Theory of multiple-detection size-exclusion chromatography of complex branched polymers

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SUMMARY:

Size exclusion chromatography (SEC) separates complex branched polymers by hydrodynamic volume, rather than by molecular weight or branching characteristics. Equations relating the response of different types of detectors are derived including band broadening, by defining a distribution function $N'(M,V_h)$, the number of chains with molecular weight M and hydrodynamic volume V_h. While the true molecular weight distribution of complex polymers cannot be determined by SEC whatever detector is used, the formalism enables multiple detection SEC data to be processed to both analyze the polymer sample and reveal mechanistic information about the polymer synthesis. The formalism also

shows how the true weight- and number-average molecular weight, $\overline{M_w}$ and $\overline{M_n}$, can be obtained from correct processing of the hydrodynamic volume distributions.

Introduction

Size Exclusion Chromatography (SEC) is a major technique for analyzing synthetic and natural polymers.[1,2] Benoit[3,4] and others[5-7] have provided evidence that SEC separation occurs by the product of $[\eta] \times M$, where $[\eta]$ is the intrinsic viscosity and M the molecular weight (MW), and not according to MW only. Zimm and others demonstrated that the presence of long-chain branches significantly changes the hydrodynamic volume at a given MW.[8,9] While for linear polymers there exists a one-to-one relation between MW and the elution time, neglecting band broadening (the conventional calibration curve), this is not the case for complex branched polymers: a sample wherein all chains have the same hydrodynamic volume may contain a distribution of molecular weights.[5,10-12] Complex branched polymers not only have a distribution of MWs but also a distribution of chain branch lengths and topologies. They are different from regular branched polymers, like regular stars or combs. Complex branched polymers are present in several important families of polymers: (i) dendritic polymers, both biopolymers (e.g. amylopectin[13,14]) and synthetic polymers[15] (ii) statistically (or randomly) branched polymers[9] such as polyethylene, poly(vinyl acetate) and polyacrylates (some biopolymers like amylose having a similar structure containing a few long-chain branches[16]).

Many of the fundamentals needed for processing SEC data for complex branched polymers

have been given by Hamielec and co-workers.[5,10-12] The objectives of the present paper are to extend the work of Hamielec and others as follows:

(a) to define distribution functions which provide the basis for interpretation of SEC and similar data for complex branched polymers; these distribution functions are a generalization of the familiar number and mass distributions for the MW of linear polymers;

(b) to show how SEC data for both linear and branched polymers using a range of different types of online detectors (refractometer, viscometry, light scattering etc.) can be expressed in a single formalism which incorporates current and potential future modes of detection;

(c) to show how this formalism enables band broadening to be taken into account for distributions from any mode of detection, thus enabling new knowledge to be obtained from the detailed shape of these distributions[17-21] (rather than just averages such as $\overline{M_{\mathrm{w}}}$ and $\overline{M_{\mathrm{n}}}$, which are usually not strongly affected by band broadening in contemporary SEC devices[22]), and

(d) to present details of how true average molecular weights, $\overline{M_{\mathrm{n}}}$ and $\overline{M_{\mathrm{w}}}$, can be obtained. For complex branched polymers, the true molecular weight distribution (MWD) cannot be obtained; thus the averages obtained from these molecular weight

distributions are not true values, while they are however calculated this way by many (including some commercial SEC software). True hydrodynamic volume distributions will be defined and can be obtained using the methods given here, leading in turn to the calculation of the true values of $\overline{M_n}$ and $\overline{M_w}$.

While the details given here are for data from SEC, the expressions derived here are equally applicable to data for linear and branched polymers using other separation techniques, particularly other chromatographic techniques, for example ion chromatography,[23,24] field flow fractionation[25,26] and capillary gel electrophoresis.[27]

For complex branched polymers assuming no band broadening, universal calibration is valid using the product $[\eta]_w \times \overline{M_n}$,[5,10-12,28] (as proved also below using the new formalism), where $[\eta]_w$ is the weight-average intrinsic viscosity and $\overline{M_n}$ the number-average molecular weight of the distribution of chains eluting at one elution time t_{el} (or equivalently elution volume); this V_h-dependent $\overline{M_n}$ will be denoted $\overline{M_n}(V_h)$ in the case of the separation of complex branched polymers assuming no band broadening. Similarly, light-scattering detectors yield $\overline{M_w}(V_h)$[5,11]. Other types of detectors will yield different types of average response (e.g. NMR[29] and online osmometry[30] which give $\overline{M_n}(V_h)$). As well known but often unappreciated, the true molecular weight distribution cannot be obtained for complex branched polymers using SEC, even using multiple detection.[2,5]

An important question to ask in the characterization of complex branched polymers is just what sort of information is desired and for what purpose. (a) Frequently, useful information

comes from the molecular weight averages, such as $\overline{M_n}$ and $\overline{M_w}$.[31,32] However, there is also useful information in detailed distribution functions, as exemplified for linear polymers in the elucidation of the mechanisms of conventional[17,20] and controlled[21] free radical polymerization and starch biosynthesis[18]. More detailed distribution functions for complex branched polymers also have the potential to yield information on the synthetic processes involved in the formation of the target polymer. (b) Another use for appropriate SEC data is simply characterization to provide a means of differentiating polymer samples. For complex branched polymers, hydrodynamic volume distributions provide an interesting tool for comparative studies. (c) A third use is to utilize such data as a basis for structure-property relations.

For linear polymers, SEC data can be expressed entirely in terms of the number molecular weight distribution $N(M)$ (or equivalently the mass distribution $W(M)$ or the SEC distribution $w(\log M)$[17,33]). $N(M)$ (sometimes also noted $P(M)$[17]) is the number distribution of chains with molar mass M; the normalization of this and all other distributions is arbitrary. The other distributions are defined by:

$$W(M) = M\,N(M) \tag{1}$$

$$w(\log M) = M^2\,N(M) \tag{2}$$

Two of the issues considered here are (1) how the true $\overline{M_n}$ and $\overline{M_w}$ can be obtained from appropriate SEC data for complex branched polymers, and (2) what is the appropriate form of the distribution function corresponding to $N(M)$ for complex branched polymers. With regard to the former, we generalize the SEC expressions presented by Hamielec et al.[5] for obtaining $\overline{M_n}$ and $\overline{M_w}$ for complex branched polymers.

We now develop the new formalism, first for the case of linear polymers and then for

branched ones. Account is taken of the variety of different detectors in current and potential use: those which are sensitive to the number of polymer chains eluting at a given elution time (such as UV or fluorescent detection where each chain bears a single fluorophore), to their mass (refractive index detector, UV when sensitive to the monomer unit), to a product of mass and a size-related parameter (viscometric detection, online osmometry[30], light scattering), to their molar mass (NMR for oligomers[29]) and to their composition or tacticity (NMR[29,34]). The branching level could also be determined by SEC-NMR, especially in the case of hyperbranched polymers, which exhibit a high branching level. Using mass spectrometry as a detector, the equations would need to be refined, since mass spectrometry not only detects but also separates the sample coming through the detector. A complete separation may thus be used for some complex branched polymers coupling to MALDI TOF MS or Electrospray MS to SEC. However, MS techniques are biased toward high molecular weight, which thus may lead to biased results even when coupled with SEC. The present development of the formalism is confined to homopolymers; extension to copolymers may require to consider variations in the refractive index increment dn/dc[2]. Using multiple detection SEC, it is important to correct for inter-detector delay; this problem is not treated in the present work since it is already detailed in the literature[35-37].

All the equations are derived taking band broadening into account, and then simplified for the case when band broadening is negligible or corrected. We thus treat four different cases depicted in Figure 1: a) linear chains (or regular branched chains) with band broadening, b) linear chains without band broadening, c) complex branched chains with band broadening, d) complex branched chains without band broadening.

The reason that it can be necessary to include the possibility of band broadening is the following. While band broadening is often considered as minimal in many modern SEC set-

ups,[22] it has been found to significantly broaden molecular weight distribution in several cases.[38,39] It has been shown[40] that this broadening is sufficient to affect the fine details of the shape of the molecular weight distribution (or hydrodynamic volume distribution), and this detailed shape can be used to provide mechanistic information.[17-21] Moreover, band broadening is quite significant as soon as low flowrates have to be used (which is the case for high molecular weights or viscous eluents, see e.g. [41]).

New Formalism for the Response of SEC Detectors with Linear Polymers

While all the results in this section are well known, the new formalism necessary for proper interpretation of multiple detection SEC of complex branched polymers can be better understood if the distribution/observable relations for linear polymers are incorporated into this new formalism which takes band broadening into account.

The SEC signal $S_{det}(t_{el})$ obtained at elution time t_{el} using a detector of type 'det' for a linear polymer is given by the Tung convolution equation:[42-44]

$$S_{det}(t_{el}) = A_{det} \int_0^\infty G(t_{el},M)\ f_{det}(M)\ N(M)\ \mathrm{d}M \qquad (3)$$

where $f_{det}(M)$ is the 'detector function' defined below for chains with MW M, $G(t_{el},M)$ is the band-broadening function (which is independent of the nature of the polymer[45]) and A_{det} is a calibration constant for the particular instrument, eluent and polymer. Detailed derivations including all the relevant constants (refractive index increment, calibration constant for each detector etc.) are developed in the appendix. All these constants must be included in any treatment of SEC data but they are ignored in the main text for sake of clarity. For the special case of no band broadening, one has:

$$G(t_{el},M) \text{ (no band broadening)} = \delta\left(\tilde{t}_{el}\,(M) - t_{el}\right) \quad (4)$$

where the Dirac function δ is defined by:

$$g(a) = \int_{-\infty}^{+\infty} \delta(x - a)\, g(x)\, \mathrm{d}x \quad (5)$$

where g is any mathematically well-behaved function and the function $\tilde{t}_{el}\,(M)$ is the 'calibration curve' relating t_{el} and M.

