# A Pseudouniversal Calibration Procedure for the Molecular Weight Determination of Cellulose

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## SYNOPSIS

Size-exclusion chromatography of carbanilated cellulose of polystyrene-divinylbenzene packing has been used to determine the molecular weight distribution of the polymer with tetrahydrofuran as the eluent. The reproducibility and the accuracy of the method is discussed in this paper with reference to depolymerized organosolvent-treated celluloses. A comparison between the results obtained from the universal calibration approach and the viscosity-average degree of polymerization, derived from the determination of the intrinsic viscosity in cupriethylene diamine solutions, is presented.

## INTRODUCTION

Although size-exclusion chromatography (SEC) presents the unique advantage of giving the molecular weight distribution ($MWD$) of a polymer in a relatively short time, this technique has never reached the status of a standard analysis in the sphere of cellulose-based compounds. This is not due to the lack of importance of the $MWD$ knowledge in the cellulose industry[1]: Information on this distribution is of interest in order to follow the anhydroglucose chain depolymerization[2] at every stage of the pulping process, as this reaction affects not only the quality and yield of the final cellulosic product, but also the degree and the rate of degradability (i.e., digestibility) of the cellulosic fines present in the liquid effluents. This is also true for viscose fiber production.

The lack of enthusiasm toward SEC is related, first, to the technique itself: SEC is reputed to be costly, difficult to handle, time-consuming, and somewhat inaccurate (problems with calibration and nonexclusion effects). Second, it is related to the difficulties associated with the substrate itself since there is often a problem of compatibility between the cellulose solvents, e.g., cadoxen, and the SEC column packing. Furthermore, the cellulose derivatization, such as nitration or acetylation, which renders the polymer soluble in an usual chromatographic solvent such as tetrahydrofuran (THF), is said to be highly degradative.[3]

In this paper, we would like to prove that SEC is indeed a reliable and accurate technique for cellulose by comparing viscosity determinations to SEC results using Benoit's concept[4,5] of universal calibration. Our analysis will be based on a series of celluloses at various degrees of polymerization derived from thermo-solvolysis of a prototype Sigma cellulose.

## MATERIALS AND METHODS

### Materials

The production of the thermo-mechano-solvolytic (TMS) celluloses has been described elsewhere.[6] To summarize the approach, Sigmacell $\alpha$-cellulose (Sigma, C-8002, lot 104F-027C) was prepared as an ethylene glycol suspension (7% w/w) and treated in a cascade reactor system. The temperature varied between 220 and 340°C, and the reaction time, between 2 and 10 min. The system was equipped with a 2 mm ID by 12 cm-long capillary that acted as a shear device when a differential pressure of 10.3 $M$Pa was applied.

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## Tricarbanilation

The tricarbanilation of the cellulose samples was carried out using the Hall and Horne method.[7] The degree of substitution was controlled through the percentage of nitrogen as determined by elemental analysis.

## Viscometry

The viscosities of the TMS celluloses were obtained in triplicate following the ASTM method. The viscosity-average degree of polymerization obtained by viscometry ($DP_{v,\text{visc},\alpha,K'}$, symbols $\alpha$ and $K'$ refer to the equation $DP^{\alpha} = K'[\eta]$) is calculated using the following equation,[8,9] which correlates it with the intrinsic viscosity (limiting viscosity number), $[\eta]$ (ml g$^{-1}$):

$$DP_{v,\text{vis},1,1.9} = 1.90[\eta] \qquad (1)$$

## High-performance Size-Exclusion Chromatography (HPSEC)

The system used consists of a Varian Vista 5000 chromatograph equipped with a variable wavelength UV-100 detector. Two columns in series thermostated at 25°C (PL Gel, 300 × 7.5 mm, particle size of 10 $\mu$m, Polymer Laboratories) were used, the first having a porosity of 10$^4$ Å, and the second, of 10$^5$ Å. The eluent, degassed and filtered (0.45 $\mu$m) THF (Anachemia, UV grade), was kept peroxide-free by bubbling inert gas through it. The CTC samples were dissolved in THF at a concentration of 1.0 mg mL$^{-1}$, filtered (0.45 $\mu$m), injected via a 10 $\mu$L loop, and eluted at 25°C at a flow rate of 1.0 mL min$^{-1}$. Detection was carried out using UV at 225 nm (254 nm gives the same peak elution volume) for the narrow distribution (polydispersity < 1.1) polystyrene standards (Polymer Laboratories) and 254 nm for the CTC samples. All data were acquired, stored, and processed on a microcomputer with the aid of a Waters software program (Maxima 820).

