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Microwave mediated rapid synthesis of chitosan

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Abstract Chitosan is synthesized by deacetylating chitin with NaOH solution under microwave irradiation. The process describes a rapid synthesis procedure in comparison to conventional methods. The microwave-synthesized chitosan was characterized by Ninhydrin test, Fourier transform-infrared spectroscopy and X-ray diffraction measurements. The experimental results show that the degree of deacetylation increased with increasing irradiation time. A degree of deacetylation of 85.3% was achieved after irradiating chitin with 45% NaOH solution in a microwave for 5.5 min at 900-watt power. This method can be very useful for synthesizing low molecular weight chitosan with rapid and clean chemistry.

1 Introduction

Chitosan is as an environment friendly polymer due to its biodegradability, easy availability and renewability. It is widely used in water treatment [1], chromatography [2], cosmetics, textiles [3] etc. It is a nontoxic biocompatible natural polymer having bacteriostatic and fungistatic activity [4–7] making it an interesting material for biomedical applications like drug delivery [8], gene delivery [9] and tissue engineering [10].

Chitosan is obtained by *N*-deacetylation of chitin, the second most widely available polysaccharide after cellulose. Chemically chitin is composed of β (1 → 4)-linked *N*-acetyl-D-glucosamine and chitosan is composed of β (1 → 4)-linked *N*-acetyl-D-glucosamine and D-glucosamine residues.

Several methods have been reported for preparation of chitosan from chitin such as alkali treatment at high temperature [11], alkali treatment at high temperature with intermittent washing with water [12], use of water miscible organic solvents [13] and enzymatic *N*-deacetylation [14]. Commercially chitosan is produced by *N*-deacetylation of chitin with highly concentrated sodium hydroxide solution (40–50% w/v) in high temperature and pressure. The process takes several hours to produce chitosan with significant degree of deacetylation (DD).

In recent years microwave chemistry has received much attention as it can speed up the reaction rate by orders of magnitude over conventional heating. In spite of it, the use of microwave irradiation for carrying out chemical reactions for biotechnological processes is few. Recently Nahar and Bora [15] used microwave irradiation for the covalent immobilization of proteins. Microwave irradiation has been used for the chemical modification of chitosan [16–18]. Peniston and Johnson [19] have patented a process to obtain chitosan from chitin, that also uses microwave treatment. In this communication, we report the simple and rapid method for the *N*-deacetylation of chitin for producing chitosan using microwave irradiation and subsequent characterization in detail.

2 Experimental

2.1 Material

Chitin with average molecular weight ~400 kD was obtained from Himedia, India. Sodium hydroxide, acetic acid

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and ninhydrin were purchased from Merck, India. Glucosamine hydrochloride was obtained from SRL, India. All other reagents were of analytical grade from Merck, India.

2.2 Deacetylation of chitin by microwave irradiation at constant power

Two grams of chitin were transferred to a 250 ml conical flask and 25 ml 45% w/v NaOH solution was added and mixed. The conical flask was placed on the centre of the turntable of the microwave oven (LG MC8083MLR microwave oven, LG electronics, India) and irradiated for 0.5, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 min at 900 watts. The products were filtered and washed with double distilled water until the pH of the filtrate became 7.0. The residue obtained after filtration was dried in a hot air oven at 50°C until constant dry weight was attained and were used for further analysis. In control experiment, deacetylation was carried out at 121°C temperature and 15 psi pressure for 4 h.

2.3 Ninhydrin test

Ninhydrin test was carried out according to the method of Prochazkova et al. [20] with slight modifications. Ninhydrin solution was prepared by dissolving 1 mg of reagent in 1 ml of methanol. Five hundred microlitre of ninhydrin solution were added to 2 mg of chitosan in 500 µl deionized water. The tubes were immediately capped, briefly shaken and incubated in a water bath at 70°C for 30 min then cooled below 30°C in a cold-water bath. The tubes were then vigorously stirred in a vortex mixture followed by a brief centrifugation. The solution was pipetted into a cuvette and absorbance recorded at 570 nm in Cary-100 UV–Vis spectrophotometer (Varian, USA). The DD values were calculated using glucosamine hydrochloride as a standard.

