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Periodate oxidation of sodium alginate in water and in ethanol–water mixture: a comparative study

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Abstract—Periodate oxidation of sodium alginate in aqueous solution as well as a dispersion in 1:1 ethanol–water was examined. The oxidation proceeded smoothly in both media, and the kinetics of oxidation was surprisingly similar. Polymer cleavage was observed in both media, but it was extensive in ethanol–water. The weight-average molar mass (M_w) of the oxidized product obtained from aqueous solution showed a gradual decrease with increase in the periodate concentration, whereas, except for very high periodate equivalent, the change in M_w was not reflected with increase in concentration of periodate in ethanol–water. The oxidized alginate obtained from the ethanol–water mixture was found to be more efficient in crosslinking proteins such as gelatin, leading to hydrogels. Oxidation of a dispersion has the advantage of generating large quantities of the oxidized alginate in higher yield with one reaction using less solvent.

Keywords: Polysaccharide; Alginate; Periodate; Oxidation; Molecular weight

The cleavage of the carbon–carbon bond employing periodates in compounds in which the two carbon atoms, each bearing an oxygen atom either as a hydroxyl or as a carbonyl group, was first observed by Malaprade.[1] The topic of periodate oxidation has been extensively reviewed.[2,3]

Alginates are an important class of polysaccharides for many biomedical applications.[4,5] Alginates, are anionic linear polysaccharides composed of (1→4)-linked β-D-mannuronate and (1→4)-linked α-L-guluronate residues in varying proportions. Although alginates are biocompatible, they are known to be resistant to biodegradation.[6] It has recently been reported that periodate-oxidized alginates are highly susceptible to biodegradation,[7] and therefore oxidized alginates have the potential to be used in a number of biomedical applications wherein biocompatibility and biodegradability are important criteria. Oxidized alginates could also function as potential nontoxic and biodegradable crosslinking agents for proteins in the preparation of hydrogels.

Periodate oxidation of polysaccharides is usually conducted in aqueous solutions. Alginates form highly viscous solutions and therefore, the reaction is usually conducted in dilute solutions.[8,9]

The objective of the present work was to examine the possibility of oxidizing alginate as a dispersion in an ethanol–water mixture with the aim of obtaining a larger quantity of the oxidized product with minimum amount of solvent in one reaction and to examine the properties of the oxidized product with that obtained by oxidation from aqueous media, especially with respect to its molecular weight and its reactivity with proteins such as gelatin. To the best of our knowledge, studies on periodate oxidation of sodium alginate as a dispersion in water–ethanol have not been reported.

The alginate used for the present study was derived from *Macrocystis pyrifera*. It contained 61% of residues

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of D-mannuronic acid and 39% of residues of L-guluronic acid. Thus, a 4% solution of alginate in water or 10% alginate as a dispersion in 1:1 ethanol–water mixture was oxidized using periodate.

We compared the degree of oxidation in aqueous as well as in ethanol–water mixture and the molecular weights of the products obtained by the two methods. The degree of oxidation was monitored by measuring the periodate consumption during reaction employing stoichiometric amounts of sodium alginate and periodate. It was found that the reaction in ethanol–water mixture proceeded smoothly as that in aqueous medium and the kinetics of oxidation in both media were surprisingly similar (Fig. 1). The ethanol–water medium also gave rise to better yield of the oxidized product (50–60%) as compared to the yield from aqueous medium, which was always within 25–35%. It has been reported that the oxidation limit of higher molecular weight alginates except at conditions at which alginate is insoluble or glycol cleavage is impaired, vary between 0.49 and 0.55 mol of periodate per hexuronic acid unit.[9] In the present study, the oxidation limit obtained after 6 h under both reaction conditions was between 0.87 and 0.89 mol of periodate per hexuronic acid unit. This can be attributed to incomplete dissolution of alginate in aqueous medium (due to the highest possible concentration employed here, 4%) or due to minimal dissolution of alginate in ethanol–water medium where hemiacetal formation is somewhat hindered. The degree of oxidation was increased to ~75% within 1 h, and thereafter the reaction was rather slow in both media. Just as in aqueous medium, periodate consumption was never complete in ethanol–water mixture. This revealed that there is still a chance for intramolecular, interresidue hemiacetal formation in both media. Hemiacetal formation between oxidized and unoxidized alginate residue prevents the complete consumption of periodate and therefore complete oxidation.[9]

