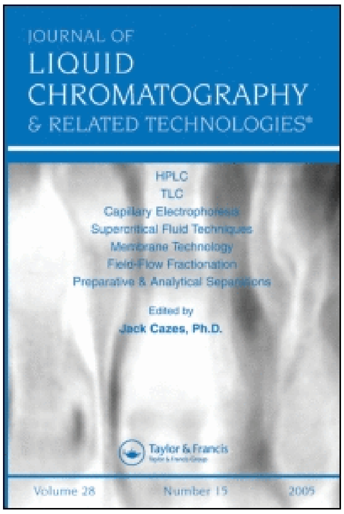

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CALIBRATION FOR INSTRUMENTAL SPREADING IN SIZE EXCLUSION CHROMATOGRAPHY BY A NOVEL RECYCLE TECHNIQUE

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ABSTRACT

So far, the best available techniques for the instrumental spreading calibration in size exclusion chromatography (SEC) provide the first moments of the spreading function, and require the measurement of several recycles. This work presents a method that enables the calculation of the spreading, making no "a priori" assumptions with regards to its shape, from the information contained in the original and first recycle chromatograms of a narrowly distributed standard only. Then, the corrected chromatogram may be obtained. The technique is tested on experimental data.

INTRODUCTION

In order to correct chromatograms obtained in SEC (or hydrodynamic chromatography) for instrumental broadening, the following integral equation due to Tung [1] is employed:

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$$z(t) = \int_{-\infty}^{+\infty} g(t,\tau)\, u(\tau)\, d\tau \qquad (1)$$

where t,τ : represent the elution time or the elution volume;

$z(t)$: is the measured chromatogram;

$g(t,\tau)$: is the time-varying, non-uniform or non-stationary spreading function; and

$u(t)$: is the corrected chromatogram.

For a fixed $\tau=\tau_1$, a function $g(t,\tau_1)$ of one independent variable is obtained. The retention time τ_1 represents the idealized elution time of the impulsive chromatogram that would be produced if a truly monodisperse polymer were injected into a chromatograph without spreading. Also $g(t,\tau_1)$ is the theoretical chromatogram that would be obtained if that same truly monodisperse polymer were injected into a real instrument with spreading.

If $u(t) = \delta(t-\tau_1)$, then $z(t) = g(t,\tau_1)$. In other words, $g(t,\tau_1)$ may be considered the non-causal response to a unit impulse applied at $\tau=\tau_1$. For this reason, the measured chromatogram $z(t)$ in eqn (1) may be thought of as the result of the process of injecting the signal $u(t)$ into a time-varying linear filter characterized by the set of impulse responses $g(t,\tau)$.

When the spreading may be assumed time-invariant, uniform or stationary (for example, because a narrowly distributed polymer is being analyzed), then

$$g(t,\tau) = g[(t-\tau),0] \qquad (2)$$

For notational convenience, we shall drop the indication of when the impulse is applied, and shall define the uniform spreading as:

$$g(t) = g(t,0) \tag{3}$$

Thus, eqn (1) reduces to the convolution integral:

$$z(t) = \int_{-\infty}^{+\infty} g(t-\tau)\, u(\tau)\, d\tau \tag{4}$$

The convolution of two signals g(t) and u(t) may be also represented symbolically as:

$$z(t) = g(t) * u(t) \tag{5}$$

Several methods have been proposed to find u(t) based on the measured chromatogram and the spreading function. For example: [1] to [4] solve the deconvolution problem of eqns (4) and (5), while in [5] to [7] the more general inverse filtering operation of eqn (1) is considered.

With the exception of some biopolymers, truly monodisperse synthetic polymers are impossible to obtain. For this reason, the problem of calibration for instrumental broadening is not trivial. Note that even if truly monodisperse polymers were available, the corresponding retention time τ would be still indetermined. For this reason, it will be necessary to specify τ at some retention time in the elution time range covered by the chromatogram z(t).

