ELSEVIER

S0032-3861(96)00458-2

*Polymer* Vol. 38 No. 1, pp. 43–51, 1997

0032-3861/97/$17.00 + 0.00

# Determination of the cation content of alginate thin films by *FT*i.r. spectroscopy

**Céline Sartori, Dudley S. Finch* and Brian Ralph**
*Department of Materials Engineering, Brunel University, Uxbridge, Middx UB8 3PH, UK*

**and Keith Gilding**
*Innovative Technologies Ltd, Road Three, Winsford Industrial Estate, Winsford, Cheshire CW7 3PD, UK*
*(Received 27 June 1995; revised 19 January 1996)*

Different sodium alginates derived from *Laminaria hyperborea*, with various mannuronate/guluronate (M/G) ratios and molecular weights, were immersed in calcium chloride solution and converted to mixed sodium/calcium salts. Thin films were obtained and studied using Fourier transform infrared spectroscopy (*FT*i.r.). *FT*i.r. spectra were recorded as a function of time and the ion exchange between sodium and calcium monitored. Peak shifts, difference in peak shapes and the appearance of new peaks were observed, and some explanations of the phenomena observed are proposed. In parallel, the samples were analysed quantitatively by atomic absorption/emission spectroscopy. From these results, peak wavenumbers derived from the *FT*i.r. spectra could be correlated quantitatively to the sodium and calcium content. Copyright © 1996 Elsevier Science Ltd.

**(Keywords: alginate; ion content; i.r. spectroscopy)**

## INTRODUCTION

Alginates are polysaccharides derived mainly from brown seaweed. They form a family of linear copolymers of $\beta$-D-mannuronate (M) and $\alpha$-L-guluronate (G), linked together by $-\beta$ 1,4 and $-\alpha$ 1,4 glycosidic bonds, with varying proportions and sequential arrangements of M and G along the chain (MM, GG or MG)[1,2] (see *Figure 1*). D-Mannuronate is in the $^4C_1$ conformation while L-guluronate is in the $^1C_4$ conformation, independently of their nearest neighbouring unit.

Alginates are well known for their ability to form gels with divalent cations such as $Ca^{2+}$ [2–4], and they have found widespread application in the food and pharmaceutical industries. The high absorbency and the haemostatic properties of alginates make them of particular use for wound dressings.

Calcium alginate is insoluble in water, whereas sodium alginate is very soluble; thus mixed salts can be prepared to obtain the desired property for any particular application. The determination of sodium and calcium content in an alginate is therefore of prime importance in order to quantify the ion exchange process.

One technique to follow the ion exchange process is infrared spectroscopy, but published work to date is limited. Important contributions include the work of Aspinall[5] who has reported the typical i.r. frequencies of functional groups commonly encountered in polysaccharides. Additional work by Mackie[6], and Filippov and Kohn[7] have shown that the M/G ratio of alginates can be calculated from i.r. spectra. Dupuy *et al.*[8] have given assignments of i.r. absorption bands for sodium alginate and have compared the infrared spectra of sodium and calcium alginate salts. They also report peak positions for high M and high G calcium alginates.

The aim of the present paper is to go beyond work published to date. The authors have tried to get a better understanding of the change in binding associated with alginate when monovalent $Na^+$ cations are exchanged with divalent cations such as $Ca^{2+}$. An attempt has been made to correlate the amount of sodium and calcium present in different alginates (by varying the M/G ratio, the molecular weight and the degree of conversion of sodium into calcium salt) with their respective infrared spectra.

## EXPERIMENTAL

The raw materials were *Laminaria hyperborea* sodium alginates, provided by Pronova Biopolymers Ltd (Norway).

The samples are referred to as:

- LF 10/60 (high G, high $\bar{M}_w$),
- LF 10/60 LS (medium G, high $\bar{M}_w$),
- LFR 5/60 (high G, low $\bar{M}_w$) and
- LFR 5/60 RB (medium G, low $\bar{M}_w$),

where G refers to guluronic acid, and $\bar{M}_w$ to weight average molecular weight.

The molecular weight ranges were given by Pronova Biopolymers and are shown in *Table 1*. They were calculated from intrinsic viscosity measurements using the Mark–Houwink–Sakurada equation[9].

* To whom correspondence should be addressed

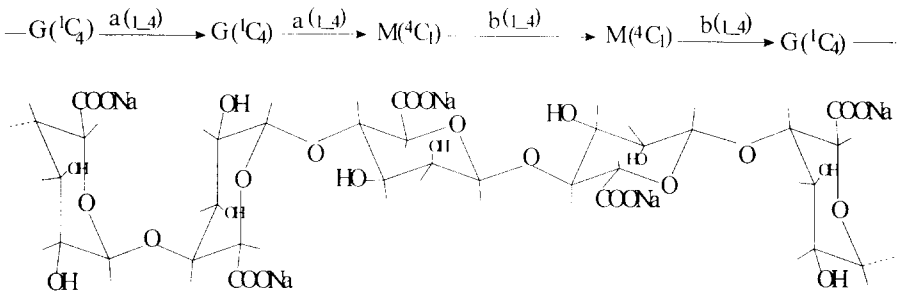

**Figure 1** Chemical structure of sodium alginate

**Table 1** Quantitative analysis of the chemical composition of alginates as a function of M/G ratio, molecular weight and immersion time in calcium chloride solution

