An investigation on the short-term biodegradability of chitosan with various molecular weights and degrees of deacetylation

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ABSTRACT

At the present work, we have studied the short-term degradation of chitosan with different degrees of deacetylation (DD) and molecular weights. The various grades of chitosan were characterized throughout DD range, molecular weight, crystallinity and swelling ratio. The biodegradation of chitosan was examined by using simulated intestinal fluid (SIF, pH 7.5) at 37 °C and 150 rpm permanent shaking to mimic a typical physiological condition. The results revealed that in high DDs no significant changes in molecular weight and DD were observed. However, molecular weight loss percentage, DD changes percentage and swelling ratio increased with decreasing the initial DD and increasing the initial molecular weight. Scanning electron microscope (SEM) images showed no significant changes in particle size after degradation. These observations demonstrated that short-term degradation of chitosan has not occurred through the β-chain scission and it can be related to the peptide bond cleavage of acetoamido side groups of the polymer.

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1. Introduction

In general, in peptide oral drug delivery systems, the encountered problem is mainly revolve around the quick denaturation of peptides and proteins in the hostile environment of the stomach as well as the intestinal enzymatic environment (Lorenzo-Lamosa, Remunan-Lopez, Vila-Jato, & Alonso, 1998). Since oral drug administration remains the easiest and the most comfortable method of drug delivery, the microencapsulation of drugs is an alternative solution to overcome the problem, allowing their slow release and protection against the acidic and enzymatic environments (Chellat et al., 2000; Wang, Ma, & Su, 2005).

The basis of our work is to study chitosan as a potential carrier in one-day oral drug delivery systems. It is reported that chitosan is a good candidate for oral drug delivery of the peptides (Hejazi & Amiji, 2003). Based on our information, Although, numerous researches have been taken on the degradation of chitosan in different aspects such as enzymatic (Chellat et al., 2000; Li, Du, & Liang, 2007; Ren, Yi, Wang, & Ma, 2005), chemical (Holme, Davidsen, Kristiansen, & Smidsrød, 2008; Huang, Wang, Huang, Zhuo, & Guo, 2007; Qun, Ajun, & Yong, 2007; Yue, Yao, Wei, & Mo, in press), irradiation (Kang, Dai, Zhang, & Chen, 2007; Lu, Wei, & Jing, 2004;

Zhang et al., 2006), thermal (Britto & Sergio, 2007) and ultrasonic degradation (Liu, Du, & Kennedy, 2007; Tsaih & Chen, 2003), there is not a good understanding about the short-term enzymatic degradation of chitosan in the simulated intestinal fluids (SIF). Since drug release is affected by the degradation rate and swelling ratio of chitosan, the aim of this work is to examine the biodegradation and swelling ratio of chitosan exposed to simulated intestinal fluids less than one day.

Also, as the properties of the chitosan are strongly dependant on its degree of deacetylation and molecular weight (Gupta & Jabrail, 2006; Hejazi & Amiji, 2003; Lamarque, Lucas, Vition, & Domard, 2005; Mirzadeh et al., 2002), effect of these two parameters was investigated on the degradation of chitosan. For this purpose, different grades of chitosan with different DDs and molecular weights were exposed to simulated intestinal conditions for 6 and 24 h and the degradation rates and swelling ratios were evaluated.

2. Experimental

2.1. Material

Three different grades of chitosan (high, medium, and low molecular weights) with the same DD of 65 from crabs shells were obtained from Fluka (Fluka Chemie AG, Buchs, Switzerland), glacial acetic acid, sodium chloride and NaOH from Merck (Darmstadt,

Germany); pepsin, pancreatin and Antibiotics (penicillin–strepto-mycin) from (Sigma).

2.2. Instruments

In this study, the following equipments have been used: A Bru-ker FRA model 106/5 infrared spectrophotometer; A Siemens D 5000 model X-ray diffraction instrument; A 2C model Ubbelohde viscometer and a Cambridge S360 scanning electron microscope.

2.3. Methods

2.3.1. Preparation of various grades of chitosan based on different DDs
Five different grades of chitosan were used in this study. Char-acteristics of these grades are presented in Table 1. Three of the chitosan grades (L55, M55 and H55) had a same DD of 55 and dif-ferent molecular weight. Also, three of them (L55, L65 and L75) had a same molecular weight and different DDs. The samples L55, L65 and L75 had a same origin with a low molecular weight. The sam-ples L55 and L75 were obtained by acetylation and deacetylation of L65, respectively. Samples M55 and H55 were obtained by acetyla-tion of medium and high molecular weight grades of chitosan with DD of 65, respectively.

