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On void time determination in thermal field-flow fractionation

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Abstract

Because of the temperature dependence of the carrier liquid density, the mass of carrier which is contained in a thermal field-flow fractionation channel depends on the cold wall temperature and on the temperature difference across the channel thickness. It is observed that the void time of the solvent peak decreases when increasing the average temperature in the channel. The void time is found to be directly proportional to the average carrier density in the channel. The determination of the void time from the knowledge of the channel geometrical volume and the measurement of the volumetric flow-rate leads to significant errors if the thermal expansion of the carrier between the temperature of the measurement and the average channel temperature is not taken into account. Recommendations are given for proper void time determinations in thermal FFF. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Field-flow fractionation (FFF) is a group of separation methods in which sample components are eluted by a flow of carrier liquid when a transverse force field is applied perpendicularly to the main direction of the flow [1]. FFF is applicable to supramolecular species (macromolecules, colloids, gels, emulsions, particles, cells). FFF is generally performed in a thin ribbon-like parallel plate channel. When interacting with the field, a sample component (also called an analyte) is compressed in

a more or less narrow layer near the accumulation plate where the flow streamlines are slowed down by the viscous friction with the wall. The displacement velocity of the analytes along the channel is then lower than the average carrier flow velocity and their degree of retention is controlled by their degree of compression near the accumulation wall, reflected by the basic FFF retention parameter, λ , defined as:

$$\frac{1}{\lambda} \equiv - \frac{dc/c}{d(x/w)} \quad (1)$$

where c is the steady-state analyte concentration, x the distance from the accumulation wall and w the channel thickness [2]. λ depends on the interaction between the analyte and the applied field. The knowledge of these interaction parameters is of paramount importance for optimizing the separation performances. When a theoretical or empirical relationship between λ and the primary analyte property (for instance, molar mass or particle size) is known,

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the sample can be characterized from its retention pattern. In fact, FFF systems appear to be efficient and relatively fast physico-chemical tools for the determination of the interaction parameters. This determination relies on the experimental determination of the analyte retention time, t_R , as well as of the so-called *void time*, t_o , which is the mean time spent by the carrier fluid in the channel. Indeed, the retention theory of FFF relates the relative retention time, t_R/t_o , to λ .

It is clear that the accuracy of the determination of λ is heavily dependent on that of t_R and t_o . The value of t_R is obtained from experimental measurements of the analyte retention time, either as the time of elution of the peak maximum or, more correctly, as that of the center-of-gravity of the peak. However, the method of determination of the void time t_o is rarely discussed in the literature because it is considered to be trivial. One of the three following methods is generally employed for this purpose: (a) measurement of the elution time of a “solvent” peak which is observed with some detectors; (b) measurement of the elution time of an injected species of low molar mass, supposedly unretained; (c) calculation from the knowledge of the geometrical channel volume, V_o , and the experimental measurement of the volumetric flow-rate of the carrier liquid, \dot{V} , as:

$$t_o = V_o / \dot{V} \quad (2)$$

These methods cannot be applied in flow FFF, where the role of the forced field is played by a cross-flow of liquid through permeable walls. Indeed, an injected small molar mass species is swept with the carrier through the semi-permeable membrane, which serves to retain the analytes, and the geometric volume is modified to an uncertain extent due to protrusion of the membrane when assembling the channel. Then, a fourth method, based on the breakthrough measurement of the void volume, V_o , has been developed and applied specifically to flow FFF [3].

The situation is apparently simpler in thermal FFF, which is that FFF method based on the application of a transverse temperature gradient to induce analyte retention, since the channel walls are impermeable which allows applying one of the three first methods above. However, because of the temperature gradient

inherent to the thermal FFF operation and of the ubiquitous temperature dependence of relevant physico-chemical parameters, some questions are raised as to the pertinent definition of the void time and as to the method to be used for the determination of the void time.

