

Deacetylation of β -Chitin. II. Factorial Experimental Design

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ABSTRACT: A 2^3 -factorial experimental design has been used to study the main, two-factor-interaction, and three-factor-interaction effects of the NaOH concentration (X_1), deacetylation time (X_2), and deacetylation temperature (X_3) on the degree of deacetylation (DD) of deacetylated products prepared from β -chitin. Because the DD values of deacetylated products increase as X_1 , X_2 , or X_3 increases, the main, two-factor-interaction, and three-factor-interaction effects for these three deacetylation conditions are positive. The sequence of the main effects on the DD values of deacetylated products is $X_3 < X_2 < X_1$. The sequence of the

two-factor-interaction effects on the DD values of deacetylated products is X_2 versus $X_3 < X_1$ versus $X_3 < X_1$ versus X_2 . The prediction equation is $\hat{Y} = 42.4 + 13.9X_1 + 9.4X_2 + 8.4X_3 + 5.8X_1X_2 + 4.9X_1X_3 + 3.7X_2X_3 + 2.6X_1X_2X_3$ (where \hat{Y} is the predicted response) © 2004 Wiley Periodicals, Inc. *J Appl Polym Sci* 93: 2423–2428, 2004

Key words: chitin; chitosan; factorial experimental design; chlorinated polyethylene (CPE); oxidized polyethylene (OPE)

INTRODUCTION

Experimental designs and their statistical analyses have been well developed and widely applied in many research areas, such as basic science, engineering, and sociology. The main advantage of experimental designs is that they can cover a larger area of engineers' experimental interest and obtain unambiguous results at a minimum cost.^{1,2} Because factorial experimental design is powerful and easy to handle, this technique is one of the most commonly used methods to determine the effects of some independent variables that significantly affect final experimental results.

In 1993, Bambrick³ studied the fusion characteristics of poly(vinyl chloride) (PVC) compounds. The dependent variables were the fusion time, fusion temperature, and fusion torque. A Rheocord System 40 torque rheometer, equipped with a Rheomix 600 bowl and roller mixing blades, was used. Moreover, a central composite design of the experiment was applied to find the optimal formulation of additives for PVC compounds through changes in the following six independent formulation variables: the amounts of the impact modifier, paraffin wax, calcium stearate, ester wax, and processing aid. In previous works,^{4,5} we applied a 2^3 -factorial experimental design to study the

main, two-factor-interaction, and three-factor-interaction effects of three independent conditions on the heat of fusion of PVC–CPE–OPE blends and the yield of polyaniline powder.

Chitin, next to cellulose, is the second most common polysaccharide on Earth. Chitosan, a deacetylated product of chitin, is a high-molecular-weight heteropolysaccharide composed mainly or fully of β -(1,4)-2-deoxy-2-amino-D-glucopyranose and partially or not at all of β -(1,4)-2-deoxy-2-acetamido-D-glucopyranose units.⁶ Chitosan is the fully or partially deacetylated form of chitin. Chitin and chitosan, which have excellent biodegradability, biocompatibility, and bioactivity, have attracted significant interest for biomedical applications, including antithrombogenic, homeostatic, immunity-enhancing, and wound-healing applications.^{7–10} Therefore, many attempts have been made to produce new biofunctional materials from chitin and chitosan.¹¹

There are three kinds of crystal structures for chitin: α , β , and γ . The crystal structure of γ -chitin, which exists in fungi and yeasts, is a combination of α -chitin and β -chitin. α -Chitin, which is rhombic, can be prepared out of the shells of crabs, shrimps, and other arthropods.¹² β -Chitin, which is monoclinic, can be produced from squid pens. β -Chitin has a more open structure (parallel chain alignment) than α -chitin (antiparallel chain alignment), which is found in crustacean exoskeletons.¹³ Therefore, it shows higher solubility and swelling than α -chitin because of the much weaker intermolecular hydrogen bonding ascribable

