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"Green" molecular weight degradation of chitosan using microwave irradiation

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ABSTRACT

Chitosan degradation in 0.1 M acetic acid solution by microwave radiation alone (without any additives and initiators) is reported. At the condition of reduced thermal effects of microwave heating degradation manifested most of typical features associated with mechanical (and or mechanochemical) degradations induced by shear forces, in this particular case oscillating molecules. At the absorbed energy dose of 9 MJ kg^{-1} chitosan in 1% w/v solution degrades within 20 min to the weight average molecular weight of 30 kDa.

Comparative evaluation of degradation yield of chain scission (G_s) with other high energy radiations such as ultraviolet- and gamma radiation showed microwave to be the least efficient, but considering the environmental impact and low running costs it presents a viable alternative to obtain targeted molecular weights of charged polysaccharides.

1. Introduction

Distinctive functional properties of chitosan can be attributed to its polycationic nature and presence of amino groups. These unique features are readily utilised in a variety of practical applications ranging from wastewater purification [1] and metal ions chelation [2] through agriculture for seeds coating to improve yield and fungal protection [3] ending up with demanding biological and pharmaceutical fields.

Examples of high level applications of chitosan include antibacterial agent [4], fat-binding agent [5], cell protection from carcinogenesis [6], hypocholesterolemic agent [7], antioxidant [8], matrix for drug release [9] artificial skin [10] and wound dressing material [11].

However, in all abovementioned applications molecular weight determines functional properties of polymer hence the ability of its precise adjust and control is a key factor.

Especially, it applies to polymers such as chitosan since its molecular weight greatly depends on the natural origin source the polymer is extracted from. Hence, depending on the final function, a further control of polymer molecular weight may be required.

There is number of well established degradation methods that could be used for polysaccharide molecular weight control. One of the earliest methods was hydrolytic method using concentrated hydrochloric acid but it is accompanied with large amount of D-glucosamine as a side product [12]. Over the years many other improved hydrolytic methods were developed e.g. using diluted hydrochloric or nitrous acid but they produced polymers with high (~20) degree of polymerisation.

Nevertheless, even the most advanced hydrolytic method will further require neutralisation and possibly desalting steps that from one side increase the processing cost and from the other introduces additional contaminants.

The enzymatic degradation of chitosan with chitinase and chitosanase group enzymes is an efficient but still expensive way to prepare low molecular weight materials [13]. Alternatively, low cost enzymes from hydrolase glycosidic (cellulase) group can be used to degrade chitosan however with limited scale-up production ability.

A few unconventional, high purity degradation product methods including gamma, ultraviolet and sonochemical degradations of chitosan have been reported earlier [14]. The main advantage of these methods over traditional chemical or biological ones is that the addition of initiator to trigger the degradation process is not required and thus any side products are generated. Additionally, in case of gamma and ultraviolet irradiations the degradation process can be design in such way that the product can

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be sterilized in the final step [15a,b] enabling direct use as biomedical products.

Finally, a limited literature exists on the microwave induced degradation of polymer and chitosan in particular. However, most refer to hydrogen peroxide assisted method where under microwave H_2O_2 molecules are cleaved producing avalanche of hydroxide radicals responsible for the degradation of polymer [16]. Alternatively, it was also reported that addition of inorganic salts can significantly contribute to the degradation of chitosan under microwave heating [17]. In recent years reports on additives free degradation of xyloglucan [18] and chitosan [19] have been published. However, the former focused on microwave superheating effect, which we have tried to minimise here. Latter one, did not take into account energy efficiency consideration as well as did not include comparison to other degradation methods.

In this paper we present "pure" (additives or initiator free) degradation of chitosan induced by microwave exposure and discuss optimisation parameters. We have also focused on the comparative study of the presented method with other non-conventional chitosan degradation methods previously published.

2. Experimental

2.1. Materials

Chitosans from shrimp shells were purchased from SigmaAdrich, UK: 50494 (Cht) 448877(MMW) and 448869(LMW). Details of Chitosans MMW_1 and LMW_1 preparation are described in supplementary information. D_2O and DCl were both acquired from SigmaAldrich. Acetic acid was supplied by Fisher Scientific UK. Other common chemicals used in this study were purchased either from SigmaAldrich, UK or Fisher Scientific, UK. Chemicals were used as received without any additional purification.

2.2. Microwave degradation of chitosan

All solutions were prepared using water from MiliQ purification system and filtered through a 0.45 μm Milipore syringe filter. Samples of chitosan in 0.1 M acetic acid solution 5 mL were exposed to microwave radiation of 2.46 GHz generated from cooled (gaseous nitrogen) and pressurized CEM Discover generator with an Explorer auto-sampler over a range of conditions. All degradation studies were performed in 10 mL reaction vessel in triplicate and immediately after irradiation samples cooled to room temperature to eliminate possible further thermal effects.

