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Aqueous Phase Size Exclusion Chromatography of Polyvinylalcohols of Different Degrees of Hydrolysis

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Summary: The aqueous-phase size exclusion chromatography (SEC) represents a suitable method for the analysis of molecular weight distribution (MWD) of polyvinylalcohols (PVOHs) of different degree of hydrolysis ranging from 72 to 98 mol-% applying the Suprema columns. Eluents of different composition were used consisting of either a mixture of water and acetonitrile for the lower degrees of hydrolysis (72 - 88 mol-%) or 0.1 M $LiNO_3$ solution for the higher degrees of hydrolysis (88 – 98 mol-%). By knowing the $\overline{M_w}$ values of commercially available PVOHs samples of a given degree of hydrolysis and using the commercial water soluble narrow distribution standards, a principle of the constant hydrodynamic equivalence ratios was applied to obtain the absolute values of the molecular weights of PVOHs of different degree of hydrolysis. The effect of a various number of extrusion cycles on MWD of different PVOHs was used to demonstrate the sensitivity of the selected SEC conditions.

Keywords: aqueous phase SEC; degree of hydrolysis; hydrodynamic equivalence ratio; polyvinylalcohol; Suprema columns

Introduction

Molecular weight distribution (MWD) and the typical molecular weight characteristics represented by weight ($\overline{M_w}$) and number ($\overline{M_n}$) averages of molecular weight, respectively, are the principal parameters controlling the application and processing properties of polymers. In the case of biodegradable polymers, the molecular weight characteristics are the measure of the polymer durability and the rate of degradation accompanied with the decrease in the molecular weight.[1] This is the reason why the precise analysis of the molecular weight of a biodegradable polymer should be performed on regular basis as one of the most crucial techniques in the field of biodegradable polymers.

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Size exclusion chromatography (SEC) is the most routinely applied technique to determine the MWD in the majority of laboratories due to its versatility, relative simplicity and acceptable cost. The common hydrophobic polymers, including polystyrene, polyacrylates and polymethacrylates, are the polymers for which the SEC analysis is straightforward without major difficulties. As recently pointed out, however, even in this case the interactivity with the column packing of commercially available SEC columns has to be taken into account as it may have a significant effect on the SEC analysis.[2]

Polyvinylalcohols (PVOHs) as the water-soluble biodegradable polymers represent the typical class of polymers for which the interactivity is an important issue in their SEC analysis. PVOHs are formed from polyvinylacetates by hydrolysis to a various degree usually ranging from 88 to 98 mol-% of vinylalcohol moieties.[3] They have to be regarded as complex copolymers differing in (1) average molecular weights and molecular weight distributions, (2) the content of vinylalcohol and vinylacetate monomer units, and (3) distribution of vinylalcohol and vinylacetate monomer units along the polymer chain. Therefore different PVOHs exhibit a various extent of hydrophobicity (vinylacetate groups) and hydrophilicity (vinylalcohol groups), which makes it difficult to identify the proper SEC conditions (selection of columns, eluent composition, etc.) to suppress the enthalpic interactions. The SEC analysis of PVOH has been studied in detail by aqueous phase chromatography applying the principle of universal calibration[4] by employing absolute molecular weight detectors.[3,5] The diversity of PVOH samples of the same degree of hydrolysis was given by Dawkins and co-workers.[6,7] The molecular weight data were decoupled from the composition distribution of partially hydrolyzed PVOH by reversed phase liquid chromatography using the gradient elution with water/tetrahydofuran and styrene/divinylbenzene stationary phase. A broad composition distribution reflects the complexity of the PVOH samples of a given average degree of hydrolysis. Another possibility for the SEC analysis of 99.6 mol-% hydrolyzed PVOHs was shown by Tacx et al.[8] by using dimethylsulfoxide as the eluent and Ultrahydrogel columns.

Since the analysis of PVOH by size exclusion chromatography has been considered as a challenging field, it certainly appears to be appropriate to show the new experimental data for a series of commercially available PVOHs and different SEC conditions than those used in the available literature. The principal goal of this contribution is to identify the suitable conditions for a simple but reliable determination of the molecular weight characteristics of PVOHs of different degree of hydrolysis. The aqueous phase SEC equipped with the Suprema columns from Polymer Standards Service was used.

