J. Pharm. Pharmacol. 1997, 49: 639–643
Received January 7, 1997
Accepted February 17, 1997

Characterization of the Block Structure and Molecular Weight of Sodium Alginates

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Abstract

Sodium alginates are widely used within the pharmaceutical sciences, yet the molecular characteristics of these materials are frequently not stated. In this study, a range of characterization techniques is applied to five sodium alginate samples and the data compared, both between techniques and with the information obtained from the manufacturer.

The mannuronic acid to guluronic acid (MG) ratio and the distribution of uronic acid residues of five sodium alginate samples have been measured using circular dichroism and NMR, with circular dichroism yielding MG ratios between 42·1 and 63·6%, depending on the grade of alginate used. The MG ratios obtained from NMR studies were in broad agreement with these values, and the technique also yielded information on the distribution of uronic acid residues within each batch; this was again found to vary considerably ($NG > 1$ values ranging from 6·9 to 17·5). It was noted that samples with similar MG ratios could have markedly different chain-distribution characteristics. The uronic acid ratio ranges obtained from the manufacturers were found to be in good agreement with those found experimentally. Intrinsic viscosity measurements were used to compare the molecular weights of the samples; values between approximately 12000 and 180000 were obtained for the different batches.

The study has enabled comparison of different methods for characterization of sodium alginate samples, highlighting their relative merits and the possible protocols that might be adopted. A critical discussion is given of the individual and combined use of these techniques and the relevance of such studies to the rational design and quality control of alginate-based pharmaceutical systems.

Alginates are polysaccharides usually derived from brown algae, in which they occur as gels with sodium, calcium, strontium, magnesium and barium ions (Haug & Smidsrød 1967). In addition, alginates are present as capsular polysaccharides in soil bacteria. The function of alginates in algae is thought to be primarily skeletal, with the gel located in the cell walls and intercellular matrix conferring the strength and flexibility necessary to withstand the force of the water in which the seaweed grows. These materials have found numerous applications in the pharmaceutical sciences, including raft-forming systems, controlled-release systems, bioadhesive systems, tablet disintegrants, suspending agents, wound dressings and implants.

Alginic acid comprises D-mannuronic (M) and L-guluronic acid (G) residues joined linearly by 1,4-glycosidic linkages. The ratio of the two residues can vary greatly and depends on numerous factors associated with the biosynthesis of the alginate. For example, in a report for the Norwegian Institute of Seaweed Research, Haug (1964) presented data for the MG ratio of alginates from various species. These results were broken down to give the MG ratio for various parts of the plants (whole plants, old fronds, new fronds, and stipes) and details of the date and place of harvesting. Seasonal variation was found, especially in Laminaria sp. where the alginates extracted contained a higher proportion of mannuronic acid in the summer.

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The wide pharmaceutical applicability of alginates is to a large extent associated with their gel-forming capacity. Alginate gels are composed of a three-dimensional network of long-chain molecules (Ross-Murphy 1991) held together at junction zones, typically considered to be guluronic acid blocks. The formation of junction zones in an alginate solution might be promoted in one of two ways: either by the presence of certain divalent cations such as calcium, or by the acidification of the solution. The presence of calcium is believed to result in the formation of an 'egg-box' structure, with Ca^{+} ions forming junctions between blocks of guluronic acid residues on adjacent chains (Grant et al 1973). Given the relationship between the gelling properties of alginates and their chemical composition, there is a clear need to consider the molecular characteristics of the alginates used in pharmaceutical products. Numerous workers have demonstrated that both the ratio and block structure of the two residues within a sample can have a profound effect on the physical properties of the alginate, particularly in terms of the tendency to bind with divalent cations (calcium being the most widely studied example) and the rheological properties of the corresponding gels (McDowell 1986; Haug & Smidsrød 1965, 1968; Grant et al 1973). However, the molecular structures of the alginates used in pharmaceutical studies are rarely specified, leading to difficulties in establishing general patterns of system behaviour and source-dependence of alginate products. The potential for product variation was exemplified in a study by Washington et al (1986), who demonstrated that alginate rafts formed from Gaviscon formulations produced from various sources had widely differing properties. In view of the variation in the

chemical structure of alginates, such difficulties will almost inevitably occur with all alginate-based dosage forms if the properties of the raw material are not characterized. In this study the molecular characteristics of a range of alginates have been studied by three methods, circular dichroism, NMR and viscosity measurement. Although these methods have previously been used on an individual basis, it is of interest to compare their relative merits using a series of alginate samples in order to facilitate the establishment of protocols for the quality-control of alginates for use in pharmaceutical systems.