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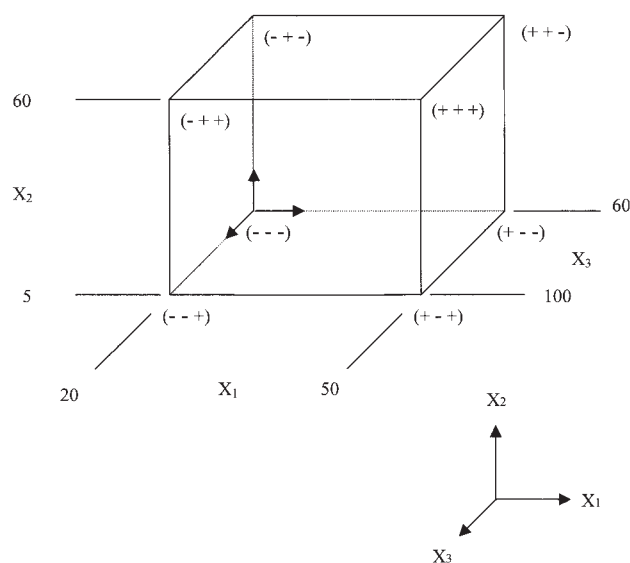


Figure 1 Diagrammatic representation of the standard ordering of a 2^3 -factorial experimental design.

to the parallel arrangement of the main chains. β -Chitin also shows higher reactivity than α -chitin during deacetylation.¹⁴

In a previous article,¹⁵ it was reported that the concentration of NaOH (X_1), the deacetylation time (X_2), and the deacetylation temperature (X_3) were three important factors affecting the degree of deacetylation (DD) of deacetylated products prepared from β -chitin. Moreover, the influences of X_1 (wt %), X_2 (min), and X_3 ($^{\circ}\text{C}$) on the DD values of deacetylated products prepared from β -chitin were studied clearly. There-

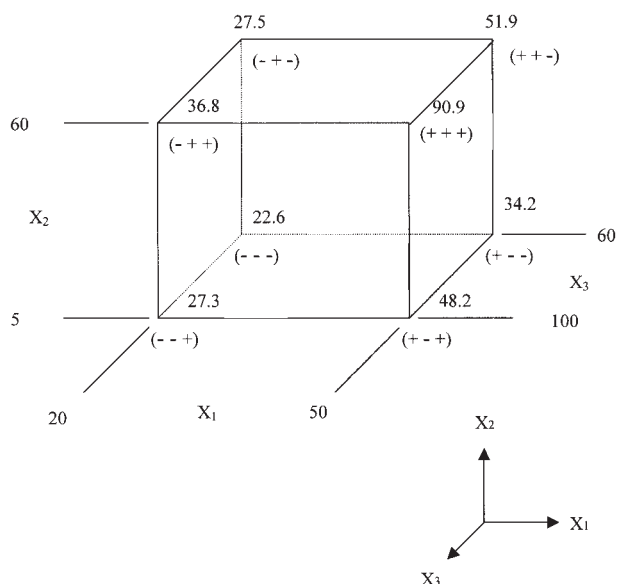


Figure 2 Diagrammatic representation of the observed DD values of deacetylated products and the standard ordering of deacetylation experiments for β -chitin.

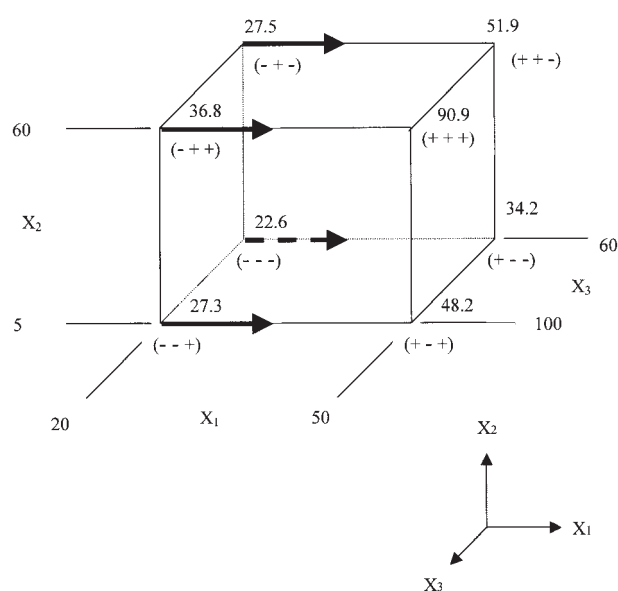


Figure 3 Determination of the main effect of X_1 on the DD values of deacetylated products.

