

# Molecular weight distribution of carrageenans studied by a combined gel permeation/inductively coupled plasma (GPC/ICP) method

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Degraded carrageenan (known as poligeenan, molecular weight: 20 kDa to 30 kDa) causes ulcerative colitis in experimental animals. In this paper, the molecular weight distributions of 29 samples of food-grade refined carrageenans were studied by high performance liquid gel permeation chromatography (GPC) directly connected to vacuum-ultraviolet inductively coupled plasma-atomic emission spectrometry (ICP) (GPC/ICP) as well as GPC/refractive index (RI) detection. All samples of food-grade carrageenan had a major broad peak of high molecular weight which eluted at around 6.5 min in both RI and ICP mode (sulphur and carbon), and each sample of them had no obvious peak of poligeenan (the detection limit was about 5%). The number average molecular weights of these carrageenans ranged from 193 kDa to 324 kDa, and the weight average molecular weights ranged from 453 kDa to 652 kDa based on RI data. Some samples had a few minor peaks which eluted around 10–12 min. These peaks came from ionic sulphate, sucrose or glucose. It was considered that if the data-sampling programme was improved, the GPC/ICP system would become a more powerful technique for evaluation of carrageenan samples containing ionic substances and sugar.

**Keywords:** carrageenan, PES, processed euclidean seaweed, poligeenan, degraded carrageenan, GPC, ICP, GPC/ICP, molecular weight distribution, sulphur

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## Introduction

Carrageenan refers to a group of closely related, high molecular weight polygalactans obtained from certain red seaweeds such as *Chondrus crispus*, *Euclidean spinosum* and *Gigartina acicularis* (Vreeman *et al.* 1980). Carrageenans are water-soluble linear polysaccharides composed of alternating  $\alpha$ -1–3 and  $\beta$ -1–4 linked D-galactose residues. The classification of these polysaccharides is based on the modification of this simple repeating disaccharide unit, which can result from the occurrence of ester sulphate and anhydride formation (Glicksman 1983, Lecacheux *et al.* 1985) (figure 1).

Kappa ( $\kappa$ ) and iota ( $\iota$ )-carrageenan can adopt a three-fold right-handed double helical structure in solution, allowing the formation of thermoreversible gels. However, lambda ( $\lambda$ )-carrageenan produces highly viscous solutions that do not gel (Lecacheux *et al.* 1985). When added to food products in a low concentration, carrageenan can act as a gelling agent, emulsifier, stabilizer or thickener with such physical properties, and so is used extensively worldwide.

Due to its frequent use in the food and pharmaceutical industries, extensive toxicological evaluations of carrageenans have been carried out. The 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated an ADI of 'not specified' (temporary) for carrageenan and PES (processed euclidean seaweed) (JECFA 1998).

The potential problem that has been identified involves degraded carrageenan (known as poligeenan), which can cause lesions in experimental animals. Poligeenan causes ulcerative colitis in rats and guinea pigs and is used in experimental models to study the effects of pharmacological agents (Delahunty *et al.* 1987, Marcus *et al.* 1989, Karlsson and Singh 1999). Therefore, it is necessary to establish whether or not food-grade products contain poligeenan.

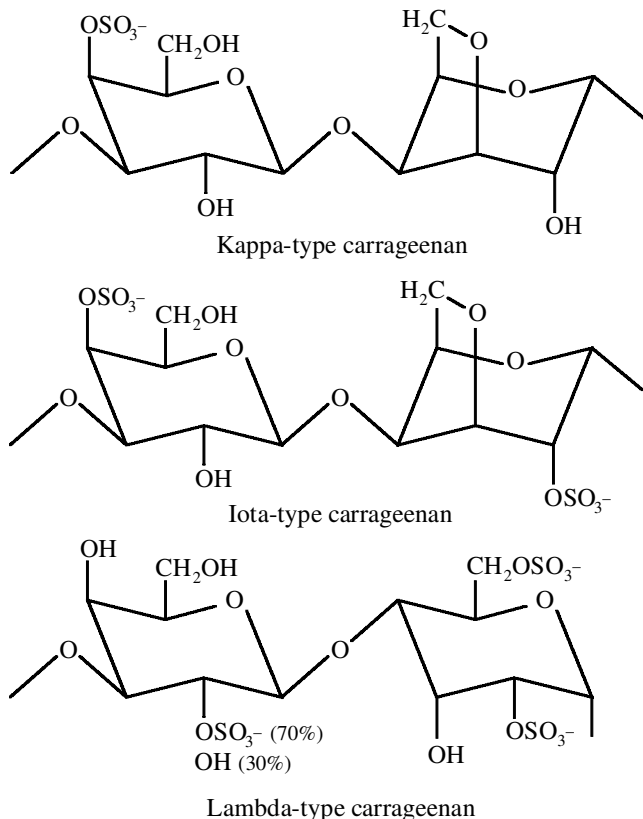


Figure 1. Ideal chemical structure of  $\kappa$ -,  $\iota$ - and  $\lambda$ -carrageenan. Carrageenans are water-soluble linear polysaccharides of alternating  $\alpha$ -1-3 and  $\beta$ -1-4 linked D-galactose residues. The classification of these polysaccharides is based on the modification of this simple repeating disaccharide unit, which can result from the occurrence of ester sulphate and anhydride formation.