Experimental Part

Polyvinylalcohols of different degree of hydrolysis in the range from 72 to 98 mol-% were kindly provided by Chemické závody, Nováky (Slovak Republic), Clariant (formerly Hoechst, Germany), Kuraray and Mitsui (both Japan). Their characteristics are summarized in Table 1. Demineralized water was used for preparation of the eluents for aqueous phase SEC. Acetonitrile, methanol and other chemicals were of analytical grade.

Table 1: Characteristics of the PVOH samples

Sample	Viscosity of 4 wt-% in water	Degree of hydrolysis	$\overline{M_w}$ a)	Producer
	mPa.s	mol-%	g/mol	
Mowiol 10-98	10	98	48 000	Clariant
Mowiol 20-98	20	98	70 000	
Mowiol 4-88	4	88	26 000	Clariant
Mowiol 8-88	8	88	49 000	
Mowiol 18-88	18	88	84 000	
Sloviol 98-10	12	98	NA	Nováky
POVAL205	5	88	NA	Kuraray
Sloviol PVAL 13	11	72	NA	Nováky
Mitsui 72.5	6	73	NA	Mitsui

a) information provided by Hoechst: B5 - Polymerisationsgrad und Molekulargewicht von Mowiol; NA – not available

The aqueous phase SEC setup consisted of a Waters In-line degasser, a Waters pump 515 equipped with a plunger washing kit, a Rheodyne 7725i injector, a 8x50 mm guard and three 8x300 mm Suprema columns (Polymer Standards Service) with particle size 10 μm and pore sizes of 100, 1000 and 3000 Å (positioned in a Waters column heater module) and a differential refractometer Waters M2410. Optionally two Suprema columns with pore sizes 1000 and 3000 Å were used. The analysis was performed at 35°C using aqueous eluents of different compositions provided in Table 2. Ethylene glycol was used as the flow marker to control the eluent flow rate between 0.8 and 1.0 ml/min. Polyvinylalcohol samples were dissolved by boiling in the eluent for around 10 min at concentrations between 0.5 and 3.0 mg/ml. The evaporated mass of water and the required volume of an organic co-eluent were added after the complete sample dissolution. The samples were filtered via a 0.45 µm filter prior to analysis. The injected volume was 200 µl. The column calibration was performed either via narrow distributed dextran (peak molecular weight, M_p, ranging from 1.8 to 1450 kDa, American Polymer Standard Corporation, Mentor, OH) or polyethylene glycol/polyethylene oxide standards (M_p ranging from 2 to 1700 kDa, Polymer Standards Service) depending on the eluent composition. The former ones were used for the pure aqueous eluent and aqueous eluent containing up to 20 vol-% of organic co-eluent and the latter standards were used at the content of the organic co-eluent above 20 vol-%.

Table 2: Eluents used for the SEC analysis of PVOH

H_2O vol-%	$LiNO_3$ mol/L	$NaNO_3$ mol/L	CH_3CN vol-%	SDS g/L	CH_3OH vol-%	NaN_3 ppm
100	-	-	-	-	-	-
95	-	-	5	-	-	-
90	-	-	10	-	-	-
80	-	-	20	-	-	-
70	-	-	30	-	-	-
50	-	-	50	-	-	-
80	-	-	-	-	20	-
100	-	-	-	2.5	-	200
100	-	0.1	-	-	-	200
100	0.1	-	-	-	-	200

SDS – sodium dodecylsulfate

Two types of PVOHs, POVAL205 and Sloviol 98-10, were re-extruded (up to 7 cycles) using a Brabender single screw extruder operating at 30 rpm with L/D ratio equal to 25, screw diameter 19 mm and compression ratio 1:3. The temperature profiles from feeding to head were 190-200-220-210 °C for POVAL205 and 200-220-230-220 °C for Sloviol 98-10, respectively.

