

전자빔조사에 의한 알지네이트 저분자화

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Depolymerization of Sodium Alginates by e-Beam Irradiation

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전자빔조사와 과산화수소를 사용하여 알지네이트(sodium alginate, SA)를 저분자화 시키는데 1.0, 2.5 MeV의 전자빔 에너지가 이용되었으며, 저분자화 수율(G_s), 분자량의 제어를 위해 2.5 ~ 20 kGy의 조사량, 0 ~ 4.5%의 과산화수소의 양을 조절하였다. 전자빔조사에 의한 저분자화 알지네이트(depolymerized sodium alginate, DSA)는 주로 1,4-glycoside결합이 끊어지며, C2-C3결합이 끊어지며 생기는 약간의 formate 말단을 가지며, 전자빔 조사 저분자화 저분자 알지네이트 말단(end group)의 화학적 구조가 단순하며, 고분자 알지네이트와 유사한 구조를 가짐을 알 수 있었다. 도입된 전자빔의 에너지와 조사량이 증가하면 알지네이트의 분자량은 감소하였으며, 알지네이트의 농도(2.0w/v%)로 높을 때 저분자화 수율이 높았다. 특히 2% 알지네이트 수용액에 20 kGy (2MeV)의 조사량과 1.5% 과산화수소를 도입하였을 때 가장 큰 저분자화 수율(7.919×10^4 mol/J)을 얻을 수 있었다.

Depolymerization of sodium alginate (SA) was carried out by electron beam irradiation in a hydrogen peroxide atmosphere. E-beam with 1.0 and 2.5 MeV of accelerating voltages were employed in this experiment. For control of molecular weight and the radiation yield of scission (G_s), the irradiation dosage of e-beam was managed in a range from 2.5 to 20 kGy while the quantity of hydrogen peroxide was adjusted in a range of 0 to 4.5%. The chemical structure of the depolymerized sodium alginate (DSA) was analyzed to have scission of 1,4-glycoside bond mainly and a few fragmentary formate end groups which may be produced by the cleavage between C2 and C3 in repeating unit of alginate. It turned out to have simple chemical structures at the DSA end groups, produced by e-beam irradiation, similar with those in the polymer SA structure. As a result, the molecular weight of SA decreased as the energy and dosage of applied e-beam increased, and the radiation yield of scission showed the best result at 2w/v% in SA concentration. The highest radiation yield of scission (7.919×10^4 mol/J) was confirmed when an irradiation dosage of 20 kGy (2 MeV) and 1.5% hydrogen peroxide were used in 2% SA aqueous solution.

Keywords: radiation yield of scission (G_s), depolymerized sodium alginate (DSA), electron beam irradiation

1. Introduction

Sodium alginate (SA) is obtained from brown algae, such as sea mustard and kelp, in a large amount, and SA as an intercellular substance, other than a component constituting the cell wall as a kind of seaweed polysaccharides, is composed of α -(1 \rightarrow 4)-L-guluronate [G] and β -(1 \rightarrow 4)-D-manuronate [M]. Having the structural repeating units of MM block, GG block, and homopolymer or heteropolymer block such as MG block, SA is a polysaccharide having α -1,4 or β -1,4

bonds in an alternating pattern between the units. As a biopolymer, SA is widely used in the medical field due to its non-toxicity and excellent biocompatibility, and SA hydrogel is a especially promising candidate for a matrix of drug delivery and cell implantation[1-3]. However, very high viscosity and low solubility in water, 2%, of polymer SA become obstructive in applying it to the pharmaceutical/medical sectors. In preceding studies, chemical treatments and enzyme reactions have been employed for depolymerizing polysaccharides. However, chemical treatments were disadvantageous in many ways including low recovery rate, excessive time for chemical reaction, difficulty in post-treatment process, disqualification for human consumption, and difficulty in waste water treatment generated during the process. Other methods using enzyme reaction also showed weaknesses in high unit-price of the enzyme, excessive time for reaction, and limitation for large scale process, besides the problems in the