Like in all previously reported methods, the technique that is proposed in this paper makes the basic assumption that

$g(t,\tau)$ is uniform in the range of elution times covered by $z(t)$. In a real situation, this hypothesis will be verified only if the molecular weight distribution of the analyzed polymer is very narrow. In this work, we shall reserve the symbol $g(t)$ to represent the spreading of a strictly uniform system, i.e., uniform in all the elution range covered by the given set of columns. Also, we shall call $h(t)$ the uniform spreading valid in a certain interval $[\tau_{1*},\tau_1^*]$ around the corresponding associated retention time τ_1. In this manner,

$$g(t,\tau) \cong h(t+\tau) \quad \forall \quad \tau \in [\tau_{1*},\tau_1^*] \tag{6}$$

Clearly, if the system is strictly uniform, then:

$$g(t,\tau) = h(t) \quad \forall \quad \tau \tag{7}$$

The existing techniques for the determination of $h(t)$ may be classified as follows:

i) Methods that assume narrowly distributed standards to be monodisperse (see [8]);

ii) Methods which employ standards of known molecular weight distribution (see [9]);

iii) Reverse flow techniques, e.g.: [10, 11]; and

iv) Recycle techniques, e.g.: [12] to [15].

The recycle techniques seem the most promising both from the experimental and the theoretical viewpoint. The first publication [12], allowed the calculation of the second moments of h(t) and u(t), but did not take into account the spreading due to the pump. An improvement to [12] is presented in [15], where the pump spreading and the calculation of up to the third moments of h(t) and u(t) are considered. The main limitations of that approach are, however, that the true shapes of h(t) and u(t) are not directly obtained, and that the measurement of several recycles is required.

This paper proposes a method which permits the determination of h(t) and u(t), without any "a priori" assumption with regards to their shapes. The method involves the injection of a narrowly distributed polymer. The required measurements are the original and first recycle chromatograms, obtained under the following conditions: when the instrument has columns, and when it is operated without the columns.

THEORY

Fig 1 shows the chromatograph configuration that is necessary for the application of a recycle technique. In recycle mode, the three way valve A is switched to the lower position, so that the detector effluent may be refed into the system.

The ideal pure fractionation model

In the ideal situation, the free volumes in the injector, the tubings, the detector and the pump are all infinitely

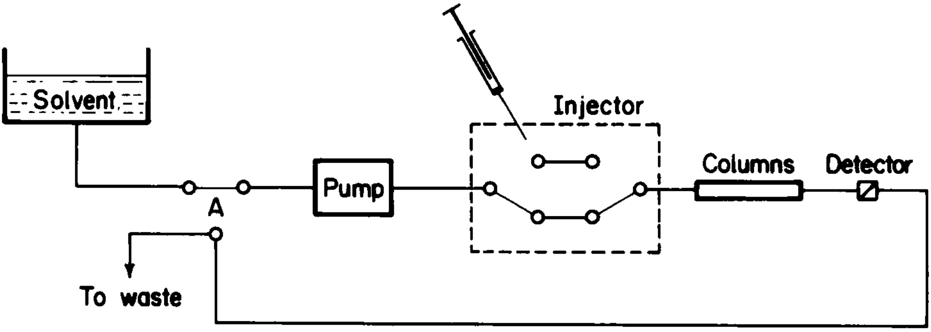

FIGURE 1: Required chromatograph configuration for the operation in recycle mode.

small; only pure fractionation is present; and no instrumental broadening occurs. Under such circumstances, the elution time for a particular molecular species in the ideal first recycle chromatogram without spreading $u_r(t)$ must exactly double the corresponding elution time in the ideal original chromatogram u(t). Also, the area under u(t) is the same as that under $u_r(t)$, because the total sample mass is constant. For the above reasons, the relationship between u(t) and $u_r(t)$ may be represented as follows:

$$u_r(t) = \frac{1}{2} u\left(\frac{t}{2}\right) \qquad (8)$$

Model with spreading

Call $h_d(t)$ the spreading due to the finite injection volume and to the broadening produced in the injector, in the detector, and in the piping between injector and detector. Let $h_c(t)$ be the spreading due to the columns alone. The total spreading h(t) is obtained by superposition of these two effects, i.e.:

$$h(t) = h_d(t) * h_c(t) \qquad (9)$$

If one assumes that no fractionation is produced outside the columns, then save a time shift, the chromatogram f(t) obtained with the instrument without the columns is representative of $h_d(t)$; i.e.:

$$h_d(t) = f(t+\theta) \qquad (10)$$

where θ is the retention time associated to f(t).

