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Rheological studies of barley (1 → 3)(1 → 4)-β-glucan in concentrated solution: investigation of the viscoelastic flow behaviour in the sol-state

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Abstract

A (1 → 3)(1 → 4)-β-D-glucan isolated from barley by a set of commercial β-glucans differing in molecular size was characterized by means of viscometry and size exclusion chromatography coupled with multi-angle laser light scattering. A η_0–M_w–c relationship ($\eta_0 = 10^{-3} + 6.04 \times 10^{-5} c M_w^{0.71} + 2.56 \times 10^{-6} c^2 M_w^{1.42} + 2.70 \times 10^{-15} c^{5.18} M_w^{3.68}$) did not reveal any evidence of association tendencies in the concentrated fresh solution either. However, rheological measurements showed a transition from typical viscoelastic behaviour to gel-like properties over a period of several hours, with a long induction period which allows rheological measurements in the sol state. In shear experiments the concentrated β-glucan solutions showed flow irregularities correlating with a Weissenberg number (N_1/τ) in the range 1–3. Repeated cycles of short-term steady shear and rest periods accelerated the sol–gel transition, whereas steady shear did not induce gelation. Barley β-glucan in freshly prepared aqueous solution behaves like a viscoelastic liquid, without any associated structures being detected even over a period of several hours, despite its ability to form solid gels. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Barley (1 → 3)(1 → 4)-β-D-glucan; Viscoelastic flow behaviour; Sol–gel transition; Flow irregularities; Shear-induced gelation

1. Introduction

The major component of cell walls of barley endosperm is (1 → 3)(1 → 4)-β-D-glucan (β-glucan), an unbranched homopolymer of D-Glc which is linked with β-(1 → 3) and β-(1 → 4) glycosidic bonds in a ratio of about 3:7 [1–4]. The linkage arrangement is not completely irregular but follows statistical rules [4–6]: consecutive blocks of (1 → 4)-linkages (mostly 2 or 3 [7], sometimes up to 14 [8]) are separated by single (1 → 3)-bonds at random. This polysaccharide is of special interest in brewing research because it is responsible for two different effects:

(a) Improper malting or mashing lead to high concentrations of β-glucan. The resulting high viscosity lowers the extract yield and affects wort run-off.

(b) During the storage period at low temperatures a gelatinous precipitate is sometimes

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formed, which blocks the filter media in the final filtration of the beer.

The latter effect is usually a problem for brewers who avoid the use of enzymes for degradation of the troublesome β-glucans, e.g., the German brewers who adhere to the German beer law [9].

The first effect is mainly caused by dissolved high-molar-mass glucan [10], i.e., β-glucan in the sol-state. The second effect is caused by the association abilities of β-glucan, which are not understood in detail, though it is generally accepted that β-glucans are responsible for the formation of gelatinous precipitates [11]. It was shown that precipitate formation can be induced, or at least promoted, by shear forces [12]. In this same context β-glucans of high molar mass are regarded as the troublesome species, as was deduced from ultra filtration experiments [13]. Low-molar-mass β-glucans seem to play a minor role in the brewing process. Most authors assume that the cellulose-like sequences of up to 14 consecutive (1 → 4)-linkages act as junction zones for association [7,11,12]. However, longer blocks of contiguous cellotriosyl residues were also believed to be responsible for insolubilization [8]. In a recent publication indications of association are interpreted from rheological measurements in dilute and semi-dilute solution as well [14]. No further discussions about gelation kinetics or the gelation mechanism were found.

Our aim was to systematically study the molecular structure in solution by viscometry and light scattering and the viscoelastic properties of β-glucan in dilute and concentrated solution in the sol state. Proceeding from this, we wanted to trace the junction of the single molecules to an infinite network, i.e., the sol–gel-transition by means of rheological measurements which allow better understanding of the gelation mechanism of β-glucan (see following paper).

2. Materials and methods

Samples and sample preparation.—The characteristics of the β-glucan samples used in this work are listed in Table 1. Apart from BG375, which was extracted according to the description below, all the samples were commercially available (Megazyme, Bray, Ireland). BG50 was degraded by sonication [15].