The requirement of a minimum viscosity, which is 5 mPa s for a 1.5% solution measured at 75°C, has been established in international specifications, including JECFA specification (JECFA 1998). This is the equivalent of a weight average molecular weight of 100 kDa (Weiner 1991). However, the presence of a limited amount of low molecular weight chains may be obscured in viscosity by the greater proportion of high molecular weight chains.

Some evaluations for the molecular weight distribution of carrageenan have been carried out by using high performance liquid gel permeation chromatography (GPC). Ekstrom (1985) tested eight samples of food-grade  $\kappa$ -carrageenan by using the system of GPC and the refractive index (RI) detector, and found the mean value of weight average molecular weights (MW) of these carrageenans was 167 kDa.

Malfait *et al.* (1990) reported that the MW of carrageenan was 303 kDa by using a similar system and that a broad peak of carrageenan was followed by a low molecular weight fraction.

Lecacheux *et al.* (1985) tried to use low angle laser light scattering (LALLS) as a detector. They reported that the number average molecular weights (MN) of food-grade carrageenans ranged from 90 kDa to 200 kDa, and their MW ranged from 340 kDa to 575 kDa by using the GPC/LALLS system. Sloomackers *et al.* (1991) used a similar system. They reported the MN of carrageenan was 142 kDa for  $\kappa$ -type, and 131 kDa for  $\lambda$ -type, and also reported the MW of carrageenan was 323 kDa for  $\kappa$ -type, and 664 kDa for  $\lambda$ -type. But the sensitivity of LALLS for low molecular weight substances was much lower than that for high molecular weight polymers.

These investigations provided important knowledge about the molecular weight distribution of carrageenan, but there were some problems in the procedures; the RI is not specific and the intensity of light scattering for low molecular weight substances is not enough for these substances, especially for the poligeenan.

Maitani *et al.* (1994, 1996) developed the GPC/ICP method, which consisted of high performance liquid gel permeation chromatography (GPC) directly connected to vacuum-ultraviolet inductively coupled plasma-atomic emission spectrometry (ICP). Vacuum-ultraviolet ICP can detect both sulphur and carbon directly. Carrageenan has a certain amount of sulphur in its molecule, and also significant amounts of carbon. Thus, the simultaneous monitoring of sulphur and carbon can reveal whether or not a substance detected is carrageenan.

In this paper, we describe the molecular weight distributions of 29 samples of food-grade refined carrageenan, and a PES, which are representative of those in Japanese market, and a poligeenan using GPC/ICP.

## Materials and methods

### Chemicals

Standard molecular weight markers of pullulans (Shodex<sup>®</sup> STANDARD P-82) were obtained from

Showa Denko (Tokyo, Japan). Pullulan is a water-soluble exocellular glucan synthesized by *Aureobasidium pullulans*, which consists of linear chains of glucopyranose units with regular alternation of two  $\alpha$ -1-4 and one  $\alpha$ -1-6 linkages (Stanford 1982). Sodium nitrate was purchased from Wako Pure Chemicals Industries (Osaka, Japan). All other chemicals were of reagent grade or of the highest grade commercially available.

### *Carrageenan solution*

Twenty-nine samples of food-grade refined carrageenan (five samples of unknown-type: U01–U05, 2 of  $\iota$ -type: I01–I02, 19 of  $\kappa$ -type: K01–K19, and three of  $\lambda$ -type: L01–L03) and a PES (P01) were obtained from various food gum producers. A poligeenan (D01) was kindly prepared and provided by FMC Biopolymer (Philadelphia, PA). These samples of 29 food-grade refined carrageenan and a PES are usually sold on the Japanese market as food additives. These samples cover almost all types of food applications containing carrageenan or PES in Japan, such as dessert jellies, puddings, ice creams, syrups, sauces, fillings, whipped creams, coffee drinks, and so on. It was considered that these samples were representative of those on the Japanese market.

Each sample powder was dissolved in ultra-pure water ( $> 18 \text{ M}\Omega \text{ cm}$ ) containing 0.05 M sodium nitrate at 80°C for 10 min with a propeller stirrer to prepare solution containing 0.1% (w/v) sample.

### *GPC/ICP study*

The GPC/ICP system was constructed according to the papers by Maitani *et al.* (1994, 1996). The high performance liquid chromatography (HPLC) system consisted of the following components: an on-line degasser (DG-980-50, JASCO, Tokyo, Japan), an HPLC pump (PU-980, JASCO), a sample injector (AS-950-10, JASCO), a column oven (CS-300H, Chromato Science, Osaka, Japan), and an RI detector (RI-930, JASCO).

After the solutions had been filtered by membrane filter (0.2  $\mu\text{m}$ ), a 300  $\mu\text{l}$  aliquot of sample solution was injected to the HPLC system equipped with a gel permeation column (Shodex<sup>®</sup> SB-804HQ, 8.0 mm

i.d.  $\times$  300 mm with Shodex<sup>®</sup> SB-G (guard column, 6.0 mm i.d.  $\times$  50 mm), Showa Denko). A sample was eluted with 0.05 M sodium nitrate (0.2  $\mu\text{m}$ , filtered) at a flow rate of 1.0 ml/min. The temperature of the column oven was set at 50°C and that of the RI detector was set at 35°C.

