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Gamma degradation studies of chitosan and sodium alginate biopolymers

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Various biopolymers have been reported to play very important roles in different fields including pharmaceutical sciences, water treatment as well as nanosciences. The properties of these biopolymers are widely affected by their molecular weight, structural and functional moieties on them. In order to improve the efficiency towards desired application, they are subjected to chemical and physical processes. One of them is radiation induced degradation or crosslinking. Radiation has been reported as one of the methods for modification of polysaccharides. In this study, chitosan and sodium alginate have been degraded using gamma radiation from ^{60}Co source with dose rate of 4.295 kGy/h, with doses upto100 kGy. The molecular weights have been determined by viscosity method. Similarly, both have been subjected to various characterization methods such as TGA-DTA, XRD, FTIR, SEM-EDX, UV-Vis and DDA (degree of deacetylation). Radiation induced degradation is confirmed through reduction in molecular weight with respect to gamma dose. Basic advantage of radiation degradation of biopolymers includes ability to promote changes in a reproducible manner, quantitatively and without introduction of chemical agents. The highlight of gamma irradiation of biopolymer is that there is no chemical waste generation.

Keywords: Gamma irradiation, Chitosan, Sodium alginate, Polymer degradation

Natural polymers and biopolymers are a class of chemicals that have attracted materials scientists due to their properties like biodegradability and non-toxicity. Radiation processing with the help of gamma radiation of natural polymers is an area of current research[1]. Chitosan and sodium alginate are polysaccharides occurring in large amounts in nature. The amino ($-NH_2$) and hydroxyl ($-OH$) groups present in chitosan shackles exhibit strong reaction sites thereby making its chemical modification feasible[2]. Chitosan is partial deacetylation of chitin. Chitin is a natural polymer that exists mainly in crustacean shells, cell walls of yeast, fungi and insects. Sodium alginate is composed of 1-4 linked blocked polymer of polyglucuronate, polymannuronate or its copolymer bound in random sequences. Due to their unique chemical, physical and biological applications chitosan as well as sodium alginate found many industrial applications in various fields[3]. Chitosan has wide applications in wastewater treatment, paper and textile industries, food processing, and agriculture as well as drug delivery agents in pharma sector. Sodium alginate behaves as a gelling agent, enwrap matrix for enzymes and cells[4].

There are three types of degradation methods: ultrasonic, ultraviolet and by gamma irradiation. Properties of chitosan depend not only on its structure but also on its molecular weight[5]. Chitosan with high molecular weight is not soluble in water but degraded chitosan will be significantly soluble in water[6]. There are ample of methods for degradation of polymers like acidic hydrolysis, enzymatic hydrolysis, oxidation by hydrogen peroxide and with the help of gamma chamber having ^{60}Co source inside it. Induced degradation of chitosan as well as sodium alginate by gamma rays has certain advantages as it can be carried out at room temperature and there is no need of chemical agents. Hence degradation of natural polymers in gamma radiation can be applied in large scale[7]. It has been reported that gamma degraded alginate can accelerate the growth of plants by increasing biological activity inside plants[8-10].

The aim of this study is to systematically study the effect of gamma doses on structural and functional properties of chitosan and sodium alginate. There are a few papers reporting effect of gamma irradiation on chitosan. However, to the best of our knowledge, there is no report of complete systematic study on gamma degradation of chitosan and sodium alginate,

followed by complete characterization. This is an effort to bridge the gap in literature.

Experimental Section

Materials and sample preparation

AR grade reagents were used as received for carrying out all the studies. Chitosan biopolymer powder with medium molecular weight having degree of deacetylation 90% was supplied by SRL chemicals Ltd., India. Similarly, sodium alginate biopolymer with medium molecular weight having degree of deacetylation 90% was purchased from SRL chemicals Pvt. Ltd., India. Acetic acid was supplied by Merck, Pvt. Ltd., India. Sodium chloride (NaCl), potassium hydrogen phthalate (KHP) was purchased from SRL chemicals Ltd., India. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) was supplied by Loba Chemie Pvt. Ltd., Mumbai.

Demineralized water from Milipore Mili Q system was used for preparation of all solutions. Gamma degraded chitosan powder with different gamma doses was dissolved in 1% (v/v) acetic acid solution.Gamma degraded sodium alginate powder with different gamma doses was dissolved in 1% Milipore deionized water.