When the recycle mode is utilized, then the important spreading due to the pump $h_p(t)$ must also be considered. Denoting with $f_r(t)$ the first recycle chromatogram produced without the columns, one has:

$$f_r(t+\theta_1) = h_p(t) * h_d(t) * h_d(t) \qquad (11)$$

where θ_1 is the retention time associated to $f_r(t)$. Replacing eqn (10) into eqn (11), one obtains:

$$f_r(t+\theta_1) = h_p(t) * f(t+\theta) * f(t+\theta) \qquad (12)$$

Thus, the pump spreading may be found through a double deconvolution of $f_r(t)$ with f(t).

Assume now that the chromatograph is fit with the columns, that a narrow polymer is injected, and that the original chromatogram z(t) and its first recycle $z_r(t)$ are available. In this case,

$$z(t) = h(t) * u(t) \tag{13}$$

$$z_r(t) = h(t) * h(t) * h_p(t) * u_r(t) \tag{14}$$

If $h_p(t)$ is obtained as explained before, then this spreading may be "extracted" from eqn (14). Call $z_c(t)$ the resultant of deconvolving $z_r(t)$ with $h_p(t)$, i.e.:

$$z_r(t) = z_c(t) * h_p(t) \tag{15}$$

In a real situation, in addition to the pure spreading without translation, a time delay d will appear in relation to the transfer of each molecular species through the pump and recycle tubing. Taking this last effect into account, eqn (8) may be rewritten as:

$$u_r(t+d) = \frac{1}{2}\, u\left(\frac{t}{2}\right) \tag{16}$$

and from eqns (14) to (16) one obtains:

$$z_c(t+d) = h(t) * h(t) * \left[\frac{1}{2}\, u\left(\frac{t}{2}\right)\right] \tag{17}$$

In what follows, the time delay d may be neglected without loss of generality. Thus, consider the Fourier transform of eqn (13), and of eqn (17) with d=0:

$$Z(w) = H(w)\, U(w) \tag{18}$$

$$Z_c(w) = H^2(w)\, U(2w) \tag{19}$$

where w is the frequency in the transform domain. Eqn (19) provides:

$$Z_c\left(\frac{w}{2}\right) = H^2\left(\frac{w}{2}\right) U(w) \tag{20}$$

Dividing eqn (18) by eqn (20) one obtains:

$$\frac{Z(w)}{Z_c\left(\frac{w}{2}\right)} = \frac{H(w)}{H^2\left(\frac{w}{2}\right)} \tag{21}$$

Define:

$$B(w) = \frac{Z(w)}{Z_c\left(\frac{w}{2}\right)} \tag{22}$$

Eqn (22) is equivalent to:

$$z(t) = b(t) * [2\ z_c\ (2t)] \tag{23}$$

where b(t) is the time domain counterpart of B(w). B(w) may be calculated directly in the frequency domain through eqn (22). Alternatively, B(w) may be obtained by antitransformation of b(t), in turn obtained through eqn (23) by a double deconvolution of z(t) with a scaled version of $z_c(t)$.