Composition of Mobile Phase

The key parameter in suppressing the enthalpic interactions in the aqueous phase SEC is the selection of the eluent composition.[5,9,10] Usually the pH is adjusted to provide the fully ionized form of a polymer in order to suppress the hydrogen bonding. The presence of salt controls the ionic interactions and the polyelectrolyte effects. The hydrophobic additives, e.g. acetonitrile or methanol, are added to the aqueous phase to suppress the effect of the hydrophobic interactions.[3] Sometimes it is suitable to increase the temperature of the aqueous eluent in order to lower its viscosity and to improve the resolution of analysis.

Some of these principles were applied in optimizing the eluent composition for the characterization of different PVOH samples by testing various eluent compositions given in Table 2. Figure 1 demonstrates an example of the critical effect of the eluent composition for 88 mol-% hydrolyzed PVOH sample (Mowiol 8-88) by the addition of acetonitrile from 0 to 50 vol-% to water. At the high acetonitrile content, the hydrophobic interactions are suppressed, which likely gives rise to hydrophilic interaction between the polymer chains and the column packing. Consequently, a delayed elution and distorted signal are observed. Upon decreasing the acetonitrile content to 30 and 20 vol-%, respectively, the signal rises and the elution shows the SEC-like behavior similar to work of Nagy.[3] At 10 vol-% the signal decreases and elution volumes are shifted to the higher values likely due to the hydrophobic interactions between column packing and acetate groups of PVOH, which lead to the complete signal suppression at 5 vol.-% or in the absence of acetonitrile in eluent, respectively. A small bump on the low elution volumes side of the peaks may be ascribed to the formation of aggregates.

It has also been observed that the elution profiles depend on the composition of the sample solvent. In order to avoid the artifacts from the solvent composition being different to the composition of eluent, all PVOH data shown further in this work were obtained by using the eluent composition as a solvent to dissolve polymer. The additional experiences (data are not shown) included (i) no concentration effect for the polymer concentrations between 0.5 and 3 mg/ml, which was intensively observed in the case of the aqueous phase SEC of polyacrylic acid,[10] (ii) finding that sodium dodecylsulphate (SDS) was not effective in screening the interactions as shown by Meehan et al.,[6] (iii) ageing of the sample solutions made in the aqueous phase, reported by Tacxs et al.[8] was not seen and a high reproducibility was achieved for the sample solutions analyzed within at least a few days period of time, (iv) a weaker suppression of the hydrophobic interactions by methanol compared to acetonitrile observed by the lower peak areas and shifted peaks to the higher elution volumes, and (v) using both $LiNO_3$ and $NaNO_3$ as the salts in the eluent composition gave the same results leading to the selection of $LiNO_3$ for the further work since this salt is considered less corrosive than $NaNO_3$.

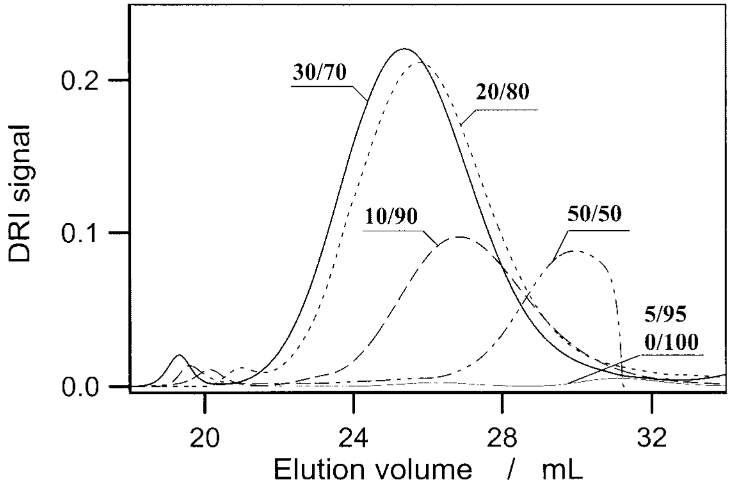

Figure 1. Elution curves of Mowiol 8-88 dissolved in water (3 mg/ml) in eluents of different volume ratio CH_3CN/H_2O. Flow rate 1 ml/min, Suprema columns 100, 1000, 3000 Å

