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Carbanilation of cereal β-glucans for molecular weight determination and conformational studies

Wei Li,[a,b] Qi Wang,[a,*] Steve W. Cui,[a] Walther Burchard[c] and Rickey Yada[b]

[a] *Food Research Program, Agriculture and Agri-Food Canada, Guelph, ON, Canada N1G 5C9*
[b] *Department of Food Science, University of Guelph, Guelph, ON, Canada N1G 2W1*
[c] *Institut für Makromolekulare Chemie, Albert Ludwigs Universität, 79104 Freiburg i. Br., Germany*

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Abstract—Cereal β-glucans can form aggregates in aqueous solution. The presence of aggregates in cereal β-glucan solutions led to inaccurate determination of molecular weights and it was believed that intermolecular hydrogen bonding caused the aggregation. To eliminate aggregates, a carbanilation method for molecular weight determination of cereal β-glucans was developed. Wheat β-glucan samples were selected for investigation. The carbanilation method can prevent intermolecular hydrogen bonding by blocking hydroxyl groups with phenyl carbamate groups. The carbanilates of cereal β-glucans were prepared by the reaction of cereal β-glucans with phenylisocyanate catalyzed by DMSO and pyridine. To avoid degradation during the carbanilation reaction, relatively mild conditions were used, which led to incomplete substitution (DS: ∼2). However, after the carbanilation reaction, the carbanilates dissolved completely in 1,4-dioxane solution without any detectable aggregates, which allowed accurate molecular weight determination. The degree of substitution (DS) of carbanilates was determined by both a nitrogen content method and an FT-IR method. The FT-IR method proved to be the more effective for DS estimation. Using this method, the converted molecular weights of cereal β-glucans were in good agreement with the results measured in 0.5 M NaOH solution, which previously was shown to be a good solvent for cereal β-glucans. After the carbanilation reaction, conformational changes of carbanilates were studied by static and dynamic light scattering techniques. The fractal dimension ($d_f = 2.27$) and the structure sensitive parameters ($\rho > 2$) suggested a porous globular structure for partially carbanilated β-glucans.

Keywords: Cereal β-glucans; Carbanilate; Molecular weight; Aggregates; Degree of substitution; Conformation; FT-IR; Light scattering

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1. Introduction

(1→3)(1→4)-β-D-Glucans are cell wall polysaccharides located in the cereal endosperm and aleurone cells. Cereal β-glucans are linear homoglucans of D-glucopyranose arranged as blocks of consecutive (1→4)-linked β-D-glucose residues separated by single (1→3)-linkages.[1] The ratio of oligosaccharide segments with two consecutive (1→4)-linkages (trisaccharides) to those with three consecutive (1→4)-linkages (tetrasaccharide) is a characteristic structural indicator of cereal β-D-glucans, which follows the order of wheat (4.2–4.5), barley (2.8–3.3), and oat (2.0–2.4).[2] This trend corresponds with the differences of their conformational and physical properties of cereal β-glucans, such as, chain stiffness, solubility, viscosity, and gelling properties. To study the structure–function relationships of cereal β-glucans, it is essential to obtain an accurate measurement of their molecular weights. Light scattering is one of the important experimental tools for the determination of the absolute molecular weights of polymers due to its high sensitivity for detecting large molecules at low concentrations. However, it is a challenge to accurately determine the molecular weights of cereal β-glucans by light scattering techniques due to severe aggregation in aqueous solutions. It is believed that the aggregation

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* Corresponding author. Tel.: +1 519 780 8029; fax: +1 519 829 2600; e-mail: wangq@agr.gc.ca

