

## Chemical Heterogeneity of Carrageenans as Shown by Fractional Precipitation with Potassium Chloride

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Three different carrageenans, isolated from *Chondrus crispus*, *Gigartina stellata*, and *Gigartina skottsbergii*, were investigated with respect to their solubility in potassium chloride solutions. The precipitation technique produces sufficiently sharp fractionation to allow the conclusion that the carrageenans cannot consist of a mixture of only two components according to the classical definition, but rather of a series of molecules of different chemical composition and, consequently, with different solubility.

A close correlation was found to exist, within each carrageenan sample, between the content of 3,6-anhydro-D-galactose of the molecules and the potassium chloride concentration at which they become insoluble. Although the average chemical composition was almost the same, the weight distribution of material with respect to the content of 3,6-anhydro-D-galactose was markedly different for the three carrageenans. This difference may explain the different gel strengths observed for the three samples.

Carrageenan is the name given to sulphate-containing polysaccharides which can be extracted with water from some algal species in the order Gigartinales.<sup>1,2</sup> Polysaccharide material, at least closely related to carrageenan, has also been extracted from a few species outside this order according to the enzymatic classification introduced by Yaphe.<sup>3</sup> In solution the carrageenans have properties between those of agar and alginate as they give both high viscosity and form gels in water. The commercial value of carrageenans is mainly connected with their gelforming properties. The gel strength depends on factors such as temperature, the concentration of carrageenan, and the amount and type of inorganic salt present. The carrageenans also form gels in milk, usually with higher gel strengths than in water. When compared under standard conditions — in water or milk — different carrageenan samples often give very different gel strengths. This difference is accounted for by the

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fact that carrageenan is not a chemically homogeneous entity. As was first shown by Smith and Cook,<sup>4</sup> carrageenan can be fractionated by precipitation with potassium chloride (0.125–0.25 M) into two fractions of different chemical composition. The terms  $\lambda$ - and  $\kappa$ -carrageenan have been used for the soluble and the insoluble fraction, respectively. The carrageenans consist of three main components: D-galactose, 3,6-anhydro-D-galactose (3,6-AG) and sulphate half-ester groups.<sup>5</sup> The insoluble fraction obtained had a high content of 3,6-AG<sup>6</sup> while the soluble fraction from some samples of *Chondrus crispus* contained only traces of 3,6-AG.<sup>6-8</sup> The gel-forming tendency has been shown<sup>4-9</sup> to be associated only with the insoluble fraction. The existence of intermediate fractions has not been investigated in any detail.

Using fractional precipitation at a fixed potassium chloride concentration, Black *et al.*<sup>10</sup> have recently shown that the carrageenans from many algal species did not give any pure  $\lambda$ -fractions as judged by their 3,6-AG contents. The purity of the  $\lambda$ -fractions from *Chondrus crispus* seemed to depend on the locality of growth and the time of harvesting. Besides a molecular-weight fractionation obtained by Smith *et al.*,<sup>6</sup> no attempts have been made to fractionate the  $\lambda$ -fraction. Recently, Dolan and Rees<sup>8</sup> were able to fractionate a  $\lambda$ -preparation with a very low 3,6-AG content into two fractions after alkali modification. The chemical structure of these fractions, and of a  $\kappa$ -fraction from the same alga, has been elucidated by the works of Rees *et al.*<sup>7,8,11</sup> There may, however, be some doubt as to the contribution of these fractions to the total carrageenan. The main questions to be answered are: does carrageenan consist 1) of only two different, chemically well-defined compounds ( $\lambda$ - and  $\kappa$ -carrageenan), 2) of three or more well-defined fractions, or 3) of a mixture of molecules with a more continuously varying chemical composition.

There are very few data in the literature concerning the correlation between the physical properties and the chemical composition of carrageenans. Experience obtained during production and practical use of carrageenans have, however, led some producers to believe that the model based on  $\lambda$ - and  $\kappa$ -carrageenan, widely accepted by scientists in the field, is an oversimplification.<sup>12</sup> The main objective of this study was to use fractional precipitation to get information on the chemical heterogeneity of some carrageenan samples and to correlate these data with gel strength measurements.

## RESULTS

Since most chemical work on carrageenan has been carried out on fractions obtained by precipitation at a fixed concentration of potassium chloride (usually between 0.125 and 0.25 M), a more detailed study on the effect of different concentrations of potassium chloride on the solubility of carrageenan was undertaken. Three carrageenan samples prepared from *Chondrus crispus* Stackh., *Gigartina stellata* (Stackh.) Batt., and *Gigartina skottsbergii* S. et G., were used. Some characteristic data of the samples are given in Table 1.

