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Comparison of conventional microparticulate and a monolithic reversed-phase column for high-efficiency fast liquid chromatography of basic compounds

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Abstract

The effect of mobile phase flow on column efficiency for a neutral compound together with weak and strong bases was compared for conventional microparticulate (3/3.5 μm and 5 μm) silica RP columns and a monolithic silica RP. For benzene, the minimum plate height (H_{min}) at optimum flow-rate (u_{opt}) for weak bases was similar for the 5 μm and the monolith phases. However, the monolith generated much flatter Van Deemter curves, such that at high flow-rate (5 ml min^{-1}) the plate height was nearly 3.5 times lower on the monolith. For weak bases analysed in unbuffered mobile phases, and stronger bases with acid phosphate buffer, increased tailing was obtained on the monolith compared with the conventional phases. Nevertheless, Van Deemter plots on the monolith still showed some advantages over particulate phases, even when asymmetry factor was included in the calculation of the plate height. However, at pH 7 considerable tailing of strong bases was found using the monolith; it is not clear whether this results from unique features of the monolith structure, or whether it is due merely to usual problems of silica activity. Van Deemter plots for conventional phases may be improved considerably by operating the column at elevated temperatures. At pH 3, these improvements are influenced to a considerable extent by increases in D_m, as shown by measurements of D_m using the Taylor–Aris procedure. However, at pH 7.0, improvements are much too substantial to be explainable wholly on this basis.
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1. Introduction

Speed of analysis is becoming increasingly important in many application areas of HPLC, such as in pharmaceutical analysis, in order to increase throughput and reduce costs. Many drugs and biomedically important compounds are bases, the analysis of which remains problematic due to poor peak shapes which are often experienced in RP chromatography. An excellent review of these problems was presented by Snyder et al. [1] and the topic has been revisited in other more recent reviews [2–4]. Despite this interest, relatively little work has been done on the effects of flow-rate and temperature on the peak shape of bases. Previously, we demonstrated that temperature may give useful selectivity effects for the separation of bases and neutral compounds. Whereas the retention of neutral solutes generally

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decreases with increasing temperature, the retention of some bases may increase. Furthermore, increased temperature (up to 60 °C) may give substantial improvement in the peak shape of bases [5]. High flow-rates appeared to be detrimental for some bases due apparently to lower values of the optimum flow-rate (u_{opt}) compared with neutrals and the steep rise in plate height with flow-rate. However, it was not possible to rationalise these results in the absence of important data such as the mobile phase diffusion coefficients of the solutes (D_m), nor was the combined influence of temperature and flow-rate on column performance studied in our previous work.

Recently, monolithic silica phases have been prepared, either in capillary tubes or in polymeric ''cladding'' materials similar to the dimensions of conventional packed HPLC columns; the latter type have become commercially available. These columns are more sparsely occupied by solid and have a larger cross sectional area of through pores, resulting in a lower flow resistance and thus the possibility of operation at high flow-rates [6,7]. However, an evaluation of the applicability of such phases to the analysis of basic compounds has not so far been reported in the literature [8].

Kirkland [9] has reviewed fast (principally isocratic) HPLC using small porous and superficially porous particle columns. Neue et al. [10] have assessed the performance of short, very small particle columns (generating moderate efficiency) in gradient elution. Unger et al. [11] compared the performance of a 5-μm column with a prototype monolithic phase from the same manufacturer. Both gave approximately equivalent performance but the monolith could be used at very high flow-rate without loss of efficiency. However, the test probes were exclusively neutral compounds.

In the present study, we have compared isocratic separations of weak and strong bases at high flow-rate using a commercial monolithic column and conventional microparticulate phases of 5 μm and sub-5 μm particle size. We measured D_m and its variation with temperature for the test probes in order to aid interpretation of the results. Our aim was to investigate choice of column and conditions to enable fast analysis of bases while maintaining high efficiency for complex separations.

2. Experimental

The HPLC system consisted of P200 pump, UV 100 detector (1 or 5 μl flow cells) operated at 254 nm (Thermo Separation Products, San Jose, USA) and 7725 valve injector with 2 μl loop (Rheodyne, Cotati, USA). Connections were made with minimum lengths of 0.010 cm I.D. tubing. These precautions were taken to minimise extra-column effects. The columns used were Inertsil ODS-3V (5 μm), 25×0.46 cm I.D. and Inertsil ODS-3 (3 μm), 10×0.46 cm I.D., both with surface area 430 m^2 g^{-1}, %C=15, from GL Sciences, Tokyo, Japan; SymmetryShield (3.5 μm), 10×0.46 cm I.D., surface area 340 m^2 g^{-1}, %C=17 (Waters, Milford, USA); Purospher Star RP-18e (3 μm) 10×0.46 cm I.D., surface area 300 m^2 g^{-1}, %C=16.5, Chromolith RP-18e, 10×0.46 cm I.D., mesopore size 13 nm, macropore (''through pore'') size 2 μm, surface area 300 m^2 g^{-1}, (both from Merck, Darmstadt, D). Column efficiency was determined using the Dorsey–Foley equation [12], $N_{df} = 41.7[t_r/w_{0.1}]^2/[A_s + 1.25]$ which has been shown, also by others, to give a reasonable estimate of true efficiency for asymmetric peaks [13]. Berthod advocated the Dorsey–Foley procedure for calculation of the efficiency of such peaks to give meaningful Van Deemter plots [14]. The asymmetry factor (A_s) was calculated at 10% of the peak height from the ratio of the widths of the rear and front sides of the peak; all measurements were made using a model 2000 data station (Trivector, Bedford, UK). All results were the mean of at least duplicate injections. Preparation of buffers was as described previously with pH measured before addition of organic solvent [15]. Temperature control was achieved by immersing the column and injector in a thermostatted water bath. A 3 m×0.5 mm I.D. length of stainless steel tubing connected between the pump and injector and also immersed in the water bath was used to preheat the mobile phase before delivery to the column. Column void volume was measured by injection of uracil. For measurement of D_m, a coiled stainless steel capillary of length 5.29 m and I.D. 0.053 cm, formed into a coil of radius 25 cm, was utilised. The I.D. of the tube was checked by weight difference when full of water and after careful drying.

