ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 321 (1999) 235–245

CARBOHYDRATE
RESEARCH

Chitosan processing: influence of process parameters during acidic and alkaline hydrolysis and effect of the processing sequence on the resultant chitosan's properties

Pankaj R. Rege, Lawrence H. Block *

Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282, USA

Received 19 December 1998; accepted 16 June 1999

Abstract

The objectives of this study were to investigate the influence of reaction temperature, processing time, and mechanical shear on the depolymerization (DP) of chitosans and to evaluate the importance of the sequencing of the deacetylation and DP processes on the resultant macromolecules and their properties. Process sequence did not alter the degree of deacetylation (DD), the intrinsic viscosity, or the molecular weight (MW). Treatment conditions affected the properties of the resultant polymer: the reaction temperature and processing time had a significant impact on the MWs and DDs of the resultant polymer. Mechanical shear, however, did not significantly affect the above properties. Furthermore, polymer crystallinity was affected by reaction temperature but not by shear or processing time. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Chitin; Chitosan; Molecular weight; Depolymerization

---

1. Introduction

Every year, approximately 100 billion tons of chitin is produced on the earth by crustaceans, mollusks, insects, fungi and related organisms. This amount is comparable to that of cellulose produced by higher plants, but chitin is not widely used by the pharmaceutical industry at present. Chitin is the last unexploited biomass on planet Earth [1]. Its limited utility, principally, is the result of its poor solubility characteristics. However, its derivative, chitosan, prepared by N-deacetylation and depolymerization of native chitin, is soluble in dilute acids when the degree of deacetylation (DD) is more than 65–70%.

In general, commercially available 'chitosans' are heterogeneous, poorly characterized materials for which the DD ranges from 60 to 90%. In addition, the molecular weights (MWs) of these commercial materials typically range from 50 to 2000 kDa. The heterogeneity of these chitosans is the result of the relatively uncontrolled commercial processing of native chitin involving both N-deacetylation and depolymerization.

An extensive review and evaluation of the published literature and our own laboratory data [S.S. Sabnis, L.H. Block, unpublished data] reveal the dependence of chitosan properties on two fundamental parameters: degree of deacetylation and degree of polymerization

---

* Corresponding author. Tel.: + 1-412-396-6362; fax: + 1-412-396-5599.

E-mail address: block@duq.edu (L.H. Block)

PII: S0008-6215(99)00172-X

(i.e., MW) [2]. These include the polymer's physicochemical properties, e.g., solubility, ionizability, reactivity [3], as well as its functionality [4] e.g., as a bioadhesive [5], wound healing agent [4], paracellular transport enhancer [6], complexing agent [7], and as an excipient for use in modified drug-delivery systems [8–10].

The main driving force in the development of new applications for chitosans lies in the fact that this cationic polymer is not only readily and economically processed from naturally abundant chitin, but also is nontoxic, biodegradable, and biocompatible [4].

The manufacture of chitosans with specific degrees of acetylation and polymerization involves both alkaline N-deacetylation and acidic depolymerization of the native chitin macromolecule. One purpose of this study was to evaluate the importance of the sequencing of these two processes on the resultant macromolecule and its properties. Furthermore, the alkaline hydrolysis of chitin (i.e., the N-deacetylation process) has been exhaustively studied [11–17]. However, the depolymerization process has not been extensively studied, notwithstanding the fact that several researchers have recognized the importance of this process [18–22]. Chitosan depolymerization by nitrous acid, following treatment with hydrochloric acid, has been reported previously by Allan and Peyron [23,24]. However, their method necessitates the use of chitosans with a relatively high degree of deacetylation in order to achieve dissolution. In addition, the removal of residual nitrous acid and its decomposition products further complicates the process. In this research, we studied the effect of depolymerization using hydrochloric acid per se and investigated the kinetics of this acidic hydrolysis process.

2. Experimental

Materials and chemicals.—Chitosans were purchased from Sigma Chemical Co., St. Louis, MO and from Fluka Chemie, Buchs, Switzerland. Reagent grades of sodium hydroxide 50% w/w solution, concentrated hydrochloric acid, glacial acetic acid, and potassium bromide (IR grade) were used as supplied by Fisher Scientific Co., Fair Lawn, NJ.

Sieve analysis and particle size distribution of chitosans.—Fifty-gram portions of chitosan were weighed and passed through a series of US standard testing sieves (6, 10, 40, 60, and 100 mesh) stacked in a sieve shaker (Tyler Portable Sieve Shaker, model 11630, W.S. Tyler Co., Salisbury, NC). The agitation was continued for 20 min, after which time the material remaining on each of the sieves was weighed. The chitosans were subsequently subjected to size reduction using a Wiley mill (model no. 3, Arthur H. Thomas Co., Philadelphia, PA) or a Comill® Quadro (model no. 1975, Quadro Engineering Inc., Canada). In the deacetylation–depolymerization experiments described below, the chitosan utilized, whether as powder or milled flakes, was the fraction that passed through a 60-mesh sieve (250 μm) and was retained on a 100-mesh sieve (150 μm).

