STUDY OF EFFICIENCY ON PACKED COLUMN SFC WITH DIFFERENT PARTICLE DIAMETERS

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I INTRODUCTION

The recent use of small particle diameter allows high performance liquid chromatography (HPLC) to achieve high efficiency for separation. However, due to the high viscosities of liquid, it oblige chromatographers to work with speciall high pressure resistant pumps (UPLC), to reach the pressures (around 1000 bars) required for maintaining classical flow rates (1 ml/min).

On the other hand, these particles allows to work with smaller column lenght (5 cm), by keeping the efficiency constant in regards to classical lenght (25 cm). In such conditions, because of the column performances, flow rates can be imrpoved in HPLC up to 5 ml/min. Consequently, analysis duration is divided by 25.

The measurement of efficiency by the theoretical plate number (N) becames not suited to compare the system performances, when both particle diameter, column length and maximun pressure change. Poppe then Desmet suggested to measure the efficiency expressed by time unit, at the maximum system flow rate allowed by the pressure limit of the pump used for different column length, by plotting $t_0.N^2$ vs N (in decroisssant order). Obviously, the N variations plotted on the x axe are due to the column lenght changes.

The two parameters used, N and t_0, are calculated from two equations, in which are included :
- the pressure maximum of the system (ΔP),
- the colum permeability (Kw), the linear speed of the mobile phase (u_0),
- the mobile phase viscosity (η),
- and for N, H the theoretical plate equivalent height

$$t_0 = \frac{K_{w.}\Delta P}{\eta . u_0^2}$$

$$N = \frac{K_{w.}\Delta P}{\eta . u_0^2 . H}$$

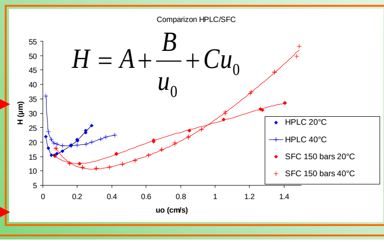
Calculation are performed because, for low flow rates (0.1 ml/min) , the column lenght that shoud be used to reach the system pressure limit in UPLC will be very high (250 cm), that could not be experimentally made.

However, it is well known that in SFC, the fluid viscosity η depend of the fluid compressibility, that hinders the calculation of t_0 and N by the previous equations, which suppose that η is constant (it is true for liquids). Cosequently, we used another type of plot N/t_0 vs u_0, by working with a column length constant. The limit linear speed will be reached for pressure of 400 bars with the Berger system we used (Minigram). The column length were 25 cm; 15 and 10 cm wth particles of 5; 3 and 2.2 micro-meters. The three columns were packed with strategy silicas (Interchim; Montluçon, France).

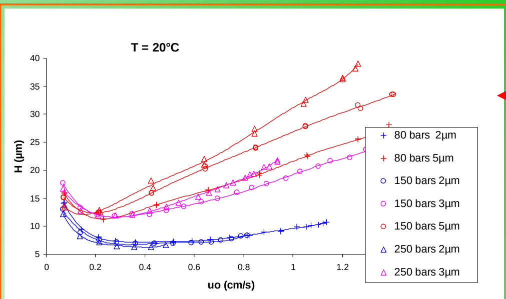
PRELIMINARY STUDIES

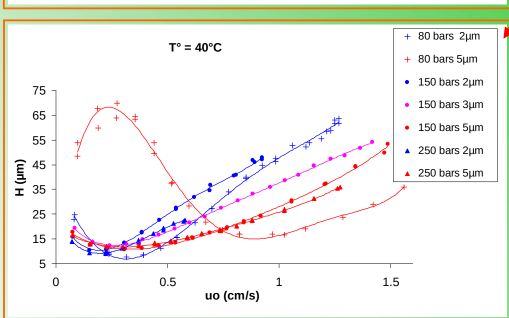
Differents points were studied before efficiency measurements. Hexyldecanebenzene was selected because it displays a retention factor around 3, allowing to minimize the extra void volume effects on the efficiency measurements, the methanol percentage was equal to 5% (modifier) to keep the retention factor around 3, the wavelenght was 210 nm with a respons time equal to 0.2 s, the compound concentration was 1000 ppm. Among different dilution solvents tested , the mixture MeOH-ACN-CH_2CH_2 was choosen to prevent loss of effciciency during injection.

First experiments were performed to check the system both in HPLC and SFC to assess its relability, then to study some classical parameters such as particle diameter, temperature and outlet pressure, by plotting H vc u.



Column : Strategy ODS 2 (5 microns, 25 cm) (Interchim)Mobile phase (HPLC) : MeOH/H_20 75/25 (v/v). Results are in agreement with known behaviors with liquid and supercritical fluids. The linear speed optimum is shifted in SFC toward higher flow rate. Temperature increase reduces the C term.





Columns : Strategy ODS 3 (3 microns; 15 cm)), Strategy ODS 2 (2.2 microns; 10 cm).

Van Deempter curves are classical. At 20°C, the particle diameter decrease induces both the A term (which decrease the minimum H value), and the C term, that leads to a flatter curve at high flow rates.