The powdery samples were suspended in 1–2 mL deionized water (including 3 mmol L^{-1} sodium azide to prevent microbial contamination) and kept in an oven for 24 h at 90 °C until they were homogeneously dissolved. They were removed from the heat immediately before use and rapidly cooled down to room temperature in a water bath.

Isolation of β-glucan from barley.—The extraction from a barley variety harvested in Northern Germany in 1996 followed McCleary's method [16] in principle, but included

Table 1
Characteristics of the β-glucan samples[a]

Code	Origin	M_w (10^3 g mol^{-1})	M_n (10^3 g mol^{-1})	M_w/M_n	$[\eta]$ (cm^3 g^{-1})	k_H
BG375	Isolated from barley	375	240	1.6	510	0.855
BG300	Megazyme	300	205	1.5	460	0.832
BG275	Megazyme	275	180	1.5	440	0.762
BG200	Megazyme	200	140	1.4	330	0.772
BG165	Megazyme	165	85	1.9	255	0.621
BG140	Megazyme	140	90	1.6	260	0.726
BG100	Megazyme	100	75	1.3	210	0.632
BG50	Sonicated BG165	40	30	1.3	115	0.565
CMBG	Carboxymethylated BG300, DS[b] 1.3	380	250	1.5	540[c]	0.258

[a] M_w = weight-average molar mass, M_n = number-average molar mass, M_w/M_n = polydispersity, $[\eta]$ = intrinsic viscosity, k_H = Huggins constant. The accuracy of the molar mass determination is ± 5% and of the intrinsic viscosities ± 3%, respectively.

[b] DS = degree of substitution.

[c] In 0.1 M $NaNO_3$.

some changes worth noting: the barley (150 g) was ground on a 0.5 mm sieve (Retsch, ZM 1000, Haan, Germany) and endogenous enzyme activity was destroyed by refluxing the flour in 80% (v/v) ethanol (750 mL) for 30 min. The flour was then washed with 96% (v/v) ethanol (750 mL) and dried at 80 °C. A suspension of the flour in phosphate buffer (0.01 M, pH 6.0) was slowly heated to 95 °C. When the starch started pasting up and thus producing a high viscosity (at 70 °C), heat-stable β-amylase (3.5 mL, Sigma, Steinheim, Germany, No. A 3306) was added. After 15 min, staining with iodine could no longer be observed, indicating the starch had been completely degraded to small dextrins. After 1 h the residue was removed by centrifugation and washed twice with water (250 mL). The washings were added to the extract and the volume reduced to 0.5 L by diafiltration (Minitan, Biomax membrane, exclusion limit 30,000 g mol^{-1}, Millipore, Bedford, UK). To the concentrated extract, ammonium sulphate was added so as to give a final concentration of 30% (w/v) and the sample thus prepared was kept over night at 4 °C. The precipitate was centrifuged and washed with 20% (v/v) and 96% (v/v) ethanol (100 mL), resuspending it with a kitchen homogenizer. It was then redissolved in deionized water (1 L) at 80 °C and filtered over celite (Sigma, acid washed) to remove hazes. To the filtrate an equal volume of 96% (v/v) ethanol was added, the precipitate was washed with 96% (v/v) ethanol and dried in vacuo. A yield of 3.55 g (2.3%) high-molar-mass β-glucan was obtained. The β-glucan content in the grain was 5.1% determined by the method described elsewhere [17].

Carboxymethylation of β-glucan.—BG300 was carboxymethylated according to the method described in [18]. The degree of substitution was determined by IGATED ^{13}C NMR spectroscopy with Me_2SO-d_6 as solvent on a Bruker MSL 300 at 75.47 MHz at 100 °C by quantitative evaluation of the glucose C_1- and the C=O-signals of the carboxymethyl group.