3. Results and discussion

3.1. General characteristics of columns

Benzene was chosen as a neutral probe to compare general column characteristics. Comparison of column performance is often based on plate height (H). A more universal index of performance is the separation impedance which takes into account pressure drop, allowing comparison of phases of different particle size. Although monolithic silica phases have a desirable low value of the separation impedance, this parameter may not be so useful for ''clad'' monolithic columns; the column we used had a recommended maximum pressure limit of 200 bar, compared with perhaps 300 bar for conventional phases [16,17]. We did not verify the pressure stability of the monolith; reduced pressure stability is likely to be due to risk of deformation of the cladding, since very close contact is necessary with the stationary phase to preserve high efficiency. Using these maximum pressures we could operate the 25 cm 5 μm column at up to 5 ml min^{-1} and the 10 cm sub-5 μm columns at up to 3 ml min^{-1}, using 30–40% acetonitrile–water mixtures and a 5-μl detector cell. The monolith generated a pressure of only 125 bar at 5 ml min^{-1}. These backpressures include the contribution of the hardware (injector, detector cell and connecting tubing) which was about 11 bar using 1 ml min^{-1} of this solvent at 30 °C. Pressure increases when using narrow connecting tubing and low volume detector cells. However, the system dead volume must be small in comparison with the peak volume, given by:

$$4V_m(1+k)/N^{0.5} \quad (1)$$

where V_m is the void volume of the column. Thus system dead volume is critical for peaks of low k (often used in fast analysis) and for short, high efficiency columns. A 5-μl cell together with small diameter tubing, was selected as a compromise between system backpressure and extra column effects. We measured an average loss of efficiency of less than 10% using a 5-μl cell compared with a 1-μl cell for critical peaks of $0<k<1$. The monolith generated only about two-thirds of the back pressure of the 5 μm phase, for columns of equal length operated at the same volumetric flow. However, the pressure advantage of the monolith over the 5 μm column disappears if both are operated at the same linear velocity (see Fig. 1 for flow inter-conversion). All columns gave reasonably symmetrical peaks for benzene ($A_s < 1.5$, see Table 1) although the value for the monolith column ($A_s = 1.43$) was significantly higher than the other phases.

Fig. 1 shows Van Deemter plots for benzene on a number of particulate columns and the monolith. Data shown in Fig. 1, gives the equivalent linear flow velocity for each column which corresponds to a flow-rate of 1 cm^3 min^{-1}, allowing interconversion of flow measurements. The monolith had significantly lower flow velocity (0.13 cm s^{-1}) than the particulate phases (0.18–0.20 cm s^{-1}) at the same volumetric flow (1 cm^3 min^{-1}), presumably because monoliths contain less solid material [6,7]. The Inertsil column, as with most modern 5 μm phases easily achieved reduced plate heights ($h = H/d_p$) for benzene in the range 2–2.5 [18,19]. The sub-5 μm phases gave somewhat higher values of h for this neutral compound, as noted also by others [9] which may be attributed to packing difficulties. In our hands, only the 3.5 μm phase gave values of h for benzene within the range for 5 μm phases although some variation may occur for different neutral compounds. None of the 10 cm columns containing sub-5 μm phases could be operated above 3 ml min^{-1} due to our imposed pressure limitation (300 bar). The monolith gave similar values of H_{min} for benzene to the 5 μm phase, however it showed a very different variation of H with flow to the particulate columns, with flow remaining practically optimum over the range 2–5 ml min^{-1}. In conventional porous particles, most of the adsorptive surface located inside the particles is accessible by slow molecular diffusion through stagnant mobile phase. Due to the construction of the monolith, which contains flow-through pores, the diffusion distance is reduced, resulting in a reduction in mass transfer effects [20]. Thus, at 5 ml min^{-1}, H is almost 3.5 times lower on the monolith than on the 5 μm phase showing a large advantage of the monolith at these high flow-rates. The monolith has clear advantages for the analysis of neutral compounds over the sub-5

