academicJournals

Vol. 8(7), pp. 211-219, 22 February, 2014
DOI: 10.5897/AJPP2013.3931
ISSN 1996-0816 © 2014

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Effect of the alkaline treatment conditions on the tableting performance of chitin obtained from shrimp heads

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Received 13 October 2013; Accepted 30 January, 2014

Chitin is a natural polymer with a potential pharmaceutical application since its exhibits some biodegradability, biocompatibility and relative non-toxicity. It is obtained from crustacean's exoskeletons by treatment with alkalis and mineral acids followed by a depigmentation step. In this work, the effect of alkaline treatment on tableting properties such as compact tensile strength and disintegration time and other polymer properties, such as acetylation degree and degree of crystallinity was evaluated. A Box Behnken Design with 17 runs, three levels and three factors (that is, reaction temperature at 30, 75 and 100°C, NaOH concentration at 0.5, 3 and 6 M and reaction time at 1, 4 and 8 h) was employed. A combination of a high temperature, alkali concentration and reaction time led to a low chitin yield, reduced its acetylation degree, increased its crystallinity and hence, decreased the tensile strength, but accelerated the disintegration time of compacts. The optimal reaction condition was achieved using an alkali concentration of 2.6 M, temperature of 70°C and a reaction time of 1 h. These conditions rendered compacts with a tensile strength of 2.3 MPa and disintegration of 28.6 min. Therefore, chitin can be used for the preparation of solid dosage forms by direct compression.

Key words: Chitin, deacetylation, alkaline hydrolysis, tableting performance.

INTRODUCTION

Chitin is the second most abundant polymer in nature found in a wide number of invertebrates such as shrimps, lobsters, insects and in the cell wall of yeast and fungi (Campana-Filho et al., 2007). Chitin has an important function comparable to that of cellulose in terrestrial plants. It is structurally identical to cellulose, except for having an acetamide group (-$NHCOOCH_3$) at the C-2 position. It occurs in nature as ordered crystalline microfibrils associated to proteins. It possesses some biodegradability, biocompatibility and non-toxicity (Cho et al., 2000; Synowiecki and Al-Khateeb, 2003). Chitin is extracted by alternating alkaline/acid treatments. The acid treatment is used to dissolve calcium carbonate, whereas the alkaline treatment eliminates proteins. It is also common to employ a final depigmentation step in the purification process (Seodi and Nada, 2007; No, 1995). In chitin, the degree of acetylation (DA) is >50% indicating the presence of some amino groups since some deacetylation might take place during the extraction process (Domard and Rinaudo 1983; Focher et al.,

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Table 1. Experimental matrix for the alkali hydrolysis conditions according to the Box Behnken Design.

Run	Temp (C)	Conc (M)	Time (h)	Yield (%)	AD (%)	DC (%)	TS (MPa)	DT (min)	TP (%)
1	75	3	4	33.9	52.3	66.8	1.32	110	0.6
2	75	3	4	47.1	46.3	62.4	1.68	90	0.08
3	100	3	8	24.5	46.0	60.4	1.68	16.6	0.05
4	100	0.5	4	33.6	52.8	62.1	1.54	28.6	1.4
5	75	0.5	1	40.0	65.3	69.0	2.18	182.8	1.5
6	75	6	1	19.4	56.3	65.3	1.6	10.2	1.6
7	100	3	1	17.6	56.1	60.7	1.53	3.8	1.3
8	75	3	4	36.4	53.4	66.3	1.71	98.7	0.5
9	30	0.5	4	42.8	63.7	36.4	1.64	263	1.8
10	30	3	1	61.0	42.4	46.8	2.53	220	1.3
11	75	3	4	34.65	48.8	65.8	1.48	96.7	0.5
12	30	3	8	34.9	48.2	54.3	2.8	52.5	1.8
13	30	6	4	28.7	55.6	55.7	2.34	38	1.5
14	75	3	4	45	52.8	69.5	1.56	91.8	0.6
15	75	0.5	8	33.8	48.4	67.0	1.61	17.9	0.8
16	100	6	4	23.4	34.7	68.7	1.75	13.7	0.08
17	75	6	8	25.5	16.2	72.5	1.37	0.9	0.2

Temp, Temperature; Conc, concentration; AD, acetylation degree; DC, degree of crystallinity; TS, tensile strength; DT, disintegration time; TP, total protein.