The acetylation and deacetylation processes used to obtain dif-ferent grades of chitosan having different DDs are described in the previous work of authors. Previous studies showed that the acety-lation and deacetylation process had no effect on the molecular weight of the chitosan samples (Taghizadeh & Davari, 2006).

2.3.2. Crystallinity
Percentage of crystallinity was measured by X-ray diffraction and was calculated according to the following equation:

$$X_c = [F_c/(F_c + F_a)] \times 100 \tag{1}$$

where F_c and F_a designate the areas of the crystalline and amor-phous peaks, respectively, and are calculated and reported analyt-ical evaluations (Wan, Creber, Pebbley, & Bui, 2003).

2.3.3. Molecular weight
The intrinsic viscosity and molecular weight of polymer were determined by viscometry, based on the viscometric constants in the Mark–Houwink equation (Wang, Bo, Li, & Qin, 1991).

$$[\eta] = kM_v^\alpha \tag{2}$$

$[\eta]$ and M_v are intrinsic viscosity and molecular weight, respec-tively. k and a are viscometry constants and Wang et al. (1991) showed that these constants are depended on the DD of chitosan:

$$k = 1.64 \times 10^{-30} DD^{44} \tag{3}$$

$$a = -1.02 \times 10^{-2} DD + 1.82 \tag{4}$$

In order to calculate the M_v, two different series of k and a was used. The first one is obtained from Eqs. 3 and 4. The second one is as-sumed to be constant and k, α in a solvent system of 0.1 M CH$_3$COOH/0.2 M NaCl at 25 °C are assumed to be 1.81×10^{-3} mL/g and 0.93, respectively, as it is reported by Roberts and Domszy (1982).

2.3.4. Degree of deacetylation (DD) measurement
Infrared spectra of samples were recorded using the KBr disc technique for which the value of the ratio of KBr to polymer was 100:1 (Domszy & Roberts, 1985; Khan, Peh, & Chang, 2002). Poly-mer and KBr were placed in an oven at 80 °C for 24 h before mix-ing. The spectra of chitosan samples were obtained within a frequency range of 4000–400 cm^{-1} and the degree of deacetylation of samples obtained by equation:

$$DD = 100 - \left[\frac{A_{1655}}{A_{3450}} 100/1.33\right] \tag{5}$$

According to Fig. 1, A_{1655} and A_{3450} were the absorbances at 1655 cm^{-1} of the amide-I band as a measure of the N-acetyl group content and 3450 cm^{-1} of the hydroxyl band as an internal stan-dard to make corrections for film thickness or for differences in chitosan concentration in powder form.

The factor '1.33' denotes the value of the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan. It is assumed that the value of this ra-tio is zero for fully deacetylated chitosan (Sabins & Block, 1997).

$$\text{Log}_{10}(DF/DE) = (A_{1655})\text{amide} \tag{6}$$

$$\text{Log}_{10}(DF/DE) = (A_{3450})\text{hydroxyl} \tag{7}$$

2.3.5. Swelling studies
The chitosan films with definite weights, thicknesses and diam-eters were prepared by casting method and placed in SIF solutions at 37 °C and 150 rpm. SIF was prepared in accordance with USP XXII. The SIF was prepared by dissolving 6.8 g of monobasic potas-sium phosphate in 250 mL of water. The solution was mixed and 190 mL of 0.2 N sodium hydroxide and 400 mL of water and 10 g of pancreatin (Sigma) were added. The pH was then adjusted with 0.2 N sodium hydroxide to 7.5 ± 0.1 and the volume was adjusted to 1 L by DD water. Antibiotics (Penicillin–streptomycin) were added to each solution to avoid bacterial contamination.

Then at the certain time intervals (6 and 24 h) swelled samples were taken out of the solutions and their surfaces were dried by fil-ter paper and weighed. The swelling ratio for each sample at cer-tain time was calculated by the following equation (Gupta & Kumar, 2000; Yun, Shao, & Yao, 1996):

$$S\% = \frac{W_t - W_0}{W_0} \times 100 \tag{8}$$

where S is the swelling ratio percentage, W_t is weight of swelled film at time t and W_0 is weight of swelled film after drying in oven at 40 °C for 24 h.