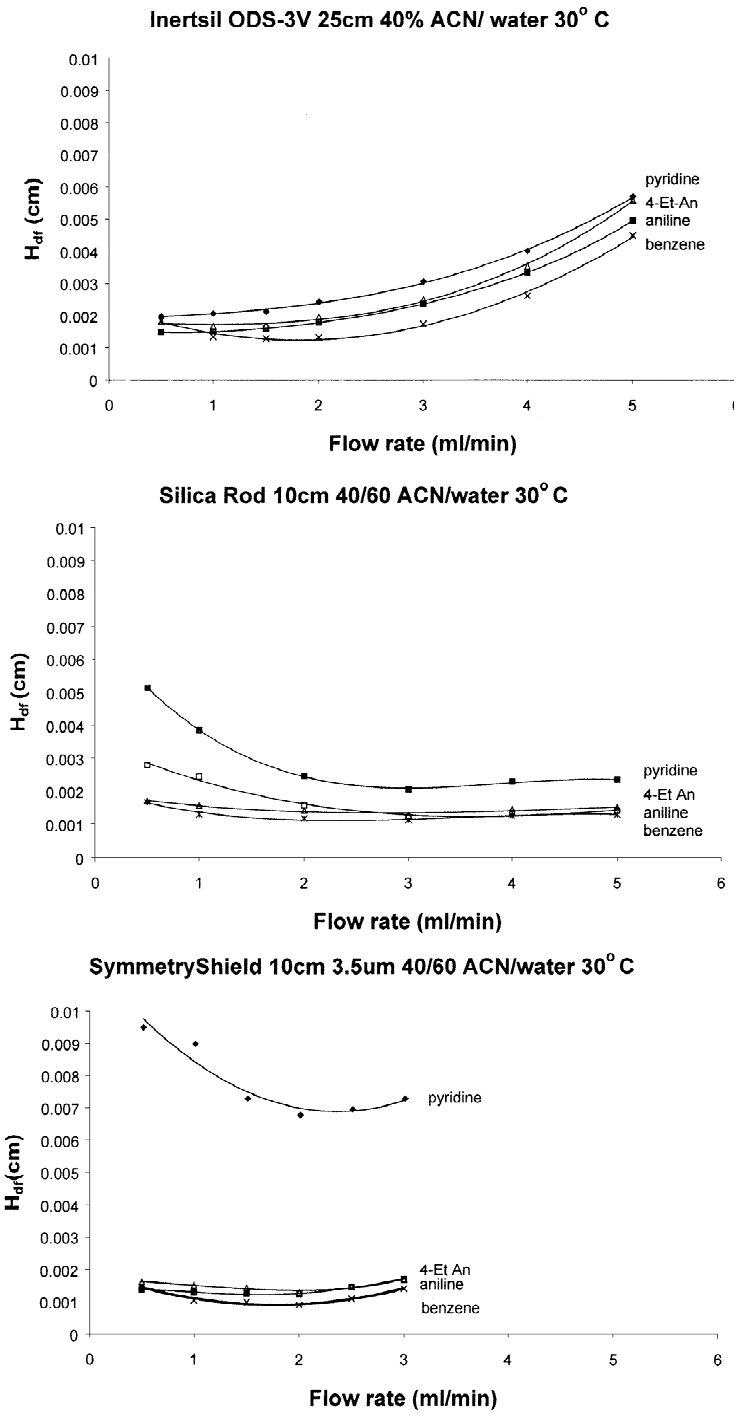

Fig. 1. Van Deemter plots for weak bases and benzene using 5 μm, monolithic and sub-5 μm RP columns, with plate height (H_{df}) calculated from the Dorsey–Foley efficiency. Detection UV at 254 nm. Injection volume 2 μl. Temperature 30 °C. Based on injections of uracil, 1 cm^3 min^{-1} is equivalent to 0.18 cm s^{-1} on Inertsil ODS-3 (5 μm and 3 μm), 0.13 cm s^{-1} on the Chromolith RP-18e column, 0.19 cm s^{-1} on Purospher Star RP-18e, 0.20 cm s^{-1} on SymmetryShield. Relative to Inertsil (taken as 1.0) the linear velocities are thus 0.72 on Chromolith, 1.06 on Purospher and 1.11 on SymmetryShield at fixed volumetric flow.

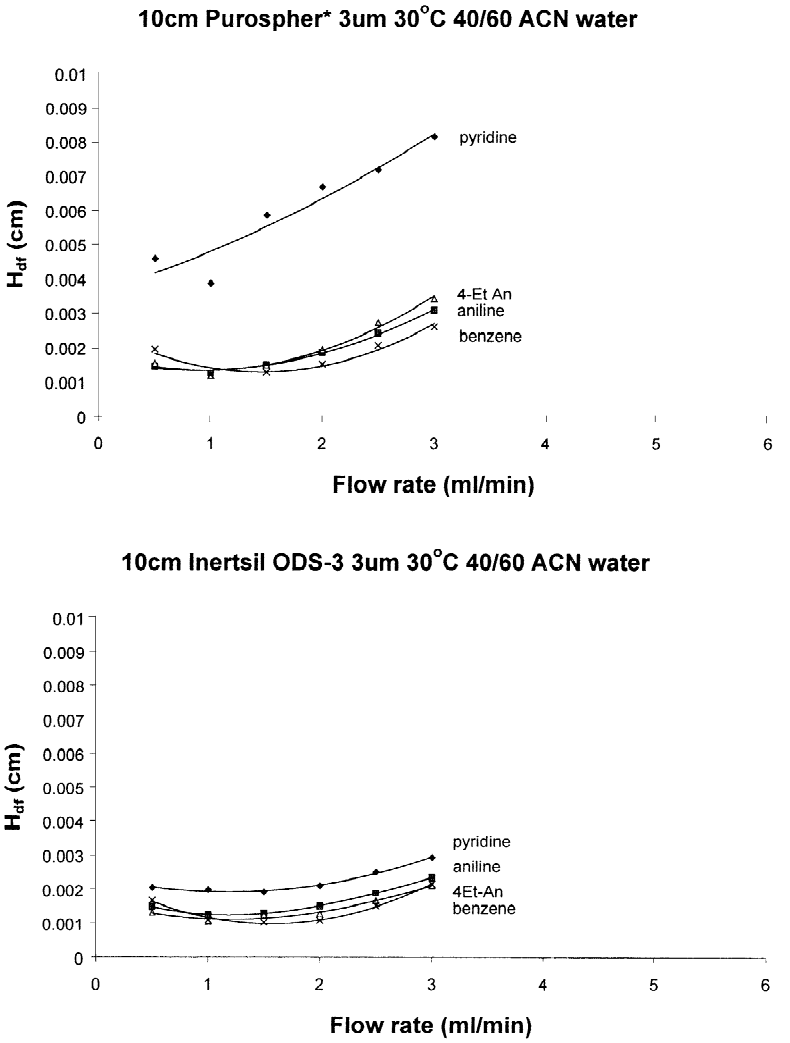

Fig. 1. (*continued*)

μm phases due to the higher back pressures that the latter generate, which restricts their length and thus the number of plates available for fast isocratic separations [16,21]. For this reason, and because the behaviour of very small particle columns for the analysis of bases is likely to parallel the performance

Table 1
Asymmetry factors for weak bases and benzene in unbuffered acetonitrile–water (40:60, v/v) at 2 ml min^{-1}

Phase	Pyridine	Aniline	4-Et Aniline	Benzene
Inertsil ODS-3 (5 μm)	1.34	1.15	1.21	1.06
Chromolith RP-18e	2.11	1.62	1.73	1.43
SymmetryShield (3.5 μm)	2.11	1.09	1.10	1.00
Purospher star RP-18e (3 μm)	2.60	1.34	1.40	1.16
Inertsil ODS-3 (3 μm)	1.43	1.19	1.15	1.06

of equivalent 5 μm phases in their behaviour with bases, these columns were investigated less rigorously, and only tested in unbuffered mobile phases. Nevertheless, short columns of very small particles can be very useful in fast gradient elution separations [10].