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of cereal β-glucans is mainly caused by intermolecular hydrogen bonding and the interference of macromolecular aggregates in molecular weight determination has been reported by many groups.[3–6] A number of physical and chemical methods have been used to eliminate the aggregates such as filtration,[7–9] heating, centrifugation,[3] or the use of different solvents.[10] However, only limited success was achieved using these methods due to the lack of effective and direct way to evaluate their effectiveness. For example, the amount of β-glucan aggregates were too small to be detected by osmotic pressure measurements used by Vårum et al.[3] The dynamic light scattering (DLS) method allows determination of size distributions over a wide range of size where the size of macromolecular aggregates are comparable to that of un-aggregated counterparts. By applying the dynamic light scattering technique to monitor the presence of aggregates, a method for eliminating the aggregates using NaOH solutions has been developed by our group, which allows the accurate determination of molecular weights.[6]

In the present study, a carbanilation method was used to chemically substitute the hydroxyl groups of cereal β-glucans by phenyl carbamate groups to prevent the formation of hydrogen bonding and hence, aggregation. To the best of our knowledge, this idea was applied for the first time to amylose and cellulose in 1961 by one of the authors[11] who compared the solution properties of these two isomeric tricarbanilated polysaccharides, which were determined by static light scattering and viscometry in dioxane, pyridine and acetone. The conformational properties were later confirmed and completed by dynamic light scattering.[12] In the meantime, the reaction of phenylisocyanate has become a routine technique for natural and bacterial celluloses.[13–21] This method may provide an alternative method for eliminating aggregation of cereal β-glucans and it is expected that the bulky derivatives will exert a similar influence on the solution properties of various cereal β-glucans. The carbanilates of cereal β-glucans could be used to investigate the conformational differences between oat, barley, and wheat β-glucans, which could provide useful information for understanding structure–conformation relationships. However, due to steric hindrance and intramolecular hydrogen bonding between neighboring carbanilate groups along the backbone chain[17] a change in the conformation of the cereal β-glucan chains may be expected, for example, considerable deviations from the chain stiffness of cellulose tricarbanilates.[22] In the present study, the carbanilation method proved to be somewhat more involved than for cellulose and required optimization. The molecular weights were determined, and the changes in the conformational properties after carbanilation were investigated. An FT-IR method was developed to determine the degree of substitution.

2. Results and discussion

2.1. Carbanilation of cereal β-glucans

The reaction conditions of cellulose with phenyl isocyanate were studied extensively, and it was reported that the fully trisubstituted products could be easily prepared.[19–21,23] However, it is also reported that degradation occurs upon derivatization of cellulose depending on reaction time, temperature, and co-reactant. Furthermore, an unskilled precipitation procedure sometimes resulted in a loss of low molecular weight fractions up to 20%.[21] These phenomena would cause inaccurate molecular weight determination of native unfractionated cereal β-glucan derivatives.

In some cases, it is difficult to achieve full substitution of cereal β-glucans without degradation. The steric hindrance may prevent the full substitution as well. For the purpose of determining molecular weight, it is not necessary to achieve complete substitution as long as the final products can dissolve fully dispersed in 1,4-dioxane (no aggregates), which allows accurate molecular weight determination by light scattering. To prevent degradation problems, relatively mild reaction conditions were used in the present study, resulting in partially substituted carbanilate products.

To correctly convert the results from these derivatives to the molecular weights of un-substituted cereal β-glucans, it is essential to determine the degree of substitution of cereal β-glucan carbanilates accurately. It is worth noting that the solvents used for the carbanilation reaction play an important role in the reaction rate and degradation. From the results of cellulose carbanilation, the efficacy of the solvents for the carbanilation reaction decreases in the order of dimethylsulfoxide (DMSO) > pyridine > *N*,*N*-dimethylformamide (DMF) ≈ *N*,*N*-dimethylacetamide (DMA).[19] The explanations for this phenomenon are controversial. It was proposed that solvents with high dielectric constants[24] or electron donor numbers[25] were particularly effective for the reactions. However, Evans et al. concluded that the rate of dissolution of cellulose during the carbanilation reaction is probably governed by the rate and extent of swelling of the cellulose in solvents.[20] Our result for cereal β-glucan carbanilation supports the latter explanation.