Equipments

Gamma degradation of chitosan and sodium alginate was carried out in gamma chamber GC-1200 supplied by Board of Radiation and Isotope Technology(BRIT), India. This unit is designed to house 185 TBq (5000 Ci) of Cobalt-60 source and it provides an irradiation rate of 4.295 kGy/h. Degraded samples with three different doses such as 0 kGy, 50 kGy and 100 kGy were characterized by various techniques such as molecular weight determination by viscometry, degree of deacetylation by conductometry, TGA-DTA, SEM, EDX, XRD, FTIR and UV-Vis. The effect of molecular weight on both the polymers were studied by using Ostwald viscometer (Sigma-Aldrich). Degree of deacetylation was studied by using conductivity meter (Equip-Tronics). The thermal stability of degraded chitosan and sodium alginate powder was determined by simultaneous DTA-TG apparatus (Shimadzu DTG-60). The surface morphology was examined by SEM (JEOL, Tokyo, Japan Model: JSM 6610LV). The elemental distribution of degraded samples was investigated by the EDX technique equipped with SEM (Secondary, backscattered, and LN2-free EDX detector). The phase structure of gamma degraded chitosan and alginate was analyzed by the XRD technique (XRD, Bruker, Elk Grove, USA, D8 Discover, X-ray source Cu, 3 KW). The presence of functional groups and elemental structure after degradationwas observed by FTIR technique with wavenumbers scanned from the range of 400 cm^{-1} to 4000 cm^{-1}. The absorbance of chitosan and sodium alginate solutions was recorded by UV-Visible Spectrophotometer model (Shimadzu UV-1900i).

Procedures

In order to carry out gamma irradiation, exactly weighed amounts of chitosan and alginate were taken in zip-lock pouches made up of plastic. They were kept in gamma chamber GC-1200 and irradiated at a dose rate of 4.295 kGy/h for desired period depending on the dose requirement. After irradiation, the characterization was carried out using various techniques.

Average molecular weight of chitosan and sodium alginate was determined by using Ubbelohde capillary type viscometer which allows the reading of flow time of the sample solution to be taken manually with the help of stopwatch. Both the biopolymers were irradiated with varying gamma doses from 0 kGy to 100 kGy. The unirradiated as well as irradiated samples were dissolved in respective solvents. 1% acetic acid was used as a solvent for chitosan while 0.1 M NaCl solution was used as a solvent for sodium alginate. The flow time of various dilutions were recorded in Ubbelohde viscometer. Each measurement was carried out at 25±0.1°CC. The molecular weights were determined using reported method based on Mark-Houwink equation[11-13].

Degree of deacetylation of unirradiated and irradiated chitosan was determined using reported method[14]. Approximately 0.1 g sample was dissolved in 25 mL of 0.1 M HCl solution by stirring overnight. It was titrated with 0.2 N NaOH solution using potentiometric titration, with the help of quinhydrone electrode. The degree of deacetylation was calculated by using following equation[15].

$$DDA = \left[\frac{203Q}{1 + 42Q}\right] \times 100$$

$$Q = \frac{N\Delta V}{m}$$

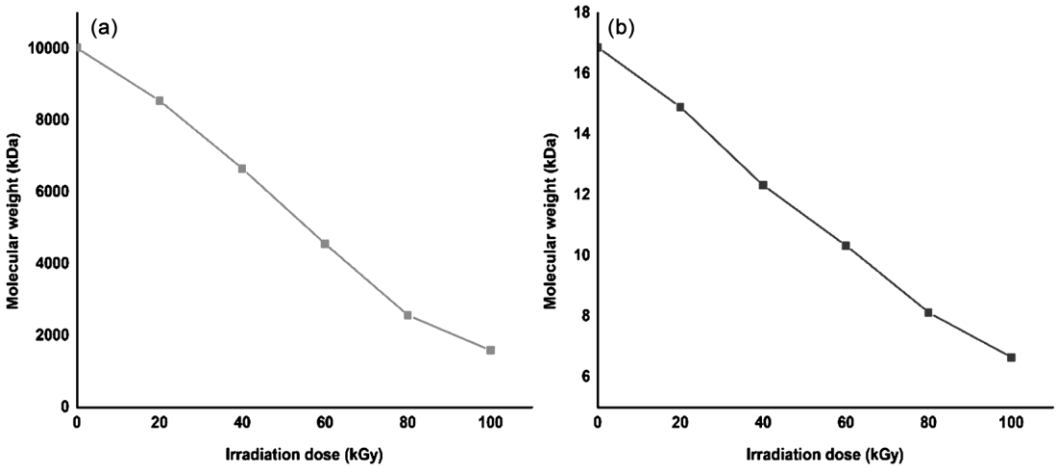

Fig. 1 — Effect of gamma dose on molecular weight of (a) chitosan (b) sodium alginate