It can be seen from the table that although the milk gel strengths are remarkably different for the three carrageenans the differences in the analytical values for the 3,6-AG and the sulphate contents are very small. The intrinsic viscosities were also similar. It is therefore clear that this kind of analysis on

Table 1. Chemical and physical properties of the carrageenan samples.

| Raw material           | % SO <sub>3</sub> Na | % 3,6-AG | [η]      | Gel strength in milk |          |
|------------------------|----------------------|----------|----------|----------------------|----------|
|                        | (w/w)                | (w/w)    | 100 ml/g | Method 1             | Method 2 |
| <i>C. crispus</i>      | 32.2                 | 23.5     | 8.7      | 280                  | 80       |
| <i>G. stellata</i>     | 33.7                 | 23.5     | 9.2      | 60                   | 30       |
| <i>G. skottsbergii</i> | 34.0                 | 20.9     | 8.4      | 20                   | 15       |

whole carrageenan samples gives no information to account for the big differences observed in the gel strengths.

Aliquots of carrageenan solutions were precipitated with potassium chloride solutions of ten different concentrations. The concentration of carrageenan in the mixture was 0.25 %, and the concentration of potassium chloride varied from 0.015 to 2.5 M. After centrifugation under standard conditions the soluble and the insoluble phases were analysed for total carbohydrate. In Fig. 1 the amount of precipitated material as percentage (w/w) of the total, is plotted against potassium chloride concentration. Decreasing the carrageenan concentration to 0.1 % gave no significant deviations from the curve obtained with 0.25 %. If the carrageenans consist of only the two components  $\lambda$ - and  $\kappa$ -carrageenan as defined by Smith and Cook, a step in the precipitation curves should occur between 0.125 and 0.25 M KCl.<sup>4</sup> Such a step was not observed in any of the curves, indicating that the carrageenans consist of more than two fractions.

To further investigate this, three main fractions were prepared from the carrageenan from *Chondrus crispus* by fractional precipitation with potassium chloride (described in Experimental). The yields of the fractions and the analytical figures for the 3,6-AG and the sulphate contents are given in Table 2.

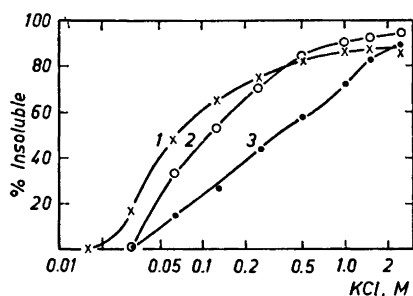


Fig. 1. Precipitation curves for carrageenan samples. 1 = *Chondrus crispus*, 2 = *Gigartina stellata*, 3 = *Gigartina skottsbergii*.

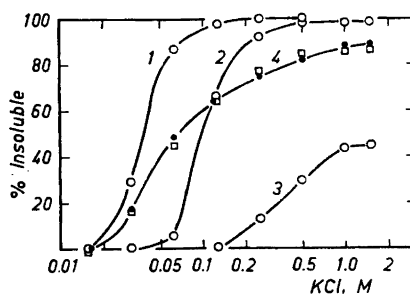


Fig. 2. Precipitation curves for fractions from *Chondrus crispus*. 1 = Fraction 1, 2 = Fraction 2, 3 = Fraction 3, 4 = Whole carrageenan: ● = Experimental points, □ = Calculated points.

Table 2. Preparation and properties of carrageenan fractions from *Chondrus crispus*.

| Fraction No. | Conc. of KCl                          | Yield* | % 3,6-AG (w/w) | % SO <sub>3</sub> Na (w/w) | [ $\eta$ ] | Gel strength in milk** |
|--------------|---------------------------------------|--------|----------------|----------------------------|------------|------------------------|
| 1            | Precipitate at 0.0625 M               | 48     | 29.2           | 24.8                       | 9.4        | 230                    |
| 2            | Precipitate between 0.0625 and 0.25 M | 20     | 27.0           | 32.2                       | 8.6        | 40                     |
| 3            | Soluble at 0.25 M                     | 31.4   | 9.0            | 39.6                       | 9.2        | 0                      |
| 3a           | Precipitate from No. 3 at 1.5 M       | 15.1   | 15.0           | 34.3                       |            |                        |
| 3b           | Soluble part of No. 3 at 1.5 M        | 16.3   | 4.0            | 40.0                       |            |                        |

\* All yields given as per cent of total recovery (ca. 90 %).

\*\* Method 2.

