel grant ITG03-169.

References

[1] C. Legido-Quigley, N. Marlin, V. Melin, A. Manz, N.W. Smith, Electrophoresis 24 (2003) 917.
[2] A. Braithwaite, F.J. Smith, Chromatographic Methods, Chapman & Hall, London, 1996.
[3] C. Legido-Quigley, N.W. Smith, D. Mallet, J. Chromatogr. A 976 (2002) 11.
[4] F. Svec, T. Tennikova, Z. Deyl (Eds.), Monolithic Materials, Journal of Chromatography Library, vol. 67, Elsevier Science, 2003.
[5] M. Petro, F. Svec, I. Gitsov, J.M.J. Frechet, Anal. Chem. 68 (1996) 315.
[6] M. Galia, F. Svec, J.M.J. Frechet, J. Polym. Sci. Part A, Polym. Chem. 32 (1994).
[7] R.E. Moore, L. Licklider, D. Schumann, T.D. Lee, Anal. Chem. 70 (1998) 4879.
[8] B.H. Xiong, L.H. Zhang, Y.K. Zhang, H.F. Zou, J.D. Wang, J. High Resolut. Chromatogr. 23 (2000) 67.
[9] I. Gusev, X. Huang, Cs. Horváth, J. Chromatogr. A 855 (1999) 273.
[10] X.A. Huang, S. Zhang, G.A. Schultz, J. Henion, Anal. Chem. 74 (2002) 2336.

[11] C. Viklund, A. Nordstrom, K. Irgum, F. Svec, J.M.J. Frechet, Macromolecules 34 (2001) 4361.
[12] A. Premstaller, H. Oberacher, C.G. Huber, Anal. Chem. 72 (2000) 4386.
[13] A. Premstaller, H. Oberacher, W. Walcher, A.M. Timperio, L. Zolla, J.P. Chervet, N. Cavusoglu, A. van Dorsselaer, C.G. Huber, Anal. Chem. 73 (2001) 2390.
[14] M. Dulay, R. Kulkarni, R. Zare, Anal. Chem. 70 (1998) 5103.
[15] G.S. Chirica, V.T. Remcho, Anal. Chem. 72 (2000) 3605.
[16] Q. Tang, M.L. Lee, J. Chromatogr. Lib. 67 (2003) 197.
[17] E. Mueller, D. Lubda, K. Derwenskus, H. Harders, Patent WO 2,001,057,516.
[18] J.R. Chen, M.T. Dulay, R.N. Zare, F. Svec, E. Peters, Anal. Chem. 72 (2000) 1224.
[19] Y. Chen, G. Gerhardt, R. Cassidy, Anal. Chem. 72 (2000) 610.
[20] M. Kato, M.T. Dulay, B.T. Bennett, J.P. Quirino, R.N. Zare, J. Chromatogr. A 924 (2001) 187.
[21] H. Oberacher, A. Krajete, W. Parson, C. Huber, J. Chromatogr. A 893 (2000) 23.
[22] D.V. McCalley, J. Chromatogr. A 965 (2002) 51.
[23] D.V. McCalley, J. Chromatogr. A 987 (2003) 17.
[24] P. Coufal, M. Cihak, J. Suchankova, E. Tesarova, Z. Bosakova, K. Stulik, J. Chromatogr. A 946 (2002) 99.
[25] V.A. Davankov, C.S. Sychov, M.M. Ilyn, K.O. Sochilina, J. Chromatogr. A 946 (2003) 67.