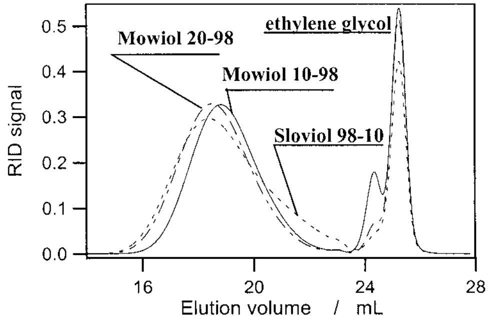

Figure 2. Elution curves of 98 mol-% hydrolyzed PVOHs in 0.1 M $LiNO_3$ containing 200 ppm NaN_3, polymer concentration 2 mg/ml, flow rate 1 ml/min, Suprema 1000 and 3000 Å

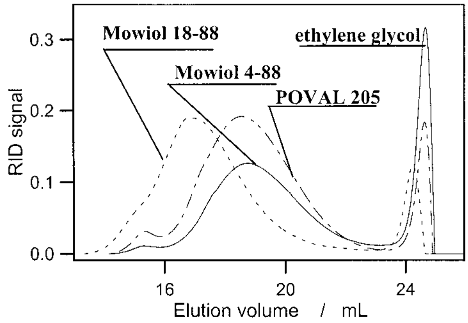

Figure 3. Elution curves of 88 mol-% hydrolyzed PVOHs in CH_3CN/H_2O = 20/80, polymer concentration 2 mg/ml, flow rate 1 ml/min, Suprema 1000 and 3000 Å

Characterization of PVOHs of Different Degree of Hydrolysis

The most suitable SEC conditions for fully hydrolyzed PVOH with the degree of hydrolysis 98 mol-% were achieved by using 0.1 M $LiNO_3$ in water containing 200 ppm of NaN_3, which follows the conditions of the work of Nagy.[3] An example of such experiments is given in Figure 2. The elution curves remind those from the organic phase SEC without a sign of adsorption or delayed retention. The SEC separation mechanism is supported by the correlation of the solution viscosity values (Table 1) with the analyzed MWD of polymers. The traces are smooth showing a slightly higher polydispersity of the sample Sloviol 98-10 from Nováky compared to the Mowiol samples from Clariant. This breadth in MWD may be caused not only by the chain-length of the polymers but also by the differences in the microstructure and chemical composition.

Two different modes of SEC analysis were found to be feasible for PVOH samples with 88 mol-% of degree of hydrolysis. The first one is the same as in work of Nagy[3] in the eluent composition CH_3CN/H_2O equal to 20/80. The elution curves in Figure 3 exhibit the SEC behavior. The same elution volumes of both Mowiol 4-88 and POVAL205 reflect the similar viscosities of 4 wt-% solutions of these polymers (Table 1). Interestingly, these partially hydrolyzed samples could be successfully analyzed under the conditions used for the fully hydrolyzed samples (Figure 2), i.e. in 0.1 M $LiNO_3$ solution containing 200 ppm NaN_3. These data are shown in Figure 4 revealing typical SEC elution curves of the same character as in Figure 3. It should be mentioned that while these partially hydrolyzed samples cannot be analyzed in pure water (Figure 1), the presence of salt provides the suitable SEC conditions for this type of PVOH to balance the hydrophobic and hydrophilic interactions in the system. The clear advantage of this approach in case of partially hydrolyzed PVOHs lays in a fast analysis along the fully hydrolyzed samples without the need to alter the conditions of analysis involving the exchange of the eluent and the equilibration of the SEC setup.

In case of special grades of PVOH with the lower degree of hydrolysis in the range of 72 mol-%, the amount of hydrophobic groups is high to provide the reasonable elution curves at the conditions used for the samples with the degree of hydrolysis 88 mol-%. At 20 vol-% of acetonitrile these samples adsorbed onto the column exhibiting a low intensity signal shifted to the high elution volumes. This situation can be improved by a further increase of acetonitrile