It was recognized for long that, in thermal FFF, the temperature dependences of the carrier thermal conductivity and of the carrier viscosity influence the shapes of the temperature profile and of the flow velocity profile and, consequently, influence analyte retention [4–7]. Moreover, the basic FFF retention parameter becomes itself temperature dependent. This seriously complicates its determination from retention time measurements. In thermal FFF, λ is expressed as [8]:

$$\frac{1}{\lambda} = (S_T + \gamma) \frac{dT}{d(x/w)} \quad (3)$$

where S_T is the Soret coefficient of the analyte in the carrier, γ the thermal expansion coefficient of the carrier and T the (absolute) temperature. In thermal FFF, the temperature dependence of λ arises primarily from that of S_T , and to a lower extent from those of γ and of the temperature gradient, dT/dx . According to Eq. (1), this temperature dependence of λ influences the shape of the transverse concentration profile of the analyte and its relative retention time, t_R/t_o . In spite of the resulting complication, methods have been developed to provide an accurate determination of S_T from the experimental value of t_R/t_o [9–11]. Furthermore, one of these methods has been employed either to obtain more accurate relationships between S_T and the molar mass, M , of polymers than those based on methods neglecting the temperature dependence of λ [12,13], or to investigate the influence of cold wall temperature on the universal calibration in thermal FFF [14].

Clearly, the accuracy of these studies is dependent on that of the experimental value of t_R/t_o . If the experimental measurement of t_R in thermal FFF does not raise specific difficulties, it is not so for that of t_o because of the temperature dependence of the carrier density. Indeed, the value of the volumetric flow-rate depends on the temperature at which its measurement is made and the value of t_o derived, for instance, from Eq. (2) becomes ambiguous. It is the purpose of the present study to investigate the

influence of the temperature dependence of the carrier density on the value of t_o and to discuss the validity of the various methods of determination of the void time in thermal FFF. Furthermore, one notes that, since FFF is preferentially performed in high retention conditions for optimum performances [15], a given error, δt_o , in the determination of t_o leads to a greater error on λ than a comparable error in t_R .

2. Theory

One considers in the following that the thermal FFF channel has a geometrical volume, V_o , a thickness, w , a breadth, b , and an effective length, L (defined as V_o/bw). These quantities are assumed to be unmodified when a temperature drop, ΔT , is applied between the two walls. In practice, the mode of construction of the thermal FFF channel is such that V_o and w are likely to be constant or that their eventual deformation is a second-order effect compared to that described below. One assumes that the carrier delivery device is providing a constant carrier flow-rate at room temperature, T_{amb} . The cold wall of the thermal FFF channel is at temperature T_c and the hot wall at temperature $T_h = T_c + \Delta T$.

2.1. Temperature profile across the thermal FFF channel

Since the carrier liquid enters the FFF channel at a uniform temperature, T_{amb} , it takes some time before a steady state transverse temperature is established through the liquid. This time can be estimated as that of diffusion of heat across distance w , i.e., of the order of w^2/α_T , where α_T is the thermal diffusivity of the carrier [13]. With typical values of w and α_T ($w = 10^{-4}$ m, $\alpha_T = 8.7 \times 10^{-8}$ m² s⁻¹ for ethylbenzene at 293 K), one gets a relaxation time of about 0.1 s. It is generally much smaller than the residence time of the carrier in the channel, which in the experiments described below is about 250 s. The steady state temperature profile can thus be considered as established all along the channel. Assuming that the carrier thermal conductivity, κ_T , varies linearly with the temperature, the temperature profile is equal to [2,4]:

$$T = T_c + \frac{1}{\omega_c} \left[\sqrt{1 + 2\omega_c \Delta T \left(1 + \frac{1}{2}\omega_c \Delta T\right)^s} - 1 \right] \quad (4)$$

with

$$s = x/w \quad (5)$$

where ω_c is the relative rate of variation of κ_T with T , i.e., $(1/\kappa_T) d\kappa_T/dT$ and κ_{T_c} the thermal conductivity at T_c . For ethylbenzene at 293 K, ω_c equals -1.77×10^{-3} K⁻¹ [7]. For vanishing ω_c , Eq. (4) becomes:

$$\lim_{\omega_c \rightarrow 0} T = T_c + s\Delta T \quad (6)$$

and the temperature profile becomes linear as expected.

2.2. Mass of carrier liquid in the thermal FFF channel

The mass, δm , of carrier liquid contained in a thin lamina of thickness δx , parallel to the plates, is $\rho[T(x)]bL \delta x$ where the carrier density, ρ , defined as the mass per unit volume, depends on T and thus on x . The mass, m , of carrier liquid contained in the channel is then:

$$m = \int_0^w \rho bL dx = V_o \langle \rho \rangle \quad (7)$$

with:

$$\langle \rho \rangle = \int_0^1 \rho ds \quad (8)$$

$\langle \rho \rangle$ is the average density of the carrier in the channel. It depends on T_c and ΔT .