Deacetylation of chitosan.—Sieved chitosan was placed in a 1000 mL three-neck round-bottom flask (diameter of flask = 12.4 cm). Sodium hydroxide (50% w/w solution, 600 mL) was added to this flask. The three-necked flask accommodated a 50 cm water-jacketed condenser, a nitrogen delivery tube, and a thermometer. The reaction was performed under a nitrogen blanket to prevent the evaporation of the solvent. An oil bath was used to maintain the required temperature (80 °C). The mixture was continuously stirred by a 1-inch oblong stir-bar on a magnetic stirrer–hot plate (model PC 351, Corning Glass Works, Corning, NY). The time of the reaction was varied to assess the effect of markedly different deacetylation reaction times on the robustness of the chitosan macromolecule and it was conducted for either 4 or 24 h in order to subject chitosans to moderate or substantial reaction conditions. At the end of the specific time period, the reaction mixture was neutralized using concentrated hydrochloric acid. Neutrality was confirmed by the measurement of pH. The precipitate was filtered using a Büchner funnel and Whatman No. 114 filter paper. The precipitate was washed several times with deionized water prior to final washing with diethyl ether. The product was then dried in a chemical hood for 12 h, followed by drying in a 60 °C hot-air oven for an additional 48 h. The degree of deacetylation of the dried powder was then determined using the IR spectroscopic procedure described below. The yield of the deacetylation reaction was approximately 50%.

Depolymerization of chitosans

Sequencing study. Previously sieved chitosan (10 g) was placed in a 1000-mL three-neck round-bottom flask. Hydrochloric acid (2.5 N, 300 mL) was then added to the chitosan powder. The reaction conditions were identical to those described above. The reaction time was varied to obtain material with different degrees of polymerization. Chitosan was subjected to the depolymerization process either for 1 or 4 h, in order to access the effect of the different depolymerization time on the robustness of the chitosan macromolecule. At the end of the specific time period, the mixture was neutralized using 5 N NaOH. The neutralized material was then subjected to vacuum filtration. The resultant solids were then washed repeatedly with deionized water until the rinse water was at a neutral pH. The wet material was then weighed and dispersed in enough deionized water to make a 10% w/v slurry. This slurry was subjected to mechanical agitation using a Lightnin' mixer (model 100426, Mixing Equipment Inc., NY) with a 1-inch marine propeller for 5 min at 1000 rpm. The resultant viscous slurry was dried in a 60 °C hot-air oven for 48 h. The horny tenacious mass formed as a result of drying was then reduced to a powder using a Waring blender (model FCI 15, Waring Products Corp., NY) for 5 min and subsequently passed through a 60-mesh sieve. The resultant powder was then transferred into glass containers and stored at ambient temperature in a glass desiccator. The yield of the depolymerization process was approximately 65%.

Identification of critical parameters during depolymerization. Previously sieved chitosan (50 g) was placed in a 1000-mL three-neck round-bottom flask. Hydrochloric acid (2.5 N, 600 mL) was then added to the chitosan powder. The reactions were conducted at 60, 80, or 100 °C. Sampling was performed at 0.5, 1, 2, 3, or 4 h. At the end of the specific time period, the mixture was neutralized using 5 N NaOH. The neutralized material was then subjected to vacuum filtration. The resultant solids were then washed repeatedly with deionized water until the rinse water was at a neutral pH. The wet material was then weighed and dispersed in enough deionized water to make a 10% w/v slurry. This slurry was either subjected to mechanical agitation using a Lightnin' mixer (model 100426, Mixing Equipment Inc., NY) with a 1-inch marine propeller for 5 min at 1000 rpm, or to no shear. The resultant products were dried in a 60 °C hot-air oven for 48 h. The horny tenacious mass formed as a result of drying was then reduced to a powder using a Waring blender (model FCI 15, Waring Products Corp., NY) for 5 min and subsequently passed through a 60-mesh sieve. The resultant powder was then transferred into glass containers and stored at ambient temperature in a glass desiccator.

Chitosan analysis

Determination of degree of deacetylation. The degree of deacetylation was determined by an IR spectroscopic method [25] using a Perkin–Elmer FTIR spectrometer (model 1605, Perkin–Elmer Corp., Norwalk, CT). Approximately 25 mg of dried chitosan was triturated with 100 mg of potassium bromide (IR grade), and the mixture was passed through a 100-mesh (150 μm) sieve. About 40 mg of the sieved mixture was then used to prepare a pellet.

Determination of the degree of polymerization. Chitosan molecular weight (viscosity average) was calculated from the classical Mark–Houwink relationship,

$$[\eta] = K_m(\mathrm{MW})^a \tag{1}$$

where $[\eta]$ is the intrinsic viscosity, $K_m = 2.14 \times 10^{-3}$, and $a = 0.657$. The values of K_m and a were previously determined by laser light-scattering techniques [26].

Polymer solutions of known concentrations were prepared in a solvent system consisting of 0.5 M acetic acid and 0.25 M sodium chloride in deionized water. The solutions were then filtered through a 5-μm nylon filter (Magna-R, Lot number 67498, Micron Separations Inc., Westboro, MA) prior to the viscosity measurements. The viscosity measurements were made, in triplicate, by recording the efflux times of the filtered solutions in Ubbelohde viscometers maintained in a constant-temperature bath at 25 ± 0.1 °C.

Determination of change in percentage crystallinity. Powder X-ray diffractograms were generated using an X'PERT System (Phillips Electronic Instruments) with Cu K_α radiation in the range 5–40° 2θ at a scan rate of 0.04 2θ/s. The powder X-ray diffraction studies with various chitosans involved the placement of about 0.5–0.7 g of the previously sieved (less than 250 µm) chitosan powder on a glass slide. The slide was subsequently mounted in place in the diffractometer after the powder surface was leveled appropriately. Diffraction data were converted into an ASCII format and the data were mathematically transformed (normalized) and analyzed. The normalized spectra were generated by dividing the intensity (counts) at each 2θ value by the sum of the intensities between 5 and 40° 2θ. The sum of square (SSQ) differences between the raw and treated material spectra were computed and used as the dependent variable in an ANOVA.

