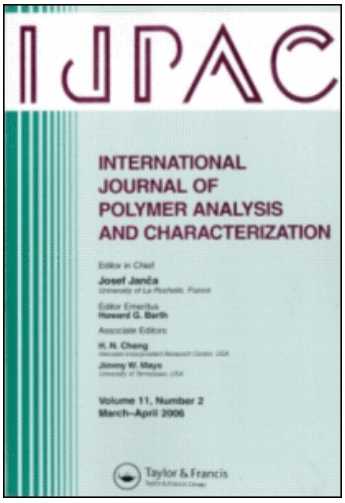

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GPC Studies of Chitosan Degradation

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Investigated were the changes in the molecular character of chitosan resulting from its degradation by the hydrolytic, radiation, and hydrolytic-enzymatic methods. It was found that under the influence of γ-^{60}Co radiation, degradation proceeds in a manner implying a random break-up of the polymer chains with a systematic decline of sample polydispersity with increasing irradiation dose. In the hydrolytic and hydrolytic-enzymatic degradation processes the differences in the resulting changes depend on chain length. While the short macromolecules degrade similarly as by radiation, the longer chains degrade less readily, which can be inferred from the molecular weight distribution curves as well as from the polydispersity.

Keywords: Chitosan, degradation of chitosan, gel permeation chromatography

INTRODUCTION

Chitin next to cellulose, is the most widespread polymer in nature. The most important source are the coats of shellfish and other sea-living organisms. Chitin also appears in the tissues of insects and cell walls of some fungi. Chemically, chitin is a polymer described as N-acetyl-2-amino-2-desoxy-D-glucose and, therefore, similar to cellulose.

Since, however, chitin is insoluble in water and in most organic solvents practical applications are limited. Nevertheless, there are certain polymers,

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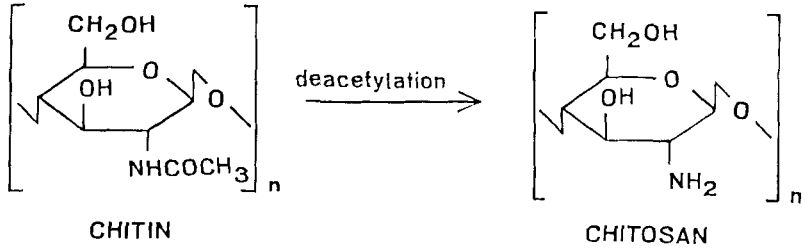
the products of chemical modification of chitin, that possess improved solubility in addition to a number of other valuable properties. Among the chitin-derived polymers, the most important is deacetylated chitin, called chitosan. In deacetylation, the N-acetyl group of the chitin is transformed into an amine group, shown in Figure 1.

Practically, the reaction of deacetylation is always incomplete and, therefore, commercial chitosan contains mers with both N-acetyl and NH_2 groups. Progress of the reaction is measured by the so-called deacetylation degree, expressed as molar percent of N-acetyl groups transformed into amine groups. The value of this index largely accounts for the properties of the resulting chitosan and beside the molecular weight, it is the most important component of the physicochemical characterization of the polymer in a molecular level [1-4].

Most of the actual and potential applications of chitosan are connected with its partial or complete degradation. The polymer undergoes degradation under the influence of certain chemical, physical or biological factors [2,5-8]. Of special value is the capability of chitosan to degrade under the influence of mammal (including human) body fluids. Chitosan preparations are characterized by bioactivity and biocompatibility [2]. In medical applications, of utmost importance is, of course, the biodegradability of chitosan, but degradability under the influence of ionizing radiation is also important, since the chitosan-containing medical materials are often sterilized by radiation [8,9].