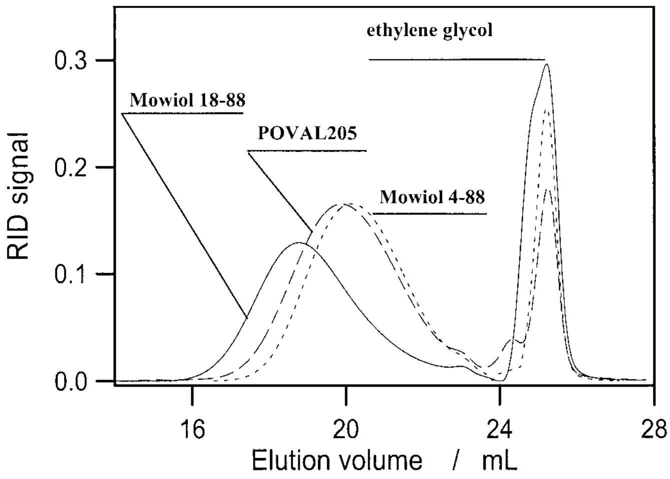

Figure 4. Elution curves of 88 mol-% hydrolyzed PVOHs in 0.1 M $LiNO_3$ containing 200 ppm NaN_3, polymer concentration 2 mg/ml, flow rate 1 ml/min, Suprema 1000 and 3000 Å

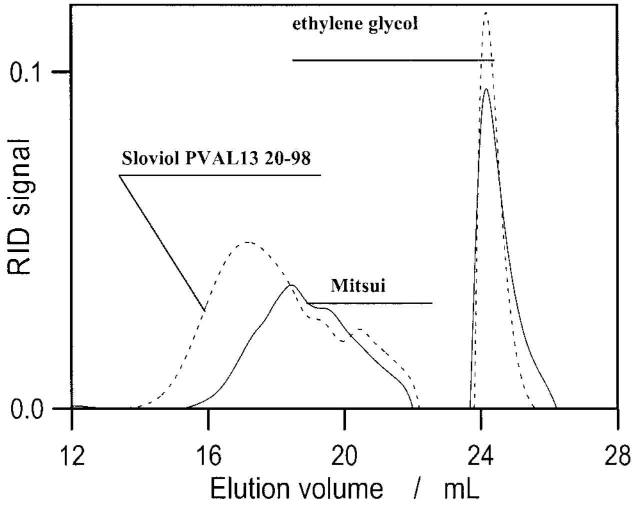

Figure 5. Elution curves of 72 mol-% hydrolyzed PVOHs in CH_3CN/H_2O = 35/65, polymer concentration 0.5 mg/ml, flow rate 0.8 ml/min, Suprema 1000 and 3000 Å

content in eluent to the amount of 35 up to 50 vol-% in water. Figure 5 depicts the elution curves for two PVOH samples with the degree of hydrolysis 72 mol-%, where the sample from Nováky, Sloviol PVAL13, is compared to the product of Mitsui. The polymodality of the elution peaks may refer to the heterogeneity of the chemical composition as well as to the polydispersity stemming from the molecular weight distribution. These two contributions cannot be distinguished from the simple SEC setup used in this work. The lower elution volume for Sloviol 98-10 than for Mitsui sample correlates with the higher viscosity of aqueous solutions of these polymers (Table 1), which is the positive feature of this elution curves reflecting the dominant effect of the polymer chain length rather than the chemical composition.

Calibration

SEC as the separation column method principally requires the calibration by relating the molar mass to the elution volume most frequently by applying the narrow polydispersity standards of the same chemical origin as the analyzed sample or by the universal calibration.[4] When the standards for the direct calibration or Mark-Houwink parameters for universal calibration are not available, the so-called equivalent calibration is often used by applying any set of the available standards (usually polystyrene in the organic phase SEC and polysaccharides - dextrans or pullulans - in the aqueous phase SEC) to which the molecular weight data of the analyzed sample are related. By this approach, obviously, the molecular weight data may significantly differ from the real values. Another option is represented by using the molecular weight detectors as the part of the SEC setup[3] providing the information on the absolute molecular weight characteristics without the need for the standards. However, the complexity involved in this technique requires time, capital and high intellectual investment in order to provide the reliable data.[11] On the other hand, the practice typically requires a simple technique providing the reasonably reliable results in a fast mode.

Absolute detectors have been used in the work on PVOHs leading to the Mark-Houwink parameters for fully and partially hydrolyzed samples.[3] Since different PVOHs even of the same degree of hydrolysis may have a different chemical distribution of monomer units as well as a different level of branching and, in addition, the columns of different suppliers used in various laboratories may exhibit a different level of interactivity, it may be not straightforward to use the universal calibration across the laboratories to obtain the information on the absolute molecular weights of PVOHs.