In the temperature ranges generally sampled in thermal FFF runs, the temperature dependence of the carrier density can be accurately expressed by a quadratic relationship:

$$\rho = r_0 + r_1 T + r_2 T^2 \quad (9)$$

The thermal expansion coefficient of the carrier, defined as:

$$\gamma \equiv -\frac{1}{\rho} \frac{d\rho}{dT} \quad (10)$$

is then given by:

$$\gamma = -\frac{r_1 + 2r_2T}{r_0 + r_1T + r_2T^2} \quad (11)$$

Combining Eqs. (4), (8) and (9), one gets:

$$\begin{aligned} \langle \rho \rangle = & r_0 + r_1 \left(T_c + \frac{1}{2} \Delta T \frac{1 + \frac{2}{3} \omega_c \Delta T}{1 + \frac{1}{2} \omega_c \Delta T} \right) \\ & + r_2 \left(T_c^2 + T_c \Delta T \frac{1 + \frac{2}{3} \omega_c \Delta T}{1 + \frac{1}{2} \omega_c \Delta T} \right. \\ & \left. + \frac{1}{3} \Delta T^2 \frac{1 + \frac{3}{4} \omega_c \Delta T}{1 + \frac{1}{2} \omega_c \Delta T} \right) \quad (12) \end{aligned}$$

This equation becomes simpler when the temperature range is sufficient narrow for the density to be assumed to vary linearly in that range. Then, when $r_2 = 0$ in Eqs. (9) and (11), one gets:

$$\rho = \rho_o [1 - \gamma_o (T - T_o)] \quad (13)$$

where ρ_o and γ_o are the values of ρ and γ at a reference temperature T_o . When, in addition, the temperature profile is given by Eq. (6), Eq. (12) becomes:

$$\langle \rho \rangle = \rho_c \left(1 - \frac{\gamma_c \Delta T}{2} \right) = \rho(T_{ave}) \quad (14)$$

where ρ_c and γ_c are the values of ρ and γ at a reference temperature T_c , and T_{ave} is the average temperature in the channel, then equal to:

$$T_{ave} = \frac{T_c + T_h}{2} = T_c + \frac{1}{2} \Delta T \quad (15)$$

When the whole channel is at the cold wall temperature, the mass, m_c , of liquid contained in it is equal to $V_o \rho_c$. Then, on heating one plate, the relative mass of liquid that is lost is equal to:

$$\frac{m_c - m}{m_c} = 1 - \frac{\langle \rho \rangle}{\rho_c} \quad (16)$$

This, for linear temperature and density profiles, becomes, according to Eq. (14):

$$\frac{m_c - m}{m_c} = \frac{\gamma_c \Delta T}{2} \quad (17)$$

For most organic liquids, γ is of the order of 10^{-3} K^{-1} . For a typical ΔT of 50 K, the relative loss of carrier mass is about 2.5%. It should be noted that this amount of liquid is leaving the heated channel through its inlet and outlet connections. It is indeed assumed in this calculation that the pressure inside the channel remains unchanged on heating. This corresponds to the conditions prevailing in thermal FFF operation since it is connected to atmospheric pressure through the outlet connection tubes and since the pressure drop along the channel and along these tubes is generally very small. One notes in passing that, if, instead of this open configuration, the inlet and outlet connections were closed, heating one plate would lead to an increase of the pressure inside the channel which would amount to:

$$\Delta P = \frac{\gamma_c \Delta T}{\beta_c} \frac{\Delta T}{2} \quad (18)$$

where β_c is the isothermal compressibility of the carrier at T_c . For most organic liquids, β is of the order of 10^{-3} MPa^{-1} , hence a ΔT of 50 K would lead to a pressure increase of 25 MPa (250 bar). Obviously, in FFF operation, the inlet and outlet connections are necessarily open. Still, the pressure inside the channel may be somewhat higher than atmospheric pressure due to the resistance to flow in the connection tubings and in the detector. However, it will be shown below that, even if the channel pressure reaches a few bars, its influence on the mass of carrier liquid contained in the channel is much lower than that of temperature.

2.3. Various possible definitions of void time

There have been various expressions used in the past for defining the void time in FFF. In systems of constant carrier density, the various void times and the resulting void volumes are found identical, except in curved channels [16] which are not considered here since thermal FFF has been up to now performed in straight channels. It is worthwhile to investigate their relationships in the case of thermal FFF with a non-constant carrier density.