| Alginate | M/G ratio | $M_w$ (g mol$^{-1}$) | Immersion time | Na (wt%) | Ca (wt%) | Na (at%) | Ca (at%) |
|---|---|---|---|---|---|---|---|
| high $\bar{M}_w$, high G | 32/68 | 60 000–00 000 | 0 | 9.3 | 0.4 | 5.0 | 0.1 |
| (LF 10/60) | | | 30 s | 3.1 | 6.7 | 1.7 | 2.1 |
| | | | 3 min | 2.7 | 8.8 | 1.5 | 2.8 |
| | | | 30 min | 0.4 | 12.4 | 0.2 | 4.1 |
| | | | 300 min | 0.2 | 12.9 | 0.1 | 4.3 |
| low $\bar{M}_w$, high G | 30/70 | 35 000–40 000 | 0 | 9.4 | 0.5 | 5.1 | 0.2 |
| (LFR 5/60) | | | 30 s | 2.5 | 7.8 | 1.4 | 2.5 |
| | | | 3 min | 2.1 | 10.6 | 1.2 | 3.5 |
| | | | 30 min | 0.3 | 12.4 | 0.2 | 4.1 |
| | | | 300 min | 0.2 | 13.1 | 0.1 | 4.3 |
| high $\bar{M}_w$, medium G | 52/48 | 60 000–70 000 | 0 | 9.3 | 0.3 | 5.0 | 0.1 |
| (LF 10/60 LS) | | | 30 s | 2.8 | 7.0 | 1.5 | 2.2 |
| | | | 3 min | 1.0 | 9.8 | 0.6 | 3.1 |
| | | | 30 min | 0.1 | 12.0 | 0.1 | 3.9 |
| | | | 300 min | 0.1 | 11.3 | 0.1 | 3.7 |
| low $M_w$, medium G | 50/50 | 35 000–40 000 | 0 | 9.6 | 0.4 | 5.2 | 0.1 |
| (LFR 5/60 RB) | | | 30 s | 2.5 | 7.6 | 1.4 | 2.4 |
| | | | 3 min | 1.7 | 9.7 | 1.0 | 3.1 |
| | | | 30 min | 0.6 | 11.4 | 0.3 | 3.7 |
| | | | 300 min | 0.1 | 12.0 | 0.1 | 3.9 |

The M/G ratios of the different samples were estimated using the method of Filippov and Kohn[7] by determining the ratio of absorbances of the bands at 1320 and 1290 cm$^{-1}$ in the *FT*i.r. spectra.

The sample preparation technique for thin alginate films was as follows: 1 wt% of sodium alginate powder was dissolved in deionized distilled water and the resulting solution poured into a polystyrene petri dish. This was then dried in an oven at 50°C for 2 h to produce a sodium alginate film. To obtain sodium/calcium alginate samples, the petri dish with the alginate solution was immersed in a calcium chloride bath (0.8 wt% $CaCl_2$ dissolved in deionized distilled water) for different lengths of time (30 s, 3, 30 and 300 min). As the two solutions come into contact with each other, an alginate gel is formed at the interface. Following ion exchange, each sample was washed for 3 min in deionized distilled water. Occasional stirring was performed in order to remove excess calcium. The samples were dried in an oven at 50°C for 2 h. The thicknesses of the films obtained were typically 10–20 $\mu$m.

Alternative methods have been used by other workers to prepare alginate gels. These include *in situ* gelation[10] where a defined supply of calcium ions is mixed with an alginate solution into which the calcium ions are released. Another technique is to cross-link the alginate using epichlorohydrin[11–13]. In the present study, we have favoured the method whereby the calcium ions are diffused in the alginate solution from a calcium chloride solution. This is more applicable to our studies since we are attempting to relate our results to alginate fibres for wound dressing applications which are routinely prepared in this manner.

Chemical analysis was carried out at Rooney Laboratories (Basingstoke, UK) on a Shandon Southern A4400 Atomic Absorption Spectrophotometer, in the emission mode for the detection of sodium, and in the absorption mode for calcium, using an air–acetylene flame. The results were obtained in weight percentages. The standard error in measured ion content is typically ±2–3%.

Infrared experiments were performed in transmission using a Nicolet 710 FTi.r. spectrometer. The spectra were averaged over 128 scans at a resolution of 4 cm$^{-1}$. Each reported wavenumber value is a mean result taken from four different samples.