2.4 FTIR analysis

A 2% w/w mixture of chitosan and potassium bromide (KBr) was ground into a fine powder using an agate mortar and subsequently compressed into a disc. Each disc was scanned at a resolution of 1 cm^{-1} over a frequency region of 450 to 4,000 cm^{-1} using a FTIR spectrophotometer (Perkin Elmer Spectrum One, USA) and the characteristic peaks of IR transmission spectra were recorded.

2.5 XRD

X-ray diffraction of powder samples was done using a Bruker D8 advance X-ray diffractometer (Bruker Axs Inc. Germany) under the following operating conditions: 40 kV and 40 mA with Cu-Kα_1 radiation at λ 1.54184 Å and acceptance slot at 0.1 mm. Approximately 20 mg of chitosan powder was spread on a sample stage, and the relative intensity was recorded in the scattering range (2θ) of 5–40° in steps of 0.04°.

2.6 Viscometric analysis

Chitosan solutions were prepared in solvent containing 0.5 M CH_3COOH and 0.25 M NaCl. The viscosities of the solutions were measured in an Ostwald viscometer at 25°C. The solution and solvent viscosity are used to calculate the relative viscosity (η_r), specific viscosity (η_{sp}) and intrinsic viscosity ([η]).

$$\text{Relative viscosity } (\eta_r) = {}^{t}/_{t_0} \quad (1)$$

$$\text{Specific viscosity } \left(\eta_{sp}\right) = \eta_r - 1 \quad (2)$$

$$\text{Reduced viscosity } (\eta_{red}) = {}^{\eta_{sp}}/_{c} \quad (3)$$

$$\text{Intrinsic viscosity } ([\eta]) = (\eta_{red}) \text{ when } c \rightarrow 0 \quad (4)$$

where t is the running time of the sample solution, t_0 is the running time of the solvent and c is the sample concentration in g/dl. The intrinsic viscosities are obtained by extrapolating η_{red} to zero concentration.

Average molecular weights of all the samples are calculated by using classical Mark–Houwink relationship

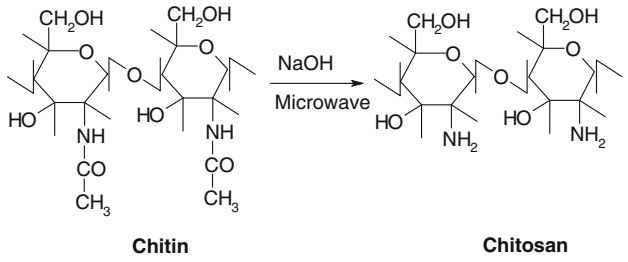
$$[\eta] = K_m M_w^a \quad (5)$$

where 'K_m' and 'a' are Mark-Houwink parameters. Values of K_m (2.14×10^{-3} dl/g) and a (0.657) were as defined by Rege and Block [21] with 0.5 M CH_3COOH-0.25 M NaCl solvent system.

3 Results and discussion

In recent years microwave irradiation has attracted a considerable amount of attention and is becoming an increasingly popular method for chemical reactions as it offers a clean, cheap, and convenient method of heating resulting in higher yields and shorter reaction times. The main advantage of microwave mediated chemistry is that it results in instantaneous 'in core' heating of materials in a homogeneous and selective manner which cannot be otherwise attained in conventional heating within a short time. Despite an increasing amount of literature on microwave chemistry, its use in biotechnology has remained limited. Here we report that microwave heating can be used to prepare a biopolymer like chitosan more efficiently than conventional heating.

In a typical experiment, chitin and highly concentrated NaOH were reacted under microwave for different time



Scheme 1 Deacetylation of chitin by microwave irradiation

periods (0.5–5.5 min). Under alkaline conditions deacetylation of chitin ($-NHCOCH_3$) to chitosan ($-NH_2$) occurs as shown in Scheme 1. The DD values of chitosan increased almost linearly with irradiation time and reached a maximum of 85.3% at 5.5 min as determined by the ninhydrin test (Fig. 1).