Experimental design

Effect of processing sequence. The effects of the deacetylation–depolymerization sequence on the resultant chitosan properties were evaluated in accordance with a full factorial experimental design. A [2 × 2 × 2 × 2] Latin square design was studied for the main effects. The independent variables were the chitosans (two different batches); the deacetylation time (4 or 24 h); the depolymerization time (1 or 4 h); and the process sequence (deacetylation followed by depolymerization or vice versa). The dependent variables were the degree of deacetylation (%), molecular weight (kDa), and the intrinsic viscosity (cps) of the resultant chitosans.

Effect of process parameters on depolymerization. A [3 × 3 × 2] full-factorial experimental design (ANOVA) was utilized to determine the effect of processing parameters on chitosan depolymerization. The independent variables were the processing time (0.5, 1, 2, 3, or 4 h); the processing temperature (60, 80, or 100 °C); and mechanical shear (1000 rpm for 5 min or no shear). The dependent variables were the degree of deacetylation (%), the molecular weight (kDa), and the change in percentage crystallinity (SSQ difference).

Data were analyzed using JMP (Version 3.1.5, SAS Institute Inc., Cary, NC).

3. Results and discussion

General considerations.—Chitosan is manufactured from commercially available chitin by alkaline and acidic hydrolysis of the chitin macromolecule [27]. Alkaline hydrolysis (i.e., N-deacetylation) results in more free amine substituents on the macromolecular backbone and an increase in the polymer's aqueous solubility, whereas acidic hydrolysis presumably breaks the ether linkages in the macromolecule, resulting in increased polymer reactivity [3]. Chitosan properties depend on two fundamental parameters: the degree of deacetylation (DD), and the degree of polymerization.

In order to obtain reproducible results, it is necessary to use starting material having similar properties. Hence, the manufacture of chitosans with specific DDs and specific MWs requires careful examination of each processing step. Although chitosans are commercially available, Table 1 provides evidence of the variability encountered in these products. The comparison of the DDs and MWs of some commercial chitosans as labeled with the values determined in our laboratory are listed in Table 1. These data indicate the necessity for more controlled chitosan processing and/or the use of more specific test procedures.

The size distributions of representative samples of chitosan powder and flakes are shown in Fig. 1. Chitosan flakes had a substantially larger median particle size (1410 µm) as compared with chitosan powder (790 µm) and hence were subjected to size reduction using a Wiley mill (model no. 3, Arthur H. Thomas and Co.) or a Comill® Quadro (model no. 1975, Quadro Engineering Inc.). Typical size distributions for the milled chitosans are shown in Fig. 1. In all cases, the material exhibited a log-normal particle size distribution. The Wiley mill was more effective than the Comill® in reducing the median diameter of the particles. Thus, all subsequent milling of chitosans employed the Wiley mill.

Deacetylation–depolymerization (DA–DP) sequence.—The study to determine the implication of the DA–DP sequence on the properties of the resulting chitosans was conducted in accordance with the ANOVA model evaluated for its main effects.

Table 1
Variability in commercial chitosans

Supplier	Lot no.	Degree of deacetylation (%)		Degree of polymerization (kDa)	
		Labeled	Determined *	Labeled	Determined *
Fluka	A	84.8	81.0 ± 0.7	150	156.6 ± 2.8
Fluka	B	89.3	89.4 ± 2.1	70	58.1 ± 2.8
Fluka	C	89.3	83.6 ± 0.6	70	404.9 ± 6.7
Fluka	D	89.3	82.4 ± 0.2	70	319.4 ± 1.7
Fluka	E	84.5	81.2 ± 0.8	400	290.2 ± 10.5
Fluka	F	83.5	79.9 ± 0.3	750	344.2 ± 8.7
Fluka	G	83.0	91.4 ± 0.7	600	422.4 ± 14.5
Fluka	H	84.3	85.3 ± 0.5	2000	461.9 ± 18.9
Fluka	I	84.4	84.4 ± 0.6	2000	332.3 ± 18.3
Fluka	J	84.4	80.0 ± 0.7	2000	356.9 ± 8.0
Fluka	K	74.1	77.6 ± 0.3	2000	370.9 ± 4.0
Sigma	L	n.a. [a]	88.1 ± 1.3	n.a.	353.8 ± 1.4
Sigma	M	n.a.	83.6 ± 0.4	n.a.	210.5 ± 10.3

[a] n.a., not available.
* $n = 3$; mean ± S.D.

Two grades of chitosan were used in the study. These chitosans differed significantly ($P < 0.05$) in their DDs. The chitosan samples were subjected to DA prior to DP or vice versa. Table 2 shows the DDs, $[\eta]$, and MWs of the resulting chitosans. The ANOVA design used the different starting material, a different processing sequence, the deacetylation level, and the depolymerization level as the independent variables, and the DDs, $[\eta]$ and MWs of the resultant chitosans as the dependant variables. The outcome is shown in Table 3. The processing sequence did not significantly affect any of the parameters evaluated. The DDs ($P > 0.77$), $[\eta]$ ($P > 0.31$), and MWs ($P > 0.37$) of the resulting chitosans were not significantly affected by the DA–DP processing sequence.

Influence of processing parameters during depolymerization

Degrees of deacetylation of the resultant chitosans: hypothesis. Energy in the depolymerization reaction may be sufficient to hydrolyze the acetyl moiety (from the acetamido groups on the polymer) in addition to hydrolyzing the ether linkages between the monomers.

If the above hypothesis is valid, some deacetylation could occur during acidic hydrolysis. Hence, we evaluated the DD of the resultant chitosans in addition to characterizing their MWs. The DDs of the chitosans are listed in Table 4.