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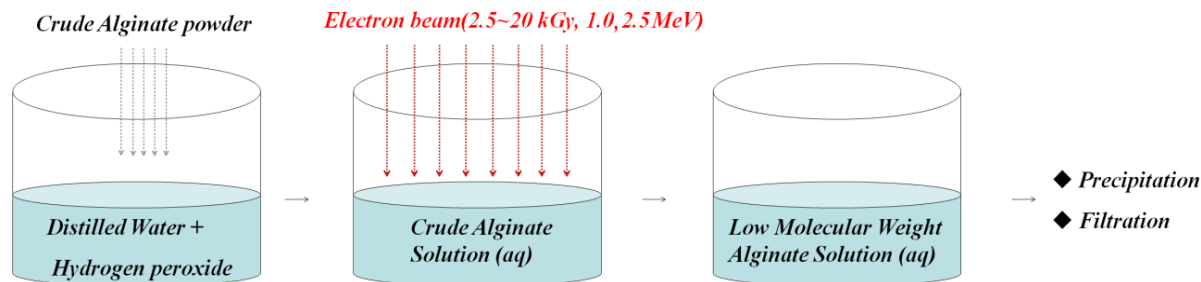
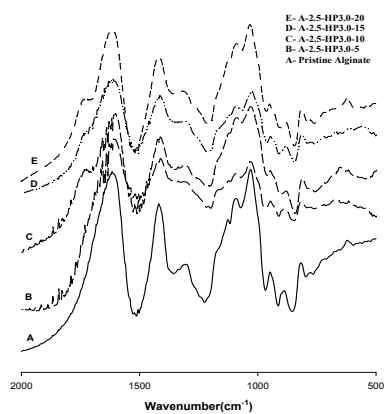
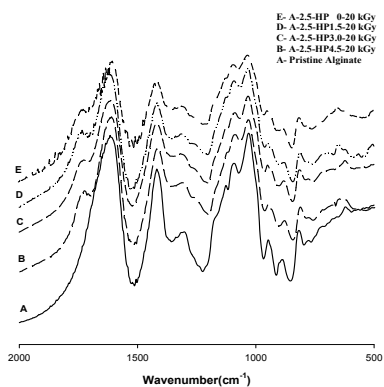


Figure 1. Depolymerization of sodium alginate by e-beam irradiation.



(a)



(b)

Figure 2. FT-IR spectra of alginates irradiated with (a) different doses, 0, 5, 10, 15 and 20 kGy at 2.5 MeV, hydrogen peroxide 3.0% and (b) different hydrogen peroxide content, 0, 1.5, 3.0 and 4.5% at 2.5 MeV and 20 kGy.

control of the molecular weight[4, 5]. Meanwhile, the depolymerization of polysaccharides using γ -ray and e-beam irradiation is disadvantageous in using expensive equipment but advantageous in having quick reaction speed, clean process, and capable of manufacturing high yield of low molecular weight SA[6].

In this study, a basic research was carried out to investigate chemical structure, molecular weight, and the radiation yield of scission of SA as a functional low molecular weight polysaccharide after depolymerizing it in a short period of time employing e-beam irradiation and hydrogen peroxide that were used to generate hydroxy radical.

2. Experimental

2.1. Materials

In this study, crude SA (Aldrich, 1800 cP) and hydrogen peroxide (30%, Samchuk Chemical, Korea) were used as reagents, and every aqueous solution used in the experiment was prepared with deionized water. The product was prepared by filtration/drying after precipitating in ethanol (Dong-yang Chemical, Korea).

2.2. e-beam irradiation and analysis

For the depolymerization reaction of SA, e-beam was irradiated on the SA solution in hydrogen peroxide atmosphere as shown in Figure 1. Using e-beam irradiation equipment (E-beam process system, EB Tech, Korea), an e-beam of 1.0 and 2.5 MeV in accelerating voltage was applied at dosages of 2.5, 5, 10, 15 and 20 kGy, respectively. Consequently, the radiation yield of scission of SA (G_s) was determined in connection with concentrations of SA solution (0.5, 1.0, 1.5, and 2.0 w/v%) and quantities of hydrogen peroxide (0 to 4.5%). Polymer degradation can be expressed in terms of molecular weight reduction due to polymer chain scission and its efficiency can be estimated by radiation yield of scission G_s (mol/J)[7].