2.3.1. Carrier replacement time

This time, $t_o^{(1)}$, is defined as that which is necessary to replace the mass, m , of carrier contained in the channel by new carrier. It is given by:

$$t_o^{(1)} = \frac{m}{\dot{m}} \quad (19)$$

where \dot{m} is the mass flow-rate of carrier. In fact, thermal FFF systems are operated in constant mass flow-rate conditions. The mass of carrier within the channel might change with the temperatures of the cold and hot plates, but the system is, in steady state conditions, operated at a constant mass flow-rate of carrier since there is no accumulation of carrier in the channel and the amount of carrier which enters the channel during a time interval must be equal to that leaving the channel in the same time interval. It was shown that this definition is the fundamental one to be used in chromatography in order to retrieve equilibrium parameters from retention time measurements [17]. Similarly, this must be the fundamental definition of void time in thermal FFF in order to obtain equilibrium thermodiffusion parameters (Soret coefficients) from retention time measurements.

The mass flow-rate is related to the volumetric flow-rate, \dot{V}_o , measured at a given temperature, T_o , by:

$$\dot{m} = \rho_o \dot{V}_o \quad (20)$$

2.3.2. Elution time of an unretained analyte

An unretained analyte is a species on which there is no specific force acting that would make it to move relatively to the carrier. Its molar fraction is constant anywhere in the channel. If the carrier concentration is not uniform because of the temperature gradient, its concentration is also non-uniform. The elution time, $t_o^{(2)}$, of such an unretained analyte is given by:

$$t_o^{(2)} = \frac{L}{\bar{u}} \quad (21)$$

where \bar{u} is the mean velocity of the unretained analyte, which is then defined, for a non-uniform carrier density, as:

$$\bar{u} = \int_0^1 \frac{\rho(s)}{\langle \rho \rangle} u(s) ds \quad (22)$$

where $u(s)$ is the carrier velocity profile. Noting that the flow velocity, u , of a lamina of thickness dx is related to the elementary mass flow-rate, $d\dot{m}$, of carrier in that lamina by:

$$u = \frac{d\dot{m}}{\rho b dx} \quad (23)$$

one gets from Eqs. (5), and (21) to (23):

$$t_o^{(2)} = \frac{bwL\langle \rho \rangle}{\dot{m}} = \frac{V_o\langle \rho \rangle}{\dot{m}} \quad (24)$$

Comparison of Eqs. (19) and (24) with help of Eq. (7) shows that:

$$t_o^{(2)} = t_o^{(1)} \quad (25)$$

The two above definitions of t_o are thus identical. It is interesting to note that this second definition of t_o is coherent with that for a species having a zero Soret coefficient. Indeed, putting $S_T=0$ in Eq. (3) and combining with Eqs. (1) and (10) shows that its concentration is given by:

$$\frac{dc}{c} = \frac{d\rho}{\rho} \quad (26)$$

Hence the relative concentration profile of an unretained analyte is equal to the relative density profile of the carrier, which justifies the use of the ratio $\rho/\langle \rho \rangle$ instead of $c/\langle c \rangle$ in the integral of Eq. (22).

2.3.3. Classical definition from the average carrier velocity

Application of a classical expression leads to a third definition of the time from the cross-sectional average carrier flow velocity, $\langle u \rangle$:

$$t_o^{(3)} = \frac{L}{\langle u \rangle} \quad (27)$$

with:

$$\langle u \rangle = \int_0^1 u ds \quad (28)$$

Comparison of Eqs. (22) and (28) clearly shows that $\langle u \rangle \neq \bar{u}$ when ρ depends on x . Hence $t_o^{(3)}$ is not equal to $t_o^{(2)}$ or $t_o^{(1)}$.

One can estimate the ratio of the relative error between these two times in a relatively simple

manner for linear density and temperature profiles and when the velocity profile is expressed by a third-degree polynomial to account for the deviation from the Poiseuille parabolic profile due to the temperature dependence of the carrier viscosity:

$$\frac{u}{\langle u \rangle} = 6[(1 + \nu)s - (1 + 3\nu)s^2 + 2\nu s^3] \quad (29)$$

where ν is the flow distortion parameter [2,5]. Using Eqs. (6), (13), (14), (22), (28) and (29), one gets:

$$\frac{\bar{u}}{\langle u \rangle} = 1 + \frac{1}{10} \frac{\gamma_c \Delta T \nu}{1 - \frac{1}{2} \gamma_c \Delta T} \quad (30)$$

Hence, the relative error made when using the void time defined by Eq. (27) instead of the true void time is about $\gamma_c \Delta T \nu / 10$. For $\Delta T = 50$ K, $\gamma_c = 10^{-3} \text{ K}^{-1}$, and a typical value of ν of -0.15 [7], this error is about -0.077% , which is negligible as it is quite lower than the experimental uncertainties.