where, m is the molecular weight of chitosan monomer and N is the normality of NaOH solution used in the titration.

Results and Discussion

Effect of gamma irradiation on molecular weight

Determination of molecular weight of polymers is one of the most important parameters in characterization of polymers. This can be done by several methods like gel permeation chromatography (GPC), viscometry *etc*[14]. Gel permeation chromatography is the most accurate method for determination of molecular weight but this method is relative and requires standards for molecular weight calibration. Hence viscometry is the simplest, easiest and quick method for determination of molecular weight[16]. Although viscometry is not the absolute method and it requires constants K and α. However, for various polymers, the values for these constants are reported in the literature for various solvent systems.

Graph of molecular weight of chitosan against irradiation dose was plotted which is shown in Fig. 1. It has been reported that with the increase in irradiation dose, the degree of crosslinking of chitosan chains and molecular weight decreases due to scission effect induced by the exposure of γ-rays. Accordingly, rupture occurs by glycosidic bonds (C-O-C) of main chain present inside chitosan but only the site of the molecule is involved in the scission reaction[17].

Determination of degree of deacetylation of chitosan

As chitosan is synthesized from chitin, the acetyl groups are converted into amino groups. However, the conversion is not quantitative. Chitosan contains

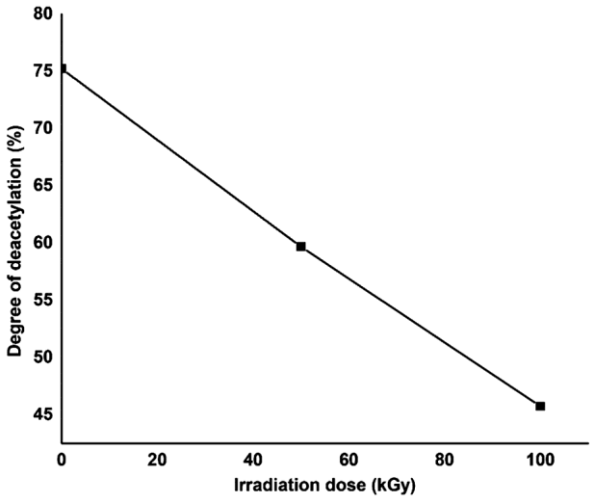

Fig. 2 — Effect of irradiation dose on DDA of chitosan

varying degree of deacetylation from 60 to 95% in general. The degree of deacetylation (DDA) influences the physical, chemical and biological properties of chitosan such as self aggregation, sorption properties, biodegradability and electrostatic characteristics[18]. The content of free amino groups in polysaccharides can be determined by degree of deacetylation. There are various methods to increase or decrease the degree of deacetylation. This can be employed by enhancing the sodium hydroxide content which helps to remove acetyl group from chitin. Several methods have been used to determine degree of deacetylation like ninhydrin test, linear potentiometric titration (LPT), ultraviolet hydrogen bromide titrimetry, infrared spectroscopy and elemental analysis[19].

Fig. 2 shows the graph plotted in between degree of deacetylation (DDA) and irradiation dose[13].It was

