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[F1002]

NMR Analysis of Non Hydrolyzed Samples of Sodium Alginate

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Abstract

Alginate, a material extracted from brown seaweeds, is a polymer of D-mannuronic acid and L-guluronic acid. The structure of alginate has carboxylic acid functionalities and is water soluble, particularly as the sodium salt, thereby possessing a wide range of commercial uses such as a rheology modifier. Here we report a simple procedure to evaluate some chemical properties of sodium alginate samples by conventional 1D- and 2D NMR experiments using mild conditions and without preliminary partial acidic hydrolysis.

Introduction

Alginic acid constitutes the major structural polysaccharide of brown seaweeds (Phaeophyta). It is a linear copolymer of 1-4 linked β-D-Mannuronic acid (M) and 1-4 linked α-L-Guluronic acid (G) residues; the two uronic acids can be arranged in heteropolymeric and homopolymeric blocks.[1-3]
Solutions of sodium alginate gel in the presence of divalent cations under very mild conditions of pH and temperature. Alginates have been utilized in several medical applications, where their hydrophilic character and ultimate solubility are advantageous. Fibrous alginate has been used for plugging cavities in dental surgery. Alginate microcapsules have been used to encapsulate various cells for product release.[4] Alginate has been formed into sponges in order to serve as scaffolding for cell transplantation [5] and has been infused into hollow fibres with endothelial cell growth factor to promote neovascularization.[6] As a medical fibre, alginate is non-toxic, non-carcinogenic, biocompatible, sterilizable and offers cheap processing technologies.
Physical properties of alginates gels are strongly correlated to the chemical composition of the polymer depending mainly on the M/G ratios. This value could be conveniently determined by NMR after preliminary partial hydrolysis by treatment in acidic solution (pH 3) at 373K for 1 to 3 hours in order to reduce the viscosity that represent a problem for the acquisition of high resolved spectra.

Results and Discussion

In this communication we report a study on the fine chemical structures of non hydrolyzed sodium alginate samples using mono and two-dimensional nuclear magnetic resonance techniques. These studies are mainly directed to the investigation of the effects produced on the starting material by a series of purification processes required for the preparation of microencapsulates for pancreatic islets transplantation.[4-6]
20 mg of sodium alginate from Macrocystis Pyrifera were dissolved in 1 ml of D_2O and analyzed in a Bruker NMR Avance 400 MHz instrument. All the spectra were recorded without the suppression of the water signal using the native or purified alginate without any preliminary treatment.

As schematized in Fig. 1 the temperature positively effects the viscosity reducing the peaks broadness and moving the DOH resonance far from the more diagnostic signals (Figure 1).
We found that at 338K the low field spectral region, assigned to the anomeric protons, can be recorded with a good resolution, giving important informations about the composition of the polymer.
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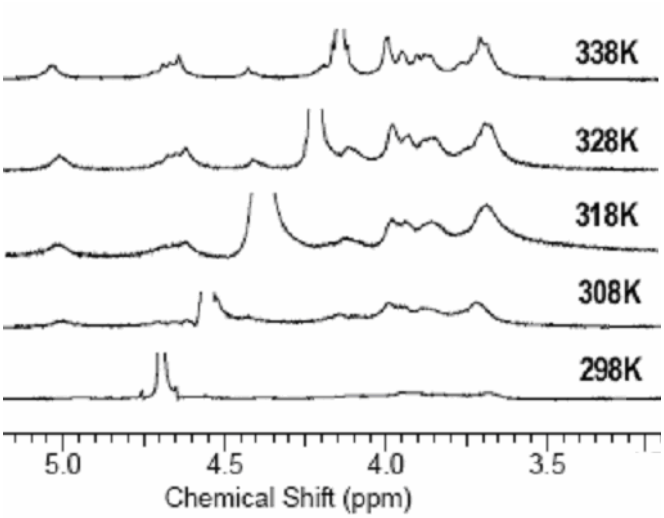

Figure 1: Effect of the temperature on the ^{1}H-NMR signals

The ^{1}H-NMR (Figure 2) spectra obtained after Fourier transformation using a Lorentz Gauss function for the resolution enhancement (LB= -1, GB = 25%) were assigned on the basis of the data previously reported in literature and the integrals are perfectly in agreement with those reported for this kind of sodium alginate.[7] The good spectral resolution allowed us to extrapolate informations on the M/G ratio as well as the molar fractions of monads, diads and some triads of nearest neighbours along the interacting copolymer chain (Table 1).