Response surfaces and statistical analysis. Response surfaces for the change in the DD as a function of processing temperature and time (with and without mechanical shear) are depicted in Fig. 2(a) and (b), respectively. The response surfaces were generated by a log-normal peak function using TableCurve 3D, Version 3.0 (SPSS Inc.). The data suggest that the DD changes proportionately with processing temperature and processing time. These graphical data are further supported by the results of the ANOVA generated using the [3 × 3 × 2] experimental design: processing temperature ($P < 0.001$) and processing time ($P < 0.001$) had a significant effect on the outcome, whereas

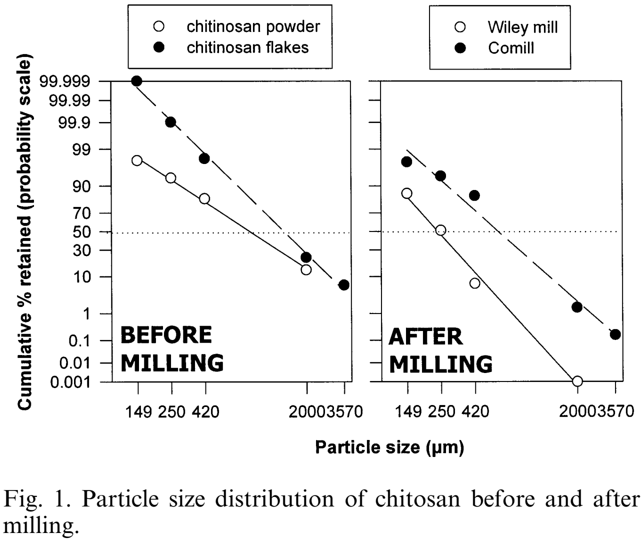


Fig. 1. Particle size distribution of chitosan before and after milling.

Table 2
Degrees of deacetylation, molecular weights, and intrinsic viscosities of resulting chitosans

No.	Lot	Sequence [a]	DA time (h)	DP time (h)	DD * (%)	MW * (kDa)	[η] * (cps)
1	SP	DA → DP	4	1	76.6 ± 0.68	6.72 ± 0.19	0.70 ± 0.01
2	SP	DA → DP	24	4	79.7 ± 1.27	18.05 ± 7.14	1.32 ± 0.33
3	SP	DP → DA	4	4	75.9 ± 0.49	17.49 ± 1.62	1.31 ± 0.08
4	SP	DP → DA	24	1	78.9 ± 0.19	3.87 ± 0.57	0.49 ± 0.05
5	SF	DA → DP	4	4	76.1 ± 0.55	11.30 ± 1.74	0.99 ± 0.09
6	SF	DA → DP	24	1	79.1 ± 0.55	11.02 ± 1.60	0.97 ± 0.09
7	SF	DP → DA	4	1	73.8 ± 2.37	2.59 ± 2.22	0.35 ± 0.20
8	SF	DP → DA	24	4	85.0 ± 0.38	11.22 ± 2.78	0.98 ± 0.16

[a] DA → DP, deacetylation prior to depolymerization and vice versa.
* Mean ± S.D.; $n = 3$.

Table 3
Effect of the sequence on the properties of resulting chitosans

	Source	DF	Sum of squares	*F* ratio	Prob > *F*
(A) Effect on DD					
	Chitosans	1	1.00	0.15	0.72
	DA time	1	51.76	7.91	0.07
	DP time	1	8.84	1.35	0.33
	Process sequence	1	0.63	0.09	0.77
(B) Effect on [η]					
	Chitosans	1	0.04	0.62	0.49
	DA time	1	0.02	0.32	0.61
	DP time	1	0.55	9.15	0.06
	Process sequence	1	0.09	1.47	0.31
(C) Effect on MW					
	Chitosans	1	1.25×10^7	0.76	0.45
	DA time	1	4.57×10^6	0.27	0.64
	DP time	1	1.43×10^8	8.68	0.06
	Process sequence	1	1.77×10^7	1.08	0.37

application of mechanical shear did not ($P > 0.84$). The two-factor interactions were non-significant when mechanical shear was one of the factors ($P > 0.05$), whereas the interaction of time and temperature was significant ($P < 0.002$). Although the overall processing effect on DD was significant, the DDs of the polymers resulting from processing at 60 and 80 °C were comparable (Student's *t*-test; $P > 0.05$). The only significant differences in the DDs occurred at the highest processing temperature (i.e., 100 °C) and the longer processing times (i.e., 3 and 4 h) (Student's *t*-test; $P < 0.05$).

Degree of polymerization (molecular weights) of the resultant chitosans. The viscosity-average MWs of the chitosans (determined by capillary viscometry) are listed in Table 5.

Response surfaces and statistical analysis. Response surfaces for the change in the MW as a function of processing temperature and time (with and without mechanical shear) are depicted in Fig. 3(a) and (b), respectively. The response surfaces were generated by a Lorentzian peak function using TableCurve 3D, Version 3.0 (SPSS Inc.). The data suggest that the MW changes inversely with processing temperature and processing time. These graphical data are supported by the results of the ANOVA generated using the [3 × 3 × 2] experimental design: processing temperature and processing time had a significant effect ($P < 0.0001$) on the outcome whereas application of mechanical shear did not ($P > 0.13$). The two-factor interactions were non-significant when mechanical shear was one of the factors ($P > 0.13$), whereas the interaction of time and temperature was significant ($P < 0.001$). Using the Student's *t*-test to compare MWs of the raw and treated material, significant differences were evident in the MWs after