Of course, this error becomes identically zero when $\Delta T = 0$, but it is interesting to note that it also vanishes when $\nu = 0$, i.e., for a parabolic velocity profile. This anecdotal but surprising result arises from the hypothesis of a linear density profile underlying Eq. (30). The error would not vanish for a non-linear density profile and a parabolic velocity profile.

2.3.4. Void time determined from the knowledge of the geometrical channel volume

When the experimental determination of the elution time of an unretained species cannot be done, the void time is sometimes computed from the knowledge of the geometrical volume of the channel and the experimental measurement of the volumetric flow-rate, \dot{V}_{meas} , at temperature T_{meas} , as:

$$t_o^{(4)} = \frac{V_o}{\dot{V}_{\text{meas}}} \quad (35)$$

This expression is essentially identical to Eq. (2) when T_{meas} is equal to the room temperature T_{amb} at which operates the carrier pump. Thus, \dot{V}_{meas} may eventually represent the nominal volumetric flow-rate delivered by the pump. From Eqs. (7), (19), (20) and (35), it comes:

$$t_o^{(4)} = t_o^{(1)} \frac{\rho_{\text{meas}}}{\langle \rho \rangle} \quad (36)$$

where ρ_{meas} is the carrier density at T_{meas} . Clearly, the void time calculated using Eq. (35) generally differs from the true void time. Assuming a linear temperature profile and a linear temperature dependence of density, the relative difference between $t_o^{(4)}$ and $t_o^{(1)}$ is obtained by combining Eqs. (13), (14) and (36):

$$\frac{t_o^{(4)} - t_o^{(1)}}{t_o^{(1)}} = \gamma_{\text{ave}} (T_{\text{ave}} - T_{\text{meas}}) \quad (37)$$

where γ_{ave} is the value of the thermal expansion coefficient at the average temperature in the channel. For a typical value of γ of 10^{-3} K^{-1} and $\Delta T = 50$ K, the error made on the true void time when using Eq. (35) with the flow-rate measurement made at the temperature of the cold wall amounts to about 2.5%. It would be larger if the cold wall is at a temperature larger than that at which the volumetric flow-rate is measured. This systematic error on t_o , is significant, and the conclusions derived from the determination of t_R/t_o may be erroneous if this error is not properly taken into account.

3. Experimental

The thermal FFF channel was homemade by cutting a void in a 100- μm thick Mylar sheet, with a tip-to-tip length of 44.7 cm and a breadth of 2.0 cm. The Mylar sheet was clamped between two nickel-plated copper bars. The channel was oriented horizontally. The top plate was heated by means of electrical resistances and the bottom bar was cooled by a flow of tap water. The carrier liquid was delivered by a syringe-type pump (Model A-99, Razel Scientific Instruments, Stamford, CT, USA). The volumetric flow-rate, measured at room temperature was selected equal to 0.1 ml min^{-1} . The pump setting was maintained unchanged in all experiments and the flow-rate was presumed constant. The sample was injected by means of a homemade six-port injection valve with a 20- μl sample loop. The signal from a refractive index detector (Model R401, Waters Associates, Milford,

MA, USA) was recorded using a data acquisition board (Model DAS-801, Keithley, Taunton, MA, USA).

Ethylbenzene was selected as carrier liquid because the thermodiffusion coefficients of some polymers, like polystyrenes, in this solvent are relatively large and because of its relatively high boiling point [4]. Experiments were conducted with cold wall temperatures ranging from 292.7 to 335.7 K and ΔT varying from 0 to 51.0 K.

The density of ethylbenzene was calculated using the Hankinson–Brobst–Thomson method recommended by Reid et al. [18] for every K between 273 and 400 K. The results were fitted according to Eq. (9) by a least mean square regression. This gave: $r_0 = 1.071568$, $r_1 = -5.03562 \times 10^{-4}$, $r_2 = -5.60774 \times 10^{-7}$ with a correlation coefficient differing from 1 by less than 10^{-5} , when ρ and T are expressed in g cm^{-3} and K, respectively. The relative thermal conductivity at the cold wall was calculated assuming that κ_T varies linearly with temperature, with $d\kappa_T/dT = -2.33 \times 10^{-4} \text{ W m}^{-1} \text{ K}^{-2}$ and $\kappa_T = 0.132 \text{ W m}^{-1} \text{ K}^{-1}$ at 293 K [7].

