



Effect of deacetylation time on the preparation, properties and swelling behavior of chitosan films

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ABSTRACT

The effect of time during the deacetylation of chitin by hot alkali was studied to ascertain the film forming ability of chitosan. Five types of chitosan films were prepared in a single step deacetylation process by varying the alkaline treatment time from 2 to 10 h. The degree of deacetylation (DD) of chitosan and chitosan films were characterized using Fourier transform infrared spectroscopy (FT-IR) and elemental (CHN) analysis methods. The DD was found not to vary significantly beyond 2 h of deacetylation and the DD values by CHN analysis was higher than by FT-IR method. The degree of crystallinity (crystallinity index, CrI) of the chitosan films was also evaluated by X-ray powder diffraction method and the film prepared with 2 h deacetylation showed lower CrI. The morphology of this film by scanning electron microscopy (SEM) shows homogeneous and continuous structure. Swelling index of the films was measured in phosphate buffer solution at physiological conditions and film prepared with 2 h deacetylation showed maximum swelling index. However, the film formed with 6 h deacetylation process has the highest DD with higher CrI and low contact angle values. This film seems to be more suitable for biomedical applications.

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1. Introduction

Chitosan is a natural polysaccharide comprising copolymers of glucosamine with *N*-acetylglucosamine and can be obtained by alkaline *N*-deacetylation of chitin, the second most abundant natural polymer after cellulose (Lisbeth Illum, 1998). Basically, the process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin leaving behind a complete amino group (NH₂) and chitosan versatility depends mainly on this high degree chemically reactive amino group (Khan, Peh, & Ch'ng, 2002). However, in order to exploit the utility of this biopolymer, it is imperative to determine its degree of deacetylation (DD), i.e. the average number of D-glucosamine units per 100 monomers expressed as a percentage (Sabnis & Block, 1997). The DD is one of the most important chemical characteristics as it influences the performance of chitosan in many of its applications (Pankaj & Lawrence, 1999). In addition, the DD which determines the content of free amino groups in the polysaccharides can be employed to differentiate between chitin and chitosan (Li, Revol, & Marchessault, 1997). For example, the increase either in temperature or strength of sodium hydroxide solution could enhance the removal of acetyl

groups from chitin, resulting in a range of chitosan molecules with different properties and hence its applications. It is therefore essential to characterize chitosan by determining its DD prior to its utilization at the developmental stage of film forming systems (Khan et al., 2002). Various analytical techniques have been used in the determination of chitosan's *N*-deacetylation (DD) and the most popular techniques being the infrared spectroscopy because, it is a convenient technique for determining deacetylation easily in solid state (Sabnis & Block, 1997). The chitosan films can be prepared from the commercially available chitosan powders (Arvani-toyannis, Nakayama, & Aiba, 1998; Mingyu et al., 2003; Tanabe, Okitsu, Tachibana, & Yamauchi, 2002; Zhang, Li, Gong, Zhao, & Zhang, 2002). However, the film forming properties were not studied in detail in terms of its crystallinity, morphology etc. (Lopes & Carla, 2007; Rong & Hurng-Dar, 1996; Mima, Miya, Iwamoto, & Yoshikawa, 1983). Hence, the present work was planned to prepare the chitosan films from deacetylated chitosan powders with different DD levels and to study the effect of time during the alkaline treatments mainly on the stability of the film by measuring its swelling index.

2. Materials and methods

2.1. Materials

Chitin (S.D. Fine chemicals Mumbai, India) with 6–8% nitrogen content and other high purity chemicals such as sodium hydroxide

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(Sisco research laboratories Pvt. Ltd., Mumbai), acetic acid (Thomas Baker chemicals Pvt. Ltd., Mumbai) etc., were procured from the local suppliers.

2.2. Experimental

Chitosan powder was prepared by deacetylating chitin by dissolving 0.5 g in 100 ml of alkaline solution with 50 Wt.% sodium hydroxide for 2 h at a constant temperature of 107 °C using a magnetic stirrer (Ilauro Lima & Claudio Airolidi, 2004). The powder were then filtered from alkaline solution and washed thoroughly with distilled water until neutral pH is obtained. The powders were dried subsequently using an oven. The different percentages of deacetylated chitosan samples were obtained by varying the deacetylation time from 2 to 10 h.