Chitosan biodegradation is brought about by enzymes produced by various micro-organisms. Chitosans are easy to biodegrade. As shown by our experiments, micro-organisms present in active sludge taken from the



CHITIN CHITOSAN

FIGURE 1 Scheme of chitin deacetylation.

waste water treatment plant of a pulp-and-paper manufacturing establishment can completely decompose chitosan in a 12-week treatment in an aqueous medium with aeration [10]. Biodegradation of chitosan can also be affected using selected enzymes, such as lysozyme present in the body fluids of mammals [6,8].

Chitosan biodegradation involves changes in its structure at the molecular, supra-molecular, and morphological levels. The investigation discussed here concerns the molecular changes being the result of ionizing radiation, hydrolytic action of a buffer solution, or a combination of the latter with lysozyme. The determinations were made using GPC (gel permeation chromatography).

EXPERIMENTAL

Materials

The material selected was shrimp chitosan (Chemopol, Complex PVT. LTD, Tada, India). The polymer had a deacetylation degree of 82.7% and contained 7.3% nitrogen and 0.44% ash. Its viscometric-average molecular weight, determined according to ref. [11] was 470,000. The commercial flake-form product was milled and screened to sift out a 0.8–1.25 mm fraction. The same material was used in all experiments.

Ionizing Radiation Degradation

The chitosan samples, weighing 20 g each, were placed in tight-sealed plastic bags and were subjected to γ-radiation from a ^{60}Co source. The activity of the radiation chamber was 20 kCi and the dose rate was 5 kGy/h. The applied doses were 5, 10, 20, 50, 100 and 150 kGy.

Hydrolytic Degradation

Degradation of chitosan flakes was carried out in a phosphate-citric buffer solution of pH 7.23 at a chitosan/buffer ratio of 1:300 under heterogeneous conditions. The test was performed in an incubator at 37°C under static conditions. All the tested samples in the baths were sterilized, prior to test, with superheated steam at 121°C for 15 min. The samples were removed

from their baths by centrifugation at predetermined time intervals, that is after 3, 7, 14, and 28 days. After removal each sample was washed first with distilled water at 50°C and then with 70% ethyl alcohol. The washed samples were then dried to a constant weight at 45°C.

Enzymatic Degradation

Enzymatic degradation was carried out similarly as hydrolytic degradation except that lysozyme, (mucopeptide N-acetyl-muramyl-hydrolase) was added. Lysozyme was a product of Merck, Germany, and its activity was 100,000 U/mg. It was added after the sample in the buffer solution had been sterilized and cooled to ambient temperature. The amount added was such as to make its concentration in the bath 300 mg/dm^3.

Similarly, as in the case of hydrolytic degradation, the preparations were removed from the bath after 3, 7, 14, and 28 days. The sample was treated similarly to the hydrolytic degradation studies.

Characterization by GPC

The samples of chitosan were characterized by GPC. The following instrumentation was used: HP 1050 chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a RI detector HP 1047A; PL-GFC 4000 A and PL-GFC 300 A columns; and PL Caliber™ GPC/SEC Software Version 5.1 (Polymer Laboratories, Ltd. Shropshire, UK).

The molecular parameters of chitosan were calculated on the basis of universal calibration method using K and a values from the Mark-Houwink equation for chitosan of deacetylation degree 80% [12]. The system was calibrated using the standards poly(ethylene oxide) and poly(ethylene glycol) of M_w from 1,400–930,000. Figure 2 represents the calibration curve. K and a values for these standards were determined in our laboratory applying the above described GPC system additionally equipped with a differential viscometer detector H 502 B (Viscotek, Houston, Texas, USA).

The samples of chitosan (10 mg each) were dissolved in 10 ml of mobile phase (0.33M acetic acid and 0.2 M sodium acetate). The solutions were filtered through Sartorius SM 12303 filters (1.2 μm) (Sartorius GmbH, Göttingen, Germany). The amount injected was 70 μL; the flow rate was 1 mL/min; temperature of the columns was 25°C.