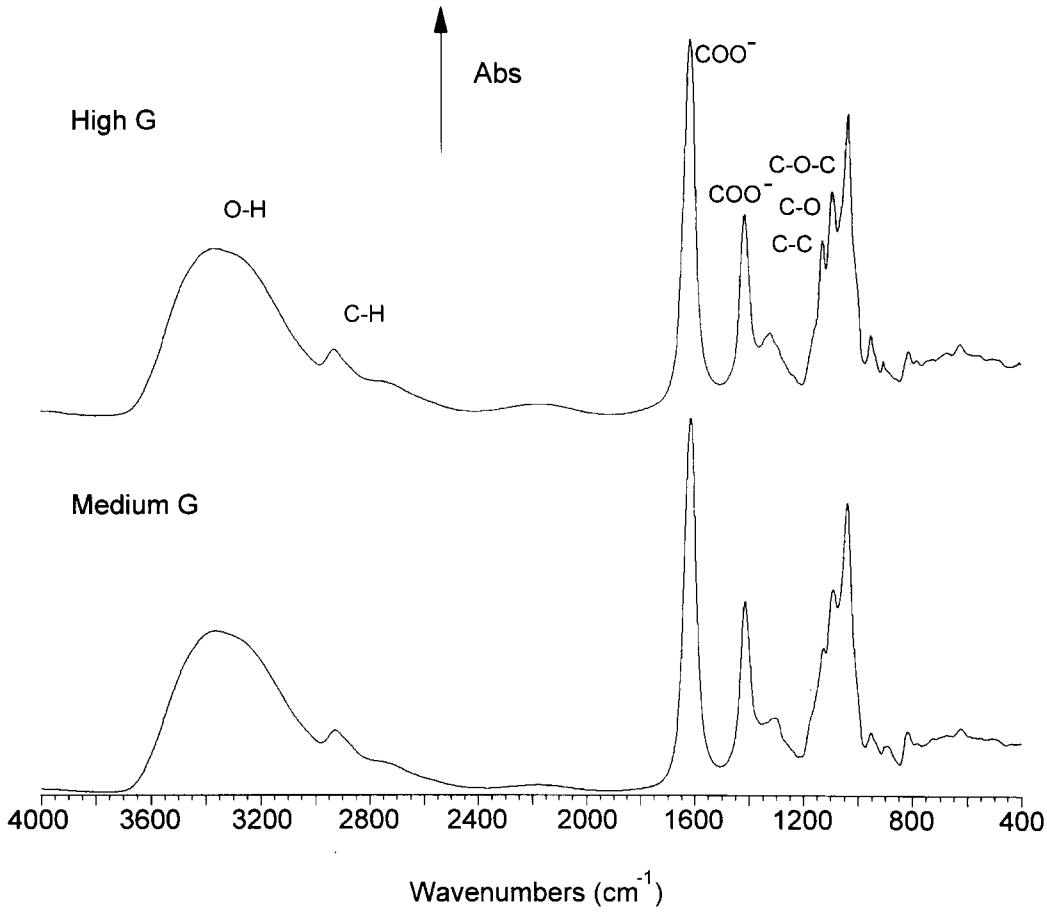


**Figure 2** *FT*i.r. spectrum of sodium alginates

## RESULTS AND DISCUSSION

### *Chemical composition*

The samples as cast contained typically 20 wt% of water (as measured by thermogravimetric analysis). Samples were analysed quantitatively for the weight percentage of sodium and calcium by atomic absorption/emission spectroscopy. These percentages are shown in *Table 1* along with theoretically calculated atomic percentages (see Appendix). Only sodium and calcium ions were assumed to be present; however, more recent studies by us suggest that chloride ions are also present.

With immersion of sodium alginate solution in a calcium chloride solution, a rapid ion exchange process takes place. More than half the ion conversion between sodium and calcium occurs within the first 30 s. Within the time scales studied, it appears that there is an initial faster rate of ion conversion with the lower molecular weight samples (LFR 5/60 and LFR 5/60 RB) which one would expect.

At 'full ion conversion' (after 300 min in $CaCl_2$), high G samples (LF 10/60 and LFR 5/60) exhibit a higher calcium content (around 4.3 at%) than medium G samples (3.8 at%). This is consistent with previous published work showing that calcium ions bind preferentially to G blocks[2–4].

Before immersion in $CaCl_2$ solution, the sodium content is close to 5 at% which suggests all potential sites for $Na^+$ are filled. After 30 s in calcium chloride solution, there is an exchange of approximately 2 $Na^+$ by 1 $Ca^{2+}$, which is the expected ion exchange between

**Table 2** Assignments of i.r. absorption bands for sodium alginate

| Wavenumber ($cm^{-1}$) | Intensity–shape | Assignment |
|---|---|---|
| 3360–3380 | very strong–broad | O–H stretching |
| 3250 | strong–shoulder | O–H stretching |
| 2930–2932 | weak–broad | C–H stretching |
| 2750 | weak–shoulder | C–H stretching |
| 1608–1611 | very strong–sharp | $COO^-$ stretching (asymmetric) |
| 1413–1414 | medium–sharp | $COO^-$ stretching (asymmetric) |
| 1317 | weak–broad | C–O stretching |
| 1294 | weak–shoulder | C–O stretching |
| 1176 | weak–shoulder | C–O stretching<br>C–C stretching<br>C–C–C bending |
| 1124–1126 | medium–sharp | C–C stretching<br>C–O stretching |
| 1087–1088 | medium–sharp | C–O stretching<br>C–O–C stretching |
| 1059 | medium–shoulder | O–H bending |
| 1030–1035 | very strong–sharp | ? |
| 997 | medium–shoulder | ? |
| 947–950 | weak–sharp | C–O stretching<br>C–C–H stretching |
| 903 | weak–sharp | ? |
| 892 | weak–shoulder | C–C stretching<br>C–C–H bending<br>C–O bending |
| 818 | weak–broad | ? |
| 781 | weak–broad | C–O internal rotation<br>C–C–O bending<br>C–C–H bending |

