Simplified Method for Estimation of Composition of Alginates by FTIR

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ABSTRACT: A modified method of preparing highly purified polymannuronic acid and polyguluronic acid was developed wherein acid hydrolysis was carried out using oxalic acid and separation was made using the difference of solubility with pH value. The simplified method using FTIR measurement to estimate the M/G ratio of alginate was investigated using Ca or Mn salts of alginate. The developed method appears to offer enhanced accuracy compared to that of previous techniques. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 1372–1377, 2004

Key words: alginates; separation techniques; FTIR; ^{13}C-NMR; gels

INTRODUCTION

Alginic acid is a constituent of brown seaweed and has a characteristic structure that consists of two uronic acids, β-D-mannuronic acid and α-L-guluronic acid. These constituents form homopolymeric regions and alternating regions in the polysaccharide. The concentrations of mannuronic and guluronic acid vary with the species, season of harvesting, and the site used of the original seaweed.[1] Generally, polymannuronic acid is similar to cellulose to form beta 1-4 linkage, whose conformation is a ribbonlike linear flexible chain. On the other hand, the conformation of polyguluronic acid is that of a hard and rigid folded polymer. It is known that the composition of the alginic acid affects the gelling properties or chemical derivatization.[2] Thus the index, called the M/G ratio, which is the ratio of constituents in the alginic acid, is especially important in industrial use.

Alginic acid has been applied in the food, pharmaceutical, and medical industries, for example. The gelling property of alginic acid is important for these applications and has been well studied.[2,3] Alginic acid makes a gel by the ionic bonding (called the "egg-box structure") between polyguluronic acid region and divalent cations, shown in Figure 1. Therefore measurement of the M/G ratio is necessary to use alginic acid.

General methods for measuring the M/G ratio are the isolation of polymannuronate and polyguluronate by partial hydrolysis, followed by colorimetry or calculation from the ^{13}C-NMR spectra.[4–8] These methods are very time consuming. During the 1970s, a simple estimation of the M/G ratio from FTIR spectra was investigated, but not established.[9,10]

In the present study, a modified method of preparing purer polymannuronic acid and polyguluronic acid is developed. Using the above homopolymers and various cations, we describe the simplified method for estimation of the M/G ratio with FTIR.

EXPERIMENTAL

Materials

Sodium alginate was purchased from Wako Pure Chemical Industries (Tokyo, Japan) and used for isolation of M-block and G-block. The sodium alginate samples were kindly provided by Kibun Food Chemifa (Tokyo, Japan) and Kimitsu Chemical Industry (Chiba, Japan) as the samples with known M/G ratio. All other chemicals used in this study were purchased from Wako Pure Chemical Industries and used as received.

Isolation of M-block and G-block from commercial sodium alginate

M-block and G-block were isolated from sodium alginate in accordance with a previously reported method,[11] which involves acid hydrolysis using oxalic acid and separation using the difference of solubility with pH value. The procedure is shown in Figure 2.

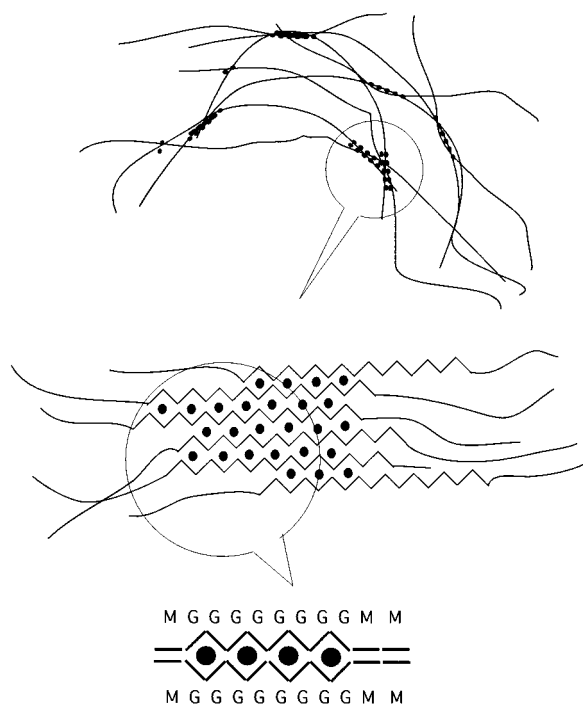*Correspondence to:* A. Takemura (akiot@mail.ecc.u-tokyo.ac.jp).