The radiation yield of scission of SA by e-beam irradiation was calculated by the following equation (1).

$$G_s = \frac{2c}{Dd} \left(\frac{1}{M_w} - \frac{1}{M_{w0}} \right) \quad (1)$$

Where G_s is radiation yield of scission (mol/J), D ; absorbed dose (Gy), d ; solution density (kg/dm^3), c ; polymer concentration (g/dm^3), M_w , M_{w0} ; weight-average molecular weight of polymer after and before irradiation respectively.

The chemical structure of DSA was analyzed by FT-IR, FT-NMR (JEOL-300 MHz) and ICP-MS (Perkin Elmer), and the molecular weight was determined by GPC-MALS (WYATT Technology corporation. Detector-MALLS: DAWN EOS-RI: OPTILAB DSP). As the eluent, 0.1 N NaNO_3 aqueous solution was used at a flow rate of 1.0 mL/min, and the temperature of column oven was 40 °C during the test.

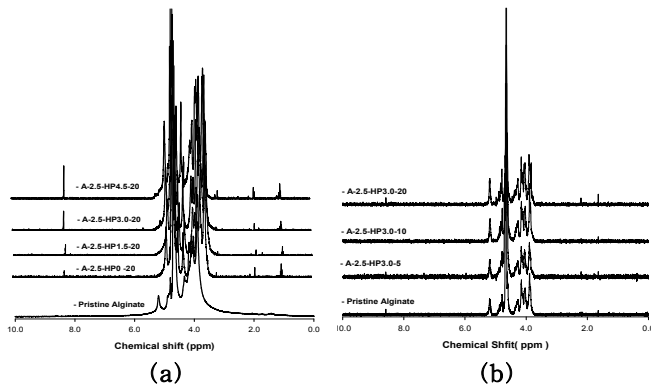


Figure 3. $^1\text{H-NMR}$ spectra of alginates e-beam irradiated with (a) different hydrogen peroxide content, 0, 1.5, 3.0 and 4.5% at 2.5 MeV and 20 kGy and (b) different doses, 0, 5, 10, 15 and 20 kGy at 2.5 MeV, hydrogen peroxide 3.0%.

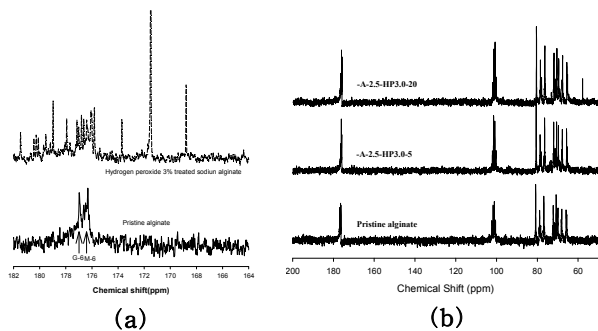


Figure 4. $^{13}\text{C-NMR}$ spectra of pristine alginate and depolymerized alginates (a) irradiated by ultrasound and (b) e-beam irradiation in different doses (2.5 MeV, hydrogen peroxide 3.0%, D_2O).

3. Results and Discussion

3.1. Structure of depolymerized sodium alginate (DSA) by e-beam irradiation

As shown in FT-IR data (Figure 2), the structure of DSA was identical with that of polymer SA including carboxylic acid ($-\text{COOH}$) 3444 cm^{-1} , C-H (2927 cm^{-1}), C=O at 1612 cm^{-1} , C-O stretching vibration at 1415 cm^{-1} . Newly generated DSA by e-beam irradiation showed a new carbonyl peak at 1750 cm^{-1} . The strength of the peak became bigger as the e-beam irradiation increased, indicating increased number of molecules having carbonyl groups at their chain ends due to the reduced molecular weight of SA. In $^1\text{H-NMR}$ data (Figure 3), no particular peaks were confirmed as evidences for new generation or disappearance in the range of typical SA structures (3.8–4.9 ppm), mannuronate and gluronate, relative to changes in the concentration of hydrogen peroxide or e-beam irradiation while new peak with very low intensity (1.98 ppm) was identified in the higher magnetic field sector, alluding new formation of $-\text{CH}_2$ by depolymerization of SA. New generation of carbonyl group by cleavage of the cyclic ring was confirmed because the intensity of peak (8.5 ppm) increased as more hydrogen peroxide was used, indicating formation of a formate structure on the molecular chain when 1,4-glycosidic bond is severed[9]. The findings are consistent with our earlier report in the DSA treated by ultrasonic wave and

