Funorans from *Gloiopeptis* species. Part II. Rheology and thermal properties

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**Abstract**

The rheology and thermal properties of funorans from the main species of the red algal genus *Gloiopeptis* – *G. furcata*, *G. tenax* and *G. complanata*, isolated by the successive cold and hot water extraction processes were comparatively investigated with special attention given to the effect of co-solutes, thermal history and galactan concentration. The galactan samples were characterized by dynamic rheometry, differential scanning calorimetry, optical density studies and X-ray analysis. Funoran gel formation is a slow process depending highly on the presence of cationic substances. The ability of metal ions to enhance funoran gelation follows the order of Li\(^+\) < Na\(^+\) < NH\(_4\)\(^+\) < K\(^+\) < Rb\(^+\) < Cs\(^+\) for monovalent and the sequence of Cu\(^{2+}\) < Mg\(^{2+}\) < Mn\(^{2+}\) < Zn\(^{2+}\) < Ca\(^{2+}\) < Sr\(^{2+}\) < Ba\(^{2+}\) for divalent cations. The Ba\(^{2+}\) ions exhibit a specific effect on the gel forming process of funoran. The ability of funoran to form very strong gels was observed for the first time, especially in the presence of Ba\(^{2+}\) ions. Anionic substances exhibit a weak inhibitive effect on the gelation of funoran, with the strongest effect observed for SCN\(^-\) and the weakest for Cl\(^-\). The galactans from *G. furcata* and *G. tenax* show similar rheological properties, latter giving solutions with somewhat higher viscosities; the presence of idealized agarose diads in the composition of *G. complanata* funorans notably enhances their gelling ability. The helical pitch of funoran (1.8 nm), determined by X-ray diffraction analysis, is slightly smaller than that of agarose.

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**1. Introduction**

The polysaccharide mixture obtained from the red algae belonging to the genus *Gloiopeptis* is widely used as thickening agent and adhesive in various applications, especially in Japan (Hirase, Araki, & Ito, 1996; Swider & Smith, 2005; Takano, Hayashi, Hara, & Hirase, 1995; Yasunaga, Koga, Takano, Kajiwara, & Urakawa, 2006; Yu et al., 2010). The main component of these polysaccharides is agarose bearing sulfate groups at 6-0 of β-D-galactopyranose units (named ‘funoran’), i.e. the polymer composing of G6S–LA (β-D-galactose-6-sulfate – 3,6-anhydro-α-L-galactose) diads according to the nomenclature proposed by Knutsen, Myslabodski, Larsen, and Usov (1994). Our previous findings have also confirmed the presence of smaller amounts of G6M–LA (6-O-methyl-β-D-galactose – 3,6-anhydro-α-L-galactose), G6S–LA2M (β-D-galactose-6-sulfate – 2-O-methyl-3,6-anhydro-α-L-galactose), G6S–L6S (β-D-galactose-6-sulfate – α-L-galactose-6-sulfate) and G–LA (β-D-galactose – 3,6-anhydro-α-L-galactose) moieties (Fig. 1a, b), acetylated residues, uronic acids and xylose in the polysaccharides from *Gloiopeptis* species (Tuvikene et al., 2015).

There are no extensive studies on the rheological properties of funoran. It has been proposed that the substitution on O-6 of β-D-galactopyranose residue does not affect the helical conformation of such polysaccharides, but instead may affect the aggregation of these helices and thus gelation (Lahaye & Rochas, 1991). The gel forming ability of funoran solutions has not been described in the literature. Instead, funorans have been found to form highly viscous solutions. It has been shown that the viscosity of funoran solutions is dependent on seaweed species, plant size and degree of
Synergy has been observed between carrageenans (Yang, Guo, He, & Ruijun, 2007) given to the in presence of various amounts of metal ions with special attention of 0.02 M Li⁺ having acetylation degree of 0.05, and HF1b (from containing galactose, 6.0% sulfur, 32.8% 3,6-anhydro-L-galactose with samples for rheological measurements were prepared by mixing a 2.2. Rheological measurements

X-ray analysis, agarose I from Sigma was used. For commercial agarose samples were from Sigma (agarose A2929) and from Dojindo Molecular Technologies, Japan (agarose III). For isolation procedures, chemical and mineral part compositions, three subsequent isolations at elevated temperatures. The detailed isolation procedures, chemical and mineral part compositions, molecular weight distributions and structural characteristics of the preparations used in this study are described in our previous work (Tuvikene et al., 2015). The present study focuses on the physical properties of these funoran preparations. The main samples used in the current study are HF1a (from G. tenax), containing galactose, 6.8% sulfur, 13.6% 3,6-anhydro-L-galactose, 4.47% uronic acids and having acetylation degree of 0.05, and HF1b (from G. tenax), containing galactose, 6.0% sulfur, 32.8% 3,6-anhydro-L-galactose with no detectable amounts of uronic acids or acetylated residues. Commercial agarose samples were from Sigma (agarose A2929) and from Dojindo Molecular Technologies, Japan (agarose III). For X-ray analysis, agarose I from Sigma was used.

