Journal of Chromatography, 637 (1993) 213–218
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 995

Short Communication

Mass transfer in open-tubular supercritical fluid chromatography

Karel Janák*, Agneta Bemgård and Anders Colmsjö

Department of Analytical Chemistry, National Institute of Occupational Health, S-171 84 Solna (Sweden)

Ingela Hägglund and Lars G. Blomberg

Department of Analytical Chemistry, University of Stockholm, Arrhenius Laboratory, S-106 91 Stockholm (Sweden)

(First received October 28th, 1992; revised manuscript received February 8th, 1993)

ABSTRACT

Solute band broadening in 50 μm I.D. open tubular columns coated with PS-264 stationary phase has been evaluated in supercritical fluid chromatography. An improved method for on-column measurements of stationary phase swelling was used. For the conditions evaluated, a significant contribution to the total solute band broadening originating from stationary phase interactions was found. Possible sources of solute band broadening in the stationary phase are discussed.

INTRODUCTION

Recently, increasing attention has been paid to physicochemical interactions in a solute–supercritical fluid–stationary phase system. Different methods have been used to study the thermodynamics of phase-transfer processes, however the application of mass spectrometric tracer pulse chromatography seems to have been particularly fruitful [1–3]. Gibbs adsorption isotherms of carbon dioxide in a stationary phase as a function of pressure or density have thus been measured in different systems [3]. The swelling of the stationary phase as a result of the interaction with supercritical fluids is of particular interest in connection with the chromatographic process. Swelling was first studied in wide-bore columns by Springston *et al.* [4]. Shim and Johnston [5] correlated the swelling of a silicone rubber in supercritical carbon dioxide with the activity of carbon dioxide. It was thereby possible to predict the solute distribution between a polymer and a fluid phase. Further, these authors pointed out the influence of swelling on the accurate estimation of solute distribution coefficients by means of elution supercritical fluid chromatography (SFC) [6].

Unfortunately, understanding of the kinetics of these processes is less developed. The validity of the Golay equation [7] for evaluation of solute band broadening in open tubular columns under

---

* Corresponding author. On leave from Institute of Analytical Chemistry, Czech Academy of Sciences, 611 42 Brno, Czech Republic.

SFC conditions is commonly accepted. However, the effects of supercritical fluid–stationary phase interactions ought to be considered as well. Possible effects of the swollen stationary phase on mass transfer were discussed but not evaluated in two papers in which a procedure for the measurement of swollen stationary phase film thickness was presented [4,8].

In this work solute band broadening in open tubular columns (OTCs) coated with PS-264 stationary phase has been evaluated in SFC. Using previously measured values for solute diffusion coefficients in supercritical carbon dioxide [9], solute band broadening originating from mobile phase diffusion has been directly estimated. Keeping extra-column effects at almost zero level by appropriate design of set-up [9], the remainder of the solute band broadening (contribution of mobile phase being subtracted) relates to the effects of mass transfer in the stationary phase. Corresponding effective diffusion coefficients of solutes were calculated. Possible contributions to solute band broadening in the stationary phase are discussed.

EXPERIMENTAL

Column preparation

A fused-silica capillary was silylated with methylhydro (3–4%) dimethyl (96–97%) polysiloxane, PS-124.5 (ABCR, Karlsruhe, Germany), using 1% solution in pentane. The dynamically coated capillary was heated at a rate of 5°C/min to 300°C and maintained at this temperature for 4 h. After rinsing with 0.5 ml of pentane and 0.5 ml of dichloromethane, the capillary was statically coated with PS-264 stationary phase (Fluka, Buchs, Switzerland) from a solution in pentane and dichloromethane (1:1) corresponding to film thickness $(d_f) = 0.5$ μm. Dicumylperoxide was added to the coating solution in the amount corresponding to 1% of stationary phase weight as a radical initiator of stationary phase cross-linking. Stationary phase immobilization was achieved by heating the column with sealed ends from 40 to 175°C at a rate of 5°C/min and keeping the temperature at 175°C for 20 min. The column was tested in GC by injection of a mixture of C_{12}–C_{17} *n*-alkanes and biphenyl and then washed with 1 ml of pentane and 1 ml of dichloromethane and retested in the same way. The decrease in k' values after rinsing corresponded to a reduction in d_f from 0.5 to 0.44 μm.