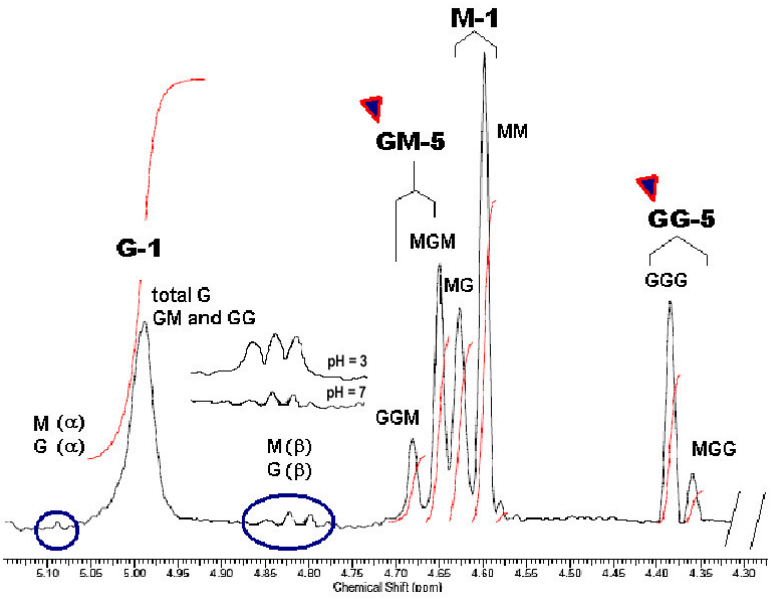

Figure 2: Assignment of the H1 and H5 signals for M and G residues

Table 1: Chemical composition of diffent samples

	M/G	monads	diads	triads
Sales specification	**1.50**	FG **0.4** FM **0.6**	FMM **0.4** FMG **0.2** FGM **0.2** FGG **0.2**	------
Native Alginate	**1.50**	FG **0.4** FM **0.6**	FMM **0.39** FMG **0.21** FGM **0.21** FGG **0.19**	FMGM **0.18** FGGM **0.03** FMGG **0.03** FGGG **0.16**
Purified Alginate	**1.50**	FG **0.4** FM **0.6**	FMM **0.39** FMG **0.21** FGM **0.21** FGG **0.19**	FMGM **0.18** FGGM **0.03** FMGG **0.03** FGGG **0.16**

The less intense resonances (circled in Figure 2) were assigned to the anomeric protons of the reducing end-groups [7] and give valuable informations on the rate and grade of hydrolysis. Using the condition reported in the present communication the areas of these signals reasonably reflect the real amount of end-groups present on the native material. We demonstrated that the temperature of 338K does not effect relevant depolimerization at neutral pH for several hours in contrast with other methods previously used to reduce the NMR sample viscosity (pH 3, 373K for 1h). Table 2 reports the ratio $M_\beta+G_\beta/M+G$ calculated by ^{1}H-NMR for different samples at different combinations of temperature and pH. The results reported below clearly demonstrate also that the purification required for the microencapsulates preparation does not produce additional depolymerization respect to the native alginate and for both the samples can be estimated a molecular weight of 17000 Da ca.

Table 2: Rate and grade of depolimerization $M_\beta+G_\beta/M+G$ calculated by NMR

$M_\beta+G_\beta$/ M + G	1h	24h	36h	48h	72h
Native Alginate pH 7, 338K	0.013	0.014	0.014	0.015	0.020
Purified Alginate pH 7, 338K	0.013	0.014	0.015	0.016	0.020
Native Alginate pH 7, 373K	0.023	0.026	0.030	0.035	0.040
Native Alginate pH 3, 373K	0.155	0.200	0.240	0.280	decomposed
Native Alginate pH 0, 298K	decomposed				

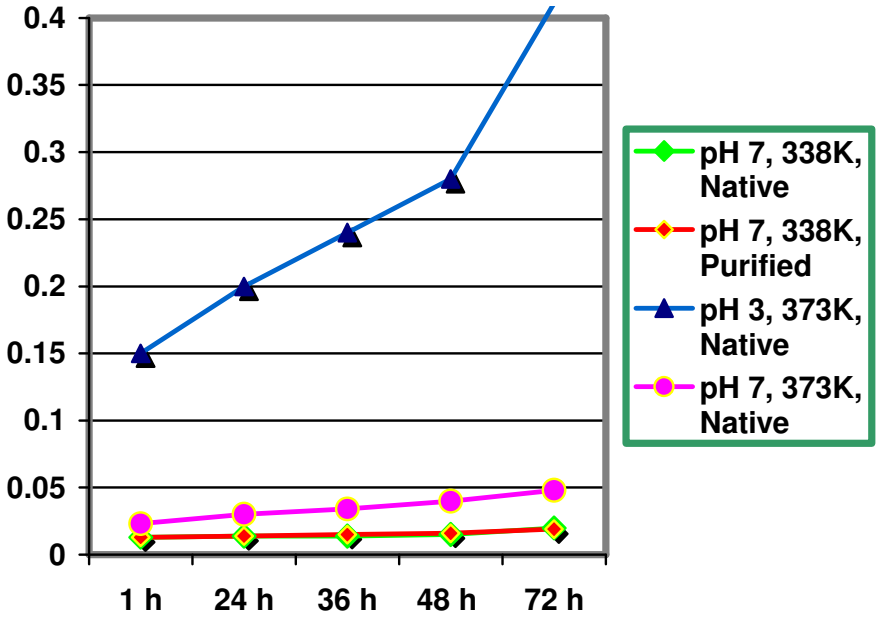

Figure 3: $M_\beta+G_\beta/M+G$ is mainly effected by the pH

Circular dichroism studies showed that the calcium ions react preferentially with the polyguluronic acid segments during the gelation process and that the concentration of poly-G chains strongly effects the characteristics and the mechanic properties of the gel.[2]