The effect of the concentration of periodate on the degree of oxidation in both media was examined by varying the molar concentration of periodate by keeping the concentration of sodium alginate constant. The degree of oxidation was found to increase with increase in the concentration of periodate, and here again the kinetics were very much similar (Fig. 2). Dialdehyde assay using the hydroxylamine hydrochloride method[10] also showed that there was no significant difference between the dialdehyde content of oxidized products obtained in water and in ethanol–water (Table 1).

The M_w of oxidized alginates was determined using HPLC–GEC.[11] Extensive polymer cleavage leading to drastic reduction in molar mass was seen in both media. There is a gradual reduction in the M_w with increase in the amount of periodate in aqueous medium due to increase in the extent of oxidation and associated polymer cleavage (Table 1). We expected the presence of ethanol would prevent polymer cleavage and give rise to oxidized alginate of high molecular weight. On the contrary, we observed drastic degradation resulting in very low molecular weight products.

Periodate oxidation of alginate in dilute solutions initially proceeds in a random manner, that only one monomeric unit in a given chain is oxidized at a given time, and the protection of either one or both neigh-

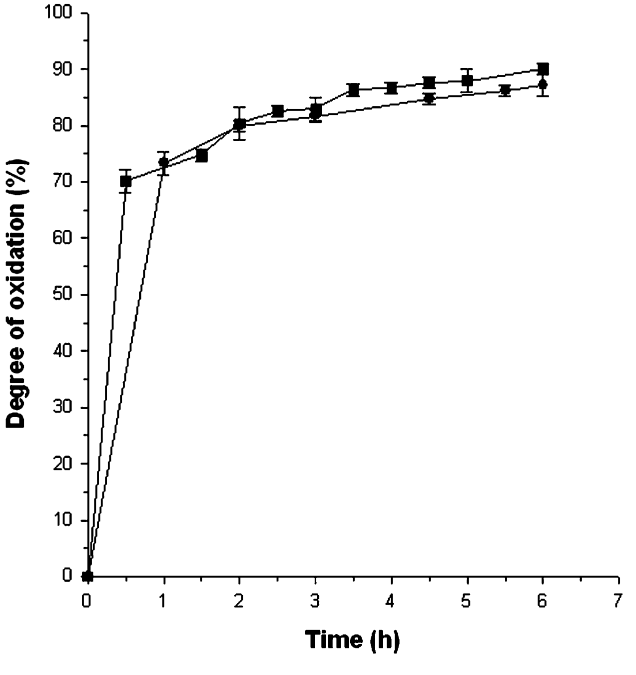

Figure 1. Variation of degree of oxidation of alginate in aqueous (■) and in 1:1 ethanol–water mixture (●) with time.

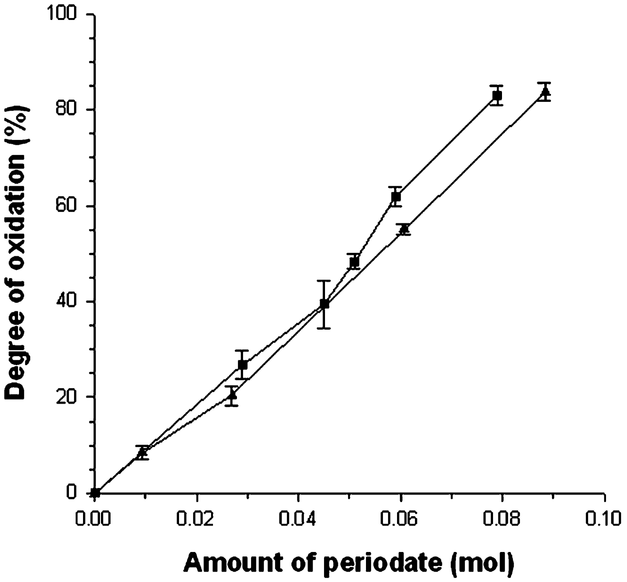

Figure 2. Variation of degree of oxidation of alginate in aqueous (■) and in 1:1 ethanol–water mixture (▲) with amount of sodium metaperiodate. Reaction time 6 h.

