

Determination of the hold-up time and column outlet density for capillary supercritical fluid chromatography

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ABSTRACT

The column hold-up time (t_u) was determined for a capillary supercritical fluid chromatographic system over a range of temperatures and densities using methane as a marker and using homologous series extrapolations. At low temperatures, t_u was found to pass through a maximum with increasing density. To establish that this phenomenon is not due to a retention effect, t_u was also calculated from flow-rate measurements. The measured hold-up times and Darcy's law were used to calculate the outlet densities for different inlet pressures at two temperatures.

INTRODUCTION

The accurate determination of the column hold-up time, t_u , is a chronic problem for chromatographers, especially when mass spectrometric detection is not available or feasible. Theoretically, the hold-up time for an open-tubular column can be calculated if the pressure drop is known [1,2]. Perrut [2] has calculated hold-up times for packed columns with 5–40 bar pressure drops. However, the pressure drops in capillary supercritical fluid chromatography (SFC) are generally too small to be determined accurately. Hence, it is usually assumed that the pressure drop is negligible and the average density is closely approximated by the inlet density. Roth and Ansorgova [3] have calculated pressure profiles for capillary SFC and found them to be nearly linear. They have also demonstrated that secondary effects (thermal effects, turbulence effects, end effects and column coiling effects) can safely be neglected under capillary SFC conditions.

Here, we report the determination of t_u on a capillary SFC system with carbon dioxide as a mobile phase using four methods. In the first method, methane was assumed to be an "unretained" solute and was used as a t_u marker. Other

compounds often used for this purpose (such as methylene chloride) were found to exhibit significant retention compared with methane, especially at low densities and temperatures. Methods 2 and 3 are based on the linearity of $\ln k'$ (capacity factor) with carbon number [4,5]. In method 2, retention times were measured for two different homologous series, alkanes and alkylbenzenes, and t_u was determined using the method of homologous series extrapolation described by Berendsen *et al.* [5]:

$$t_{R,n+1} = At_{R,n} - (A - 1)t_u \quad (1)$$

where $t_{R,n}$ is the retention time of the n th member of a homologous series and $t_{R,n+1}$ is the retention time for the next member of the series. A plot of $t_{R,n+1}$ vs. $t_{R,n}$ gives the slope A and the intercept $-(A - 1)t_u$. The hold-up time is determined from

$$t_u = \frac{\text{intercept}}{1 - \text{slope}} \quad (2)$$

In method 3, retention data for a homologous series of alkanes is fitted directly to the non-linear equation

$$t_R = t_u + t_u \exp a \exp bM \quad (3)$$

where M is the number of methylene groups and t_u, a

and b are determined by fitting retention data for each density and temperature.

Using each of these methods, we found that t_u passes through a maximum with increasing density at low column temperatures. The maximum occurs near the critical density. This result was contrary to expectation, as t_u decreases with increasing pressure for both gas and liquid chromatography. In order to verify that the maximum was not an artifact caused by solute retention, in method 4, we used flow-rate measurements to determine t_u . The column-outlet volumetric flow-rate was measured and the mass flow-rate, \dot{m} , was determined from

$$\dot{m} = \rho_A F \quad (4)$$

where ρ_A is the density of the mobile phase in g/cm^3 under ambient conditions, as calculated from the extended BWR equation of state [6], and F is the measured volumetric flow-rate in cm^3/min . Then t_u is calculated from [1]

$$t_u = \frac{V_0 \rho_i}{\dot{m}} \quad (5)$$

where ρ_i is the column inlet density in g/cm^3 and V_0 is the column void volume in cm^3 . With minimal error, the inlet density has been substituted for the spatial average density in eqn. 5 (see below).

In this study, we compared the column hold-up times obtained by the four methods outlined above and used the t_u values to calculate the column outlet density using equations derived from Darcy's law [1].

The derived expression for t_u is

$$t_u = \int_0^{t_u} dt = \frac{B}{(v_0 \rho_0)^2} \int_{\rho_0}^{\rho_i} D_t(\rho) d\rho \quad (6)$$

where v is the velocity, ρ is the density and $D_t(\rho)$ is the temporal distribution function; the subscripts i and o refer to inlet and outlet conditions, respectively. For open-tubular columns, B is a constant which can be calculated:

$$B = \frac{\pi r^2}{8} \quad (7)$$

where r is the column radius. Making use of the equation

$$L = \int_0^L dx = \frac{B}{v_0 \rho_0} \int_{\rho_0}^{\rho_i} D_x(\rho) d\rho \quad (8)$$

where L is the column length and $D_x(\rho)$ is the spatial distribution function, $v_0 \rho_0$ can be eliminated from eqn. 6, resulting in

$$t_u = \frac{L}{B} \cdot \frac{\int_{\rho_0}^{\rho_i} D_t(\rho) d\rho}{\left[\int_{\rho_0}^{\rho_i} D_x(\rho) d\rho \right]^2} \quad (9)$$

The density distribution functions, $D_x(\rho)$ and $D_t(\rho)$, have been calculated for the given densities and temperatures in previous work [6]. An iterative procedure was used to solve this equation for the column outlet density. The outlet pressure corresponding to this density was then calculated using the extended BWR equation of state.