For these reasons we focused our attention on the fine characterization of the main G containing blocks. From the analysis of the COSY correlations map is possible to assign two different patterns of coupled protons: one has been assigned to the GGG frame and the other to the MGM frame (Figure 4).

On the basis of these assignments the NOE correlations (Figure 5) indicate for the polyguluronic segment a ribbon-like conformation stabilized by an intramolecular hydrogen bond, a suitable conformation for the chelation of divalent cations and the formation of the "egg-box" structure hypothesized for the alginate gels.

The NOE effect evidenced also a dipolar correlation between H1 and H5 that probably indicates a spatial correlation between two adjacent poly-G chains suggesting a supramolecular organization also in the sodium alginate.

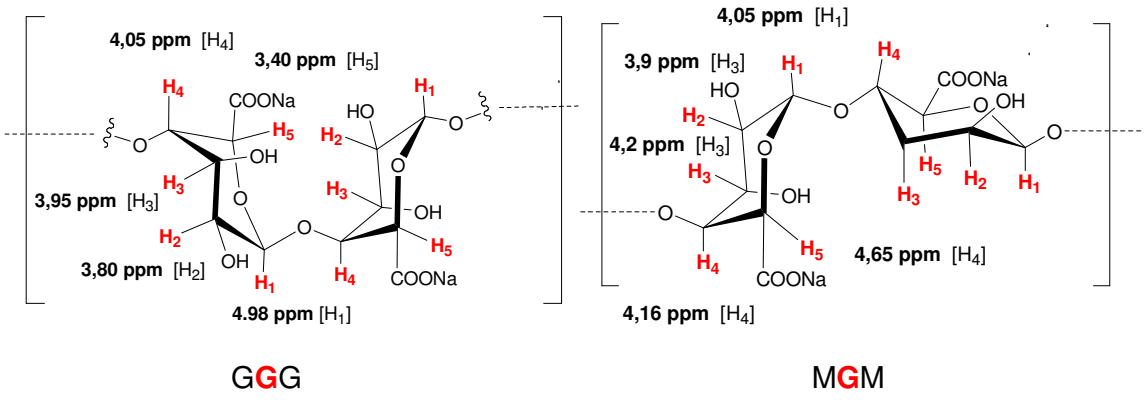

Figure 4: Assignment of coupling patterns in guluronic monomers by COSY experiments

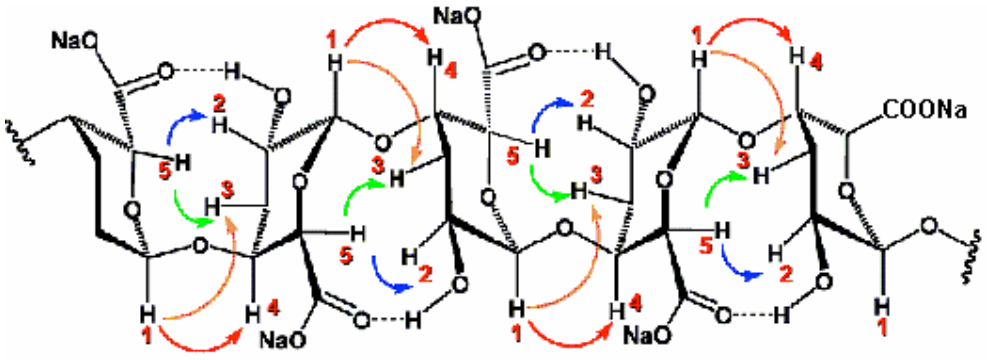

Figure 5: NOESY correlations in poly-G chain confirm a ribbon-like conformation

Finally, in order to compare the chemical composition of the final microcapsules respect to the native material, we realized the complete degeling by treatment with sodium citrate. The resulting sodium alginate solution, after liophilization, was dissolved in D_2O and analyzed by NMR showing a composition similar to the corresponding starting material, indicating that the gelation involves all the components of the copolymer even if the alternating chains play no direct role in the gel formation.

Conclusion

In conclusion we described a fast and simple procedure to evaluate some chemical properties of sodium alginate samples by conventional 1D- and 2D NMR experiments using mild conditions and without preliminary partial acidic hydrolysis. This conditions allowed us to calculate more accurately the amount of the reducing end-groups estimating the molecular weight of the polymer.

Acknowledgments

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