Determination of molar mass and molar mass distribution.—The samples were fractionated on four size-exclusion chromatography (SEC) columns with decreasing exclusion limit (TSK-Gel PW_{XL} G3000, G4000; G5000, G6000; Toso Haas, Stuttgart, Germany) using filtered (Anodisc 47, pore size 0.02 (m, Whatman, Maidstone, England) sodium nitrate solution (0.1 mol L^{-1}) with sodium azide (3 mmol L^{-1}) as carrier liquid and sample solvent. The columns were coupled with a multi-angle laser light scattering photometer (MALLS) sensitive to molar mass (DAWN-F, Wyatt Technology Corp., Santa Barabara, CA, USA) equipped with a He–Ne-laser ($\lambda = 632.8$ nm) and a differential refractometer (DRI) sensitive to concentration (Shodex RI SE 51, Showa Denko, Tokyo, Japan). For determination of the refractive index increment (dn/dc), eight barley glucan solutions in the concentration range 10^{-3} to 10^{-4}% (w/w) were directly injected into the DRI. The (dn/dc)-value was obtained by graphical evaluation of the measured refractive indices versus the concentrations.

The columns were operated at 77.5 °C at a flow rate of 0.5 mL min^{-1}. The eluate was cooled down to 25 °C in a water bath before analysis with MALLS and DRI.

Polymer degradation by sonication.—Ultrasonic reduction of the molar mass was carried out with a W-450 sonifier (Branson, Heusenstamm, Germany) with a 19 mm titanium resonator at the medium power setting. The sample solution (1% (w/w) in deionized water) was kept at a temperature of 65 °C. The abraded titanium particles from the resonator were removed by centrifugation.

Viscometry.—The viscometric measurements for determining intrinsic viscosities $[\eta]$ were carried out with an Ubbelohde capillary (Type 536 13, Schott, Hofheim, Germany) at 25 ± 0.1 °C. The flow times were measured with a Viscoboy 2 (Lauda, Lauda-Königshofen, Germany). Deionized water with sodium azide (3 mmol L^{-1}) was used as solvent. Prior rheological experiments ensured that the measurements took place within the Newtonian region at the chosen concentrations.

Rheological experiments.—Oscillatory measurements were carried out with a Rheometrics Fluids Spectrometer (RFS 8500, Rheometric Scientific, Bensheim, Germany). The rheometers employed for shear measurements were a Universal Dynamic Spectrometer (UDS 200, Physica, Stuttgart, Germany) and two ARES rheometers with normal force detection (Rheometric Scientific, Bensheim, Germany) equipped with a 10 and 2000 g transducer, respectively. All rheometers were fitted with cone-plate measuring tools. The measurements were carried out at 25 °C, unless otherwise noted.

Determination of protein content.—The protein content was calculated from the nitrogen content (obtained from elemental analysis) multiplied by the factor 6.25.

Sugar analysis.—For polysaccharide hydrolysis the dried samples (50 mg) were first treated with ice-cold 72% sulfuric acid (1 mL) and kept at 30 °C for 1 h. The mixture was then diluted with water (28 mL) and further hydrolysed at 120 °C in an autoclave. After dilution to 100 mL, the acid hydrolysate was injected (20 µL) into a borate complex anion-exchange chromatography system (BTA 2118 Biotronik, Maintal, Germany). The borate buffer was directly mixed from its components potassium tetraborate (1 M), boric acid (0.5 M) and distilled water with a ternary pump system (Kontron 325, Kontron, Neufahrn, Germany). For elution of the sugars a linear gradient from 20% potassium tetraborate, 20% boric acid, 60% water to 70% potassium tetraborate, 20% boric acid, 10% water over 30 min was employed. The column was then equilibrated for a further 15 min with the starting buffer before starting the next cycle. For analysis the sugars were subjected to a post column reaction with copper–bicinchoninate [19] and the absorption measured at 546 nm. The chromatography software MTZ2 (Kontron, Neufahrn, Germany) was used for quantitative evaluation.

3. Results and discussion

Purity of the samples.—The protein contents of the commercial samples and the isolated β-glucan BG375 were both close to the limit of sensitivity, i.e., < 0.5%. The glucose purity of the commercial samples was higher (97.5%) than of BG375 (95.9%). Xyl was the main contaminant.