The formation of chitosan from chitin by microwave irradiation was confirmed by FTIR spectroscopy (Fig. 2). Chitosan has a characteristic band at $\sim$3,450 cm^{-1} which can be attributed to $-NH_2$ and –OH stretching vibration [22]. Chitin has a sharp band at 1,377 cm^{-1} which is due to the symmetrical deformation or rocking of the CH_3 group and the band at 1,626 cm^{-1} is attributable to the stretching of CN vibration of the superimposed C=O group linked to –OH group by hydrogen bonding [23, 24]. Due to high deacetylation these peaks are weakly visible in the chitosan spectra. Further in the chitosan spectra the peak at $\sim$1,645 cm^{-1} indicated that the hydrogen interactions are less accentuated and the hydroxyl groups exist freely due to removal of acetyl group [25].

The conversion process was further confirmed by XRD analysis (Fig. 3). The characteristic sharp peak of chitin at

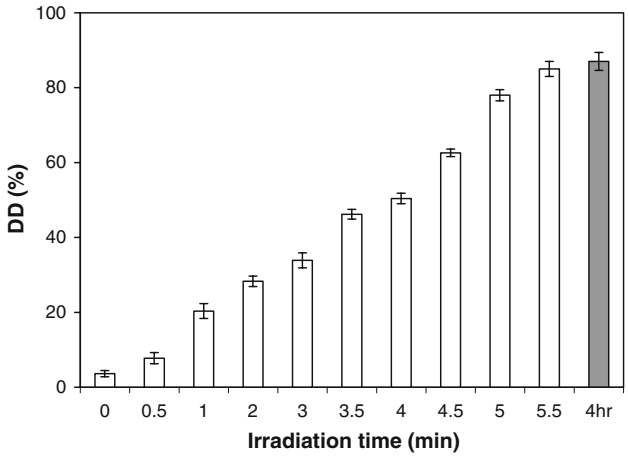


Fig. 1 DD values of chitosan after microwave irradiation for 0.5, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 min at 900 W (hollow columns) and conventional heating (solid dark column), as determined by the ninhydrin test. The DD value of Chitosan obtained at 5.5 min is comparable to that obtained at 4 h at 121°C and 15 psi (conventional heating)

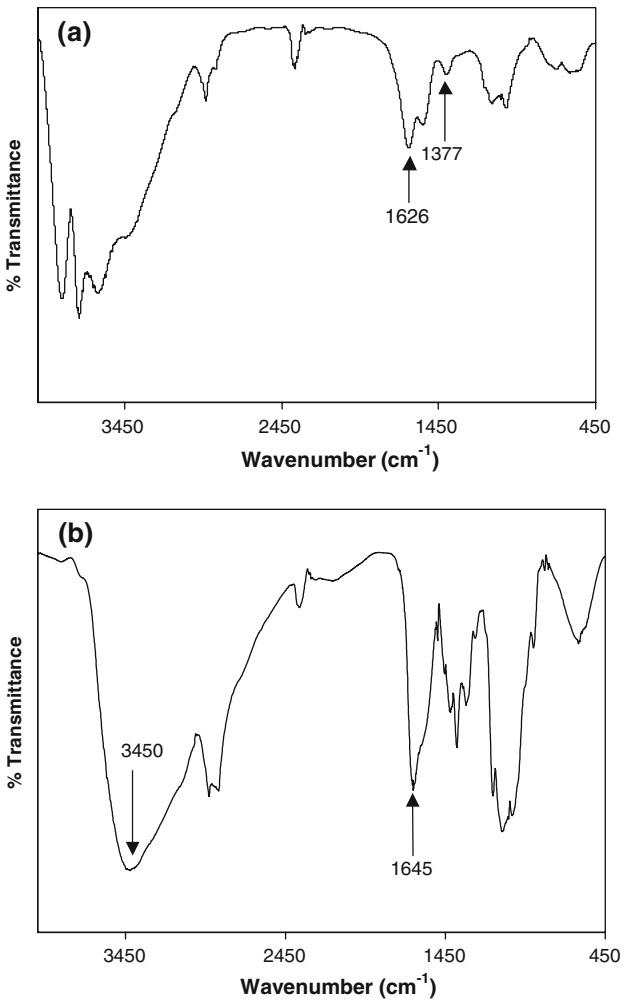


Fig. 2 FT-IR spectrum of (**a**) chitin and (**b**) chitosan formed after 5.5 min of microwave irradiation of chitin at 900 watt

2θ of 9.24° and 19.2° decreased considerably and became broad after 5.5 min of microwave irradiation. It gave clear indication of the formation of chitosan as described by Zhang et al. [26].