Table 1. Degree of oxidation, dialdehyde content and M_w of the product obtained by periodate oxidation of sodium alginate at different periodate concentrations in ethanol–water mixture and in aqueous solution (values in parentheses) for 6 h at 25 °C (the M_w of parent sodium alginate is 489,000 ± 50,000)

Periodate equivalent (%)	Degree of oxidation (%)	Dialdehyde content (%)	M_w (g/mol)
29.3	27.4 ± 0.4 (27.4 ± 0.2)	20.4 ± 2 (26.2 ± 1)	28,790 ± 1440 (69,620 ± 3480)
50.0	48.0 ± 0.4 (49 ± 0.5)	43 ± 2 (47.3 ± 1)	31,240 ± 1560 (56,430 ± 2820)
65	57.48 ± 0.2 (61.8 ± 0.2)	55.0 ± 1 (58.0 ± 1)	30,245 ± 1510 (43,060 ± 2150)
94.5	87.0 ± 0.3 (87.1 ± 1.0)	83.7 ± 2 (84.2 ± 4)	11,340 ± 570 (22,930 ± 1150)

bouring units ensues immediately, before the next oxidative attack on the chain occurs.[9] Oxidation is usually accompanied by extensive cleavage of the chains. Periodate-induced degradation of alginate occurs by two mechanisms; one fast, not mediated by hydroxyl free-radical, and the other slow, due to low concentrations of hydroxyl radicals. The free radical-independent degradation has been attributed to the cleavage of infrequent and unusual monomers in the alginate resulting in the breakage of the main chains.[12] Free radical-mediated polymer cleavage is due to the generation of radicals during oxidation of phenolic impurities present in alginate and aliphatic alcohols such as propanol have been shown to inhibit this cleavage by acting as a free-radical scavenger.[9,12,13]

We believe the extensive polymer cleavage seen in ethanol–water mixture is due to the generation of more reactive hydroxyethyl radicals during oxidation[14] (as opposed to the more stable and less reactive hydroxypropyl radicals when propanol was incorporated in the reaction medium) along with hydroxide radicals cleaving the glycosidic bonds in alginate. It is seen that although there is drastic reduction in the M_w from the parent value in this medium, this reduction is not reflected with respect to increasing periodate concentrations except for very high periodate equivalent. This is attributed to the presence of excess ethanol in the reaction medium predominantly influencing the polymer cleavage. We reason that at very high periodate equivalent, the effect will be synergic in both the free radical-mediated and the free radical-independent cleavage of the alginate chains.

Since oxidized alginates could function as potential nontoxic and biodegradable crosslinking agents for proteins, we examined the gelation reaction between oxidized alginate prepared by two different methods with gelatin using dynamic viscosity measurements. We employed a 10% solution of oxidized alginate prepared by both methods using 65% periodate equivalent and 10% solution of gelatin. Gelation was studied in the presence of 0.1 M phosphate-buffered saline (pH 7.4).

Crosslinking is due to Schiff's base formation between the ε-amino groups of lysine or hydroxylysine side groups of gelatin and the aldehyde groups on oxidized alginate. The gelation of gelatin using the oxidized product obtained from aqueous medium was rather sluggish. However, when we used the oxidized product obtained from ethanol–water mixture, the gelation occurred rapidly (Fig. 3). This rapid gelation is attributed to a viscosity effect due the lower M_w of the product obtained by oxidation in ethanol–water in comparison with the product obtained from aqueous medium. The gelation leading to the three-dimensional network is rather rapid pointing to the possibility of constructing an injectable in situ forming scaffold for cell or drug delivery.