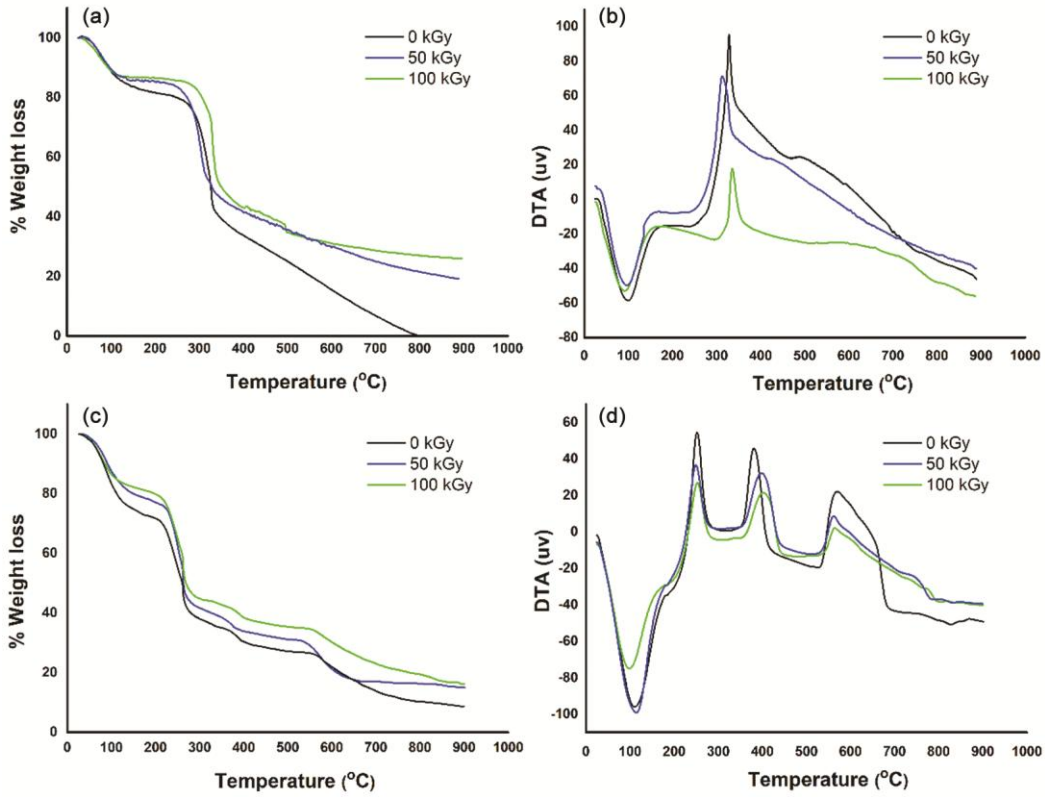

Fig. 3 — TGA curves of (a) chitosan, (c) sodium alginate and DTA curves of (b) chitosan, (d) sodium alginate at different gamma doses.

observed that the DDA goes on decreasing upon irradiation. This can be attributed to cleavage of the C-C bond of chitosan with acetyl group.

Thermal studies

Effect of gamma irradiation on the thermal stability was studied using TGA and DTA analyses. Fig. 3 depicts the TGA and DTA curves of chitosan and alginate at different gamma doses. Fig. 3(a) shows TGA curve of chitosan with two step degradation, first degradation is observed around 100ºC with a weight loss of 11.04%, 10.00% and 8.434% for 0 kGy, 50 kGy and 100 kGy irradiation, respectively with a corresponding endotherm in DTA indicating the loss of moisture. The second degradation is observed around 250 to 300ºC with weight loss of 40.24%, 37.48% and 36.76% for 0 kGy, 50 kGy and 100 kGy respectively[20]. Sharp exotherms were observed around 300ºCindicating the possible disruption of polysaccharide ring of chitosan. The overall weight loss for 0 kGy chitosan was 100% whereas 50 kGy and 100 kGy have 76.74% and 73.91%, respectively. There was a gradual increase in thermal stability of chitosan with increase in the irradiation dose probably due to formation of more stable chitosan oligomers[21]. Fig. 3(c) depicts the TGA spectra of alginate biopolymer with first degradation around 100ºC with weight loss of 12.33% for 0 kGy and 8.16%, 7.84% for 50 kGy, 100 kGy respectively. Second degradation is observed around 200ºC with weight loss of 35.33%, 34.60% and 33.46% for 0 kGy, 50 kGy and 100 kGy, respectively. Whereas DTA shows a sharp peak around 400ºC indicating the disintegration of guluronic chains of alginate[13]. There was a regular decrease in overall weight loss for alginate with 90.06% weight loss for 0 kGy and 85.52%, 81.23% for 50 kGy and 100 kGy alginate respectively[22-24].