The precipitation curves of the fractions are given in Fig. 2, curves 1, 2, and 3. By comparing the amount precipitated with the potassium chloride concentrations used during the preparation, it can be seen that the fractionation was fairly sharp. The contamination of material belonging to the other fractions was only about 10 %. Using the precipitation curves for fractions 1, 2, and 3 and the corresponding yields of the fractions, a theoretical precipitation curve for the original carrageenan sample may be calculated. The calculated curve is given in Fig. 2, curve 4, and may be compared with the experimental curve for the unfractionated sample. The good agreement between the calculated points and the experimental curve indicates that the solubility of the carrageenan molecules in mixture is essentially the same as in the more homogeneous fractions.

It was of interest to compare the precipitation curve of the sample with the curve for the mixture of the two extreme fractions in a proportion giving the same 3,6-AG content as the original sample. Fraction 3 was subfractionated as described in the experimental section. One part of fraction 3b ("λ"-carrageenan) and two parts of fraction 1 ("κ"-carrageenan) were mixed. The precipitation curves of this mixture and its two components are given in Fig. 3. A theoretical curve was calculated, and even if the results indicate that a certain coprecipitation of the "λ"-fraction (3b) takes place, the results clearly demonstrate that the original sample cannot be composed of a mixture of the two extreme fractions only, but must contain molecules of an intermediate solubility.

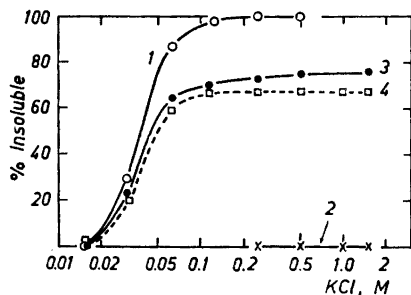


Fig. 3. Precipitation curves for extreme fractions from *Chondrus crispus*, and a mixture of these fractions to yield a 3,6-AG content equal to original sample. 1 = Fraction 1, 2 = Fraction 3b, 3 = Experimental curve for the mixture, 4 = Calculated curve for the mixture.

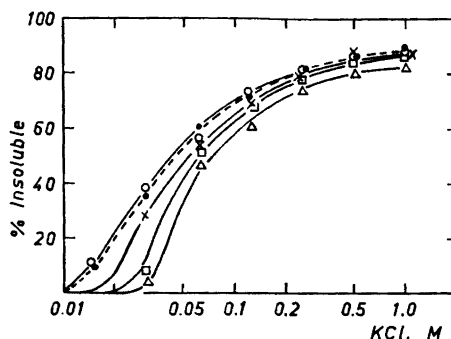


Fig. 4. Precipitation curves for degraded carrageenans with different intrinsic viscosities from *Chondrus crispus*.  $\circ$ :  $[\eta] = 8.7$ ,  $\bullet$ :  $[\eta] = 7.0$ ,  $\times$ :  $[\eta] = 6.2$ ,  $\square$ :  $[\eta] = 5.2$ ,  $\triangle$ :  $[\eta] = 3.5$ .

It is well known that the solubility of a polymer molecule depends not only on its chemical composition but also upon its molecular weight. As the polysaccharides are polydisperse with respect to molecular weight, any chemical fractionation may be accompanied by a molecular weight fractionation. The intrinsic viscosities of fractions 1, 2, and 3 were determined, and the results are shown in Table 2. The intrinsic viscosities of the three fractions were equal within the experimental error, indicating that a molecular weight fractionation only occurred to a very small extent by precipitation with potassium chloride.

In order to compare the precipitation curves for carrageenan samples of different molecular weights, four carrageenan samples with different intrinsic viscosities were prepared by degradation at pH 2.5 at 50°C, and precipitation curves were determined. It can be seen from Fig. 4 that the curves are identical for the two highest intrinsic viscosities, while there exists a significant difference between these curves and the curves representing samples with the lower intrinsic viscosities. The differences in the intrinsic viscosities of the degraded samples are, however, much larger than the differences among the three original carrageenan samples. The precipitation curves for the latter samples may therefore be compared without making any corrections for differences in intrinsic viscosity.

As the carrageenans clearly must consist of more than two components, it was intended to perform a more detailed study of the distribution of molecules with different chemical composition. The results given above demonstrate that the precipitation curves may be used for such a study because 1) the carrageenan molecules, when in mixture, behave independent of each other; *i.e.* that the solubility of carrageenan molecules of a certain composition is not affected by the existence of molecules of another solubility (Fig. 3), and 2) the solubility must be correlated more to the chemical composition of the

molecules than to their molecular weight (Table 2). The difference between the three precipitation curves in Fig. 1 may then be explained only as a difference in the distribution of molecules with different solubilities and, consequently with different chemical composition.