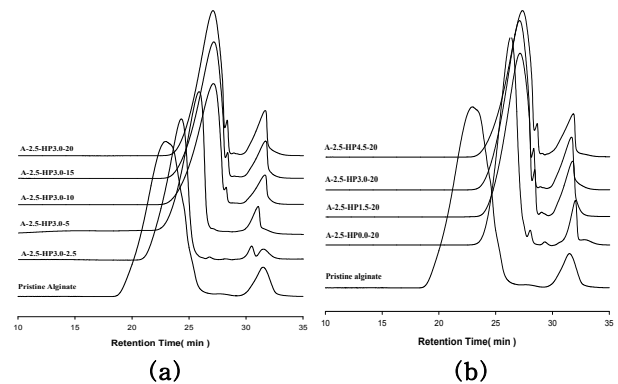


Figure 5. GPC chromatograms of pristine alginate and e-beam irradiated alginates with (a) different doses (2.5 MeV, hydrogen peroxide 3.0%) (b) different hydrogen peroxide content (2.5 MeV, 20 kGy).

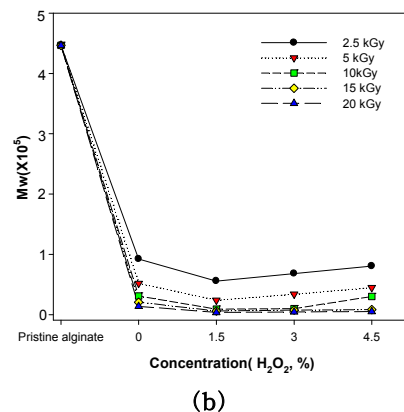
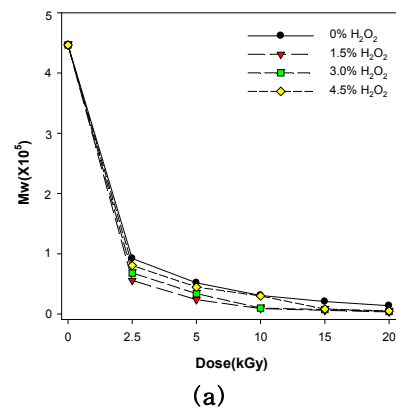


Figure 6. Plots of (a) dose (kGy) vs. molecular weights and (b) concentration of hydrogen peroxide (%) vs. molecular weights of DSA (2 w/v% 2 MeV).

hydrogen peroxide showed diverse end structures, and DSA treated by e-beam irradiation had similar chemical structures with the repeating unit of the original polymer SA[8]. Similar results were found in the $^{13}\text{C-NMR}$ spectrum (Figure 4b). Unlike multiple chemical shifts shown in Figure 4a (169–181 ppm), only the intensity of gluronic acid (G6) peak at 176.9 ppm decreased while new peak of aliphatic carbon was identified in the high magnetic sector (58.0 ppm). Others showed the same chemical shifts with those in polymer SA.