1990). When the degree of acetylation is <50%, this material is regarded as chitosan and becomes soluble in acid solutions (Khor and Lim, 2003; Signini and Campana-Filho, 1999).

Currently, natural or semisynthetic excipients such as microcrystalline cellulose, starch, lactose, calcium diphosphate, mannitol and sorbitol are used to prepare tablets. They are mostly used as diluents for drugs when formulating compacts for immediate release of drugs. These products are prepared by direct compression, dry granulation or wet granulation. However, only microcrystalline cellulose and sorbitol can be used as single excipients for direct compression and dry granulation due to their good mechanical properties. For this reason, it is important to look for new biodegradable and biocompatible excipients as alternative materials to produce tablets. It is possible that the extraction conditions of chitin affect some physicochemical properties varying its tableting performance. Therefore, the objective of this work is to study the effect of alkaline treatment on tableting properties such as tensile strength and disintegration time of compacts and other polymer properties such as degree of acetylation and degree of crystallinity using a Box Behnken Design.

MATERIALS AND METHODS

Shrimp exoskeletons were purchased from Comerpes SA (Cartagena, Columbia). Sodium hydroxide (lot B064398119) was obtained from Merck (Darmstadt, Germany), concentrated hydrochloric acid (lot 3024) and sodium hypochlorite (lot 1791) was purchased from JM chemicals (Medellin, Columbia).

Experimental design

A Box Behnken Design (BBD) with 17 runs was used to assess the effect of alkaline hydrolysis conditions such as temperature (30, 75 and 100°C), concentration (0.5, 3.0 and 6.0 M) and reaction time (1, 4 and 8 h) on yield, acetylation degree, crystallinity and tableting-related properties such as compact tensile strength and disintegration time. A central point in 5 replicate was added to estimate the curvature of the design. The conditions and levels of the alkaline treatment are shown in Table 1. The statistical analysis was conducted using the Design Expert® software 8.04 (Stat-Easy Inc., Minneapolis).

Production of chitin

Approximately, 20 g of dry exoskeletons were milled on a cutting mill (Model 3, Willey, Arthur Thomas Co., Philadelphia, USA), passed through a No. 16 mesh sieve and hydrolyzed at the conditions shown in Table 1 using a heating mantle (P&P, Medellin, Columbia) coupled with a round bottom flask and a two-decked condenser. The solid-to-NaOH solution ratio was (1:10). The dispersion was then neutralized with a 3 M HCl, filtered and dried in an oven (U50, Memmert, Schawabach, Germany) at 100°C for 3 h. The dry material was then treated with a 3 M HCl at 25°C for 24 h to decompose proteins, fats and pigments. This dispersion was then neutralized with a 3 M NaOH, vacuum filtered and treated with a 15% w/v sodium hypochlorite for 24 h at a 1:2 solid-to-NaClO ratio for bleaching. The resulting material was then washed until a conductivity of <20 µS/cm was reached and filtered. The cake thus obtained was dried at 60°C for 24 h and passed through a No. 100

mesh sieve.

Acetylation degree (AD)

Approximately, 1.5 mg of sample was mixed with ~200 mg of dry KBr (previously dried at 110°C for 4 h) with an agate mortar and pestle. The powdered sample was compressed into a pellet using a 13 mm flat-faced punch and die tooling, fitted on a portable press at a dwell time of 5 min. A Perkin-Elmer spectrophotometer (Perkin Elmer, CA, USA) equipped with the Spectra software (Spectrum BX, vs. 5.3.1, Perkin Elmer, Inc, CA, USA) was used to obtain the spectrum between 400 and 4000 cm^{-1}. The resolution, interval length and number of scans employed were 16, 2.0 and 16 cm^{-1}, respectively. The acetylation degree was found by applying the method of Baxter and collaborators by taking the ratio of the absorbance of the FT-IR bands obtained at 1660 and 3450 cm^{-1}, respectively (Baxter et al., 1992).

$$DA = 100 - \frac{\frac{A_{1660}}{A_{3450}}}{1.33} * 100 \quad (1)$$

where A_{1660} and A_{3450} correspond to the type I amide and hydroxyl stretching band, respectively.