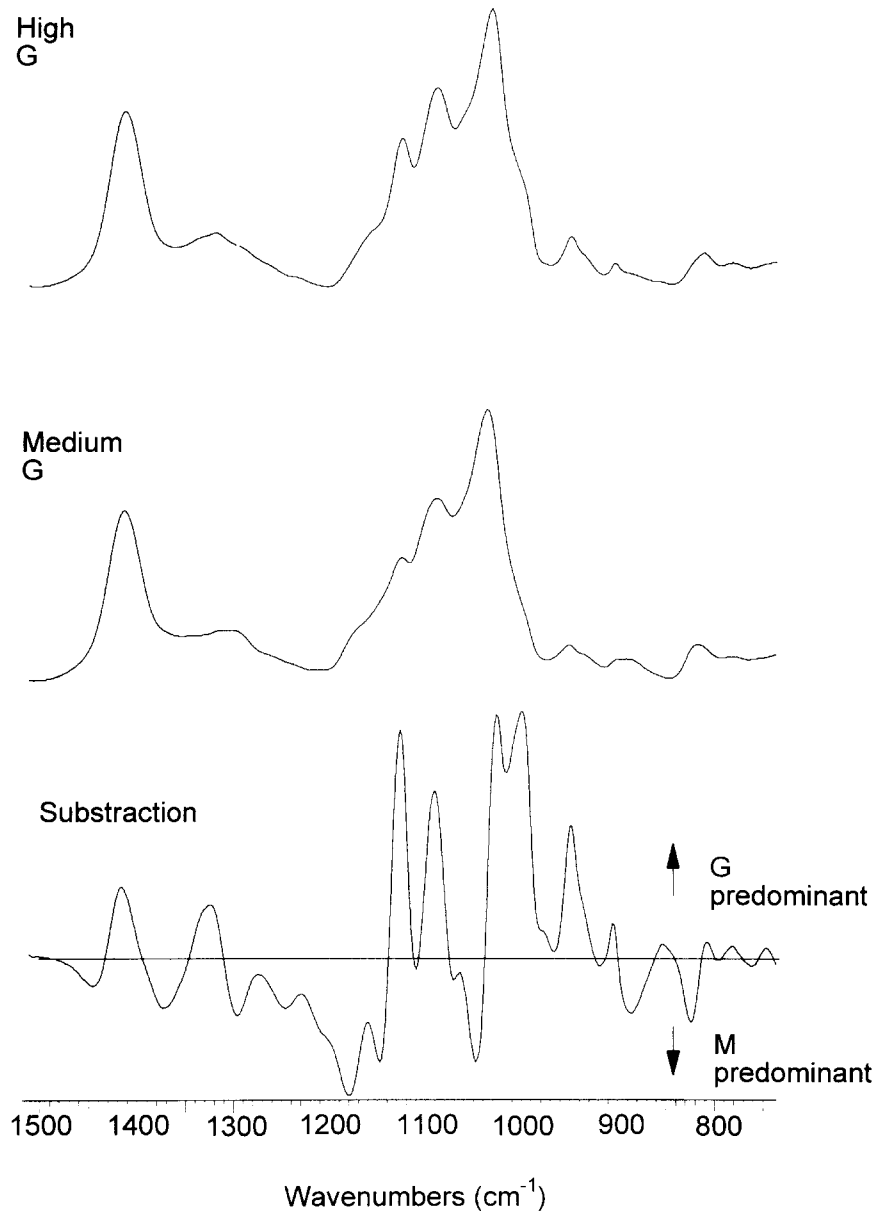


**Figure 3** Fingerprint region of sodium alginate spectra

a monovalent and a divalent cation. However, at longer immersion times in $CaCl_2$, there is a greater ion exchange, leading to an average of 4 at% of calcium ions. The excess positive charge due to $Ca^{2+}$ must be balanced; we strongly suspect this is achieved by retention of chloride ions in the alginate.

FT*i.r. spectroscopy*

*Sodium alginate spectra.* Typical spectra as a function of M/G ratio are shown in *Figure 2*. With reference to previous studies on the i.r. spectra of polysaccharides[5], and more particularly on alginates[8], *Table 2* gives detailed absorption band assignments for sodium alginate.

As previously observed[6,7], the i.r. spectra of alginates containing different M/G ratios show some variation in relative band intensities.

*Figure 3* shows the fingerprint region and difference spectrum ($COO^-$ asymmetric stretching peak around 1610 $cm^{-1}$ was used as reference) from *Figure 2*. For the high G alginate (LF 10/60 or LFR 5/60), the bands or shoulders around 1410, 1320, 1130, 1090, 1020, 1000 and 950 $cm^{-1}$ are of greater intensity than for the medium G alginate (LF 10/60 LS or LFR 5/60 RB). By contrast, the bands or shoulders at 1440, 1370, 1300, 1250, 1180, 1150, 1050, 890 and 820 $cm^{-1}$ are most pronounced for the medium G alginate. Therefore, the first set of wavenumbers is characteristic of guluronate whilst the second set is typical of mannuronate. No obvious differences could be observed in the i.r. spectra as a function of molecular weight.

*Effect of sodium/calcium ion exchange on i.r. alginate spectra*

*Alginate LFR 5/60.* The i.r. spectra of sodium alginate LFR 5/60 (high G, low $\bar{M}_w$) immersed for different times in calcium chloride solution are shown in *Figure 4*. For the same source of alginate, peak shifts,

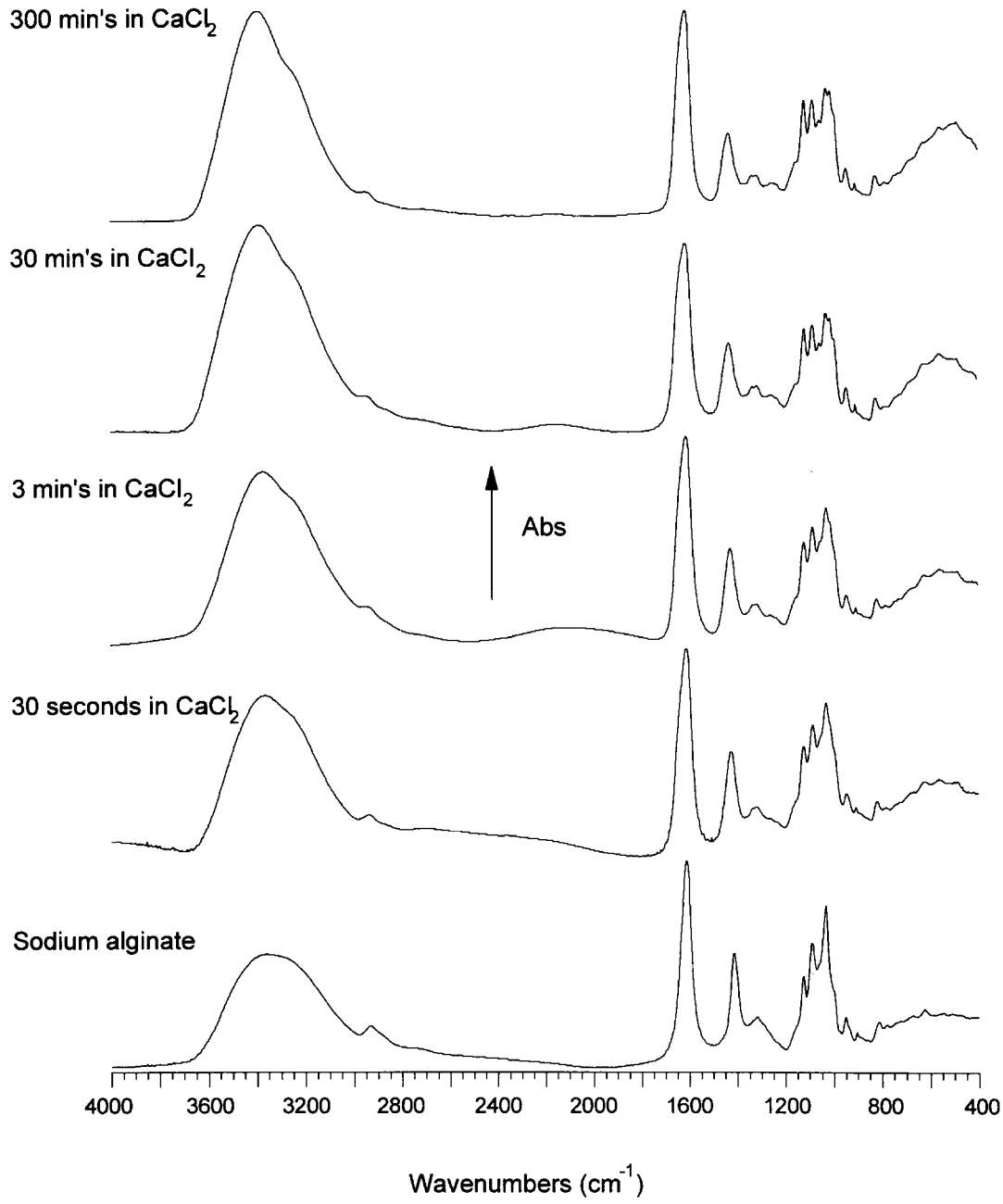


**Figure 4** *FT*i.r. spectra of sodium alginate LFR 5/60 immersed for different lengths of time in calcium chloride solution

differences in peak shapes and the appearance of new bands were observed as the calcium content increased. Therefore, *FT*i.r. alginate spectra can give direct information on the ion exchange process. Only the major peak variations are described below.