Assuming that precipitation with potassium chloride gives the same sharp fractionation of the carrageenans from *Gigartina stellata* and *G. skottsbergii* as was the case for the one from *Chondrus crispus*, the three curves can be regarded as the integral distribution curves for molecules with different solubilities in potassium chloride. Such distributions are often given as differential distribution curves, where the amount of material with a certain solubility is seen more easily. The curves in Fig. 1 were differentiated graphically, and the quantity  $dP/d(\log c_{\text{KCl}})$ , where  $P$  is the amount of precipitate and  $c_{\text{KCl}}$  is the molarity of potassium chloride, was plotted as a function of the potassium chloride concentration (Fig. 5). These curves thus represent the weight distribution of molecules with respect to their solubility in potassium chloride solution. The three curves are very different; the main tendency is that the amount of material with low solubility decreases and the amount of material with intermediate or high solubility increases when compared in the order *Chondrus crispus*, *G. stellata*, *G. skottsbergii*. It is to be noted that a small part of the molecules is not included in the curves, as this fraction does not precipitate even at the highest potassium chloride concentration. In the three samples used, these fractions correspond to 12, 6, and 10 %, respectively, of the total weight of the carrageenans.

As was pointed out above, a correlation should exist between the chemical composition and the solubility in potassium chloride. It is evident from the analytical figures for the sulphate and 3,6-AG content of the fractions (Table 2) that molecules rich in 3,6-AG and poor in sulphate have the lowest solubility in potassium chloride. As the relative variation in the 3,6-AG content of the fractions is by far the largest, and as this quantity can be measured easily by a colorimetric method, it was attempted to find a correlation between the 3,6-AG content of the molecules and the potassium chloride concentration at which they precipitate.

The precipitation curve given in Fig. 1 was determined by using one aliquot of the carrageenan solution for each KCl-concentration. The 3,6-AG content was determined in all the soluble and insoluble fractions, and the results are given in Fig. 6 as % 3,6-AG of total carbohydrate in the two fractions, plotted

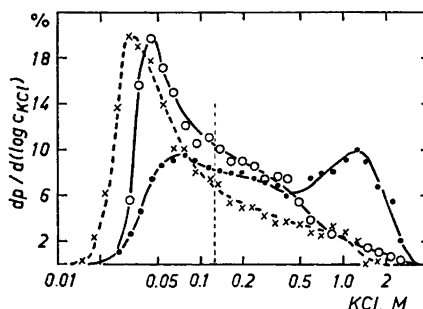


Fig. 5. Differential distribution of material with different solubilities from  $\times =$  *Chondrus crispus*,  $\circ =$  *Gigartina stellata*, and  $\bullet =$  *Gigartina skottsbergii*.

against the potassium chloride concentration. The composition of fractions precipitated in the range between two successive potassium chloride concentrations,  $i$  and  $i + 1$ , can be calculated according to the formula

$$3,6\text{-AG}_{i,i+1} (\%) = \frac{Q_{i+1} - Q_i}{P_{i+1} - P_i} \times 100$$

where  $Q_i$  and  $P_i$  represent the amount of 3,6-AG and carbohydrate, respectively, precipitated at the potassium chloride concentration  $i$ . The value of  $3,6\text{-AG}_{i,i+1}$  was calculated for several potassium chloride concentrations using values taken from the smooth curves in Figs. 1 and 6. The calculation was done independently for the soluble and the insoluble phase. The results are shown in Fig. 7 where the values for  $3,6\text{-AG}_{i,i+1}$  are placed on the arithmetic mean of the potassium chloride concentrations.

Fig. 7 shows that the correlation between the 3,6-AG content of the molecules and the potassium chloride concentration at which they precipitate is remarkably good. The analytical data for the fractions (Table 2) show that the sulphate content increases as the 3,6-AG content decreases, and it seems reasonable to assume that differences both in sulphate and 3,6-AG content may influence the solubility of the molecules. This assumption is substantiated by the fact that different correlations were obtained for the three different carrageenan samples. In spite of this, it is possible to use the experimentally obtained correlations between the 3,6-AG content and the potassium chloride concentration to construct the differential distribution curves for molecules with different 3,6-AG contents. The quantity  $dP/d(3,6\text{-AG})$  was calculated for different 3,6-AG contents by combining values taken from Figs. 7 and 5. The value  $dP/d(3,6\text{-AG})$  was plotted as a function of the 3,6-AG content in Fig. 8.

