OVERVIEW ON KINETICS OF DEGRADATION PROCESSES FOR CHITIN AND CHITOSAN

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Abstract

This study provides a brief overview on kinetics of chitin and chitosan degradation. The expression of degradation was used for any changes in molecular weight or degree of acetylation, DA. Degradation of the polymers may occur in solid state or in solution. These polymers are sensitive to environmental atmosphere. The degradation in nature and solid state may result from the action of chemicals (oxygen, impurities such as metal ions and salts), bio-chemicals (micro-organisms such as bacterial enzymes), or energy generated from Sun (ultraviolet/ visible and thermal). The kinetics of degradation in solid state depends on degree of crystallinity, molecular weight, and the degree of acetylation, DA. Amorphous polymers are more susceptible to degradation than that of crystalline ones.

Degradation of chitin/ chitosan by chemical reagents (acids, alkali, oxidative reagents) in solution was random process. In presence of air, oxygen or oxidative reagents (H_2O_2, HNO_2, $NaNO_2$, $K_2S_2O_8$ and $NaBO_3$) free radicals was observed and the degradation process was initiated by generated free-radicals. Degradation of the polymers by enzymes did not follow a single kinetics model. Some enzymes followed Michaelis-Menton kinetics model at low substrate concentration. The interaction of the polymers with mechanical energy obeyed first-order kinetics. Free-radical mechanism was more possible for photo-degradation.

Introduction

Chitin and chitosan, two naturally occurring polysaccharides, are susceptible to degradation in nature [1-3]. In laboratory, the degradation processes performed by using chemical, bio-chemical reagents or applying energy [4-8]. In this study, the expression of degradation was used for any changes in molecular weight or degree of acetylation, DA, in different processes (deacetylation, acetylation, decomposition, depolymerization, and fragmentation). Two types of degradation processes are important for chitin/ chitosan: main chain scission (scission of β, 1, 4-D-glucosic linkage or depolymerization); and side chain reaction (N-deacetylation). Various properties of the two polymers depend on two fundamental parameters: the degree of acetylation and the degree of polymerization [9]. Alkaline hydrolysis (N-deacetylation) converted N-acetyl groups into free amine groups on the macromolecular backbone and an increase in aqueous solubility and reactivity of the polymer, whereas acidic hydrolysis breaks ether linkages of the macromolecule chains; facilitates preparation of its derivative [10]; preparation of its solution and some cases increase its performance and efficiency. The degradation processes occurred by using chemical, bio-chemical

reagents or applying energy. The degradation occurring in nature can be either desirable or un-desirable. The degradation in laboratory mainly performs to increase their efficiency, performance and applications.

The polymers obtained from some sources possess high molecular weight and use of high-molecular weights generates practical limitations in processing. In addition, preparation of their derivatives using high molecular weight is a difficulty. This is because they generate high solution viscosities. Furthermore, various micro-organisms cause main or side chain scission due to their biodegradation behavior. The effectiveness of the polymers in various applications depends on their molecular weights and chemical structure (DA). As a result, reduction of their molecular weights through a degradation process is a solution. In addition, knowledge on their biodegradation process enables one to predict and estimate (in some extend) their chemical structure, morphology or molecular size. Degradation process may change their chemical structure; properties; efficiency; performance; and applications. Thus: optimum conditions (such as time, temperature, concentration of reagent, or polymer concentration) for the two reactions can be achieved through studies on their kinetics of reactions; a better understanding of kinetics of deacetylation and depolymerization reactions would be yielded in useful and desirable information for production of chitin and chitosan with specific DA and M_w; and the cost of processing can be minimized through studies on their kinetics. The objective of this study was an overview of the literature data on kinetics of chitin and chitosan degradation.

Degradation Methods

Degradation methods are classified as follows: (1) chemicals: hydrolysis with acid or alkali and oxidative; (2) bio-chemicals using specific enzymes, chitinase and chitosanase, and non-specific enzymes such as lysozyme, lipases, papin, pepsin, pronase and etc; and (3) energy generating from: mechanical [shear force; vibration energy (ultrasound); turbulent flow; cavitation forces; or high pressure (microfluidization)]; photons (conventional, laser, gamma, x-ray); charged particles and neutrons; and heat sources.