2.2. Rheological measurements

To ensure complete solubilization of the polysaccharide, the samples for rheological measurements were prepared by mixing a salt solution with a solution of the polymer. Freeze-dried funoran preparations were solubilized in water by boiling water bath to prepare 3.0% (w/w) polymer solutions. After complete dissolution, the hot salt solution was added to obtain 1.5% polysaccharide solutions containing a specified amount of cations. The mixture was stirred vigorously, heated for additional 1 min and immediately used for dynamic rheology measurements.

Dynamic rheology measurements were carried out using a HAAKE MARS II (Thermo Scientific) rheometer. A parallel plate geometry (35 mm diameter, 1 mm gap) and 1% strain amplitude that was well within the measured linear viscoelastic region was used in all the determinations. The hot (80°C) polymer solution was loaded on the preheated plate, the edge of the sample compartment was covered with a moistened sponge in order to minimize water evaporation. After an equilibration time of 3 min, the cooling step was performed at a constant rate of 2°C/min and a fixed frequency of 1 Hz from 70°C to 15°C followed by an isothermal measurement step at 15°C for 120 min. The gelling temperature (Tg) was defined as the crossover point of the storage (G’) and loss (G”) moduli (i.e. tan δ = 1). After the isothermal step, frequency sweeps were recorded over the range 0.01–10 Hz. The sample was subsequently heated from 15°C to 85°C using the same parameters as in the cooling step. The melting temperature (Tm) was determined at the point where tan δ = 1.

Continuous-shear flow curves were obtained by using a concentric cylinder measuring system (CC25-DIN) at 15°C. The samples were allowed to equilibrate for 15 min prior to the measurement. The viscosity was measured versus shear rate that was continuously varied from 0.1 to 1000 1/s within 10 min time.

The relative viscosity of 0.05% funoran solutions (ηrel) at 20°C and 70°C was measured by using a Cannon-Fenske capillary viscometer. The samples for the viscosity measurements were prepared by dissolving the freeze-dried polysaccharide in water in a boiling water bath.

2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a Micro-DSC III system (Setaram, Caluire, France). 1.5% polymer solutions in 0.12 M CaCl₂ or 0.25 M KCl (0.8 ml) were loaded into stainless steel cells, heated to 85°C to erase the thermal history, cooled to a specified temperature (1, 5, 15 or 25°C) at a rate of 1°C/min, kept at that temperature for 120 min and then reheated to 85°C at 1°C/min. Transition enthalpies (per gram dry weight of funoran) and temperatures were determined using the built-in DSC software.

2.4. Optical density measurements

Optical density measurements were performed on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a thermoelectric cell holder S-1700 at 400 nm against water as a blank. The hot (~90°C) galactan solution was inserted into a 2-mm cuvette, sealed and kept in the cuvette holder at 85°C for 5 min to allow sample equilibration. Thereafter, with continuous absorbance reading, the temperature was decreased at a rate of 2°C/min to 15°C, followed by the isothermal hold for 2 h after which the temperature was increased (2°C/min) to 95°C. The results were normalized so that the absorbance at 85°C before the cooling step was zero.

2.5. X-ray analysis

X-ray diffraction analysis was performed on a SmartLab (Rigaku™, Tokyo, Japan) diffractometer (CuKα radiation, 9 kW
rotating anode source, camera radius 200 mm) by using point focus optics (CBOf). An X-ray detector Pilatus 100 K (Dectris Ltd., Baden, Switzerland) was used for recording of two-dimensional (2D) transmission scattering patterns. The air scattering was decreased by a primary beam trap located nearby the sample position. The configuration of sample holder and primary beam trap enabled to record scattering pattern in the interval of scattering angle 20 starting at 7. Small angle X-ray scattering (SAXS) optics of Smart-Lab was used for recording one-dimensional (1D) transmission patterns below 7 (20). Si and Ag benenate powders were used for calibration of camera radius. Diffractometer software 2DP was used for integration of the 2D scattering pattern and converting to an equivalent slit recorded 1D-scattering pattern.