Powder yield

It was determined on a dry basis dividing the amount of chitin that resulted from each run by 20 and multiplying by 100%.

Degree of crystallinity (DC)

It was determined by powder X-ray difractometry conducted over a 5 to 45° 2θ range. A Rigaku difractometer (Miniflex II, Rigaku, Americas, Woodlands, TX) operated at 40 kV and 30 mA equipped with a monochromatic CuKα (α_1=1.5460 Å, α_2= 1.54438 Å) X-ray radiation was employed for the analysis. The sweep speed and step width were 0.5° 2θ/min and 0.008°, respectively. The DC was calculated from the Peakfit software (Seasolve, Inc, Framingham, MA) by separating the crystalline and amorphous scattering radiation using the baseline selection tool.

Protein content (Kjeldahl method)

~0.2 g of sample, a catalyzer (potassium sulfate, copper sulfate and selenium oxide) and 10 ml of concentrated sulfuric acid were added to a round bottom flask and heated up at 100°C for 1 h. This solution was then distilled (~100 ml) in a Buchi automatic distiller and the ammonium borate, thus formed was titrated with 0.01 N HCl. The total protein content was obtained by: Nitrogen×6.25.

Compact tensile strength (TS)

Cylindrical compacts of ~100 mg and 6.5 mm in diameter were made at ~430 psi and a dwell time of 1 s. A single punch tablet press (060804 Compac, Indemec, Itagui, Columbia) equipped with a load cell (LCGD-10K, Omega Engineering, Inc., Stamford, CT) was employed. The data of crushing strength values obtained on a hardness tester (UK 200, Vankel, Manasquan, NJ) were transformed to tensile strength according to the Fell and Newton model (Fell and Newton, 1970).

Compact disintegration time (DT)

Tablets, each weighing ~100 mg, were made on a single punch tablet press (060804 Compac, Indemec, Itagui, Columbia) at ~100 psi using a 6.5 mm round flat-faced punches and die set and a dwell time of 1 s. Three replicate were tested in distilled water at 37°C employing a Hanson disintegrator (39-133-115, Hanson Research Corporation, Northridge, CA, USA) operating at 30 strokes/min.

RESULTS AND DISCUSSION

Surface models

The multiple regression coefficients were higher than 0.9354 indicating that >93.54% of the experimental variance of the properties studied is explained by the surface models and the remaining experimental variation is attributed to random errors (Tables 2 and 3). Therefore, these high r^2 indicate that the cubic models are good predictors for the properties studied and these models described very well the relationship between the three factors (NaOH concentration, temperature and reaction time). These models were also validated by the lack of fit test. Further, the goodness of fit statistic test evaluated whether the variation due to lack of fit of the model was small enough to be accepted as a negligible portion of the pure error. The results showed that the experimental variations observed for the properties studied could be attributed to randomized errors since for all models, the lack of fit was >0.05 and thus the cubic models with three factors can be considered as accurate. The fitted models are:

$1/yield = 0.027+0.013A+0.008B-0.0014C+0.0004AB-0.007AC-0.004BC+0.004B^2+0.007C^2-0.01AB^2$

$AD = 51.2-8.43B-14.25C-3.98\ AC-5.8BC-3.8C^2+13.2\ A^2C-7.95AB^2$

$DC = 66.6+5A+0.45B-3.18AB-11.6\ A^2+1.3B^2+6.0A^2B+4.7\ AB^2$

$TS = 1.55-0.53\ A-0.21B-0.2C-0.12AB-0.03AC+0.36\ A^2-0.089\ B^2+0.23\ C^2+0.43\ A^2B+0.31A^2C+0.36\ AB^2$

$DT = 99.3-63.9\ A-53.7\ B-41.1\ C+52.5\ AB+45.1\ AC+38.9\ BC-15.7\ B^2-28.3C^2$

The analysis of variance is shown in Tables 2 and 3. Results indicate that at least one factor was significant for all properties studied. Since only temperature was significant for the degree of crystallinity (DC) and compact tensile strength (TS), it is deducted that these two properties are related and thus, a large arrangement of chitin crystallites had a large contribution on the resulting strength of the tablets. This means that high polymer crystallinity is required for a compact to have a

Table 2. ANOVA analysis of chitin properties.