Table 4
Degrees of deacetylation of chitosans

Expt.	Processing temperature (°C)	Processing time (h)	Mechanical shear [a]	DD [b] (%)
Raw material				81.0 ± 0.7
1	60	0.5	Yes	78.3 ± 0.2
2	60	1	Yes	78.5 ± 0.7
3	60	2	Yes	79.0 ± 0.7
4	60	3	Yes	80.1 ± 0.4
5	60	4	Yes	79.6 ± 0.7
6	60	0.5	No	79.2 ± 1.4
7	60	1	No	79.7 ± 0.2
8	60	2	No	79.9 ± 0.5
9	60	3	No	80.2 ± 0.2
10	60	4	No	80.4 ± 0.7
11	80	0.5	Yes	79.0 ± 0.5
12	80	1	Yes	79.3 ± 0.3
13	80	2	Yes	80.3 ± 0.3
14	80	3	Yes	81.7 ± 0.2
15	80	4	Yes	83.5 ± 0.6
16	80	0.5	No	79.4 ± 0.3
17	80	1	No	79.3 ± 0.3
18	80	2	No	79.8 ± 0.9
19	80	3	No	81.1 ± 0.3
20	80	4	No	82.0 ± 0.8
21	100	0.5	Yes	80.1 ± 0.2
22	100	1	Yes	80.6 ± 0.1
23	100	2	Yes	83.8 ± 1.8
24	100	3	Yes	86.4 ± 0.4
25	100	4	Yes	87.0 ± 0.6 [c]
26	100	0.5	No	81.2 ± 0.5
27	100	1	No	80.0 ± 0.7
28	100	2	No	82.3 ± 0.7
29	100	3	No	86.1 ± 1.5 [c]
30	100	4	No	86.8 ± 0.9 [c]

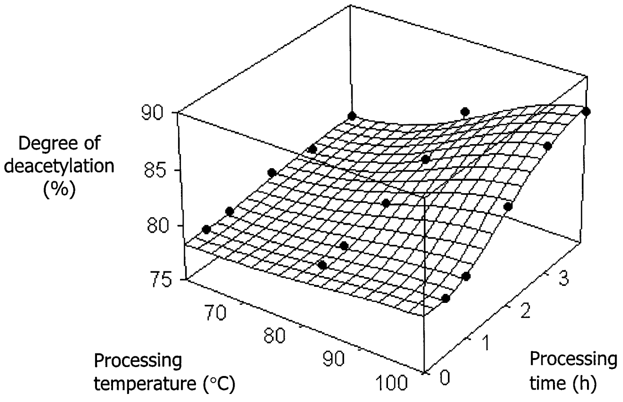
[a] Yes, 1000 rpm for 5 min ($N_{Re} \approx 10^5$). No, mechanical shear not used.
[b] Mean ± S.D.; $n = 3$.
[c] $P < 0.05$ (Student's t-test).

treatment irrespective of treatment time or temperature ($P < 0.05$).

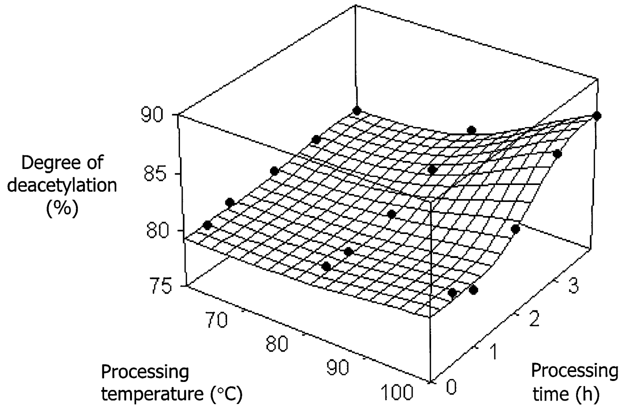
Difference in crystallinity of the resultant chitosans. Problematic aspects of X-ray diffractometry and its application to partially crystalline chitosan. Traditionally, the X-ray diffraction technique, when used for partially crystalline polymeric material, yields qualitative information. As no two X-ray diffraction spectra are achieved under identical conditions, a simple comparison of spectral data would not yield meaningful results. Furthermore, the spectra of these materials are diffuse and characterized by an irregular (i.e., rough) baseline, making comparisons between selected peaks prone to error.

New method for analysis of X-ray diffraction data. In order to overcome these limitations, we have developed a novel approach involving comparisons of the entire spectra rather than a few specific peaks. Spectral comparisons were made after the spectra were subjected to a normalization procedure. Briefly, the spectral data was converted into an ASCII format, and the intensity at each 2θ value was divided by the cumulative spectral intensity. Representative raw spectra (as produced by the X-ray diffraction system) and their normalized counterparts are shown in Fig. 4(A) and (B), respectively. Although the superimposed 'raw' X-ray spectra suggest significant differences, the normalized spectra look quite similar, indicating no significant effect of mechanical shear on the crystallinity of the chitosans. The sum of squares of the spectral differences (i.e., $SSQ_{shear} - SSQ_{no\ shear}$) were calculated and used as the dependent variable in a subsequent ANOVA.