4. Results and discussion

In order to verify the influence of the temperature in the channel on the void time, experiments were conducted by injecting ethylbenzene. Although the sample and the carrier liquid were the same, a solvent peak was observed at the void time with the differential refractometer, possibly because of a slight difference in the amount of some low molar mass impurity between them. A variation in this void time was observed as a function of T_c and ΔT . The void time was computed from the experimentally elution time by removing the dead time spent in the connections between injection valve and channel inlet and between channel outlet and detector. The dead time was determined by connecting the inlet tubing to the outlet tubing by means of a zero-dead volume connector and measuring the elution time at the same volumetric flow-rate delivered by the pump at room temperature as in the thermal FFF runs.

The values of the corrected void time are reported in Fig. 1 as a function of the average temperature in the channel, T_{ave} , defined by Eq. (15). Clearly, the void time decreases with increasing average tempera-

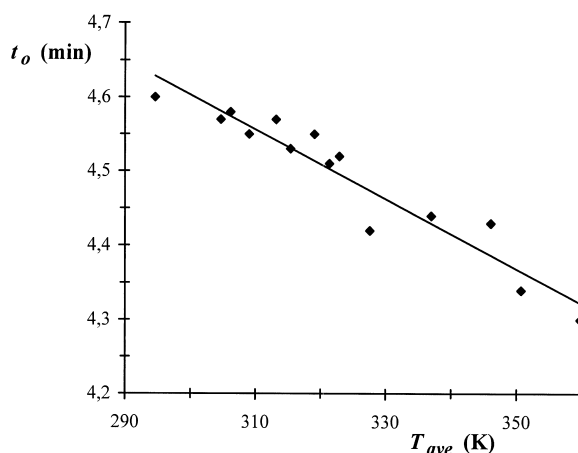


Fig. 1. Variation of the void time (corrected for connection dead time) versus average channel temperature, T_{ave} . The solid line represents the least mean square linear regression fit.

ture. This trend is consistent with the fact that the mass of carrier liquid contained in the channel decreases with increasing temperature. For a constant mass flow-rate of carrier, less time is required to replace the amount of liquid in the channel when its mass is lower. The (t_o, T_{ave}) data points fit reasonably a linear plot as seen on Fig. 1 showing the least mean square regression line. This can be expected from the discussion above. Indeed, it can be assumed that the mean carrier density in the channel is equal to the density of the average temperature as expressed by Eq. (14) (the relative difference between these two quantities for ethylbenzene with $T_c = 293$ K and $\Delta T = 50$ K is 0.027%). Then, from Eqs. (13) and (24), one gets:

$$t_o = \frac{V_o}{\dot{m}} \rho_o [1 - \gamma_o (T_{\text{ave}} - T_o)] \quad (38)$$

where γ_o is the thermal expansion coefficient at some reference temperature T_o . For a constant mass flow-rate, this equation predicts that t_o decreases linearly with increasing T_{ave} . Let a and b be the parameters of the least mean square linear regression of the (t_o, T_{ave}) experimental data points:

$$t_o = a - bT_{\text{ave}} \quad (39)$$

Combining this equation with Eq. (38) allows to derive the thermal expansion coefficient at T_o as:

$$\gamma_o = \frac{1}{\frac{a}{b} - T_o} \quad (40)$$

Using the regression coefficients of the linear plot in Fig. 1 ($a=6.0119$ min and $b=4.6948 \times 10^{-3}$ min K^{-1}) and taking T_o as the average of all experimental T_{ave} values in Fig. 1, i.e., $T_o=323.4$ K, one gets $\gamma_o=1.045 \times 10^{-3}$ K^{-1} , which compares quite favorably with the value directly computed at this temperature from Eq. (11), which gives $\gamma=1.062 \times 10^{-3}$ K^{-1} at 323.4 K. This good agreement clearly demonstrates that the thermal expansion of the carrier liquid is responsible for the variation of t_o . In fact, it can be proposed to use plots like the one in Fig. 1 for the accurate experimental determination of thermal expansion coefficients.