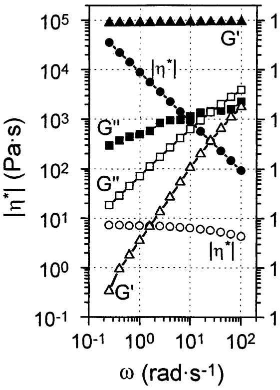

Fig. 1. Complex viscosity $|\eta^*|$, storage modulus G' and loss modulus G'' vs. angular frequency ω of a freshly prepared BG165, 6% (w/w) solution (open symbols) and the same sample 3 days later (solid symbols).

Changes in rheological behaviour of concentrated β-glucan solutions with time.—Fig. 1 shows oscillation measurements of a fresh β-glucan solution (BG165, 6% w/w) with all the features of a viscoelastic liquid. The same solution three days later behaves like a typical gel. In the viscoelastic liquid state a frequency-independent complex viscosity $|\eta^*|$ (Newtonian behaviour), a storage modulus G' with a slope of 2 and a loss modulus G'' with a slope of 1 with deviations in the non-Newtonian region are observed [20,21]. In the gel state the complex viscosity has a slope of -1, G' is independent of the frequency and G'' is more than one decade below G' [22]. With rheological oscillation experiments the change can be traced over time as shown in Fig. 2. After an induction period a sharp increase of G' and $|\eta^*|$ sets in, whereas changes in G'' are minor. The induction period proved to be long enough to perform rheological measurements

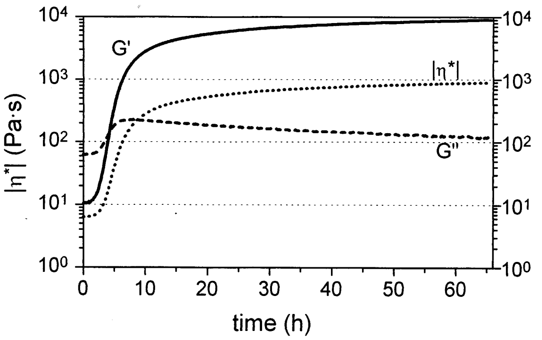

Fig. 2. Changes in storage modulus G', loss modulus G'' and complex viscosity $|\eta^*|$ in an oscillatory time experiment at 10 rad s^{-1}. BG165, 6% (w/w), 25 °C.

(at least 10 min) in the sol-state without any interference from the onset of gelation. The induction period increases with decreasing concentration.

The gelation proved to be completely thermoreversible, i.e., heating of the gelled sample at 80 °C restored the viscoelastic liquid behaviour shown in Fig. 1. This setting–melting cycle was repeatable at least ten times without any detectable changes. This observation led to the concept of minimizing association by performing the measurements above the gel melting temperature (SEC) or with sample solutions which were heated to 80 °C and cooled down immediately before use (MALLS, rheology, viscometry).

Light scattering and viscometry.—To understand macroscopic flow and association behaviour, precise knowledge of both chemical and steric structure is necessary. This part of the paper deals with the rheological aspects in the sol-state. Hence the conformational information in solution, accessible via light scattering and viscometry, is more important. By coupling a size-exclusion chromatography system (SEC) with a multi-angle laser light-scattering photometer (MALLS) and a concentration detector (here: refractive index DRI) the weight-average molar mass M_w, the number-average molar mass M_n and the polydispersity M_w/M_n are obtained. Detailed information about the apparatus is given in [23]. The refractive index increment of β-glucan used for calculating the molar masses was determined as $(dn/dc) = 0.130$.

As discussed above, the SEC was run at 77.5 °C to favour the measurement of single molecules rather than associated structures.

The intrinsic viscosities were obtained by extrapolation of viscometric data to zero concentration according to the Huggins equation (Eq. (1)) with $\eta_{\text{spec}} = (\eta_{\text{solution}}/\eta_{\text{solvent}}) - 1$ and the Huggins constant k_H.

$$\frac{\eta_{\text{spec}}}{c} = [\eta] + k_H[\eta]^2 c \qquad (1)$$

With a set of intrinsic vicsosity values for different molar masses a Mark–Houwink relationship (Eq. (2)) can be obtained by graphical evaluation.