Source	Sum of squares	Df	Mean square	F- value	p-value, α=0.05
Yield					
Model	0.0018	9	0.0002	11.6	0.002
A-Temp	0.0007	1	0.0007	40.8	0.001
B-Conc	0.0005	1	0.0005	27.1	0.001
C-Time	1.65×10^{-5}	1	1.65×10^{-5}	0.97	0.357
AC	0.0002	1	0.0002	11.8	0.011
C^2	0.0002	1	0.0002	11.9	0.011
AB^2	0.0002	1	0.0002	10.8	0.013
Residual	0.0001	7	1.69×10^{-5}	-	-
Lack of fit	5.9×10^{-5}	3	1.97×10^{-5}	1.3	0.384
Pure error	5.95×10^{-5}	4	1.49×10^{-5}	-	-
Cor total	0.0019	16	-	r^2	0.9372
Degree of crystallinity					
Model	1254.3	7	179.2	18.6	0.000
A-Temp	100	1	100	10.4	0.010
B-Conc	0.8	1	0.8	0.08	0.778
AB	40.3	1	40.3	4.2	0.071
A2	569.3	1	569.3	59.1	<0.000
A2B	72.6	1	72.6	7.5	0.023
AB2	43.7	1	43.7	4.5	0.062
Residual	86.7	9	9.6	-	-
Lack of fit	60.8	5	12.2	1.9	0.28
Pure error	25.9	4	6.5	-	-
Cor total	1341	16	-	r2	0.9354

large TS. Therefore, reaction conditions that produced chitins with a large crystalline component allowed the polymer chains to get closer forming a tight hydrogen bond network, which is boosted upon compaction. As a result, more contact points are formed between particles resulting in tablets of good strength and extended disintegration times. Likewise, soft alkaline conditions let to a product which rendered tablets of a high strength and hence, it was more tortuous for water to penetrate and disrupt the particle-particle interaction and hydrogen bonding in those tablets.

The reaction temperature and NaOH concentration were significant for chitin yield. Basically, NaOH not only hydrolyzed, but denaturalized associated proteins and fats and as a result, a low chitin yield (<25%) was obtained when harsh alkali conditions (that is, 100°C, 3 M and 8 h) were employed. On the contrary, a large chitin yield (~61%) was obtained when mild conditions (that is, 30°C, 3 M and 1 h) were employed.

The models surface plots are depicted in Figure 1. In general, high NaOH concentrations and high reaction temperatures led to a chitin with a low acetylation degree, low yield and high crystallinity which in turn, formed weak compacts and rapid disintegration times.

Degree of acetylation

During the alkaline treatment, degradation of associated proteins and fats along with partial chitin deacetylation took place. Further, a high deacetylation occurred by using harsh reaction conditions such as alkali concentration ≥6 M and temperatures higher than 75°C. Therefore, a high temperature and high NaOH concentration deacetylated chitin independent of the reaction time. Further, the heterogeneous alkaline hydrolysis implied a major deacetylation in the amorphous regions of the polymer leaving partially intact the crystalline native regions of the parent chitin. Therefore, the alkali hydrolysis of the non-crystalline fraction removed the acetamide groups, especially if they are the crystallite surface.

Table 3. ANOVA analysis of chitin tableting properties.