The curves in Fig. 8 now represent the weight distribution of material, as per cent of total, with respect to the 3,6-AG content. As was pointed out

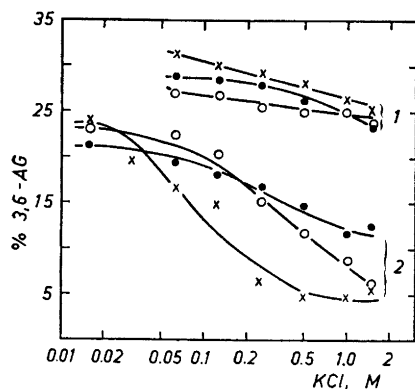


Fig. 6. 3,6-AG content (% of total carbohydrate) of the soluble and insoluble fractions. 1 = Insoluble, 2 = Soluble,  $\times$  = *Chondrus crispus*,  $\circ$  = *Gigartina stellata*,  $\bullet$  = *Gigartina skottsbergii*.

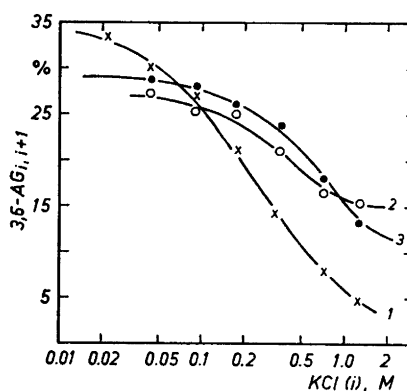


Fig. 7. The calculated 3,6-AG content of molecules precipitating at different potassium chloride concentrations.  $\times$  = *Chondrus crispus*,  $\circ$  = *Gigartina stellata*,  $\bullet$  = *Gigartina skottsbergii*.

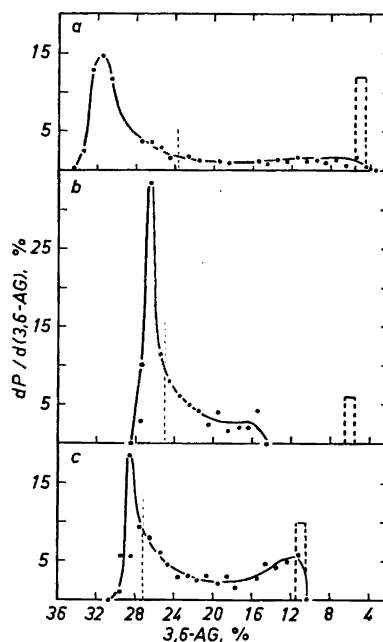


Fig. 8. The differential weight distribution of material with different 3,6-AG content. a) = *Chondrus crispus*, b) = *Gigartina stellata*, c) = *Gigartina skottsbergii*.

for the integral curve (Fig. 1) all three carrageenans contain a certain amount of material which could not be precipitated by KCl. This material was disregarded by the construction of the curves in Fig. 7, but must be considered of importance in connection with the distribution of molecules with respect to their 3,6-AG content. These fractions have therefore been included in Fig. 8 as columns, the area indicating their contribution to the total amount and the abscissa their mean 3,6-AG content.

This figure shows that all the three carrageenans contain molecules with 3,6-AG contents varying over a very broad range. The distribution is by far the broadest for the carrageenan from *Chondrus crispus* as there is a peak in the distribution curve around 32 % of 3,6-AG and a significant amount of material at all 3,6-AG values down to 4 %. The amount of material not precipitated is also high and has a low 3,6-AG content compared to the other carrageenans. The main peaks in the distribution curves for the two other carrageenans are located at lower 3,6-AG values than that from *Chondrus crispus*. The difference in the mean value of the 3,6-AG content is very small (Table 1) in spite of the rather big difference in the amount of material with a high 3,6-AG content. This may be explained from Fig. 8 by considering the varying amount of material with an intermediate composition.

Returning now to the difference among the milk gel strengths of the samples (Table 1), this may be explained qualitatively by assuming a correlation between the gel strength and the solubility of the molecules in potassium chloride. The figures for the fractions (Table 2) show that such a correlation exists, at least within a certain sample, since the gel strength dropped mark-



edly when the solubility of the fractions increased. The distribution curves of the three samples (Fig. 5) show that the amount of material with low solubility decreases in the same order as the milk gel strength. The amount of material with a very low solubility in potassium chloride therefore seems to be of prime importance in determining the gel strength of a sample.