Table 1. Properties of Depolymerized Alginate by E-beam Irradiation (1.0, 2.5 MeV, 2.0 w/v% Aqueous Solution)

Samples	H ₂ O ₂ content%	e-beam dose (kGy)	Mw	Mn	PDI (Mw/Mn)	η_{inh}^a (dL/g)	$G_s \times 10^{-4}$ (mol/J)
Native Alginate	-		446,467	226,167	1.974	6.44	-
A-2.5-HP0-2.5	0	2.5	92,187	46,768	1.971	1.88	1.989
A-2.5-HP0-5.0	0	5.0	51,786	26,310	1.968	1.36	1.972
A-2.5-HP0-10	0	10.0	30,809	15,688	1.964	1.09	1.745
A-2.5-HP0-15	0	15.0	20,709	10,573	1.959	0.96	1.773
A-2.5-HP0-20	0	20.0	13,717	7,033	1.950	0.87	2.041
A-2.5-HP1.5-2.5	1.5	2.5	55,671	28,277	1.969	1.41	3.632
A-2.5-HP1.5-5	1.5	5.0	23,817	12,147	1.961	1.00	4.591
A-2.5-HP1.5-10	1.5	10.0	9,055	4,672	1.938	0.81	6.248
A-2.5-HP1.5-15	1.5	15.0	5,948	3,098	1.920	0.77	6.387
A-2.5-HP1.5-20	1.5	20.0	3,617	1,918	1.886	0.74	7.919
A-2.5-HP3.0-2.5	3.0	2.5	68,102	34,572	1.970	1.57	2.875
A-2.5-HP3.0-5	3.0	5.0	33,917	17,261	1.965	1.13	3.147
A-2.5-HP3.0-10	3.0	10.0	9,832	5,065	1.941	0.82	5.745
A-2.5-HP3.0-15	3.0	15.0	7,501	3,885	1.931	0.79	5.046
A-2.5-HP3.0-20	3.0	20.0	4,394	2,312	1.901	0.75	6.508
A-2.5-HP4.5-2.5	4.5	2.5	80,533	40,867	1.971	1.73	2.351
A-2.5-HP4.5-5	4.5	5.0	44,794	22,769	1.967	1.27	2.320
A-2.5-HP4.5-10	4.5	10.0	30,032	15,294	1.964	1.08	1.794
A-2.5-HP4.5-15	4.5	15.0	8,278	4,279	1.935	0.80	4.565
A-2.5-HP4.5-20	4.5	20.0	5,171	2,705	1.912	0.76	5.520
A-1.0-HP1.5-2.5	1.5	2.5	81,310	41,260	1.971	1.74	2.324
A-1.0-HP1.5-5	1.5	5.0	84,418	42,830	1.971	1.78	1.110
A-1.0-HP1.5-10	1.5	10.0	71,987	36,539	1.970	1.62	0.673
A-1.0-HP1.5-15	1.5	15.0	40,133	20,409	1.966	1.21	0.873
A-1.0-HP1.5-20	1.5	20.0	40,909	20,802	1.967	1.22	0.641

^aInherent viscosity of solution was measured at a concentration of 0.5 g/dL in distilled water at 35 °C