Evaluation of Literature Data on Kinetics of Degradation

Degradation in Nature

Chitin and chitosan are sensitive to environmental atmosphere. The action of chemicals (oxygen, impurities such as metals, metal ions or salts); bio-chemicals (micro-organisms such as bacterial enzymes); or energy generated from sunlight (ultraviolet/ visible and thermal) may cause degradation of chitin and chitosan in nature and in solid state. The kinetics of degradation in solid state depends on degree of crystallinity, molecular weight, and the degree of acetylation, DA. Amorphous polymers are more susceptible to degradation than that of crystalline ones. In general, kinetics behavior of chitin is affected by physical state of chitin/ chitosan. The accessibility of glucosidic linkages of chitin/ chitosan macromolecules to H+, other chemical reagent or energy in solid state depends on chemical structure of the polymers (DA, sequence of co-monomer units); morphology and hydrogen bonds. Thus, all glucosidic bonds are not equally accessible and reactive [11-13]. The stability of the polymers in solid state depends on their environments. The polymers can be protected from degradation in inert atmosphere when they are relatively far from enzymes, or other impurities.

Degradation in Laboratory

Chemical degradation

Hydrolysis by acids and alkalis. Chitin extracted from crab shell was deacetylated (C_{NaOH} = 50%; T= 87-99 °C; t =1- 7 h; 3% $NaBH_4$) [11]. The reaction was second order with respect to acetamide group and OH^-. The reaction was pseudo-first order when sodium hydroxide was in excess [14]. The kinetics equation was:

$K.t = 2.303 \log C_0/ C$

where t is reaction time; K, a constant; C. the fractional concentration of acetamide groups at time t; and C_0, the original concentration of the acetamide group. The deacetylation of β-chitin extracted from squid pens (C_{NaOH} = 40%; T= 80°C; t = 3 h, under N_2 stream) was faster than that of α- chitin (extracted from shrimp), and β-chitin shown more reactivity than α- chitin [15]. In alkaline medium bellow 100°C, two reaction mechanisms are dominant: oxidative degradation in presence of oxygen; and alkaline degradation or deacetylation in which chitin converts into chitosan. The mechanism of deacetylation with NaOH was SN_2 [16]. Roger & Block [9] noted that the deacetylation of chitosan in acidic hydrolysis conditions was zero-order.

Acid hydrolysis of chitosan with DA between 0- 62 in dilute and concentrated HCl was investigated [16, 17]. These authors noted that two reactions occurred as follows: (a) cleavage of glucosidic linkage or depolymerization process and cleavage of N-acetyl linkage or deacetylation one. The rate of depolymerization was found to be equal to the rate of deacetylation in dilute acid, whereas in concentrated acid the rate of depolymerization was more than 10 times higher than the rate of deacetylation one. Chito-oligosaccharides with different degree of polymerization were prepared by using high concentration of acid at different reaction conditions (time, temperature) {18, 19]. The results indicate that the rate of degradation at high concentration of acid was significantly high. In addition, the temperature was another major parameter affecting the rate of reaction. The results indicate that the rate of degradation at high concentration of acid is high. In addition, the temperature is another major parameter affect the rate of reaction. This result indicates that main chain scission reaction occurred. Rege & Block [9] have shown the order of decetylation and depolymerization processes did not affect the results of DA, [η], or the M_w of chitosans samples, i.e. it does not matter whether one deacetylates first and then depolymerizes, or vice versa. Deacetylation of chitosan under acidic hydrolysis condition may also take place. Roger & Block [9] noted that the deacetylation of chitosan (change in DA versus time) in acidic hydrolysis conditions was zero-order, whereas depolymerization (change in molecular weight versus time) was first order. Reaction temperature significantly affected the rate of deacetylation and depolymerization process.