In a real system with band broadening, $G(t_{el},M)$ is the distribution of elution times of a perfectly monodisperse distribution in M.

The function $f_{det}(M)$ is different for each detector. For a refractometer (which is mass-sensitive) one has:

$$f_{DRI}(M) = M \quad (6)$$

For a number-sensitive detector (either UV or fluorescence with a single fluorophore per chain), one has:

$$f_{NS}(M) = 1 \quad (7)$$

For molecular-weight sensitive detectors (light scattering, viscometer etc.), one has to consider that, at one particular elution time t_{el}, a mixture of different molecular weights elute through the detector and this detector can thus only yield a molecular weight average [case a) of Figure 1]. The function $G(t_{el},M)$ is however likely to be the same as the one used for mass- and number-sensitive detectors, since the band broadening in the detector cells is commonly negligible compared to band broadening before the detectors (axial dispersion in the columns etc.)[46] Band broadening can also be important after the viscometer, which is therefore always located at the end of the detectors line to overcome this problem.

Expression for the signal of the viscometer

While the software provided with a commercial online viscometer often processes the data to give an intrinsic viscosity and a molecular weight, this data processing uses the signal from two detectors: the viscometer and a concentration detector (e.g., refractometer). We here consider these signals separately, which is necessary to obtain the general formulae which, for example, can be used to correct band broadening, including for the case of complex branched polymers. An online viscometric detector measures a pressure drop between a line containing the polymer solution and one containing the pure eluent,[47] and this ratio is proportional to the specific viscosity $\eta_{sp} = (\eta/\eta_0) - 1$, where η is the viscosity of the solution and η_0 that of the pure eluent. In a sample in an increment of elution time from the SEC separation, one has a mixture of species with different MWs. From the Einstein impenetrable sphere result,[48] if a component i with mass concentration (mass per unit volume) c_i in a solution has a specific viscosity η_{sp}^i (that is the specific viscosity of a solution containing only this component at this mass fraction), then:

$$\eta_{sp} = \sum_i \eta_{sp}^i \tag{8}$$

Thus at a particular elution time, one has:

$$S_{visc}(t_{el}) = \int_o^\infty \eta_{sp}(M)\mathrm{d}M \tag{9}$$

where $\eta_{sp}(M)$ is the specific viscosity of a sample of chains with molar mass M.

The intrinsic viscosity $[\eta]$ of a mixture is given by $\eta_{sp}/\Sigma c_i$ (in the limit of low concentration). Furthermore, the mass in an increment $G(t_{el},M)\ \mathrm{d}M$ is $G(t_{el},M)\ M\ N(M)$ (assuming for the moment that $N(M)$ is normalized). Thus the intrinsic viscosity can be expressed as:

$$\eta_{sp}(M) = [\eta(M)]\ G(t_{el},M)\ M\ N(M) \tag{10}$$

where the volume as other constants is omitted.

The signal of the viscometer can then be expressed as a function of $[\eta(M)]$, the intrinsic viscosity of a chain of molecular weight M (treated as a continuous rather than a discrete variable, so that an integral replaces the summation):

$$S_{\mathrm{visc}}(t_{\mathrm{el}}) = \int_0^\infty G(t_{\mathrm{el}},M)\ [\eta(M)]\, M\, N(M)\, \mathrm{d}M \tag{11}$$

For the viscometer, the function $f_{\mathrm{visc}}(M)$ is thus:

$$f_{\mathrm{visc}}(M) = [\eta(M)]\, M \tag{12}$$

$f_{\mathrm{visc}}(M)$ is proportional to the hydrodynamic volume V_{h} (Equation (14)). $G(t_{\mathrm{el}},M)$ can be determined using the concentration-sensitive detector and deconvolution methods. Baumgarten et al. have given a comprehensive and critical review of the different methods used to correct band broadening.[46] Castro et al. proposed recently a new method using capillary electrophoresis to obtain the true (i.e. unbroadened) molecular weight distribution;[41] in the latter method, G can be any sufficiently flexible function, e.g. an exponentially modified gaussian. Equation (11) gives the means to correct band broadening on the online viscometer signal separately.

On combining the viscometer signal with the refractometer signal without correcting band broadening, an average intrinsic viscosity is determined:

$$\frac{S_{\mathrm{visc}}(t_{\mathrm{el}})}{S_{\mathrm{DRI}}(t_{\mathrm{el}})} = [\eta(t_{\mathrm{el}})] = \frac{\int_0^\infty [\eta(M)]\, G(t_{\mathrm{el}},M)\, M\ N(M)\, \mathrm{d}M}{\int_0^\infty G(t_{\mathrm{el}},M)\, M\ N(M)\, \mathrm{d}M} \tag{13}$$

This average intrinsic viscosity, $[\eta(t_{\mathrm{el}})]$, of the mixture of chains eluting at t_{el}, is thus proved here to be the mass-weighted average of the intrinsic viscosities of the components eluting at

t_{el}, as also stated by Balke and Hamielec et al.[2,5]

The viscometer can also yield a molecular weight, using a universal calibration curve. The universal calibration curve is established by injecting standards of known molecular weight and intrinsic viscosity (or known Mark-Houwink-Sakurada parameters in the eluent) to relate the product $[\eta] \times M$ to the elution time via a polynomial function. If band broadening is not corrected, the signal of the detector at t_{el} corresponds to the response to a mixture of chains with different hydrodynamic volumes (Figure 1 a). It is thus necessary to introduce an average hydrodynamic volume of the chains eluting at $t_{el.}$ Flory gives the following expression for the hydrodynamic volume of a chain of intrinsic viscosity $[\eta(M)]$ and molecular weight M:[49]

$$V_h = \gamma\, M\, [\eta(M)],\ \gamma = \frac{4}{3}\pi \frac{1}{6^{3/2}\Phi} \tag{14}$$

where Φ is the Flory constant. Φ is indeed known to vary with polymer architecture.[28]

The number-average hydrodynamic volume of the chains eluting at t_{el} is defined as:

$$\overline{V}_{h,n}\left(t_{el}\right) = \frac{\int_0^\infty G\left(t_{el}, M\right) V_h \; N(M)\, \mathrm{d}M}{\int_0^\infty G\left(t_{el}, M\right) N(M)\, \mathrm{d}M} \tag{15}$$

Equation (11), (14) and (15) can be used to express the viscometer signal as follows:

$$S_{visc}(t_{el}) = \overline{V}_{h,n}\left(t_{el}\right) \int_0^\infty G\left(t_{el}, M\right) N(M)\, \mathrm{d}M \tag{16}$$

If one uses a refractometer in conjunction with a viscometer, then combining Equation (16) with Equation (3) and (6) leads to:

$$\frac{S_{\text{visc}}(t_{\text{el}})}{S_{\text{DRI}}(t_{\text{el}})} = \overline{V}_{\text{h,n}}(t_{\text{el}}) \frac{\int_0^\infty G(t_{\text{el}}, M) N(M) \, \mathrm{d}M}{\int_0^\infty G(t_{\text{el}}, M) M \; N(M) \, \mathrm{d}M} \tag{17}$$

Combining the signal of a viscometer, a refractometer and using an universal calibration curve, one thus determines at each elution time a number-average molecular weight defined as:

$$\overline{M_{\text{n}}}(t_{\text{el}}) = \frac{\int_0^\infty G(t_{\text{el}}, M) M \; N(M) \, \mathrm{d}M}{\int_0^\infty G(t_{\text{el}}, M) N(M) \, \mathrm{d}M} \tag{18}$$

through the following relation:

$$\frac{S_{\text{visc}}(t_{\text{el}})}{S_{\text{DRI}}(t_{\text{el}})} = \frac{\overline{V}_{\text{h,n}}(t_{\text{el}})}{\overline{M}_{\text{n}}(t_{\text{el}})} \tag{19}$$

If band broadening is corrected or negligible (Figure 1 b), then the ratio of the signals of the viscometer and the refractometer gives simply:

$$\frac{S^*_{\text{visc}}(t_{\text{el}})}{S^*_{\text{DRI}}(t_{\text{el}})} = \frac{V_{\text{h}}}{M} \tag{20}$$

where $S^*_{\text{det}}(t_{\text{el}})$ corresponds to the signal of a detector corrected for band broadening. The true molecular weight distributions of linear (or regular branched) homopolymers can thus be obtained using universal calibration with an online viscometer and a refractometer if the band broadening is corrected or negligible.

Note that $\overline{M}_{\text{n}}(t_{\text{el}})$ can also be obtained from the ratio of the signal of a mass-sensitive detector (Equation (3) and (6)) to that of a number-sensitive detector (Equation (3) and (7)):

$$\frac{S_{\mathrm{DRI}}(t_{\mathrm{el}})}{S_{\mathrm{NS}}(t_{\mathrm{el}})} = \overline{M}_{\mathrm{n}}(t_{\mathrm{el}}) \tag{21}$$

or

$$\frac{S^{*}_{\mathrm{DRI}}(t_{\mathrm{el}})}{S^{*}_{\mathrm{NS}}(t_{\mathrm{el}})} = M \tag{22}$$

The latter equation has already been used to determine molecular weight, e.g. ref. [50].

Expression for the signal of light-scattering detectors

For a light-scattering (LS) based detector, using the Rayleigh relation[51] and assuming the injected concentration to be low enough, the function $f_{\mathrm{LS}}(M)$ can be expressed as:

$$f_{\mathrm{LS}}(M) = M^2\, P(\theta) \tag{23}$$

where $P(\theta)$ is the form factor. The signal of the light scattering is:

$$S_{\mathrm{LS}}(t_{\mathrm{el}}) = \int_0^{\infty} G(t_{\mathrm{el}},M)\, M^2\, P(\theta)\, N(M)\, \mathrm{d}M \tag{24}$$

Equation (24) can be used to correct band broadening on the light-scattering signal.