2.3.6. Degradation studies
Chitosan powders were meshed by molecular sieves (100–200). Seven milliliters of degradation medium (SIF) was added to 0.125 g

Table 1
Initial properties of different grades of chitosan.

Sample code	DD	Crystallinity (%)	$[\eta]$	$\overline{M_v} \times 10^{-5 \text{ a}}$	k^{b}	a^{b}	$\overline{M_v} \times 10^{-5 \text{ b}}$
L55	55	34	430 ± 17	6.03 ± 0.27	3.8×10^{-6}	1.259	24.94 ± 0.8
L65	65	30	471 ± 15	6.66 ± 0.23	3.9×10^{-5}	1.157	13.10 ± 0.37
L75	75	20	513 ± 8	7.29 ± 0.13	2.9×10^{-4}	1.055	8.30 ± 0.13
M55	55	22	743 ± 7	10.86 ± 0.12	3.8×10^{-6}	1.259	38.51 ± 0.31
H55	55	23	871 ± 8	12.89 ± 0.13	3.8×10^{-6}	1.259	43.71 ± 0.32

[a] Molecular weight obtained by using k and a reported by Roberts and Domszy (1982).
[b] Molecular weight obtained by using k and a calculated with equations, reported by (Wang et al. (1991).
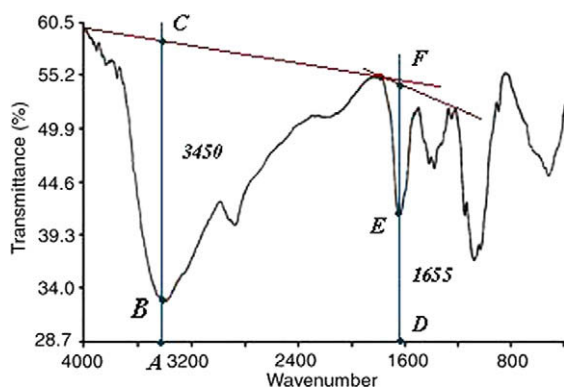
Fig. 1. IR spectrum of chitosan showing two baselines ('a' and 'b') for calculating the amide-I band absorbance for the ratio A1655/A3450.

chitosan and incubated at 37 °C under continuous shaking at 150 rpm. After a predetermined incubation time (6 and 24 h), the samples were washed with distilled water and then dried in oven for 24 h.

Degradation rate was obtained by measuring the changes of molecular weight loss percentage and DD changes percentages, as following:

$$\text{Molecular weight loss percentage} = \frac{(M_{v,o} - M_{v,t})}{M_{v,o}} \times 100 \qquad (9)$$

which $M_{v,o}$ and $M_{v,t}$ are the initial molecular weight and molecular weight after exposure to degradation medium for t hours, respectively.

Degree of deacetylation changes percentages

$$= \frac{(DD_t - DD_o)}{DD_o} \times 100 \qquad (10)$$

which DD_o and DD_t are the initial DD and DD after exposure to degradation medium for t hours, respectively.

2.3.7. Morphological studies

In order to study the morphology of the chitosan powders before and after biodegradation and pursuing chain fragmentation during the degradation, scanning electron microscopy (SEM; Cambridge S360) was applied.

3. Results

3.1. Characteristics of initial polymers

Intrinsic polymer viscosity of synthesized grades of chitosan is presented in Table 1. As can be seen, the intrinsic viscosity of chitosan samples increases with the increase in the DD with the same molecular weight because of the polyelectrolyte effect, which results from the electrostatic repulsion force of ionic groups along chitosan chain in dilute acid solution. This force makes the molecular dimension of the chitosan coil expand as the DD increases (Wang et al., 1991).

In order to eliminate the effect of DD on the calculated M_v from intrinsic viscosity, Wang et al. (1991) proposed Eqs. 3 and 4 to calculate k and a as a function of DD. Comparison of the M_v calculated by using k and a obtained from this equations (M_v^b) and M_v calculated by using constant k and a (M_v^a) reported by Roberts and Domszy (1982) revealed that differences between the M_v^b of samples L55, L65 and L75 are too big than the M_v^a (Table 1). As mentioned before, samples L55, L65 and L75 had a same origin with low molecular weight and previous studies of authors showed no

degradation after deacetylation and acetylation process. Therefore, the most proper viscometry constants (k and a), in this case, are the ones which result in the closer calculated M_v for these samples. Accordingly, these equations are not useful in this case and we decided to use constant k and a to calculate the M_v.

In order to reduce the effect of differences in M_v, in degradation studies, molecular weight lost percentage is used as described in Section 2.3.6.