## Molecular Weight Calculations

The definitions used are the following:

- The viscosity-average molecular weight $MW_{v,\text{SEC},\alpha}$:

$$MW_{v,\text{SEC},\alpha} = \left(\frac{\sum n_i MW_i^{\alpha+1}}{\sum n_i MW_i}\right)^{1/\alpha} \qquad (2)$$

where $\alpha$ is the exponent in the Mark–Houwink equation $[\eta] = K \cdot MW^{\alpha}$ and $n_i$ is the number of molecules having a molecular weight equal to $MW_i$.

- The weight-average molecular weight $MW_w$ ($j = 1$)

$$MW_j = \left(\frac{\sum n_i MW_i^{j+1}}{\sum n_i MW_i^{j}}\right) \qquad (3)$$

- Concerning the Maxima 820 software, the weight-average molecular weight $MW_w$ ($k = 1$) was calculated as shown below, once the integration parameters—mainly the baseline definition and the number of slices to be used—had been specified:

$$MW_k = \frac{\sum A_i MW_i^{k}}{\sum A_i MW_i^{k-1}} \qquad (4)$$

where $A_i$ is the area under the chromatogram curve of the $i$th slice whose corresponding molecular weight at the midpoint elution volume—determined from an input calibration curve—is $MW_i$.

These formulae can also be used for the number-average $MW_n$ and the Z-average molecular weight $MW_z$ with $j$ or $k$ equal to 0 or 2, respectively.

It is clear that the Maxima calculations could be performed only by assuming that the detector's response is proportional to the weight, $\Delta w$, of the sample eluting through the detector cell at any time, $\Delta t$. When using UV detection, this assumption leads to the hypothesis that the molar extinction coefficient (L mol$^{-1}$ cm$^{-1}$) is inversely proportional to the molecular weight of the analyzed polymer. In other words, the weight extinction coefficient (L g$^{-1}$ cm$^{-1}$) is a constant for a given polymer, whatever its degree of polymerization. When the cellulose carbanilation is successfully carried out (i.e., the degree of substitution is effectively 3), the chromophoric groups become uniformly distributed, making this assumption a reasonable inference. Even for organosolv lignin, this hypothesis caused an error of only 5% in the $MW$ determination by HPSEC.[10]

## RESULTS AND DISCUSSION

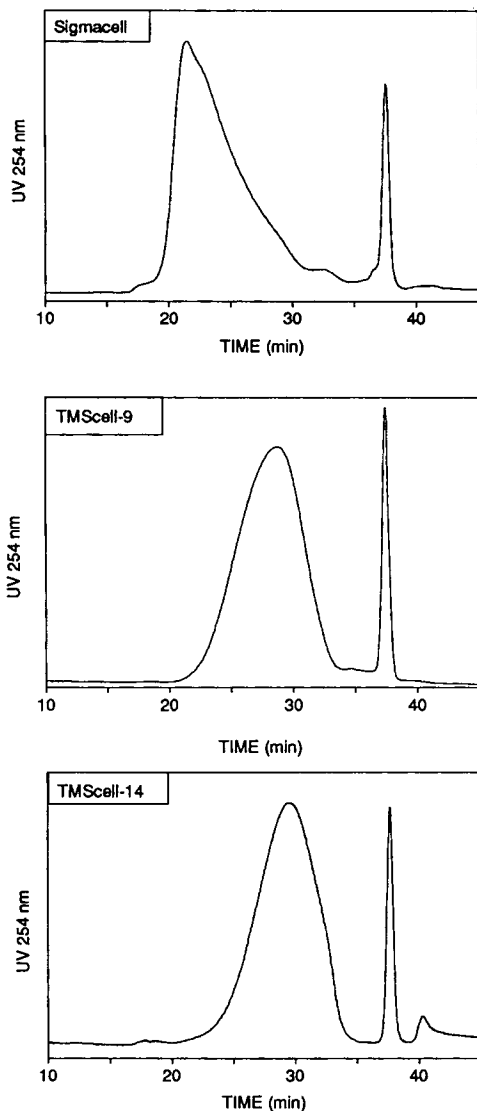Figure 1 depicts the chromatograms of the initial Sigmacell, a middle-range and a low-range degree of