The eluate from HPLC was introduced continuously via the RI detector to the nebulizer tube of the ICP (LIBERTY II controlled by Plasma 96 software, Varian, Mulgrave Victoria, Australia). The atomic emission intensities of sulphur (wavelength, 180.7 nm), carbon (wavelength, 193.0 nm) and RI were collected every 0.1 s using a personal computer. Collected data were integrated for 0.6 s by the SIC 480 Data Station (System Instrument, Tokyo, Japan) and stored. GPC/ICP chromatograms were represented on the display of a personal computer by using stored data and a program developed for this GPC/ICP study according to the method of Maitani *et al.* (1994, 1996).

### *Molecular weight distribution*

The average molecular weights of these carrageenan samples were determined by using stored data and the GPC data processing program (SIC 480 Data Station, System Instrument). As standard molecular weight markers, six molecular weights of pullulans (5.6–788 kDa) were used. Using the GPC data processing program, the calibration curve in RI base was obtained.

### *Detection limit for poligeenan contamination in carrageenan*

A sample of carrageenan (No. L03) and poligeenan were mixed to prepare each sample powder in which the poligeenan content was 0, 1, 3, 5, 10, 33, 50% (w/w) respectively. Prepared powders were applied to the GPC/ICP system as described above.

### *Ion-chromatography*

Each sample powder was stirred in ultra-pure water ( $> 18 \text{ M}\Omega \text{ cm}$ ) for 10 min with a propeller stirrer to extract ionic compounds from the sample. After

solutions had been filtered by filter paper and membrane filter, a 25  $\mu\text{l}$  aliquot of sample solution was injected to the IC system (Dionex 4500i with AMMS-MPIC (anion micro membrane suppressor for mobile phase ion-chromatography) as suppressors, Dionex, Sunnyvale, CA, USA) equipped with IonPac NG1 (Dionex) as the guard column, and IonPac NS1 (Dionex) as the separating column. A sample was eluted with 17% (w/v) acetonitrile, 2 mM TBAOH (tetrabutylammonium hydroxide) and 1.2 mM sodium carbonate solution at a flow rate of 1.0 ml/min. The sulphate contents of sample solutions were determined by using the peak area of conductivity detector (Yamada *et al.* 1991).

### Analysis of sugar content

Each sample powder was stirred in ultra-pure water ( $> 18 \text{ M}\Omega \text{ cm}$ ) for 10 min with a propeller stirrer to extract sugars from sample. After the solutions had been filtered by filter paper and membrane filter, a 10  $\mu\text{l}$  aliquot of sample solution was injected to the HPLC system (LC-900, JASCO) equipped with a Shodex<sup>®</sup> SZ5532 column (Showa Denko). A sample was eluted with 80% acetonitrile solution at a flow rate of 1.0 ml/min. The temperature of the column oven was set at 60°C and that of the RI detector was set at 35°C. The sugar contents of sample solutions were determined by using the peak area of RI detector.

## Results and discussion

### Some typical elution profiles of carrageenans

In this paper, the molecular weight distributions of 29 samples of food-grade refined carrageenan were studied. A typical elution profile of carrageenan (sample No. L03) is shown in figure 2. This sample had only one major broad peak of high molecular weight which eluted around 6.5 min in RI. The changes in chromatograms of sulphur and carbon by ICP corresponded to the changes by RI. These results suggested that the major peak was carrageenan.

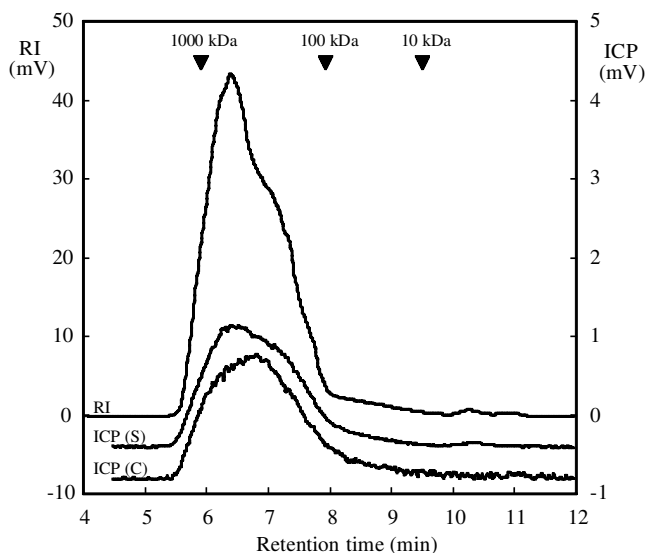


Figure 2. A typical elution profile of carrageenan with only one major peak. A carrageenan powder (sample No. L03) was dissolved in ultra-pure water containing 0.05 M sodium nitrate to prepare a solution containing 0.1% carrageenan. A 300  $\mu\text{l}$  aliquot of sample solution was injected to the HPLC system equipped with a gel permeation column (Shodex<sup>®</sup> SB-804HQ). The sample was eluted with 0.05 M sodium nitrate at a flow rate of 1.0 ml/min. The eluate from HPLC was introduced continuously via the RI detector to the nebulizer tube of ICP. The atomic emission intensities of sulphur, carbon and RI were collected every 0.1 s, and integrated for 0.6 s.