The results obtained from XRD showed the influence of molecular weight and DD on crystallinity of powder structures of the prepared polymers. As it can be seen in Table 1, the crystallinity percentage of polymers increased by increasing the DD which suggests that the introduction of the acetyl groups breaks up the crystal zone in chitosan making it less ordered in structure (Ren et al., 2005). The effect of molecular weight on crystallinity of chitosan has not shown to be significant.

3.2. Swelling study

In Table 2 swelling ratio of samples as a function of DD and molecular weight are presented. Data from swelling ratio showed that for the samples with the same DD, by increase in molecular weight, swelling ratio of chitosan increased. This can be due to the higher chain-relaxation ability in high-molecular weight chitosan as a result of increasing the entanglement of the polymeric chain (Mi, Wong, Shyu, & Chang, 1999).

In contrast, the swelling ratio of chitosan decreased by increase in DD. It is 216% for L55 and decreased to 115% for L75. As mentioned before, introducing acetyl groups in chitosan chains has broken the crystalline zone and so more water has diffused into the polymeric matrix leading to higher swelling ratio (Ren et al., 2005).

3.3. Molecular weights loss percentage after degradation process

As shown in Fig. 2, after 6 h, the molecular weight loss percentages of samples with the same DD (55) increased and then became almost constant. Molecular weight loss percentages of the other samples increased gradually by the rate lower than the samples with DD of 55.

The reduction in molecular weight of L55, M55 and H55 after 24 h is 17.5 ± 3.5, 19.43 ± 6.7 and $23.3 \pm 8.8\%$, respectively. In Fig. 3b, a comparison can be made between the swelling ratios and molecular weight loss percentages of these samples. As it is evident, swelling ratio and molecular weight loss percentage show similar trend as a function of initial molecular weight. By increasing in swelling ratio, polymeric chains are more accessible to SIF enzymes and thus samples with higher swelling ratio exhibit higher degradation rates.

Chitosan with different DDs has shown different degradation behavior. The degradation rate is 5.7 ± 4, 9.4 ± 6 and $17.5 \pm 3.5\%$ for L75, L65 and L55, respectively. But both swelling ratio and molecular weight reduction have a similar trend as a function of the initial DD, as well as initial molecular weight.

Table 2
Swelling ratio and DD percentage change after 24 h degradation of different grades of chitosan.

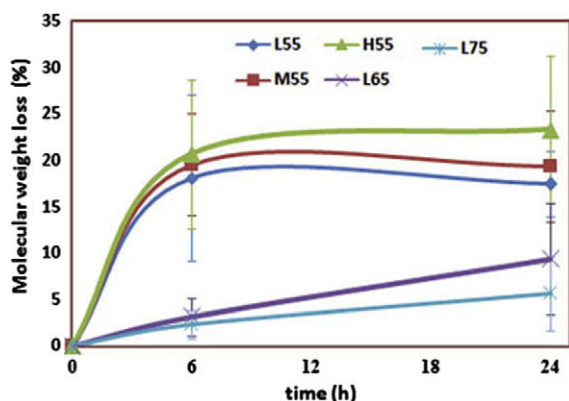| Samples	DD (%)	Swelling ratio (%)	DD change after degradation (%)
L75	74	115 ± 42	2.23 ± 1.4
L65	64	188 ± 40	2.29 ± 0.7
L55	56	216 ± 12	22.8 ± 2.2
M55	55	229 ± 14	36.9 ± 2.6
H55	56	300 ± 0	38.7 ± 5.6

Fig. 2. Molecular weight loss percentage in chitosan as a function of degradation time.

3.4. DD changes after degradation

The data obtained from FTIR spectra showed that after degradation of chitosan, DD of samples increased. Variations in DD of different samples after 24 h degradation are listed in Table 2, showing 38.7 ± 5.6, 36.9 ± 2.6, 22.8 ± 2.2, 2.3 ± 0.7 and $2.2 \pm 1.4\%$ for H55, M55, L55, L65 and L75, respectively. Increase in DD after degradation can be related to cleavage of peptide-like acetoamido groups (NHCOCH$_3$) at the presence of peptidase enzymes presented in SIF. This cleavage of peptide-like acetoamido groups leading to deacetylation of chitosan during degradation.

Fig. 4 indicates a comparison of the results presented in Table 2 and Fig. 2. DD percentage variations and swelling ratio showed the same trend as a function of DD and molecular weight of samples, as well as molecular weight loss percentages.