Finally, to determine the molecular weights of chitosan formed we carried out viscometric analysis. Viscometric analysis requires a polymer to be soluble in a suitable medium. The solubility of chitosan in acidic buffer is dependent on DD values. The chitosan formed after 4.0, 4.5, 5.0 and 5.5 min of microwave irradiation revealed good solubility and were therefore taken for viscometric analysis. We found that intrinsic viscosity of the samples decreased with increasing microwave irradiation time (Fig. 4). Table 1 shows that the molecular weight decreased as DD of chitosan increased with microwave irradiation time. Further we observed that molecular weight of chitosan prepared by microwave irradiation showed more decrease than that prepared by conventional heating technique. Liu et al. [17] observed a similar phenomenon while preparing *N*-phthaloyl chitosan using microwave

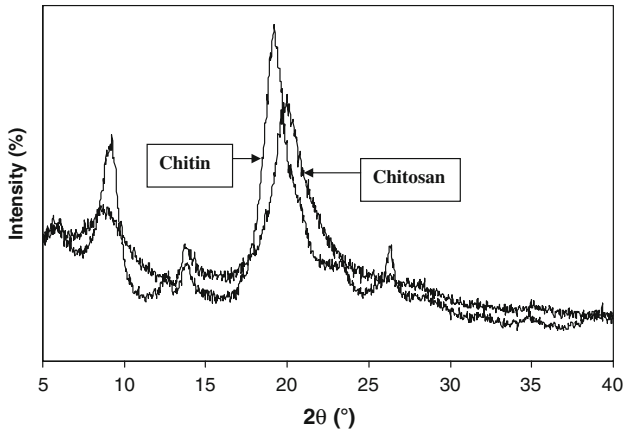


Fig. 3 Comparison of X-ray diffractograms of chitin and chitosan formed after 5.5 min microwave irradiation. Decrease of peaks at 2θ of 9.24° and 19.2° indicates the formation of chitosan from chitin due to microwave irradiation

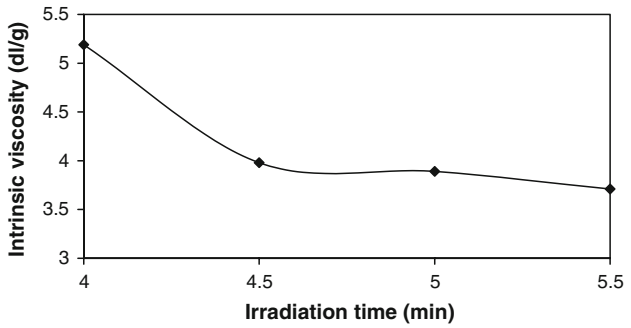


Fig. 4 Intrinsic viscosity of chitosan solutions formed after microwave irradiation of 4.0, 4.5, 5.0 and 5.5 min, respectively

Table 1 Molecular weight and DD of chitosan as a function of different microwave irradiation time

Iradiation time (min)	Mw (kD)	DD (%)
4.0	142.06	49.2
4.5	94.84	62.5
5.0	91.59	76.2
5.5	85.22	85.3
Conventional heating (121°C, 4 h)	244.34	87.0

irradiation and attributed this to the fact that microwave accelerated splits of chitosan chains. Therefore this method can be very useful for synthesizing low molecular weight chitosan with rapid and clean chemistry.

4 Conclusion

Conventionally chitosan is prepared by deacetylation of chitin using high temperatures. In this study we used microwave for deacetylation of chitin. The successful conversion of chitin to chitosan was confirmed by detailed characterization with FTIR, XRD and viscometric analysis. The in core heating through microwave can raise the temperature and speeds up the reaction by many folds over conventional heating. When chitin is reacted with a 45% w/v NaOH solution under microwave, the deacetylation reaction occurs very fast leading to a DD of 85.3% in as low as 5.5 min whereas conventional heating takes about 4 h to achieve this DD. We foresee the design of a microwave reactor using this simple, fast and reproducible method for continuous synthesis of chitosan with high DD.

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