The light scattering can yield a molecular weight if one determines the form factor $P(\theta)$. If band broadening is significant but not corrected (Figure 1 a), then one takes a z-average form factor into account:

$$\overline{P(\theta)}_{\mathrm{z}} = \frac{\int_0^{\infty} G(t_{\mathrm{el}},M)\, P(\theta)\, M^2\, N(M)\, \mathrm{d}M}{\int_0^{\infty} G(t_{\mathrm{el}},M)\, M^2\, N(M)\, \mathrm{d}M} \tag{25}$$

If the band broadening is not corrected, the signal of the light scattering can be expressed by combining Equation (24) and (25):

$$S_{LS}(t_{el}) = \overline{P(\theta)}_z \int_0^\infty G(t_{el},M)\, M^2\, N(M)\, dM \tag{26}$$

Combining the signals of the light scattering and the refractometer, one obtains:

$$\frac{S_{LS}(t_{el})}{S_{DRI}(t_{el})} = \overline{P(\theta)}_z \frac{\int_0^\infty G(t_{el},M)\, M^2\, N(M)\, dM}{\int_0^\infty G(t_{el},M)\, M\, N(M)\, dM} \tag{27}$$

The weight-average molecular weight determined at each elution time is thus defined as:

$$\overline{M}_w(t_{el}) = \frac{\int_0^\infty G(t_{el},M)\, M^2\, N(M)\, dM}{\int_0^\infty G(t_{el},M)\, M\, N(M)\, dM} \tag{28}$$

$$\frac{S_{LS}(t_{el})}{S_{DRI}(t_{el})} = \overline{P(\theta)}_z\, \overline{M}_w(t_{el}) \tag{29}$$

If band broadening is corrected or negligible, the ratio of the signal of the light scattering and the refractometer is then no longer a function of average form factors and molecular weights:

$$\frac{S^*_{LS}(t_{el})}{S^*_{DRI}(t_{el})} = P(\theta)\, M \tag{30}$$

To deduce $\overline{M}_w(t_{el})$from the light-scattering signal which is the same as M in the case of negligible or corrected band broadening, one needs to know $\overline{P(\theta)}_z$, which is $P(\theta)$ without band broadening. Three different methods are used. The use of multiple-angle laser light scattering (MALLS) makes it possible to extrapolate to θ = 0 ° where the form factor is unity.[52] Low-angle laser light scattering (LALLS) uses an angle small enough to consider that the form factor is equal to 1 (there is however a critical molecular weight above which $P(\theta)$ decreases to values significantly below 1).[53] Triple detection uses the 90° angle,

RALLS, and calculates the form factor using relations given by Flory[49,51,54]:

$$P(\theta) = 2\frac{e^{-x} - (1-x)}{x^2} \tag{31}$$

with:

$$x(\theta) = \frac{8}{3} R_g \left(\frac{\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \right)^2 \tag{32}$$

where n is the refractive index of the eluent, λ is the wavelength of the laser and the radius of gyration R_g is obtained from:

$$R_g = \frac{1}{6^{1/2}} \left(\frac{M[\eta]}{\Phi} \right)^{1/3} \tag{33}$$

In the case of triple detection (θ = 90°):

$$\mathrm{x}(90°) = \frac{4}{3} R_g \left(\frac{\pi n}{\lambda} \right)^2 \tag{34}$$

In the case of triple detection, assuming the form factor $P(\theta)$ is equal to 1, an approximate molecular weight is calculated using RALLS (Equation (29) and (30)). This approximate MW value allows to estimate the radius of gyration R_g (using as well the intrinsic viscosity obtained from an online viscometer, Equation (33)) and then a better approximation of $P(\theta)$ (Equation (31) and (34)). More and more accurate approximations of the form factor and the molecular weight can be calculated through iterations until the values obtained are considered constant. If one does not correct for band broadening and if one proceeds through the iterations described above, then the numerical value for R_g in Equation (33) is calculated from the weight-average molecular weight, $\overline{M}_w(t_{el})$ (Equation (28)) and the weight-average viscosity from the viscometer signal (Equation (13)). If band broadening is not corrected, the

iteration process will be based on ill-defined R_g values and it appears thus of particular importance to correct all detector signals for band broadening before calculating any molecular weight by multiple-detection SEC techniques.

LALLS, MALLS and triple detection are the light-scattering methods most commonly used to determine molecular weight, and combine the light-scattering detector signal together with the signal of a concentration-sensitive detector, generally a refractometer. One can theoretically also deduce a molecular weight from the MALLS data alone: instead of extrapolation at $\theta = 0$ °, one then uses the slope of some relation between the Rayleigh ratio and the angle. The z-average radius of gyration and z-average molecular weight are then obtained.[52,55]

Molecular weight distribution

Taking now the specific case of refractometer detection without band broadening (Figure 1 b)) when Equation (4) is applicable, substituting this result and Equation (6) into Equation (3), and changing variable of integration from M to t_{el}, yields (using Equation (5)) the result (derivation detailed in appendix):

$$S_{DRI}^{*}(t_{el}) = \left. \frac{M\ N(M)}{\mathrm{d}\tilde{t}_{el}(M)/\mathrm{d}M} \right|_{\tilde{t}_{el}(M)=t_{el}} \tag{35}$$

By definition of w(log$M$) (Equation (2)

$$w(\log M) = \left. \frac{S_{DRI}(t_{el})}{\mathrm{d}\log M / \mathrm{d}\tilde{t}_{el}(M)} \right|_{\tilde{t}_{el}(M)=t_{el}} \tag{36}$$

The simplest case is obtained for a linear calibration curve and no band broadening:

$$\tilde{t}_{el}(M) \text{ (linear calibration)} = a + b \ln M \tag{37}$$

The SEC distribution is then simply the familiar result: $w(\log M) = S_{DRI}(t_{el})$.

Response of SEC Detectors for Complex Branched Polymers

General equations for detector responses

For complex branched polymers, even without band broadening, at a given elution time one may detect polymer chains having the same V_h, but different M as depicted in Figure 1 d).[5,10,12] To take this into account, we define $N'(M,V_h)$ as the number distribution of chains with molar mass M and hydrodynamic volume V_h; this distribution corresponds to $N(M)$ for linear polymers. There is a corresponding definition for the mass distribution $W'(M,V_h) = M\ N'(M,V_h)$. To take band broadening into account, the function $G(t_{el},M)$ is replaced by $G'(t_{el},V_h)$. $G'(t_{el},V_h)$ is the distribution of elution times of a perfectly monodisperse distribution in V_h. We thus assume in this case that band broadening is not significantly affected by the structure of the polymer chain. The general equation for the signal of a detector, replacing Equation (3), is thus:

$$S_{det}(t_{el}) = \int_0^\infty G'(t_{el},V_h) \left(\int_0^\infty f'_{det}(M,V_h)\ N'(M,V_h)\ dM \right) dV_h \qquad (38)$$

Equation (6) and (7) for number-sensitive detectors (UV, fluorescence detecting end-groups etc.), refractometer and light-scattering detectors remain unchanged, except that formally one replaces $f_{det}(M)$ by $f'_{det}(M,V_h)$, because none of the signals from these various detectors have a dependence on V_h.

It is important to be aware that, except where specifically stated, the inclusion of $G'(t_{el},V_h)$ enables signals for all detectors to be corrected explicitly for band broadening. Again it is emphasized that while band-broadening corrections for modern SEC devices have little effect on averages such as $\overline{M}_n$,[22] they are essential to take into account if one wants detailed

mechanistic information from the actual shape of the various distribution functions. If one knows the broadening function $G'(t_{el},V_h)$, then a number of procedures have been given[46] for numerically inverting the convolution equation, Equation (38). The form of $G'(t_{el},V_h)$ for a given SEC set-up can be obtained using ultra-narrow standards[46] or a new procedure[41] using broad standards which is widely applicable.

One can readily extend the definition of the distribution function $N'(M,V_h)$ to take account of detectors which can measure, for example, branching fraction ϕ (which could be determined by NMR as a function of hydrodynamic volume), in which case one would define a distribution $N'(M,\phi,V_h)$. Similar extensions for composition can also be made (possible dn/dc variations have then also to be considered).

While complex branching prevents from having a unique relation between M and t_{el}, a unique relation still exists between V_h and t_{el} as long as universal calibration holds (Figure 1 d)). However, band broadening also precludes obtaining a unique relation between V_h and t_{el} (Figure 1 c)).

Expression for the signal of the viscometer

The viscosity-sensitive detector is more complex. Recall that this detector measures a pressure drop ratio, which is proportional to the specific viscosity η_{sp}. In a sample in an increment of elution time from the SEC separation, one has a mixture of species for a complex branched polymer, particularly chains with different MWs. From the Einstein impenetrable sphere result at a particular elution time, one has:

$$S_{visc}(t_{el}) = \int_0^\infty \int_0^\infty G'\left(t_{el},V_h\right)\eta'_{sp}\left(M,V_h\right)\mathrm{d}M\ \mathrm{d}V_h \tag{39}$$

where $\eta'_{sp}(M,V_h)$ is the specific viscosity of a sample of chains with molar mass M and

hydrodynamic volume V_h. The intrinsic viscosity $[\eta]$ is given by $\eta_{sp}/\Sigma c_i$ (in the limit of low concentration). Furthermore, the mass in an increment $G'(t_{el},V_h)\ dM\ dV_h$ is $G'(t_{el},V_h)\ M\ N'(M,V_h)$ (assuming for the moment that $N'(M,V_h)$ is normalized). Thus the intrinsic viscosity can be expressed as:

$$\eta_{sp}(M,V_h) = [\eta(M)]\ G(t_{el},V_h)\ M\ N'(M,V_h) \tag{40}$$

where the volume as other constants is omitted.

$$S_{visc}(t_{el}) = \int_0^\infty \int_0^\infty G'\left(t_{el},V_h\right)\left[\eta'\left(M,V_h\right)\right] M\ N'\left(M,V_h\right) dM\ dV_h \tag{41}$$

Equation (41) enables one to correct the viscometer signal for band broadening.