2.3. FT-IR spectroscopy

Fourier transform infrared spectroscopy (Spectrum one, Perkin-Elmer, USA) was used to analyze the functional groups of the various deacetylated chitosans in order to find out the possible interactions between hydroxyl and amide groups, which are the major functional groups in determining degree of deacetylation. The deacetylated chitosan powders are made in the form of pellets using KBr and analyzed between 400 and 4000 cm^{-1} .

2.4. CHN elemental analysis

The CHN analysis of deacetylated chitosans was carried out using CHNS/O analyzer (2400 Series II, Perkin-Elmer, USA) and the DDs were calculated from the following equation (Xuan, Lirong, & Wei, 2003).

$$\text{DD} = \left(1 - \frac{\text{C/N} - 5.145}{6.861 - 5.145} \right) \times 100 \quad (1)$$

where, C/N is carbon to nitrogen ratio.

2.5. X-ray powder diffraction

X-ray powder diffraction studies were carried out for both chitin and deacetylated chitosans using a X-ray powder diffractometer (XD-D1, Shimadzu, Japan) of Cu $K\alpha$ radiation ($\lambda = 1.54 \text{ \AA}$) with the scanning rate of 0.05°/step and with 2θ ranging from 5° to 30° in order to study the crystallinity index (CrI) of the polymer before and after the treatments at (0 2 0) and (1 1 0) planes. The crystallinity indices were determined by the following equations.

$$\text{CrI} = [I_{020} - I_{\text{am}}/I_{020}] \times 100 \quad (2)$$

$$\text{CrI} = [I_{110} - I_{\text{am}}/I_{110}] \times 100 \quad (3)$$

Where I_{020} and I_{110} are the maximum intensities of diffraction peaks at (0 2 0) and (1 1 0) planes and I_{am} is the intensity of amorphous diffraction region (Zhang, Xue, Xue, Gao, & Zhang, 2005).

2.6. Preparation of films

Chitosan films were prepared by solvent casting method by taking known amount of deacetylated chitosan powder and dissolving in 0.3 M acetic acid. The castings were made by pouring the chitosan solution into teflon coated glass moulds and subsequently kept in an oven at 50 °C in order to dry. The chitosan films were removed from the moulds and neutralized with 1 Wt.% NaOH solution for 30 min and washed thoroughly with distilled water and dried subsequently. The dimensions of the films were measured using a digital micrometer (Digimatic Micrometer, Mitutoyo – Japan).

2.7. Swelling index measurements

The dried films of chitosan were immersed in phosphate buffer solution of pH 7.4 at ambient temperature until swelling equilibrium was attained. The weight of swollen sample (W_s) was measured after removing the surface water with blotting paper. Swelling index (SI) was then calculated on the basis of the weight of swollen and dry films (W_d) using the following equation.

$$\text{SI} = [(W_s - W_d)/W_d] \times 100 \quad (4)$$

2.8. Contact angle measurements

Static contact angles of chitosan films were measured using a contact angle goniometry (GBX-Digidrop, France). Five microlitres of distilled water were dropped onto the surface of the films before measuring. The contact angle data was obtained from the images of water droplets on film surface.

2.9. SEM analysis

Surface morphology of all the chitosan films was analyzed using scanning electron microscopy (FEI-Quanta 200, Netherlands).

3. Results and discussion

3.1. Mechanism of deacetylation

The process of deacetylation causes the removal acetamido (CH_3CONH) group during hot alkali treatments leaving more amino (NH_2) groups and this takes place in the amorphous region of chitin, then proceeds from the edge to the inside of the crystalline region (Tolaimatea et al., 2000). The chemical formula of chitin and chitosan is shown in Fig. 1.

3.2. Degree of deacetylation

Fourier transform infrared spectroscopy (FT-IR) spectroscopy studies were carried out to evaluate the DD of deacetylated chitosan samples. From the infrared spectra as shown in the Fig. 2, the large and intense bands located at 3700 and 3000 cm^{-1} can be attributed to axial OH and NH group vibrations, which are more

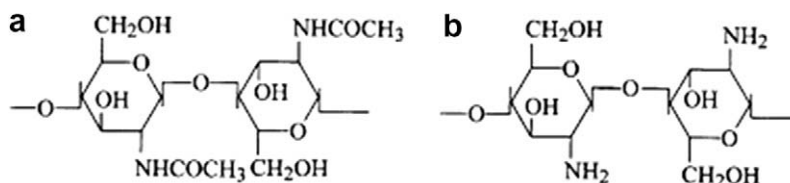


Fig. 1. Structures of chitin (a) and chitosan (b).