Figure 1 Egg-box structure of G-block.

The expected crude polymannuronic acid, portion C, included a small amount of polyguluronic acid, so we repeated the precipitation for further purification; the crude polymannuronic acid, portion D, was thus successfully produced. These obtained fractions were characterized by ^{13}C-NMR and GPC.

^{13}C-NMR measurement

^{13}C-NMR measurements were performed to determine the M/G ratio values of the obtained M-block, G-block, and their original alginate. Samples (50 mg) were dissolved in 1.0 mg of D$_2$O with one drop of 15M NaOD solutions and submitted for measurement. An acetonitrile solution was added to the sample as an internal standard ($\delta = 117.8$ ppm). Their NMR spectra were recorded on a JEOL alpha 500 (125 MHz; JEOL, Tokyo, Japan), using a ^{1}H(X)-tunable probe. The inverse-gated decoupling method was selected as an irradiation mode to eliminate the nuclear Overhauser effect for the quantitative measurement. A series of 25,000–60,000 scans were accumulated to obtain spectra showing good S/N ratio.

The chemical shifts obtained were assigned to mannuronate and guluronate in each block, with respect to previous studies,[4–8] as follows (in ppm):

$\delta_{MM-1} = 98.47$, $\delta_{MM-2} = 68.78$, $\delta_{MM-3} = 70.06$, $\delta_{MM-4} = 76.05$, $\delta_{MM-5} = 74.40$, $\delta_{MM-6} = 173.99$, $\delta_{GG-1} = 99.71$, $\delta_{GG-2} = 63.55$, $\delta_{GG-3} = 67.71$, $\delta_{GG-4} = 78.99$, $\delta_{GG-5} = 68.58$, $\delta_{GG-6} = 174.21$.

Preparation of alginate salts

Three kinds of alginate salts (i.e., calcium, barium, and manganese) were prepared. An alginate sample (50 mg) was dissolved in deionized water to obtain 2.5 wt % aqueous solution of sample. Then the obtained alginate sample solution was charged to a 0.5M CaCl$_2$, BaCl$_2$, or MnCl$_2$ solution and allowed to remain overnight. The precipitated alginate salts were collected by centrifugation, followed by lyophilization.