$$[\eta] = K_\eta \, M_W^a \qquad (2)$$

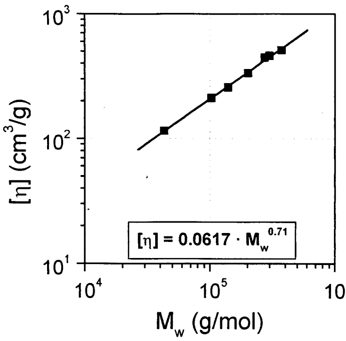

Fig. 3. Plot of intrinsic viscosities $[\eta]$ vs. weight-average molar masses M_w of β-glucan. Linear regression leads to the Mark–Houwink relationship in aqueous solution at 25 °C and a polydisperity (M_w/M_n) of 1.3–1.6.

The exponent a gives information about the coil shape: $a < 0.5$ indicates a very compact system, e.g., glycogen, $a = 0.5$ indicates β-conditions, $0.5 < a < 1$ expanded coils, $a = 2$ rigid rods.

The experimental data and the fitted function are shown in Fig. 3. The Mark–Houwink relationship for β-glucan in water at 25 °C is $[\eta] = 0.0617\ M_w^{0.71}$ with $M_w/M_n = 1.2$–1.6 for all employed data. This result is in good agreement with that reported in Ref. [24]. The Mark–Houwink exponent $a = 0.71$ indicates an expanded coil. The expansion is more likely to be attributable to chain stiffness than to excluded volume because a good solvent prevents gelation.

From the intrinsic viscosities the critical concentration $c^*_{[\eta]}$ can be calculated (Eq. (3)) assuming that at $c^*_{[\eta]}$ the whole solvent volume is filled with unperturbed single coils. According to Einstein, $2.5/[\eta]$ is the density of the polymer coil when the coil is spherical.

$$c^*_{[\eta]} = \frac{2.5}{[\eta]} \times 100 \text{ in \% (w/v)} \qquad (3)$$

The critical concentration $c^*_{[\eta]}$ of BG50 (lowest molar mass) is about 2% and of BG375 (highest molar mass) about 0.5%, i.e., all the rheological experiments were indeed carried out in concentrated solution. The results of both light scattering and viscometry (Table 1) were highly reproducible and independent of sample preparation, provided that the samples were heat treated before use.

The observed good correlation between intrinsic viscosities and molar mass is incompatible with associated structures because the cluster size should depend on concentration and the sample history [25,26]. The negligible extent of association in freshly heated solutions is supported by the similar degrees of polymerisation (DP) of the sample BG300 (DP = 1840) and its carboxymethylated derivative CMBG (DP = 1430), in which association should be electrostatically and sterically hindered. The slightly smaller DP of CMBG may be caused by degradation due to the strongly basic reaction conditions during carboxymethylation.

η_0–M_w–c relationship of barley β-glucan.—The results described above indicate the absence of associates in fresh, diluted β-glucan solutions but this may not be true for concentrated solutions. In the transition from diluted to concentrated solution a sharp increase of zero shear viscosity η_0 is observed for every polymer system due to the onset of entanglement in the polymer chains above the critical concentration c^* (provided that the molar mass exceeds the entanglement molar mass). A suitable measure for the segment density in solution is the overlap parameter $c[\eta]$ [27]. A linear relationship between log η_0 and log $(c[\eta])$ is found for $c[\eta] < 1$ (dilute solution) and for $c[\eta] > 10$ (concentrated solution) with a transition for $1 \leq c[\eta] \leq 10$ (moderately concentrated solution). If the Mark–Houwink relationship, the Huggins constant k_H and the slope n of log η_0 versus log $(c[\eta])$ in the concentrated state are given, a η_0–M_w–c relationship valid for a wide range of molar masses and concentrations of the form

$$\eta_{spec} = \frac{\eta_0}{\eta_{solvent}} - 1 = c[\eta] + k_H(c[\eta])^2 + B_n(c[\eta])^n \quad (4)$$

can be calculated [28]. It allows prediction of the zero shear viscosity η_0 of a polymer solution with a concentration c with a simple single measurement of the intrinsic viscosity $[\eta]$.