From eqns (21) and (22) one has:

$$H(w) = B(w)\ H^2\left(\frac{w}{2}\right) \tag{24}$$

The calibration problem would be solved if H(w) could be calculated through the non-linear functional eqn (24), from the knowledge of B(w) only. This problem will be analyzed in a following section. Note finally that if h(t) is known, then u(t) can be calculated through:

$$z(t) = \int_{-\infty}^{+\infty} h(t-\tau)\ u(\tau)\ d\tau \tag{25}$$

The basic characteristics of b(t) and B(w)

Consider some conditions that must be verified by B(w) and b(t). Note first that since the areas under z(t) and $z_c(t)$ are equal, then $Z(0) = Z_c(0)$. Therefore, from eqn (22) one must have:

$$B(0) = 1 \tag{26}$$

i.e., the area under b(t) is unity. Now differentiate eqn (24) with respect to w. This gives:

$$H'(w) = B'(w)H^2\left(\frac{w}{2}\right) + B(w)\,H\left(\frac{w}{2}\right)\,H'\left(\frac{w}{2}\right) \tag{27}$$

Since H(0) = 1, bearing in mind eqn (26), and considering w = 0 in eqn (27), then it follows that:

$$B'(0) = 0 \tag{28}$$

For any transform pair x(t) <==> X(w), the k-th order moment of x(t) defined by:

$$m_k = \int_{-\infty}^{+\infty} t^k\, x(t)\, dt \qquad (k = 0,1,2,\ldots) \tag{29}$$

may be calculated from:

$$(-j)^k\, m_k = \left.\frac{d^k H(w)}{dw^k}\right|_{w=0} \tag{30}$$

Thus, the mean value of b(t)that we shall call b, may be obtained through:

$$b = \frac{m_1}{m_0} = \frac{B'(0)}{B(0)} = 0 \tag{31}$$

i.e., b(t) has zero mean.

The solution to equation (24)

It is easy to show that if $H(w)$ is a solution to eqn (24), then:

$$H_1(w) = H(w)\exp(sw) \tag{32}$$

(where s is a complex) is also a solution.

Consider first s pure imaginary and equal to jT. Call $h(t)$ and $h_1(t)$ the inverse transforms of $H(w)$ and $H_1(w)$, respectively. Eqn (32) indicates that if $h(t)$ is a solution, then any time-shifted version of it: $h_1(t) = h(t+T)$ is also a solution.

All viable solutions for eqn (24) must provide real functions when inverse transforming $H(w)$ or $H_1(w)$ into the time domain. For this reason, $|H(w)|$ and $|H_1(w)|$ are both even. This would not be the case if s were a complex $(\alpha+jT)$ with $\alpha\neq 0$ however, and for this reason, all non pure imaginary values of s should be rejected. This is so because if $|\exp[(\alpha+jT)w]| = \exp(\alpha w)$ is multiplied by an even $|H(w)|$, then $\exp(\alpha w).|H(w)|$ is not even.

The uncertainty with respect to the time origin of $h(t)$ is in fact inherent to the chromatographic problem, and is equivalent to the indetermination of the retention time τ_1 in $g(t,\tau_1)$. Because the calibration is performed with narrowly distributed polymers instead of monodisperse, the normal practice is to associate the retention time τ to some measure of central tendency of the corresponding distribution. Similarly, the time origin of $h(t)$ may for example be chosen as the mean value of that curve. In this case, it can be shown that the application of the so defined spreading in eqn (25) will generate a corrected chromatogram u(t) with its mean coincident with that of z(t). Another possibility is to assign t=0 at the maximum (mode) of h(t). This selection is intuitively appealing because in this manner the hypothetical impulse associated with h(t) is assumed to occur at the instant in time when the polymer concentration is at its maximum.

We shall now derive a formula that is based on eqn (24), and allows the calculation of H(w) from the knowledge of B(w) only. Consider the identity:

$$H(w) = H(w) \cdot \frac{H^2(\frac{w}{2})}{H^2(\frac{w}{2})} \cdot \frac{H^4(\frac{w}{4})}{H^4(\frac{w}{4})} \cdots \qquad (33)$$

$$H(w) = \prod_{i=0}^{\infty} \frac{[H(\frac{w}{2^i})]^{(2^i)}}{[H(\frac{w}{2^{i+1}})]^{(2^{i+1})}} \qquad (34)$$

But from eqns (22) and (24), one may write:

$$[B(\frac{w}{2^i})]^{(2^i)} = \frac{[H(\frac{w}{2^i})]^{(2^i)}}{[H(\frac{w}{2^{i+1}})]^{(2^{i+1})}} \qquad (i = 0,1,2,\ldots) \qquad (35)$$

Replacing eqn (35) into eqn (34) one obtains:

$$H(w) = \prod_{i=0}^{\infty} [B(\frac{w}{2^i})]^{(2^i)} \qquad (36)$$

Eqn (36) provides the basis for the numerical solution of H(w). It may be shown that if H(w) is infinitely differentiable at w=0, the residual obtained when truncating the infinite product in eqn (34) at a finite value of i, will become arbitrarily small as $i \to \infty$. For this reason, a truncated version of eqn (36) may be used with satisfactory results.