Source	Sum of Squares	Df	Mean square	F Value	p-value,α=0.05
Compact tensile strength					
Model	2.69	11	0.25	9.5	0.011
A-Temp	1.12	1	1.12	43.4	0.001
B-Conc	0.17	1	0.17	6.5	0.051
C-Time	0.16	1	0.16	6.2	0.055
A^2	0.53	1	0.53	20.7	0.006
C^2	0.22	1	0.22	8.5	0.033
A^2B	0.37	1	0.37	14.5	0.013
A^2C	0.19	1	0.19	7.2	0.044
AB^2	0.26	1	0.26	9.9	0.026
Residual	0.13	5	0.03	-	-
Lack of fit	0.03	1	0.03	1.15	0.344
Pure error	0.10	4	0.03	-	-
Cor total	2.82	16	-	r^2	0.9542
Compact disintegration time					
Model	99062.4	8	12382.8	142.5	< 0.0001
A-Temp	32614.6	1	32614.6	375.3	< 0.0001
B-Conc	23058.8	1	23058.8	265.3	< 0.0001
C-Time	13521.9	1	13521.9	155.6	< 0.0001
AB	11035.5	1	11035.5	127	< 0.0001
AC	8127.02	1	8127.02	93.5	< 0.0001
BC	6052.8	1	6052.8	69.7	< 0.0001
B2	1043.0	1	1043.0	12.0	0.009
C2	3385.6	1	3385.6	39	0.0002
Residual	695.2	8	86.9	-	-
Lack of fit	448.2	4	112.0	1.81	0.29
Pure error	247.1	4	61.8	-	-
Cor total	99757.6	16	-	r2	0.9930

Degree of crystallinity

Since upon alkali hydrolysis, chitin swells and NaOH accesses the C-2 acetamide linkage, the net result is an increase of the crystallinity of the samples. This is due to deacetylation taking place in the swollen crystallites. It is possible that the hydrolysis process disrupted the intermolecular hydrogen bond pattern of chitin disturbing the regularity of lateral packing between chains, especially of the most accessible amorphous regions. Once samples are washed and regenerated, the crystalline structure of chitin is increased. During swelling, the intersheet and intra-sheet hydrogen bonds are broken and the crystalline state is disrupted. Once chitin has been solubilized the microfibrillar morphology is lost and the alkali uptake causes swelling without chain scission leading to decrystallization. During swelling, the surface chains are unhinged from the underlying microfibrils and in the subsequent washing treatments, these chains recrystallize on top of the remaining microfibrils.

Chitin yield

In general, chitin yield ranged from 17.6 to 61% on a dry basis. A high yield was obtained at low temperatures and alkali concentrations. This trend was observed independent of the reaction time. In all cases, the total protein content can be considered as negligible. It is possible that during the alkali hydrolysis polymer, degradation is slower in crystalline regions than in amorphous regions. Likewise, at harsh alkaline reaction conditions, deproteinization increased strongly with increasing alkali

Table 4. Validation runs of the optimized alkaline reaction conditions.

Run	Yield (%)	AD (%)	TS (MPa)	DT (min)	DC (%)
Model	21.7	61.8	1.9	30.0	64.1
Experimental	24.6	59.6	2.3	28.6	53.2

AD, Acetylation degree; DC, degree of crystallinity; TS, tensile strength; DT, disintegration time.

concentration rendering a low yield of chitin and virtually no protein left over. This is explained by the increased accessibility of C-2 acetamide linkages to hydroxyl groups due to swelling.

Compact tensile strength and disintegration time

Mild and moderate reaction conditions led to a product with a low acetylation degree, but a more tight hydrogen bond network. This was reflected on a material with better mechanical properties compared to those chitins produced under harsh conditions. On the other hand, large alkali concentrations led to a chitin that rendered tablets of decreased tensile strength. It is plausible that this phenomenon was due to the increased powder crystallinity. For this reason, a high deacetylation eases the formation of inter and intramolecular hydrogen bonds which is needed for the formation of hard compacts. Therefore, more crystalline products are less prompt to form hydrogen bonds with incoming water molecules and thus disintegration times were slower than those of less crystalline chitins.

Validation runs

The optimal reaction conditions were found from the grid search of the models to render a chitin with a degree of acetylation from 50 to 90%, tensile strength >5 MPa and disintegration time <30 min. These responses are expected if a low temperature and concentration and small reaction times are employed. Therefore, compacts with the best tableting properties were obtained when an alkali concentration of 2.6 M, temperature of 70°C and reaction time of 1 h were employed. A validation run was conducted at these conditions and the resulting properties were compared to the theoretical values obtained by the surface models (Table 4). The models with three factors obtained by the BBD experimental design accurately predicted the properties studied.