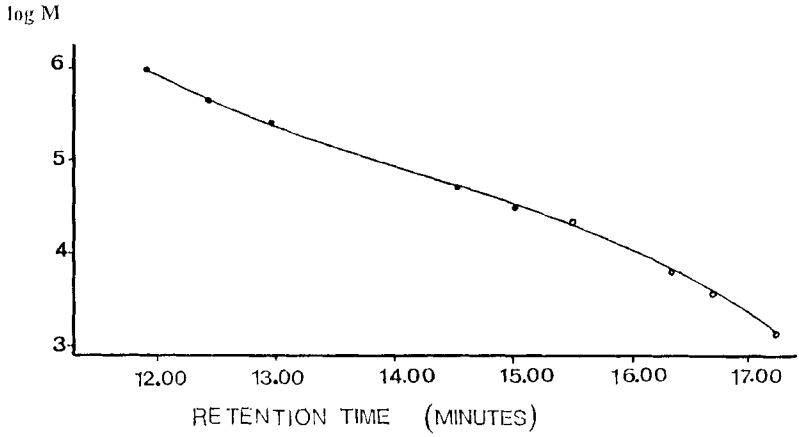


FIGURE 2 Calibration curve for poly(ethylene oxide) (●) and poly(ethylene glycol) (○) standards.

DISCUSSION OF RESULTS AND CONCLUSIONS

The process of degradation by radiation is illustrated by Figure 3; the changes taking place in the process are numerically characterized in Table I.

The shape of the molecular weight distribution (Fig. 3), and also the changes in polydispersity expressed by the ratio M_w/M_n suggests that degradation by radiation is associated with a random, fully statistical breaking-up of the chains with the reduction of M_n and M_w values and polydispersity. The process implies that degradation is independent of the macromolecular structure and chemical composition of the polymer chains. This interpretation is corroborated by the systematic decline in the values of M_w/M_n, disappearance of the highest fractions, and "smoothing" of the molecular weight distribution curve.

In the hydrolytic degradation in the citric-phosphate buffer medium, the process differs from the above, especially in its first phase. The susceptibility to degradation of lower and higher fractions of the polymer is noticeable and no significant changes, during degradation, in the low-molecular part of the MWD curves, presented in Figure 4, are observed. Similarly, as in the degradation by radiation (Fig. 3), it was observed that with progressing degradation, the curves show a gradual shift towards lower molecular weight values.

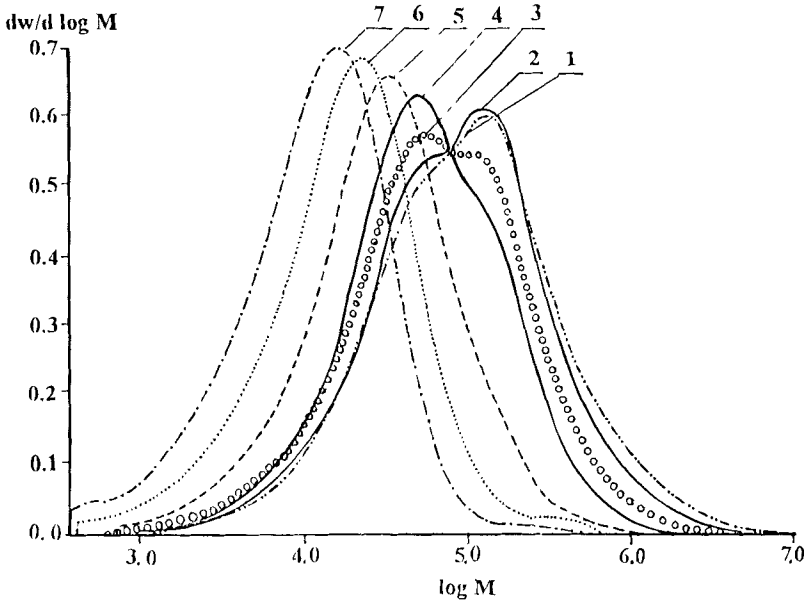


FIGURE 3 Molecular weight distribution curves of degraded chitosan caused by ionizing radiation. 1. initial sample; 2. sample irradiated with 5 kGy; 3. 10 kGy; 4. 20 kGy; 5. 50 kGy; 6. 100 kGy; 7. 150 kGy.