The major peak which eluted around 6.5 min in RI had a shoulder, and each sample of food-grade refined carrageenans we tested had a similar shape of a major peak in RI. These facts suggested that each sample contained at least two components, which had different molecular weights. But, considering our GPC condition, 0.1% of sample content might be higher against the column we used. Namely, the shoulder recognized in RI might be caused as a result of an over-loading of solutes on the column.

Some samples of carrageenans had a few additional peaks eluted between 10 and 12 min. As shown in figure 3, sample No. I01 had three additional peaks in RI which were eluted at 10.2 min, 10.9 min and 11.6 min. In ICP, however, this sample had one additional sulphur peak corresponding to the peak at 10.2 min by RI, and one additional carbon peak corresponding to the peak at 10.9 min. Therefore, these facts suggested that these two additional peaks (retention time, 10.2 and 10.9 min) of RI were not

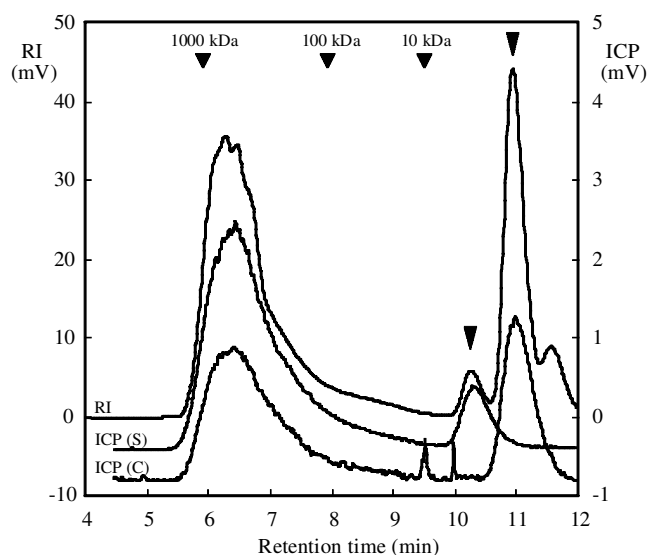


Figure 3. A typical elution profile of carrageenan with several peaks. A carrageenan powder (sample No. I01) was dissolved in ultra-pure water containing 0.05 M sodium nitrate to prepare a solution containing 0.1% carrageenan. See the legend for figure 2.

carrageenan, because carrageenan had a certain amount of sulphur and carbon in its molecule.

Regarding the peak eluting at 10.2 min by RI, it seemed to come from ionic sulphate because of the absence of a carbon peak. In fact, when we applied a 300  $\mu$ l aliquot of ammonium sulphate (10 ppm as sulphur) to the GPC/ICP system, there was only one peak eluting at 10.2 min in both RI and sulphur. In addition, we studied the relationship between actual ionic sulphate contents of some samples determined by ion-chromatography and area of the sulphur peak in ICP and RI chromatograms. The ionic sulphate contents of these samples were correlated with the areas of sulphur peak in ICP chromatograms ( $r = 0.987$ ) and the peak areas of RI ( $r = 0.965$ ). These facts suggested that the peak which eluted at 10.2 min in sample No. I01 came from ionic sulphate.

Similarly, regarding the peak which eluted at 10.9 min, it seemed to come from sucrose or another saccharide, namely glucose. In fact, sucrose had only one peak which eluted at 10.9 min in both RI and ICP (carbon). The sugar contents of these samples were correlated with the peak areas on the ICP chromatogram (carbon) ( $r = 0.998$ ) and RI corresponding to the sugar peak ( $r = 0.976$ ).

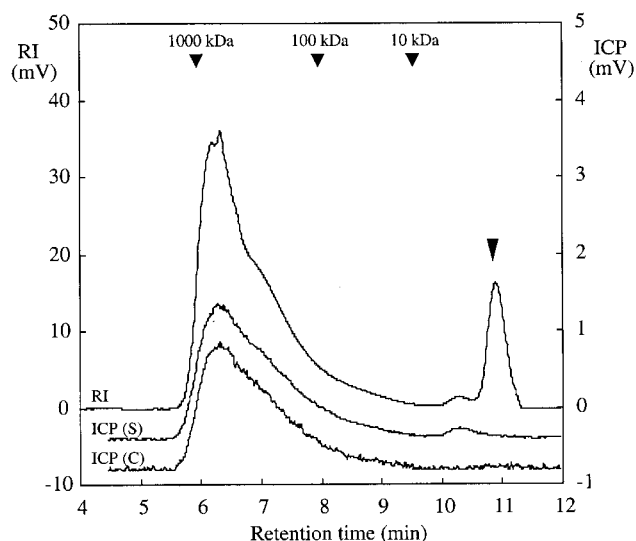


Figure 4. A typical elution profile of carrageenan giving RI peak without ICP peak. A carrageenan powder (sample No. K10) was dissolved in ultra-pure water containing 0.05 M sodium nitrate to prepare a solution containing 0.1% carrageenan. See the legend for figure 2.