#### DISCUSSION

The terms  $\lambda$ - and  $\kappa$ -carrageenan were originally introduced by Smith and Cook<sup>4</sup> to designate the soluble and insoluble fractions, respectively, obtained by fractionation of carrageenan at a definite potassium chloride concentration (0.125 M). The evidence presented by these authors strongly suggested that in this particular sample of carrageenan, a remarkably sharp fractionation was obtained. Gel strength, and a high 3,6-AG content, was associated with the  $\kappa$ -fraction, while the  $\lambda$ -carrageenan was devoid of gelifying properties and contained only a very small fraction of 3,6-AG units (around 2 %). Later investigations<sup>7,8,13</sup> have further emphasized the distinct difference in 3,6-AG contents and fostered the idea of a low 3,6-AG content as a purity criterion for the  $\lambda$ -fraction. A dualism is thus introduced into the original, physical definition of the two fractions. A semantic question then arises as to the definition and use of the terms  $\lambda$ - and  $\kappa$ -carrageenan. They may be used either 1) as names for the soluble and insoluble fractions obtained by KCl-precipitation as originally introduced by Smith and Cook, or 2) as names of carrageenan fractions of a definite, or at least of a closely related, chemical composition.

Obviously, our results are inconsistent with the idea of a fractionation, at a definite potassium chloride concentration, to give two components homogeneous with respect to their solubility properties in potassium chloride solution. The results plotted in Fig. 5 clearly demonstrate that in all our samples, fractions will be precipitated at any KCl-concentration within a considerable range on both sides of the 0.125 M KCl introduced by Smith and Cook.<sup>4</sup> If the same concentration is marked out on the correlation curves in Fig. 7, the intersection values represent the 3,6-AG content of the fractions precipitated at this KCl-concentration from the three different carrageenens. These values are then transferred to the abscissa of Fig. 8 and represented in the figure by vertical lines. The part of the curves to the left of this line then represents the weight distribution of material, with respect to the 3,6-AG content, of the material precipitated in a 0.125 M potassium chloride solution, *i.e.* in the  $\kappa$ -carrageenan according to the original definition. In all the three samples there is a rather narrow peak in the distribution curve in this area, and it seems reasonable to associate the term  $\kappa$ -carrageenan with this material. It should be noted that, if defined in this way, the different  $\kappa$ -carrageenans will be different both with respect to chemical composition and to range of distribution. For this particular *Chondrus*-carrageenan, it would seem more appropriate to isolate a  $\kappa$ -fraction by precipitation at a lower KCl-concentration corresponding, in Fig. 8, to about 30 % 3,6-AG, a procedure actually adopted for the preparation of fraction 1 of Table 2. This does, however, conflict with the  $\kappa$ -definition.

The soluble fraction, *i.e.* the curve to the right of the line in Fig. 8, should then represent the  $\lambda$ -carrageenan according to the classical definition. This fraction contains material precipitating over a wide range of KCl-concentrations and, in addition, some material not precipitated by KCl. The 3,6-AG content of this material also shows a considerable variation, and it is noteworthy that even the fractions completely soluble in KCl are considerably higher in 3,6-AG than those reported in the literature. Even if we were to accept the definition of the insoluble fraction as  $\kappa$ -carrageenan, it seems extremely doubtful to assign a definite name to the heterogeneous mixture remaining in the soluble phase. Rees<sup>7</sup> has previously pointed out that for some carrageenans the sharp  $\kappa/\lambda$ -fractionation is not obtained. Black *et al.*<sup>10</sup> recently demonstrated that the two fractions, prepared according to the method of Smith and Cook, varied considerably in 3,6-AG and sulphate contents both from species to species as well as among different samples from the same species. Their results are thus in complete accordance with what should be expected according to the data presented above.

The results of Fig. 8 would seem to justify a subdivision of the fraction soluble above 0.125 M KCl into two parts: a) the fraction soluble at all KCl-concentrations, which, apart from the higher 3,6-AG content, is the one most closely related to the classical  $\lambda$ -carrageenan, b) the fraction precipitated with KCl at concentrations above 0.125 M KCl, which will be called the intermediate fractions in the following discussion. It should be stressed that neither of these fractions are homogeneous with respect to chemical composition, and whether this system is adequate will have to await further investigations.

The other alternative, using the terms for compounds of a definite chemical composition, was very recently introduced by Anderson and Rees.<sup>11</sup> The structures they suggested allow some variation with respect to the degree of sulphate esterification thus allowing the chemical composition to vary within certain limits. The most striking feature for the present discussion is probably that the  $\lambda$ -carrageenan should not contain any 3,6-AG. According to the results of Black *et al.*, and to those presented in this paper, it seems extremely difficult to fulfill this condition in any of the carrageenans investigated. Obviously, the structural formulae suggested by Anderson and Rees cannot be considered representative for  $\lambda$ - and  $\kappa$ -carrageenans isolated according to the original method. The two nomenclature systems are thus inconsistent. Unfortunately, no information is available as to the solubility of the fractions used by Rees *et al.* or their contribution to the total polymer weight. To suggest a nomenclature which will reconcile the chemical and physical data is therefore not possible. The decision will have to await further investigations on the structure of a variety of fractions duly characterized by means of detailed information on their solubility properties and their contribution to the polymer weight.