X- ray diffraction studies

The effect of gamma irradiation on X ray diffractograms of chitosan and alginate were studied. In native chitosan, two peaks were obtained at 2θ values of 10.24° and 19.67° while for alginate, the 2θ values for two peaks were 13.04° and 24.67° (Fig. 4). In case of irradiated samples, no shifting of peaks was observed in chitosan and alginate which indicates that after conversion from polymer to oligomer form, the structural units remain same. Average crystallite size was calculated by Scherrer equation and it was

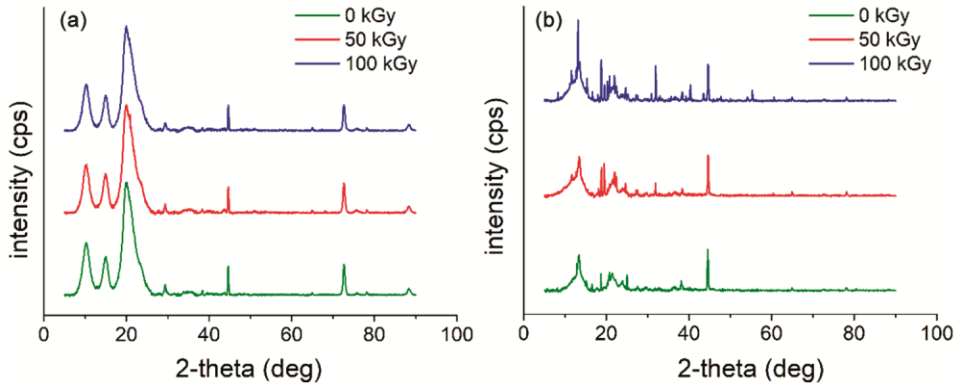

Fig. 4 — XRD pattern of original and gamma irradiated polymers at different doses (a) Chitosan (b) sodium alginate

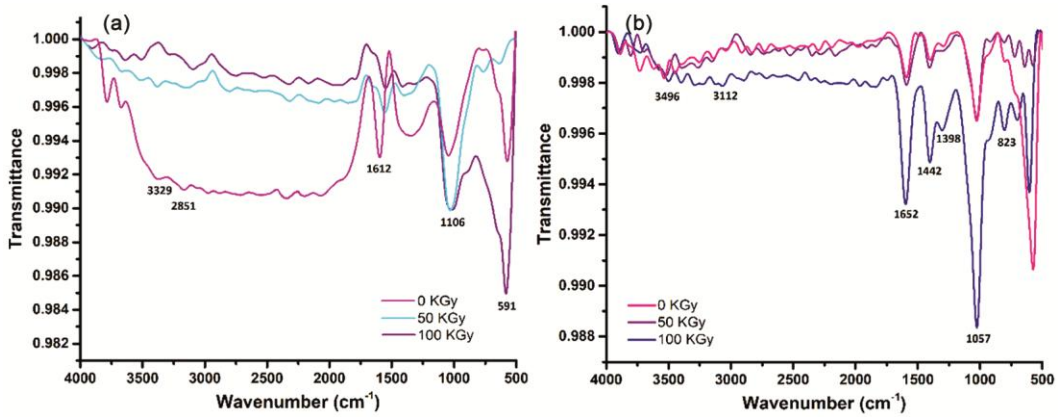

Fig. 5 — FTIR spectra of (a) Chitosan (b) Sodium alginate at various gamma irradiation doses

observed, that native polymer has larger crystalline size which goes on decreasing with the dose of irradiation. The crystallite size of chitosan decreases in the range of 36.58 nm to 12.20 nm whereas sodium alginate crystalline size decreases from 72.59nm to 24.20nm for varying irradiation dose from 0 to100kGy. The decrease in crystallite size was due to change in full width at half-maximum (FWHM) value as dose increases. This is mainly due to intrinsic defects in the polymeric crystals which are dominant over gamma irradiation[25].