However, a peak which eluted around 11 min was sometimes observed, even if the carrageenan sample did not contain sugar (figure 4). Although sample No. K10 did not contain sugar and had no peak corresponding to sugar on ICP (carbon), there was a peak on RI which eluted around 11 min. Based on RI detector measurements, it is well known that some peaks of counter-cations are observed (Ekstrom 1985, Lecacheux *et al.* 1985). Therefore, it was considered that this peak which eluted around 11 min in RI in figure 4 came from counter-cations.

#### Average molecular weights of carrageenans

All samples of food-grade refined carrageenan had a major broad peak of high molecular weight in both RI and ICP (sulphur and carbon). Although some samples had a few minor peaks which eluted around 10–12 min (figure 3), the peaks were excluded from the calculation of average molecular weight because they came from ionic substances or mono- or disaccharides as mentioned above. MW calculations were performed between 5.00 min and 9.35 min which corresponded to 3000 kDa and 10 kDa, respectively.

Table 1. Mean of average molecular weights in types of carrageenan.

| Type    | Mean of average molecular weights <sup>a</sup> |          |          |
|---------|--|----------|----------|
|         | n  | MN (kDa) | MW (kDa) |
| Unknown | 5  | 262      | 563      |
| Iota    | 2  | 230      | 519      |
| Kappa   | 19   | 233      | 521      |
| Lambda  | 3  | 253      | 518      |
| Average |  | 245      | 530      |

<sup>a</sup> Calculated between 5.00 min (3000 kDa) and 9.35 min (10 kDa).

The results of weight average molecular weights of carrageenans based on RI are summarized in tables 1 and 2. The number average molecular weights (MN) of these carrageenans ranged from 193 kDa to 324 kDa, and their weight average molecular weights (MW) ranged from 453 kDa to 652 kDa. In addition, their mean MN was 245 kDa, and their mean MW was 530 kDa. These average molecular weight values were similar to those reported previously by some authors (Ekstrom 1985, Lecacheux *et al.* 1985, Malfait *et al.* 1990, Sloomackers *et al.* 1991).

Carrageenan is classified into three groups ( $\kappa$ -,  $\iota$ -,  $\lambda$ -type) based on the difference in the repeating disaccharide unit (figure 1). The mean MN for these types of carrageenans were 233, 230 and 253 kDa for  $\kappa$ -,  $\iota$ - and  $\lambda$ -type carrageenans, and the mean MW were 521, 519 and 518 kDa for  $\kappa$ -,  $\iota$ - and  $\lambda$ -types, respectively. There was not much difference in average molecular weight among carrageenan types (table 1).

When the PES product was tested, a similar elution profile was also obtained (figure 5). The MN of PES was 243 kDa and the MW was 531 kDa (RI base), which was nearly equal to the mean MN and MW of food-grade refined carrageenans, respectively (table 2).

On the other hand, a poligeenan had a clearly different elution profile from other samples in both RI and ICP (sulphur and carbon) (figure 6). The major peak was observed around 9 min, which corresponded to 20 kDa. Carrageenans degraded under certain acid conditions are called poligeenan. So, commonly-called poligeenan has a variety of molecular weight distributions depending on the degradation condition. Although it was supposed that this sample must have contained an amount of ionic sulphate or other ionic compounds, the average molecular weight of this poligeenan was calculated between 5.00 min and

12.0 min. The MN of poligeenan was 0.7 kDa and the MW was 28 kDa (RI base) (table 2).

### Detection limit for poligeenan contamination in carrageenan

On the use of carrageenans in the food and pharmaceutical industries, the major problem identified is the presence of degraded carrageenan (known as poligeenan) which can cause lesions in laboratory animals (Wakabayashi *et al.* 1978, Watt and Marcus 1981, Delahunty *et al.* 1987, Marcus *et al.* 1989, Karlsson and Singh 1999). The weight average molecular weight of a poligeenan given to the animals was 20–30 kDa. Therefore, it is important to know whether or not food-grade products contain the poligeenan.

At first, we prepared the sample powders of carrageenan that contained 0, 1, 3, 5, 10, 33, 50% (w/w) poligeenan, and obtained the RI chromatogram of each sample (figure 7). The lowest line indicates the sample containing 0% poligeenan, and the highest line shows the sample containing 50% poligeenan (figure 7). Poligeenan is eluted around 8.7 min in this chromatogram. We could recognize the poligeenan peak in the sample containing 10% poligeenan, and the slight peak in the sample containing 5% poligeenan. In addition, we prepared the differential curve of each elution profile (RI) described in figure 7 between 8.0 and 9.5 min (figure 8). The differential curve of the each sample containing not less than 10% poligeenan showed the sigmoid shape and the weak sigmoid curve was observed in the sample containing 5% poligeenan. These facts suggested that the detection limit of poligeenan contamination was about 5% (w/w) in our GPC system.