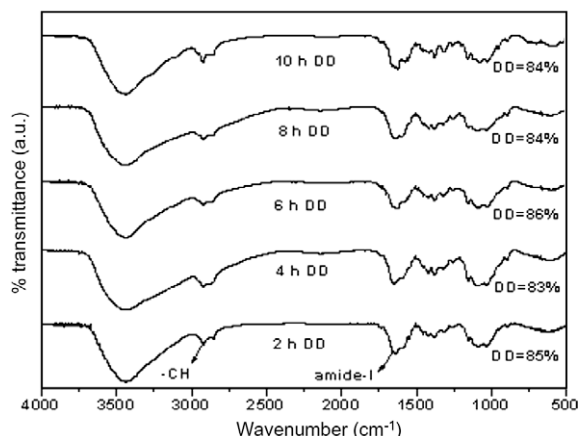


Fig. 2. FT-IR spectra of chitosan with different DDs.

evident in the chitosan spectra. On the other hand, the absorption band at 3480 cm^{-1} can be assigned to the hydrogen bond between OH on carbon-5 of the biopolymer structure with the carbonyl acetamide group. The other absorptions at 3260 and 3100 cm^{-1} are associated with the intermolecular hydrogen bond to acetamide vibration. The axial carbon-hydrogen bond that appears in the $3000\text{--}2800\text{ cm}^{-1}$ range is more intense for the chitin biopolymer. The symmetrical and asymmetrical carbon-oxygen-carbon ring gives rise to the bands located at 1051 cm^{-1} and 1161 cm^{-1} , respectively. The absorptions at 1420 cm^{-1} and 1380 cm^{-1} are assigned as CH_2 and CH bending bands respectively (Ilauro Lima & Claudio Airolidi, 2004). The DD was calculated by the absorbance ratios of amide-I band and OH^{-1} band at 1655 cm^{-1} and 3450 cm^{-1} using the following Eqs. (5)–(7) according to various methods reported (Baxter, Dillon, Taylor, & Roberts, 1992; Domszy & Roberts, 1985; Sabnis & Block, 1997).

$$\text{DD} = 100 - \frac{A_{1655}}{A_{3450}} \times 115 \quad (5)$$

$$\text{DD} = 100 - \frac{A_{1655}}{A_{3450}} 100/1.33 \quad (6)$$

$$\text{DD} = 97.67 - \left(26.486 \frac{A_{1655}}{A_{3450}} \right) \quad (7)$$

where, A corresponds to the absorbance of respective bands.

The variation of DD with time of deacetylation was calculated by the above methods as shown in Fig. 3. The percentage of deacet-

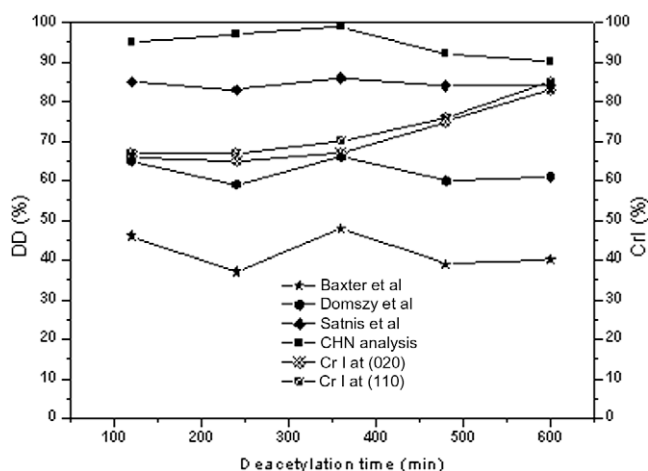


Fig. 3. Variation of % DDs and % CrIs with respect to various methods.

ylation obtained by a single step alkaline treatment corresponds to 80–85% (Chang, Tsai, Lee, & Fu, 1997), and the method proposed by Sabnis (Sabnis & Block, 1997) seems to be more appropriate for the calculation of DD for infrared spectra. From the same figure, it was also observed that marginal increase in DD taking place up to 6 h which may be due to the bulk acetamido groups getting reduced