The proposed method

The proposed technique involves narrowly distributed standards and may be summarized as follows:

a) Find the chromatograms without columns $f(t)$ and $f_r(t)$.

b) Find the chromatograms with columns $z(t)$ and $z_r(t)$.

c) Find the spreading due to the pump $h_p(t)$, through eqn (12).

d) Obtain the corrected first recycle chromatogram $z_c(t)$, through eqn (17). Note that an accurate determination of the dead time d is not required [see f) below].

e) Obtain $B(w)$ through eqn (22) or eqn (23).

f) Adjust $B(0) = 1$ and $b = 0$. This operation makes unnecessary the exact positioning of all intermediate curves intervening in the calculation of $b(t)$.

g) Solve for $H(w)$ through eqn (36).

h) Find $h(t)$ by inverse transformation of $H(w)$, adopting the time origin at some measure of central tendency of the former function.

i) Solve for $u(t)$ with eqn (2).

j) Assign τ at some measure of central tendency of $u(t)$.

EXAMPLE OF APPLICATION

Consider the calibration of a WATERS Ass. ALC/GPC 244 size exclusion chromatograph, fitted with the following set of μ-styragel columns: 500, 1000, 10000, 100000 and 1000000 Å. The carrier solvent was tetrahydrofurane (THF) at 1 ml/min. A UV-visible absorbance detector set at 254 nm was utilized. A polystyrene standard of a nominal weight average molecular weight Mw=9000 was analyzed. The chromatograph was linked to a Digital Minc 11 process computer for the data acquisition and baseline correction. Via floppy disks, the baseline-corrected chromatograms were transfered to a VAX 11/780 computer for the calculation of h(t) and u(t). The deconvolutions were carried out following the technique described in [7]. The forward and inverse discrete Fourier transforms were solved numerically through a fast Fourier transform algorithm. All computer programs were written in FORTRAN 77.

Fig 2 shows the required data, i.e.: the chromatograms obtained with and without the columns after discretization, normalization and baseline correction. The time axis is scaled in computer sampling instant numbers, with a sampling period of 7 secs.

The breadth of f(t) is very small compared to that of $f_r(t)$. For this reason, the spreading $h_d(t)$ was neglected, and the pump spreading $h_p(t)$ straightly adopted as a conveniently shifted version of the first recycle chromatogram $f_r(t)$, with t=0 assigned at its maximum.