3.2. Analysis of weak bases in unbuffered mobile phases

Analysis of weak bases such as aniline and pyridine is possible in unbuffered mobile phases. These low pK_a compounds are likely to be unionised in organic solvent–water mixtures, and thus peak tailing may not be greatly affected by the presence or absence of buffer components, at least on relatively inert phases prepared from high purity silica [22]. In addition, it has been shown that reproducible results can be obtained for weak bases in unbuffered mobile phases as long as sufficient time is allowed for equilibration of the column with the mobile phase [23].

For aniline and 4-Ethylaniline, acceptable peak shapes ($A_s < 1.5$) were obtained on all columns apart from the monolith, which gave somewhat greater tailing ($A_s = 1.62$ and 1.73) for these compounds respectively (see Table 1). The greater asymmetry on the monolith is likely to be due at least partially to the greater asymmetry shown even for benzene (see above). Pyridine clearly presents a more severe challenge for all columns, with only the Inertsil phases yielding $A_s < 1.5$. Despite higher peak tailing on the monolith and the use of the Dorsey–Foley method for calculation of plate height (which takes into account peak A_s—see Section 2), relatively favourable Van Deemter profiles of all the solutes tested in the unbuffered mobile phase were obtained on the monolith (see Fig. 1), compared with the conventional phases. The flat Van Deemter profiles and the low back pressures generated are clear advantages of the monolith for the separations of these compounds. The Van Deemter plots obtained for weak bases, which are likely to be unionised in the mobile phase, are broadly comparable with those obtained by other workers for neutral compounds, despite some increased tailing of these compounds [11]. The separation of the mixture on the monolith at relatively high flow-rate is shown in Fig. 2.

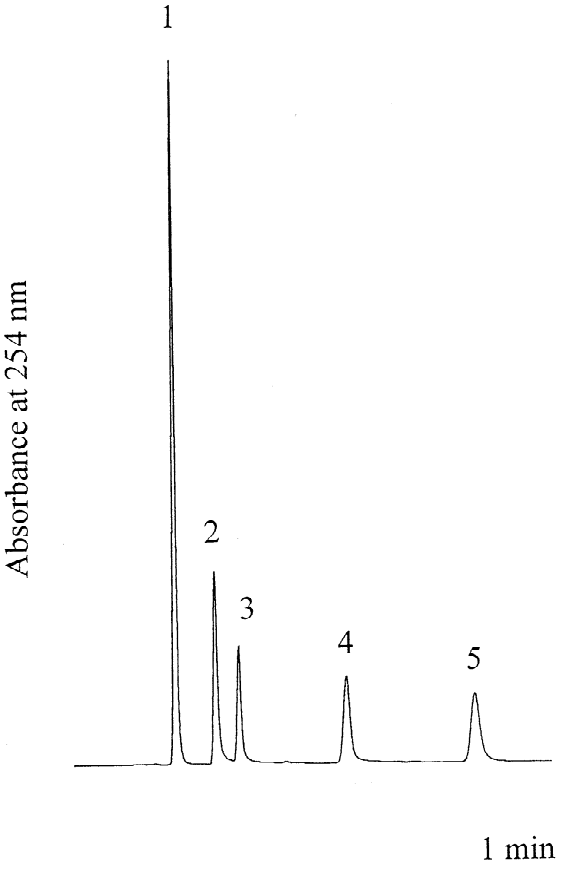

Fig. 2. Rapid analysis of weak bases and benzene using a monolithic silica RP column. Mobile phase acetonitrile–water (40:60, v/v). Flow-rate 5 cm^3 min^{-1} (corresponding to 0.65 cm s^{-1}). Other conditions as Fig. 1. Peak identities: 1 = uracil, 2 = pyridine, 3 = aniline, 4 = 4-Ethylaniline, 5 = benzene.

3.3. Analysis of stronger bases in buffered mobile phases

Weak bases can give peak shape problems, however, ionised strong bases can give additional detrimental interactions in RP separations. Whereas most bases are analysed in buffered mobile phases, there is no necessary connection with performance in unbuffered solvents [15,18,19]. Tests in unbuffered mobile phases of low pK_a compounds may indicate the hydrogen bonding ability of a phase, whereas for stronger bases, ion-exchange interactions with dissociated silanol groups are likely to contribute to peak tailing. Thus, we have shown that good peak shape of pyridine in unbuffered mobile phases does not

inevitably indicate good peak shape for stronger bases analysed in buffered mobile phases. For example, the high value of H and A_s for pyridine on SymmetryShield is an anomaly on this phase, which otherwise gives generally very good peak shapes for many strong bases, as reported previously [15]. Studies in mobile phases buffered at different pH are also important, using a variety of test compounds, since the performance of a given phase relative to others may differ at different pH and with different test compounds [15,18,19]. Previously, we demonstrated improved peak shape for strong bases at elevated temperatures, using constant flow [5] and decided to look further at the combined effects of flow and temperature. The stronger bases quinine (pK_a 8.5) and nortriptyline (pK_a 10) were included in the tests. Pyridine (pK_a 5.2) was retained to compare its behaviour in a buffered mobile phase (it should be at least partially protonated in acidic buffer/organic modifier), and benzene was kept as a neutral control. A chemometric study has indicated that pyridine, quinine and nortriptyline may probe a reasonable range of the activity of RP columns to different bases [24].

Fig. 3a shows van Deemter curves for the analysis of these compounds on the 5 μm phase, with an acidic phosphate buffer/acetonitrile mobile phase over a range of temperatures from 20 to 60 °C. It is worth considering briefly the variation in H for the neutral compound with temperature and flow. For convenience, benzene data are abstracted from Fig. 3a and shown in Fig. 4. We showed previously that on the same phase, (Inertsil ODS-3), efficiency at constant flow (1 ml min^{-1}) for benzene hardly varied with temperature [5], although elevated temperatures are expected to increase efficiency [25]. Clearly, our previous results were attributable to movement of u_{opt} from about 1 ml min^{-1} at 20 °C to 2.5 ml min^{-1} at 60 °C, due to changes in D_m with temperature. These results show similarities with those obtained for neutral compounds with capillary LC columns by others [26] and discussed by Snyder et al. [25].