FT-IR characterization

Chitin chains are organized in sheets where they are tightly held by a number of intra-sheet hydrogen bonds. This tight network is dominated by the strong C-O…..N-H hydrogen bonds where the hydrogen bonds are distributed in two sets with half occupancy in each set. First, a carbonyl group bonds to the hydroxyl group on C-6 and there is also a second hydrogen bond between the OH group on C-3 and the ring oxygen (Guo et al., 2002). The amide groups also forms strong inter molecular hydrogen bond networks (-NH/O]C and –OH/O]C) (Figure 2).

Compared to the original sample, in the chitin spectra, two absorption peaks are observed at 3270 and 3110 cm^{-1} (Figure 3) and were assigned to the N-H stretching by the intermolecular C(2)NH/O]C(7) H-bonds and the O-H stretching by the intermolecular C(6)OH H-bonds, respectively (Liu et al., 2008). Further, a splitting of the amide I vibration at 1660 and 1630 cm^{-1} (minor shoulder) which are assigned to the C=O stretching hydrogen bonded to the N-H groups of the adjacent chain appear bifurcated by forming an additional shoulder attributed to intramolecular hydrogen bonding C=O….$HOCH_2$ (Binias et al., 2007). In contrast, the amide II band is shown at 1560 cm^{-1} and it was attributed to the deformation in the CONH plane (Lamarque et al., 2007). Other minor vibrational bands occurred at 2930 (CH_3 symmetric stretching), 1418 (CH_2 bending and CH deformation), 1375 (C-CH_3 amide stretching), 1314 (amide II and CH_2 wagging), 1155 (C-O-C antisymmetryc bridge stretching), 1073 (C-O-C stretching in ring), 1029 (CO stretching), 896 (C-O-C β-linkage) and 752 cm^{-1}(OH out of plane) (Li et al., 1997).

Compared to the native chitin, the OH bands (3500 to 3200 cm^{-1}) presented great changes. Further, minor changes were observed at the following bands: C-O-C (1000 to 1100 cm^{-1}), C=O (1670 to 1620 cm^{-1}) and the NH amide group (1560 cm^{-1}). The absence of the bands at 1660 and 1630 cm^{-1} in the original native shrimp heads might be due to proteins associated to native chitins. During the alkali hydrolysis, the amide I band at 1660 cm^{-1} gradually increased and was splitted (1630 cm^{-1}). Likewise the band at 1560 cm^{-1} was intensified indicating the prevalence of CONH (amide II). Further, the bands at 3270 and 3110 cm^{-1} were absent in the original sample and were intensified when harsh alkaline conditions were employed.