3.5. Morphological studies

SEM micrographs of chitosan particles for L55 before and after 6 and 24 h degradation process are shown in Fig. 5. After 6 and 24 h degradation, no significant changes in size or morphology of particles were observed. It shows that maybe chitosan in SIF medium, within short degradation time, shows no fragmentation and bulk degradation. It is suggested that perhaps the most part of molecular weight loss may be due to increase in DD during exposure to SIF. While the acetoamido groups (NHCOCH$_3$) on the polymer chain have been converted to amine groups (NH$_2$) during exposure to SIF, the DD has increased. Molecular weight of acetoamido group is higher than amine; therefore, the presence of higher amount of amine groups has decreased total molecular weight of polymer. It can be confirmed by comparison the DD change percentage and molecular weight loss percentage of different chitosan grades after 24 h exposure to SIF which changes in a same manner. Samples L75 and L65 both showed low amounts of DD change percentage and molecular weight loss percentage. In contrast, samples L55, M55 and H55 showed high amount of DD change percentage and molecular weight loss percentage.

As a consequence, it can be concluded that short-term degradation of chitosan has occurred on the side groups of polymer chain more than the polymer backbone.
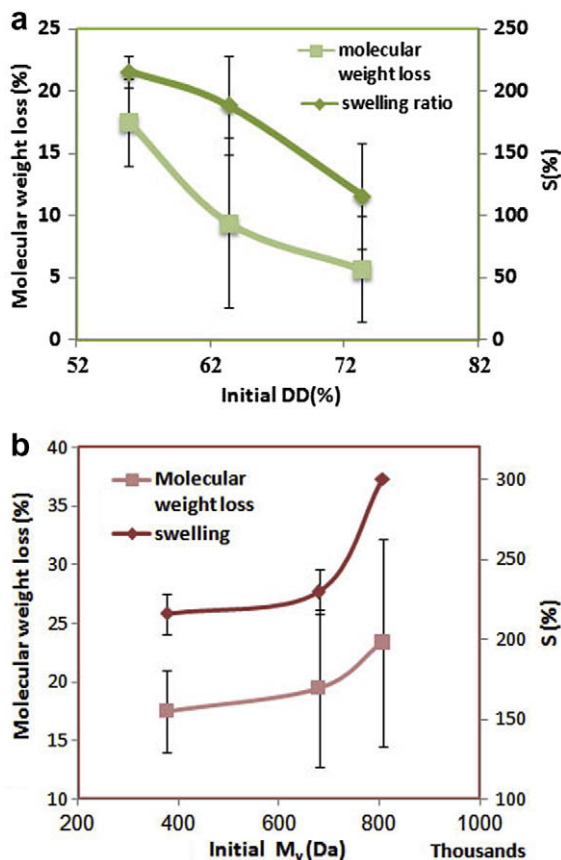

Fig. 3. Molecular weight loss percentage and swelling ratio as a function of initial DD (a) and molecular weight (b).
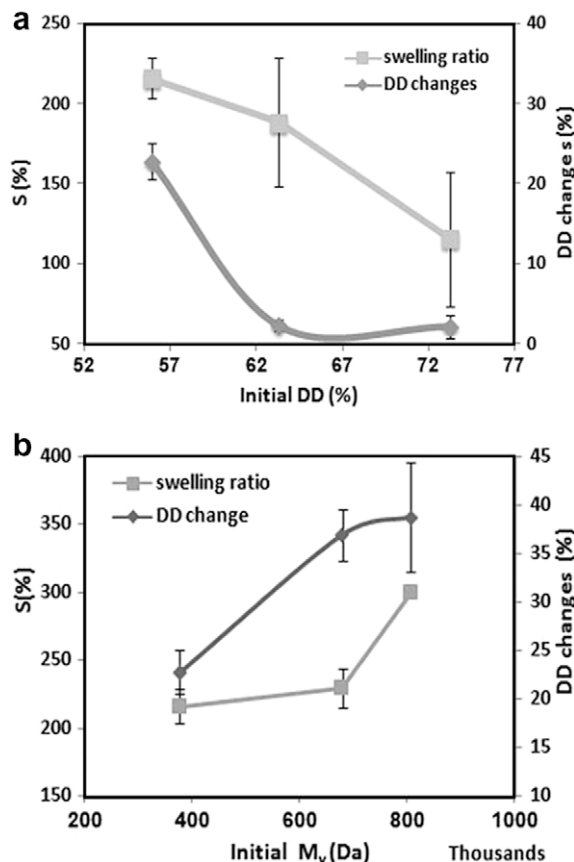