In acid medium at temperate bellow 100°C, hydrolysis of glucosidic bonds is dominant reaction (de-polymerization). The degradation of the polymers in presence of acid was initiated by protonation (H_3O^+) of glucoside linkage; and followed by scission of protonated linkage. The depolymerization reaction in acid medium was SN_1 [16].

The activation energies for depolymerization of two almost fully deacetylated chitosan (DA= 0.2 and DA < 0.03) were found to be 152.2 ± 8.1 and 158.1 ± 9.8 $kJ.mol^{-1}$ K^{-1}, respectively. The activation energies of two partially N-acetylated chitosans (DA= 47 and DA= 62) were determined to be 130.4 ± 2.5 and 134.3 ± 3.1 $kJ.mol^{-1}$ K^{-1}, respectively. This data indicate that the activation energy does not change significantly as a function of DA. The energies of activation for deacetylation and depolymerization processes occurring during acidic hydrolysis were calculated in accordance with Arrhenius equation [9]. The energies of activation for deacetylation (during acidic hydrolysis) were 47.7 $kJ.mol^{-1}$ K^{-1} (with shear) and 48.4 $kJ.mol^{-1}$ K^{-1} (without shear), respectively.

The energies of activation for depolymerization were 20.7 and 28.5 kJ.mol^{-1} K^{-1}, with or without mechanical shear, respectively.

A derivative of chitosan, N-succinyl-chitosan was depolymerized with 7.5 M HCl at room temperature or with 3.3 M HCl at 40°C. The molecular weight (M_w) of the products were determined by size-exclusion chromatography–multi angle light scattering (SEC–MALS) and viscometry [19]. At 40 °C, the elution profiles were quickly shifted to small molecules after reaction for 5 min. Therefore, at 40 °C, the depolymerization proceeds rapidly, and it was difficult to control the depolymerization process. However, at room temperature the process of degradation can be controlled. For example, moderate or low molecular weight chitosan can be prepared using 7.5 M HCl at room temperature. The chemical structure of the fragment obtained from hydrolysis was principally similar to the original one as examined by ^{1}H NMR spectroscopy [20].

Oxidative degradation. The degradation for partially acetylated chitosan by hydrogen peroxide followed first- order kinetics (1/[η] or 1/ M versus time was linear) with low concentrations of H_2O_2 [21]. The degradation was independent of chemical composition of chitin/ chitosan (DA). The oxidative degradation of chitosan by $NaBO_3$ was random process and average molecular weight decreased with an increase in $NaBO_3$ concentration; reaction time; but the DA of chitosan was not changed significantly within reaction time [22]. They produced a series of chitosan samples with molecular weight ranging from 880 kDa to 90 kDa by changing the amount of sodium per-borate. The value of DA was changed from original value of 0.11 to DA= 0.14. The rate of depolymerization of chitosan in dilute aqueous HCl solutions by nitrous acid was independent of molecular weight of chitosan, first order with respect to the concentrations of both nitrous acid and glucosamine moieties, not catalyzed by either hydrogen or chloride ion, and Arrhenius temperature dependent [23].
In oxidative degradation by potassium per-sulphate, the latter component as an initiator decomposes into two anionic free radicals

$$S_2O_8^{-2} \rightarrow 2\ .SO_4^{-}.$$

The anionic radical abstracted a hydrogen atom from one of glucosidic ring [24]. Subsequently the macromolecule chain at position of losing hydrogen divided into smaller macromolecules [see Scheme 3 of Ref. 24]. In oxidative degradation by hydrogen peroxide, hydroxyl free radical was formed by decomposition of hydrogen peroxide. Hydroxyl free radicals abstracted hydrogen atoms from glucosamine residues of chitosan. Subsequently macromolecule chain was split into two smaller species (at position of losing hydrogen similar to oxidative degradation by per-sulfate). Chitosan with reducing units donates a hydrogen atom to a molecule and dissociate a molecule such as abstraction of hydrogen atom from hydroxyl free radical [25].