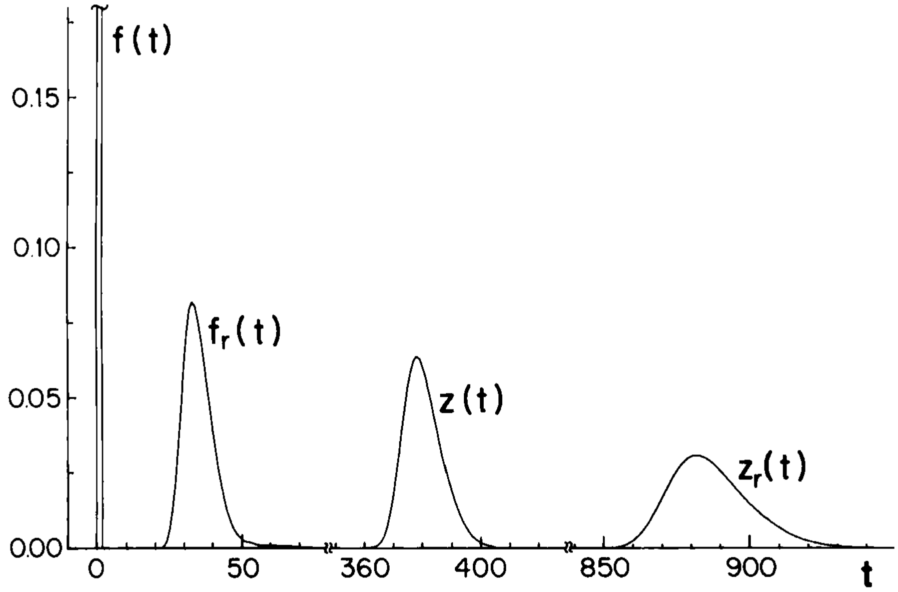

FIGURE 2: Basic required data for the application of the proposed method, i.e.: the baseline corrected and normalized chromatograms f(t), $f_r(t)$, z(t) and $z_r(t)$.

Fig 3 shows $z_r(t)$ and its deconvoluted version $z_c(t)$. A scaled version of $z_c(t)$: 2 $z_c(2t-d)$ is shown in Fig 3 superimposed with z(t). [Note that 2 $z_c(2t)$ would appear to the right of z(t)]. Then, b(t) is obtained by deconvolution, and represented with zero mean in Fig 3. Because z(t) and 2 $z_c(2t-d)$ are very similar to each other, this operation is particularly ill-conditioned, and therefore a robust deconvolution method must be employed.

B(w) was obtained by transformation of b(t), and H(w) calculated by application of eqn (36). The moduli of B(w) and H(w) are represented in Fig 4.

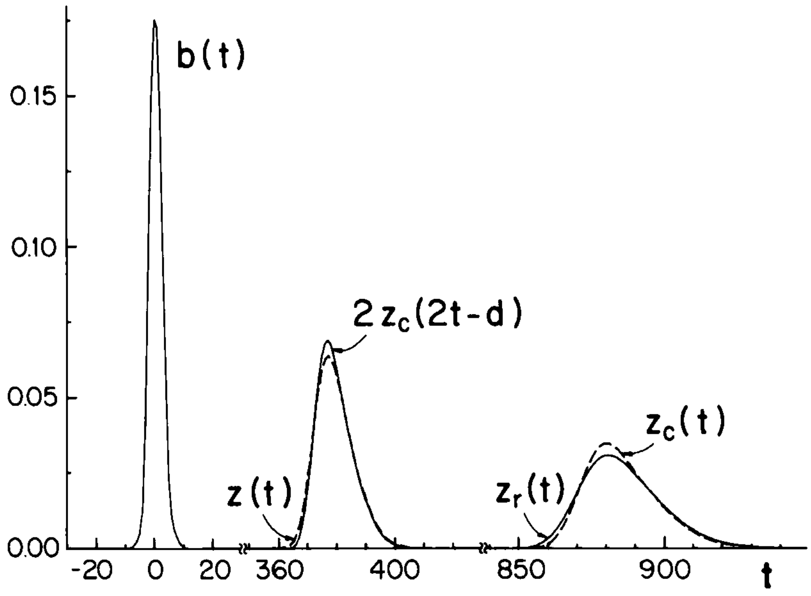

FIGURE 3: In the right hand side, $z_r(t)$ is compared with its deconvoluted version $z_c(t)$. Then, a scaled version of $z_c(t)$ is compared to z(t) (center figure). These last two curves provide the means of obtaining b(t) (left curves).