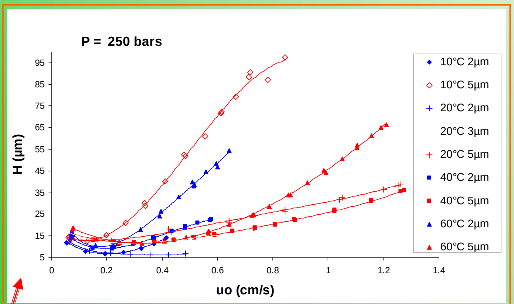
The effect of outlet pressure seems reduced from 5 to 2.2 microns particles. With 5 microns particles, the pressure increase defavors efficiency, probably by reducing the diffusion coefficient in the mobile phase D_m. With 2.2 microns particules, the importance of the diffusion coefficient seems became minor.

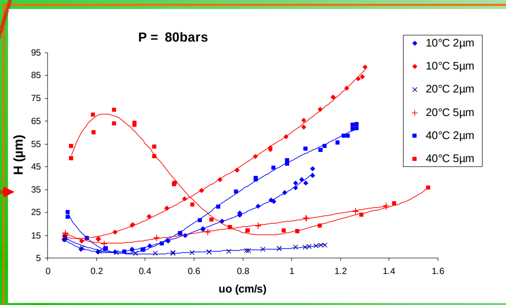
At 40°C and 80 bars, the low density of the mobile phase leads to a loss in efficiency with the 5 micron particles, up to 0.8 cm/s. The pressure change effect act in the same way.

With 5 micron particles, whatever the pressure, 8 and 25 MPa, the increase in temperature (between 10 and 20°C) strongly favors efficiency, wheras this positive effect is lower between 20 and 40°C. However, between 40 and 60°C (at 25 MPa), it leads to loss ef effciciency for the higher flow rates.

With 2.2 micron particles, the increase in temperature from 10 to 20 °C also favors efficiency at high flow rates (flat curves). However, the temperature increase from 20 to 40 or 60°C reduces efficiency for high rates. Because the optima values seems unchanged, the effect of temperature only occurs on the C term. Because this effect seems greater at 25 MPa than at 8 MPa it also seems be not depends on the diffusion coefficient in the mobile phase.

In conclusion, greater efficiencies are reached by using 2.2 micron particles at 20°C, whatever the pressure. However, for lower or higher temperatures, the use of 2.2 micron particles do not favor efficiency for the flow rates higher than 4 ml/min.

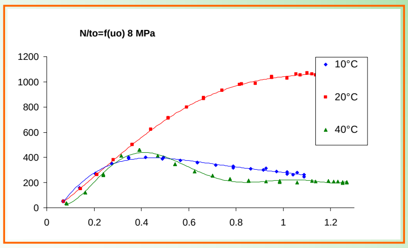


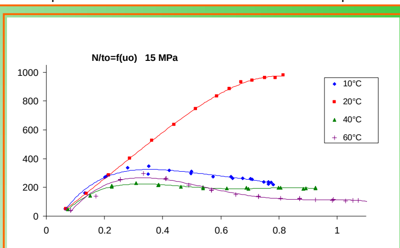


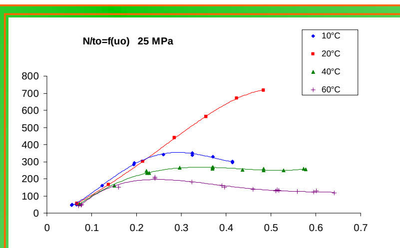
COMPARISON OF CHROMATOGRAPHIC SYSTEMS HPLC vs SFC

Instead curves $t_0/N^2 = f(N)$ used in UPLC, we plotted curves N/t_0 vs u_0 for the 2.2 micron particles, at three pressure. As expected from previous results ($H = f(u_0)$), better results are obtained at 20°C. Values are ranged from 750 at 25 MPa to 1100 at 8 MPa. These values are in the same range order than the one obtained in UPLC (1) at the optimum of the performances of these liquid systems. The optima are reached for higher flow rates, despite the slight decrease in theoretical plate number induced by the flow rate increase (see shema $H = f(u_0)$. Besicly, the reduction of t_0 due to the flow rate increase explains the great increase of N/t_0 at 20°C.

For other temperatures, optima are reached for linear speed around 0.4 ml/min, that correspond to a flow rate of 3 ml/min. Lower is the pressure are higher are the values.







CONCLUSIONS

These first studies show that efficiency variations in SFC on packed columns are not identical to the one observed in HPLC. The increase in temperature do not provide efficicient improvement whatever the particle diameter. The 5 micron particles seem (in the studied conditions, 5% of methnol) well suited for analyses at temperaturearound 40/60°, whereas the 2.2 micron particles display greater performances at 20°C. The use of small particle diameter would offer very high performances, such as it does in HPLC. At the optimum conditions, the theoretical plate number reached is equal to 240.00 by meter of column, with particles of 2.2 microns, and 92000 with particles of 5 microns. At optimum H values, which do not obviously correspond to maxima for N/t_0, the reduced equivalent plate height (h) are closed for the two particle sizes, h = 2.8 with 2.2 microns (20°C; 25 MPa) and h = 2.5 with 5 microns particles (40°C; 15 MPa), but the pressure drop are stongly lower than the one in HPLC, 95 bar for 2.2 microns, and 20 bar for 5 microns particles. Obviously, such values warrant the particle mecanic stability and a long column duration by usinf supercritical fluids.

(1) D.T.T Nguyen, D. Guilarme, S. Rusdaz, J.L. Veuthey, J. Chromatogr. A 1128 (2006) 105