Precipitation with potassium chloride is a well established property of carrageenan, and it seems reasonable to suggest that this technique should be used to characterize whole and fractionated carrageenans. A distribution curve for the polymer molecules with respect to their 3,6-AG content (*cf.* Fig. 8) would be very suitable for this purpose. There are, however, some disadvantages inherent in the method used to obtain this type of curve; the correlation be-

tween the 3,6-AG content and the potassium chloride concentration at which the molecules precipitate is different for different samples and must consequently be determined for each sample to be investigated. This is quite laborious, as a very high analytical accuracy is required. For most purposes sufficient information may be obtained just by determining the precipitation curve (*cf.* Fig. 1). This curve can be used to characterize not only the original samples but the distribution of material in their component fractions. If a quantitative interpretation is attempted it should be noted that the calibration factor used in the phenol-sulphuric acid colour reaction varies with the composition of the fraction. The factor was about 40 % higher for a fraction of the  $\lambda$ -type than for one of the  $\kappa$ -type, most probably because of the high sulphate content of the former.

Although, in the previous part of this discussion, the main emphasis has been put on the correlation between the 3,6-AG content and the solubility in potassium chloride, the importance of the sulphate to the solubility properties should not be underestimated. The fact that the correlation between solubility and 3,6-AG content is different for the three samples investigated (*cf.* Fig. 7) demonstrates that solubility is not controlled solely by the 3,6-AG content, and it seems reasonable to associate this deviation with variations in the sulphate content or distribution.

If, within a certain carrageenan sample, there exists a correlation between the 3,6-AG content and the sulphate content of the molecules, the distribution curve with respect to the sulphate content could be found by changing the abscissa axis in Fig. 8 according to this correlation. Painter<sup>14</sup> showed that, in  $\kappa$ -carrageenan, the sulphate could be located in any of the possible positions of the two sugar units. No attempt was made to determine the quantitative distribution among the positions. An analysis of the distribution in the total amount of sulphate should therefore be connected with an investigation of the position in the sugar.

Since molecules with a low solubility in potassium chloride solutions have been shown to give the main contribution to the gel strength, a precipitation curve should be a good characteristic where properties such as gel strength are of interest. The significance of the "intermediate fractions", *i.e.* fractions which precipitate only at high potassium chloride concentrations, should be pointed out as they determine the properties of the carrageenans to a large extent. These fractions have a relatively high content of 3,6-AG as compared to the soluble fraction, but give a very small contribution to the gel strength of the sample (Table 2). The effect of the intermediate fractions therefore is to increase the mean value of the 3,6-AG content as compared to a " $\lambda$ "-fraction, but to have the same negligible contribution to the gel strength. Table 3 demonstrates this effect in the three samples investigated. The amount of intermediate fractions increases from 23 % in *C. crispus* to 63 % in *G. skottsbergii* while the gel strength decreases markedly in the same order (*cf.* Table 1). The total 3,6-AG content of a carrageenan sample can therefore not be readily correlated to the gel strength.

On account of the large variations observed by Black *et al.*, the data presented in this report should not be considered as representative exponents for the three species in question. More information is needed in this field, and

Table 3. Solubility of carrageenan samples in potassium chloride (data from Fig. 1).

| Raw material           | Precipitate<br>at 0.125 M | Amount of material in per cent of total<br>Prec. between<br>0.125 and 2.5 M | Soluble<br>at 2.5 M |
|------------------------|---------------------------|---|---------------------|
| <i>C. crispus</i>      | 65                        | 23  | 12                  |
| <i>G. stellata</i>     | 54                        | 40  | 6                   |
| <i>G. skottsbergii</i> | 27                        | 63  | 10                  |

work is in progress to use the precipitation technique in comparing carrageenan samples prepared from different raw materials.

### EXPERIMENTAL

**Materials.** Carrageenans from three different algal species were used. The carrageenans from *Gigartina stellata* (harvested in Norway) and from *Chondrus crispus* (harvested on the east coast of Canada) were commercial samples and contained no detectable excess of potassium salts. The third sample was prepared from *Gigartina skottsbergii* (obtained from Argentina) in the following way: 50 g of algal material were extracted 4 times with 2 l of 0.02 M sodium acetate at 80°C. A fifth extraction failed to remove more material from the algae. The combined extracts were centrifuged and evaporated in vacuum to approximately 2 l. KCl solution was added to 0.1 M and the carrageenan precipitated with 4 l of ethanol. The precipitate was washed 2 times with 30% (v/v) ethanol saturated with KCl, 3 times with 70% ethanol, 3 times with ethanol and 3 times with ether before drying overnight at 40°C. The yield of carrageenan was 31.5 g.