For the viscometer, the function $f'_{visc}(M,V_h)$ is:

$$f'_{visc}(M,V_h) = [\eta'(M,V_h)]\ M \tag{42}$$

If one combines the viscometer signal with the refractometer one without correcting for band broadening, an average intrinsic viscosity is determined:

$$\frac{S_{visc}\left(t_{el}\right)}{S_{DRI}\left(t_{el}\right)} = [\eta'(t_{el})] = \frac{\int_0^\infty \int_0^\infty \left[\eta'\left(M,V_h\right)\right] G'\left(t_{el},V_h\right) M\ N'\left(M,V_h\right) dM\ dV_h}{\int_0^\infty \int_0^\infty G'\left(t_{el},V_h\right) M\ N'\left(M,V_h\right) dM\ dV_h} \tag{43}$$

This average intrinsic viscosity, $[\eta(t_{el})]$, of the mixture of chains eluting at t_{el}, is the mass-weighted average of the intrinsic viscosities of the components. Note that the dependence from average viscosity on t_{el} prevents from comparing $[\eta'(t_{el})]$ measured on different SEC set-ups or even using different columns.

The viscometer combined with a refractometer can also yield a molecular weight, using a universal calibration curve. If band broadening is not corrected, one needs to introduce the

number-average hydrodynamic volume of the chains eluting at t_{el}:

$$\overline{V'}_{h,n}(t_{el}) = \frac{\int_0^\infty \int_0^\infty G'(t_{el}, V_h) V_h \; N'(M, V_h) \, dM \, dV_h}{\int_0^\infty \int_0^\infty G'(t_{el}, V_h) N'(M, V_h) \, dM \, dV_h} \tag{44}$$

Equation(38), (41) and (44) can be used to express the viscometer signal as follows:

$$S_{visc}(t_{el}) = \overline{V'}_{h,n}(t_{el}) \int_0^\infty \int_0^\infty G'(t_{el}, V_h) N'(M, V_h) \, dM \, dV_h \tag{45}$$

If one uses a refractometer in conjunction with the viscometer, then combining Equation (45) with Equation (38) and (6) yields:

$$\frac{S_{visc}(t_{el})}{S_{DRI}(t_{el})} = \overline{V'}_{h,n}(t_{el}) \frac{\int_0^\infty \int_0^\infty G'(t_{el}, V_h) N'(M, V_h) \, dM \, dV_h}{\int_0^\infty \int_0^\infty G'(t_{el}, V_h) M \; N'(M, V_h) \, dM \, dV_h} \tag{46}$$

Combining the signal of a viscometer, a refractometer and using a universal calibration curve, one thus determines at each elution time a number-average molecular weight defined as:

$$\overline{M'}_n(t_{el}) = \frac{\int_0^\infty \int_0^\infty G'(t_{el}, V_h) M \; N'(M, V_h) \, dM \, dV_h}{\int_0^\infty \int_0^\infty G'(t_{el}, V_h) N'(M, V_h) \, dM \, dV_h} \tag{47}$$

through the following relation:

$$\frac{S_{visc}(t_{el})}{S_{DRI}(t_{el})} = \frac{\overline{V'}_{h,n}(t_{el})}{\overline{M'}_n(t_{el})} \tag{48}$$

If band broadening is corrected or negligible, then all the chains detected at one elution time have the same hydrodynamic volume. This one-to-one relation between hydrodynamic volume and elution time makes it possible to express the ratio of the signal of the viscometer

and the refractometer as:

$$\frac{S^*_{\mathrm{visc}}\left(t_{\mathrm{el}}\right)}{S^*_{\mathrm{DRI}}\left(t_{\mathrm{el}}\right)} = \frac{V_{\mathrm{h}}}{\overline{M}_{\mathrm{n}}\left(V_{\mathrm{h}}\right)} = [\eta(V_{\mathrm{h}})] \tag{49}$$

The combination of the signals of the refractometer and the viscometer thus allows the determination of a weight-average intrinsic viscosity:

$$[\eta(V_{\mathrm{h}})] = \frac{\int_0^\infty \int_0^\infty \delta\left(\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) - t_{\mathrm{el}}\right)\left[\eta'\left(M, V_{\mathrm{h}}\right)\right] M\ N'\left(M, V_{\mathrm{h}}\right) \mathrm{d}M\ \mathrm{d}V_{\mathrm{h}}}{\int_0^\infty \int_0^\infty \delta\left(\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) - t_{\mathrm{el}}\right) M\ N'\left(M, V_{\mathrm{h}}\right) \mathrm{d}M\ \mathrm{d}V_{\mathrm{h}}} \tag{50}$$

When band broadening is corrected or negligible, combining the signal of a viscometer, a refractometer and using an universal calibration curve, one thus determines at each elution time a number-average molecular weight defined as:

$$\overline{M}_{\mathrm{n}}(V_{\mathrm{h}}) = \frac{\int_0^\infty M\ N'\left(M, V_{\mathrm{h}}\right) \mathrm{d}M}{\int_0^\infty N'\left(M, V_{\mathrm{h}}\right) \mathrm{d}M} \tag{51}$$

In contrast to $\overline{M}'_{\mathrm{n}}(t_{\mathrm{el}})$ and $[\eta'(t_{\mathrm{el}})]$ (Equation (47) and (43)), the quantities $\overline{M}_{\mathrm{n}}(V_{\mathrm{h}})$ and $[\eta(V_{\mathrm{h}})]$ can be compared even if obtained from different SEC systems, as long as universal calibration is valid.

With complex branched polymers, it is possible to determine the hydrodynamic volume but not the true molecular weight distribution using SEC even after correction of band broadening. In the case where band broadening has been corrected for, to obtain meaningful results, universal calibration has to be applied using an online viscometer to obtain $\overline{M}_{\mathrm{n}}(V_{\mathrm{h}})$. If some Mark-Houwink-Sakurada (MHS) parameters of the complex branched polymer are

used to obtain the intrinsic viscosity, the situation is completely different. The use of MHS parameters instead of on-line viscometry will yield different values, since it refers to the intrinsic viscosity of an equivalent chain, while the online viscometer yields the average intrinsic viscosity [$\eta(V_h)$]. The MHS relation is an empirical relation that relates a weight-average intrinsic viscosity to the viscometric-average molecular weight:

$$[\eta(V_h)] = K\ \overline{M}_v\left(V_h\right)^{\alpha} \tag{52}$$

where K and α are the MHS parameters and $\overline{M}_v$ is the viscometric-average molecular weight. It is important to note here that $\overline{M}_v$ is a dimensionless quantity because of its empirical nature. By using Equation (52) and (14), the following relationship is obtained:

$$V_h = \frac{4}{3}\ \pi\ \frac{K\ \overline{M}_v\left(V_h\right)^{\alpha}\ \overline{M}_n\left(V_h\right)}{6^{3/2}\ \Phi} \tag{53}$$

Thus, it is not possible to obtain physically meaningful values of molecular weight using MHS parameters for complex branched polymers. Note that also MHS parameters vary with branching topology and distributions[28] and thus they are very likely to be quite inaccurate for complex branched polymers.[56]

Furthermore, as in the case of linear polymers (Equation (21) and (22)), the ratio of the signals of a mass-sensitive and number-sensitive detectors yield the number-average MW:

$$\frac{S_{DRI}\left(t_{el}\right)}{S_{NS}\left(t_{el}\right)} = \overline{M'}_n\left(t_{el}\right) \tag{54}$$

or

$$\frac{S^*_{DRI}\left(t_{el}\right)}{S^*_{NS}\left(t_{el}\right)} = \overline{M}_n\left(V_h\right) \tag{55}$$

Expression for the signal of light-scattering detectors

For a light-scattering based detector, the Rayleigh relation leads to the same expression of $f'_{LS}(M,V_h)$ as the function $f_{LS}(M)$ in Equation (23). The signal of a light scattering detector can thus be expressed as:

$$S_{LS}(t_{el}) = \int_0^\infty \int_0^\infty G'(t_{el},M)\, M^2\, P(\theta)\, N'(M,V_h)\, dM\, dV_h \tag{56}$$

Equation (56) enable band broadening to be corrected on the light-scattering signal.