Cereal β-glucans swell well in DMSO at 60 °C, but not in pyridine, even at higher temperatures. On the other hand, pyridine is an important additive to catalyze the reaction. However, the use of DMSO caused severe degradation during the carbanilation reaction of cereal β-glucan samples (data not shown). Therefore, pyridine was used as the carbanilation solvent in the present study. To promote the swelling of cereal β-glucans in pyridine, a small amount of DMSO, at a concentration below which degradation occurred, was added. However, the addition of DMSO also promoted the trimerization of phenyl isocyanate.[20] The trimer phenyl isocyanate did not dissolve in methanol and co-precipitated with the cereal β-glucan carbanilates. More serious is the occurrence of diphenylurea as a byproduct, formed due to traces of water, which is only sparingly soluble in methanol. It can be removed only by repeated re-precipitation in methanol. The methyl-phenylurethanes are easily soluble in methanol and are removed without problems. Incomplete purification would lead to an overestimation of the nitrogen content, which is used for calculation of the degree of substitution. The trimerization also consumes more phenyl isocyanate for the reaction.

To avoid loss of low molecular weight fractions during precipitation, only five fractions of wheat β-glucans were used for the carbanilation reaction in the present study, which had a narrow molecular weight distribution (polydispersity $M_w/M_n < 1.1$ for most fractions). Therefore, the loss of cereal β-glucan fractions during precipitation, if any, imparts a negligible influence on the molecular weight distribution. However, care must be taken to ensure that glucan carbanilate chains are not removed during the preparation. Wood et al.[21] reported precipitation of cellulose tricarbanilate (CTC) in methanol-fractionated CTC, if the original cellulose had a DP less than 40. Even 5% loss of CTC exerted a large influence on the number average molecular weight of the samples. However, the influence on the weight average molecular weight was negligible.

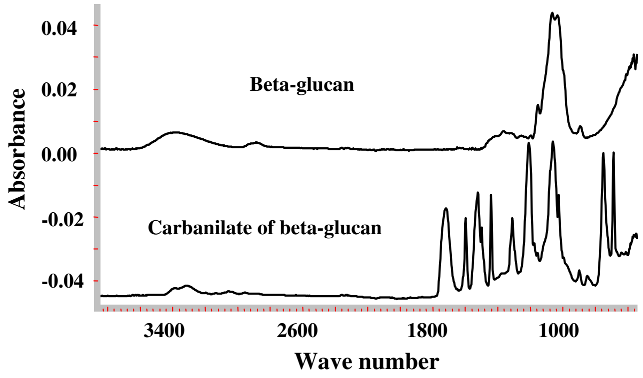
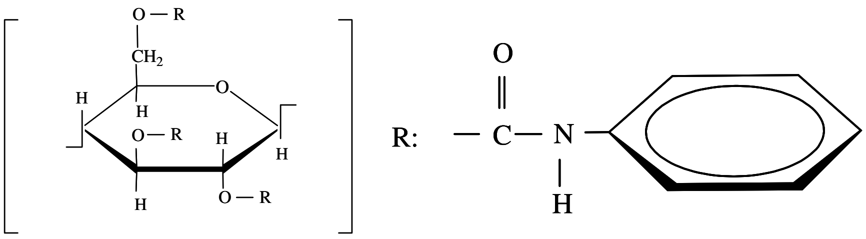


Figure 1. FT-IR spectra of cereal β-glucans and their derivatives.

The reactions of cereal β-glucans with phenylisocyanate were monitored by FT-IR spectroscopy. The FT-IR spectra of wheat β-glucan Fraction 1 before and after carbanilation are shown in Figure 1 as an example and are compared with the primary structure of the β-glucan carbanilate (Fig. 2). The functional groups and corresponding wave numbers and the assignment of the bands are presented in Table 1. After reaction, the additional functional groups (C=C, N–H, C=O, C–H) are shown in the fingerprint region of the carbanilate spectra. The absorbance in the 3600–3000 cm^{-1} region corresponded to hydrogen bonding of –OH groups.[26] A drastic decrease of intensity in this region indicated the substitution of –OH groups (Fig. 3). The remaining peaks in this region implied incomplete substitution. The absorbance in the 3600–3000 cm^{-1} region was used to calculate the degree of substitution as discussed later.