Fig. 3a indicates that u_{opt} is lower for all bases than for benzene, giving rise to poorer efficiency for these compounds when analysed at usual flow-rates; a lower value of u_{min} may be indicative of a larger C term. H_{min} is not reached for pyridine, quinine or nortriptyline even at 0.2 ml min^{-1} at 20 °C. Also, the curve for quinine at high flow is considerably steeper than for benzene.

Table 2 shows increased A_s for the stronger bases on the 5 μm phase, and slightly increased tailing of the weak base pyridine, which at pH 3 should be partially protonated and thus may experience detrimental ionic interactions.

We considered that the differing shape of the Van Deemter curves for individual bases might at least partially be due to reduced solute mobile phase diffusion (D_m) of the larger analytes quinine and nortriptyline [5]. Flatter Van Deemter curves for all solutes at higher temperature should be obtained due to increases in D_m with temperature [27]. D_m can be approximated by the use of various equations [21], however we instead measured values using the Taylor–Aris procedure as described by Li and Carr (see Appendix A, [27]). Values for D_m are shown in Table 3; there appears to be no significant effect of pH on the results, although we felt initially that different protonation and hydration of species at different pH might have some influence. The larger molecules quinine and nortriptyline show diffusion coefficients about half those of the smaller pair of molecules benzene and pyridine. Slower diffusion should contribute to higher mobile phase mass transfer terms resulting in lower optimum flow-rates and steeper curves at high flow-rate for these compounds. We estimated the effects of these values of D_m on plate height vs. flow curves by use of an empirical equation where the coefficients of the Van Deemter equation are approximated by fitting values from a large body of experimental data of different (mostly neutral and weakly acidic) solutes in different mobile phases, with various retention factors, [21]:

$$H = 1.5d_p + D_m/u + 0.167u(d_p^2)/D_m \quad (4)$$

This equation is approximate, and neglects the effect of k on the B and C terms. Fig. 5 shows the predicted curves based on values of D_m in Table 3. Linear velocity and volumetric flow-rate for the column were interconverted using uracil t_0 measurements. Comparison with the actual results (Fig. 3a) shows that pyridine and benzene behave much more differently than would be expected from their similar

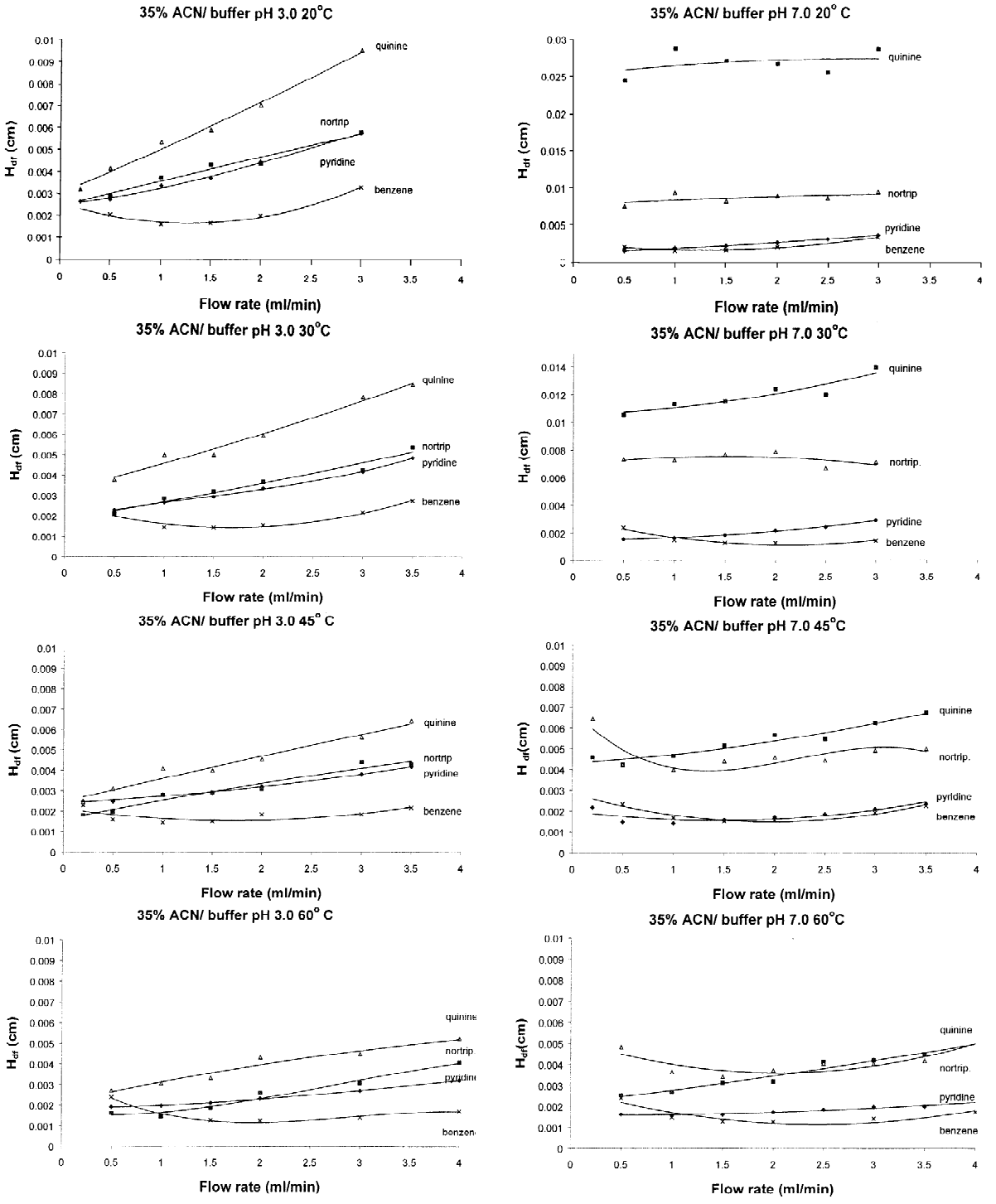

Fig. 3. (a) Temperature variation of Van Deemter plots including stronger basic solutes using Inertsil ODS-3 (5 μm). Mobile phase acetonitrile–0.083 *M* phosphate buffer pH 3.0 (35:65, v/v). (b) Mobile phase acetonitrile–0.083 *M* phosphate buffer pH 7.0 (35:65, v/v). 1 cm^3 min^{-1} corresponds to a linear flow velocity of 0.18 cm s^{-1}.