To make films suitable for X-ray diffraction analysis, 2% polysaccharide solutions in water (for funoran) or in DMSO (dissolved at 60–100 °C) were poured into teflon moulds and the solvent was evaporated slowly in an air circulation thermostat at 60 °C. The evaporated films (thickness 0.17–0.20 mm) were repeatedly washed with methanol to remove traces of DMSO (if needed) and stretched in 75% ethanol (v/v) in regular intervals to 2 times the original length at room temperature.

To make pure Na+–funoran preparations for X-ray analyses, the polysaccharide solution was dissolved in water at 95 °C followed by the addition of dry NaCl to make 0.25% funoran solution in 10% NaCl. The solution was stirred overnight at room temperature, dialyzed (molecular weight cutoff 12 kDa) against water to remove free salts, diluted to 0.1% polymer content and passed through amberlite IR120, Na+–form. The obtained solution was concentrated under vacuum at 40 °C and freeze-dried. The method allowed preparing pure Na+–funoran samples with metal to sulfate molar ratio of 1.1 containing negligible amounts of other cations.

3. Results and discussion

3.1. Aggregation behaviour

For the polysaccharide fractions obtained by the successive extraction steps from G. tenax, the ηrel of dilute solutions was measured at 20 and 70 °C (Fig. 2a). As expected, the cold water extracted preparations revealed higher viscosities compared to those obtained by the hot water isolation procedures. The latter were also characterized by significantly smaller peak average molecular weight (Mp) values (Tuvikene et al., 2015). As a rule, the ηrel values measured at 70 °C were 3–8% lower than those measured at 20 °C. This could indicate the aggregation behaviour of these polysaccharides or a change in chain hydration or conformation. Surprisingly, an opposite tendency was observed for the preparations CF1a and CF2b which appeared to be more viscous at high temperatures (Fig. 2a). This could indicate the need of high temperatures for these samples to achieve more complete hydrated states whereas at lower temperatures the solubility of these preparations is significantly impaired. It should be noted that even the diluted solutions of these preparations remained rather turbid and it was not possible to remove the haziness with prolonged stirring at high temperatures or by conventional centrifugation techniques. The partial solubility of these samples is connected with their structural characteristics – CF1a and CF2b are among the preparations containing the lowest amounts of 3,6-AG and have relatively high acetylation degrees (AD). Although the amount of uronic acids is also high in these preparations (4.89 and 4.57%, respectively), the poor solubility is evidently not connected with the presence of uronates, as these also appear in high quantities in the highly soluble CF1b.

The lowest viscosities were observed for the preparations isolated in the first hot water extraction step by precipitation in saline alcoholic solution (HF1b) while the subsequent isolation steps afforded samples (HF2b, HF3b) with slightly higher viscosities. The organic solvent used in the precipitation had a strong influence on the rheological properties of the funoran samples. Use of more hydrophilic solvents, like ethanol, led to more homogeneous preparations with higher viscosities by precipitation without the aid of salts (HF1a) whereas the less polar organic solvents, e.g., 2-propanol, gave less polydisperse preparations with slightly lower viscosities by precipitation in saline alcoholic solutions (HF1b, see Fig. 2a).

The molecular weight distributions were very similar for the HF1b (from G. tenax) isolated by ethanol and 2-propanol, but the latter was slightly less polydisperse. Nevertheless, continuous-shear flow curves revealed significant differences in the rheological properties between these samples with shear-thinning behaviour being especially evident for the ethanol-precipitated sample (Fig. 3a, b).

The overall tendency of the G’ and G” values measured for 1.5% funoran sols agrees with the ηrel results of their dilute solutions (Fig. 2a, b). Although such correlation is coincidental and not necessarily expected, it could be explained by a higher molecular weight of essentially the same polymer leading to both a higher sol viscosity and a higher elastic modulus of the gel. G’ was larger than G” for the cold water extracted preparations, whereas an opposite

![Fig. 2. Relative viscosities (ηrel) of 0.05% G. tenax funoran sols measured at 20 °C (a) and at 70 °C (b)].

![Fig. 3. Shear rate (γ) dependence of the viscosity (η) for G. tenax funoran (HF1b) 1.5% sols. Sample obtained by precipitation in saline ethanol (a) or saline 2-propanol (b).]
trend was noted for the samples obtained by the hot water process, with a notable exception being HF1a. It should be noted that none of these funoran samples formed self-standing gels at 1.5% polymer concentrations in pure water even after ageing overnight at 4 °C.