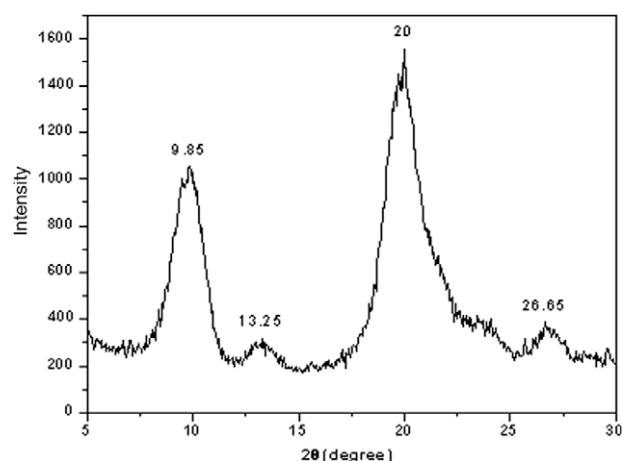


Fig. 4. X-ray diffraction pattern of chitin before deacetylation.

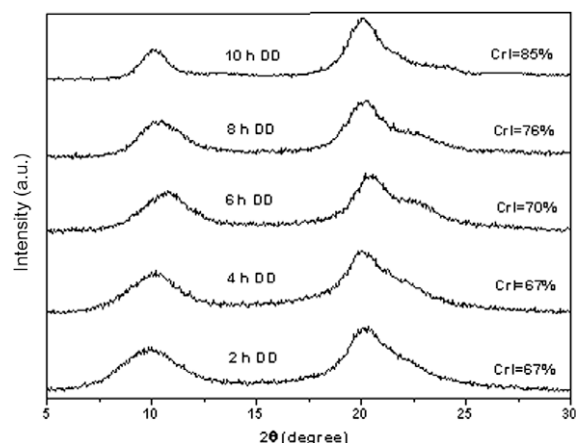


Fig. 5. X-ray diffraction pattern of deacetylated chitosans.

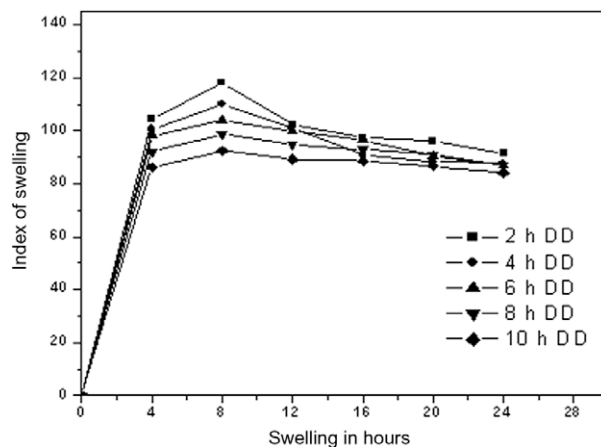


Fig. 6. Swelling index of chitosan films at 7.4 pH.

Table 1

Comparison of DDs with contact angle of the chitosan films.

Deacetylation time (h)	DD (%)		Contact angle (degrees)
	FT-IR	CHN	
2	85	95	82
4	83	97	81
6	86	99	75
8	84	92	81
10	84	90	80

by increasing the number of amino groups. After 6 h, the DD values are tend to decrease due to the high free amino group within the molecular chain of chitosan to form cations which combine with the water in the alkaline solution increase viscosity. This hinders the stirring rate of alkaline solution resulting reduction in DD values.

Apart from the FT-IR spectroscopic methods, another method for the determination of DD of chitosans is elemental analysis using the Eq. (1) as mentioned earlier. The elemental analysis method is relatively fast, and the DDs were calculated for all the

deacetylated chitosans using the above formula for various reaction times. The DD values by FT-IR as well as CHN analysis was presented in Fig. 3. It was noticed that the DD value from FT-IR spectra is less as compared to the DD obtained from elemental analysis, with a maximum difference in the DD value of 14% corresponding to 4 h DD sample. This was due to the moisture adsorption of chitosan during the sample preparation of FT-IR study. Since chitosan is hygroscopic in nature, the adsorption moisture would have resulted difference in determining the intensity of OH absorption in the hydroxyl band. Similar difference in the DD values between these two methods were also reported (Taghizadeh & Davari, 2006). Overall, the DD seems to be not varying much with the deacetylation time beyond 2 h and the method by Sabnis (Sabnis & Block, 1997) is more appropriate in finding DD from 2 h reaction.