fore, in this study, we applied a 2^3 -factorial experimental design [three independent variables with high (+) and low (-) levels] to study the main, two-factor-interaction, and three-factor-interaction effects of these three independent deacetylation variables on the DD values of deacetylated products prepared from β -chitin. A prediction equation is also provided.

EXPERIMENTAL

Materials

β -Chitin with 0.3% ash and a DD value of 6% was supplied by Ohka Enterprises Co., Ltd. (Kaohsiung,

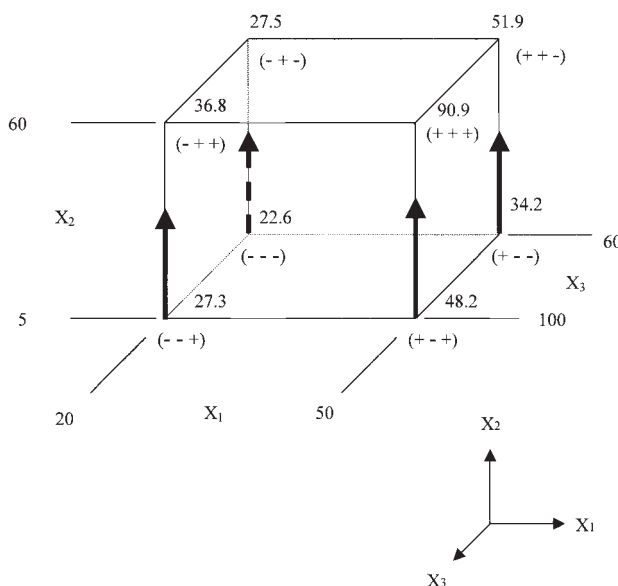


Figure 4 Determination of the main effect of X_2 on the DD values of deacetylated products.

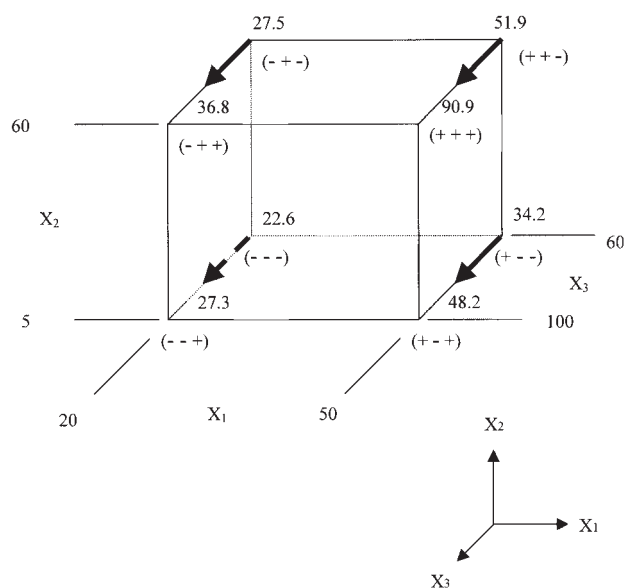


Figure 5 Determination of the main effect of X_3 on the DD values of deacetylated products.

Taiwan). It was extracted from squid pen. Its viscosity was about 155 mPa S (0.5 g of β -chitin was dissolved in a 5% LiCl solution via stirring for 6 h and then was measured with a viscometer). The material was light yellow, and the particle size was about 40-mesh. NaOH was purchased from Union Chemical Works, Ltd. (Hsinchu, Taiwan). All the reagents were used without further purification or treatment.