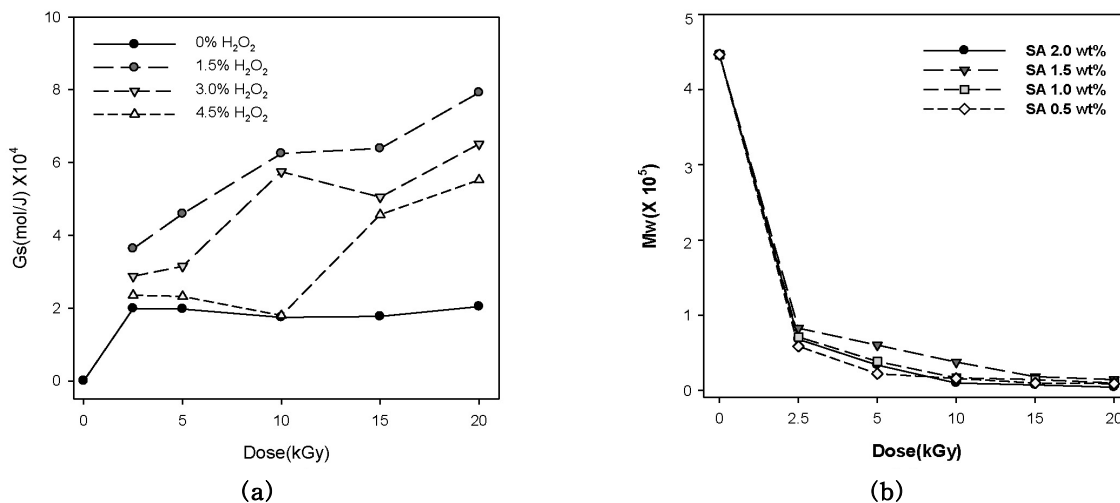


Figure 7. Plots of (a) dose vs. G_s in different hydrogen concentration (2 w/v% SA solution) and (b) dose vs. Mw in different concentration of sodium alginate aqueous solution (2 MeV).

3.2. Molecular weight/radiation yield of scission and viscosity of DSA by e-beam irradiation

The radiation yield of scission of SA was calculated by equation (1),

and GPC-MALS was employed to determine the molecular weight (Mw, Mn) and polydispersity (PDI) of DSA, showing the results in Table 1 and Figures 5 to 7(a). The energy of e-beam (1.0, 2.5 MeV)

Table 2. Properties of DSA According to the Concentration of Solution (1.0, 2.5 MeV)

Samples	solution con. (w/v%)	H ₂ O ₂ content%	e-beam dose (kGy)	Mw	Mn	PDI (Mw/Mn)	η_{inh}^a (dL/g)	$G_s \times 10^{-4}$ (mol/J)
A-2.5-HP3.0-2.5		3.0	2.5	68,102	34,572	1.970	1.57	2.875
A-2.5-HP3.0-5		3.0	5.0	33,917	17,260	1.965	1.13	3.147
A-2.5-HP3.0-10	2.0	3.0	10.0	9,832	5,065	1.941	0.82	5.745
A-2.5-HP3.0-15		3.0	15.0	7,501	3,885	1.931	0.79	5.046
A-2.5-HP3.0-20		3.0	20.0	4,394	2,312	1.901	0.75	6.508
A-1.0-HP3.0-2.5		3.0	2.5	99,956	50,702	1.971	1.98	1.794
A-1.0-HP3.0-5		3.0	5.0	52,563	26,704	1.968	1.37	1.939
A-1.0-HP3.0-10	2.0	3.0	10.0	57,225	29,064	1.969	1.43	0.880
A-1.0-HP3.0-15		3.0	15.0	65,771	33,392	1.970	1.54	0.499
A-1.0-HP3.0-20		3.0	20.0	55,671	28,277	1.969	1.41	0.454
A-2.5-HP3.0-2.5		3.0	2.5	82,864	42,047	1.971	1.76	1.135
A-2.5-HP3.0-5		3.0	5.0	60,333	30,638	1.969	1.47	0.828
A-2.5-HP3.0-10	1.5	3.0	10.0	37,802	19,229	1.966	1.18	0.699
A-2.5-HP3.0-15		3.0	15.0	18,378	9,393	1.957	0.93	1.004
A-2.5-HP3.0-20		3.0	20.0	14,494	7,426	1.952	0.88	0.964
A-2.5-HP3.0-2.5		3.0	2.5	71,210	36,146	1.970	1.61	1.363
A-2.5-HP3.0-5		3.0	5.0	38,579	19,622	1.966	1.19	1.368
A-2.5-HP3.0-10	1.0	3.0	10.0	16,825	8,606	1.955	0.91	1.652
A-2.5-HP3.0-15		3.0	15.0	14,494	7,426	1.952	0.88	1.285
A-2.5-HP3.0-20		3.0	20.0	9,832	5,065	1.941	0.82	1.436
A-2.5-HP3.0-2.5		3.0	2.5	58,779	29,851	1.969	1.45	1.706
A-2.5-HP3.0-5		3.0	5.0	22,263	11,360	1.960	0.98	2.465
A-2.5-HP3.0-10	0.5	3.0	10.0	16,048	8,213	1.954	0.90	1.735
A-2.5-HP3.0-15		3.0	15.0	9,832	5,065	1.941	0.82	1.915
A-2.5-HP3.0-20		3.0	20.0	9,055	4,672	1.938	0.81	1.562