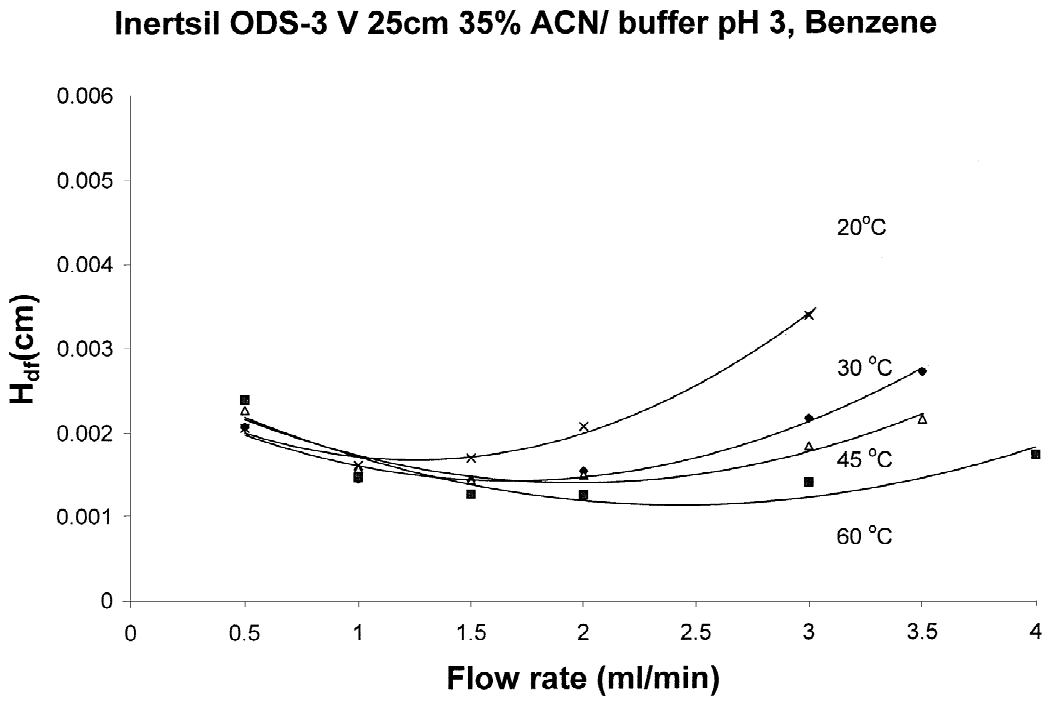

Fig. 4. Temperature variation of Van Deemter plots for benzene. Data abstracted from Fig. 3(a), 1 cm^3 min^{-1} corresponds to a linear flow velocity of 0.18 cm s^{-1}.

D_m values, and the same is true for nortriptyline and quinine. Both pyridine and quinine give significantly higher values of H than predicted from D_m values, which can be attributed to detrimental stationary phase interactions. The higher B term shown in the actual results for benzene could be due to surface diffusion (longitudinal diffusion of the solute in the stationary phase along the walls of the pores) [25,28]. In spite of differences between actual and predicted values for individual compounds, the general improvement in the Van Deemter curves by raising the temperature from 30 to 60 °C using pH 3 buffer is roughly in line with that predicted by changes in D_m. Clearly, at pH 3, a higher D_m value leads to higher plate count at high flow-rate, in line with theory.

Fig. 3b shows similar results obtained using pH 7 buffer. Pyridine is unprotonated at pH 7 and now shows behaviour more similar to benzene, in line with that predicted by D_m values. However, the high plate height of quinine and nortriptyline at 20–30 °C, and the very large improvements in performance after raising the temperature to 60 °C, are not predictable based on D_m. As shown previously [5], the reduction in plate height at elevated temperature can be at least partially attributed to significant reduc-

Table 2
Asymmetry factors for weak and strong bases, and benzene in buffered mobile phase. For Inertsil ODS-3 mobile phase acetonitrile–0.0833 *M* phosphate buffer pH 3.0 (35:65, v/v), 2 ml min^{-1}, 30 °C. For Chromolith RP-18e, mobile phase acetonitrile–0.077 *M* phosphate buffer pH 3.0 (30:70, v/v), 2 ml min^{-1}, 30 °C

Phase	Pyridine	Quinine	Nortriptyline	Benzene
Inertsil ODS-3 (5 μm)	1.50	1.61	1.37	1.04
Chromolith RP-18e	2.19	2.34	1.85	1.36

Table 3
Diffusion coefficients ($\times 10^{-5}$ cm^2 s^{-1}) in (a) acetonitrile–0.0833 *M* phosphate buffer pH 3.0 (35:65, v/v) and (b) acetonitrile–0.0833 *M* phosphate buffer pH 7.0 (35:65, v/v) at various flow-rates and temperatures

Substance	Temperature (°C)		Flow rate (ml min^{-1})			
			0.1	0.15	0.2	0.3
Benzene	a	30	1.2	–	1.2	1.3
		60	2.2	–	–	–
	b	30	1.3	–	–	–
		60	2.2	–	–	–
Quinine	a	30	0.47	0.50	0.54	0.74
		60	0.94	–	–	–
	b	30	0.48	–	–	–
		60	0.85	–	–	–
Pyridine	a	30	1.1	1.1	–	–
		60	2.1	–	–	–
	b	30	1.2	–	–	–
		60	2.2	–	2.2	–
Nortriptyline	a	30	0.54	–	–	–
		60	1.0	–	–	–
	b	30	0.50	–	–	–
		60	0.93	0.95	1.0	–

tions in peak asymmetry of bases at pH 7 when the temperature is raised. The improvement with temperature is so great that at 60 °C almost identical results in terms of plate height are obtained for pH 7 buffer and pH 3 buffer, whereas at 20 °C, pH 3 gives greatly superior results for the strong bases. It is clear that interaction with silanols is the major influence on *H* at pH 7. It is likely that the pK_a of both analytes and silanols changes as a function of temperature, and that these changes contribute to the observed behaviour [5]. Further work including experimental measurement of these pK_a changes with temperature is necessary to aid interpretation of the results.