Instrumentation

Solute band broadening was measured on an apparatus slightly modified from that described for measurements of diffusion coefficients in supercritical carbon dioxide [9] (Fig. 1). A Suprex SFC/200A supercritical fluid chromatograph (Pittsburgh, PA, USA) was equipped with a pneumatically actuated Valco injection valve Model C14W (Valco Instruments, Houston, TX, USA) with a 0.06-μl internal loop. A fused-silica column, 6.67 m × 50 μm I.D., coated with methylpolysiloxane PS-264, $d_f = 0.44$ μm, was connected to the injection valve via a flow splitter, the other end being connected to a variable-flow restrictor 0.40 m × 9 μm (R_1) via a low-dead-volume butt connector, MVSU 004 (SGE, Austin, TX, USA). A back-pressure μLC-500 micropump (Isco, Lincoln, NE, USA) controlling mobile phase velocity by adjustment of the pressure drop over the variable-flow restrictor was connected to this restrictor and a restrictor, 0.35 m × 9 μm (R_2), ending in the jet of the flame ionization detector via a low-dead-volume ZT.5 Valco T-piece. As was proven in a previous paper [9], this apparatus exhibited no measurable extra-column effects. The chromatograph was connected to an ELDS 900 laboratory data

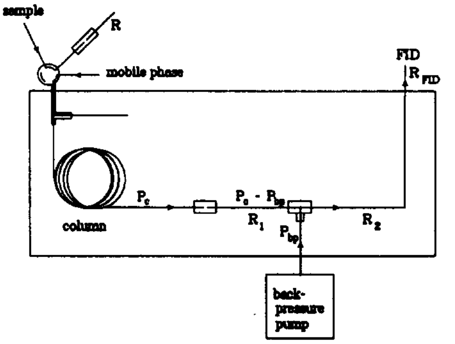

Fig. 1. Apparatus scheme for the band broadening measurement. R_1, R_2 and R_{FID} are restrictors; P_c and P_{bp} are column pressure and back-pressure, respectively; $P_c - P_{bp}$ is pressure drop over the variable flow restrictor R_1.

system (Chromatography Data System, Kungshög, Stenhamra, Sweden).

Measurement of the stationary phase swelling

The thickness of the swollen stationary film was measured by the on-column chromatographic method [4]. A modified procedure using the apparatus for measurement of diffusion coefficients in supercritical fluids [9] was used (Fig. 2). The column effluent (F_C) was split between an empty tube (F_T), 2.24 m × 50 μm, connected to a flame ionization detector via a variable-flow restrictor, R_1, and to the flame ionization detector (F_s) via a restrictor, 0.15 m × 5 μm (R_s). The flow-split ratio (C) was calculated from the corresponding areas of peaks of the same solute eluted from the column through the R_s restrictor (area A_C) and from the empty tube through the variable-flow restrictor R_1 (area A_T). Thus $C = (F_s/F_T) = (A_C/A_T)$. The column was prepared from the same batch of fused-silica capillary as the empty tube.

Measuring the hold-up time in the empty tube, t_m^T, and calculating dead time in the column, t_m^C, according to Guardino *et al.* [10] using C_6–C_{13} *n*-alkanes, the film thickness of the swollen stationary phase, d_f^*, was obtained from the formula:

$$d_f^* = r_T\left[1 - \sqrt{(1+C)\frac{L_T t_m^C}{L_C t_m^T}}\right] \tag{1}$$

where r_T is the radius of the empty tube and L_T and L_C are the lengths of the empty tube and the column, respectively.

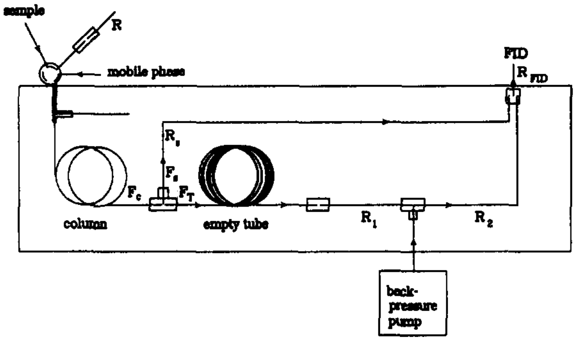

Fig. 2. Apparatus scheme for the measurement of the stationary phase swelling. R_s, R_1, R_2 and R_{FID} are restrictors and F_C, F_s and F_T are column flow, effluent split flow and empty tube flow, respectively.

Band-broadening measurements

Broadening of the solute zone in the column was estimated for C_8–C_{13}, C_{15} *n*-alkanes, dodecanone and biphenyl by injection of their solution in pentane, 10 mg/ml, on the column at 100 atm, 125°C, using 50–100 ms time split and a flow-split ratio of 1:4 to 1:10. Mobile phase velocity was adjusted to 28.8–41.2 mm/s by a back-pressure device [9] using supercritical carbon dioxide. The mixture was injected six or seven times at five different velocities.

Data handling

Chromatograms were registered at a speed of 4.55 Hz. An optimization programme was obtained by means of a least-squares fit of the measured values (H_i, u_i) to the Golay equation [7,11]. For capacity ratio the mean of all capacity ratios for each solute at all applied mobile phase velocities, and for column radius the difference $r_T - d_f^*$, were substituted into the Golay equation.