As the immersion time in $CaCl_2$ solution increases, the O–H stretching peak ($\approx 3380\,cm^{-1}$) becomes narrower and of greater intensity. This is characteristic of an increase in intramolecular bonding. By contrast, the O–H shoulder (at $3250\,cm^{-1}$), corresponding to intermolecular binding, does not greatly change with $CaCl_2$ immersion time. Deconvolution and curve fitting of the O–H stretching region confirmed this observation.

The $COO^-$ peaks at $\approx 1610$ and $1420\,cm^{-1}$ (asymmetric and symmetric stretch, respectively) become broader with higher $Ca^{2+}$ percentage. The $COO^-$ symmetrical stretch peak (around $1420\,cm^{-1}$) exhibits a large shift to higher wavenumbers, as well as a decrease in intensity. The ion content as a function of wavenumber for this band is plotted in *Figure 5*. This peak is specific to ionic binding. As calcium ions replace sodium ions in the alginate blocks, the charge density, the radius and the atomic weight of the cation are changed, creating a new environment around the carbonyl group. Hence a peak shift would be expected.

In the region $1150-1000\,cm^{-1}$, one can observe a set of three sharp peaks. The first two peaks have been assigned to C–C and C–O stretching, however, no assignment is available for the third peak. All these peaks show shifts towards lower wavenumbers as the calcium content increases. The ion content as a function of wavenumber has not been plotted for reasons of brevity. The shift to lower frequencies is indicative of a weakening in the C–C and C–O bonds, most likely

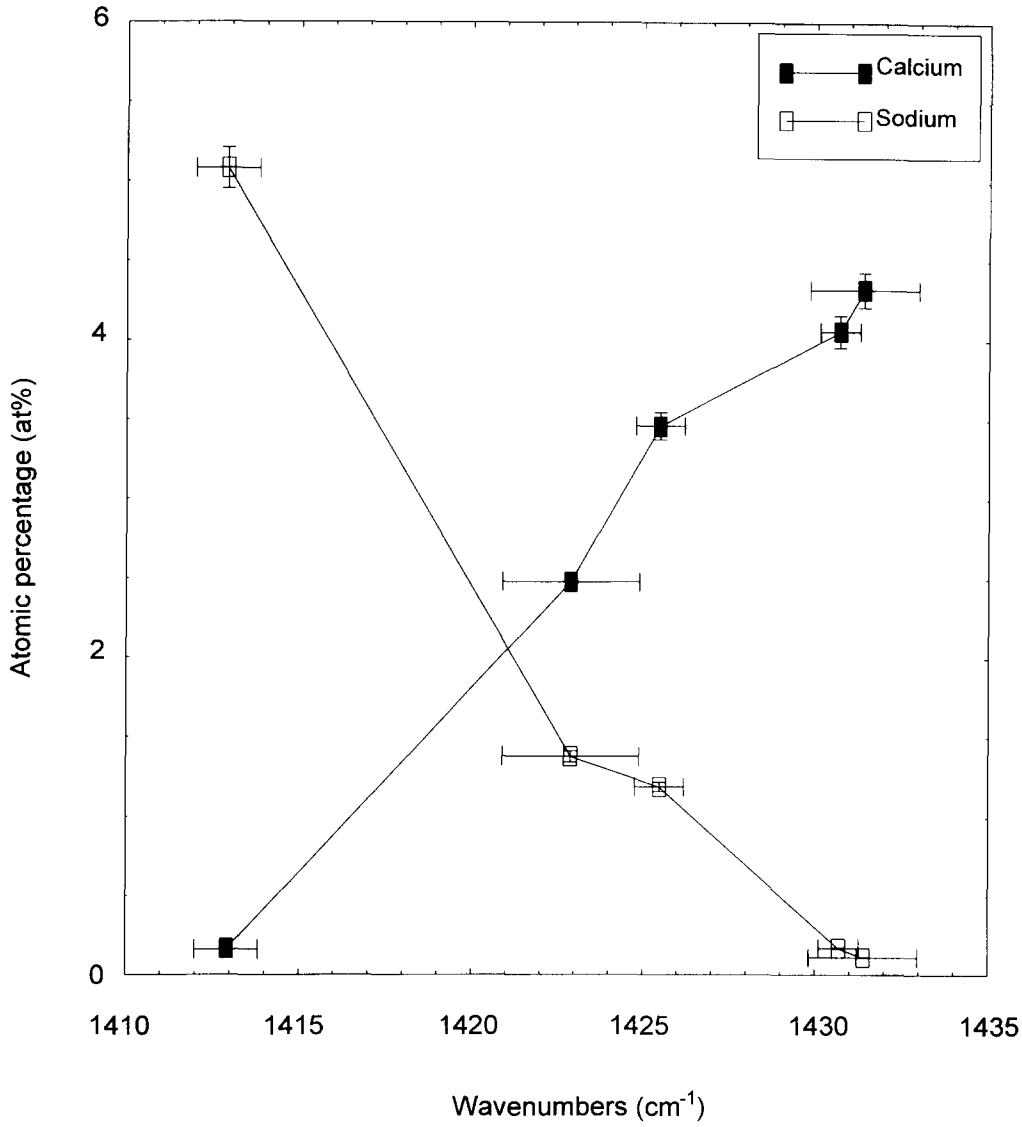


**Figure 5** Ion content as a function of wavenumber (around 1420 $cm^{-1}$) for LFR 5/60

due to these bonds being shared with the calcium ion. The ratio of peak intensities at 1088 and 1125 $cm^{-1}$ (peaks associated with the guluronic blocks) changes from 1.4 for pure sodium alginate to 1.0 for pure calcium alginate. This suggests a change in the binding within these blocks as calcium is introduced.