β-Glucan carbanilate

Phenyl isocyanate group

Figure 2. The structure of cereal β-glucan carbanilates.

Table 1. FT-IR spectrum of cereal β-glucans and their derivatives; wave numbers and intensities of functional groups

Wave number (cm^{-1})	Functional groups	Intensity
3600–2500	O–H stretching	Broad, strong
3000–2800	C–H stretching, symmetric, asymmetric	Sharp, occasionally double overlapping with O–H
1760–1730	C=O stretching	Strong
1600	C=C stretching	Strong
1550–1510	N–H deformation	Strong
1470–1460	C–H scissoring, asymmetric	Strong
1305–1200	N–H deformation	Medium
1150–1070	C–O–C stretching, asymmetric	Strong
1200–1000	C–O stretching	Strong
770–690	C–H out-of-plane, asymmetric	Strong

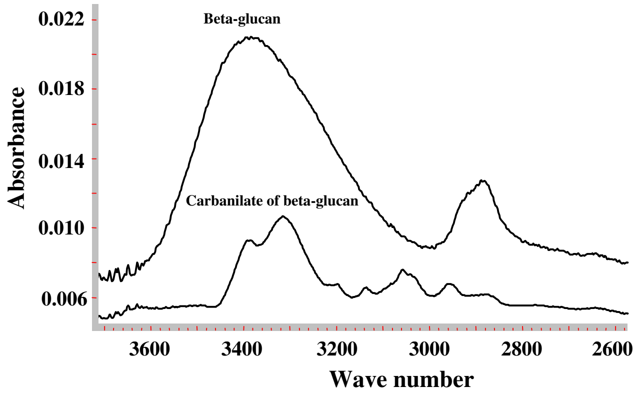


Figure 3. The FT-IR absorbance of –OH groups of cereal β-glucans and their derivatives.

2.2. Determination of the degree of substitution and molecular weight

The commonly used method for determining the degree of substitution (DS) of carbanilates is the nitrogen content method. Impurities due to traces of nitrogen containing by-products may overestimate the degree of substitution, which would result in an underestimation of molecular weight. Such impurities include trimer phenylisocyanurate from trimerization of phenylisocyanate (11.76% N), diphenylurea (13.08% N) from the reaction of phenylisocyanate with water, and methyl carbanilate from the reaction of methanol with phenylisocyanate. With the exception of methyl carbanilate (9.40% N) these by-products may co-precipitate in methanol with the carbanilates of cereal β-glucans. The higher nitrogen content of the by-products causes overestimation of degree of substitution.[20]

A new method for determination of degree of substitution was developed in the present study using FT-IR spectroscopy. With the progress of FT-IR techniques, its use for quantitative analysis is widely accepted, for example, the degree of esterification of pectin was obtained by FT-IR analysis.[27–29] As described in the previous section, the absorbance of hydrogen bonding of –OH groups in 3600–3000 cm^{-1} region were selected for DS determination. The potential by-products did not have absorbance in this region, thus did not interfere with the measurements. One problem for this method is that large intensity variations existed among experiment replicates. This is caused by the inevitable density difference of the test samples during packing the samples to the ATR sampling cell. To solve this problem, a reference peak was chosen in the 1150–940 cm^{-1} region, which corresponds to both the C–O–C asymmetric stretching and C–O stretching frequencies of the sugar rings in the backbone of cereal β-glucan molecules and their derivatives. Because there were no changes of the backbone during the carbanilate reaction, when the peak areas of $\nu_{\text{–OH}\cdots}$ band were normalized to this reference peak, reproducible results were obtained. On the other hand, there are limitations to this method. The presence of water would interfere the peaks in this region; therefore, complete removal of moisture is essential. Furthermore, this method assumed a linear relationship between the degree of substitution and the intensity decrease. The lack of standard curve may influence the accuracy of this method.