The introduction of lysozyme augments the molecular weight changes as shown in Figure 5. The enzyme readily affects the lower fraction, while the high fractions are less susceptible to changes, which is particularly clear in the first phase of the degradation process. There are basic differences between the after-three-days values of M_n presented in Tables II and III. It is also worth noting that throughout the process, the polydispersity remains practically at a constant level. As can be seen, enzymatic degradation of the

TABLE I Molecular Characteristics of Degraded Chitosan Caused by Ionizing Radiation

No.	*Dose [kGy]*	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n
1	0	38	270	7.1
2	5	35	198	5.7
3	10	27	138	5.1
4	20	27	102	3.8
5	50	16	55	3.4
6	100	9	32	3.6
7	150	6	19	3.2

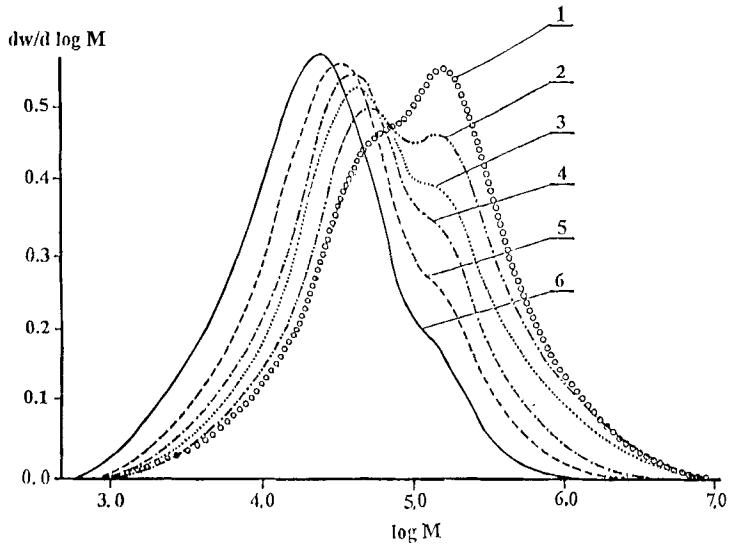


FIGURE 4 Molecular weight distribution curves of hydrolytically degraded chitosan. 1. initial sample; 2. sample after sterilization (15 min at 121°C); 3. after 3 days of degradation; 4. after 7 days; 5. after 14 days; 6. after 28 days.

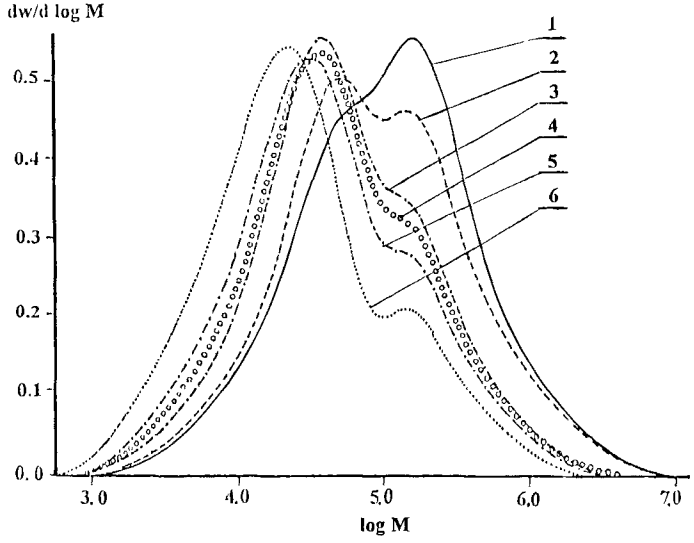


FIGURE 5 Molecular weight distribution curves of enzymatically degraded chitosan. 1. initial sample; 2. sample after sterilization (15 min at 121°C); 3. after 3 days; 4. after 7 days; 5. after 14 days; 6. after 28 days.