**Figure 1** HPSEC chromatograms of (a) Sigmacell; (b) TMScell-9 $(DP_{v,\text{vis},1,1.9} = 133)$; and (c) TMScell-14 $(DP_{v,\text{vis},1,1.9} = 90)$.

polymerization TMS cellulose. All the samples are eluted within the fractionation volume, and compared to the broad distribution of the starting material, the treated samples appear to have a narrower $MWD$. The peak at 37 min corresponds to products from the cellulose carbanilation procedure that were not removed during the Soxhlet washing and are, obviously, not taken into account for the molecular weight calculations.

Although according to Eq. (1) the exponent $\alpha$ in the Mark–Houwink equation for cellulose in CED equals 1, the viscosity-average $DP$ obtained by viscometry $(DP_{v,\text{vis},1,1.9})$ should be identical to the $DPw$ as determined by computerizing the raw chromato-

graph data (in this case, $DP_w = DP_{v,\text{SEC},1}$). Figure 2(a) shows the $DPw$ obtained directly from the polystyrene calibration curve $(DP_{w,\text{PS}})$ as a function of $DP_{v,\text{vis},1,1.9}$. We can notice a significant deviation from the expected linear relationship (the dashed median), especially for the high $DP$ samples.

To employ the principle of universal calibration proposed by Benoit and co-workers[4] we had to adapt the Maxima 820 calculation procedure since it is impossible to incorporate the hydrodynamic volume values, either for the polystyrene standards (i.e., to construct the universal calibration curve) or for the cellulose samples so as to determine their average molecular weights. Thus, we have constructed a pseudouniversal calibration curve, which is, in fact, a calibration curve based on the hydrodynamic volume principle but valid only for the CTC samples, obtained by substituting a corrected $MW$ $(MW_{\text{PS,CTC}})$, for the actual $MW$ of the polystyrene standards $MW_{\text{PS}}$. The corrected value is expressed as
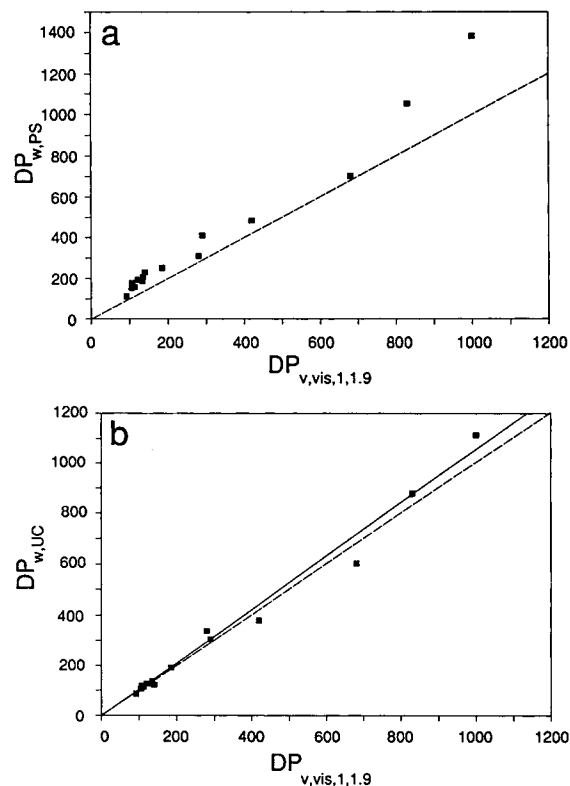


**Figure 2** Weight-average $DP$ obtained from (a) the polystyrene calibration curve $(DP_{w,\text{PS}})$ and (b) the pseudouniversal calibration curve $(DP_{w,\text{UC}})$, in relation with the corresponding $DP$ obtained by viscometry using Eq. (1) $(DP_{v,\text{vis},1,1.9})$. The dashed lines represent the theoretical relationship: $DP_{v,\text{SEC},1} = DP_{v,\text{vis},1,1.9}$. The solid line (b) represents the least-squares linear regression.