Furthermore, this agreement indicates that the key parameter in controlling the value of t_o is the average temperature, T_{ave} , in the channel and not the actual T_c and ΔT values. A high T_c and a low ΔT will provide the same value of t_o as a relatively low T_c and high ΔT if T_{ave} is the same in both cases, provided of course that the mass flow-rate is kept constant. The range of variations of t_o in the experiments is relatively important and amounts to about 7% as seen on Fig. 1. Clearly, this is significant and the determination of t_o from the knowledge of the geometrical volume of the channel will lead to serious errors if it is not corrected for the variation in carrier density between the temperature at which the pump is operating and the average temperature in the channel. This is especially true when the cold wall temperature is varied to study its influence on thermodiffusion or when experiments are performed at a high T_c to satisfy solubility requirements, such is the case, for instance, in polyolefin analyses.

There is some scatter of the data points in Fig. 1 around the regression line. Each data point is the mean of three experimental measurements in identical temperature conditions and the scatter observed in Fig. 1 is larger than that due to the repetition of the experiments. It is suspected that this comes principally from variations of the room temperature over the duration of the experiments and, thus, from slight variations in the carrier mass flow-rate delivered by the pump.

The void time t_o should remain constant if it is

properly corrected by the average carrier density in the channel, according to Eq. (24). For a given experimental set of T_c and ΔT , the average carrier density has been computed by means of Eq. (12). In Fig. 2 are plotted the variations of $t_o/\langle\rho\rangle$ versus T_{ave} . The scatter of the data noted in Fig. 2 is obviously still observed in this plot, but there is no trend of systematic variation of $t_o/\langle\rho\rangle$ which appears constant whatever T_{ave} . This is in accordance with Eq. (24) for a given mass flow-rate. Again, this demonstrates that the void time is directly proportional to the average carrier density in the channel, and, thus, to the mass of carrier contained in the channel.

As mentioned in the Section 2, the true void time can be obtained by measuring the elution time of an unretained analyte. One may question the validity of the assumption that a low molar mass analyte is truly unretained. Indeed, it is likely that, when placed in a temperature gradient, the mixture of such an analyte with the carrier will lead to a partial segregation effect, characterized by a Soret coefficient. Typically, values of S_T of mixtures of low molar mass compounds are of the order of a few 10^{-3} K^{-1} [19]. In the high λ limit, it was shown that t_R/t_o can be expressed as [20]:

$$\frac{t_R}{t_o} = 1 - \frac{\nu}{10\lambda} \quad (41)$$

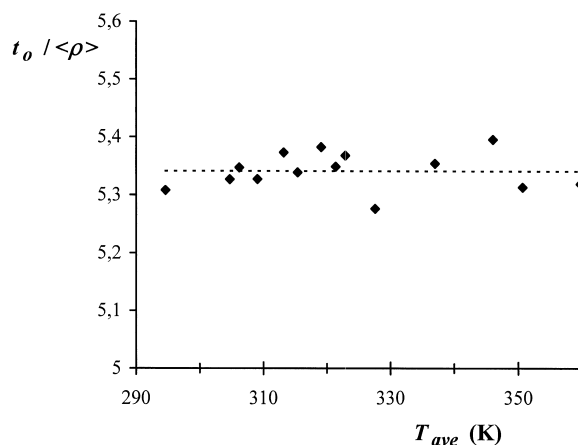


Fig. 2. Variation of the experimental void time corrected for connection dead volume and normalized by the average carrier density in the channel calculated using Eq. (12), $t_o/\langle\rho\rangle$, versus average channel temperature, T_{ave} . The dotted line corresponds to the mean value of $t_o/\langle\rho\rangle$.

Combining this equation with Eq. (3), and assuming a linear temperature gradient, the relative difference, $\Delta t/t_o$, between the elution time of a low molar mass probe of t_o and the true void time is equal to:

$$\frac{\Delta t}{t_o} = \frac{\nu S_T \Delta T}{10} \quad (42)$$

If the Soret coefficient of this probe is $S_T = 3 \times 10^{-3} \text{ K}^{-1}$, $\Delta T = 50 \text{ K}$ and $\nu = -0.17$ for ethylbenzene, the relative difference becomes -0.26% . This is an order of magnitude smaller than the error made by using Eq. (35) for determining t_o and is generally smaller than the experimental uncertainties on t_o . Therefore, even if the extent of the Soret effect of a low molar mass analyte is not known, the elution time of such an analyte can be taken as a reasonable measure of t_o .