A light scattering detector can yield molecular weight if one determines the form factor. If band broadening is not corrected, then one has to take a z-average form factor into account:

$$\overline{P(\theta)}_z = \frac{\int_0^\infty \int_0^\infty G'\left(t_{el},M\right) P(\theta)\, M^2\, N'\left(M,V_h\right) dM\, dV_h}{\int_0^\infty \int_0^\infty G'\left(t_{el},M\right) M^2\, N'\left(M,V_h\right) dM\, dV_h} \tag{57}$$

If the band broadening is not corrected, the signal of the light scattering can be expressed as:

$$S_{LS}(t_{el}) = \overline{P(\theta)}_z \int_0^\infty \int_0^\infty G'(t_{el},M)\, M^2\, N'(M,V_h)\, dM\, dV_h \tag{58}$$

Combining the signal of a light scattering detector with a refractometer, one obtains:

$$\frac{S_{LS}\left(t_{el}\right)}{S_{DRI}\left(t_{el}\right)} = \overline{P(\theta)}_z \frac{\int_0^\infty \int_0^\infty G'\left(t_{el},M\right) M^2\, N'\left(M,V_h\right) dM\, dV_h}{\int_0^\infty \int_0^\infty G'\left(t_{el},M\right) M\, N'\left(M,V_h\right) dM\, dV_h} \tag{59}$$

One thus determines at each elution time a weight-average molecular weight defined as:

$$\overline{M'}_{\mathrm{w}}(t_{\mathrm{el}}) = \frac{\int_0^{\infty}\int_0^{\infty} G'(t_{\mathrm{el}}, M)\, M^2\, N'(M, V_{\mathrm{h}})\, \mathrm{d}M\, \mathrm{d}V_{\mathrm{h}}}{\int_0^{\infty}\int_0^{\infty} G'(t_{\mathrm{el}}, M)\, M\, N'(M, V_{\mathrm{h}})\, \mathrm{d}M\, \mathrm{d}V_{\mathrm{h}}} \tag{60}$$

via the equation:

$$\frac{S_{\mathrm{LS}}(t_{\mathrm{el}})}{S_{\mathrm{DRI}}(t_{\mathrm{el}})} = \overline{P(\theta)}_{\mathrm{z}}\ \overline{M'}_{\mathrm{w}}(t_{\mathrm{el}}) \tag{61}$$

If band broadening is corrected or negligible, the ratio of the signal of the light scattering and the refractometer is then a function of less complex average form factors and molecular weights:

$$\frac{S^*_{\mathrm{LS}}(t_{\mathrm{el}})}{S^*_{\mathrm{DRI}}(t_{\mathrm{el}})} = \overline{P(\theta)}_{\mathrm{z}}\ \overline{M}_{\mathrm{w}}(V_{\mathrm{h}}) \tag{62}$$

where

$$\overline{M}_{\mathrm{w}}(V_{\mathrm{h}}) = \frac{\int_0^{\infty} M^2\, N'(M, V_{\mathrm{h}})\, \mathrm{d}M}{\int_0^{\infty} M\, N'(M, V_{\mathrm{h}})\, \mathrm{d}M} \tag{63}$$

and

$$\overline{P(\theta)}_{\mathrm{z}} = \frac{\int_0^{\infty} P(\theta)\, M^2\, N'(M, V_{\mathrm{h}})\, \mathrm{d}M}{\int_0^{\infty} M^2\, N'(M, V_{\mathrm{h}})\, \mathrm{d}M} \tag{64}$$

The expressions stated by Hamielec and co-workers[5,11] are thus proved to give the correct results for universal calibration and light scattering of complex branched polymers. The rigorous derivation given here explicitly expresses the hydrodynamic volume and form factor and makes it possible to correct for band broadening. Assuming no band broadening or correcting it, LALLS, MALLS or triple detection can then be applied to obtain $\overline{M}_{\mathrm{w}}(V_{\mathrm{h}})$.

There are no apparent reasons for LALLS not to be valid for complex branched polymers. The situation is far more complex for MALLS and triple detection. First, the validity of the equations allowing one to calculate or extrapolate $P(\theta)$ can be questioned in the case of complex branched polymers. For example, the form factor of 6-arm stars or comb polymers has a significantly different dependence on R_g compared to linear chains.[57] The scattering of light from polymers in solution is essentially the same physical process for complex branched polymers as for linear ones. The scattered intensity is related to the polymers' form factor, in effect its average density profile. If we have many different chains present in a sample, we will obtain the average form factor, or equivalently the average R_g^2 if we expand the form factor about small angles. Equation (31), (32) and (34) are valid for linear and branched polymers. However, the validity of the expression of R_g in Equation (33) is questionable for complex branched polymers.

Second, the expression used for $P(\theta)$ in triple detection (see Equation (31) to (33) in the case of linear chains) uses the molecular weight, M, and the intrinsic viscosity $[\eta]$ to calculate hydrodynamic volume. The product $[\eta] \times M$ has dimensions of volume, and though strictly this volume is related to the hydrodynamic radius, it is reasonable for a linear polymer to replace it with the radius of gyration; both almost certainly vary in the same way with degree of polymerization. In the case of the separation of complex branched polymers, the input is $\overline{M}_w(V_h)$ and $[\eta(V_h)]$ if band broadening is corrected or negligible. However, as seen previously when dealing with a viscometer signal, the hydrodynamic radius is only obtained through the product $\overline{M}_n(V_h) \times [\eta(V_h)]$. Using $\overline{M}_w(V_h)$, the hydrodynamic radius is overestimated and thus $P(\theta)$ is underestimated ($P(\theta)$ is a monotonic decreasing function of x).

The numerical value of $\overline{M}_w(V_h)$ obtained by triple detection may thus be overestimated (when the molecular weight is high enough to have $P(\theta)$ different from one). As shown above, in the most general case of complex branched polymers and significant band broadening, $[\eta] \times M = V_h$ is replaced by the number-average hydrodynamic volume $\overline{V'}_{h,n}(t_{el})$ (Equation (44) and (45)). It is essential that this number-average is over the polymers physically present in the viscometer, whose molecular weight may vary both due to band broadening and to actual variations in the polymer microstructure.

We can obtain an estimate of the average size of polymers in a sample from either light scattering (we would obtain $\sqrt{\left(R_g^2\right)_n}$) or from a viscometer (which would yield $\overline{V'}_{h,n}{}^{1/3}$). The broader the distribution of polymer properties present, the less likely these two estimates are to agree, and the less useful is Equation (33). In cases of weak branching (polyethylene, polyacrylates, amylose etc.), one might simply allow for a Flory constant (Φ in Equation (33)) specific to branched polymers, and this may even be all that is necessary for statistical branching, since the variation of the Flory constant with statistical branching have been measured to be relatively minor.[28] Another new effect is present when we consider the variation of $\overline{V}_{h,n}$ with degree of polymerization for complex branched polymers. For hyperbranched polymers, both experimental and theoretical evidence point to different scaling behaviors of the number-average hydrodynamic volume.[19,58] The packing of short-chain branches into the polymer may make volume grow slowly with degree of polymerization, so that the intrinsic viscosity shows a maximum. Simulations and mean-field theory also predict qualitatively different variations in $\overline{V'}_{h,n}$ at different sizes, as for example, the repulsions between the chains become more important as more are added.[19] Although

more work is needed to establish the relations between branching structure and different measures of the size of a hyperbranched polymer, it seems likely that the combination of light scattering and viscometry will prove an informative probe of the structure of hyperbranched polymers such as amylopectin.

Hydrodynamic volume distributions

In the following, it is considered that band broadening is negligible or has been corrected for. True molecular weight distributions cannot be obtained for complex branched polymers, even using multiple detection SEC. Raw chromatograms prevent any comparison, since they depend on a type of column at a given time. In order to obtain comparable data, one can use some arbitrary distributions, e.g. distribution of linear equivalent molecular weight.[59] As discussed in the literature[60] but presently rarely implemented, from SEC one can also obtain true distributions expressed explicitly in terms of hydrodynamic volume. Using a refractometer, one can define the distribution $w(\log V_h)$ that corresponds to $w(\log M)$ in Equation (2) and (36) as:

$$w(\log V_h) = V_h\, W(V_h) = \left. \frac{S^*_{DRI}(t_{el})}{\mathrm{d}\log V_h \,/\, \mathrm{d}\tilde{t}_{el}(V_h)} \right|_{\tilde{t}_{el}(V_h)=t_{el}} \tag{65}$$

The derivation is given in the appendix. The number distribution $N(V_h)$ can be readily shown to be obtained from

$$N(V_h) = \frac{W(V_h)}{\overline{M}_n(V_h)} \tag{66}$$

Thus the number distribution $N(V_h)$ can be obtained with a mass-sensitive detector (refractometer), universal calibration and an online viscometer.

The value of V_h corresponding to a particular SEC elution time can be obtained through Equation (49) establishing the universal calibration curve from monodisperse standards and online viscometry. Instead of a viscometer, one can also use the Mark-Houwink-Sakurada parameters K and α for the standards (which then have to be linear or regularly branched), the hydrodynamic volume in one's particular SEC set-up at the elution time at which this standard elutes can then be found from:

$$V_h = \frac{4}{3}\,\pi\,\frac{K\,M^{\alpha+1}}{6^{3/2}\,\Phi} \tag{67}$$

It is preferable to determine V_h directly using online viscometry without relying on the Mark-Houwink-Sakurada empirical relation.

Instead of using online viscometry, the number distribution $N(V_h)$ can also be obtained from a number-sensitive detector and universal calibration. Following the same type of calculations as the one previously described, the signal of this detector can be expressed as:

$$S_{NS}^{*}(t_{el}) = \left.\frac{V_h\;N(V_h)}{\mathrm{d}\tilde{t}_{el}(V_h)/\mathrm{d}\log V_h}\right|_{\tilde{t}_{el}(V_h)=t_{el}} \tag{68}$$

Note that while $M\,N(M) = W(M)$, $V_h\,N(V_h)$ does not correspond to $W(V_h)$; as shown in Equation (66), $W(V_h) = \overline{M}_n(V_h)\,N(V_h)$.

$\overline{M}_n$ and $\overline{M}_w$ for the whole molecular weight distribution of complex branched polymers

The number hydrodynamic volume distribution for a complex branched polymer is given by:

$$N(V_h) = \int_0^\infty N'(M,V_h)\,\mathrm{d}M \tag{69}$$

with a similar definition for the mass distribution:

$$W(V_h) = \int_0^\infty M\,N'(M,V_h)\,\mathrm{d}M \tag{70}$$

It is useful to determine the true average MWs for the whole MW distribution (MWD). The expression for $\overline{M}_n$ for a complex branched polymer is:

$$\overline{M}_n = \frac{\int_0^\infty \int_0^\infty M\,N'(M,V_h)\,\mathrm{d}M\,\mathrm{d}V_h}{\int_0^\infty \int_0^\infty N'(M,V_h)\,\mathrm{d}M\,\mathrm{d}V_h} = \frac{\int_0^\infty W(V_h)\,\mathrm{d}V_h}{\int_0^\infty N(V_h)\,\mathrm{d}V_h} \tag{71}$$

whence

$$\frac{1}{\overline{M}_n} = \frac{\int_0^\infty \frac{W(V_h)}{\overline{M}_n(V_h)}\,\mathrm{d}V_h}{\int_0^\infty W(V_h)\,\mathrm{d}V_h} \tag{72}$$

which corresponds to the expression quoted by Hamielec.[5]

While the true MWD, $N(M)$ or $W(M)$, cannot be obtained for a complex branched polymer by any size separation technique, it is possible to obtain the true $\overline{M}_n$ using multiple detection SEC and universal calibration: for example, using a refractometer yielding $W(V_h)$ and a viscometer yielding $\overline{M}_n(V_h)$. Any mass-sensitive detector could theoretically replace the refractometer, also yielding $W(V_h)$; instead of a viscometer, one could use a number-sensitive detector, yielding $N(V_h)$ directly ($\overline{M}_n(V_h)$ is then not used in the calculation).