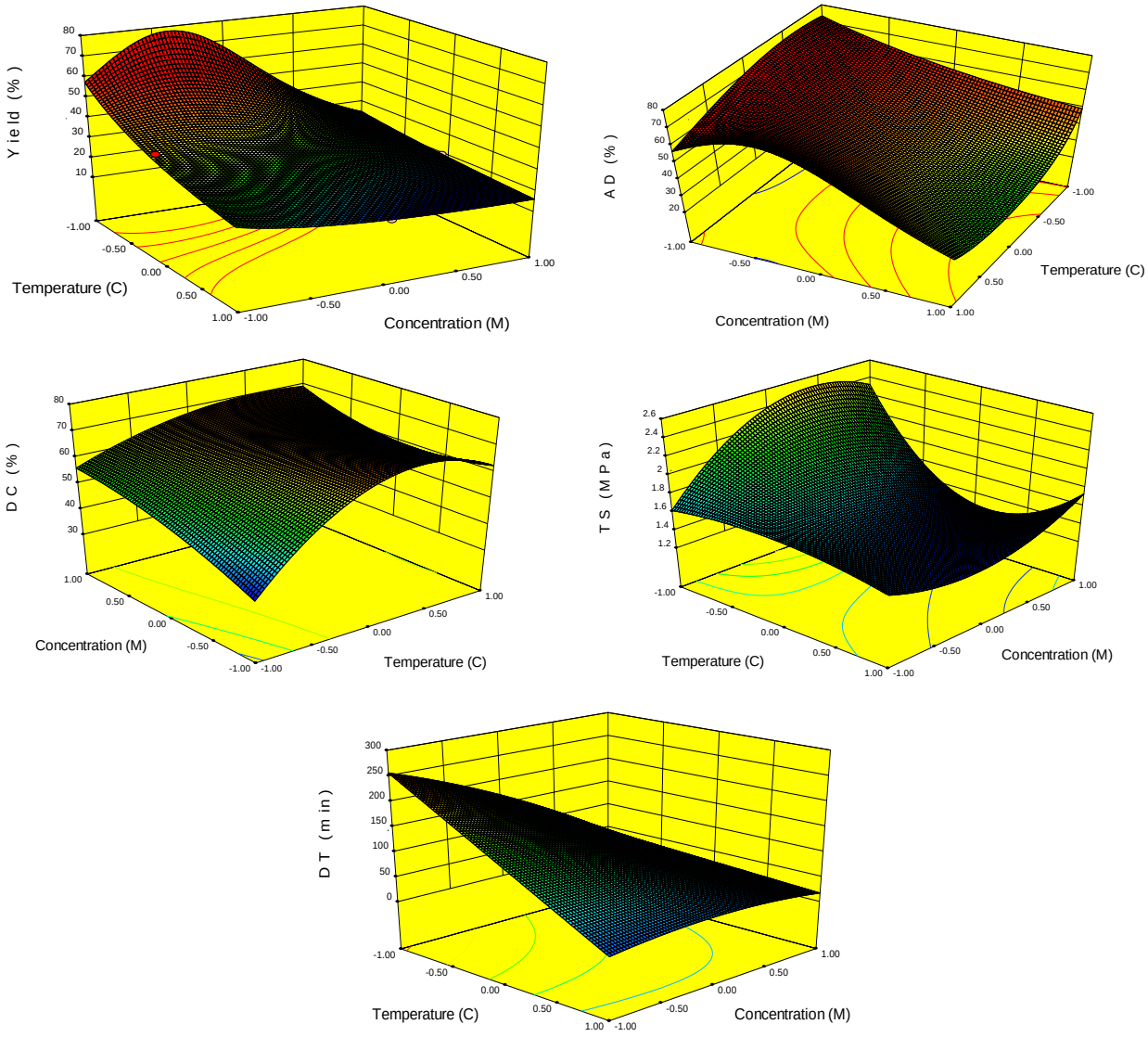

Figure 1. Surface plots of the properties studied according to the BBD. AD, Acetylation degree; MW, molecular weight; DC, degree of crystallinity; TS, tensile strength; DT, disintegration time.

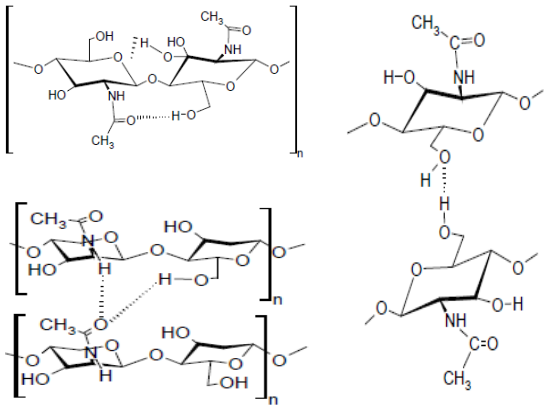

Figure 2. Intra and intermolecular hydrogen network pattern between chitin monomers.