### 3.2. The effect of K⁺ and Ca²⁺ on gelling properties

The rheological properties of funoran solutions have been found to be somewhat similar to those observed for λ-carrageenan (Watase et al., 2000), and so far no strong gel forming ability has been demonstrated for funoran. We investigated the effect of various cations on the rheological properties of funorans isolated by the successive extraction procedures. The two main fractions, HF1a and HF1b from *G. tenax*, were studied in detail. The effect of various amounts of KCl or CaCl₂ on the rheological properties of these preparations is shown in Fig. 4. At high salt concentrations, funoran forms gels of moderate strengths, especially in the presence of Ca²⁺ ions (Fig. 4a, c). The gel formation process of funoran was rather slow; even at 15 °C the G' values did not reach plateau during the 120 min of ageing time. An eventual plateau probably always exists, but over the limited measurement time it is possible to differentiate between the low salinity samples that were still evolving, and the high salinity samples that reached an apparent plateau within 120 min. In some measurements at low cation concentrations, equilibration of the elastic moduli of funoran gels (1.5%) took more than 12 h at 15 °C. The gelling capacity of HF1a (Fig. 4a, b) was notably lower than that of HF1b (Fig. 4c, d), although the Mp of the latter was smaller. While higher cation concentrations tended to increase G' values, the addition of smaller amounts of salt (e.g. 0.02 M CaCl₂) decreased markedly G' of 1.5% sol of HF1a. The opposite was noted for HF1b, similarly to the results described in the literature for *G. furcata* funorans, where the presence of 0.02 M KCl was found to slightly increase G' of the 2% polysaccharide sol (Watase et al., 2000). Thus the significance of the isolation conditions on the rheological properties of funoran sols is clearly evident.

HF1b was easily dissolved in cold water giving transparent solutions (Fig. 5) with no noticeable discolouration. Due to the low viscosity, relatively concentrated polysaccharide solutions containing 6–8% funoran could be readily prepared without the need of heating. By introducing specified amounts of cations, it is possible to induce slow gelation of funoran at room temperature. No changes were observed in the transparency of the solutions during the gelling process, indicating that the gel formed is structurally rather homogeneous and only limited aggregation takes place. Such properties could be advantageous in applications where the use of elevated temperatures must be avoided and the clarity of the gelled system is of importance. Conversely, although HF1a is also soluble in cold water, its aqueous solutions were turbid (Fig. 5) and more viscous even after prolonged heating, making it difficult to prepare solutions of >4% polysaccharide content. Such differences in the rheological properties can be associated with the higher Mp value and the higher chemical heterogeneity (presence of uronic acids, acetylated residues, xylose, low 3,6-AG content) of HF1a.

Ca²⁺ had a markedly higher gel-inducing effect than K⁺. In the presence of 0.1 M CaCl₂ and 0.2 M KCl, the G' values measured for HF1b from *G. tenax* after 120 min of ageing at 15 °C were 1320 and 825 Pa, respectively. With increasing the salt concentration, the G' values increased in the studied concentration range (up to 0.25 M for KCl and up to 0.12 M for CaCl₂), whereas G'' increased to a maximum value (at 0.15 M for KCl at 0.04 M for CaCl₂) and then gradually decreased with further increase in the salt content (Fig. 5a, b). In the absence of added salts G' was lower than G'', but even small additions of salt led to G' > G''.

At 0.12 M CaCl₂, both G' and G'' increased and showed a similar polysaccharide concentration dependence (Fig. 7a). The increase was especially notable over polymer concentrations in the range 0.3–1.5%. For the 1.5% funoran gels, the G' and G'' showed characteristics (1510 Pa and 16.8 Pa) typical for gels of moderate strengths. Increasing the funoran content to 3.5% increased both G' and G'' by about factor 5. It is known that temperature and thermal history significantly alter macromolecular association in polysaccharide solutions (Iijima, Hatakeyama, Takahashi, & Hatakeyama, 2007), thus affecting their rheological properties. With increasing temperature, the plateau (pseudo-equilibrium) value of G' gradually decreased, while the plateau value of G'' depended weakly on the temperature (Fig. 7b). At 35 °C very weak gels were obtained (G' = 19.2 Pa) and the gelling process was rather slow with G'
exceeding $G''$ after 72 min of ageing. No gel formed at temperatures above 35 °C, $G''$ only showed a marked decrease close to this temperature. It is known that the temperature dependence of the storage elastic modulus of cold-setting thermoreversible gels is monotonous when the binding energy is small, while with an increase in the binding energy an initial increase in the elastic modulus develops, followed by a decrease an eventual melting (Nishinari et al., 1992). Of the galactan family, the charged $\kappa$-carrageenan belongs to the former group with a small binding energy. $\kappa$-carrageenan carries one negative charge per idealized diad unit, seems to belong in the former group with a small binding energy.