Materials and Methods

Materials

The alginate samples used were obtained from Pronova Biopolymers, Drammen, Norway. Five samples (LFR 5/60, LF 120M, LF 10/40RB, LF 20/200, LF 200DL) were used as received. These five samples were chosen because they reflect a range of characteristics, including varied composition and viscosity. In particular, whereas four are classified as medium-viscosity products, LFR 5/60 is a low viscosity grade.

Circular dichroism

Sodium alginate solutions (3 mg mL^{-1}) were prepared in deionized water and were adjusted to pH 7 by the addition of 1 M sodium hydroxide. The circular dichroism (CD) spectra were obtained by use of a Jasco J600 spectropolarimeter equipped with a thermostatted cell holder which maintained the sample temperature at 25°C. The wavelength-region between 185 and 260 nm was scanned because this was the region of interest identified by Morris et al (1975). The values for the peak/trough ratio were calculated from the spectra and from these values the relative amounts of mannuronic and guluronic acid residues were calculated with reference to the calibration values given by Morris et al (1975).

Nuclear magnetic resonance

At the concentrations convenient for ^1H NMR analysis, alginate solutions are reasonably viscous which will cause line-broadening in the NMR spectrum. To reduce the viscosity it is necessary to perform a mild partial hydrolysis of the alginate samples before analysis. The method outlined below is based on the work of Haug et al (1966, 1967) and Grasdalen et al (1979), although a novel adaptation of the established hydrolysis protocol has been used here.

The sodium alginate samples (200 mg) were weighed into a 250-mL round-bottomed, two-necked flask containing distilled water (100 mL) and the sodium alginate was partially dissolved by shaking vigorously for 2 min. The pH was adjusted to 5·2 by dropwise addition of either 1 M sodium hydroxide or 1 M hydrochloric acid and the suspension was again shaken for 2 min to dissolve the remaining sodium alginate. The solution temperature was then increased to boiling point and left to boil under reflux, under a nitrogen atmosphere, for 15 min. After cooling to room temperature (21°C) the pH of the solution was adjusted to 3·6 with 0·05 M sulphuric acid; it was then heated under reflux, again under nitrogen, for a further 25 min. After cooling to room temperature the solution was divided into two portions which were transferred to stoppered round-bottomed flasks and freeze-dried overnight.

The freeze-dried solid was then prepared for analysis. The contents of one of the round-bottomed flasks, containing approximately 100 mg freeze-dried solid, were dissolved in 5·0 mL deuterium oxide (99·8% D or better) containing 0·5% w/w trimethylsilyl-3-propionic acid d4-2,2,3,3, sodium salt (TSP) by warming gently as necessary. Saturated disodium EDTA solution (0·3 M; 120 μL) was added to complex any di- and trivalent cations present and the solution pH was adjusted to 4·2 by use of approximately 1% w/w sodium deuteroxide solution.

A dummy sample tube containing 0·50 mL D_2O and TSP was placed in the magnet, the probe temperature was set to 90°C and left to stabilize for 40 min. After tuning for optimum resolution the NMR tube containing the alginate solution (0·50 mL) was placed in the probe and left for 20 min for the solution temperature to stabilize. After re-tuning of the spectrometer the proton spectrum was recorded. The baseline was corrected and the spectrum integrated at 400 MHz, at 90°C, using 300 pulses, a pulse angle of 28°, a spectral width of 12 ppm, and zero pulse delay.