$$MW_{\text{PS,CTC}} = \left( [\eta]_{\text{PS}} \cdot \frac{MW_{\text{PS}}}{K_{\text{CTC}}} \right) 1/\alpha_{\text{CTC}} + 1 \quad (5)$$

where

- $[\eta]_{\text{PS}}$ is the intrinsic viscosity of the polystyrene standard having the molecular weight $MW_{\text{PS}}$. We used the following Mark–Houwink relation for the polystyrene in THF at 25°C: $[\eta]_{\text{PS}} = K_{\text{PS}} \cdot MW_{\text{PS}}^{\alpha_{\text{PS}}} = 0.0112 \cdot MW_{\text{PS}}^{0.72}$ (Ref. 11).

- $K_{\text{CTC}}$ and $\alpha_{\text{CTC}}$ are the Mark–Houwink constants for CTC in THF at 25°C: $K_{\text{CTC}} = 0.0053$ and $\alpha_{\text{CTC}} = 0.84$.[12]

Figure 2(b) presents the relation between $DP_{w,\text{UC}}$ and $DP_{v,\text{vis,1,1,9}}$—each point is the average of two injections—and the linear regression obtained using the least-squares method. The slope was found to be $1.04 \pm 0.03$ and $r^2 = 0.98$; an acceptable correlation but with a slight deviation of the slope from unity.

Among the parameters affecting the reproducibility and the accuracy of the average degree of polymerization are those

- Concerning the derivatization: The effectiveness of the substitution ($DS$ is taken equal to 3 for all the calculations) and the recovery of the small $DP$ molecules that can affect the $MWD$.

- Concerning the apparatus: Flow-rate accuracy (during the analysis) and precision (between two analyses) are the most important factors together with the linearity of the detector and the aging of the columns.

- Concerning the data analysis: Besides the number of slices and of points per second taken into account, the definition of the baseline (peak start and peak end) is of importance since it may vary from one experimentalist to another.

As the variation in the flow rate between two consecutive series of experiments, i.e., with a pump shutoff, can be checked by injecting a PS standard and corrected by translating the whole series of chromatograms according to the retention time shift, we only have to consider the flow rate accuracy for estimating the error on the molecular weight calculations.

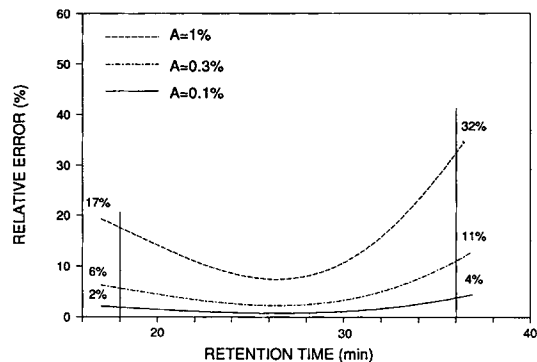The relative error on the molecular weight determination can be estimated by derivatizing the



**Figure 3** Relative error on the molecular weight determination as a function of the retention time according to different flow-rate accuracies (A).

third-order polynomial equation that describes the calibration curve employed. Figure 3 presents this result as a function of the retention time according to three different flow-rate accuracies: $A = 1\%$, $A = 0.3\%$, and $A = 0.1\%$. It is evident that an inexpensive pump having an $A$ value of 1% is not suitable for size-exclusion chromatography. A 0.3% flow-rate accuracy, which corresponds to most of the HPLC pumps including the one used for this work, gives a quite satisfactory relative error that is between 6 and 11% for the practical fractionation interval (6 and 8% for the interval of interest). As the corresponding relative error varies only between 2 and 4% in the $MW$ range under study, a high-quality pump ($A = 0.1\%$) happens to be a worthwhile piece of equipment in any HPSEC installation.

Besides showing the importance of the pump characteristics on the molecular weight determination, the calculation of the relative error induced by the variation in the flow-rate legitimates the equivalence between $DP_{w,\text{UC}}$ and $DP_{v,\text{vis,1,1,9}}$, since the relative error on the viscosity measurements, done in triplicate, is 3%.