With $k_H = 0.703$, $B_n = 5.57 \times 10^{-6}$, $n = 5.18$ and $[\eta] = 0.0617 M_w^{0.71}$ the η_0–M_w–c relationship for β-glucan is:

$$\eta_{spec} = (6.04 \times 10^{-5} c M_w^{0.71} + 2.56 \times 10^{-6} c^2 M_w^{1.42} + 2.70 \times 10^{-15} c^{5.18} M_w^{3.68}) / 10^{-3}$$

Fig. 4 is a plot of the measured data and the calculated function. For comparison, some η_0–M_w–c relationships of different non-associating polymer–solvent systems are also shown [29,30]. If β-glucan had a tendency to associate in concentrated solutions the final slope should be markedly higher than actually observed ($n = 5.18$). In our working group a slope of 7.0 for ι-carrageenan and a slope of 15.3 for κ-carrageenan in deionized water as solvent were observed in concentrated solution at room temperature [31], both being examples of associating systems. From this it may also be concluded that fresh β-glucan solutions do not exhibit significant energetic chain interactions in the concentrated state. However, for oat β-glucan, a final slope of 3.9 was reported [32]. A possible explanation is that the maximum value of $c[\eta]$ was 7, whereas in this work the $c[\eta]$ scale was extended to 50.

Shear and oscillation experiments of fresh concentrated β-glucan solutions.—Comparison of shear and complex viscosity may suggest associations and molecular clusters which are sensitive to shear forces. Cox and Merz empirically found that in polymer solutions that are devoid of energetic interactions shear

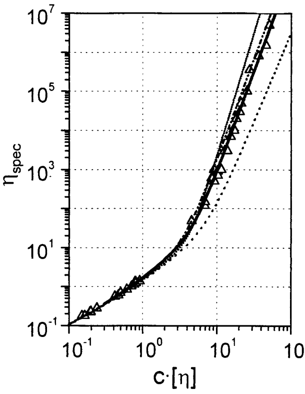

Fig. 4. Plot of zero shear viscosity (expresses as the specific viscosity η_{spec}) vs. overlap parameter $c[\eta]$ for β-glucan. The solid line is the calculated function of the η_0–M_w–c relationship. As reference, some η_0–M_w–c relationships of non-associating polymer–solvent systems are also plotted (. . . . polystyrene in toluene [32], ...polystyrene in *t*-decalin [32], -··-·· polyurethane in *N*,*N*-dimethylformamide [33]).

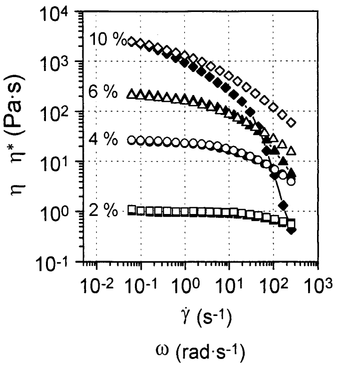

Fig. 5. Comparison of shear viscosity η as a function of shear rate γ (solid symbols) and complex viscosity $|\eta^*|$ as a function of angular frequency ω (open symbols) for BG300 at different concentrations.

and oscillatory flow curves are identical [33]. Deviations from this rule are found for instance in aqueous polyacrylamide solutions [34] and xanthan solutions [35]. For low concentrations of β-glucan BG300 (2 and 4%) the flow curves are identical. At higher concentrations (6 and 10%) shear viscosity decreases more rapidly than the complex viscosity in the non-Newtonian region, whereas zero shear and zero complex viscosity are identical (Fig. 5). It is important to note that the shear viscosity of the 10% solution declines with a slope of less than -1 at shear rates $>30\ \mathrm{s}^{-1}$ (the limiting value must be >-1, e.g., for narrowly dispersed polystyrene in toluene the observed slope is -0.83 [36], the calculated value is -0.82 [37]). The reason is sample loss (Fig. 6) due to flow irregularities, i.e., deviations from laminar shear flow between cone and plate [38]. The strong decrease of the viscosity is virtual because the shear stress and the normal stress difference N_1 run through a maximum (Fig. 7).