Viscosity measurements

Flow-rheology measurements were made on the alginate solutions by means of a Carrimed CSL500 controlled-stress rheometer (TA Instruments, Surrey, UK). A range of alginate concentrations up to 2·5% w/v was used. The rheometer was used with cone and plate measuring geometry; the cone had a diameter of 4·0 cm and a cone angle of 2°. The temperature of the plates was maintained at 20°C by use of the Peltier temperature-control system housed in the rheometer. A sample (approximately 0·6 mL) was placed on the bottom plate by means of a syringe. Each solution was measured at least three times with the plates being cleaned and fresh sample loaded each time. For each sample, the strain was measured as the sample was subjected to a stress increasing from 0 to 15 N m^{-1} in 2 min.

Results and Discussion

Circular dichroism

The circular dichroism spectra for the five alginate samples are shown in Fig. 1. Circular dichroism is an optical technique which measures the absorption of polarized light as a function of wavelength. For the technique to be applicable to a particular system there must be a chiral molecule present which has a UV absorption in the range 190–750 nm. Morris et al (1975) showed that all D-uronic acid glycosides give a positive band at 212 nm as their principal spectral feature and that L-uronic acid glycosides give a corresponding negative band. This difference in sign is because the configuration of the C5 carbon atom of the sugar affects the position of the oxygen atom in the ring relative to the chromaphore (the carboxyl group in this case). The position of the hydroxy group on the carbon atom at position C4 on the ring is also close enough to the carboxyl group to interact with it directly. The difference in configuration at C5 results in the bands in the $n \rightarrow \pi^*$ region of the spectrum being of opposite sign. The opposite configuration of the monomers at C4 causes the principal bands of the spectrum to be offset, thus the positive and negative bands are clearly separated. These two configuration differences result in the $n \rightarrow \pi^*$ transitions occurring at approximately 212 and 200 nm, giving a trough and a peak, respectively. The relative

proportions of guluronic and mannuronic acid residues can be calculated from the ratio of the peak height (P) and trough depth (T) (Morris et al 1975). By measuring the peaks and troughs for each sample the P/T ratios were calculated for each alginate; the results are shown in Table 1. From these P/T ratios the percentage of guluronic acid residues present could be found by reference to the values provided by Morris et al (1975), whereby the P/T ratios were compared with the uronic acid composition; these values fit the equation:

$$\ln(P/T) = -0{\cdot}0387(\%\ \text{guluronic acid}) + 1{\cdot}1519\ (r = 0{\cdot}980) \quad (1)$$

The CD values were in broad agreement with the nominal values given by Pronova, although they were generally towards the lower limits quoted.

Nuclear magnetic resonance

Typical NMR spectra for LFR 5/60, LF 120M and LF 200DL are shown in Fig. 2. The NMR peak assignments for mannuronic and guluronic acid were proposed by Penman & Sanderson (1972) and Grasdalen et al (1979); their assignments were used in this study. It is also possible to detect peaks from two or three linked residues. The significance of this analysis is that the block structure, and the MG ratio, may be ascertained. The numerical data presented in Table 2 are the average values obtained from two separate hydrolyses for each of the five samples under investigation. For each of the samples the fraction of guluronic acid and mannuronic acid residues are given (as F(G) and F(M)). Also shown are the relative amounts of the individual diads and triads, i.e. fractions comprising two or three identical uronic acid residues. The average G block length for chain lengths greater than one unit ($N_{G>1}$) is also given. This is calculated as follows. If a sample has the structure:

MMGMGGMGGGMMGGMGGGMGGM

then the average (fractional) length of the G blocks is given by the fraction of G residues divided by the (fractional) number of G blocks. The number of G blocks will be given by the number of MG doublets, as each will represent the end of a chain, hence:

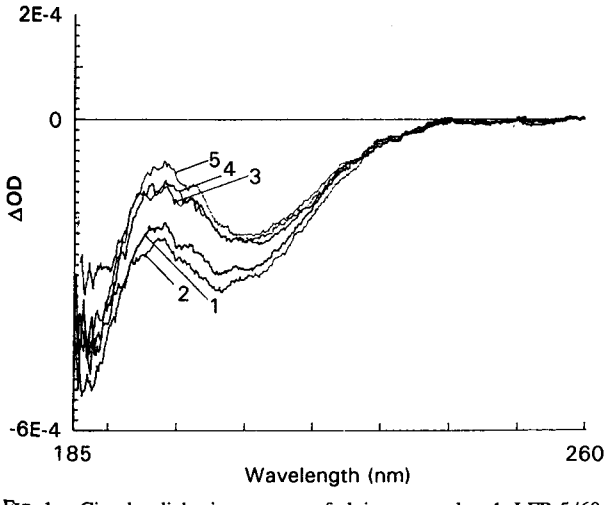

FIG. 1. Circular dichroism spectra of alginate samples: 1, LFR 5/60; 2, LF 20/200; 3, LF 200DL; 4, LF 10/40RB; 5, LF 120M.

Table 1. The amount of guluronic acid in alginate samples determined by circular dichroism compared with the nominal values provided by the manufacturer (Pronova).

Alginate sample	P/T ratio	Amount of guluronic acid	
		Circular dichroism	Manufacturer's values
LFR 5/60	0·32	59·2	65–75
LF 120M	0·62	42·1	35–45
LF 10/40RB	0·50	47·7	45–55
LF 20/200	0·27	63·6	65–75
LF 200DL	0·42	52·2	55–65

$$NG = F(G)/F(MG) \quad (2)$$

which gives an average block length of 2, with F(G) = 0·6 and F(MG) = 0·3. Hence to find the average block length for (G > 1), the number of residues in blocks > 1 is divided by the number of blocks of length > 1 G residues which is given by:

$$N_{G>1} = (F(G) - F(MGM))/F(GGM) \quad (3)$$

It should be noted that in the example given above, the assumption is made that the number of G blocks is given by MG and the number of blocks > 1 G residue will be given by GGM. This is true in the example given above but had the chain ended in, for example, MGGG, the last G block would not have been included. However, on chains the length of a typical alginate molecule such errors will be extremely small. This analysis is of considerable use as it enables comparison of the proportion of G ratios that are present as blocks; an equivalent expression is available for $N_{M>1}$. As the properties of the alginate may be highly dependent on the distribution of residues within the chain in addition to the absolute proportion of M and G residues, it is helpful to have a measure of this distribution. It is, for example, interesting to note that although the alginates LFR 5/60 and LF 20/200 have similar values of F(G) (0·672 and 0·670, respectively) the values of $N_{G>1}$ show considerably larger proportionate differences (17·5 and 13·6, respectively), suggesting that in LFR 5/60 the guluronic acid blocks are longer than in LF 20/200. As previously mentioned, the block distribution may be a determining factor in the properties of the alginates, hence such information may be of considerable use in interpreting product behaviour. The values obtained for the guluronic acid content of the alginates are in good agreement with the nominal values given by Pronova (shown in Table 1) and are slightly larger than the values found using circular dichroism.

Viscosity measurements

While the use of alginates is almost invariably associated with their rheological properties, the intrinsic viscosity of alginate solutions can be used as a means of estimating the molecular weight, although other techniques such as light scattering are also available (Smidsrød & Haug 1968). The relationship between the intrinsic viscosity and molecular weight of alginate samples in 0·1 M NaCl can be approximated (Smidsrød & Haug 1968; Smidsrød 1970; Matsumoto & Mashiko 1990) by:

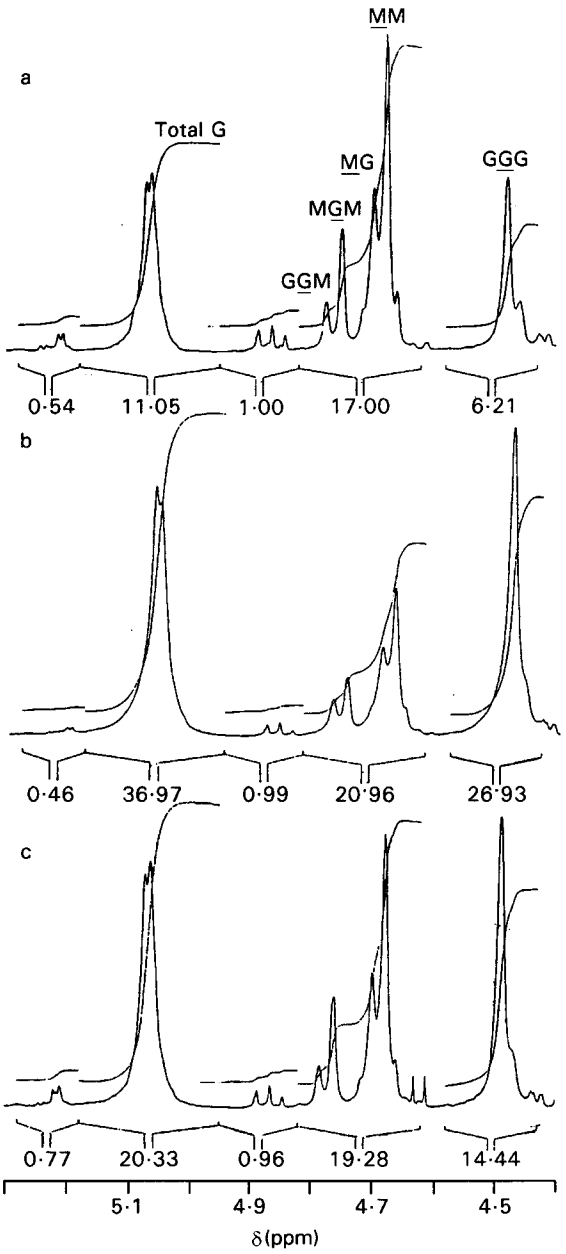

FIG. 2. ^{1}H NMR spectra of (a) LF 10/40RB, (b) LF 20/200 and (c) LF 200DL, including representative peak assignments. The figures under the traces are integration values.

$$[\eta] = 2{\cdot}0 \times 10^{-5} M_w \qquad (4)$$

This relationship should only be regarded as yielding an estimate of the degree of polymerization as the equation does not take into account the MG ratio or block structure of the alginate. The intrinsic viscosity is affected not only by the molecular weight of the alginate but also by the flexibility of the polymer chains. The flexibility of the chains is determined by the chemical composition (i.e. the block structure) because parts of the chains containing predominantly G blocks are less flexible than those containing predominantly M blocks which are in turn stiffer than areas of roughly alternating M and G (Smidsrød et al 1973). However, Matsumoto & Mashiko (1990) have suggested that the intrinsic viscosity is more closely related to the molecular weight of the sample than to the block structure (although the same is not the case for gel systems), hence the viscosity might be used as a reasonable guide to the molecular weight. The flow curves for the alginates under the conditions used here showed little deviation from Newtonian flow, hence the viscosity was obtained from the slope of the stress scans. The intrinsic viscosity of an alginate solution can be determined using equation 5 (Haug & Smidsrød 1962) and is found by plotting either of the terms against concentration; it is possible to extrapolate both lines to zero and estimate the intrinsic viscosity of the solution:

$$[\eta] \equiv \lim_{c\to 0}(\eta_r - 1)/C \equiv \lim_{c\to 0} \ln \eta_r / C \qquad (5)$$

where η_r is the viscosity of the solution relative to the solvent (with $\eta_r - 1$ equal to the specific viscosity η_{sp}) and C is the concentration in g (100 mL)$^{-1}$. The values of (ln η)/C were plotted against concentration as shown in Fig. 3 and the intercepts used to calculate $[\eta]$. An estimate of the molecular weight can then be obtained (Table 3), although it should be emphasized that, given the approximate nature of the method and the use of water as a solvent in this example, these values can only be regarded as comparative. It can be seen that four of the alginates had molecular weights of the same order of magnitude, whereas one (LFR 5/60) had a considerably lower molecular weight, as expected from the manufacturer's information.