Similarly to many carrageenans, the melting temperature $T_m$ of funoran gels is highly dependent on the cation concentration. $T_m$ of G. tenax 1.5% funorans gelled in 0.09–0.25 M KCl or 0.02–0.12 M CaCl$_2$ varied over a wide temperature range, 34.0–46.5 °C (Fig. 8a). Conversely, the significant variations in the polymer contents between 0.3 and 3.5% only slightly affected the $T_m$ of funoran gels (46.0–48.5 °C) containing 0.12 M CaCl$_2$ (Fig. 8b). Interestingly, the $T_m$ remained fairly stable for 1.5% funoran samples gelled in 0.12 M CaCl$_2$ at different temperatures; $T_m$ was found to be 47.5 °C for the one aged at 10 °C and 46.5 °C for the samples aged at 15–35 °C.

The determination of the gelation temperature $T_g$ was complicated as for the majority of the salt conditions tested, gelation did not occur during cooling but was rather observed during isothermal ageing at 15 °C. In the studied salt concentration range, the values of $T_g$ could only be assigned to HF1b gelled in 0.25 M KCl ($T_g = 16$ °C) and in 0.12 M CaCl$_2$ ($T_g = 18$ °C).

### 3.3. The effect of various cations on rheological and optical properties

It is known that the presence of co-solutes and counter ions can notably influence the rheological properties of various anionic polysaccharides (Morris, Rees, & Robinson, 1980; Tuvikene et al., 2010). The main rheological characteristics of the funorans (HF1b) from the studied Gloiopeltis species in the presence of various cations are presented in Fig. 9. Taking into account the charge density of the counter ion (i.e., considering that the effective molar concentration of the divalent cations is two times lower than that of the monovalent cations), the ability of funoran to form gels was considerably high salt concentrations (0.25 mol/L). Although the optical density of funoran gelled in 0.12 M BaCl$_2$ was comparable to that of agaroses gelled in pure water, the increase in optical density during the ageing process of funoran at 15 °C was notably larger. The effect of Ba$^{2+}$ ions on the gelation processes of algal galactans has not been thoroughly studied. Nevertheless, it has been...
Among the studied Gloiopeltis species, funorans from *G. furcata* have somewhat lower $G'$ values than those from *G. tenax*. While the $G'$ values of the polysaccharides from the sporophytic form of *G. complanata* were relatively similar to those for *G. furcata* polysaccharides, the funorans originating from the gametophytic form of *G. complanata* were characterized by significantly higher $G'$ (Fig. 9). It is known that the presence of LA2M residues usually reduces the gelling capacity of agar type polysaccharides (Armsén & Galatas, 2000). Because the HF1b from *G. tenax* contained significant amounts of these moieties while only a small amount was present in the respective fraction from *G. furcata*, the differences in the rheological properties between these preparations could rather be attributed to the molecular weight characteristics than to the structural variations. This is in accordance with the Mp values of these samples (679 kDa for *G. furcata* funoran and 776 kDa for the one from *G. tenax*) observed in our previous work (Tuvikene et al., 2015). It is important to note, that although the structural features of the polysaccharides from *G. furcata* (HF1b) were the closest to the idealized funoran repeating sequence (containing only small amounts of G6M and LA2M residues), the HF1b from *G. tenax* was the closest to ideal funoran as virtually all of its 3-linked residues were sulfated at O-6. On the other hand the rheological properties of *G. complanata* funorans could be notably affected by the presence of noticeable amounts of G and G6M moieties. Interestingly, in the presence of Ba$^{2+}$ ions the $G'$ values of *G. complanata* funorans remained lower than those for *G. furcata* and *G. tenax* specimens while the samples containing the other cationic species showed higher $G'$ for those from *G. complanata* (Fig. 9). This could be explained by the impaired gelling ability of agarose related diads (G–LA, G6M–LA) in solutions containing Ba$^{2+}$ ions.

It has been found that the funorans from *G. tenax* have about 50% higher viscosity than those from *G. furcata* (Sand et al., 1973). This agrees with the results of the current study. The complex viscosity ($\eta'$) of HF1b of 1.5% *G. tenax* funoran in pure water at 1 Hz was 0.36 Pa s whereas the value for *G. furcata* funoran was only 0.16 Pa s. In the presence of 0.25 M NaCl or LiCl, the respective values were 9.07 and 86.8 Pa s for *G. furcata* and 15.2 and 100.2 Pa s for *G. tenax*. The basis of these differences is probably caused by the mutual effect of the structural features and molecular weight characteristics of these polysaccharides.