FTIR measurements

FTIR spectra of alginate samples were recorded in absorbance with 64–128 scans at a resolution of 4 cm^{-1} using a Nicolet Magna 860 FTIR spectrometer (Nicolet Analytical Instruments, Madison, WI) ,equipped with DTGS detector. All alginate samples were prepared for measurement in the form of KBr pellets. Triplicated KBr pellets were used for measurement and the mean value of absorbance strength at the appropriate bands was taken for the estimation of the sample's M/G ratio.
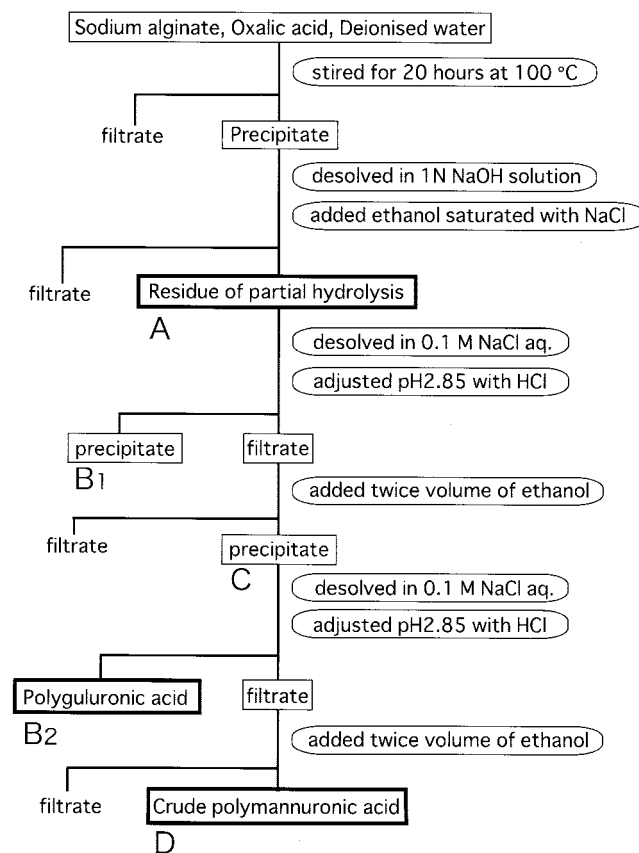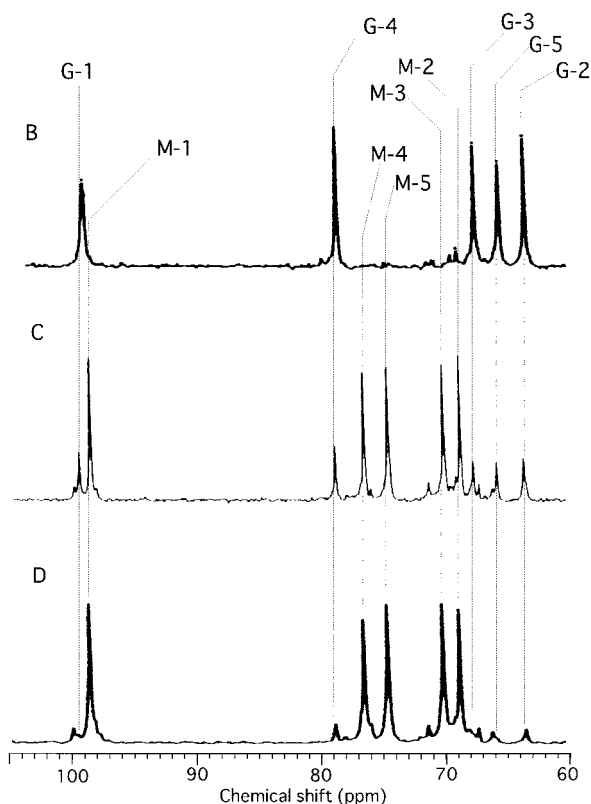

**Figure 2** Procedure of polymannuronic and polyguluronic acid isolation.

Figure 3 C1–C5 region of ^{13}C-NMR spectra for precipitates B, C, and D.

RESULTS AND DISCUSSION

Preparation and characterization of polymannuronic acid and polyguluronic acid

Polymannuronic acid (portion C in Fig. 2) and polyguluronic acid (portion B1 in Fig. 2) were obtained in 15.0 and 16.0% yields by use of a previously reported method.[11] Figure 3 shows ^{13}C-NMR spectra of these fractions. Assignments of peaks were determined according to the published literature.[11] Portion C is largely homo-polymannuronic acid, but a slight amount of polyguluronic acid was detected. Further purification of portion C produced portion D. From Figure 3, the higher purity of portion D is clear because the C5 peak has disappeared and the other C1–C4 and C6 peaks are assigned as MG blocks and no GG blocks are observed. Figure 4 shows the ^{13}C-NMR spectra of the anomer region. It is known that peaks assigned as C1, which is considered to be most affected by the sequence of M and G, appear in four distinct peaks.[12] In this region the residual G block in portion C disappeared in portion D. It is concluded that the scarcely observed peaks of polyguluronic acid are not derived from G block but from MG or GM blocks.