EXPERIMENTAL

The instrument used was a Model 501 capillary supercritical fluid chromatographic system with a flame ionization detector from Lee Scientific (Salt Lake City, UT, USA). The injection valve with a 200-nl sample loop was pneumatically operated. The sample was split on injection with a splitting ratio of *ca.* 20:1.

The capillary column was supplied by Lee Scientific. The stationary phase was SB-Octyl-50 with a film thickness of $0.25 \mu\text{m}$. The column was $10 \text{ m} \times 50 \mu\text{m}$ I.D. A 30-cm frit restrictor was attached between the end of the column and the detector.

The mobile phase was supercritical-grade carbon dioxide obtained from Matheson Gas Products (Baltimore, MD, USA). Other gases (helium, used for valve actuation; nitrogen, used as a make-up gas; hydrogen and air, used for the flame ionization detector) were obtained from Roberts Oxygen (Rockville, MD, USA). Methane, used as the marker for the first t_u determination method, was obtained from natural gas piped into the laboratory. The alkanes and alkylbenzenes used for the homologous series studies were obtained from various standard sources. It was not deemed necessary to use highly purified solutes for these studies since trace impurities are resolved during the chromatographic process and easily identified.

The detector temperature was set at 325°C; the range and attenuation were adjusted for each set of conditions. The signal output was recorded on a Linear strip-chart recorder.

All experiments were performed under isothermal, isobaric (*i.e.*, non-programmed) conditions. Settings were selected to provide measurements at 10 K intervals from 320 to 380 K and 0.05 g/cm³ density increments from 0.2 to 0.7 g/cm³. Pressure settings were determined by calculating each pressure from the extended BWR equation of state for the selected temperature and density.

Replicate injections (usually three) were performed at each temperature and density setting. The system was allowed to equilibrate between each change of settings.

The low column flow-rates in capillary SFC are difficult to measure accurately. The solubility of carbon dioxide and its diffusion into the connective tubing make the determination of flow-rates with the usual soap-bubble flow meter semi-quantitative at best. A flow meter modified to minimize these problems was devised using a standard 50-ml buret with side-arm (Fig. 1). The glass side-arm is connected to the flame ionization detector directly to prevent diffusion of carbon dioxide through the connective tubing. The stopcock is closed after introduction of the bubble to prevent dissolution of the carbon dioxide in the soap solution and the flow meter is stoppered to minimize the effects of air currents and pressure fluctuations. Flow-rate measurements were made at 310, 320 and 360 K, with the detector flame extinguished. The reproducibility was significantly improved with the modified flow meter, but the relative uncertainty is still about 7%.

RESULTS AND DISCUSSION

Selected results of the t_u determinations are given in Table I and Fig. 2. With a few exceptions, there is very good agreement among the values obtained using the first three methods. The t_u values obtained by fitting the alkane data and the alkylbenzene data by method 2 are also in good agreement. The hold-up times calculated using method 3 are in excellent agreement with those calculated using method 2. Method 2 has the advantage of being a linear fit but the disadvantage of requiring retention data for a series of sequential homologs. It also has

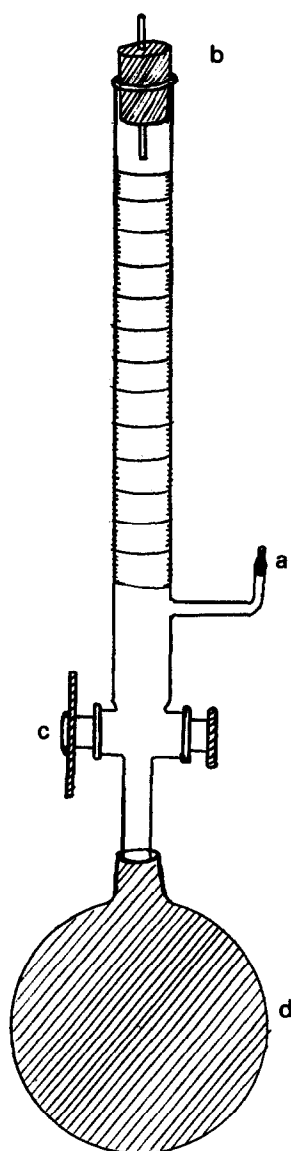


Fig. 1. Modified soap-bubble flow meter: (a) glass side-arm; (b) stopper with capillary; (c) stopcock; (d) rubber bulb containing soap solution.