3.3. Crystallinity index measurement (Crl)

The degree of crystallinity for chitin and chitosan biopolymers was evaluated through X-ray powder diffraction method. The diffraction pattern of chitin before deacetylation as shown in Fig. 4, which exhibits four diffraction peaks at 9.85°, 13.25°, 20° and

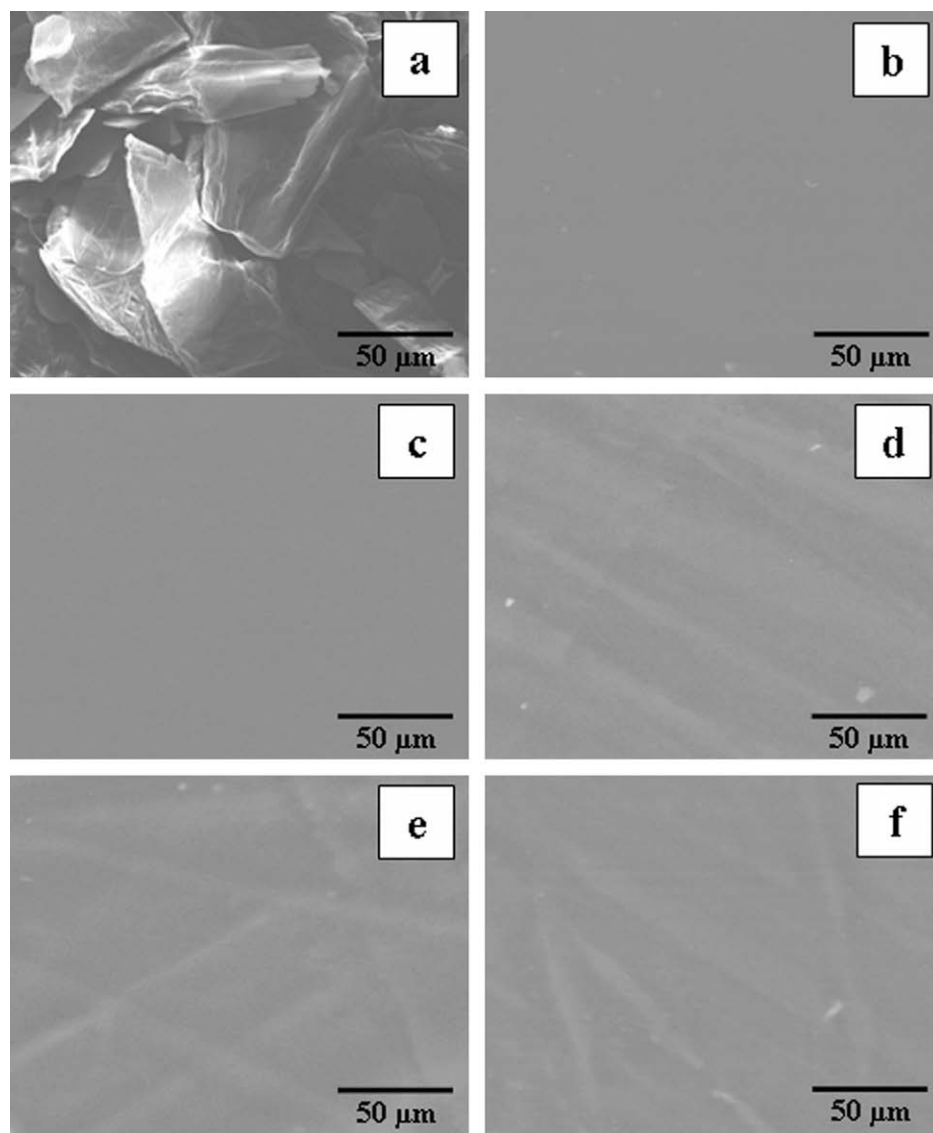


Fig. 7. SEM morphology of deacetylated chitosan powder and chitosan films. (a) chitosan powder (b) 2 h DD (c) 4 h DD (d) 6 h DD (e) 8 h DD (f) 10 h DD.