The slight deviation from unity obtained for the slope may also be explained based on the uncertainty about the value of 1 for the Mark–Houwink coefficient $\alpha$ of cellulose solutions in cupriethylenediamine. There exists some controversy over the ($\alpha$, $K'$) values, and new sets have been suggested by various authors. Table I summarizes the results obtained when calculating—using Lotus 1-2-3—the viscosity-average degree of polymerization [Eq. (2)] on the Sigmacell HPSEC molecular weight distribution ($n_i$ as a function of $MW_{i,\text{UC}}$), together with the viscosity-average by viscometry ($DP_{v,\text{vis},\alpha,K'}$). These calculations were carried out using the values of $\alpha$ and $K'$, (a) suggested in the standard method SCAN C15 : 62 (Ref. 13):

**Table I  Viscosity-average Degrees of Polymerization of Sigmacell Obtained by HPSEC ($DP_{v,SEC,\alpha}$) and Viscometry ($DP_{v,vis,\alpha,K'}$) Using Different Mark–Houwink Coefficients and Their Ratio**

| $(\alpha, K')$[a] | $DP_{v,SEC,\alpha}$ | $DP_{v,vis,\alpha,K'}$ | $\dfrac{DP_{v,SEC,\alpha}}{DP_{v,vis,K'}}$ |
|---|---|---|---|
| $(1, 1.9)$[b] | $DP_{w,UC} = 1114$ | 1000 | 1.11 |
| $(0.905, 0.75)$[c] | 928 | 735 | 1.26 |
| $(0.90, 1.65)$[d] | 924 | 1825 | 0.51 |

[a] These symbols refer to the equation $DP^\alpha = K'[\eta]$.
[b] Ref. 8.
[c] Ref. 13.
[d] Ref. 14.

$$DP^{0.905}_{v,vis,0.905,0.75} = 0.75[\eta] \qquad (6)$$

and (b) proposed by Evans and Wallis[14]:

$$DP^{0.90}_{v,vis,0.9,1.65} = 1.65[\eta] \qquad (7)$$

Ideally, the constants $\alpha$ and $K'$ should give values for which the ratio $DP_{v,SEC,\alpha}/DP_{v,vis,\alpha,K'}$ is equal to 1. It is evident that the use of these more recent sets of $(\alpha, K')$ does not bring any significant improvement in the ratio.

## CONCLUSION

In comparative studies when the molecular weights (molar masses of IUPAC) of a given polymer are expressed in terms of PS molecular weight equivalents, the great merits of the polystyrene (PS) standards calibration procedure in performing the SEC calculations are its rapidity and its flexibility. Nonetheless, this procedure could give poor results, specifically when the compounds studied do not have a solvated structure similar to that of polystyrene. This appears to be the case for our CTC samples. In fact, since the exponent $\alpha$, according to the standard ASTM method for the Mark–Houwink equation for cellulose in CED, equals 1, the viscosity-average $DP$ should be identical to the weight-average $DP$ determined by SEC. However, the experimental results indicate that $DP_{v,vis,1,1.9}$ is always less than $DP_{w,PS}$, the difference being greater for the less depolymerized samples. On the other hand, as the use of an input calibration curve constructed with PS

standards is the only way to perform SEC calculations when commercially available or easy to produce narrow distribution standards of the sample under study do not exist and when a weight detector, such as LALLS or an on-line viscometer, is unavailable or unsuitable, the principle of universal calibration developed by Benoit and co-workers offers a satisfactory solution.

This comparative study on the $MW$ of different TMS celluloses, obtained either by viscometry in CED or by SEC in THF after carbanilation, has led to the following conclusions.

1. Assuming the viscosity-average $MW$ by viscometry is obtained without a bias—i.e., the Mark–Houwink coefficients for cellulose in CED have been correctly chosen—it appears that

   • the carbanilation procedure is nondegradative, DMF as the reaction medium gives results similar to those obtained with pyridine (data not shown) but with greater simplicity of operation,

   • THF is an appropriate solvent for cellulose tricarbanilate and offers a fair compatibility between the derivative, the solvent, and the column packing,

   • the hypothesis that the signal provided by the UV detector is proportional to the weight of the eluted cellulose sample is satisfactory,

   • it is quite easy to modify the software in order to employ the principle of universal calibration.