Guiochon et al. have suggested that in linear chromatography (i.e. where sample overload is not occurring) that tailing may be due to slow kinetics of ''strong'' sites together with fast kinetics of a non-selective surface [29,30]. Increasing the temperature may increase the kinetics of the slow sites to approach that of the fast sites, giving an improvement in peak shape for the strong bases. However, the Van Deemter curves of strong bases might be expected to be much steeper at values of u above u_{opt} than is indicated by our results, if slow kinetics was the main reason for poor peak shape.

The monolith gave higher asymmetry factors for the bases using an acidic mobile phase than shown by the 5 μm column (see Table 2). However, at 30 °C the Van Deemter plot (Fig. 6) stills shows advantages at high flow-rate over the 5 μm phase when operated at a similar temperature, even when the Dorsey–Foley equation was used to calculate *H*. We investigated if elevated temperature might give similar improvements with the monolith as with the conventional phases. However, these experiments were limited due to (the manufacturer's recommended) maximum operating temperature of 45 °C for the monolith; the restriction is likely to be due to the risk of deformation of the organic polymer cladding. Formation of voids between cladding and stationary phase could cause serious deterioration in performance. Such problems do not occur with conventional columns, although the chemical stability of the phase may be an issue with both types of material. At 45 °C, the performance of the monolith was hardly different from that at 30 °C. This is only a small temperature range, however if diffusion distances are low in monolithic phases giving rise to small mass transfer terms, then increase in D_m might not be expected to give the same overall proportional improvement in performance as with conventional phases.

The monolith column gave more significant peak

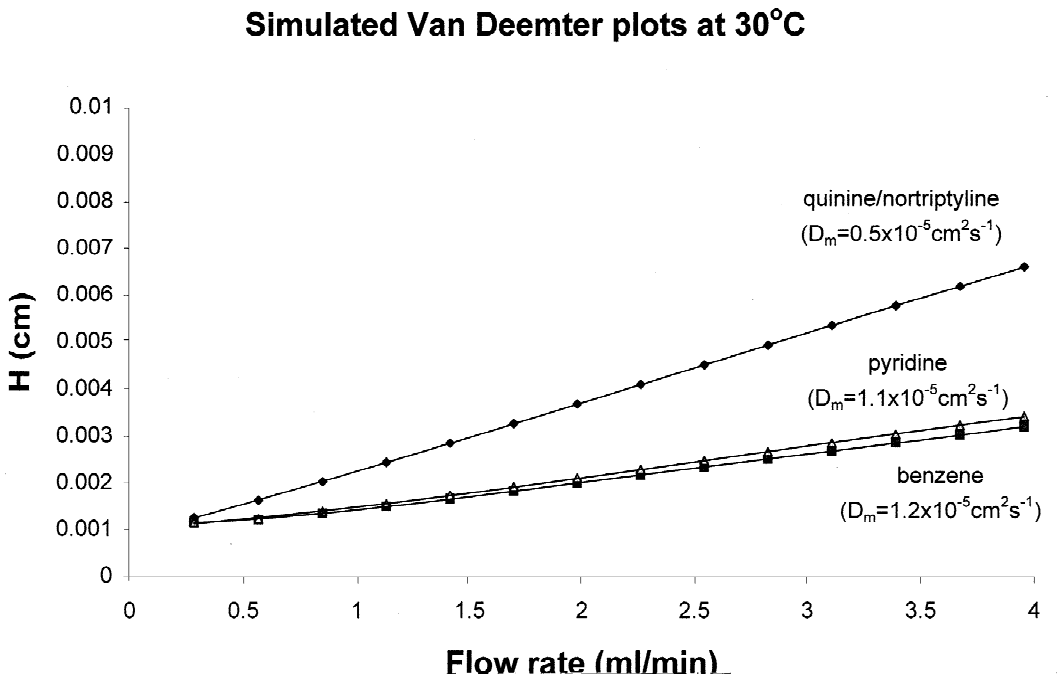

Fig. 5. Van Deemter plots simulated using Eq. (4). Upper plot: using $D_m = 0.5 \times 10^{-5}$ cm^2 s^{-1} for quinine/nortriptyline, pyridine = 1.1×10^{-5} cm^2 s^{-1}, benzene = 1.2×10^{-5} cm^2 s^{-1}. Lower plot: using $D_m = 0.95 \times 10^{-5}$ cm^2 s^{-1} for quinine/nortriptyline, 2.2×10^{-5} cm^2 s^{-1} for pyridine/benzene.

advantages for neutral solutes are apparent even over sub-5 μm conventional columns, if high plate counts are required in isocratic separations, due to the high back pressures generated by these very small particle columns. The advantages of the monolithic columns tested are somewhat less for basic compounds. For weak bases, and strong bases analysed under acidic conditions at room temperature, plate heights at high flow-rate can still be lower than for conventional columns, despite increased tailing on the monolith. However, much poorer peak shapes were obtained for strong bases using the monolith at pH 7: it is uncertain whether this may be a consequence of the different construction of monoliths or merely problems of high silica ''activity'' observed also with many conventional columns.

Plate count at high flow-rate can be improved considerably on conventional columns by using temperatures above ambient; some improvement can be attributed to increase in D_m with temperature, although at pH 7 the effect of higher temperature on silanol interactions appears to be a significant cause of this improvement.

The long-term stability of all the phases at elevated temperature is an important issue which has not been investigated, although no significant deterioration was noted over the relatively short period of use in the present study [31].