Taking the peak at 1030 $cm^{-1}$, the shoulders on each side become more pronounced, suggesting stronger O–H bending vibration and stronger binding of the calcium to the G blocks. There is also a new peak appearing at 1010 $cm^{-1}$ (see *Figure 6*). Peaks relating to metal–oxygen–metal bonds[14] are commonly observed in this region. This could, therefore, correspond to a partial covalent bonding between calcium and oxygen atoms. This is currently under further investigation using a wider range of cations.

*Alginates LF 10/60, LF 10/60 LS and LFR 5/60 RB.* Similar observations in terms of peak shifts and band broadening have been made for alginates LF 10/60, LFR 5/60 and LFR 5/60 RB. The variations in ion content as a function of peak shift for the band centred at 1420 $cm^{-1}$ are presented in *Figure 7*. The fingerprint region for high and medium G alginate samples are immersion in $CaCl_2$ solution for 300 min is shown in *Figure 8*.

As alginate samples are converted from pure sodium to pure calcium types, the ratio between the absorbance at 1088 and 1125 $cm^{-1}$ decreases from 1.4 down to 1.0 for high G samples, and from 1.5 down to 1.3 for medium G samples. Therefore, the decrease in absorbance ratio is less pronounced for LF 10/60 LS and LFR 5/60 RB. The new peak around 1010 $cm^{-1}$ appearing for the high G samples is less prominent for the medium G samples. This peak is most likely associated with calcium binding to G blocks.

With reference to the peak at around 1030 $cm^{-1}$, the shift in wavenumber varies from 1030 to 1025 $cm^{-1}$ for high G samples, and from 1035 to 1030 $cm^{-1}$ for medium G alginates. This again reinforces the observation that calcium is bound differently within the M and G blocks.

Other changes in the *FT*i.r. spectra can be observed (e.g. the peaks at 1300 and 1315 $cm^{-1}$ become more pronounced with a greater immersion time in $CaCl_2$ solution, and there is a small peak appearing at around 970 $cm^{-1}$ for medium G samples) all of which are clearly

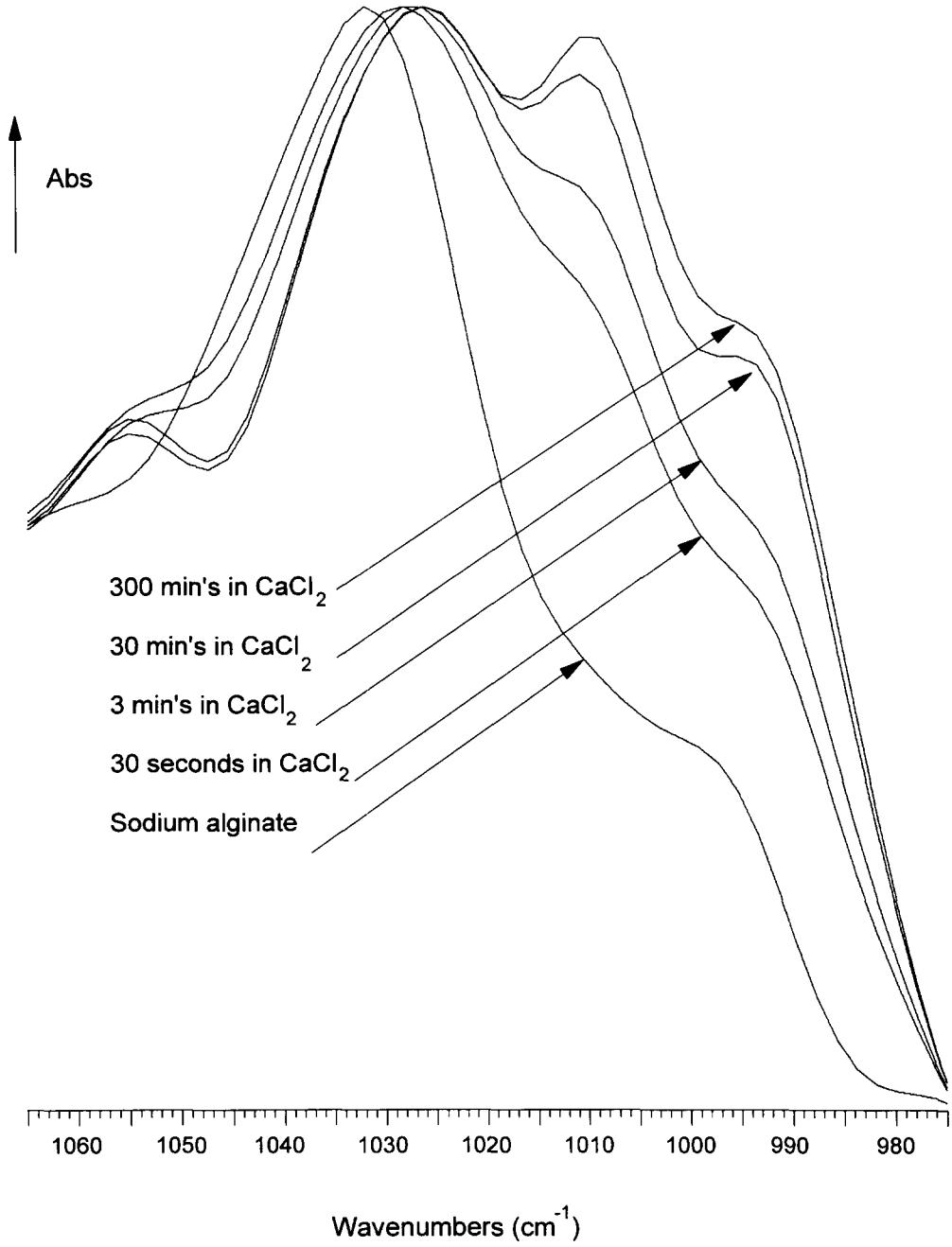


**Figure 6** Fingerprint region of sodium alginate LFR 5/60 immersed for different lengths of time in $CaCl_2$ solution

related to the interaction between the calcium ion and specific binding sites.