RESULTS AND DISCUSSION

Accurate estimation of the film thickness of swollen stationary phases is a prerequisite for evaluation of stationary phase contribution to total solute band broadening. The on-column chromatographic method is considered to be less precise than mass spectrometric tracer pulse chromatography [6]. However, it does not necessitate any special instrumentation, and precision can be increased by avoiding manipulation of the columns during measurements. Thus, an accurate ratio of volumetric flows can be measured, which is not the case if the columns are reconnected to the restrictor (possible flow change in the low-dead-volume connector). Unfortunately, none of these methods is able to distinguish between the adsorbed layer of carbon dioxide on the surface of the stationary phase and the swollen stationary phase film thickness itself. As one to three monolayers of carbon dioxide were proven to be sorbed on the surface of common adsorbents [12], a stagnant layer should be supposed on the surface of a polymeric stationary phase film as well.

The film thickness of the swollen stationary phase at 100 atm, 125°C, was estimated to be 1.28 μm (S.D. = 0.09 μm, $n = 10$), which corresponds to a swelling factor of 1.9 (S.D. = 0.3). This is somewhat higher than that found by Springston *et al.* [4] for SE-30 at 89 atm, 40°C, which was 1.0 ± 0.6. The difference might originate from different degree of cross-linking. In this case, 88% immobilization degree has been obtained, which is sufficient to get stable stationary phase film and keep the column efficiency. Thus, possible changes in the stationary phase diffusivity due to excessive cross-linking were avoided.

In Table I, effective solute diffusion coefficients in the stationary phase measured in SFC [$D_s(\text{eff.})^{SF}$] are summarized together with corresponding solute diffusion coefficients in the supercritical fluid phase (D_m^{SF}). Variation in measurements of swollen film thickness (R.S.D. = 7%) gives a relative standard deviation for effective solute diffusion coefficients of 14%. The standard deviation of solute capacity ratios was less than 2.7%, which had almost no effect on the variation of solute effective diffusion coefficients, R.S.D. < 2.5%. Compared with the effective solute diffusion coefficients in the same stationary phase estimated from data measured by GC [$D_s(\text{eff.})^G$] [11] and the data obtained with similar polysiloxane phases [13], the values measured in SFC are at least one order of magnitude lower. Similarly, Horká *et al.* [14] showed that the diffusion in the stationary phase in OTLC is dependent on the type of mobile phase used. In theoretical calculations of the effect of stationary phase film thickness on efficiency in open tubular SFC [15,16] and LC [16], the stationary phase diffusion was assumed to be independent of the mobile phase. Such an assumption may produce misleading conclusions.

Evaluation of solute band broadening in the stationary phase under GC conditions revealed a significant contribution of mobile phase–stationary phase film interphase resistance to the total solute band broadening [11]. Including this effect, the extended Golay equation can be written:

$$H = B/u + C_m u + (C_s + C_i)u \tag{2}$$

where:

$$C_s = \frac{2k'd_f^{*2}}{3(1+k')^2 D_s} \tag{3}$$

$$C_i = \frac{2k'd_f^*}{(1+k')^2 k_d} \tag{4}$$

C_m, C_s and C_i are resistance to the mass transfer in the mobile phase, stationary phase and in the interphase between these phases, respectively. D_s is the solute diffusion coefficient in the swollen stationary phase and k_d is the desorption rate constant. In a previous paper [11], values of solute diffusion coefficients independent of film thickness were obtained (D_s^G) and desorption rate constants (k_d^G) were calculated for the same stationary phase as used here. Similarly, using GC values of solute diffusion coefficients independent of stationary phase film thickness,

TABLE I

SOLUTE DIFFUSION COEFFICIENTS IN STATIONARY PHASE PS-264 AT 125°C AND 100 atm (1 atm = 101 325 Pa)

Solute	D_m^{SF} $\times 10^3$ (mm^2/s)	$D_s(\text{eff.})^{SF}$ $\times 10^3$ (mm^2/s)	k_d^{SF} (mm/s)	$D_s(\text{eff.})^G$ $\times 10^3$ (mm^2/s)	D_s^G $\times 10^3$ (mm^2/s)	k_d^G (mm/s)
C_{11}	47	0.0474	0.115	0.54	1.35	2.90
C_{12}	45	0.0472	0.115	0.49	1.33	2.50
C_{13}	44	0.0488	0.119	0.51	1.33	2.67
C_{15}	41	0.0225	–	–	–	–
C_{12} on	43	0.0715	–	–	–	–
Biphenyl	50	0.0072	0.017	0.70	2.12	3.37

desorption rate constants for SFC conditions (k_d^{SF}) were calculated. Compared with GC conditions, k_d^{SF} is affected not only by solute interaction with stationary phase, but by solute solubility in the mobile phase as well. Lower solubility of biphenyl compared with the *n*-alkanes used in supercritical carbon dioxide corresponds to lower k_d^{SF} values. However, possible effects of a stagnant layer of supercritical carbon dioxide are included in these values.