The definition of $\overline{M}_w$ in the case of complex branched polymers is:

$$\overline{M}_w = \frac{\int_0^\infty \int_0^\infty M^2\,N'(M,V_h)\,\mathrm{d}M\,\mathrm{d}V_h}{\int_0^\infty \int_0^\infty M\,N'(M,V_h)\,\mathrm{d}M\,\mathrm{d}V_h} \tag{73}$$

which can be re-written as

$$\overline{M}_{\mathrm{w}} = \frac{\int_0^{\infty} \overline{M}_{\mathrm{w}}(V_{\mathrm{h}})\, W(V_{\mathrm{h}})\, \mathrm{d}V_{\mathrm{h}}}{\int_0^{\infty} W(V_{\mathrm{h}})\, \mathrm{d}V_{\mathrm{h}}} \tag{74}$$

which also corresponds to the expression quoted by Hamielec.[5] The true $\overline{M}_{\mathrm{w}}$ of the whole distribution can thus be obtained by multiple detection SEC using universal calibration (using online viscometry or the Mark-Houwink-Sakurada relation) with a refractometer yielding $W(V_{\mathrm{h}})$, plus a light-scattering detector yielding $\overline{M}_{\mathrm{w}}(V_{\mathrm{h}})$.

The true $\overline{M}_{\mathrm{n}}$ and $\overline{M}_{\mathrm{w}}$ of the whole MWD of a complex branched polymer can thus be obtained by multiple detection SEC using the hydrodynamic volume distributions. It is very important to emphasize that the data treatment used in many current commercial SEC software packages considers that the separation is complete and that at each elution time only one MW is detected and measured. The $\overline{M}_{\mathrm{n}}$ and $\overline{M}_{\mathrm{w}}$ values are thus calculated by these software packages by using some distribution of $\overline{M}_{\mathrm{n}}(V_{\mathrm{h}})$ and $\overline{M}_{\mathrm{w}}(V_{\mathrm{h}})$ rather than the actual hydrodynamic volume distributions $W(V_{\mathrm{h}})$ and $N(V_{\mathrm{h}})$. Some current commercial SEC software packages thus yield incorrect $\overline{M}_{\mathrm{n}}$ and $\overline{M}_{\mathrm{w}}$ values for complex branched polymers.

Statistically branched polyacrylates have been analyzed by multiple detection SEC[62,63] and all the data treatment presented here have been successfully tested and will be presented in a forthcoming publication.[64] The importance of band broadening and complex branching in this specific case will be discussed.

Conclusions

As is well known, but not universally appreciated, data from size separation detectors such as

SEC yield distributions in terms of hydrodynamic volume, rather than in terms of molecular weight. A new formalism for expressing observables obtained by SEC or other size separation technique of complex branched polymers such as polyacrylates, polyethylene and starch has been developed. The new formalism is based on a multi-variable distribution function $N'(M,V_h)$ giving the number distribution of polymers with molar mass M and hydrodynamic volume V_h (the formalism using this distribution function could be extended to additional independent variables such as composition in a copolymer or degree of branching). We also make use of the corresponding mass distribution $W'(M,V_h) = M\ N'(M,V_h)$. The generalized convolution equation is derived relating this distribution to the various observables using multiple detection SEC, such as online light scattering, viscometry, number-sensitive detection and mass-sensitive detection (refractometer etc.). These detectors yield signals as a function of elution time which are generically written as $S_{det}(t_{el})$, encapsulated in Equation (38). This formalism shows how data from the various detectors can be corrected for band broadening, which is important for new techniques[17-21] which enable mechanistic information to be deduced from the detailed form of appropriate distributions. The formalism shows (e.g. in Equation (65)) how the distributions in terms of hydrodynamic volume can be obtained from appropriate data. The formalism also shows how data from appropriate detectors can be treated to yield quantities of interest such as the distribution for the dependence of number-and weight- average molecular weights on hydrodynamic volume, e.g. in Equation (51) and (63). The new formalism is also used to rigorously derive expressions whereby the true overall $\overline{M}_n$ and $\overline{M}_w$ can be determined from such data (Equation (72) and (74)), and these expressions show that some current commercial software for obtaining $\overline{M}_w$ and $\overline{M}_n$ from SEC data will not yield correct results for complex branched polymers.

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Appendix A. Case of linear polymers

All the derivations and equations presented in the main text are detailed here including all the constants that need to be used and known to process SEC signals.

In the case of linear or regular branched polymers, the dependence of the particular detector on mass $f_{det}(M)$ is:

- for a mass sensitive-detector (refractometer): $f_{DRI}(M) = A_{DRI} \frac{dn}{dc} M$,

- for a number sensitive-detector (UV or fluorescence detector): $f_{NS}(M) = A_{NS}$,

- for the viscometer: $f_{visc}(M) = A_{visc} [\eta] \frac{M}{V_{inj}}$,

- for a light-scattering based detector at angle θ: $f_{LS}(M) = A_{LS} M P(\theta) \frac{M}{V_{inj}}$,

where A_{det} is the calibration constant for each detector, dn/dc is the refractive index increment, and V_{inj} is the injection volume. Note that the dn/dc is not a constant in the case of copolymers

with a distribution of compositions.

The refractometer signal is expressed as:

$$S_{\mathrm{DRI}}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^\infty G(t_{\mathrm{el}},M)\, M\, N(M)\, \mathrm{d}M \tag{75}$$

In the case of no band broadening, a change in variable from M to t_{el} yields:

$$S^*_{\mathrm{DRI}}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^\infty \delta\left(\tilde{t}_{\mathrm{el}}(M) - t_{\mathrm{el}}\right) \frac{M\, N(M)}{\mathrm{d}\tilde{t}_{\mathrm{el}}(M)/\mathrm{d}M} \mathrm{d}t_{\mathrm{el}} \tag{76}$$

or

$$S^*_{\mathrm{DRI}}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^\infty \delta\left(\tilde{t}_{\mathrm{el}}(M) - t_{\mathrm{el}}\right) \frac{M^2\, N(M)}{\mathrm{d}\tilde{t}_{\mathrm{el}}(M)/\mathrm{d}\log M} \mathrm{d}t_{\mathrm{el}} \tag{77}$$

Thus by definition of the Dirac function

$$S^*_{\mathrm{DRI}}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \left.\frac{M^2\, N(M)}{\mathrm{d}\tilde{t}_{\mathrm{el}}(M)/\mathrm{d}\log M}\right|_{\tilde{t}_{\mathrm{el}}(M)=t_{\mathrm{el}}} \tag{78}$$

The molecular weight distribution obtained with a refractometer is thus:

$$w(\log M) = M\, W(M) = M^2\, N(M) = \left.\frac{S^*_{\mathrm{DRI}}(t_{\mathrm{el}})}{A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \mathrm{d}\log M \Big/ \mathrm{d}\tilde{t}_{\mathrm{el}}(M)}\right|_{\tilde{t}_{\mathrm{el}}(M)=t_{\mathrm{el}}} \tag{79}$$

Using the same procedure, a number sensitive detector yields:

$$W(M) = M\, N(M) = \left.\frac{S^*_{\mathrm{NS}}(t_{\mathrm{el}})}{A_{\mathrm{NS}}\, \mathrm{d}\log M \Big/ \mathrm{d}\tilde{t}_{\mathrm{el}}(M)}\right|_{\tilde{t}_{\mathrm{el}}(M)=t_{\mathrm{el}}} \tag{80}$$

Similar calculations lead to the expression of the signal of the viscometer and light scattering:

$$S^*_{\mathrm{visc}}(t_{\mathrm{el}}) = \frac{A_{\mathrm{visc}}}{V_{\mathrm{inj}}} \left. \frac{[\eta]\, w(\log M)}{\mathrm{d}\tilde{t}_{\mathrm{el}}(M)/\mathrm{d}\log M} \right|_{\tilde{t}_{\mathrm{el}}(M)=t_{\mathrm{el}}} \tag{81}$$

$$S^*_{\mathrm{LS}}(t_{\mathrm{el}}) = \frac{A_{LS}}{V_{\mathrm{inj}}} \left. \frac{P(\theta)\, w(\log M)}{\mathrm{d}\tilde{t}_{\mathrm{el}}(M)/\mathrm{d}\log M} \right|_{\tilde{t}_{\mathrm{el}}(M)=t_{\mathrm{el}}} \tag{82}$$

where A_{LS} is often noted K:

$$A_{\mathrm{LS}} = K = \frac{2\,\pi^2\, n^2}{\lambda^4\, N_{\mathrm{A}}} \left(\frac{\mathrm{d}n}{\mathrm{d}c}\right)^2 \tag{83}$$

The molecular weight can also be determined from the ratio of the signals of a mass-sensitive detector to a number-sensitive detector:

$$\frac{S^*_{\mathrm{DRI}}(t_{\mathrm{el}})}{S^*_{\mathrm{NS}}(t_{\mathrm{el}})} = \frac{A_{\mathrm{DRI}}\, \mathrm{d}n/\mathrm{d}c}{A_{\mathrm{NS}}} M \tag{84}$$

Appendix B. Case of complex branched polymers without band broadening

In the case of complex branched polymers, the dependence of the particular detector on mass and hydrodynamic volume $f'_{det}(M,V_h)$ is not changed for the weight- or number-sensitive detectors.