The DS values of cereal β-glucan carbanilates and their converted molecular weights to the non-substituted glucans are summarized in Table 2. Higher values of DS were obtained by the nitrogen content method compared to the FT-IR method. In the cereal β-glucan fractions, the DS values were less than 3, indicating incomplete substitution. The DS measured by FT-IR method showed molecular weight dependence, that is the low molecular weight fractions had a higher degree of substitution than the others. This probably is due to the fact that smaller molecules are better dispersed in pyridine and furnish less steric barrier to the substitution, which favors the carbanilation reaction. The DS, measured by nitrogen content analysis, did not show molecular weight dependence. It may be caused by the interference of the by-products, which was co-precipitated with the β-glucan carbinilates. Higher molecular weight fractions may have a higher ability to entrap the by-products, and this masks a higher DS.

Figure 4 shows the molecular size distribution of carbanilate of wheat β-glucan Fraction 1 in 1,4-dioxane solution measured by dynamic light scattering. The uni-model distribution indicated there was no observable aggregation present in dioxane solution. Although full substitution was not achieved, the steric hindrance of the bulky substituted groups may have prevented the formation of hydrogen bonding between the remaining hydroxyl groups. The aggregate-free solutions of

Table 2. Degree of substitution (DS) and molecular weight (M_w) of β-glucan carbanilates and the molecular weight of corresponding β-glucans calculated by using DS obtained from nitrogen method (M_{wc1}) and FT-IR method (M_{wc2})

Sample	DS1 (N%)	DS2 (FTIR)	M_w (Da)	M_{wc1} (Da)	M_{wc2} (Da)	$M_{w\,ref}$ (Da)
Car-F1	2.30	1.70	1,022,100	379,800	454,500	475,200
Car-F2	2.79	1.91	829,100	271,800	345,000	367,900
Car-F3	2.44	1.97	640,500	229,000	261,300	277,800
Car-F4	2.70	2.27	532,200	178,100	199,400	194,200
Car-F5	2.72	2.03	163,300	54,500	65,600	54,600

$M_{w\,ref}$ is M_w measured in 0.5 M NaOH solution.[30]

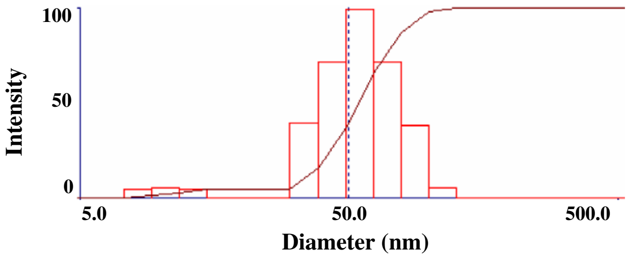


Figure 4. Molecular size distribution of cereal β-glucan carbanilates in 1,4-dioxane. solution measured by dynamic light scattering.

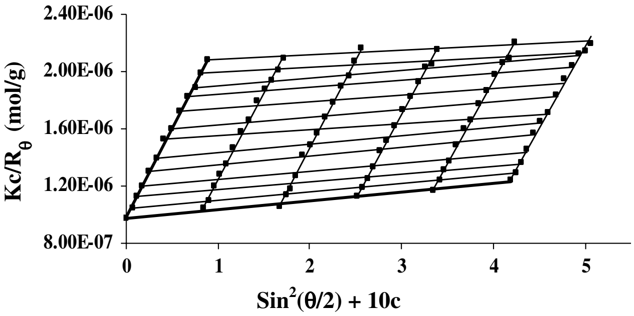


Figure 5. The Zimm plot of cereal β-glucan carbanilates in 1,4-dioxane solution measured by static light scattering.

carbanilates provided the possibility of accurate molecular weight determination and the conformational study by light scattering techniques. For comparison, the converted molecular weights of wheat β-glucan fractions are shown in Table 2, together with those measured in 0.5 M NaOH solution.[30] Good agreement with the converted molecular weights by the FT-IR method was obtained.