Response surfaces for the sums of square differences as a function of processing temper-

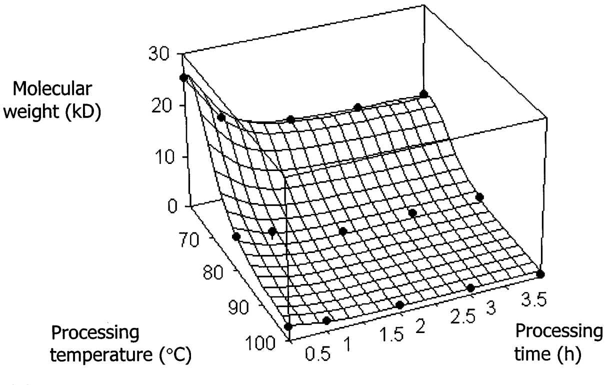


(a) r^2 = 0.985, Fit Std Error = 0.48, Fstat = 66.3

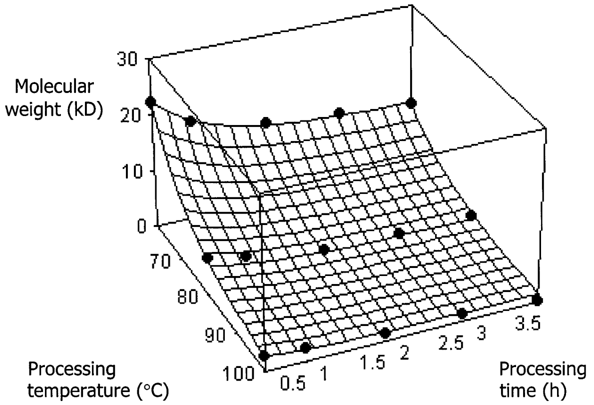


(b) r^2 = 0.981, Fit Std Error = 0.46, Fstat = 50.7

Fig. 2. (a) Degree of deacetylation as a function of process temperature and time (mechanical shear used, $N_{\mathrm{Re}} \approx 10^5$). (b) Degree of deacetylation as a function of process temperature and time (no mechanical shear used).



(a) r^2 = 0.995, Fit Std Error = 0.71, Fstat = 203.9



(b) r^2 = 0.995, Fit Std Error = 0.50, Fstat = 1297.4

Fig. 3. (a) Molecular weight as a function of process temperature and time (mechanical shear used, $N_{\mathrm{Re}} \approx 10^5$). (b) Molecular weight as a function of process temperature and time (no mechanical shear used).

ature and time are depicted in Fig. 5. The response surfaces were generated by an exponential function using TableCurve 3D, Version 3.0 (SPSS Inc.). The data suggest that the spectral differences between treated (i.e., mechanical shear applied) and untreated spectra (i.e., no mechanical shear) (as indicated by their sums of squares differences) increase with increasing processing temperature and processing time. These data are supported by the results of the ANOVA generated using the [3 × 3 × 2] experimental design: processing temperature had a significant effect ($P < 0.025$) on the outcome, whereas processing time ($P > 0.052$) and the application of mechanical shear did not ($P > 0.953$).

Kinetics of the depolymerization process (acidic hydrolysis). The degrees of acetylation (i.e., 100 − DD) and the MWs were plotted against the processing time at each processing temperature in order to evaluate the kinetics of the change in DDs and DPs during this acidic hydrolysis. The data were modeled using zero-, first- and second-order kinetic models. Changes in the DD were best modeled via zero-order kinetics (Fig. 6(A)), whereas changes in the DP followed first-order kinetics (Fig. 6(B)). Reaction temperature significantly affected the rate for the deacetylation process. The energies of activation for both processes occurring during this acidic hydrolysis were calculated in accordance with the Arrhenius equation. The energies of activation for deacetylation (during this acidic hydrolysis) were 47.7 kJ/deg mol (with shear) and 48.4 kJ/deg mol (without shear), respectively. The energies of activation for depolymerization were 20.7 and 28.5 kJ/deg mol, with or without mechanical shear, respectively.