In conclusion, periodate oxidation of alginate as a dispersion in ethanol–water mixture has the potential to prepare large quantities of oxidized product in a single step. Multigram quantities of the oxidized product can be prepared with ease. By doing the reaction in ethanol–water mixture, the quantity of solvent needed was small, which facilitated the recovery of the oxidized product. The product yield was also higher in this medium as compared to water. Other polysaccharides such as hyaluronic acid, carboxymethyl cellulose, gum arabic, etc., which form very viscous solutions in water, could also be susceptible to oxidation as dispersions in ethanol–water, enabling one to prepare larger quantities of the oxidized product.

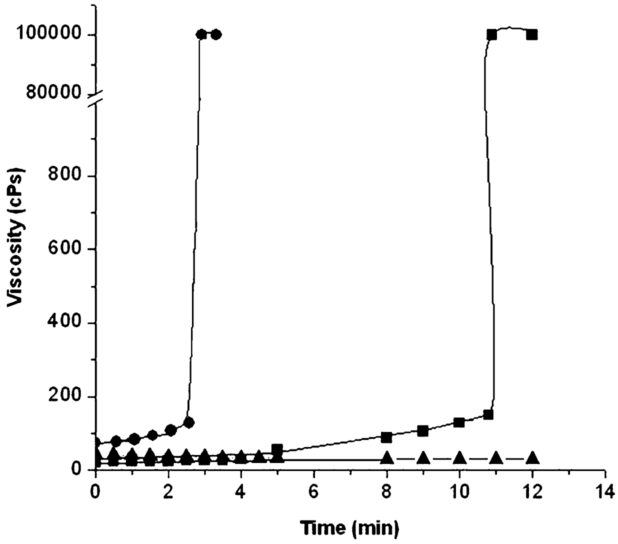

Figure 3. Viscosity change at 37 °C during gel formation between 10% solution of oxidized alginate obtained by periodate oxidation in aqueous (■) and in 1:1 ethanol–water mixture (●) using 65% periodate equivalents and 10% solution of gelatin in the presence of 0.1 M PBS. Control 10% gelatin solution (▲).

1. Experimental

1.1. Materials

Sodium alginate (Medium viscosity grade, viscosity of 2% solution 3500 cps at 25 °C), gelatin (Bloom 300, Type A, M_w 100,000) and sodium metaperiodate were obtained from Sigma Chemical Co., St. Louis, MO, USA. Dialysis tubing (Spectra/Por®, MWCO 3500) was from Spectrum Laboratories Inc., CA, USA. Dextran ($M_w/M_n < 1.2$) standards of various molecular weights and glucose were from Polymer Laboratories (Amherst, MA, USA). PBS (pH 7.4, 0.1 M) was prepared by dissolving 17.97 g of disodium hydrogen phosphate, 5.73 g of monosodium hydrogen phosphate and 9 g of sodium chloride in 1 L of distilled water. Ethanol was distilled before use. All other reagents were of analytical or equivalent grade. Double-distilled water was employed throughout.

1.2. General methods

The extent of periodate oxidation in water and in ethanol–water mixture was followed by iodometric titration of the residual periodate present in the reaction mixture.[15] Briefly, 5 mL of aliquot of the reaction mixture was neutralized with 10 mL of 10% $NaHCO_3$ solution, and iodine was liberated by the addition of 20% potassium iodide solution (2 mL). This was kept under dark for 15 min. The amount of excess periodate in the reaction mixture was estimated by titrating liberated iodine against standard sodium thiosulfate solution using starch as indicator. All experiments were done in triplicate.

The dialdehyde content was measured according to the procedure adopted by Zhao and Heindel.[10] Values reported are average of a minimum three estimations.

M_w of polymers was determined by using HPLC–GEC.[11] A 30 cm × 0.75 cm TSK-G4000 PW column (Toyo Soda, Tokyo, Japan) preceded by a 2-μm filter (Rheodyne, CA) was equipped with a Hitachi pump (Model L-6000), a precision injection valve (Rheodyne, 50 μL sample loading) and a differential refractometer (R401 Waters Associates, France) connected to a computer for sample detection. Dextran standards and glucose were injected at a concentration of 1 mg/mL to establish the selectivity curve of the column. Injection volume was 50 μL for all analyses. The mobile phase was 100 mM $NaNO_3$ aqueous solution (pH 7) at a flow rate of 1 mL/min. A plot of $\log M_w$ versus K_d was used for the determination of the molar mass of the alginate derivatives.