the disadvantage of having errors in both “ x ” and “ y ”, thus making the errors in t_u difficult to estimate. A non-linear fitting routine must be available in order to use method 3. We used SAS NLIN for this purpose. A series of homologs is necessary for this fit, but the series need not be sequential. Also, the

TABLE I
COLUMN HOLD-UP TIMES

Temperature (K)	ρ_i (g/cm ³)	Column hold-up time (min)				
		Method 1	Method 2		Method 3	Method 4
			a	b		
310	0.25	15.90	15.26			
	0.30	20.40	19.90			17.63
	0.35	24.90	24.80			
	0.40	26.00	25.95			20.68
	0.45	26.38	26.33			
	0.50	26.40	26.38			25.07
	0.55	26.39	26.30			
	0.60	25.40	25.70			27.11
	0.65	24.50	24.44	23.79	23.81	
0.70	21.81	21.80	21.20	21.20		22.55
320	0.20	12.10	12.19			12.48
	0.25	12.60	12.62	12.42	12.43	
	0.30	13.50	13.47	13.20	13.16	13.51
	0.35	14.50	14.35	14.34	14.33	
	0.40	15.50	15.39	15.27	15.28	15.38
	0.45	16.21	16.17	16.00	16.01	
	0.50	16.55	16.36	16.37	16.35	15.76
	0.55	16.50	16.32	16.37	16.36	
	0.60	15.95	15.86	15.88	15.88	15.01
0.65	14.80	14.66	14.77	14.76		
0.70	12.95	12.79	12.87	12.91	12.94	
360	0.20	7.72	7.79	7.40	7.42	8.06
	0.25	7.12	7.27	7.11	7.10	
	0.30	7.00	7.90	6.83	6.83	7.21
	0.35	6.80	6.78	6.73	6.75	
	0.40	6.63	6.58	6.58	6.64	6.29
	0.45	6.40	6.34	6.26	6.31	
	0.50	6.10	6.08	6.04	6.05	5.76
	0.55	5.71	5.68	5.62	5.66	
	0.60	5.22	5.20	4.93	5.15	4.84

errors are contained in "y" and errors in t_u are estimated by the program.

Using method 4, the calculated hold-up times are not in particularly good agreement with the results from the other methods. However, these measurements do follow the same trend and also exhibit a maximum in t_u near the critical density for low temperatures. The discrepancies are probably due to the reproducibility and accuracy of the flow-rate measurements. Mass flow-rates are plotted in Fig. 3. It can be seen that a small error in the flow-rate determination leads to a large error in t_u . Also, V_0

was assumed to be constant for this calculation, whereas it may change slightly with temperature and density of the mobile phase.

Eqn. 8 and the hold-up times determined by method 2 were used to calculate the column outlet densities. The extended BWR equation of state was then used to calculate the column outlet pressures with the assumption that the residence time of the mobile phase in the restrictor is negligible. The calculated outlet densities and pressures for 320 and 360 K are given in Table II.

The effect of the density drop on $\ln k'$ was

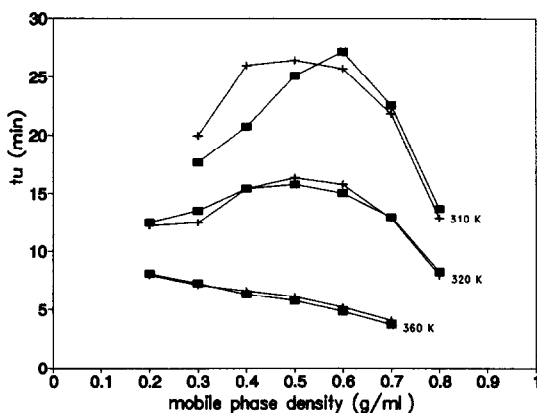


Fig. 2. Hold-up times versus mobile phase density at 310, 320 and 360 K. ■ = Method 1; + = method 4.

determined by comparing the $\ln k'$ values using the inlet density:

$$\ln k'(\rho_i) = \ln k'_0 + a\rho_i + b\rho_i^2 \quad (10)$$

and using the temporal average density:

$$\ln k'(\langle \rho \rangle_t) = \ln k'_0 + a\langle \rho \rangle_t + b\langle \rho^2 \rangle_t \quad (11)$$

where a and b are arbitrarily chosen constants. These results for $\ln k'_0 = 9.55$, $a = -29.38$, $b = 19.90$ are given in Table III. The pressure drops were found to be very small and the density drops insignificant. Therefore, the effect of using the inlet