Conclusions

The study has compared three methods of characterizing alginates for a range of samples. Circular dichroism is a rapid and simple means of assessing the MG ratio, although the method used did not enable characterization of the block structure of the alginates. NMR requires partial hydrolysis, thus in terms of sample preparation is more complex, but yields more detailed information, particularly with regard to the block structure of the alginates. Although on balance NMR appears to be the method of choice for the characterization of alginate block structure, it should be stressed that circular dichroism is also extremely useful for monitoring interactions between these polymers and other molecules, notably cations (Haug & Smidsrød 1968). Intrinsic viscosity measurements enabled assessment of the molecular weight of the samples,

Table 2. Average guluronic and mannuronic acid composition values as determined by NMR.

Alginate	F(G)	F(M)	F(GG)	F(MM)	F(GM)	F(GGG)	F(GGM)	F(MGM)	$N_{G>1}$
LFR 5/60	0·672	0·328	0·582	0·238	0·090	0·546	0·035	0·055	17·5
LF 120M	0·424	0·576	0·266	0·419	0·158	0·221	0·046	0·112	6·9
LF 10/40RB	0·509	0·491	0·334	0·315	0·175	0·282	0·053	0·122	7·4
LF 20/200	0·670	0·330	0·562	0·223	0·108	0·517	0·045	0·063	13·6
LF 200DL	0·551	0·449	0·419	0·318	0·131	0·384	0·036	0·096	12·7

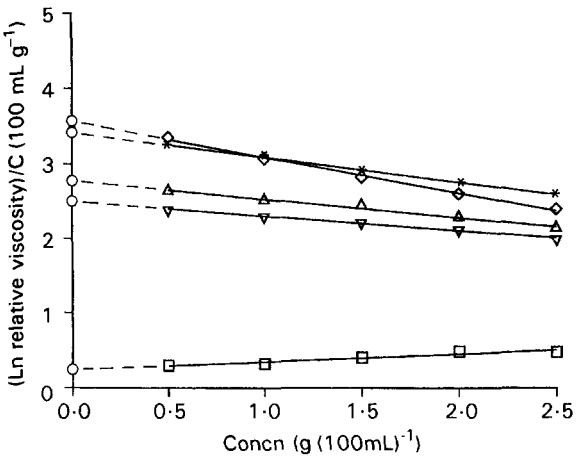

FIG. 3. Concentration-dependence of relative viscosity for alginate samples. □, LFR 5/60; ◇, LF 20/200; *, LF 200DL; ▽, LF 10/40RB; △, LF 120M; ○, extrapolated value.

Table 3. Intrinsic viscosity and molecular weight of alginate samples (approximated to the nearest thousand).

Alginate	$[\eta]$ (100 mL g^{-1})	Mol. wt. (approx.)
LFR 5/60	0·242	12 000
LF 120M	2·774	139 000
LF 10/40RB	2·4981	124 000
LF 20/200	3·5676	178 000
LF 200DL	3·4217	171 000

although certain assumptions are associated with the method outlined here, and these render the measurements approximate.

However, such information is of use in terms of basic characterization and comparison between samples to assess, for example, possible degradation on processing or storage. The molecular characteristics of alginates are of primary importance in terms of understanding their functionality in products. It might not always be necessary to conduct specific studies such as those outlined here if, for example, approximate (or specific) information is available from the manufacturer. However, given the structural complexity of alginates, it is almost invariably desirable to obtain as much of the information outlined above as possible, as, for example, use of the MG ratio alone might be misleading, given that it yields no information on the block distribution. Such information, quoted in the literature, would be extremely helpful to facilitate comparison between studies and to control the quality both of raw materials and of the corresponding products.

Acknowledgements

The authors would like to thank Dr A. Drake (University College, University of London) for help with the circular dichroism studies, Dr E. S. Pepper (SmithKline Beecham, Herts) for help with the design, execution and interpretation of the NMR studies and Dr Sushil Chauhan (SmithKline Beecham, Herts) for helpful comments. Thanks are also due to Pronova for kind supply of alginate samples.

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