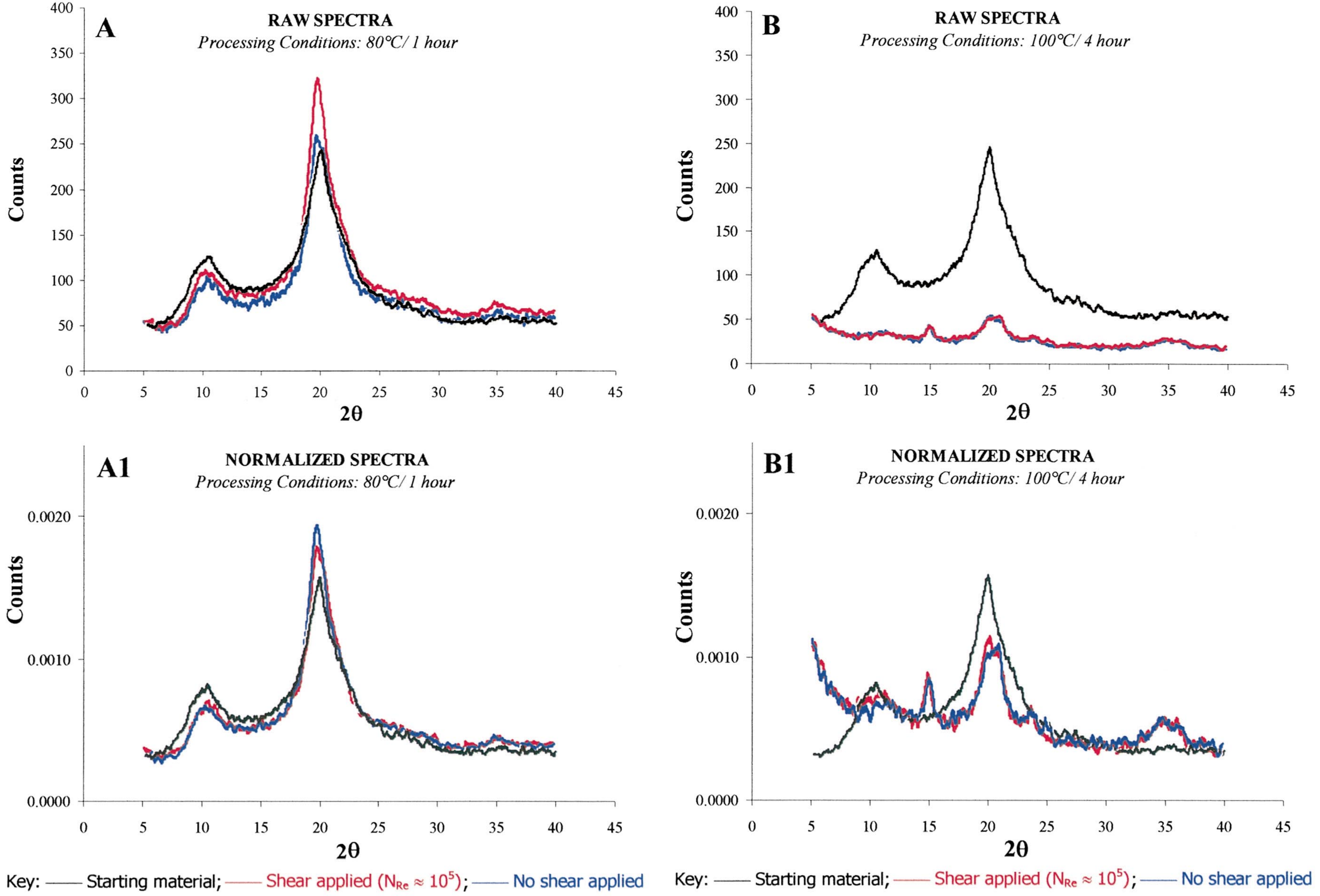


Fig. 4. X-ray diffractograms for chitosan.

Table 5
Molecular weights of chitosans

Expt.	Processing temperature (°C)	Processing time (h)	Mechanical shear [a]	Molecular weights [b] (kDa)
Raw material				156.6 ± 2.8
1	60	0.5	Yes	22.5 ± 2.7 [c]
2	60	1	Yes	16.5 ± 0.7 [c]
3	60	2	Yes	13.1 ± 0.5 [c]
4	60	3	Yes	12.5 ± 0.3 [c]
5	60	4	Yes	12.2 ± 0.3 [c]
6	60	0.5	No	22.2 ± 0.5 [c]
7	60	1	No	17.4 ± 0.8 [c]
8	60	2	No	14.3 ± 1.1 [c]
9	60	3	No	13.2 ± 0.4 [c]
10	60	4	No	12.2 ± 0.8 [c]
11	80	0.5	Yes	6.6 ± 0.1 [c]
12	80	1	Yes	5.9 ± 0.3 [c]
13	80	2	Yes	2.9 ± 0.2 [c]
14	80	3	Yes	3.1 ± 0.1 [c]
15	80	4	Yes	3.0 ± 0.2 [c]
16	80	0.5	No	6.7 ± 0.1 [c]
17	80	1	No	5.4 ± 0.3 [c]
18	80	2	No	3.2 ± 0.1 [c]
19	80	3	No	3.1 ± 0.1 [c]
20	80	4	No	3.1 ± 0.1 [c]
21	100	0.5	Yes	2.7 ± 0.1 [c]
22	100	1	Yes	2.1 ± 0.1 [c]
23	100	2	Yes	1.5 ± 0.1 [c]
24	100	3	Yes	1.5 ± 0.1 [c]
25	100	4	Yes	0.5 ± 0.1 [c]
26	100	0.5	No	2.7 ± 0.1 [c]
27	100	1	No	2.4 ± 0.1 [c]
28	100	2	No	1.6 ± 0.3 [c]
29	100	3	No	1.4 ± 0.1 [c]
30	100	4	No	0.4 ± 0.1 [c]

[a] Yes, 1000 rpm for 5 min ($N_{Re} \approx 10^5$). No, mechanical shear not used.
[b] Mean ± S.D.; $n = 3$.
[c] $P < 0.05$ (Student's t-test).

4. Conclusions

Based on the premise that chitosan functionality depends upon the specific degrees of deacetylation and polymerization of the macromolecules, it becomes necessary to determine

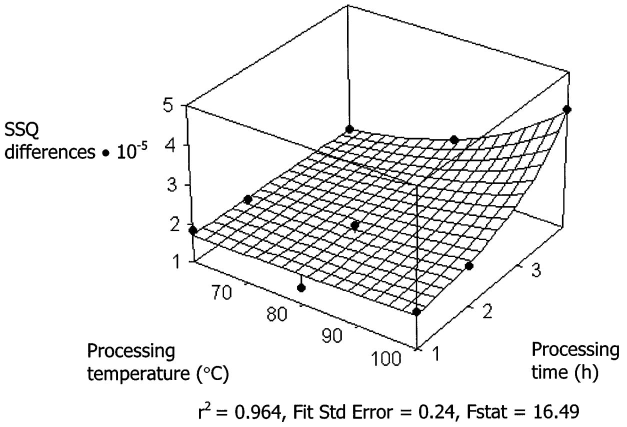


Fig. 5. SSQ differences between raw and treated spectra as a function of process temperature and time.