Biochemical degradation

Chitin prepared from several biological sources and present in excess in the reaction mixtures with chitinase (as β-glucosidase) yielded in a linear Lineweaver-Buke plot (1/V versus 1/substrate concentration). The result was in good agreement with an enzyme obeying Michaelis-Menten Kinetics [26]. The degradation rates of several types of chitins (native chitin, acid- treated, acid and alkali-treated, freshly molted and pure chitin) as substrate by chitinase in vivo and in vitro were determined and compared [27]. The rate of degradation in vivo for untreated chitin (native chitin) was maximum (440 mg chitin decomposed/ day), whereas in pure chitin was minimum (25 mg chitin decomposed/ day). In vitro the degradation rates were varied from 25 to 42 mg/ day/ 10^{10} cells at 22 °C.
Specific (chitinase and chitosanase) and non-specific enzymes (hemicellusases, lysozyme, papain, lipases, glucanases, proteases etc.) enzymes were found to cleave glycosidic linkages in chitin and chitosan [28- 39]. The degradation by some non-specific proteases (pesin, papain and pronase) [40], hen egg white and human lysozyme [41] followed Michaelis-Menten kinetics. The rate increased

with an increase in DA [41]. The degradation of fully N-acetylated chitosan was easier and faster than less acetylated and lysozyme did not hydrolysis completely N-deacetylated chitosan [42]. There was an optimum value for DA, where the degradation rate was maximum value [41]. The rate increased exponentially with an increase in DA. The exponent for DA was between 3.5 and 4.5. An optimum degradation rate was found with the DA of 80 [43]. Difference in degradation rate may be due to difference in substrate solubility [43-45].

Physical degradation

Moderate to extensive mechanical degradation of polymers by shear forces followed a random scission model [46]. The rate was determined as:

$$-d(1/M) / dt = k [1/ M_L - 1/ M]$$

where k is first-order degradation rate constant; M_L is the limiting molecular weight of the polymer, M is the molecular weight of the fragments, and t is the exposure time. The rate of degradation for chitosan by irradiation with ultrasound followed first-order kinetics [47], similar to degradation by shear force. The rate was faster at the beginning and decreased gradually with time. The rate increased with a decrease in chitosan concentration and with an increase in molecular weight of original sample [47]. Chain scission versus residence time for chitosan degradation by microfluiziation process was a bi-linear [48]. The initial slope was larger than the second one. Wavelength shorter than 340 nm (280- 340 nm) yielded chain scission in chitosan and chitin. The rate increased with a decrease in wavelength (between 260 and 340 nm) and intensity of incident light [49, 50]. Chain scission versus irradiation time was sigmoidal for fragmentation of chitosan by high intense laser light [51]. The initial slope was low, followed by high rate and a final period was slow rate.

Conclusions

Degradation of chitin and chitosan in nature depends on their chemical structure (DA, sequence); morphology (degree of crystallinity), molecular weight, MWD, and the activity of chemical and biochemical (enzyme) reagents. The amorphous region was more susceptible to degradation than that of crystalline region in solid state. Degradation of chitin and chitosan in laboratory depends on above-mentioned parameters; extraction and purification procedures; and reaction conditions. The degradation of the polymers with different mode of actions yielded in a rapid reduction of molecular weights; and followed by a slow decrease. Degradation of chitin/ chitosan by chemical reagents (acids, alkali, oxidative reagents) was random (more possible). In presence of air, oxygen or oxidative reagents (H_2O_2, HNO_2, $NaNO_2$, $K_2S_2O_8$ and $NaBO_3$) free-radical was generated. The rate of degradation increased with an increase in temperature and concentration of chemical reagents. In general, degradation of the polymers by enzymes did not follow a single kinetics model. Some enzymes followed Michaelis-Menton kinetics model at low substrate concentration. Substrate inhibition was observed at high concentrations of the substrates. Gaussian model can be used for mechanical degradation. The interaction of the polymers with mechanical energy obeyed first-order kinetics. Free-radical mechanism was more possible for photo-degradation.

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