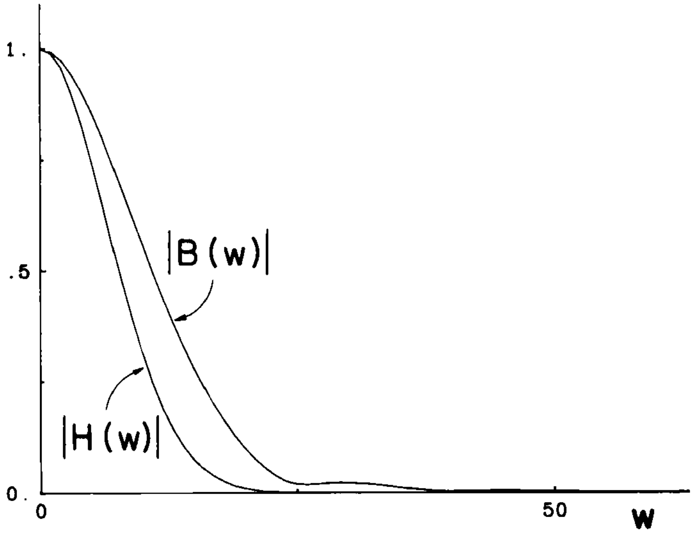

FIGURE 4: Moduli of B(w) and H(w).

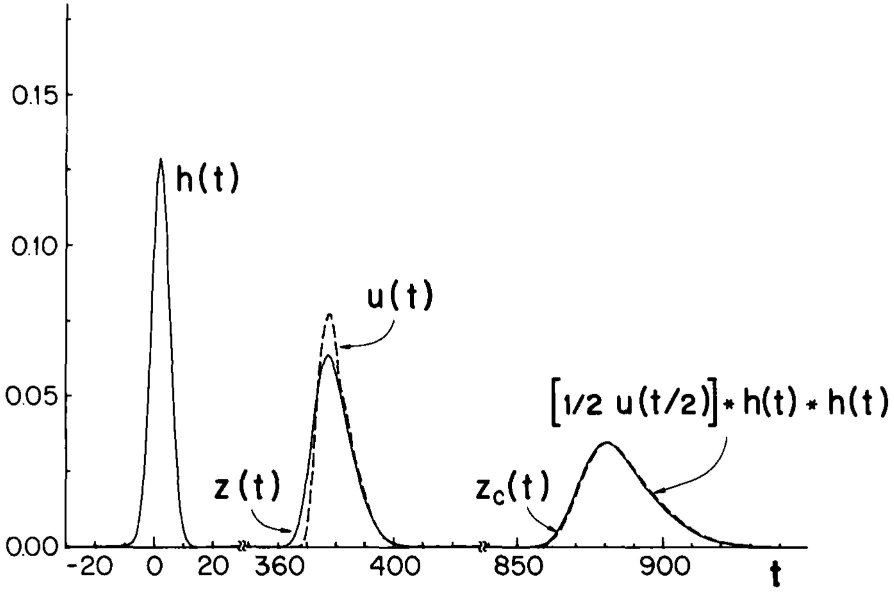

FIGURE 5: Illustrates the obtained h(t), the resulting u(t) and the validation check.

The spreading h(t) was obtained by inverse transformation of H(w), and the time origin specified at its maximum (see Fig 5). Through eqn (25), u(t) can be calculated. This corrected chromatogram is also represented in Fig 5, and the time to its maximum utilized to estimate the associated retention time $\tau = \tau_1$.

Finally, the calculations may be verified as follows. From eqn (16), the corrected first recycle chromatogram $z_c(t)$ should be equal to the resultant of convoluting twice a scaled version of the estimated input u(t) with the spreading h(t). The said comparison is shown in the right hand side of Fig 5, and confirms the validity of the proposed technique.