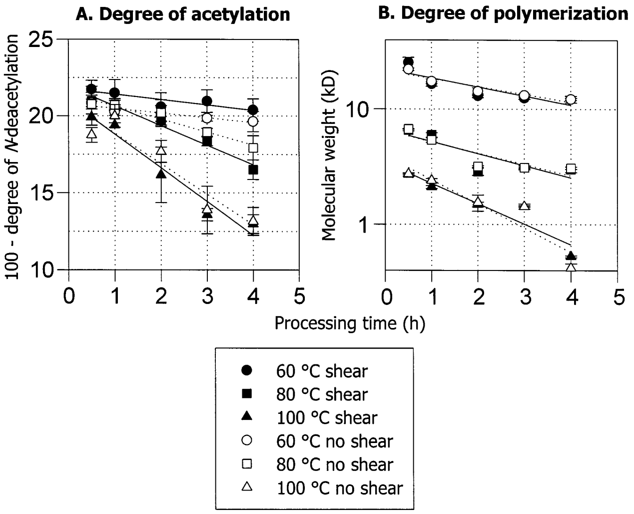


Fig. 6. Kinetic profiles.

whether the sequence of the DA and DP processes affects the resultant molecules. The influence of processing parameters on the kinetics of the depolymerization process (acidic hydrolysis) were also studied. This study shows that this DA–DP sequence does not affect the DD, [η], or the MW of the resultant chitosans. Thus, it does not matter whether one deacetylates first and then depolymerizes, or vice versa. Furthermore, although processing time and temperature significantly affected the properties of the resultant chitosans, application of mechanical shear did not. In order to manufacture chitosans with specific degrees of deacetylation and depolymerization, a better understanding of the kinetics of these reactions is necessary if process optimization and processing cost reduction are to be achieved.

Acknowledgements

The authors acknowledge Dr Shobhan Sabnis (American Cyanamid, Animal Health Formulations Research, Princeton, NJ) for his constructive comments and helpful discussions during the course of this research. Thanks are also due to Professors Pradeep P. Phulé and Jean R. Blachère (Department of Material Sciences, School of Engineering, University of Pittsburgh, Pittsburgh, PA) for providing access to the Phillips X'PERT X-ray diffractometer and for their expert discussions.

References

[1] Y. Machida, T. Nagai, in D.D. Breimer, D.J.A. Crommelin, K.K. Midha. (Eds.), *Topics in Pharmaceutical Sciences*, 1989, pp. 211–221.
[2] L.H. Block, *Chitin/chitosan isolierung, charakterisierung, anwendung*, Lübeck, Germany, 1997.
[3] S.S. Sabnis, *Ph.D. Dissertation*, Duquesne University, Pittsburgh, PA, 1996, pp. 78–80.
[4] J. Akbuga, *Int. J. Pharm. Adv.*, 1 (1995) 3–18.
[5] C. Lehr, J.A. Bouwstra, E.H. Schacht, H.E. Junginger, *Int. J. Pharm.*, 78 (1992) 43–48.
[6] N.G.M. Schipper, K.M. Vårum, P. Artursson, *Pharm. Res.*, 13 (1996) 1686–1692.
[7] A.A. Al-Angary, *Saudi Pharm. J.*, 2 (1994) 157–162.
[8] S.S. Sabnis, P.R. Rege, L.H. Block, *Pharm. Develop. Tech.*, 2 (1997) 243–255.
[9] P.R. Rege, D.J. Shukla, L.H. Block, *Proc. Int. Symp. Control. Rel. Bioact. Mater.*, Vol. 25, 1998, pp. 930–931.
[10] D.J. Shukla, P.R. Rege, L.H. Block, *Pharm. Res.*, 15 (1998) S426.
[11] K.L.B. Chang, G. Tsai, J. Lee, W.R. Fu, *Carbohydr. Res.*, 303 (1997) 327–332.
[12] T. Sannan, K. Kurita, Y. Iwakura, *Polymer J.*, 9 (1977) 649–651.
[13] K. Kurita, T. Sannan, Y. Iwakura, *Makromol. Chem.*, 178 (1977) 3197–3202.
[14] G.C. East, Y. Qin, *J. Appl. Polym. Sci.*, 50 (1993) 1773–1779.
[15] S. Miyakazi, K. Ishii, T. Nadai, *Chem. Pharm. Bull.*, 29 (1981) 3067–3069.
[16] A. Domard, M. Rinaudo, *Int. J. Biol. Macromol.*, 5 (1983) 49–52.
[17] S. Mima, M. Miya, R. Iwamoto, S. Yoshikawa, *J. Appl. Polym. Sci.*, 28 (1983) 1909–1917.
[18] G.A.F. Roberts, J.G. Domszy, *Int. J. Biol. Macromol.*, 4 (1982) 374–377.
[19] J. Wyatt, *LC–GC*, 1 (1997) 160–168.
[20] B. Wu, S. Zhou, W. Wang, *Biopolymers*, 35 (1995) 385–392.
[21] A.-L. Kjøniksen, B. Nyström, C. Iversen, T. Nakken, O. Palmgren, T. Tande, *Langmuir*, 13 (1997) 4948–4952.
[22] R.G. Beri, J. Walker, E.T. Reese, J.E. Rollings, *Carbohydr. Res.*, 238 (1993) 11–26.
[23] G.G. Allan, M. Peyron, *Carbohydr. Res.*, 277 (1995) 257–272.
[24] G.G. Allan, M. Peyron, *Carbohydr. Res.*, 277 (1995) 273–282.
[25] S.S. Sabnis, L.H. Block, *Polym. Bull.*, 39 (1997) 67–71.
[26] S.S. Sabnis, *Ph.D. Dissertation*, Duquesne University, Pittsburgh, PA, 1996, pp. 127–132.
[27] L.H. Block, S.S. Sabnis, US Patent 5,830,883 (1998); *Chem. Abstr.*, 129 (1998) 335793a.