In Ref. [39] the onset of flow irregularities is correlated with elastic forces expressed as the Weissenberg number We:

$$\mathrm{We} = \frac{N_1}{\tau} \qquad (5)$$

When elastic forces dominate (at $\mathrm{We} \approx 3$), flow irregularities first appear as deviations from laminar shear flow and are enhanced in sample loss.

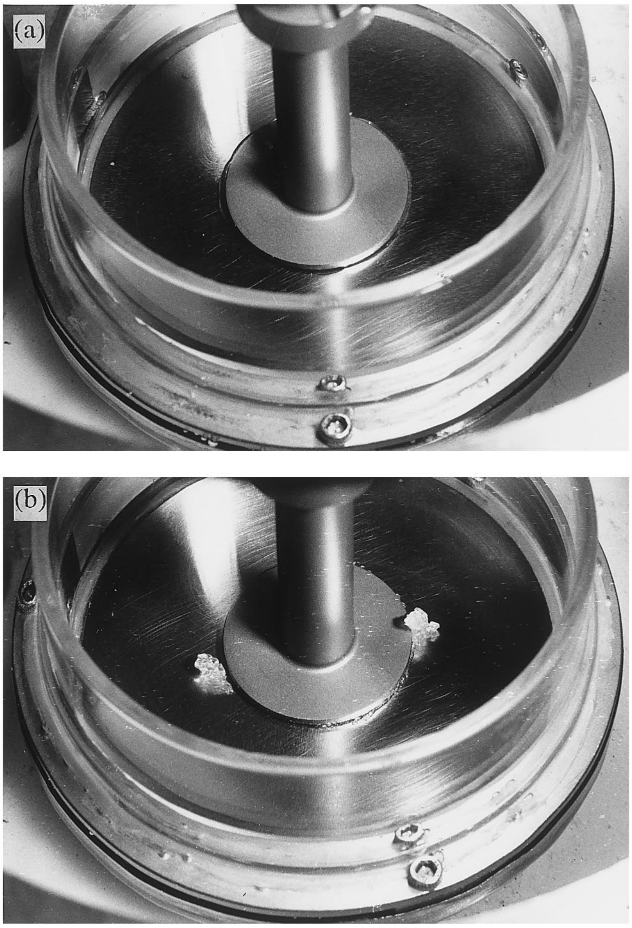

Fig. 6. BG300 10% (w/w) in cone-plate geometry at 1 s^{-1} (a) and at 10 s^{-1} showing flow irregularities ans sample loss (b).

Flow irregularities of β-glucan occurred independently of molar mass, concentration or temperature. The shear stress turns to negative slopes at lower shear rates than the first normal stress difference because it is more sensitive to sample loss. Therefore the Weissenberg number was calculated from the maximum shear stress value and the conjugated N_1 value. The onset of flow irregularities in β-glu-

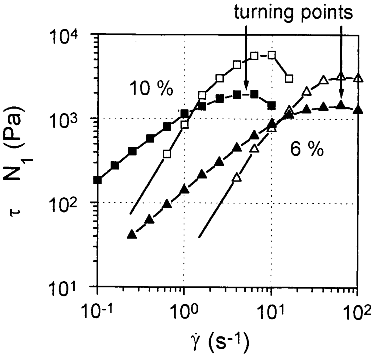

Fig. 7. Shear stress τ (solid symbols) and first normal stress difference N_1 (open symbols) for BG300 in 6 and 10% (w/w). The turning points of the shear stress curves indicate sample loss in the measuring system.

Table 2
Shear stress values at the turning points $\tau_{\max}$, the related first normal stress difference values N_1 and the Weissenberg numbers $\mathrm{We} = N_1/\tau_{\max}$ as a measure of elastic forces in laminar shear flow

Sample and conditions			$\tau_{\max}$ (Pa)	N_1 at $\tau_{\max}$ (Pa)	We
BG300	10% (w/w)	25 °C	1970	5630	2.9
BG300	10% (w/w)	55 °C	2050	3770	1.8
BG300	10% (w/w)	85 °C	1790	2060	1.2
BG300	8% (w/w)	25 °C	1950	2980	1.5
BG300	6% (w/w)	25 °C	1450	3210	2.2
BG165	10% (w/w)	25 °C	2680	3870	1.4
BG100	10% (w/w)	25 °C	2730	2420	0.9
CMBG	10% (w/w)	25 °C	2660	3780	1.4

can solutions occurs at $\mathrm{We} \approx 1$–3 (Table 2). To ensure that this effect is merely attributed to elasticity and not to β-glucan-specific properties, the carboxymethylated β-glucan CMBG was tested in the same manner. This polymer, whose intermolecular interactions via hydrogen bonding should be suppressed, also showed sample loss at $\mathrm{We} = 1.42$.