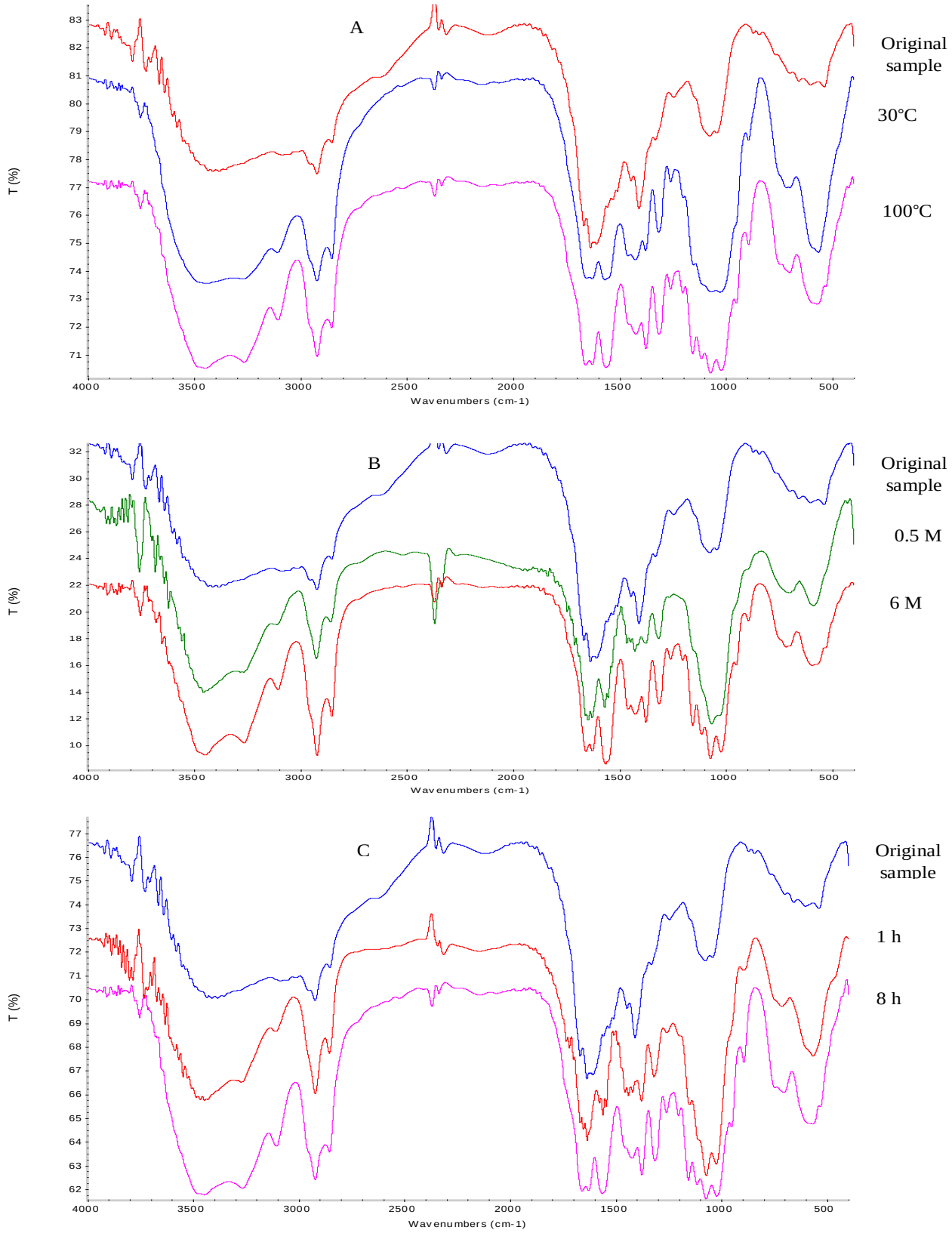

Figure 3. Effect of temperature (A), NaOH concentration (B) and reaction time (C) on the FT-IR chitin bands at 3 M NaOH for 1 h; 30°C for 4 h and 100°C and 3 M, respectively.

Conclusions

The alkaline treatment caused hydrolysis of acetamido groups and deacetylation, forming a different intra and intermolecular hydrogen bonding. At a high alkaline concentration or high temperature native chitin samples obtained from shrimp heads underwent extensive deacetylation. Harsh hydrolysis conditions led to a reduction of compact tensile strength and fastened disintegration time making this material able to be used as excipient for the development of pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors greatly thank the Committee for the Development of Research (CODI) and its Sustainability Strategy (2013-2014) for their financial support.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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