We evaluated the elution profiles (RI) of all samples using this method mentioned above, and summarized the detection of poligeenan in all samples (table 2). No sample of food-grade refined carrageenan had an obvious peak of poligeenan, but some of them had the weak sigmoid shape in the differential curve of RI chromatogram. This suggested that some food-grade refined carrageenan might contain about 5% poligeenan. Our concern with this result was whether this fraction came from poligeenan or other polysaccharides, because the RI detector was not specific. ICP can detect sulphur directly, and both carrageenan and poligeenan had a certain amount of sulphur in the molecule (figure 1). So, it was expected that the

analysis of the ICP (sulphur) chromatogram would reveal whether or not the fraction came from de-graded carrageenan. Although there was no peak in the ICP (sulphur) chromatograms and no sigmoid shape in their differential curve, unfortunately it was

considered that the sensitivity of ICP was not sufficient to evaluate the presence of peaks in this region where the content of a substance is low (data not shown). Thus, we are developing a new system that improves the sensitivity of ICP. After accomplishing

Table 2. Summary of average molecular weights and detection of poligeenan, sulphate and sugar in carrageenans by GPC/ICP.

| Type             | Sample No. | Ionic sulphate <sup>a</sup> (%) | Sugar <sup>b</sup> (%) | Average molecular weight <sup>c</sup> |                 | Detection by GPC/ICP  |                    |                         |
|------------------|------------|---------------------------------|------------------------|---------------------------------------|-----------------|-----------------------|--------------------|-------------------------|
|                  |            |                                 |                        | MN (kDa)                              | MW (kDa)        | Sulphate <sup>d</sup> | Sugar <sup>e</sup> | Poligeenan <sup>f</sup> |
| Unknown          | U01        | 2.73                            | 0.0                    | 217                                   | 516             | +                     | —                  | —                       |
|                  | U02        | n.d.                            | n.d.                   | 240                                   | 509             | +                     | —                  | ±                       |
|                  | U03        | n.d.                            | n.d.                   | 231                                   | 560             | +                     | +                  | —                       |
|                  | U04        | n.d.                            | 48.0                   | 324                                   | 652             | +                     | +                  | —                       |
|                  | U05        | n.d.                            | n.d.                   | 300                                   | 580             | +                     | +                  | —                       |
| Iota             | I01        | 2.42                            | 18.0                   | 231                                   | 537             | +                     | +                  | —                       |
|                  | I02        | n.d.                            | n.d.                   | 229                                   | 501             | +                     | —                  | —                       |
| Kappa            | K01        | n.d.                            | n.d.                   | 274                                   | 557             | —                     | +                  | —                       |
|                  | K02        | 0.10                            | n.d.                   | 290                                   | 547             | —                     | +                  | —                       |
|                  | K03        | n.d.                            | 0.0                    | 193                                   | 453             | —                     | +                  | —                       |
|                  | K04        | n.d.                            | n.d.                   | 219                                   | 472             | —                     | +                  | —                       |
|                  | K05        | n.d.                            | n.d.                   | 207                                   | 488             | +                     | +                  | —                       |
|                  | K06        | 2.17                            | 30.9                   | 217                                   | 542             | +                     | +                  | —                       |
|                  | K07        | n.d.                            | n.d.                   | 204                                   | 495             | +                     | —                  | —                       |
|                  | K08        | 2.70                            | n.d.                   | 217                                   | 525             | +                     | —                  | ±                       |
|                  | K09        | n.d.                            | n.d.                   | 243                                   | 532             | +                     | —                  | —                       |
|                  | K10        | n.d.                            | 0.0                    | 211                                   | 486             | +                     | —                  | —                       |
|                  | K11        | n.d.                            | 0.0                    | 236                                   | 527             | +                     | —                  | ±                       |
|                  | K12        | 0.27                            | 18.4                   | 318                                   | 578             | +                     | +                  | —                       |
|                  | K13        | n.d.                            | n.d.                   | 247                                   | 501             | —                     | +                  | —                       |
|                  | K14        | n.d.                            | n.d.                   | 202                                   | 510             | +                     | +                  | —                       |
|                  | K15        | n.d.                            | n.d.                   | 221                                   | 498             | —                     | —                  | —                       |
|                  | K16        | n.d.                            | n.d.                   | 260                                   | 591             | +                     | +                  | —                       |
|                  | K17        | 3.26                            | n.d.                   | 223                                   | 520             | +                     | —                  | —                       |
|                  | K18        | n.d.                            | n.d.                   | 224                                   | 514             | +                     | —                  | —                       |
|                  | K19        | n.d.                            | 18.3                   | 226                                   | 561             | +                     | +                  | —                       |
| Lambda           | L01        | n.d.                            | 10.3                   | 260                                   | 526             | +                     | +                  | —                       |
|                  | L02        | 2.53                            | n.d.                   | 232                                   | 523             | +                     | —                  | —                       |
|                  | L03        | 0.29                            | n.d.                   | 266                                   | 505             | —                     | —                  | —                       |
| PES <sup>g</sup> | P01        | n.d.                            | n.d.                   | 243                                   | 531             | +                     | +                  | —                       |
| Poligeenan       | D01        | n.d.                            | n.d.                   | 0.7 <sup>h</sup>                      | 28 <sup>h</sup> | n.d.                  | n.d.               | n.d.                    |

n.d.: Not determined.

<sup>a</sup> Value of ion-chromatography analysis.

<sup>b</sup> Value of sugar analysis.

<sup>c</sup> Calculated between 5.00 (3000 kDa) min and 9.35 min (10kDa).