There is no difference in the spectra of portions B1 and B2, which were determined as highly pure homo-polyguluronic acid.
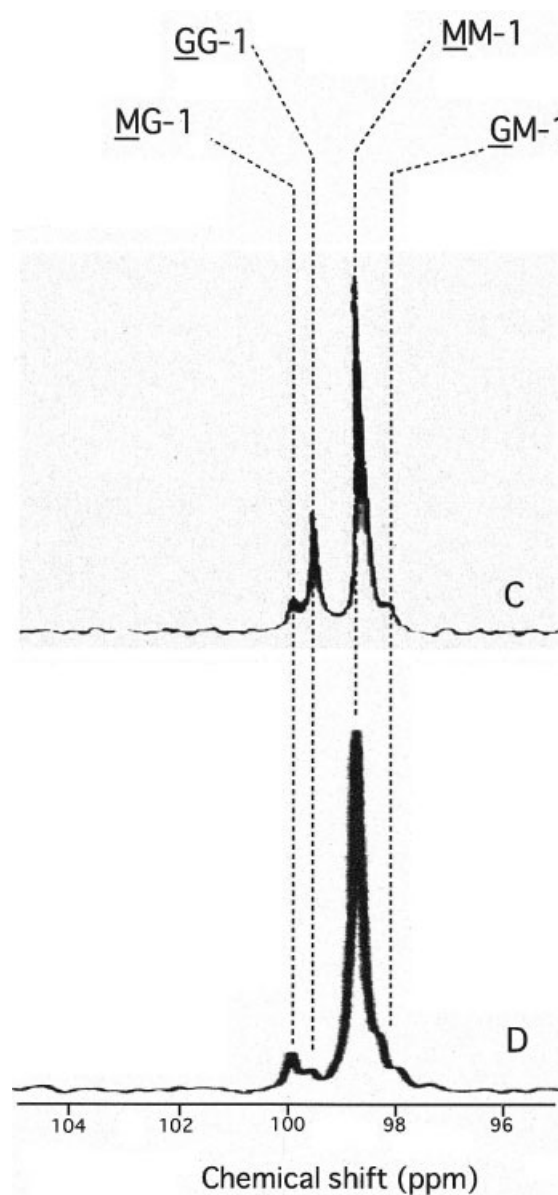
Figure 4 ^{13}C-NMR spectra of anomer region for precipitate C (top), and D (bottom).

Table I shows the degree of polymerization for polyuronic acid from GPC using polyethylene glycol as the molecular weight (MW) standard. The degree of

TABLE I
Degree of Polymerization (DP) of Hydrolyzates Calculated from GPC Curve

Hydrolyzate	Retention time (min)	$\overline{DP}^a$
Sodium alginate (Wako)	9.994	346
Portion A	10.549	169
Portion B (polyguluronic acid)	10.453	191
Portion D (polymannuronate)	10.869	116

^{a} These values are calculated as the molecular weight of polymer unit (uronate) ($\sim$ 200).
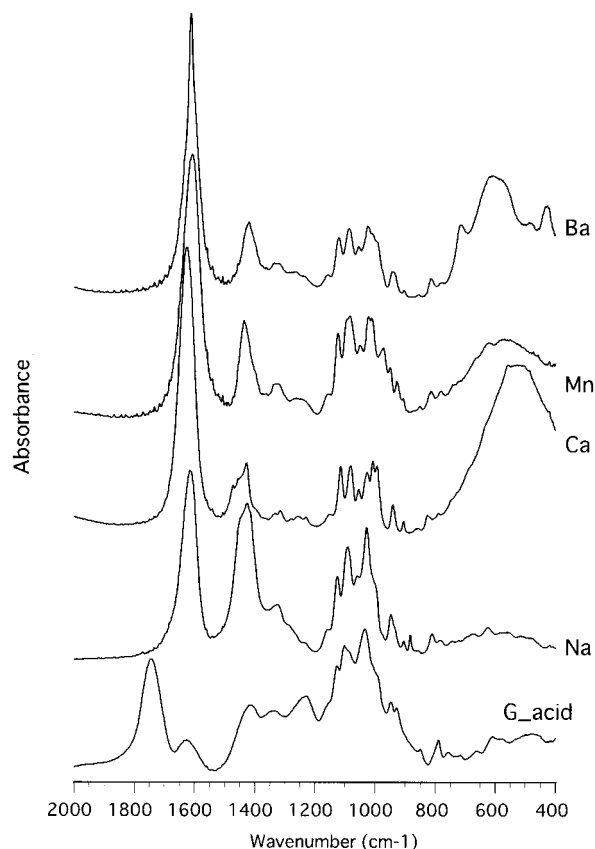
Figure 5   IR spectra of polyguluronates.

polymerization (DP) of polymannuronic acid and polyguluronic acid was determined as 116 and 191, respectively. Polymannuronic acid has a lower MW than that of polyguluronic acid, which reflects the properties of acid hydrolysis. It is known that acid hydrolysis proceeds without significant loss of DP of polyalginic acid, by comparing the MW of sodium alginate and its hydrolyzed material.