Viscosity change during gelation reaction between oxidized alginate and gelatin was determined by using a programmable viscometer (Brookfield, Model DV-II+, Brookfield Laboratories, MA, USA) at 37 °C using spindle SLV-31 and small sample adapter (10 mL) at 200 rev/min. Briefly, 5 mL of 10% solution of oxidized alginate obtained by two methods using 65% periodate equivalents in PBS was mixed with equivalent quantity of 10% solution of gelatin in the adapter. A 10% solution of gelatin alone was used as control. The gelation reaction was monitored by following the change in viscosity in the viscometer using the Wingather 32® software (Brookfield Engineering Laboratories, Inc. MA, USA) until it became infinity at gelation point.

1.3. Periodate oxidation of alginate

1.3.1. Oxidation in aqueous medium. Sodium alginate (20 g) was dissolved in 400 mL of distilled water by prolonged magnetic stirring in a beaker. Different quantities of sodium metaperiodate dissolved in 100 mL of distilled water were added to the solution and were stirred magnetically in the dark at 25 °C for 6 h. The solution was then dialyzed against distilled water (2.5 L) with several changes of water until it was free from periodate (48 h). Complete removal of periodate was ensured by testing the dialyzate for the absence of turbidity or precipitate with an aqueous solution of silver nitrate. The solution was then frozen at −78 °C, lyophilized and stored in a desiccator in the refrigerator at 4 °C. Yields were always within 25–35%.

1.3.2. Oxidation in ethanol–water mixture. Briefly, 20 g of sodium alginate was dispersed in 100 mL of ethanol. Different amounts of sodium metaperiodate in 100 mL of water were then added to the reaction mixture to get different percent oxidized alginates. This was then stirred magnetically in the dark at 25 °C for 6 h. The solution was dialyzed against distilled water (2.5 L) as before, lyophilized and stored in a desiccator at 4 °C. Yield ranged from 50% to 60%.

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References

1. Malaprade, L. *Bull. Soc. Chim.* **1928**, *43*, 683–696.
2. Bobbit, J. M. *Adv. Carbohydr. Chem.* **1956**, *11*, 1–41.
3. Guthrie, R. D. *Adv. Carbohydr. Chem.* **1961**, *16*, 105–158.
4. Dumitriu, S. Polysaccharides as Biomaterials. In *Polymeric Biomaterials*; Dumitriu, S., Ed., 2nd ed.; Marcel Dekker: New York, 2002; pp 1–61.
5. Shapiro, L.; Cohen, S. *Biomaterials* **1997**, *18*, 583–590.

6. Lansdown, A. B. G.; Payne, M. J. *J. R. Coll. Surg. Edinburgh* **1994**, *39*, 284–288.
7. Bouhadir, K. H.; Lee, K. Y.; Alsberg, E.; Damm, K. L.; Anderson, K. W.; Mooney, D. J. *Biotechnol. Prog.* **2001**, *17*, 945–950.
8. Lee, K. Y.; Bouhadir, K. H.; Mooney, D. J. *Macromolecules* **2000**, *33*, 97–101.
9. Painter, T.; Larsen, B. *Acta Chem. Scand.* **1970**, *24*, 813–833.
10. Zhao, H.; Heindel, N. D. *Pharm. Res.* **1991**, *8*, 400–402.
11. Lesieur, S.; Grabielle-Madelmont, C.; Paternostre, M.; Ollivon, M. *Chem. Phys. Lipids* **1993**, *64*, 57–82.
12. Scott, J. E.; Tigwell, M. J. *Carbohydr. Res.* **1976**, *47*, 105–117.
13. Kang, H.-A.; Jeon, G.-J.; Lee, M.-Y.; Yang, J.-W. *J. Chem. Technol. Biotechnol.* **2002**, *77*, 205–210.
14. Oldfield, F. F.; Cowan, D. L.; Sun, A. Y. *Neurochem. Res.* **1991**, *16*, 83–87.
15. Guthrie, R. D. *Methods Carbohydr. Chem.* **1953**, *1*, 432–447.