Here we present a simple calibration method, which should lead to reliable molecular weight characteristics of PVOH samples based on the constant hydrodynamic equivalence ratio recently applied to evaluate the molecular weight of block copolymers.[12] This method simplifies the universal calibration principle by assuming that the calibration curves of two polymers are strictly parallel in the given good solvent for both types of polymers, i.e. the Mark-Houwink parameters a are similar. The error estimation of this approach[12] points out that the maximum error introduced by this approach is acceptable for the practical purposes of the SEC analysis even if the a values are relatively dissimilar. The premise behind this approach is to know any moment of the MWD of the analyzed polymer A, M_A, and to relate this value to the same moment of the polymer standard B, M_B, eluted at the same elution volume via the hydrodynamic equivalence ratio r_B according to equation 1: [12]

$$M_A = r_B M_B \qquad (1)$$

The r_B value depends on the eluent composition and in fact the only prerequisite is to know any moment M_A of the MWD of the analyzed type of the polymer. Since the $\overline{M_w}$ values of the Mowiol samples of different degree of hydrolysis are known (Table 1), this allows for the estimation of the constant hydrodynamic equivalence ratio to, for example, the dextran calibration curve. The MWD of two Mowiol samples with the degree of hydrolysis 98 mol-% (Mowiol 10-98 and Mowiol 20-98) and two Mowiol samples with the degree of hydrolysis 88 mol-% (Mowiol 4-88 and Mowiol 18-88), calibrated toward the dextran standards, are shown in Figure 6. The obtained $\overline{M_w}$ values, related to the dextran calibration, i.e. the M_B values in eq. (1), are contained in Table 3 together with the absolute $\overline{M_w}$ values, i.e. the M_A values in eq. (1), provided by the supplier. The calculated hydrodynamic equivalence ratio for 98 mol-% hydrolyzed samples gives the value equal to 1.2 (rounded to one decimal point). In the case of 88 mol-% hydrolyzed samples, this value is close to 2.2. The same values obtained within each group of the degree of hydrolysis indicate the consistency and reliability of the used approach. It means that the dextran calibration gives 1.2 and 2.2-times lower molecular weights for 98 mol-% and 88 mol-% hydrolyzed PVOHs, respectively, than are the real values. Consequently, by assuming the similar microstructure of PVOHs of the same degree of hydrolysis from different suppliers, this enables to obtain the information about the absolute molar mass of the PVOHs from Nováky (Sloviol 98-10) and Kuraray (POVAL205). $\overline{M_w}$ of fully hydrolyzed sample Sloviol 98-10 is in the range of 70 000 g/mol with the polydispersity index ~ 9 (polydispersity index for Mowiol samples is ~ 3) and $\overline{M_w}$ for partially hydrolyzed sample POVAL205 is around 25 000 g/mol with the polydispersity index ~ 5. These values are certainly more precise than the equivalent molecular weights to dextran values (particularly in case of 88 mol-% hydrolyzed samples) and they point out that the principle of hydrodynamic equivalence ratio[12] is applicable also for the aqueous phase SEC.

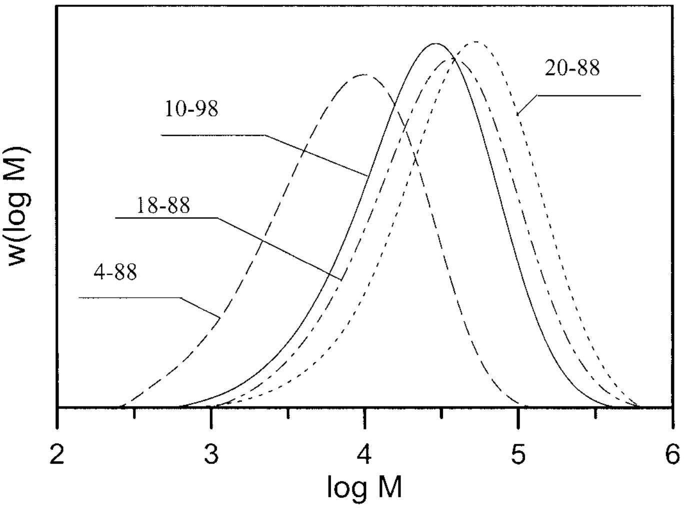

Figure 6. MWD of the Mowiol samples of different molecular weights and degree of hydrolysis