Difference in FTIR spectra of alginic acid and its salts

Determination of the M/G ratio using FTIR was developed previously,[9,10] where, using G-rich and M-rich sodium alginates, the distinctive peaks of the two alginates under investigation were selected and the relative peak strengths were determined as the M/G ratio. However, use of this method could lead to a substantially large error. In the present study three salts, whose ionic crosslinking strengths are different, were prepared to investigate the M/G ratio by FTIR.

Figure 5 shows the FTIR spectra of polyguluronic acid and polyguluronates. Peaks of divalent polyguluronate are sharper than those of polyguluronic acid or sodium polyguluronate. This sharpness of divalent polyguluronates reflects the limited mobility of molecules fixed by

the "egg-box" structure, which produces hidden peaks compared to those of sodium guluronate. On the other hand, the spectra of polymannuronic acid and polymannuronates showed little change in this band (not shown). This result suggests that polymannuronate does not undergo a strong interaction with divalent cations like the egg-box structure in polyguluronates. The above results indicate that ionic crosslinking in the G-block enhances the clarity of the FTIR spectrum.

Estimation of composition of alginates by FTIR

To visualize the relationship between FTIR spectra and the M/G ratio of the sample, the ratio of absorbance readings was plotted against mannuronate concentration.

Figure 6 shows the FTIR spectra of Ca salts of model mixtures prepared in various mannuronate concentrations. Some peaks shift in correspondence to the M concentration. As the concentration increases, the absorbance readings at 905, 940, 990, and 1120 cm^{-1} become lower; and at 960, 1035, 1100, 1150, and 1175 cm^{-1} the readings become higher. The variation of concentration causes shifting of the peak, which is assignable to the absorbance of OH bending, at 1030 cm^{-1}. The absorbance at 1080 cm^{-1} has a slight change with the variation of M/G ratio of the samples. Because this absorbance is assignable to C—O—C stretching of the six-membered ring in both of mannuronic and guluronic acid, we thus selected this absorbance as the standard and plotted the ratio of absorbance readings against mannuronate concentration. Figure 7 shows the linear relation between the ratio of absorbance readings at 1030 and 1080 cm^{-1}
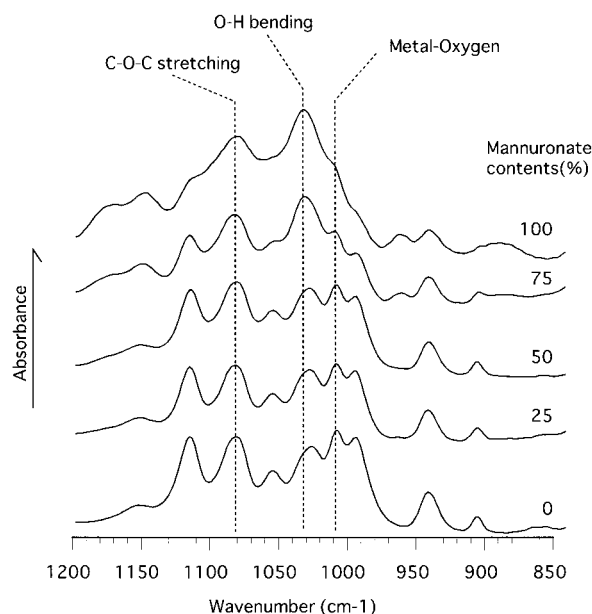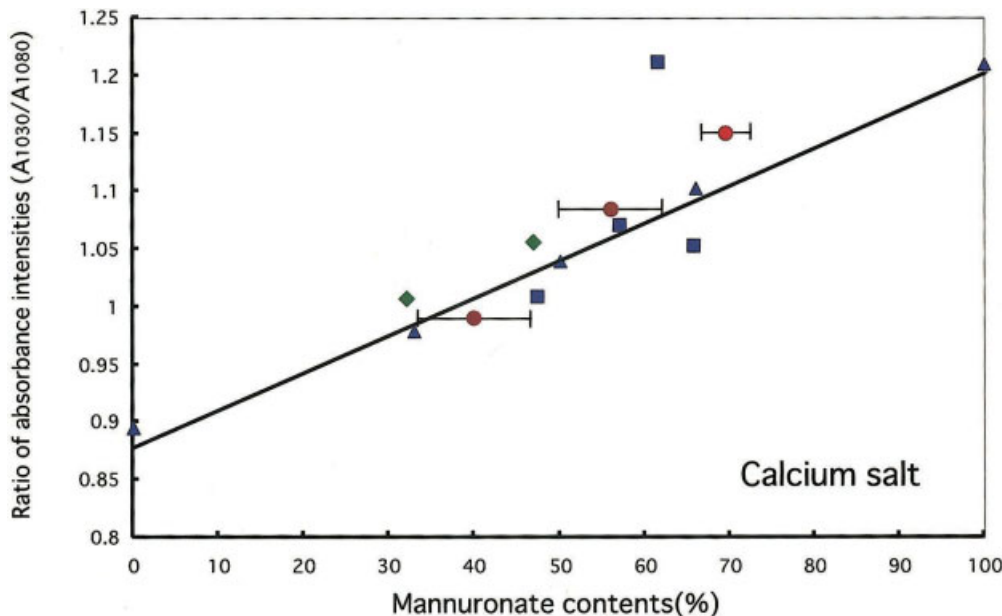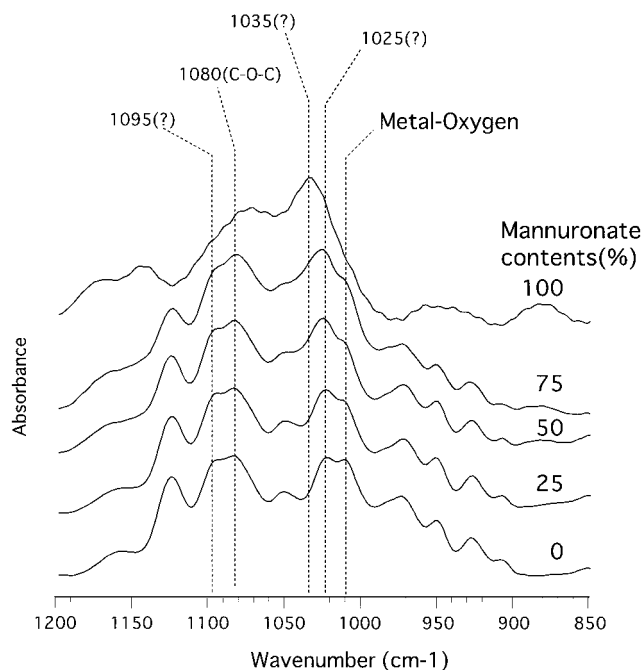

Figure 6   IR spectra of fingerprint region for calcium salt of MG mixtures.

Figure 7 Ratio of specific absorbance intensities related with mannuronate contents: ▲, model mixture; ■, model materials (M/G ratio is known); ●, samples provided by Kimitsu; ◆, samples provided by Kibun Food Chemifa.

and the mannuronate concentration of the model mixture and commercial alginate samples with known M/G ratio. In the case of other absorbance combinations, a clear relationship was not observed. We found that the absorbance at 1030 cm^{-1} directly reflects the change of mannuronate concentration of calcium alginate.


Figure 8 IR spectra of fingerprint region of manganese salt of MG mixtures.