FT-IR spectra

FT-IR spectra of chitosan and sodium alginate before and after gamma radiation at different doses were recorded. Fig. 5(a) shows FT-IR spectra of chitosan with a broad peak at 3329 cm^{-1} and 2851 cm^{-1} indicating the O-H and N-H stretching vibrations of chitosan biopolymer, respectively[22-24]. A strong peak at 1612 cm^{-1} was observed indicating the traces of N-acetyl group of chitin for 0 kGy chitosan whereas a decrease in intensity is observed for the same in 50kGy and 100 kGy irradiated chitosan. The reason for this can be attributed to the decrease in the degree of deacetylation of chitosan biopolymer. This is in accordance with the results obtained for DDA.A characteristics skeletal vibration of -C-O-C- bond was observed at 1106 cm^{-1} and C-H stretching vibration was observed at 1465 cm^{-1} wavenumber[20]. A broad spectrum was observed for 0 kGy chitosan (native chitosan), whereas a sharp spectrum was observed for 50 kGy and 100 kGy chitosan. The reason of sharpness in the spectra may be attributed to the radiation mediated degradation of chitosan resulting in oligomers[21]. On gamma irradiation, the peak at 1106 $cm^{-1}$was found to reduce in intensity. This is a clear indication that the C-O-C linkage is being cleaved due to gamma rays.

Fig. 5(b) shows FTIR spectra of alginate with weak bands at 1442 cm^{-1} and 1398 cm^{-1}, corresponding to O-H bending vibrations and C-C-H stretching vibrations of pyranose rings, respectively. A distinct characteristic peak of mannuronic acid at 823 cm^{-1} is observed with a peak of C-O stretching vibration at

1057 cm^{-1}. A broad band stretching vibration at 3496 cm^{-1} is observed for hydrogen bonded O-H group with a weak peak at 3112 cm^{-1} of C-H stretching vibration. An intense band at 1652 cm^{-1} was observed indicating the asymmetric stretching vibration of carboxylate group. The spectra of 100 kGy were more intense as compared to that of 0 kGy and 50 kGy alginate due to gamma degradation resulting in breaking in chains of the copolymer[8]. Gamma irradiation has shown effect on all of these bands thereby decreasing their intensity.

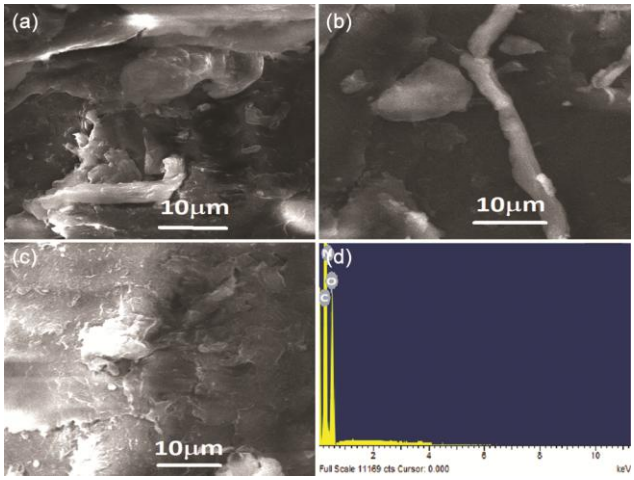

Fig. 6 — (a) native chitosan (b) Gamma-irradiated chitosan (50kGy) (c) Gamma-irradiated chitosan (100kGy) (d) EDS spectrum of chitosan.

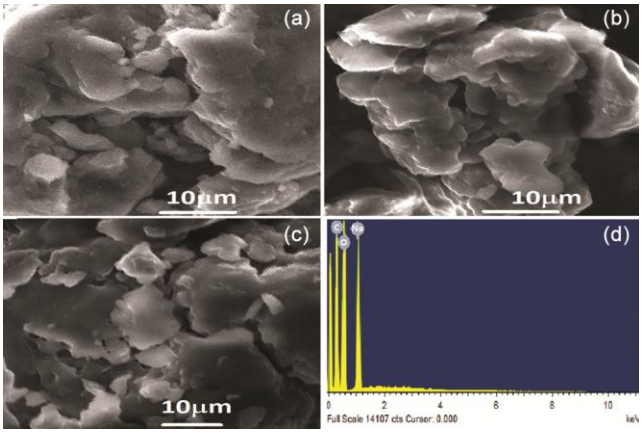

Fig. 7 — (a) native alginate (b) Gamma-irradiated alginate (50kGy) (c) Gamma-irradiated alginate (100kGy) (d) EDS spectrum of alginate.

Surface morphology and elemental analysis

The SEM micrographs before and after chitosan and alginate irradiation are shown in Fig. 6 and 7, respectively. Considering the morphology of polymers, the size of the particles and surface has significant difference after irradiation. It was observed that with increase in gamma dose the surface of the chitosan becomes more rough, where as in alginate the size of the particles are gradually reduced[26]. Similar EDS spectra of unirradiated and irradiated polymers show that there is no change in the elemental composition of the polymers[27,28].