Shear-induced gelation of concentrated β-glucan solutions.—For highly diluted β-glucan solutions, such as beer containing 0.04% (w/w) β-glucan on average [40], formation of a gelatinous sediment can be induced by shear forces employing a homogenizer [13]. Formation reaches completion some two weeks after exposure, indicating this a very slow process. In concentrated solution it was possible to cause remarkable changes in the rheological behaviour within less than 1 h.

When BG300 was exposed to repeated cycles of slow shear flow and rest periods a stepwise transition into gel-like behaviour could be demonstrated by creep tests (Fig. 8). A creep test consists of two phases: the creep phase, where a constant stress is applied, and the recovery phase, where the stress is zero. The deformation profile in the creep phase mainly depends on the shear viscosity of the sample. A low viscosity is reflected in high deformation values. In the recovery phase elastic forces are detectable. High elasticity causes high strain recovery. In the case of an elastic solid the strain is recovered to 100%, provided that the network structure is not affected by the test conditions. A low stress was applied, resulting in small deformations. The creep test with the fresh solution shows a typical viscoelastic behaviour, i.e., a nearly linear increase of the deformation with time and a relatively small strain recovery of 26%. Then the sample was subjected to a shear period of 2 min with a shear rate of 2 s^{-1} and a rest period of 10 min. Three of these cycles were repeated and a creep test was carried out between each cycle. After the third cycle (about 50 min after the beginning) the sample shows gel-like behaviour, i.e., small deformation indicating a strong increase in viscosity and 100% strain recovery.

On the other hand, application of a constant shear stress on a fresh sample over one hour does not alter the solution properties significantly (Fig. 9). For the shear-induced gelation it thus seems to be essential for the molecules to be orientated by shear forces and for them to have a chance to form lateral intermolecular interactions resulting in a network structure during rest periods. In the steady shear experiment the hydrogen bond cross-links are likely to be disrupted while still

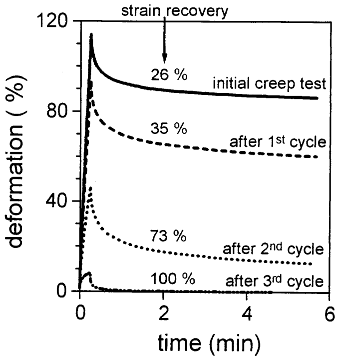

Fig. 8. Creep recovery tests with BG300, 10% (w/w) (creep time 15 s, 153 Pa, recovery time 5.5 min). A cycle consists of 2 min shearing at a rate of 2 s^{-1} and a 10 min rest period. After the third cycle the creep recovery curve indicates gel-like properties.

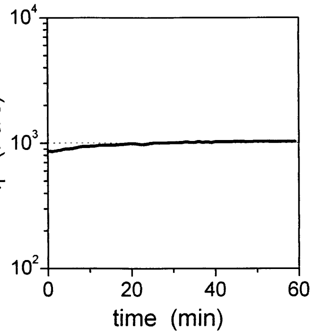

Fig. 9. Shear viscosity η as a function of time during application of a constant shear stress (982 Pa), BG300, 10% (w/w).

in an incipient state. This additionally supports the hypothesis that it is elastic forces and not shear-induced association that cause the flow irregularities.

The shear-induced gelation of β-glucan is the intermediate between the sol-state, apparently free of chain interactions, and the gel formation, a thermodynamically driven spon-taneous process of hydrogen bond association. The next part of this paper will report on rheological and kinetic investigations of the gelation of β-glucans and will also discuss aspects of the gelation mechanism.

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