Deacetylation experimental design

A 250-mL conical Erlenmeyer flask was used for all deacetylation experiments. This reactor was equipped with a thermometer. A stirrer was put in the reactor to ensure proper mixing. β -Chitin (5 g) and a 100-mL NaOH solution were used in the deacetylation experiments.

X_1 , X_2 , and X_3 were chosen as the independent variables for the factorial experimental design. Two levels, high (+) and low (-), were also defined for each independent variable. Thus, a 2^3 -factorial exper-

TABLE I
Main Effect of X_1 on the DD Values of the Deacetylated Products

Effect of X_1 individual comparisons	X_2	X_3
$(90.9 - 36.8) = 54.1$	60	100
$(48.2 - 27.3) = 20.9$	5	100
$(51.9 - 27.5) = 24.4$	60	60
$(34.2 - 22.6) = 11.6$	5	60

Average (main effect of $X_1 = (54.1 + 20.9 + 24.4 + 11.6)/4 = 27.8$.

TABLE II
Main Effect of X_2 on the DD Values of the Deacetylated Products

Effect of X_2 individual comparisons	X_1	X_3
$(90.9 - 48.2) = 42.7$	50	100
$(36.8 - 27.3) = 9.5$	20	100
$(51.9 - 34.2) = 7.7$	50	60
$(27.5 - 22.6) = 4.9$	20	60

Average (main effect of $X_2 = (42.7 + 9.5 + 7.7 + 4.9)/4 = 18.7$.

imental design would have eight runs, the first in the standard order being --- and the last in the standard order being +++. For X_1 , 50 and 20 wt % were chosen as the high and low levels, respectively. For X_2 , 60 and 5 min were chosen as the high and low levels, respectively. For X_3 , 100 and 60°C were chosen as the high and low levels, respectively. The DD values of deacetylated products prepared from β -chitin were studied as the dependent variable here.

Determination of DD

The cross-polarization/magic-angle-spinning ^{13}C NMR spectrum was recorded at 100.624 MHz with a Bruker (Germany) Advance DPX-400 spectrometer at a spinning rate of 6200 Hz. The NMR spectrum represents an optimized 3-s pulse delay, a 10-ms contact time, and 1000 scans. The chemical shifts with respect to the intensities were measured in parts per million. The DD values of the deacetylated products prepared from β -chitin were related to the ratio of the signal intensities of methyl on acetyl groups (I_{CH_3}) and the first anomeric carbon (I_{C_1}) obtained from the ^{13}C -NMR spectrum.¹⁶ The following equation was used to calculate the DD values of the deacetylated products:

$$\text{DD (\%)} = \left(1 - \frac{I_{\text{CH}_3}}{I_{\text{C}_1}}\right) \times 100\% \quad (1)$$

RESULTS AND DISCUSSIONS

Figure 1 shows the standard figure of a 2^3 -factorial experimental design. Figure 2 shows the DD values of

TABLE III
Main Effect of X_3 on the DD Values of the Deacetylated Products

Effect of X_3 individual comparisons	X_1	X_2
$(90.9 - 51.9) = 39.0$	50	60
$(48.2 - 34.2) = 14.0$	50	5
$(36.8 - 27.5) = 9.3$	20	60
$(27.3 - 22.6) = 4.7$	20	5

Average (main effect of $X_3 = (39.0 + 14.0 + 9.3 + 4.7)/4 = 16.8$.