UV-Vis spectra

UV-Vis spectra of native chitosan and alginate were compared with the gamma irradiated chitosan and alginate at different doses to evaluate the change in the λ_{max} as well as the molar absorptivity[29]. It was observed that similar adsorption patterns were obtained as noticed from the λ_{max} for native and irradiated polymers (Fig. 8). In case of irradiated chitosan samples, the peaks were obtained between 250 nm and 280 nm. Slight shift in λ_{max} values with increase in intensity were observed. The two peaks are assigned to carbonyl and carboxyl group. Shifting of peaks can be due to change in degree of deacetylation[30].The trend obtained refers towards the degradation of polymeric chain of chitosan into the oligomeric form. The fragments of chitosan with C=O group generated due to gamma irradiation. This can also be noticed from the colour changes in chitosan and also

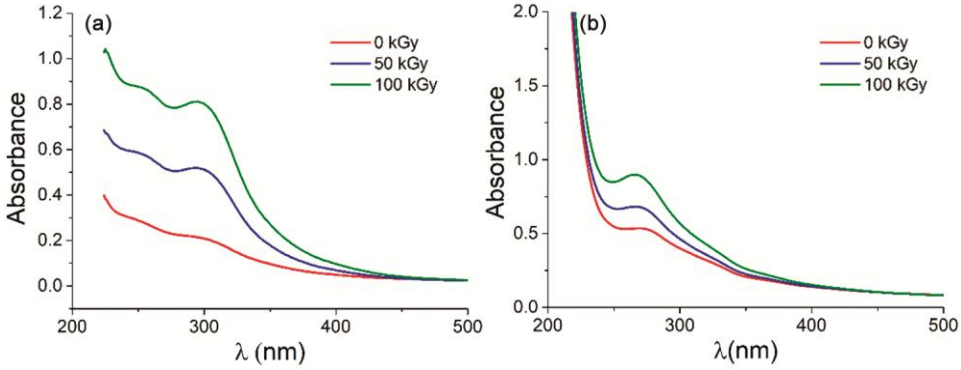

Fig. 8 — UV-Vis spectra of original and gamma irradiated polymers at different doses (a) chitosan and (b) sodium alginate

confirmed from FTIR analysis. Similarly, alginate has been converted into smaller oligomeric after gamma irradiation. Absorption band at 265nm has appeared and its intensity increases with exposure dose[31]. This peak can be due to double bonds formed after the main chain scission of the polymer followed by the ring opening[32].

Conclusion

In this paper, chitosan and sodium alginate were degraded by gamma radiation. Firstly, we studied the effect of molecular weight and degree of deacetylation on degraded polymers. The results show that as we increase the irradiation dose the molecular weight exponentially decreases. Similarly, degree of deacetylation goes on decreasing as we increase the dose. Gamma degraded chitosan and sodium alginate were further characterized. Thermal study shows that there is overall weight loss for 0 kGy chitosan with 100%, whereas 50 kGy and 100 kGy have 76.74% and 73.91% respectively. There was a gradual increase in thermal stability of chitosan with increase in the irradiation dose probably due to formation of more stable chitosan oligomers. In case of alginate, there was a regular decrease in overall weight loss for alginate with 90.06% weight loss for 0 kGy and 85.52%, 81.23% for 50 kGy and 100 kGy alginate, respectively. XRD analysis depicts that native polymer has larger crystalline size which goes on decreasing with the dose of irradiation.FTIR analysis shows, a broad spectrum was observed for 0 kGy chitosan (native chitosan) whereas a sharp spectrum was observed for 50 kGy and 100 kGy chitosan. For alginate, the spectra of 100 kGy was more intense as compared to that of 0 kGy and 50 kGy alginate due to gamma degradation resulting in breaking in chains of the copolymer. SEM images shows with increase in gamma dose the surface of the chitosan becomes rougher, whereas in alginate the size of the particles are gradually reduced. UV-Vis spectra of both the polymers were recorded. In case of irradiated chitosan samples, the peaks were obtained between 250 nm and 280 nm. Hence radiation degradation of biopolymers with the help of gamma include ability to promote changes reproducibly and quantitatively without introduction of chemical changes.