Table 3. Determination of the hydrodynamic equivalence ratio, r_B

Mowiol sample	$(\overline{M_w})_A$ Clariant	$(\overline{M_w})_B$ Dextran cal.	r_B
10-98	48 000	41 000	1.17
20-98	70 000	60 000	1.17
4-88	26 000	12 000	2.17
18-88	84 000	40 000	2.10

It should be emphasized that these ratios are strictly valid only for the given conditions of analysis and it is advisable to control them always after the conditions of analysis are modified. In case of the special grades with the low degree of hydrolysis around 70 mol-% the samples can be compared only on the relative basis as the equivalent molecular weights toward the PEO standards. Thus $\overline{M_w}$ of Mitsui polyvinylacohol is 30 000 while that of PVAL13 from Nováky is 82 000 g/mol.

Stability of PVOH During Extrusion Assessed by SEC

PVOHs are susceptible to the auto-accelerated thermal degradation during melt-processing due to the release of acetic acid.[13] Figures 7 and 8 depict the initial study of MWDs of polyvinylalcohols Sloviol 98-10 and POVAL205, respectively, determined after a different number of cycles in an extruder.

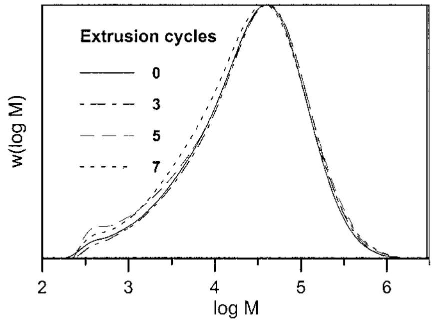

Figure 7. Effect of the number of extrusion cycles on the MWD of Sloviol 98-10

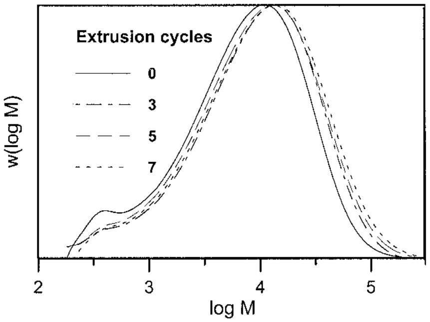

Figure 8. Effect of the number of extrusion cycles on the MWD of POVAL 205

The MWD of the fully hydrolyzed Sloviol 98-10 remain stable without observable changes with the $\overline{M_w}$ value around 68 000 g/mol until the 7th cycle, where a slight change in the MWD appeared in the low molecular weight side of distribution. Apparently the MWD of partially hydrolyzed POVAL205 sample exhibits changes already after the 3rd cycle indicating the lower stability of this type of PVOH to the processing conditions. The $\overline{M_w}$ values increase from around 25 000 up to 42 000 g/mol after the 7th cycle. The chemistry operating behind the shift of the MWD to the higher values has been investigated by using the analytical techniques complementary to SEC and will be discussed in our future work. At this stage the data in Figures 7 and 8 were selected to demonstrate that SEC can be used as a sensitive tool to follow the minor changes in MWD of PVOHs during their melt-processing.

Conclusion

This contribution deals with the specific features of the aqueous phase SEC in the determination of the MWD of polyvinylalcohols of different degree of hydrolysis between 72 and 98 mol-%. Generally, the aqueous phase SEC has not been frequently used due to a number of possible artifacts, which may negatively influence the results and lead to the incorrect conclusions. In this work the practical guidelines are provided to suppress the non-size exclusion phenomena (mainly enthalpic interactions) in the characterization of the MWD of polyvinylalcohols by using Suprema columns from Polymer Standards Service. The simple principle of the hydrodynamic equivalence ratio can be considered as a valid calibration means in acquiring the information about the real molecular weight characteristics also in the aqueous phase SEC.

Acknowledgement

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