The $T_m$ of 1.5% funoran samples gelled in the presence of various monovalent (0.25 mol/L) and divalent cations (0.12 mol/L), except Ba$^{2+}$ ions, remained in the range 36–56 °C for those from *G. furcata* and *G. tenax* and between 46 and 66 °C for the preparations from *G. complanata* specimens (Fig. 9). The occurrence of unsulfated diads (G–LA, G6M–LA) can notably affect the $T_g$ and $T_m$ values of funoran preparations. It was established that these moieties are responsible for the significantly increased $T_g$ and $T_m$ values in funorans from the both *G. complanata* specimens which were found to contain notable amounts of G6M–LA together with smaller quantity of agarose diads (G–LA). This agrees with the literature results, where it has been shown that the $T_g$ of agar type polysaccharides is affected by the methoxylation degree with a greater methoxylation at C-6 corresponding to a higher gelling temperature while methoxylation of the rest of the carbons usually reduces both the gelling temperature and gel strength (Armsén et al., 2000; Guiseley, 1970). Furthermore, the *G. complanata* gametophyte which was found to contain more G6M and less LA2M residues than was observed in the sporophytic form of this alga, contained the polysaccharides with higher $T_g$, $T_m$ and $G'$ values (Fig. 9).

However it was not clarified whether the G–LA would have more pronounced effect on these characteristics compared to G6M–LA in the case of funoran samples.

The heating curves of *G. complanata* funorans revealed two step melting. This was especially notable for the sporophytic form of

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**Fig. 9.** Shear storage ($G'$) and loss ($G''$) moduli, melting ($T_m$) and gelling ($T_g$) temperatures of 1.5% funoran HF1b fractions from *G. furcata* (■), *G. tenax* (▲), *G. complanata* gametophytic (■) and sporophytic (▲) forms measured after 120 min of ageing at 15 °C in the presence of different monovalent (0.25 mol/L) or divalent (0.12 mol/L) cations introduced as chlorides.

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**Fig. 10.** Optical density during cooling and heating for 1.5% galactan sols. Scanning rate: 2 °C/min. Cooling and heating cycles were separated by isothermal hold at 15 °C for 120 min. Absorbance measured at 400 nm in 2-mm cuvette for agarose A2929 in water (1), agarose III in water (3), and *G. furcata* funoran HF1b solubilized in 0.12 M BaCl$_2$ (2) or in 0.12 M SrCl$_2$ (4).
G. complanata, but was not evidenced in the case of G. furcata or
G. tenax polysaccharides (Fig. 11). The initial melting step was
observed at the lower temperatures (by 10–15 °C) than the
measured \( T_m \) values determined on the basis of rheometry. Such
melting properties can be caused by the presence of notable
amounts of unsulfated 3-linked residues in the preparations from
G. complanata, but may also be the result of the more polydisperse
nature of these preparations. Our previous studies have revealed
the higher polydispersity of HF1b from G. complanata sporophyte
compares to that from the gametophytic form of this seaweed,
while those from G. furcata and G. tenax were the least polydisperse
among these fractions (Tuvikene et al., 2015). The notably higher \( G^\prime \)
values for the funorans from the G. complanata sporophytic form
also agree with these findings (Fig. 9).

Thermal hysteresis between the setting and melting was
observed for funorans in the presence of added salts (Fig. 9).
The largest hysteresis (\( T_m - T_g = 41.5–48 °C \)) was noted for samples
gelled in the presence of \( \text{Ba}^{2+} \) ions. Such high values are close to
those often observed for agaroses. The preparations containing Cs
ions, especially those from G. furcata, were characterized by the
lowest hysteresis (\( T_m - T_g = 24.5–33 °C \)). The average hysteresis
values for the polysaccharides from the studied Gloiopeltis species
increases in the row: G. furcata < G. tenax < G. complanata
sporophyte < G. complanata gametophyte.

Fig. 12 shows the changes in the storage \( (G') \) and loss \( (G'') \)
moduli as a function of frequency after ageing of 1.5% funoran (HF1a
from G. tenax) sols for 120 min at 15 °C. The sample without the
added salt revealed \( G'' > G' \) for frequencies lower than 0.046 Hz,
indicating viscoelastic solution behaviour. The crossover frequency,
which corresponds to the average relaxation time of entanglements,
can be attributed to the temporary association of funoran
chains during short oscillation periods. The presence of salts
(0.25 mol/L) induced a gel-like behaviour with \( G' > G'' \) over
the experimental frequency range (0.01–10 Hz) and no crossover
occurred. In these cases both dynamic moduli showed higher
values with increasing oscillation frequency. Compared to other
alkali metal ions, Cs\(^+\) ions led to weaker frequency dependence of
\( G' \) at low frequencies.