It has been assumed up to now that the change in the average carrier density in the channel is entirely due to variations in temperature. In some cases, the thermal FFF channel is pressurized in order to increase the boiling point of the carrier for selecting an elevated hot wall temperature [21,22]. If P_{ch} is the pressure in the channel, the average carrier density at P_{ch} is equal to that at or near the atmospheric pressure, P_o , amplified by a factor $[1 + \beta(P_{ch} - P_o)]$. For a 15-bar increase of the channel pressure [22] and a typical value of β of 10^{-4} bar^{-1} , the resulting correction is 0.15%. This correction is usually negligible. Its effect on t_o is, in any case, much smaller than that resulting from the increase of the average channel temperature that the pressurization allows.

5. Conclusion

The results of the experimental measurements of the void time when varying the cold wall temperature and the temperature difference across the walls of a thermal FFF channel clearly show, in agreement with the equations developed in Section 2, that the void time depends significantly on the average carrier density, hence on the average temperature in the channel.

This average temperature influences also the accuracy of the void time determination by the break-

through method, which, although mainly developed for application to flow FFF [3], is sometimes used in thermal FFF. Regarding this influence, three points can be noted. First, because the breakthrough method is based on the appearance of the first tracer molecules or particles moving through the channel at the fastest flow velocity, it is preferentially performed without applying the transversal field, i.e., in isothermal conditions in a thermal FFF channel. In these conditions, it is obvious that the influence of the temperature gradient on the void time is not manifested, which necessarily leads to an error in void time determination unless the constant temperature at which this determination is performed is equal to the average temperature at which the thermal FFF retention or separation occurs. Second, if the void time determination was performed under application of the temperature gradient by means of a low diffusivity species, as suggested to minimize lateral diffusional transport [3], there would be a possibility that such a high molar mass species exhibits a relatively large thermophoretic mobility. The first eluting molecules would then, in average, move at a lower velocity than that of the fastest flow streamline, failing to allow a correct estimation of the latter. Third, even if it was not so, one would be able to relate this latter velocity to the average carrier velocity, which, as seen above, leads to an erroneous determination of the void time. Therefore, for all these reasons, the breakthrough method does not allow a correct estimation of the thermal FFF void time.

Accordingly, the main conclusions which can be drawn from the present study are presented below.

1. For accurate determinations of the basic FFF retention parameter, λ , from retention times measurements, the influence of the temperature dependence of carrier density in the channel must be taken into account.
2. The true void time, t_o , can be experimentally measured from elution time of the center-of-gravity of the solvent peak.
3. Alternatively, the elution time of a low molar mass solute can be used as a measure of t_o . Even if the Soret coefficient of this void time marker in the carrier liquid is not known, its influence on t_o is most likely negligible.
4. If it is not possible to use a low molar mass

solute, the void time can be estimated from the knowledge of the geometrical volume of the channel, V_o , and from the volumetric carrier flow-rate, \dot{V}_{meas} , measured at the temperature T_{meas} as:

$$t_o = \frac{V_o}{\dot{V}_{\text{meas}}} \frac{\langle \rho \rangle}{\rho_{\text{meas}}} \quad (43)$$

where ρ_{meas} is the carrier density at T_{meas} and $\langle \rho \rangle$, the average carrier density in the channel given by Eq. (12). Alternatively, t_o can be approximated by:

$$t_o = \frac{V_o}{\dot{V}_{\text{meas}}} \frac{1}{1 + \gamma_{\text{ave}}(T_{\text{ave}} - T_{\text{meas}})} \quad (44)$$

where γ_{ave} is the thermal expansion coefficient of the carrier at the average temperature, T_{ave} , in the channel (i.e., at $T_c + \Delta T/2$). Eq. (44) is based on the assumption that the carrier density varies linearly in the T_{meas} to T_{ave} temperature range. Eventually, \dot{V}_{meas} can be taken as the volumetric flow-rate delivered by the pump (provided it is accurate) and T_{meas} as the temperature at which the pump operates (usually room temperature).

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Personal note

I first met Professor Josef Huber in the early seventies during his visits to the laboratory where I was a Ph.D. student. I was struck by the real attention that this well-renowned pioneer of high performance liquid chromatography was paying to a beginner in the field and it was a great pleasure and honour for me that he accepted to be member of my thesis defence jury. I have often appreciated the great clarity of his presentations and I have been impressed by his leadership qualities, especially when he was chairing scientific meetings or conducting official, sometimes politically difficult, discussions devoted to the development of the cooperation

between Eastern and Western European chromatographers.

Professor Huber left us with the souvenir of a man of great human qualities with a vast culture.

Michel Martin

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