26.65° as reported (Webster, Osifo, Neomagus, & Grant, 2006). Of the four peaks, the peaks at 9.85° and 20° are the two prominent peaks in the diffraction pattern, which confirms the partial crystallinity of the polymer. After deacetylation process for 2 h, only two broad peaks have appeared at 10° and 20.3° with a little shift in the diffraction angles as shown in Fig. 5 and the CrI calculated from the intensities of these peaks using Eqs. (1) and (2) are plotted as a function of reaction time as shown in Fig. 3. The broad peaks appear as a result of change in the unit cell dimension. The CrI for 2 h DD is 67%, which remains the same for 4 h DD, but increases subsequently to 85% (as shown in Fig. 5) with increasing deacetylation time. This may be due to the penetration of alkali into the chitin crystallites which cleavage the acetyl groups conceivably modifying the initial order of the chitin molecules to enhance the crystallinity of the polymer (Focher, Beltrame, Naggi, & Torri, 1990).

3.4. Swelling index measurement

Swelling is one of the most important properties of chitosan films which characterize its uses for biomedical application. Swelling studies were carried out by soaking the films in phosphate buffer solution of pH 7.4 for the period up to 24 h and the swelling index was calculated using the Eq. (4) as mentioned earlier. The variation of SI calculated using Eq. (4) with time is shown in Fig. 6 and it can be easily seen that all the five films have attained the equilibrium state after certain period of time and thereafter decreased slowly to reach a steady value. Since chitosan is hydrophilic and diffusion of water into chitosan is faster, the films begin to swell prior to degradation. So, during the initial stages of hydration process, bond cleaving and degradation of chitosan also occurs, but the swelling surpasses the degradation (Ren, Yi, Wang, & Ma, 2005). Similar profile of swelling reaching a maximum and then a slow decrease with time has also been reported for chitosan films. This decrease in swelling after attaining equilibrium has been attributed to due to the buffer salts, residual acetic acid etc. (Eduardo et al., 2006). From the same figure, it was also observed that the film formed with 2 h DD showed maximum index of swelling due to the presence of amino groups which interacted with hydrophilic group in the water. However, the rest of the films showed less SI, which may be due to the influence of other parameters such as, the nature of polymer, polymer chain flexibility, molecular mass, crystal structure and chemical composition along with DDs (Taghizadeh & Davari, 2006).

3.5. Contact angle measurements

The surface wettability of chitosan films were measured by contact angle analysis using water. The contact angles of all the chitosan films are listed in Table 1 and from the table, it was observed that there is no significant difference in the surface wettability of chitosan films with varying deacetylation time. This confirms that DD has no significant effect on the wettability of chitosan film (Cao et al., 2005). Generally, the smaller is the contact angle the better is the surface wettability and as the chitosan film formed after 6 h treatment shows lowest contact angle, it may be more suitable for biomedical application.

3.6. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was carried out to examine the morphology of deacetylated chitosan powder as well as films prepared in this study as shown in Fig. 7. The micrograph of deacetylated chitosan powder shows irregular flakes morphology as shown in Fig. 7(a). All chitosan films formed in this study

are clear and colorless. The film prepared from 2 h deacetylated chitosan showed homogeneous and uniform structure and thus the film is suitable for further evaluation as shown in Fig. 7(b). The film prepared by 4 h deacetylated chitosan also showed similar morphology as given in Fig. 7(c). The film prepared from 6 h deacetylated chitosan showed slightly undulating and with finely distributed striations as shown in Fig. 7(d). The films prepared from 8 h and 10 h deacetylated chitosans showed fine striations in different directions are shown in Figs. 7 (e) and (f). The presence of striation lines were attributed to the periodic nature of the cholesteric helix (Dong et al., 2006). The crystallinity index (CrI) of the films for 2 h DD is 67%, which remains the same for 4 h DD, but CrI was found to increase subsequently from 70% to 85% (as shown in Fig. 5) with increasing deacetylation time. So the increase in crystallinity may be cause of the striations in the SEM of the said films. Although the striations are observed in these films, the surface of the films was relatively smooth.

4. Conclusions

Five types of chitosan films were prepared in a single step deacetylation process by varying the deacetylation time. The DD values calculated by different methods from FT-IR spectra shows variation and found to be lower when compared with the CHN analysis. The DD was found not to vary significantly beyond 2 h indicating that the process not only depends on the time but also depends on other parameters such as strength of alkaline solution, chitin to solution ratio etc. The chitosan film formed with 2 h deacetylation has the maximum swelling index and contact angle with lower crystallinity. However, the film formed after 6 h DD has the highest DD with higher CrI and low contact angle values. The effect of deacetylation time thus seems to play a role in improving the properties of the chitosan film for biomedical applications with suitable swelling index, contact angle and crystallinity.

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