3.4. The effect of anions on rheological properties

Anions have only slight effect on the rheological properties of
funoran solutions. The \( T_m \) of the most pure funoran fraction (HF1b
from G. furcata) remained in the range 41–44 °C for 1.5% funoran
gels containing various potassium salts at 0.25 M K\(^+\) concentration
and increased in the order SCN\(^−\), I\(^−\) < SO\(_4^{2−}\) < Br\(^−\) < Cl\(^−\). A similar
sequence has been observed for \( \kappa \)-carrageenan, in which case an-
ions have been found to differ in their capacity to stabilize poly-
saccharide helix in the sequence Cl\(^−\) < NO\(_3^{−}\) < Br\(^−\) < SCN\(^−\) < I\(^−\),
whereas the iodide and thiocyanate anions impede aggregation
and gel formation (Zhang, Picuill, & Nilsson, 1991). While the \( G' \)
values measured in the beginning of the isothermal step also
increased in the same order as \( T_m \), the respective values obtained at
the end of the isothermal hold increased in a different order:
SCN\(^−\) < Br\(^−\) < SO\(_4^{2−}\), I\(^−\) < Cl\(^−\). Thus, among the studied anions,
thiocyanate exhibits the strongest influence over the rheological
properties of funoran by decreasing \( G' \) and increasing \( G'' \) values for
gels aged for 120 min at 15 °C (for KSCN: \( G' = 832 \text{ Pa}, G'' = 20 \text{ Pa} \); for
KCl: \( G' = 1004 \text{ Pa}, G'' = 15 \text{ Pa} \)).

3.5. Thermal properties

The DSC cooling and heating curves of G. tenax funoran (HF1b)
solutions in the presence of various cations are shown in Fig. 13. The
exothermic peak temperature (\( T_{ex} \)) shifted to notably higher

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Fig. 11. Dependence of the shear storage modulus \( (G') \) of 1.5% funoran in 0.12 M CaCl\(_2\) on the temperature during heating for HF1b from G. furcata (■), G. tenax (△) and G. complanata sporophytic (○) and gametophytic (■) forms.

Fig. 12. Frequency dependence at 15 °C of the shear storage \( (G') \) and loss \( (G'') \) moduli of 1.5% G. tenax funoran (HF1a) in the presence of various alkali metal chlorides (0.25 mol/L) recorded at 1% strain.

Fig. 13. Cooling and heating DSC curves of G. tenax 1.5% funoran (HF1b) samples solubilized in 0.25 M NaCl, 0.25 M KCl or 0.12 M CaCl\(_2\) solutions. The characteristic shoulders are indicated with arrows.
temperatures following addition of alkali metal chlorides in the order of Na⁺ < K⁺ < Ca²⁺ with the respective \( T_{ex} \) values of 11.1, 18.5 and 21.5 °C. This sequence is in accordance with the rheology results obtained under similar conditions (cooling and heating rates 1 °C/min) where the observed \( T_g \) values were about 3 °C higher \( (T_g = 24.5 °C \) for funoran containing 0.12 M CaCl₂). The endothermic peak temperatures \( (T_m) \) followed the same order as \( T_{ex} \) and were 35.8, 38.3, 40.6 °C, respectively. The corresponding \( T_m \) values from the rheological measurements were found to be about 5 °C higher \( (T_m = 45.5 °C \) for funoran containing 0.12 M CaCl₂). The DSC heating curves of the studied \( G. \) tenax funoran samples revealed a characteristic shoulder at around 3 °C lower temperature than their respective \( T_m \) values. These shoulders were especially notable for the samples containing NaCl or KCl (Fig. 13) and were not present in the heating profile of \( G. \) tenax polysaccharide in rheological measurements (Fig. 11). The shoulders are probably caused by the presence of notable amounts of \( 1,2,3 \)-trisubstituted residues in HF1b from \( G. \) tenax. The methylation at O-2 of 3,6-anhydro-\( \alpha-L \)-galactopyranose moieties limits the number of hydrogen bonds available for the formation of gel network, thus reducing the gelling ability of the polysaccharide (Armisen & Galatas, 2000) and lowering the \( T_m \) value.