As has been recently found in OTCs coated with Carbowax 20M, a stagnant layer of mobile phase has a significant influence on solute band broadening under LC conditions [14]. Accordingly, further extension of the Golay equation for evaluation of all contributions is necessary:

$$H = B/u + C_m u + (C_s + C_i + C_{SM})u \qquad (5)$$

where C_{SM} is the contribution to mass transfer from the stagnant layer, which can be expressed according to ref. 17 as:

$$C_{SM} = \frac{2(1 - \Phi + k')^2 d_f^{*2}}{3(1 - \Phi)(1 + k')^2 D_{SM}} \qquad (6)$$

where Φ is the part of the mobile phase in the swollen stationary phase film and D_{SM} is solute diffusion coefficient in the stagnant layer. Separate evaluation of C_i and C_{SM} terms is not possible from these experiments. The total contribution of the stationary phase to solute band broadening was estimated and the percentage contributions of resistance to solute mass transfer in mobile and in stationary phases at a velocity, 20 mm/s, commonly used in SFC were calculated from the measured data and are expressed in Table II. The contribution of the stationary phase to solute band broadening is commonly considered to be low under SFC conditions [15,16], but in the present work mass transfer in the stationary phase contributed about 50% to the total solute band broadening.

Further progress in the understanding of mass transfer effects in the stationary phase under SFC conditions can be achieved if a non-retained solute with good solubility in supercritical carbon dioxide is used for estimation of stagnant layer contributions. By subtracting this contribution to solute band broadening in columns differing in film thickness, contribution of interphase resistance can be obtained.

TABLE II

EFFECTS OF THE MASS TRANSFER TERMS ON HEIGHT EQUIVALENT TO A THEORETICAL PLATE (HETP) IN SFC AT 125°C, 100 atm, AND VELOCITY 2 cm/s CALCULATED ACCORDING TO EQN. 5

Column: 6.67 m × 50 μm; PS-264, $d_f^* = 1.28$ μm

Solute	HETP (mm)	k'	$C_m \times u$ (%)	$(C_s + C_i + C_{SM}) \times u$ (%)
C_{12}	0.17	1.94	38	59
C_{13}	0.17	2.76	46	52
C_{15}	0.23	5.62	44	54
C_{12} on	0.14	3.76	61	36

In summary, it was found that, for the conditions evaluated, effective diffusion in the stationary phase was one order of magnitude lower when applying SFC conditions than when GC conditions were applied. As a consequence, the contribution to band broadening from mass transfer in the stationary phase was higher than reported earlier. Thus, unacceptable band broadening would appear at lower film thicknesses than previously thought.

REFERENCES

1 M.I. Selim and J.R. Strubinger, *Fresenius' Z. Anal. Chem.*, 330 (1988) 246.
2 M. Roth, *J. Microcol. Sep.*, 3 (1991) 173.
3 C.R. Yonker and R.D. Smith, *J. Chromatogr.*, 550 (1991) 775.
4 S.R. Springston, P. David, J. Steger and M. Novotny, *Anal. Chem.*, 58 (1986) 997.
5 J.-J. Shim and K.P. Johnston, *AIChE J.*, 35 (1989) 1097.
6 J.-J. Shim and K.P. Johnston, *AIChE J.*, 37 (1991) 607.
7 M.J.E. Golay, in V.J. Coates, H.J. Noebels and I.S. Fagerson (Editors), *Gas Chromatography*, Academic Press, New York, 1958, p. 36.
8 M. Novotny and P. David, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 647.
9 K. Janák, I. Hägglund, L.G. Blomberg, A.K. Bemgård and A.L. Colmsjö, *J. Chromatogr.*, 625 (1992) 311.
10 X. Guardino, J. Albaigés, G. Firpo, R. Rodriguez-Viñals and M. Gassiot, *J. Chromatogr.*, 118 (1976) 13.
11 A. Bemgård, L. Blomberg and A. Colmsjö, *Anal. Chem.*, 61 (1989) 2165.
12 J.F. Parcher and J.R. Strubinger, *J. Chromatogr.*, 479 (1989) 251.

13 J.M. Kong and S.J. Hawkes, *J. Chromatogr. Sci.*, 14 (1976) 279.
14 M. Horká, M. Krejcí and V. Kahle, *J. Microcol. Sep.*, 4 (1992) 305.
15 S.M. Fields, R.C. Kong, M.L. Lee and P.A. Peaden, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 423.
16 P.J. Schoenmakers, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 278.
17 S.J. Hawkes, *J. Chromatogr.*, 68 (1972) 1.