**Intrinsic viscosity.** The viscosity was determined in a capillary viscometer, Ubbelohde No. 2. Five different carrageenan concentrations between 0.5 and 0.05% were used; all the solutions contained 0.1 M NaCl. The extrapolation of the reduced viscosity against concentration to determine the intrinsic viscosity was performed on a semi-logarithmic paper where the curves were linear at low concentrations of carrageenan.

**Precipitation curves.** The precipitations were carried out by mixing equal volumes of potassium carrageenate solutions (0.5% w/v) and solutions of potassium chloride. When a higher concentration than 1.5 M of potassium chloride in the mixture was required, one part of carrageenan solution (1%) was mixed with three parts of potassium chloride solution. The mixture was stirred thoroughly for 1 min, allowed to stand for 30 min, and then centrifuged for 30 min at 26 000 g. The precipitate was dissolved in water, and the amount of material present in the resultant solution and in the original centrifugate was determined by the phenol-sulphuric acid method.<sup>15</sup> The amount of 3,6-AG in the two phases was determined by a colorimetric method according to Yaphe.<sup>16</sup> An optical density of 0.1 at 555 m $\mu$  was found to correspond to 3.92  $\mu$ g 3,6-AG per ml. The optical density of the phenol-sulphuric acid reaction depended to some extent on the composition of the carrageenan or the carrageenan fraction. An optical density of 0.1 at 485 m $\mu$  was found to correspond to 12.4, 11.8, and 12.8  $\mu$ g/ml for the Norwegian, Canadian, and Argentine samples, respectively. These values were used for calculating the precipitation curves.

**Preparation of fractions.** 10 g of carrageenan prepared from *Chondrus crispus* was dissolved in 2 l of water and precipitated with 2 l of 0.125 M KCl solution. The mixture was stirred with a high speed mechanical stirrer during the slow addition of potassium chloride (5 min) and for another 5 min. After standing for 1 h the mixture was centrifuged for 30 min at 16 000 g. The precipitate was collected, washed and dried as before (Fraction 1). A solution of 3 M potassium chloride was added to the centrifugate while stirred to obtain a concentration in the mixture of 0.25 M. After centrifuging, the precipitate

was washed and dried (Fraction 2). The centrifugate was evaporated in vacuum to a small volume, dialysed against water to decrease the concentration of KCl to approximately 0.1 M, precipitated with two volumes of ethanol, washed and dried (Fraction 3). A 0.5 % solution of Fraction 3 was precipitated with an equal volume of 3 N KCl solution. The precipitate and the soluble fraction were named 3a and 3b, respectively. The fractions were analysed for 3,6-AG and for sulphate by the barium chloranilate method.<sup>17</sup>

*Gel strength.* As different methods for gel strength determination are not equally suitable for every type of gel, there is no standardised method in general use. A very simple method giving reproducible results and a reasonable difference between the highest and the lowest readings was used in this investigation. The apparatus consisted of a two-armed balance with a cup containing the gel on one side, and an empty cup on the other side. After equilibration of the balance, a cylindrical plunger was mounted to touch the surface of the gel. Water was poured into the empty cup at a constant rate until the plunger penetrated the gel surface. The gel strength is given as this amount of water in gram. All the gel strengths were measured in milk. As variations in the quality of milk may cause non-reproducible results, dry skimmed milk of a standard composition was used in all the experiments. One part of carrageenan was mixed with 15 parts of the milk powder and suspended in 150 parts of cold water. The mixture was heated on a boiling water bath with occasional shaking until all the carrageenan particles dissolved. The warm milk solution was poured into small cups which were placed in a tap water bath. The cups were elongated with tape around the top in such a way that the level of the solution could be kept approximately 5 mm above the upper edge. After standing overnight, the tape was removed and the upper part of the gel was cut off by means of a fine wire. The gel strength was measured on the fresh surface immediately after cutting. Two sets of plungers and cups were used:

Method 1. A plunger with a diameter of 24 mm and a cup with a volume of 65 ml and an outer diameter of 60 mm

Method 2. A plunger with a diameter of 12 mm and a cup with a volume of 7 ml and an outer diameter of 24 mm.

The second method required only 75 mg of carrageenan for the determination, but as the first gave larger differences between the samples, this method was used when sufficient material was available. The gel strength (when expressed as g/cm<sup>2</sup>) was not the same for the two methods, possibly because the distance between the cylinder and the wall was very small in Method 2. Comparison between different samples can thus only be done within each method.

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