<sup>d</sup> + indicates the presence of peaks in both of RI and ICP (S). — indicates a peak in neither RI nor ICP (S).

<sup>e</sup> + indicates the presence of peaks in both of RI and ICP (C). — indicates a peak in neither RI nor ICP (C).

<sup>f</sup> ± indicates around the detection limit (about 5%). — indicates below the detection limit.

<sup>g</sup> Processed eucheuma seaweed.

<sup>h</sup> Calculated between 5.00 min (300 kDa) and 12.00 min (10 Da).

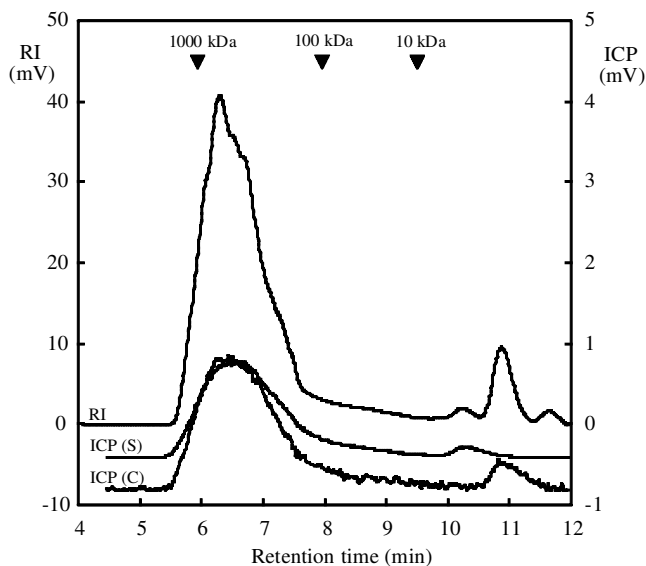


Figure 5. Elution profile of processed eucheuma seaweed (PES). A PES powder (sample No. P01) was dissolved in ultra-pure water containing 0.05 M sodium nitrate to prepare a solution containing 0.1% PES. See the legend for figure 2.

this improvement, it will be revealed whether or not the peak comes from poligeenan.

A tail region after the major peak in GPC is another concern. In our cases, all samples of food-grade refined carrageenans had a tail region after a major broad peak. Therefore, it is necessary to decide whether this tail region is the tail of a high molecular weight substance, or a low molecular weight substance *per se*. Lecacheux *et al.* (1985) showed that the tail was significantly decreased by re-injection of the major peak fraction. But the tail depends closely on the individual GPC system.

Although it is known that poligeenan has harmful effects on laboratory animals (Wakabayashi *et al.* 1978, Watt and Marcus 1981, Delahunty *et al.* 1987, Marcus *et al.* 1989, Karlsson and Singh 1999), it is not clear if the sample of poligeenan we tested has such harmful effects, because there are many different molecular weight distributions of poligeenan due to the state of acid degradation. Therefore, studies to establish which molecular weight fractions have harmful effects on animals are urgently required.

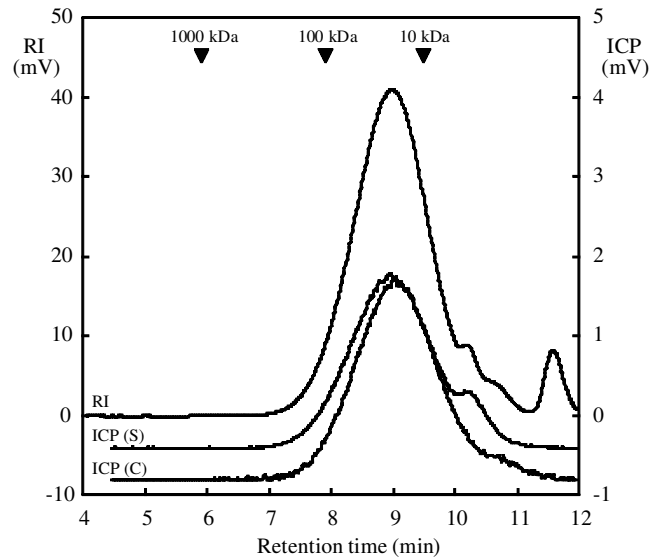


Figure 6. Elution profile of poligeenan. A poligeenan powder (sample No. D01) was dissolved in ultra-pure water containing 0.05 M sodium nitrate to prepare a solution containing 0.1% poligeenan. See the legend for figure 2.

#### Improvement of GPC/ICP system

In this paper, RI detection as well as ICP instrumentation were used to compare both chromatograms and to perform quantitative analysis. Because of the limit of a pressure against the flow cell of the RI detector, a wide-diameter tube was used to connect RI and ICP. Thus, substances separated by the GPC column may have been diffused. As the result, peaks in ICP chromatograms might become broader.

Furthermore, in this study, only one element (sulphur or carbon) was monitored in each GPC/ICP run due to the requirement of the data-sampling program. The improvement for sequential multi-element monitoring is now in progress. When it is completed, the measurement of sulphur to carbon ratio (figure 1) will clarify the type of carrageenan tested and the monitoring of alkali and alkali earth metals will reveal the true form of the RI peak which was not detected by the present ICP study (figure 4).