Figure 8 shows the FTIR spectra of Mn salts of model mixtures prepared in various mannuronate concentrations, although these spectra are not clearer than those of Ca salts. However, several peaks vary in intensity with the change of M/G ratio of the sample. The peak at about 1030 cm^{-1} shows a shift similar to that in the spectra of Ca salts. The peak at 1095 cm^{-1} is not observed in the spectra of Ca salts. This absorbance intensity also varies with the M/G ratio of the samples; nevertheless, its assignment was not determined. This peak influences the peak at 1080 cm^{-1} used as the standard in the spectra of Ca salts. In this case, therefore, we attempted to find the absorbance combination, which shows good agreement with the M/G ratio of the samples, without the standard. Figure 9 shows the relation between the ratio of absorbance readings at 1010 and 1025 cm^{-1} and the mannuronate concentration of the samples. These peaks are attributed to the metal–oxygen interaction and OH bending of guluronate, respectively. The commercial alginate samples with known M/G ratio show a tendency similar to that of the model mixtures. Therefore this absorbance combination can be applied to estimate the M/G ratio of alginate.

CONCLUSIONS

A modified method of preparing highly purified poly-mannuronic acid and polyguluronic acid was developed wherein acid hydrolysis was carried out using
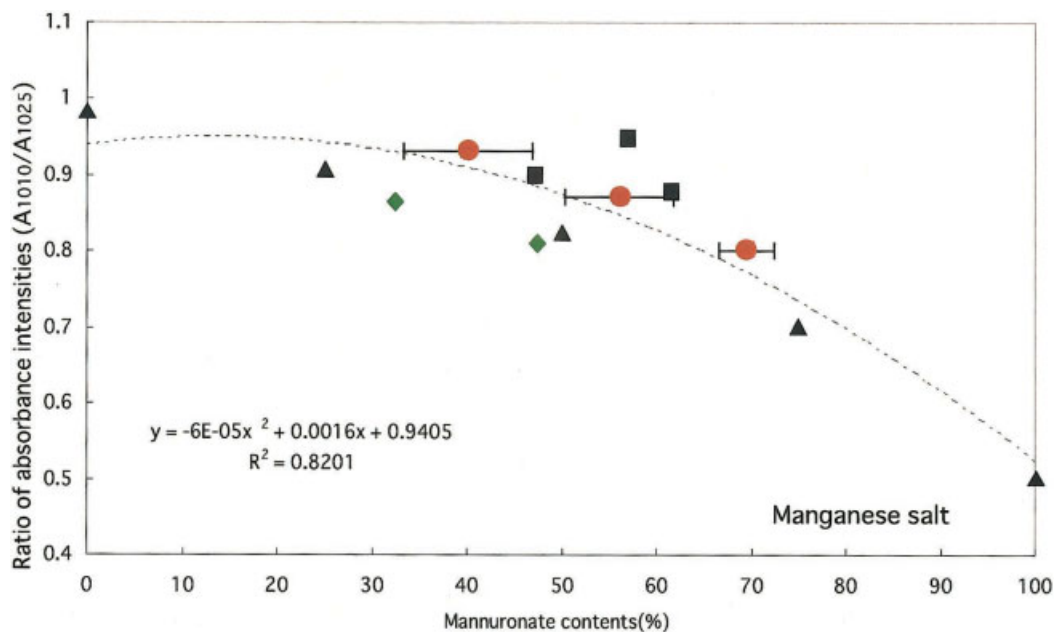
Figure 9 Ratio of specific absorbance intensities related with mannuronate contents. For manganese salts: ▲, model mixture; ■, model materials (M/G ratio is known); ●, samples provided by Kimitsu; ◆, samples provided by Kibun Food Chemifa.

oxalic acid and separation was made using the difference of solubility with pH value.

A simplified method using FTIR measurement to estimate the M/G ratio of alginate was investigated; Ca or Mn salts of alginate showed characteristic peaks, which reflect the M/G ratio of the sample, in the FTIR spectra.

The ratio of specific absorbance combinations (A_{1030}/A_{1080} and A_{1010}/A_{1025} in the spectra of Ca and Mn salts, respectively) was in substantial agreement with the M/G ratio of the model mixtures. Commercial alginate samples with known M/G ratios showed good agreement with the calibration curve.

The FTIR technique reported in this study would appear to offer a simplified estimation method of the M/G ratio of alginate.

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