^aInherent viscosity of solution was measured at a concentration of 0.5 g/dL in distilled water at 35 °C

and the dosage exerted large impact on the depolymerization of SA while moderate use of catalyst (hydrogen peroxide) increased the radiation yield of scission effectively. In obtaining SA with lower molecular weight by hydrogen peroxide in the depolymerization reaction, the dosage of hydrogen peroxide did not have a significantly influence on the rate. DSA prepared with 1.5% hydrogen peroxide showed the lowest molecular weight, an average molecular weight (Mw) of 3617, and the highest radiation yield of scission as G_s of 7.919×10^4 mol/J. Use of excessive hydrogen peroxide (3.0 to 4.5%) may cause elimination of radicals already generated in a large quantity at the terminal portion of DSA in low molecular weight by additional radicals, causing recombination effect. The viscosity of DSA was showed a similar pattern, exhibiting the lowest intrinsic viscosity value at A-2.5-HP1.5-20 as 0.74 (dL/g). Moreover, most DSA showed PDI around 2.0, and intrinsic viscosity also was confirmed to have a similar tendency as the molecular weight, resulting in uniform depolymerization of SA in solution.

3.3. DSA's radiation yield of scission by e-beam irradiation relative to the concentration of solution

Tests were conducted to investigate the correlation between the radiation yield of scission of SA by e-beam irradiation and the concentration of polymer SA solution in order to find optimum concentrations of the catalyst (hydrogen peroxide) and the reaction solution. The results of radiation yield of scission relative to the concentration of polymer SA solution are shown in Table 2. As shown in Table 2 and Figure 7(b), the radiation yield of scission increased as the dosage of e-beam irradiation became intensified when the concentration of reaction solution was 2.0 w/v%, the energy of e-beam as 2.5 MeV, and a hydrogen peroxide concentration of 3.0%. When the concentration of reaction solution is lower than the above, relatively low radiation yield of scission was resulted. The radiation yield of scission became even lower when a low dosage of e-beam energy, 1.0 MeV, was applied to the lowly concentrated reaction solution. The radiation yield of scission tended to decrease as the dosage of e-beam irradiation became intensified when the energy of the applied e-beam was 1.0 MeV. When considering the correlation between

the irradiation energy of e-beam and the penetration depth of e-beam, the depth showed different values relative to the energy. In case of e-beam having relatively low energy of 1.0 MeV, low e-beam was transmitted, resulting in a low concentration of radical formation within the penetration depth, so that subsequent low radiation yield of scission (G_s) was resulted. Lower the concentration of the reaction solution was, larger the radiation yield of scission became. Meanwhile, at 2.5 MeV e-beam energy, a high level of the radiation yield of scission (G_s) was confirmed in a highly concentrated reaction solution of polymer SA while resulting in a decreased radiation yield of scission in the lowly concentrated SA solution due to the small quantity of SA within the depth of penetration. The radiation yield of scission showed different results depending on the concentration of aqueous solution and the energy of transmitted e-beam, showing a clear inverse relationship between the applied dosage of e-beam irradiation and the molecular weight of SA.

4. Conclusion

In depolymerization of SA by e-beam irradiation, different levels of the radiation yields of scission were observed relative to the energy intensity of e-beam, irradiation dosage, quantity of hydrogen peroxide, and concentration of SA aqueous solution. The chemical structure of the depolymerized sodium alginate (DSA) was analyzed to have scission of 1,4-glycoside bond mainly and a few fragmentary formate end groups which may be produced by the cleavage between C2 and C3 in repeating unit of alginate. It turned out to have simple chemical structures at the DSA end groups, prepared by e-beam irradiation, similar with those in the polymer SA structure. As a result, the molecular weight of SA decreased as the energy and dosage of applied e-beam increased, and the radiation yield of scission showed the best result at a high concentration of SA (2

w/v%). The most effective products in depolymerization of the alginate were obtained with 1.5% hydrogen peroxide content and the radiation yield of scission (G_s) is increased with increasing the applied e-beam dose up to 7.919×10^4 mol/J.

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