Appendix A. Measurement of D_m

In the Taylor–Aris method, solute is injected into a capillary tube and the bandspreading measured. Axial (longitudinal) diffusion of solutes is small and can be ignored; thus the width of the band is controlled by the radial diffusion of the solutes in the tube against the parabolic flow profile [27]. The diffusion coefficient is calculated from the equation:

$$(\sigma_t)^2 = (d_t)^2 / 96\, D_m t_o \quad (2)$$

where σ_t is the peak standard deviation (in time units), t_o is the time taken to pass through the tube and d_t is the inner diameter of the tubing. The higher the value of D_m, the narrower the peak. The effects of secondary flow can be ignored if $F < F_{\text{trans}}$, where

$$F_{\text{trans}} = (518\, R_{\text{coil}} t_o D_m \eta/\rho)^{0.5} \quad (3)$$

and F is the volumetric flow-rate, F_{trans} is the transitional flow, R_{coil} is the radius of the coil, η and ρ are the viscosity and density of the solvent respectively. We used a wide bore capillary tube and a water bath to thermostat the coil, which allowed use of a coil radius sufficiently large to ensure a transitional velocity within range of the operation of the HPLC pump. Clearly, the transitional velocity is proportional to the square root of D_m for a given mobile phase, and thus lower flow-rates should be required for measurement of low values of D_m. For a coil of dimensions as specified in Section 2, Eq. (3) predicts a value of the transitional flow for a compound with $D_m = 0.5 \times 10^{-5}$ cm^2 s^{-1} of approximately 0.22 and 0.16 ml min^{-1} at 30 °C in pure water and pure acetonitrile respectively, and 0.34 and 0.26 ml min^{-1} for a compound with $D_m = 1.2 \times 10^{-5}$ cm^2 s^{-1}. D_m values measured at 30 and 60 °C in acetonitrile–phosphate buffer pH 3 and acetonitrile–phosphate buffer pH 7 at 0.1 ml min^{-1} are shown in Table 3. In some cases, D_m was measured at higher flow-rate in order to determine whether significant variation occurred with flow, which would be indicative that the critical flow had been exceeded. Benzene has a relatively high D_m at 30 °C (1.2×10^{-5} cm^2 s^{-1}) and the calculated value hardly varies over the range 0.1–0.3 ml min^{-1} at pH 3. Using the same flow range however, D_m for quinine (with relatively low D_m) apparently increases from 0.47 to 0.74 cm^2 s^{-1}, indicative of secondary flow effects. It appears that 0.1 ml min^{-1} is suitable for all four compounds. Increasing the temperature from 30 to 60 °C gives an approximate doubling of the value of D_m; this increase is attributable both to increased molecular motion and reduction in the viscosity of the mobile phase. No significant differences were obtained using a pH 7 buffer, within experimental error. Only small differences were found in the values of D_m when measured using PEEK instead of stainless steel tubing, which indicates that interaction of the compounds with the tube surface was probably not a significant source of error in the measurements.

References

[1] M.A. Stadalius, J.S. Berus, L.R. Snyder, LC–GC 6 (1988) 494.

[2] S.D. Rogers, J.G. Dorsey, J. Chromatogr. A 892 (2000) 57.
[3] D.V. McCalley, LC–GC 17 (1999) 440.
[4] J. Nawrocki, J. Chromatogr. A 656 (1997) 29.
[5] D.V. McCalley, J. Chromatogr. A 902 (2000) 311.
[6] N. Ishizuka, H. Minakuchi, K. Nakanishi, N. Soga, N. Tanaka, J. Chromatogr. A 797 (1998) 133.
[7] H. Minakuchi, N. Ishizuka, K. Nakanishi, N. Soga, N. Tanaka, J. Chromatogr. A 828 (1998) 83.
[8] R.J. Vervoort, A.J. Debets, H.A. Claessens, C.A. Cramers, G.J. de Jong, J. Chromatogr. A 897 (2000) 1.
[9] J.J. Kirkland, J. Chromatogr. Sci. 38 (2000) 535.
[10] U.D. Neue, J.L. Carmody, Y.F. Cheng, Z. Lu, C.H. Phoebe, T.E. Wheat, in: Advances in Chromatography, Vol. 41, Marcel Dekker, New York, 2001, p. 93.
[11] B. Bidlingmaier, K.K. Unger, N. von Doehren, J. Chromatogr. A 832 (1999) 11.
[12] J.P. Foley, J.G. Dorsey, Anal. Chem. 55 (1983) 730.
[13] W.W. Yau, S.W. Rementer, J.M. Boyajian, J.J. de Stefano, J.F. Graff, K.B. Lim, J.J. Kirkland, J. Chromatogr. 630 (1993) 69.
[14] A. Berthod, J. Liq. Chromatogr. 12 (1989) 1187.
[15] D.V. McCalley, J. Chromatogr. A 844 (1999) 23.
[16] U.D. Neue, in: R.A. Meyers (Ed.), Encylopedia of Analytical Chemistry, Wiley, Chichester, UK, 2000, p. 11450.
[17] D. Lubda, Merck, personal communication.
[18] D.V. McCalley, J. Chromatogr. A 738 (1996) 169.
[19] D.V. McCalley, J. Chomatogr. A 769 (1997) 169.
[20] T.B. Tennikova, R. Freitag, J. High Resolut. Chromatogr. 23 (2000) 27.
[21] U.D. Neue, HPLC Columns, Wiley–VCH, New York, 1997.
[22] D.V. McCalley, J. Chromatogr. A 664 (1994) 139.
[23] M. Kele, G. Guiochon, J. Chromatogr. A 869 (2000) 181.
[24] R.G. Brereton, D.V. McCalley, Analyst 123 (1998) 1175.
[25] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, Wiley, New York, 1997.
[26] J.S. Yoo, J.T. Watson, V.L. McGuffin, J. Microcol. Sep. 4 (1992) 349.
[27] J. Li, P.W. Carr, Anal. Chem. 69 (1997) 2530.
[28] R.W. Stout, J.J. Destefano, L.R. Snyder, J. Chromatogr. 282 (1983) 263.
[29] T. Fornstedt, G. Zhong, G. Guiochon, J. Chromatogr. A 741 (1996) 1.
[30] G. Gotmar, T. Fornstedt, G. Guiochon, J. Chromatogr. A 831 (1999) 17.
[31] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.