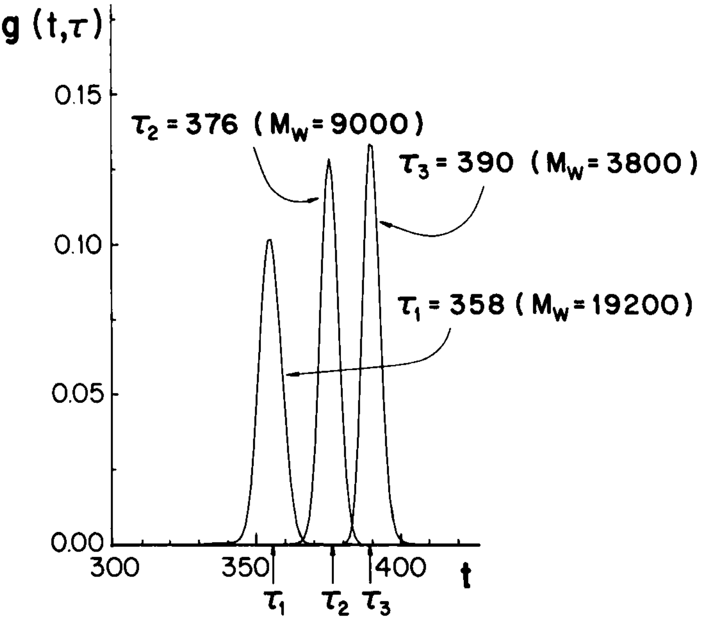

FIGURE 6: $g(t,\tau_i)$, (i = 1,2,3) for the three analyzed standards.

The procedure was repeated with two other polystyrene standards of Mw=3800 and 19200. The three resulting calibrations are represented in Fig 6.

DISCUSSION

This paper has presented a novel recycle technique for the spreading calibration in SEC. Its main advantage is that no 'a priori' assumptions with regards to the shapes of u(t) or h(t) are necessary.

Strictly speaking, the developed approach is valid only with continuous time functions and continuous Fourier transforms. The problem must be solved numerically through discrete approximations however, and for this reason some care must be taken on how to perform the operations.

The correction for instrumental broadening of the chromatograms obtained for each calibration standard should be prior to the calibration of the time axis in terms of molecular weights. This is so because the elution time of the arithmetic mean of u(t) should be made to correspond with the weight average molecular weight of the analyzed standard; or the elution time of the harmonic mean of u(t) made to correspond with its number average molecular weight.

If the proposed technique is applied to several calibration standards, then a set of h(t) functions with their corresponding retention times is obtained. This information provides the basis for the obtention of the non-uniform spreading $g(t,\tau)$. The determination of this function, and its further use in relation to the more general correction of eqn (1), will be the subject of a future communication.

The key tool for the numerical solution to the proposed problem is provided by eqns (22) and (36). These expressions allow the obtention of h(t) based on the measured chromatogram and on a corrected version of the first recycle chromatogram. In the given procedure, at least two deconvolutions must be performed, and some of these operations are particularly ill-conditioned. In such cases, the use of a robust numerical method is essential.

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REFERENCES

1) Tung, L.H., J. Appl. Polym. Sci., 10, 375 (1966).

2) Balke, S.T. and Hamielec, A.E., J. Appl. Polym. Sci., 13, 1381 (1969).

3) Pierce, P.E. and Armonas, J.E., J. Polym. Sci. C, 21, 23, (1968).

4) Alba, D. and Meira, G.R., J. Liq. Chromatogr., 6, 2411 (1983).

5) Chang, K.S. and Huang, Y.M., J. Appl. Polym. Sci., 16, 329 (1972)

6) Ishige, T., Lee, S.-I. and Hamielec, A.E., J. Appl. Polym. Sci., 15, 1607 (1971).

7) Alba, D. and Meira, G.R., J. Liq. Chromatogr., 7, 2833 (1984).

8) Hess, M. and Kratz, R.F., J. Polym. Sci., 10, 375 (1966).

9) Husain, A., Hamielec, A.E. and Vlachopoulos, J., J. Liq. Chromatogr., 4, 459 (1981).

10) Tung, L.H., Moore, J.C. and Knight, G.W., J. Appl. Polym. Sci., A-2, 10, 1261 (1966).

11) Tung, L.H. and Runyon, J.R., J. Appl. Polym. Sci., 13, 1397 (1969).

12) Waters, J.L., J. Polym. Sci.,part A-2, 8, 411 (1970).

13) Grubisic-Gallot, Z., Marais, L. and Benoit, H., J. Polym. Sci., Polym. Physics Edition, 14, 959 (1976).

14) Gruneberg, J. and Klein, J., J. Liq. Chromatogr., 3, 1593 (1980).

15) McCrakin, F.L. and Wagner, H.L., Macromolecules, 13, 685 (1980).