Fig. 4. DD changes percentages and swelling ratio as a function of initial DD (a) and molecular weight (b).
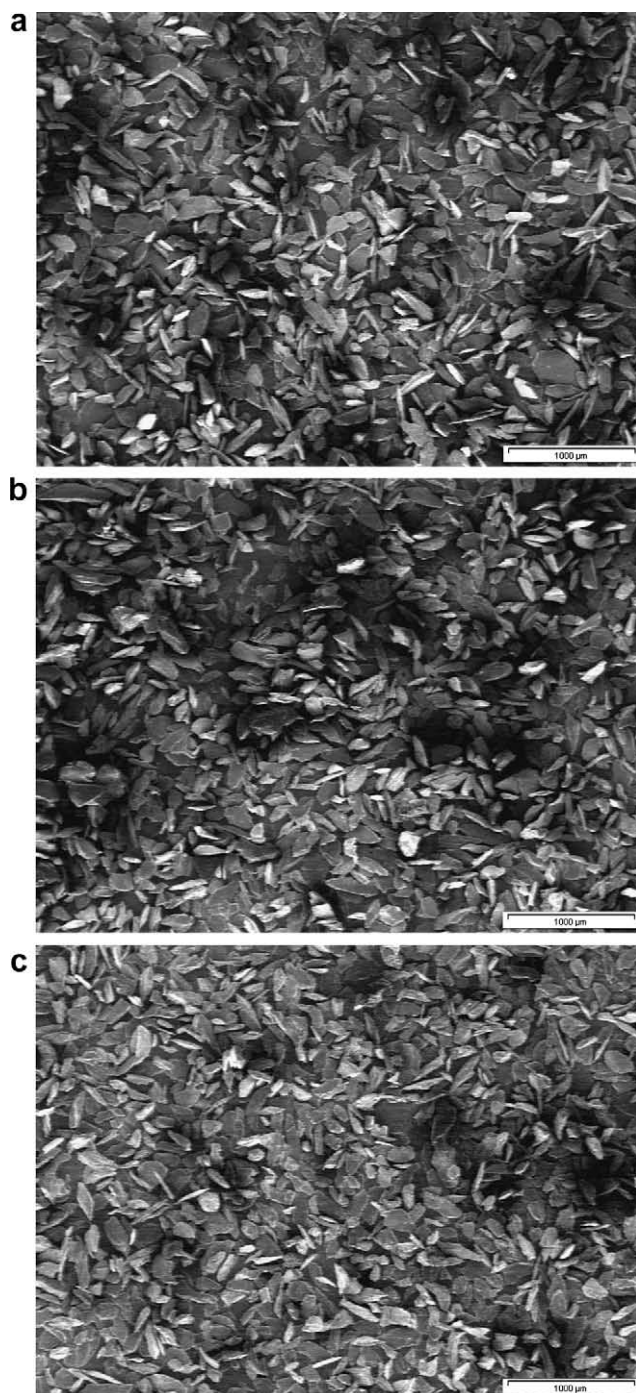
Fig. 5. SEM image of chitosan (a) before degradation, (b) after 6, and (c) after 24 h.

cleavage of peptide-like bonds of acetoamido (–NHCOCH₃) groups on the chitin part of chains.

Swelling ratio and degradation studies indicated that both increased in samples with chain fragmentation was observed. It can be concluded that most part of weight loss of chitosan in the short-term degradation is related to deacetylation of chitosan rather than β-scission of backbone chains.

Base on our observations, the properties of chitosan carriers, specially their DD, change gradually while they pass the intestine tract. As, the DD of the chitosan can affects on the carrier properties such as swelling ratio, mucoadhesion and drug release profile which have a great influence on the performance of the chitosan carrier, it is suggested to consider the above parameters while designing a chitosan drug carrier for one-day oral drug delivery applications.

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4. Conclusion

In order to investigate the short-term degradation behavior of chitosan exposed to the SIF, the molecular weight and DD changes, morphology of the powders and swelling ratio of the chitosan films were evaluated.

The data from viscometry revealed that exposure to the SIF medium caused a weight loss in chitosan samples. In case of samples beyond degree of deacetylation of 55, the reduction was not so significant. FTIR spectra showed that after degradation, DD of chitosan samples increased, which may be related to enzymatic

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