2.3. Conformational properties of cereal β-glucan carbanilates

A Zimm plot of wheat β-glucan carbanilate Fraction 1, measured by static light scattering, is shown in Figure 5 as an example. The weight average molecular weight (M_w), radius of gyration (R_g), and second virial coefficient (A_2) extracted from Zimm plots are listed in Table 3. The positive values of the second virial coefficients of the fractions indicated 1,4-dioxane as a good solvent for the cereal β-glucan carbanilates.

Due to the addition of the large phenyl carbamate groups to the backbone chain the molecular weight increased largely (2–3 times). As expected, the increase of the radius of gyration was not as high as that of the molecular weight, which is caused by the coil-like conformation of the chains. Applying the scaling concept, the exponent ν in equation: $R_g = KM_w^{\nu}$ was obtained from the logarithmic plot of M_w versus R_g (Fig. 6). A value of $\nu = 0.45$ was obtained for the carbanilates of wheat β-glucans, which is considerably lower than $\nu = 0.62$ for wheat β-glucan in 0.5 M NaOH solution.[30] The lower ν value of carbanilates may reflect a branched conformation for the cereal β-glucan carbanilates, which would be in contradiction to the non-substituted β-glucans in 0.5 M NaOH. For the moment, we can only safely state a fractal dimension of $d_f = 1/\nu = 2.27$ compared to that in 0.5 M NaOH of $d_f = 0.161$, which is typical for coiled linear chains in a good solvent with some indications for chain stiffness.

The conformational changes of carbanilates were also studied by dynamic light scattering. The angular dependence and concentration dependence of the apparent diffusion coefficients are demonstrated by the dynamic Zimm plot (Fig. 7). Each concentration line (linear fitted) was extrapolated to zero angle, whereas each angle line (linear fitted) was extrapolated to zero concentration. The intercept of the extrapolation lines corresponds to the translational diffusion coefficient D. Applying the Stokes–Einstein equation, the hydrodynamic radii R_h were calculated and are summarized in Table 3. Combined with the results from static light scattering measurement, the structure sensitive parameter ρ (defined as the ratio of R_g/R_h) was obtained and also listed in Table 3. For monodispersed random coils, $\rho = 1.50$ in a θ solvent was predicted and $\rho = 1.78$ in a good solvent. For rigid rods, values of $\rho > 2$ are to be expected.[31] However, a broad molecular weight distribution can increase the ρ-values by about 15%; in a similar manner, a porous structure would give a much larger ρ-value as a result of partial solvent draining. The ρ-values of all carbanilate samples were larger than 2 as shown in Table 3; this would suggest a rod-like conformation of the β-glucan carbanilates in 1,4-dioxane solution, which contradicts the conclusion derived from fractal dimension data. The fractal dimension d_f of carbanilate of wheat β-glucans was 2.27, which clearly indicated a globular-like structure similar to a branched or aggregated conformation. A stiff conformation is indicated by fractal dimension of $d_f = 1.0$ –1.5. Thus, a stiff linear

Table 3. Summary of static and dynamic light scattering results of carbanilates of wheat β-glucan fractions analyzed by Zimm plots and dynamic Zimm plots

Sample	$M_w \times 10^5$ (Da)	$A_2 \times 10^4$ (cm^3 mol/g^2)	R_g (nm)	R_h (nm)	ρ
Car-F1	10.22 ± 0.07	2.50 ± 0.28	69.52 ± 0.58	31.85 ± 0.05	2.18
Car-F2	8.29 ± 0.06	2.03 ± 0.34	54.00 ± 0.67	26.82 ± 0.30	2.02
Car-F3	6.41 ± 0.04	2.52 ± 0.09	50.76 ± 0.64	20.60 ± 0.20	2.46
Car-F4	5.32 ± 0.06	3.11 ± 0.07	48.90 ± 0.10	20.5 ± 0.15	2.39
Car-F5	1.63 ± 0.02	4.50 ± 0.21	28.60 ± 1.30	12.61 ± 0.22	2.27