TABLE II Molecular Characteristics of Hydrolytically Degraded Chitosan

No.	*Time of Degradation*	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n
1	0	38	270	7.1
2	sterilized	32	265	8.3
3	3 days	27	220	8.1
4	7 days	21	121	5.8
5	14 days	17	83	4.9
6	28 days	11	48	4.4

lower polymer fractions is similar in character to hydrolytic degradation and degradation by radiation; whereas, degradation of the high fractions is fairly stable under the process conditions.

The hydrolytic and enzymatic degradation processes yield a certain amount of oligoaminosaccharides that are soluble in the buffer solution used in the experiment. The yield of oligoaminosaccharides is important in that the compounds are decisive for the bioactivity of chitosan during degradation. It has been found that these substances can accelerate the healing of wounds [13]. The biological activity of the various commercial forms of chitosan can also be used for plant protection [14].

Hydrolytic degradation of chitosan is characterized by low total concentration of oligoamidosaccharides (20 mg/dm^3) being formed, while during enzymatic degradation, the oligoaminosaccharides concentration rises to 60 mg/dm^3 and depends on the molecular weight of chitosan used. Obviously, for a lower initial molecular weight, higher aminosaccharides concentration is observed.

Consistently with these results, is our observation that there is a weight loss of chitosan as the degradation process progresses. This is largely

TABLE III Molecular Characteristics of Enzymatically Degraded Chitosan

No.	*Time of Degradation*	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	M_w/M_n
1	0	38	270	7.1
2	sterilized	32	265	8.3
3	3 days	22	156	7.1
4	7 days	20	131	6.6
5	14 days	17	115	6.8
6	28 days	11	76	6.9

dependent on the form of chitosan and on process conditions. Thus, chitosan weight loss after 28 days was 6–8% during hydrolytic degradation and up to 20% in enzymatic degradation.

Generally it can be stated that the processes of chitosan degradation distinctly differ from one another. When evaluating by means of GPC the degradation caused by ionizing radiation, the influence of supramolecular structure is unnoticeable. This can be ascribed to the fact that the high energy of ionizing radiation acts throughout the total volume of the sample and statistical breaking-up of polymer chains does not depend on the polymer supramolecular structure. The continuous decrease of polydispersity (Table I) results from the proportionality of the number of breaks to the length of the macromolecular chain. The course of chemical and chemical-enzymatic degradation depends mainly on polymer chain accessibility to degrading factors. In turn, the accessibility depends on the degree of supramolecular structure, as well as on the presence of N-acetyl group aggregation among macromolecular chains [15]. The higher the content of crystalline structure and the lower the degree of deacetylation, the more difficult are both chemical degradation and enzymatic digestibility. Thus, macromolecules of the amorphous part of a polymer, having a high degree of deacetylation, are particularly exposed to the degradation action of buffer or enzyme solutions.

Selectivity of degradation occurring in chemical and enzymatic degradation can result from the lower content of smaller macromolecules in the crystalline structure and also from the higher probability of the presence of N-acetyl group aggregation among the longer macromolecules.

The observed increase of polydispersity after sterilization can be interpreted as a result of the rapid acceleration of degradation of short- and average-length chains during the short but significant temperature rise. Most of the polymer degradation processes for both natural and synthetic polymers occur with a simultaneous decrease of polydispersity. It is worth noting that the observed stability of polydispersity in the case of enzymatic degradation (Table III) occurs also during deacetylation of chitin [16]. In the described process, the degree of polymerization has dropped by about 50% while the polydispersity remained at the same level (4.8) in spite of the drastic conditions required in the course of the deacetylation process. The above example can be considered as indirect evidence of the effect of structure on chitin and chitosan processing.

Acknowledgements

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