As above, although it was shown that the sensitivity of ICP was not enough to evaluate the presence of a peak at the retention time of degraded carrageenan,

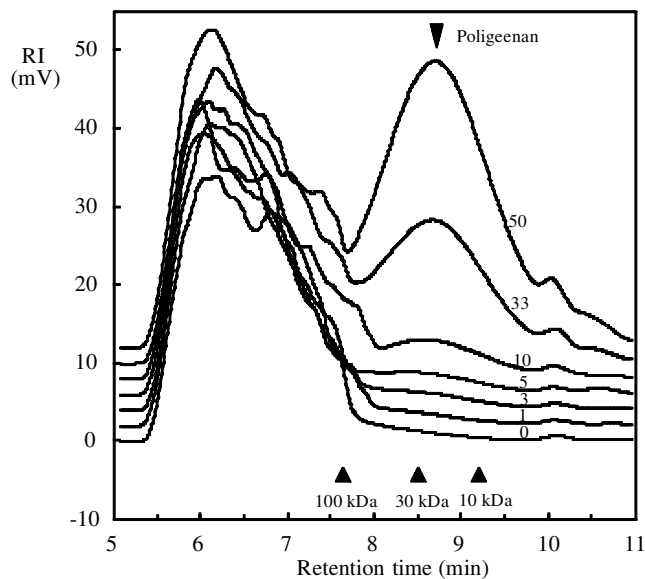


Figure 7. Detection limit for poligeenan contamination in carrageenan (elution profile). A sample of carrageenan (No. L03) and poligeenan was mixed to prepare each sample powder whose poligeenan contents were 0, 1, 3, 5, 10, 33, 50% respectively. Prepared powders were dissolved in ultra-pure water containing 0.05 M sodium nitrate to prepare a solution containing 0.1% mixed sample. The lowest line indicates the sample containing 0% poligeenan, and the highest line shows the sample containing 50% poligeenan. See the legend for figure 2 (RI detection only).

the detailed evaluation of poligeenan contamination in carrageenan seems possible when these improvements are established.

## Conclusions

We tested the molecular weight distributions of 29 samples of food-grade refined carrageenan, a PES and a poligeenan by the GPC/ICP system. All samples of food-grade refined carrageenan had a major broad peak of high molecular weight eluted around 6.5 min in both RI and ICP (sulphur and carbon), and each sample had no obvious peak of poligeenan (the detection limit was about 5%). The MN of these carrageenans ranged from 193 kDa to 324 kDa, and the MW from 453 kDa to 652 kDa based on RI data. Some samples had a few minor peaks which eluted around 10–12 min. It was deduced that these peaks

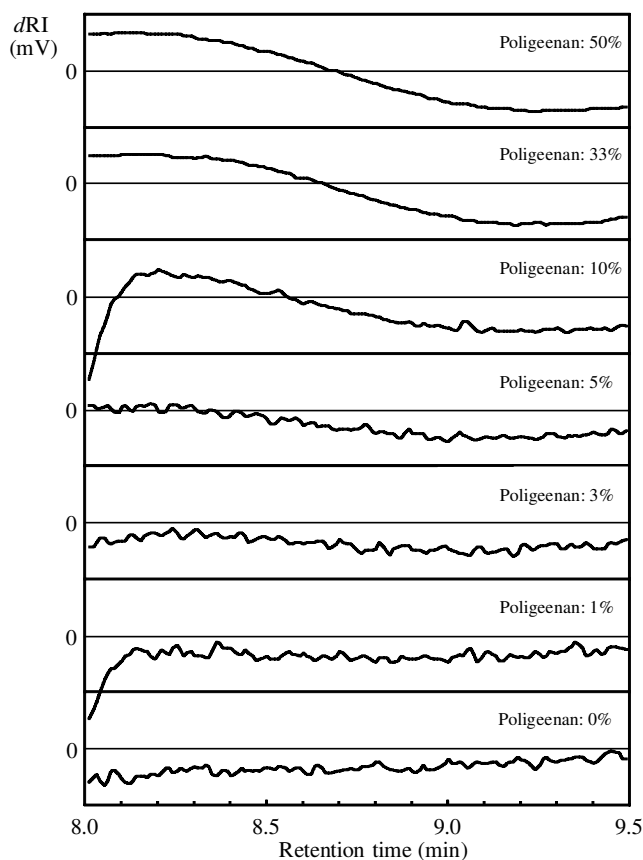


Figure 8. Detection limit for poligeenan contamination in carrageenan (differential curve). The differential curve is shown of each elution profile described in figure 7 between 8.0 and 9.5 min.

came from ionic sulphate, sucrose or glucose. It was considered that after the sensitivity of ICP and the data-sampling program are improved, the GPC/ICP system will become a more powerful technique for evaluation of carrageenan samples containing ionic substances and sugar.

A PES sample showed an elution profile similar to refined carrageenan, which had a major broad peak of high molecular weight. The MN of PES was 243 kDa and the MW was 531 kDa (RI base). An elution profile of poligeenan was clearly different from those of other samples in both RI and ICP (sulphur and carbon). The major peak was observed around 8.7 min, which corresponded to 20 kDa. The MN of poligeenan was 0.7 kDa and the MW was 28 kDa (RI base).

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