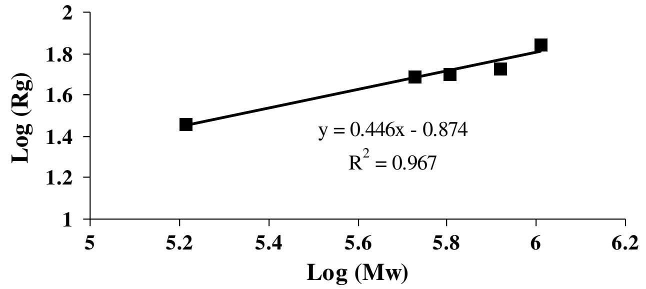


Figure 6. Double logarithmic plot of the molecular weight versus the radius of gyration of cereal β-glucancarbanilates in 1,4-dioxane solution.

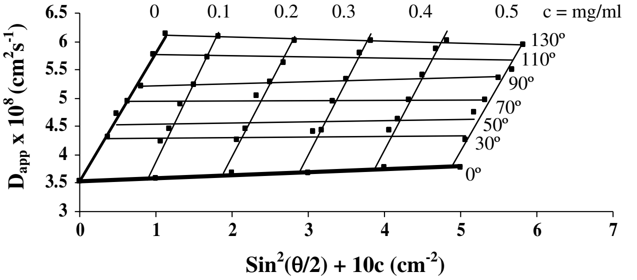


Figure 7. The dynamic Zimm plot of cereal β-glucan carbanilates in 1,4-dioxane solution measured by dynamic light scattering.

conformation has to be discarded. In the current experimental condition the effect of polydispersity has to be excluded in view of the measured low polydispersity. In addition, intermolecular aggregation was not detected. The observation of a large fractal dimension (indicating globular structure) and large ρ-parameter value can be reconciled if a porous globular structure is taken into consideration. A porous globular structure allows partial draining of the solvent that leads to a decrease in the hydrodynamic radius and increase of the ρ-parameter. However, the question still remains why the incompletely carbanilated β-glucan can have a globular structure although the measurements of the non-substituted β-glucan clearly exhibited the expected coil behavior in a good solvent with fractal dimension of $d_f = 1.61$. Ongoing work has been to prepare carbanilates from other cereal β-glucans, that is, oat and barley β-glucans. A better understanding of the conformational property of glucan carbanilates is expected by the comparison of molecular parameters obtained from all three β-glucan carbanilates, and from those obtained for cellulose tricarbanilates.

3. Conclusions

Intermolecular hydrogen bonding led to aggregation of cereal β-glucans in water resulting in inaccurate molecular weight determination. Intermolecular hydrogen bonding can be eliminated by the carbanilation method, when all or most of the hydroxyl groups were chemically substituted by phenyl carbamate groups. After the carbanilation reaction, the carbanilates were uniformly dispersed in a non-aggregated form in 1,4-dioxane, which allowed accurate molecular weight determination. The degree of substitution of carbanilates was determined by both elementary nitrogen content determination and a newly developed FT-IR method. The FT-IR method proved to be better in the present study. The molecular weight data obtained from the molecular weights of the carbanilates were in good agreement with the results measured in 0.5 M NaOH solution. Conformational analysis via the fractal dimension and the structure sensitive parameters ($\rho = R_g/R_h$) suggested a porous globular structure for partially carbanilated β-glucans. Further study on other cereal β-glucans (oat and barley) is underway to verify this hypothesis.

4. Experimental

4.1. Materials

Five fractions of wheat β-glucans differing in molecular weights were used for the carbanilation reaction. These fractions were obtained from the purified wheat β-glucan samples using the gradient ammonium sulfate precipitation method as described previously.[32,33]

4.2. Preparation of wheat β-glucan carbanilates

DMSO (0.5 mL) was added in a glass vial containing 100 mg β-glucan sample, and kept in room temperature for 10 min, then put it in an oil bath at 60 °C. When the β-glucan sample became clear (around 30–60 min), 5 mL anhydrous pyridine was added. The mixture was stirred until it dispersed very well (~60 min). The suspension was heated to 100 °C for 10 min. As 0.65 mL phenyl isocyanate was added, a quick reaction started, and the suspension became a clear solution with a slightly golden color. After 60 min, the solution was cooled to room temperature, and then 1.5 mL MeOH was added to consume the non-reacted phenyl isocyanate. The final solution had a β-glucan carbanilate concentration of about 5% (w/v).