2. Since the Mark–Houwink parameters $K'$ and $\alpha$ are strongly correlated together, very important errors could have arisen from their experimental determination. However, it appears from this work that there is a fairly good correlation between $DP_{w,UC}$ and $DP_{v,vis,\alpha,K'}$ when the ASTM values are used instead of the more recent ones.

With every manufacturer's generation, the equipment for liquid chromatography ameliorates in reliability and becomes more accessible. The improvement in column and hardware technology has now almost closed the gap that has, for 10 years, existed between liquid chromatography and gas chromatography. This work, therefore, aims to promote size-exclusion chromatography as a standard method for process control in the cellulose industry.

## NOMENCLATURE

| | |
|---|---|
| $A$ | flow-rate accuracy |
| CTC | cellulose tricarbanilate |
| $d$ | polydispersity |
| $DP$ | degree of polymerization |
| $DP_n$ | number-average $DP$ |
| $DP_w$ | weight-average $DP$ |
| $DP_{n,PS}$ | number-average $DP$ obtained using the PS calibration curve |
| $DP_{w,PS}$ | weight-average $DP$ obtained using the PS calibration curve |
| $DP_{n,UC}$ | number-average $DP$ obtained using the pseudouniversal calibration curve |
| $DP_{w,UC}$ | weight-average $DP$ obtained using the pseudouniversal calibration curve |
| $DP_{v,vis,\alpha,K'}$ | viscosity-average $DP$ obtained by viscometry, symbols $\alpha$ and $K'$ refer to the equation $DP^\alpha = K'[\eta]$ |
| $DP_{v,SEC,\alpha}$ | viscosity-average $DP$ calculated on the HPSEC chromatogram using the Mark–Houwink coefficient $\alpha$ |
| $[\eta]$ | limiting viscosity number (mL g$^{-1}$). |
| $MW$ | molecular weight (g) |
| $MW_n$ | number-average $MW$ |
| $MW_w$ | weight-average $MW$ |
| $MW_{PS}$ | $MW$ used for the construction of the PS calibration curve (PS equivalent $MW$) |
| $MW_{PS,CTC}$ | $MW$ used for the construction of the pseudouniversal calibration curve (CTC equivalent $MW$) |
| PS | polystyrene |
| TMScell | thermo-mechano-solvolytic cellulose |

## REFERENCES

1. T. Rantanen, P. Farm, and J. Sundquist, *Paperi Puu*, **68**(9), 634–636 (1986).
2. J. D. Cosgrove, B. C. Head, and T. J. Lewis, in *Cellulose and Its Derivatives*, J. F. Kennedy, Ed., Horwood, Chichester, UK, 1985, pp. 142–152.
3. I. Kossler, J. Danhelka, M. Netopilik, M. Samkova, and G. Katuscakova, *Svensk Papperstidning*, **18**, R137–R140 (1981).
4. Z. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci. B*, **5**, 753 (1967).
5. J. I. Wadsworth, L. Segal, and J. D. Timpa, *Adv. Chem. Ser.*, **175**, 178–186 (1973).
6. G. Garnier, J. Bouchard, R. P. Overend, E. Chornet, and P. F. Vidal, *Can. J. Chem. Eng.*, **68**, 113–118 (1990).
7. D. M. Hall and J. R. Horne, *J. Appl. Polym. Sci.*, **17**, 3727–3732 (1973).
8. B. Browning, *Methods in Wood Chemistry*, Vol. II, John Wiley, New York, 1967, Chap. 25, pp. 519–557.
9. ASTM Standard D1795-62, Standard Test Method for Intrinsic Viscosity of Cellulose.
10. W. Lange, O. Faix, and O. Beinhoff, *Holzforschung*, **37**(2), 63–67 (1983).
11. M. Kolynsky and J. Janca, *J. Polym. Sci. Polym. Chem. Ed.*, **12**, 1181–1191 (1974).
12. J. Danhelka, I. Kossler, and V. Bohackova, *J. Polym. Sci. Polym. Chem. Ed.*, **14**, 287–298 (1976).
13. SCAN Standard C15 : 62, Viscosity of Cellulose in Cupriethylenediamine Solution (CED).
14. R. Evans and A. F. A. Wallis, *J. Appl. Polym. Sci.*, **37**, 2331–2340 (1989).