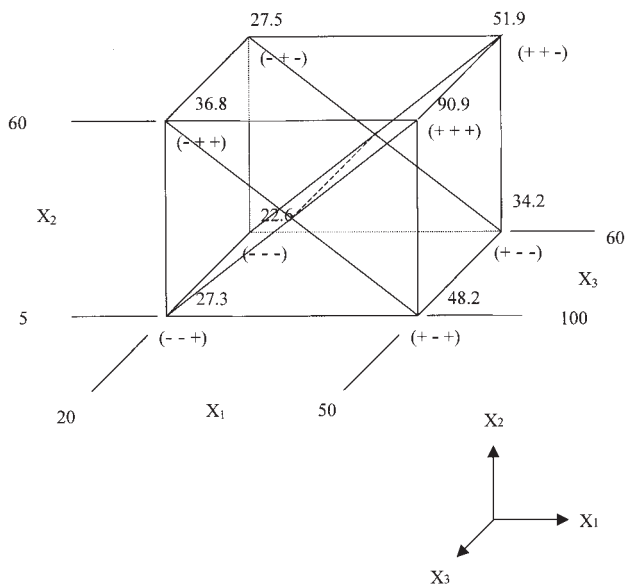


Figure 6 Determination of the interaction effect of X_1 versus X_2 on the DD values of deacetylated products.

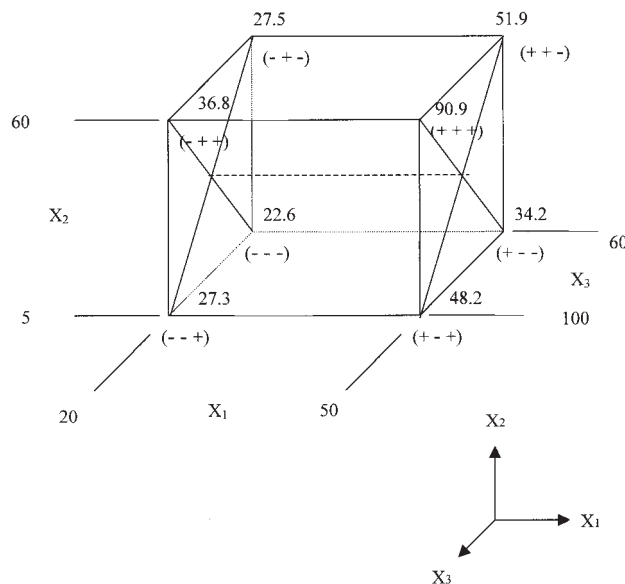


Figure 8 Determination of the interaction effect of X_2 versus X_3 on the DD values of deacetylated products.

deacetylated products and the standard ordering of the deacetylation experiments. Figures 3–5 present the determinations of the main effects of X_1 , X_2 , and X_3 , respectively. The main effect of the controlled independent variable is the average of the difference between the values at the high level (+) and the values at low level (–). Tables I–III illustrate the results of the main effects of X_1 , X_2 , and X_3 , respectively. Comparing the results of these three tables, we find that the sequence of the main effects on the DD values of deacetylated products is $X_3 < X_2 < X_1$.

Figures 6–8 present the determinations of the interaction effects of X_1 versus X_2 , X_1 versus X_3 , and X_2 versus X_3 , respectively. The two-factor-interaction effect of X_1 versus X_2 is equal to half the difference $[(39.3 - 16.3)/2 = 11.5]$ between the average X_1 effect with a concentration of 50 wt % $[(54.1 + 24.4)/2 = 39.3]$ and the average X_1 effect with a concentration of 20 wt % $[(20.9 + 11.6)/2 = 16.3]$. The interaction effect of X_1 versus X_3 is equal to half the difference $[(37.5 - 18.0)/2 = 9.8]$ between the average X_1 effect with X_3

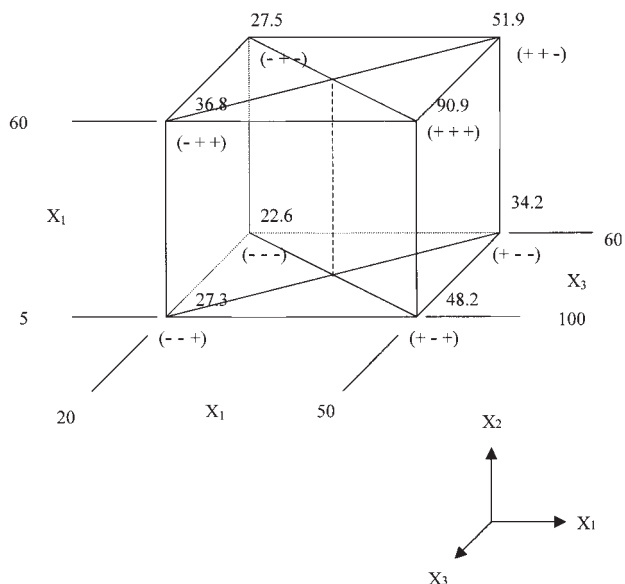


Figure 7 Determination of the interaction effect of X_1 versus X_3 on the DD values of deacetylated products.