The DSC curves indicated rather slow gelling process (peak area on cooling and heating is very different) of funoran as was also confirmed by the rheological measurements. Cooling the solutions down to 1 °C allowed to calculate the gel formation enthalpies for the preparations containing 0.25 M NaCl, 0.25 M KCl or 0.12 M CaCl₂ \((-7.47, -19.3 \) and \(-14.1 \) J/g, respectively). The gel melting enthalpies for these samples measured after 120 min of ageing time were significantly higher (27.0, 27.9 and 25.5 J/g, respectively) in absolute values indicating the notable association of the polysaccharide chains during the isothermal course as was also noted in the rheology measurements. \( T_m \) was not dependent on the thermal history of the samples, whereas the gel melting enthalpies slightly decreased with increasing temperature of curing (curing time = 120 min) in the range 1–25 °C. It was also noted, that the broadness of the endothermic peak was virtually unaffected by the curing temperature.

### 3.6. Conformation of funoran chains

It has been proposed that the helical conformation of agar-type polysaccharides is not fundamentally altered by introduction of substitutions on O-6 and O-4 of \( \beta-D \)-galactopyranose and O-2 of 3,6-anhydro-\( \alpha-L \)-galactopyranose residues (Lahaye et al., 1991; Rees & Welsh, 1977). However, these structural variations may affect the gelling properties of the galactan by interfering with aggregation behaviour of the helices. The presence of detectable amounts of xylose, uronic acids and acetylated residues, which are likely distributed randomly along the funoran chain, may not only affect the aggregation behaviour of its helices, but can also induce asymmetry in the helix geometry.

As the ability of algal galactans to form crystalline and oriented fibres is higher for specimens that yield clear sols and gels (i.e. containing less aggregated chains), water was used as a solvent for funorans, while DMSO allowed us to prepare clearer agarose films.

The X-ray scattering pattern of agarose showed three reflections: a weak and broad reflection, corresponding to the second order layer lines, at around 0.9–1.2 nm; one broad symmetric reflection at 0.46 nm; a clear meridional reflection at 0.327 nm (Fig. 14a). SAXS measurements (patterns not shown) of agarose and funoran did not reveal any reflections below 2\( \theta = 9° \). These reflections indicate that the threefold helix has a pitch \( (P) \) of about \( 2.0 \pm 0.1 \) nm, with an axial advance \( h \) of 0.65 ± 0.02 nm. Although these values are slightly higher compared to the parameters determined by Arnott et al. (1974), the difference is within the standard deviation. The X-ray pattern of a funoran sample (HF1b from \( G. \) tenax, Na⁺-form) recorded in two different directions in diffraction space: in meridional direction (parallel to sample stretch direction, \(-\)) and in equatorial direction (\(+\)). The labels above the peak positions show the corresponding lattice periods in nanometres. The insets show the two-dimensional scattering pattern recorded in meridional direction.

![Fig. 14](image)

**Fig. 14.** X-ray diffraction patterns of agarose (a) and funoran HF1b from \( G. \) tenax, Na⁺-form (b) recorded in two different directions in diffraction space: in meridional direction (parallel to sample stretch direction, \(-\)) and in equatorial direction (\(+\)). The labels above the peak positions show the corresponding lattice periods in nanometres. The insets show the two-dimensional scattering pattern recorded in meridional direction.

### 4. Conclusions

The sulfation at G-6 in funoran notably affects rheological properties, but also contributes to the small conformational changes, resulting in a slightly decreased helical pitch \( (P = 1.8 \) nm) compared to agarose. Funoran does not gel in the absence of added ions but is capable of forming strong gels in the presence of
relatively high amounts of inorganic cations. The ability of alkali metals to promote funoran gelation increases with increasing ionic radius. A similar tendency was found for alkaline earth metals, with Ba\(^{2+}\) ions showing a specific effect and markedly increasing gel strength. Anions do not notably impede the gel forming process of funoran. SCN\(^{-}\) had the strongest effect among anions, as it slightly decreased \(G'\) and increased \(G''\) values. Funoran gelation is a slow process with the rate of the gelation being highly dependent on the cation species and concentration.

Because of the presence of agarose diads in \textit{G. complanata}, the rheological properties of the funorans from this seaweed species are somewhat different to those of funorans from \textit{G. furcata} and \textit{G. tenax}. The good solubility in cold water, slow gelling and ability to form strong transparent thermoreversible gels in the presence of various cations makes the pure funoran (one of the main polysaccharide fractions from \textit{Gloiopeltis} species, HF1b) a promising hydrocolloid for applications where the use of elevated temperatures must be avoided and a transparent gel is preferred.

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