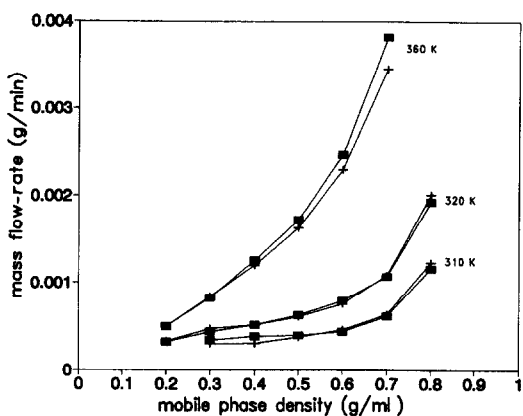


Fig. 3. Mass flow-rates versus mobile phase density at 310, 320 and 360 K. ■ = Method 1; + = method 4.

TABLE II
CALCULATED PRESSURE AND DENSITY DROPS

Temperature (K)	ρ_i (g/cm ³)	P_i (bar)	$\Delta\rho$ (g/cm ³)	ΔP (bar)
320	0.25	82.74	0.003	0.38
	0.30	88.65	0.004	0.38
	0.35	93.07	0.005	0.40
	0.40	96.70	0.006	0.42
	0.45	100.12	0.006	0.45
	0.50	103.78	0.006	0.48
	0.55	108.19	0.005	0.52
	0.60	114.25	0.004	0.58
	0.65	123.42	0.003	0.72
	0.70	138.00	0.003	0.94
360	0.20	97.32	0.002	0.54
	0.25	113.27	0.002	0.63
	0.30	127.45	0.003	0.71
	0.35	140.58	0.003	0.79
	0.40	155.39	0.004	0.89
	0.45	166.70	0.004	1.01
	0.50	181.40	0.004	1.14
	0.55	198.64	0.004	1.31
	0.60	220.01	0.003	1.59

TABLE III
CALCULATED CAPACITY FACTORS (k')

Temperature (K)	ρ_i (g/cm ³)	$\ln k'(\rho_i)$	$\ln k'(\langle \rho \rangle_t)$
320	0.20	4.47	4.49
	0.25	3.45	3.48
	0.30	2.53	2.56
	0.35	1.71	1.75
	0.40	0.98	1.03
	0.45	0.36	0.40
	0.50	-0.16	-0.13
	0.55	-0.59	-0.57
	0.60	-0.91	-0.90
	0.65	-1.14	-1.13
360	0.70	-1.26	-1.26
	0.20	4.47	4.49
	0.25	3.45	3.47
	0.30	2.53	2.55
	0.35	1.71	1.73
	0.40	0.98	1.01
	0.45	0.36	0.38
	0.50	-0.16	-0.15
	0.55	-0.59	-0.57
	0.60	-0.91	-0.90

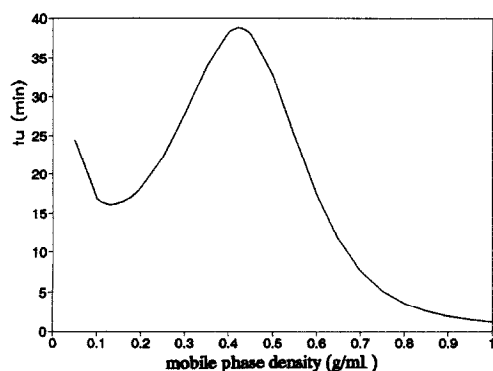


Fig. 4. Hold-up times calculated from eqn. 9 for 320 K, assuming $\rho_0 = 0.994\rho_i$.

density instead of the temporal (or spatial) average density is seen to be minimal.

In order to examine the behavior of t_u over a full range of densities, including those normally encountered in gas and liquid chromatography, a constant relative density drop was assumed ($\rho_0 = 0.994\rho_i$) and t_u was calculated at 320 K from eqn. 9 (see Fig. 4). This curve has the same general pattern in the vicinity of the critical density although the maximum is exaggerated. A minimum is also predicted at lower density. This behavior of t_u is peculiar to dense gases. As expected, the equation predicts a decrease in t_u with increasing pressure for both ideal gases and liquids.

CONCLUSIONS

Measurements of t_u as a function of inlet density for several temperatures indicate that, at low supercritical temperatures, the hold-up time increases with increasing density, reaches a maximum at or near the critical density, then decreases as the inlet density is further increased. At higher temperatures, t_u decreases with increasing density. This behavior, which is related to the dependence of the density distribution functions, $D_x(\rho)$ and $D_t(\rho)$, on the isothermal compressibility and viscosity of the mobile phase [1,6], can be predicted from eqn. 9, provided that both the inlet and outlet densities are known. Alternatively, it can be confirmed from eqn. 5, provided that sufficiently accurate mass flow-rates are available.

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