## CONCLUSIONS

This work has shown that significant changes can be observed in i.r. spectra for alginates (peak shapes as well as band shifts), as a function of immersion time in calcium choride solution. The exchange of sodium for calcium changes the local binding between the ion and the polysaccharide chain. Evidence appears to show a change from 'pure ionic bonding' involving $COO^-$ groups to a mixture of ionic and covalent bonding involving other functional groups (C–O and C–C in particular). It is shown that preferential binding to G blocks occurs which is consistent with previously published work.

From a careful study of these i.r. spectra, and from the use of atomic absorption/emission spectroscopy, a quantitative correlation has been shown between ion content and peak shifts. The ability to quantify ion content from the i.r. spectrum should provide a valuable tool for analysing unknown sodium/calcium contents. This is an important property since the sodium and calcium percentages will strongly influence the swelling and healing properties of the alginate.

## ACKNOWLEDGEMENTS

The authors wish to thank Dr V. Self and Dr D. Waters, Chemistry Department, Brunel University, for helpful discussion and valuable comments, as well as Mr M. Beth for his calculations.

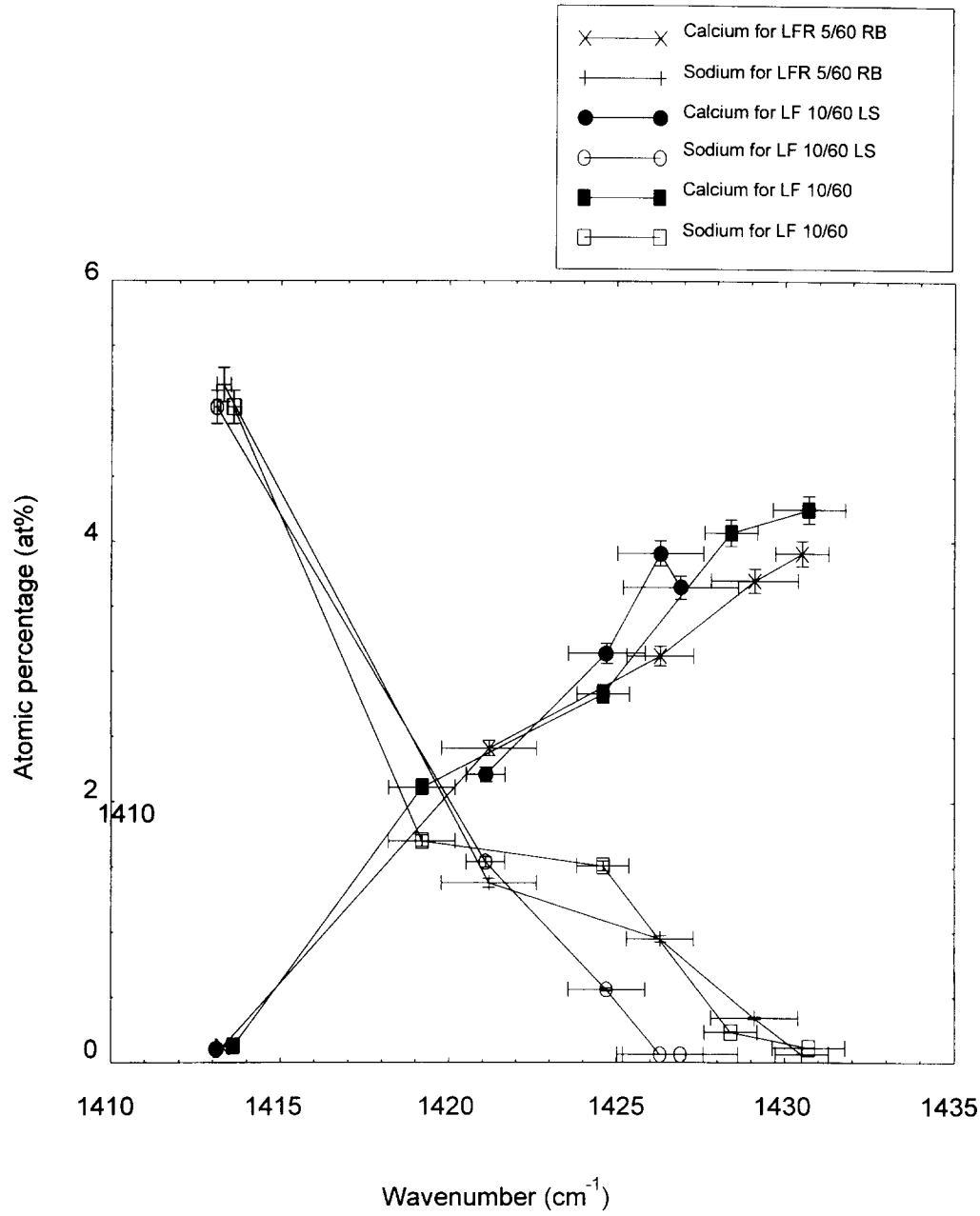


**Figure 7** Ion content as a function of wavenumber (around 1420 $cm^{-1}$)

## REFERENCES


1 Skjåk-Bræk, G. *Biochem. Soc. Trans.* 1992, **20**, 27
2 Gacesa, P. *Carbohydr. Polym.* 1988, **8**, 161
3 Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C. and Thom, D. *FEBS Lett.* 1973, **32**, 195
4 Bryce, T. A., McKinnon, A. A., Morris, E. R., Rees, D. A. and Thom, D. *Faraday Discuss. Chem. Soc.* 1974, **57**, 221
5 G.O. Aspinall (Ed.) 'The Polysaccharides', Vol. 1, Academic Press, New York, 1982
6 Mackie, W. *Carbohydr. Res.* 1971, **20**, 413
7 Filippov, M. P. and Kohn, R. *Chem. Zvesti* 1974, **28**, 817
8 Dupuy, B., Arien, A. and Perrot Minnot, A. *Art. Cells, Blood Subs., Immob. Biotechnol.* 1994, **22**, 71
9 Martinsen, A., Skjåk-Bræk, G. and Smidsrød, O. *Carbohydr. Polym.* 1991, **15**, 171
10 Draget, K. I., Ostgaard, K. and Smidsrød, O. *Carbohydr. Res.* 1991, **14**, 159
11 Moe, S. T., Elgsaeter, A., Skjåk-Bræk, G. and Smidsrød, O. *Carbohydr. Polym.* 1993, **20**, 263.
12 Moe, S. T., Skjåk-Bræk, G., Elgsaeter, A. and Smidsrød, O. *Macromolecules* 1993, **26**, 3589
13 Moe, S. T., Skjåk-Bræk, G. and Smidsrød, O. *Food Hydrocolloids* 1991, **5**, 119
14 Perry, C. C., Li, X. and Waters, D. N. *Spectrochim. Acta* 1991, **47A**, 1487