- for the viscometer: $f'_{\mathrm{visc}}(M,V_{\mathrm{h}}) = A_{\mathrm{visc}}\,[\eta(V_{\mathrm{h}})]\,\frac{M}{V_{\mathrm{inj}}}$,

- for a light-scattering based detector at angle θ: $f'_{\mathrm{LS}}(M,V_{\mathrm{h}}) = A_{\mathrm{LS}}\, M\, P(\theta) \frac{M}{V_{\mathrm{inj}}}$,

In the case of no band broadening, the refractometer signal is expressed as

$$S_{\mathrm{DRI}}{}^*(t_{\mathrm{el}}) = A_{\mathrm{DRI}}\, \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^\infty \int_0^\infty \delta\left(\tilde{t}_{\mathrm{el}}(V_{\mathrm{h}}) - t_{\mathrm{el}}\right) M\, N'(M,V_{\mathrm{h}})\, \mathrm{d}M\, \mathrm{d}V_{\mathrm{h}} \tag{85}$$

Using the definition of the weight hydrodynamic volume distribution (Equation (70)):

$$S_{\mathrm{DRI}}^{*}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^{\infty} \int_0^{\infty} \delta\left(\tilde{t}_{\mathrm{el}}(V_{\mathrm{h}}) - t_{\mathrm{el}}\right) W(V_{\mathrm{h}})\, \mathrm{d}V_{\mathrm{h}} \tag{86}$$

A change in variable from the hydrodynamic volume V_{h} to elution time t_{el} results in:

$$S_{\mathrm{DRI}}^{*}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^{\infty} \delta\left(\tilde{t}_{\mathrm{el}}(V_{\mathrm{h}}) - t_{\mathrm{el}}\right) W(V_{\mathrm{h}}) \frac{\mathrm{d}t_{\mathrm{el}}}{\mathrm{d}\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) / \mathrm{d}V_{\mathrm{h}}} \tag{87}$$

or

$$S_{\mathrm{DRI}}^{*}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \int_0^{\infty} \delta\left(\tilde{t}_{\mathrm{el}}(V_{\mathrm{h}}) - t_{\mathrm{el}}\right) V_{\mathrm{h}}\, W(V_{\mathrm{h}}) \frac{\mathrm{d}t_{\mathrm{el}}}{\mathrm{d}\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) / \mathrm{d}\log V_{\mathrm{h}}} \tag{88}$$

Using the definition of the Dirac function δ it becomes:

$$S_{\mathrm{DRI}}^{*}(t_{\mathrm{el}}) = A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \left. \frac{V_{\mathrm{h}}\, W\left(V_{\mathrm{h}}\right)}{\mathrm{d}\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) / \mathrm{d}\log V_{\mathrm{h}}} \right|_{\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) = t_{\mathrm{el}}} \tag{89}$$

which can also be expressed as

$$w(\log V_{\mathrm{h}}) = V_{\mathrm{h}}\, W(V_{\mathrm{h}}) = V_{\mathrm{h}}\, \overline{M}_{\mathrm{n}}(V_{\mathrm{h}})\, N(V_{\mathrm{h}}) = \left. \frac{S_{\mathrm{DRI}}^{*}\left(t_{\mathrm{el}}\right)}{A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} \mathrm{d}\log V_{\mathrm{h}} \Big/ \mathrm{d}\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right)} \right|_{\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) = t_{\mathrm{el}}} \tag{90}$$

Similarly, the signal of the number-sensitive detector can be expressed as:

$$S_{\mathrm{NS}}^{*}(t_{\mathrm{el}}) = A_{\mathrm{NS}} \left. \frac{V_{\mathrm{h}}\, N\left(V_{\mathrm{h}}\right)}{\mathrm{d}\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) / \mathrm{d}\log V_{\mathrm{h}}} \right|_{\tilde{t}_{\mathrm{el}}\left(V_{\mathrm{h}}\right) = t_{\mathrm{el}}} \tag{91}$$

The ratio of the signals of the viscometer and the refractometer can thus be expressed as:

$$\frac{S_{\mathrm{visc}}^{*}\left(t_{\mathrm{el}}\right)}{S_{\mathrm{DRI}}^{*}\left(t_{\mathrm{el}}\right)} = \frac{A_{\mathrm{visc}}}{A_{\mathrm{DRI}} \frac{\mathrm{d}n}{\mathrm{d}c} V_{\mathrm{inj}}\, \gamma} \frac{V_{\mathrm{h}}}{\overline{M}_{\mathrm{n}}\left(V_{\mathrm{h}}\right)} \tag{92}$$

By a similar derivation, the expression obtained for the signal of a light-scattering detector is

$$\frac{S^*_{\mathrm{LS}}\left(t_{\mathrm{el}}\right)}{S^*_{\mathrm{DRI}}\left(t_{\mathrm{el}}\right)} = \frac{A_{\mathrm{LS}}}{A_{\mathrm{DRI}}\dfrac{\mathrm{d}n}{\mathrm{d}c}V_{\mathrm{inj}}}\,\overline{P}(\theta)_{\mathrm{z}}\,\overline{M}_{\mathrm{w}}\left(V_{\mathrm{h}}\right) \tag{93}$$

The ratio of the signals of the mass-sensitive and the number-sensitive detectors can be expressed as:

$$\frac{S^*_{\mathrm{DRI}}\left(t_{\mathrm{el}}\right)}{S^*_{\mathrm{NS}}\left(t_{\mathrm{el}}\right)} = \frac{A_{\mathrm{DRI}}\,\mathrm{d}n/\mathrm{d}c}{A_{\mathrm{NS}}}\,\overline{M}_{\mathrm{n}}\left(V_{\mathrm{h}}\right) \tag{94}$$

References

[1] S. Mori, H. G. Barth "*Size Exclusion Chromatography*", 1st ed., Springer-Verlag, Berlin 1999.

[2] S. T. Balke, "Characterization of complex polymers by SEC and HPLC", in: *Modern methods of Polymer Characterization*, 1st ed., H. G. Barth and J. W. Mays, Eds., John Wiley & Sons, New York / Chichester / Brisbane / Toronto / Singapore 1991, vol. 113, p. 1.

[3] Z. Grubisic, P. Rempp, H. Benoit, *J. Polym. Sci. Part B Polym. Lett.* **1967**, *5*, 753.

[4] Z. Grubisic, P. Rempp, H. Benoit, *J. Polym. Sci. Part. B Polym. Phys.* **1996**, *34*, 1707.

[5] L. K. Kostanski, D. M. Keller, A. E. Hamielec, *J. Biochem. Biophys. Methods* **2004**, *58*, 159.

[6] C. Jackson, Y. J. Chen, J. W. Mays, *J. Appl. Polym. Sci.* **1996**, *61*, 865.

[7] T. Sun, R. R. Chance, W. W. Graessley, D. J. Lohse, *Macromolecules* **2004**, *37*, 4304.

[8] C. D. Thurmond, B. H. Zimm, *J. Polym. Sci.* **1952**, *8*, 477.

[9] W. Burchard, *Adv. Polym. Sci.* **1999**, *143*, 113.

[10] A. E. Hamielec, A. C. Ouano, L. L. Nebenzahl, *J. Liq. Chromatogr.* **1978**, *1*, 527.

[11] A. Hamielec, *Pure Appl. Chem.* **1982**, *54*, 293.

[12] A. E. Hamielec, A. C. Ouano, *J. Liq. Chromatogr.* **1978**, *1*, 111.

[13] J. R. Daniel, R. L. Whistler, H. Roeper, "Starch", in: *Ullmann´s Encyclopedia of Industrial Chemistry*, 7th ed., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim 2005, vol. A25.

[14] G. Galinsky, W. Burchard, *Macromolecules* **1997**, *30*, 6966.

[15] B. Voit, *J. Polym. Sci. Pol. Chem.* **2005**, *43*, 2679.

[16] Y. Takeda, S. Tomooka, S. Hizukuri, *Carbohydr. Res.* **1993**, *246*, 267.

[17] P. A. Clay, R. G. Gilbert, *Macromolecules* **1995**, *28*, 552.

[18] J. V. Castro, C. Dumas, H. Chiou, M. A. Fitzgerald, R. G. Gilbert, *Biomacromolecules* **2005**, *6*, 2248.

[19] C. J. C. Watts, A. Gray-Weale, R. G. Gilbert, *Biomacromolecules* **2006**, *submitted*.

[20] J. M. Asua, S. Beuermann, M. Buback, P. Castignolles, B. Charleux, R. G. Gilbert, R. A. Hutchinson, J. R. Leiza, A. N. Nikitin, J. P. Vairon, A. M. van Herk, *Macromol. Chem. Phys.* **2004**, *205*, 2151.

[21] Y. Guillaneuf, D. Bertin, P. Castignolles, B. Charleux, *Macromolecules* **2005**, *38*, 4638.

[22] S. T. Balke, T. H. Mourey, *J. Appl. Polym. Sci.* **2001**, *81*, 370.

[23] P. R. Haddad, P. E. Jackson "*Ion Chromatography - principles and applications"*, 1st ed., Elsevier, Amsterdam - Oxford - New York - Tokyo 1990.

[24] K. Koch, R. Andersson, P. Aman, *J. Chromatogr. A* **1998**, *800*, 199.

[25] S. Lee, P. O. Nilsson, G. S. Nilsson, K. G. Wahlund, *J. Chromatogr. A* **2003**, *1011*, 111.

[26] A. Litzen, K. G. Wahlund, *Anal. Chem.* **1991**, *63*, 1001.

[27] H. Cottet, J. P. Biron, J. Taillades, *J. Chromatogr. A* **2004**, *1051*, 25.