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References

1 Gryczka U, Dondi D, Chmielewski A G, Migdal W, Buttafava A & Faucitano A, *Radiat Phys Chem*, 78 (2009) 543.
2 Gad Y H, *Radiat Phys Chem*, 77 (2008) 1101.
3 Nagasawa N, Mitomo H, Yoshii F & Kume T, *Polym Degrad Stab*, 69 (2000) 279.
4 Hien N Q, Phu D V, Duy N N & Lan N T K, *Carbohydr Polym*, 87 (2012) 935.
5 Gonçalves C, Ferreira N & Lourenço L, *Polymers*, 13 (2021) 2466.
6 Pandit A, Indurkar A, Deshpande C, Jain R & Dandekar P, *Carbohydr Polym Technol Appl*, 2 (2021) 100033.
7 Chmielewski A G, *Radiat Phys Chem*, 79 (2010) 272.
8 Ravi Kumar M N V, *React Funct Polym*, 46 (2000) 1.
9 Leonhardt J, Arnold G, Baer M, Langguth H, Gey M & Hübert S, *Radiat Phys Chem*, 25 (1985) 887.
10 Yue W, Yao P, Wei Y & Mo H, *Polym Degrad Stab*, 93 (2008) 1814.
11 Kang B, Dai Y, Zhang H & Chen D, *Polym Degrad Stab*, 92 (2007) 359.
12 Kasaai M R, *Carbohydr Polym*, 68 (2007) 477.
13 Fertah M, Belfkira A, Dahmane E, Montassir, Taourirte M & Brouillette F, *Arab J Chem*, 10 (2017) S3707.
14 Renata C, Diana J, Bozena R, Piotr U & Rosiak J, *Progress Chem Appli Chitin*, 17 (2012) 2186.
15 Tandekar S, Saravanan D, Korde S & Jugade R, *Mater Today Proc*, 29 (2020) 726.
16 Gupta K C & Jabrail F H, *Int J Biol Macromol*, 38 (2006) 272.
17 Mario A, Nilia P, Cristina C, Jose L, Manuel R, Robin Z, Piedad G & Alicia C, *J Rad Res App Sci*, 8 (2015) 190.
18 Rabiul H, Murshid I & Tarun K M, *Int J Adv Eng Appl*, 6 (2013) 4.
19 Chudinova Y V, Kurek D V & Varlamov V P, *Prog Chem Appl Chitin Der*, 21 (2016) 18.
20 Gomase V, Jugade R, Doondani P, Deshmukh S, Saravanan D & Pandey S, *Int J Biol Macromol*, 223 (2022) 636.
21 Mahmud M, Naziri M I, Yacob N, Talip N & Abdullah Z, *AIP Conference Proceedings*, 1584 (2014) 136.
22 Tandekar S, Pande M, Shekhawat A, Fosso-Kankeu E, Pandey S & Jugade R, *Minerals*, 12 (2022) 874,
23 Shekhawat A, Kahu S, Saravanan D & Jugade R, *Int J Biol Macromol*, 104 (2017) 1556.
24 Shekhawat A, Kahu S, Saravanan D & Jugade R, *Carbohydr Polym*, 172 (2017) 205.
25 Gili M, Pares F, Nery A, Guillermo N, Marquez E & Olegario E, *Mater Res Express*, 6 (2020) 125552.
26 Rao R N, Rao V T, Reddy R & Rao S, *J Radiat Res Appl Sci*, 8 (2015) 621.
27 Baysal K, Aroguz A, Adiguzel Z & Baysal B, *Int J Biol Macromol*, 59 (2013) 342.
28 Gokila S, Gomathi T, Sudha P & Anil S, *Int J Biol Macromol*, 104 (2017) 1459.
29 Kowalonek J, *Int J Biol Macromol*, 103 (2017) 515.
30 Muley A, Shingote P, Patil A, Dalvi S & Suprasanna P. *Carbohydr Polym*, 210 (2019) 289.
31 Wasikiewicz J, Yoshii F, Nagasawa N, Wach R & Mitomo H, *Radiat Phys Chem*, 73 (2005) 287.
32 Lee D, Choi W, Byun M, Park H, Yu Y & Lee C, *J Agri Food Chem*, 51 (2003) 4819.