## APPENDIX: DETAILED CALCULATION OF ATOMIC PERCENTAGE

For each M or G block, consider there is on average '$x$' atoms of sodium and '$y$' atoms of calcium. The molecular weight of sodium is 23 $g\,mol^{-1}$, and that of calcium is 40 $g\,mol^{-1}$. Theoretically, there are six carbon atoms, six oxygen atoms, seven hydrogen atoms and one ion site per G or M block, giving a molecular weight of 175 g, excluding the mass of the ion(s). However, because alginate is a natural polymer, the M and G block are irregular, and the molecular weight is in practice closer to 200 (again excluding the mass of the ion).

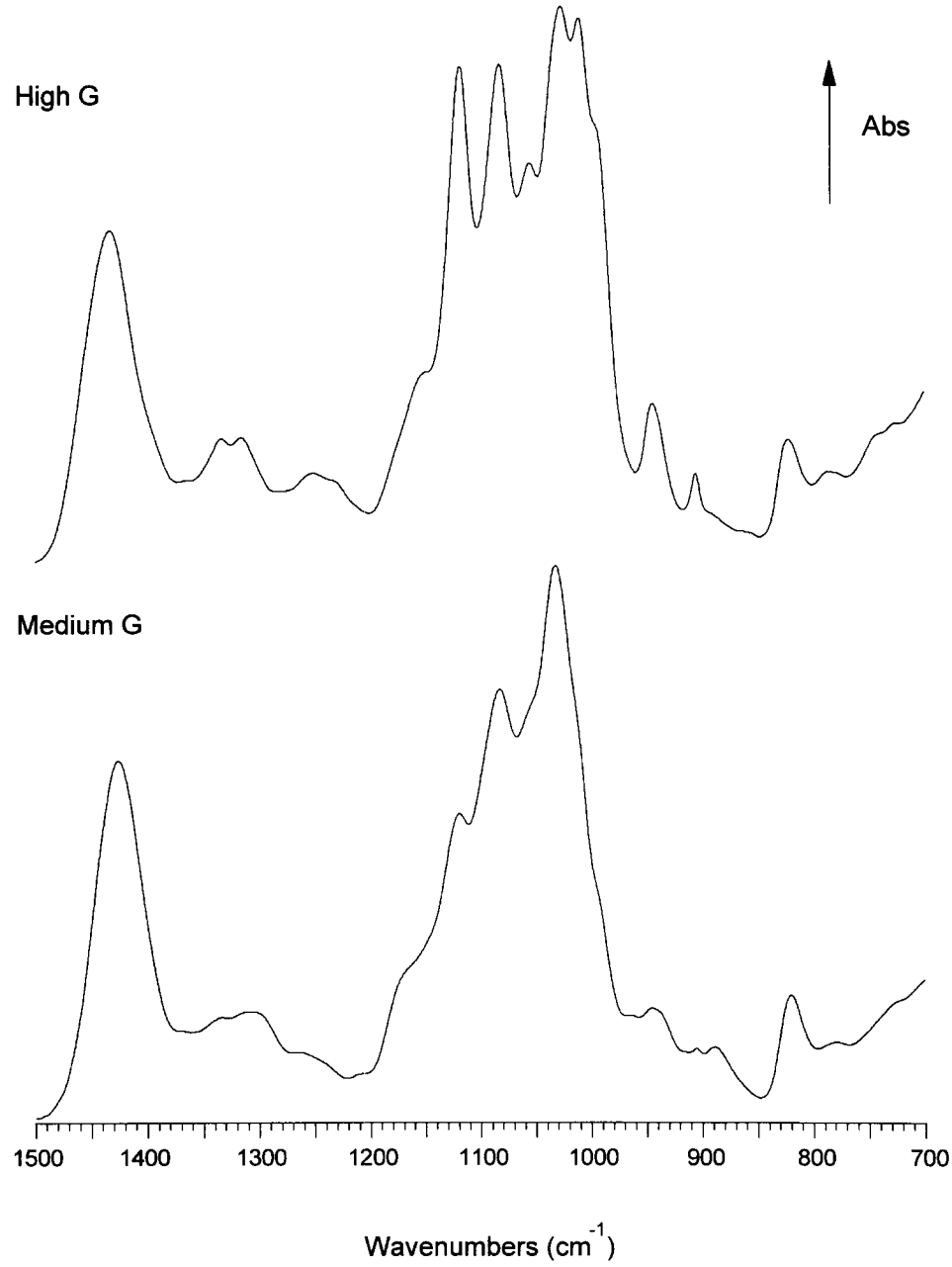


**Figure 8** Fingerprint region of sodium alginates immersed for 300 min in $CaCl_2$ solution

Let us call 'Na' the experimental weight percentage of sodium and 'Ca' the experimental weight percentage of calcium. The dry matter is equivalent to 80% of the total weight.

For a particular alginate, after a given immersion time in $CaCl_2$ solution, Na and Ca are given by:

$$\mathrm{Na} = (0.8 * 23x * 100)/(200 + 23x + 40y) \quad \text{and}$$

$$\mathrm{Ca} = (0.8 * 40y * 100)/(200 + 23x + 40y)$$

which rearranged gives:

$$y = 36.8\mathrm{Ca}/(588.8 - 7.36\mathrm{Ca} - 7.36\mathrm{Na}) \quad \text{and}$$

$$x = (32y - 0.4y\mathrm{Ca} - 2\mathrm{Ca})/0.23\mathrm{Ca}$$

The atomic percentage of sodium and calcium are then given by:

$$\text{Sodium At\%} = 100x/(x + y + (19 * 200/175)) \quad \text{and}$$

$$\text{Calcium At\%} = 100y/(x + y + (19 * 200/175))$$