The solution was slowly added to 800 mL MeOH for precipitation. The precipitate was washed with the same volume of MeOH. After the removal of MeOH, the residue was dried in an air oven at 60 °C. For further purification, the residue was dissolved in 20 mL acetone, centrifuged (10,000*g*, 20 min) and the supernatant was evaporated until the volume was close to 5 mL. The solution was precipitated into water. The precipitate was recovered by filtration and finally dried in vacuum oven at 80 °C for 3 h.

4.3. Determination of degree of substitution

4.3.1. Nitrogen content method. Nitrogen content was analyzed using NA2100 Nitrogen and Protein Analyzer (Thermo Quest, Milan, Italy). The degree of substitution of carbanilates was calculated based on the following formula:

$$\mathrm{DS} = 162 \times \mathrm{N}\%/(14 - 119.12 \times \mathrm{N}\%).$$

4.3.2. FT-IR spectroscopic method. FT-IR spectra were obtained using a FTS 7000 Fourier transform infrared spectrometer equipped with a DTGS detector (DIGILAB, Randolph, MA). A Golden-gate Diamond single reflectance ATR was used for sampling. The spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1} (mid infrared region). 128 scans at a resolution of 4 cm^{-1} were averaged. To avoid the interference of water, all samples were dried in a vacuum oven at 80 °C for 3 h first and then moved to a vacuum desiccator containing a desiccant (phosphorous pentoxide powder) and kept for 24 h before the measurement. The absorbance of hydrogen bonding of –OH group in the 3000–3400 cm^{-1} region ($\nu_{\mathrm{-OH}\cdots}$ band) was used for calculation. The decreased intensity of the absorbance of hydrogen bonding after carbanilation is supposed to be proportional to the degree of substitution. Therefore,

$$\mathrm{DS} = 3 \times (A_0 - A_1)/A_0$$

where A_0 is the peak area of $\nu_{\mathrm{-OH}\cdots}$ band of original cereal β-D-glucans; A_1 is the peak area $\nu_{\mathrm{-OH}\cdots}$ band of cereal β-D-glucan carbanilates.

4.4. Light scattering

A 35 mW helium neon laser (Melles Criot Laser Group, Carlsbad, CA, USA) with a wave length of 632.8 nm was focused on to a precision cylindrical cell (quartz, diameter 25 mm) containing a sample solution. Both static and dynamic measurements were conducted using a Brookhaven light scattering instrument including a precision Goniometer, a photomultiplier and a 128-channel BI-9000AT digital autocorrelator (Brookhaven Instruments, Holtsvile, NY, USA). Both static and dynamic measurements were carried out in the angular range of 30–140° and data was extrapolated to zero degree to yield desired parameters. Toluene was used as a reference with the Rayleigh ratio of $1.398 \times 10^{-5}\ \mathrm{cm}^{-1}$ in the static light scattering measurements. The refractive index increment, $\mathrm{d}n/\mathrm{d}c$, was determined as 0.158 mL/g for β-glucan cabanilates in 1,4-dioxane solution. For dynamic measurement, the translational diffusion coefficient (D) was obtained by using cumulant analysis. The hydrodynamic radius (R_h) was calculated by applying Stokes–Einstein relation for sphere particles: $D = kT/6\pi\eta R_\mathrm{h}$, where T is absolute temperature, k is the Boltzmann constant, and η is the solvent viscosity. All the light scattering measurements were performed at 25 °C. 1,4-Dioxane was used as the solvent for cereal β-glucan cabanilates. In the experiments, dynamic light scattering was used to measure hydrodynamic radius, whereas the static light scattering allowed the determination of weight average molecular weight M_w, radius of gyration R_g, and the second virial coefficient A_2.

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