[28] B. S. Farmer, K. Terao, J. W. Mays, *Int. J. Polym. Anal. Charact.* **2006**, *11*, 3.

[29] K. Hatada, T. Kitayama, "Online SEC/NMR analysis of polymers", in: *NMR spectroscopy of polymers*, 1st ed., Springer-Verlag, Berlin 2004, chap. 8, p. 199.

[30] U. Lehmann, W. Kohler, W. Albrecht, *Macromolecules* **1996**, *29*, 3212.

[31] J. D. Ferry, "Molecular theory for undiluted amorphous polymers and concentrated solutions; networks and entanglements", in: *Viscoelastic properties of polymers*, 3rd ed., John Wiley and Sons Inc., New York 1980, chap. 10, p. 224.

[32] T. Fukuda, *J. Polym. Sci. Pol. Chem.* **2004**, *42*, 4743.

[33] D. W. Shortt, *J. Liq. Chromatogr.* **1993**, *16*, 3371.

[34] I. Kraemer, H. Pasch, H. Haendel, K. Albert, *Macromol. Chem. Phys.* **1999**, *200*, 1734.

[35] T. P. Davis, M. D. Zammit, *Macromol. Symp.* **1996**, *111*, 243.

[36] M. D. Zammit, T. P. Davis, *Polymer* **1997**, *38*, 4455.

[37] R. Thitiratsakul, S. T. Balke, T. H. Mourey, *Int. J. Polym. Anal. and Charact.* **1996**, *2*, 345.

[38] S. T. Popovici, W. T. Kok, P. J. Schoenmakers, *J. Chromatogr. A* **2004**, *1060*, 237.

[39] W. Lee, H. Lee, J. Cha, T. Chang, K. J. Hanley, T. P. Lodge, *Macromolecules* **2000**, *33*, 5111.

[40] K. Y. van Berkel, G. T. Russell, R. G. Gilbert, *Macromolecules* **2005**, *38*, 3214.

[41] J. V. Castro, R. M. Ward, R. G. Gilbert, M. A. Fitzgerald, *Biomacromolecules* **2005**, *6*, 2260.

[42] L. H. Tung, *J. Appl. Polym. Sci.* **1966**, *10*, 1271.

[43] L. H. Tung, *J. Appl. Polym. Sci.* **1966**, *10*, 375.

[44] L. H. Tung, J. C. Moore, G. W. Knight, *J. Appl. Polym. Sci.* **1966**, *10*, 1261.

[45] L. H. Tung, J. R. Runyon, *J. Appl. Polym. Sci.* **1969**, *13*, 2397.

[46] J. L. Baumgarten, J. P. Busnel, G. R. Meira, *J. Liq. Chromatogr. Relat. Technol.* **2002**, *25*, 1967.

[47] M. A. Haney, *J. Appl. Polym. Sci.* **1985**, *30*, 3037.

[48] W.-M. Kulicke, C. Clasen, "Chapter 4: The intrinsic viscosity", in: *Viscometry of polymers and polyelectrolytes*, 1st ed., Springer Verlag, Berlin Heidelberg 2004, p. 41.

[49] P. J. Flory, "Configurational and frictional properties of the polymer molecule in dilute solution", in: *Principles of Polymer Chemistry*, 1st ed., Cornell University Press, Ithaca, New York 1953, chap. XIV, p. 595.

[50] Y. Takeda, S. Shibahara, I. Hanashiro, *Carbohydr. Res.* **2003**, *338*, 471.

[51] P. J. Flory, "Determination of molecular weights", in: *Principles of Polymer Chemistry*, 1st ed., Cornell University Press, Ithaca, New York 1953, p. 266.

[52] P. J. Wyatt, *Anal. Chim. Acta* **1993**, *272*, 1.

[53] A. C. Ouano, W. Kaye, *J. Polym. Sci. Pol. Chem.* **1974**, *12*, 1151.

[54] Y. Huang, H. Peng, J. W. Y. Lam, Z. D. Xu, F. S. M. Leung, J. W. Mays, B. Z. Tang, *Polymer* **2004**, *45*, 4811.

[55] M. Netopilik, *J. Chromatogr. A* **1998**, *793*, 21.

[56] L. Couvreur, G. Piteau, P. Castignolles, M. Tonge, B. Coutin, B. Charleux, J. P. Vairon, *Macromol. Symp.* **2001**, *174*, 197.

[57] P. M. Toporowski, J. Roovers, *Macromolecules* **1978**, *11*, 365.

[58] L. Garamszegi, T. Q. Nguyen, C. J. G. Plummer, J. A. E. Manson, *J. Liq. Chromatogr. Relat. Technol.* **2003**, *26*, 207.

[59] R. M. Ward, Q. Gao, H. de Bruyn, D. J. Lamb, R. G. Gilbert, M. A. Fitzgerald, *Biomacromolecules* **2006**, *7*, 866.

[60] A. Huber, W. Praznik, "Molecular Characteristics of Glucans: High-amylose cornstarch", in: *Strategies in Size Exclusion Chromatography*, M. Potschka and P. L. Dubin, Eds. 1996, 635, p. 351.

[61] A. D. McNaught, A. Wilkinson "*Compendium of Chemical Terminology"*, 2nd ed., Blackwell Science Ltd., Oxford 1997.

[62] P. Castignolles, *Ph.D. Thesis*, University Pierre and Marie Curie, Paris, 2003,

[63] M. Gaborieau, *PhD Thesis*, University Louis Pasteur, 2005, http://www-scd-ulp.u-strasbg.fr/theses/theselec.html.

[64] M. Gaborieau, J. Nicolas, M. Save, B. Charleux, J.-P. Vairon, R. G. Gilbert, P. Castignolles, *Macromolecules*, *submitted*.

FIGURE

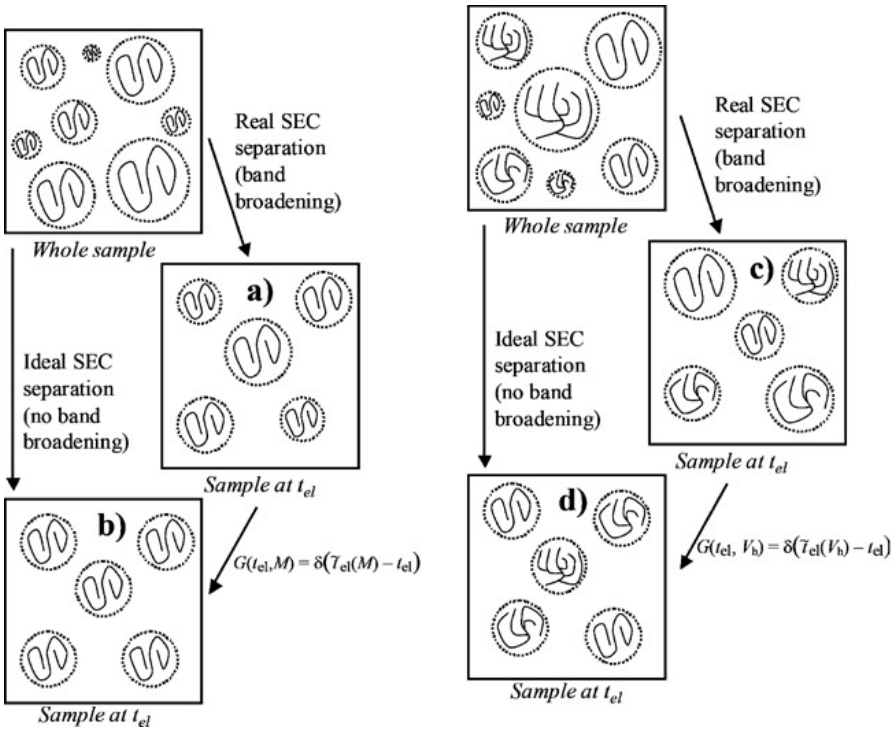

Figure 1: Influence of the band broadening and of the branching on the separation by hydrodynamic volume in SEC and on the population of chains present in a detector at the elution time t_{el}. The case of linear (or regularly branched) polymer is depicted on the left part of the Figure while the case of complex branched polymers is depicted on the right.

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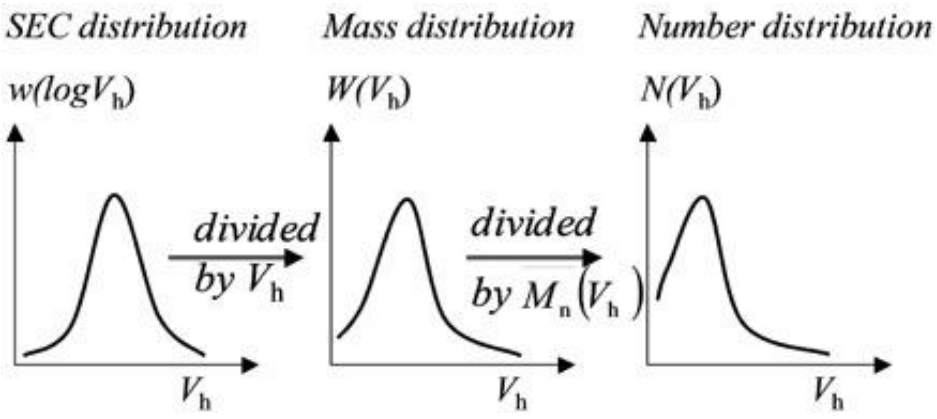

SEC of complex branched polymers (polyethylene, polyacrylates, starch etc.) cannot yield the true molecular weight distributions but can yield hydrodynamic volume distributions. The equations relating the response of different detectors are derived, by defining a number distribution $N'(M,V_h)$ of chains which have molecular weight M and hydrodynamic volume V_h. The true $\overline{M}_w$ and $\overline{M}_n$ can be obtained from correct processing of the hydrodynamic volume distributions.