Thermal degradation was performed by immersing microwave vials with 5 mL of chitosan samples in Grant Instruments Ltd SUB6 water bath at 89 °C for a specified time periods.

2.3. Determination of molecular weight of microwave-degraded chitosan

2.3.1. Static light scattering

Weight-average molecular weight (M_w) was determined by multi-angle static laser light scattering on a BI200S setup (Brookhaven Instruments) equipped with an Innova 70C Argon ion laser with cut off filter at 488 nm. Samples (Cht) after microwave treatment where precipitated with 1 M NaOH, centrifuged, washed with deionised water until neutral pH, lyophilised and re-dissolved in 0.1 M acetic acid followed by filtration through 0.45 and 0.22 μm Milipore syringe filters. Intensity of the scattered light was measured over the angular range 30–150° and evaluated according to Zimm treatment [20]. Refractive index increment for chitosan in 0.1 M acetic acid solution was determined as 0.1758 mL g^{-1}.

2.3.2. GPC

GPC measurements were performed by in-house chromatograph equipped with ERC 7515A DR refractive index detector. Two TSK gel columns in series (6000, 5000) were used with 0.1 M citric acid/sodium azide solution as solvent at a flow rate 0.5 mL/min. Samples after microwave treatment where precipitated with 1 M NaOH, centrifuged, washed with deionised water until neutral pH, lyophilised and re-dissolved in 0.1 M citric acid/sodium azide solution followed by filtration through 0.45 μm Milipore syringe filter. Concentration of injected sample was 0.3% w/w. The columns were calibrated with PEG standards.

2.4. Structure analysis of microwave-degraded chitosan

UV/VIS spectra of sample solution (Cht) just after microwave exposure were recorded using a Varian Cary 5000 spectrometer from 200 to 700 nm, at a rate 300 nm/min and a data acquisition interval of 0.5 nm.

Infrared spectra were recorded with a Bruker Tensor FTIR 27 spectrometer on powder samples (lyophilised solutions) at a resolution of 4 cm^{-1} over an accumulation of 32 scans. The degree of deacetylation was determined as a ratio of the amide peak at 1550 cm^{-1} to reference peak at 2877 cm^{-1} in relation to the respective intensity ratio of 100% acetylated chitin [21].

^1H NMR spectra of chitosan samples dissolved in 0.5 M DCl/D_2O solution were recorded at ambient temperature using a Bruker Avance III 400 MHz spectrometer. The degree of deacetylation was calculated as a ratio of the integral acetyl group methyl protons to all D-glucopyranose ring protons as described by Hirai et al. [22].

Degree of deacetylation, determined by ^1H NMR and FTIR spectroscopy, and weight average molecular weight as determined by light scattering of the Chitosan used in this study were respectively 95% and 66 kDa. Overlap concentration (c^*) in 0.1 M acetic acid, calculated as $1^*[\eta]^{-1}$ [23] from the intercept at zero concentration of Kreamer and Huggins plots was 0.12 g dL^{-1}, giving a reduced concentration (c/c^*) regime of studied samples between 2 and 16.

3. Results and discussion

3.1. Microwave degradation efficiency – general overview

Fig. 1 shows the change in chitosan M_w of 1% w/v solutions subjected to a microwave intensity of 100 W as a function of time (adsorbed energy). M_w was found to decrease by a factor of 2 within 10 min, decreasing by an overall factor of 3 after 40 min. Upon microwave irradiation the chitosan solution reached a equilibrium temperature of 89 ± 2 °C within 1 min and by way of comparison chitosan solution was exposed to conventional thermal treatment at 89 °C where it can be seen that the rate of molecular weight degradation is significantly lower when compared with microwave treatment. This leads us to conclude that in microwave irradiation thermal effects are not the only degradation mechanism involved, the most likely concurrent process being mechanical shear induced by molecular vibrations [24].

Change in M_w of chitosan solution subjected to microwave radiation was also monitored by gel permeation chromatography (Fig. 2). Here, we can observe similar trend of molecular weight decrease with increase of absorbed energy dose – as elongation of retention time. Shortening in σ of Gaussian peaks with irradiation time (~molecular weight decrease) also indicates narrowing of molecular weight distribution to reach value of 2.0 for the last sample.

Apparent discrepancy in M_w between SLS and GPC measurements for initial and first degraded sample it is due to several