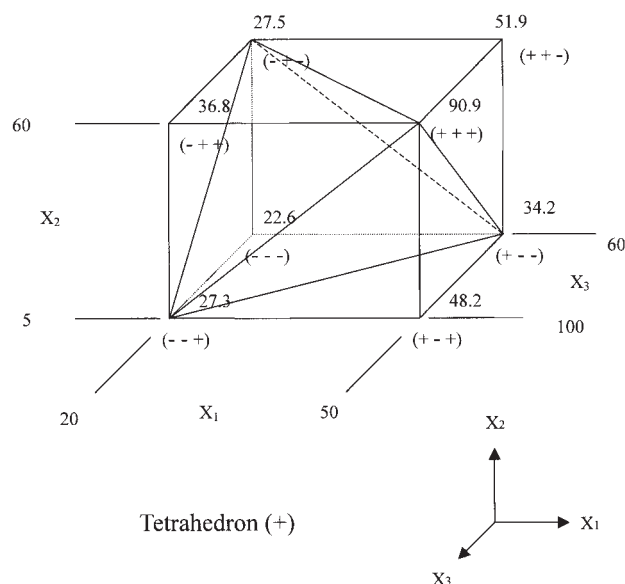


Figure 9 Determination of the three-factor-interaction effect of X_1 versus X_2 versus X_3 on the DD values of deacetylated products [(+)-tetrahedron].

= 100°C [(54.1 + 20.9)/2 = 37.5] and the average X_1 effect with $X_3 = 60^\circ\text{C}$ [(24.4 + 11.6)/2 = 18.0]. Similarly, the interaction effect of X_2 versus X_3 is equal to half the difference [(26.1 - 11.3)/2 = 7.4] between the average X_2 effect with $X_3 = 100^\circ\text{C}$ [(42.7 + 9.5)/2 = 26.1] and the average X_2 effect with $X_3 = 60^\circ\text{C}$ [(17.7 + 4.9)/2 = 11.3].

We can consider the individual comparisons of the effect of X_1 . There are two available measurements from the experiment that can be used to estimate the three-factor-interaction effect, X_1 versus X_2 versus X_3 , one for each value of X_3 : $X_3 = 100^\circ\text{C}$ [(54.1 - 20.9)/2 = 16.6] and $X_3 = 60^\circ\text{C}$ [(24.4 - 11.6)/2 = 6.4]. Half this difference [(16.6 - 6.4)/2 = 5.1] is defined as the three-factor-interaction effect of X_1 versus X_2 versus X_3 . The difference between these two estimates is a measure of consistency for each value of X_2 : $X_2 = 100$ min [(54.1 - 24.4)/2 = 14.9] and $X_2 = 60$ min [(20.9 - 11.6)/2 = 4.7]. Half this difference [(14.9 - 4.7)/2 = 5.1] is the same result mentioned previously.

The same results will also be obtained from the effect of individual X_2 comparisons or individual X_3 comparisons. As for the main effects and the two factor-interaction effects, the estimate of the three-factor-interaction effects can be obtained from the difference between the average of vertices of (+)-tetrahedron (Fig. 9) and the average of vertices of (-)-tetrahedron (Fig. 10), that is, (90.9 + 34.2 + 27.3 + 27.5)/4 - (48.2 + 51.9 + 22.6 + 36.8)/4 = 5.1.

Table IV summarizes the main, two-factor-interaction, and three-factor-interaction effects of the DD values of deacetylated products. The sequence of the main effects on the DD values of deacetylated prod-

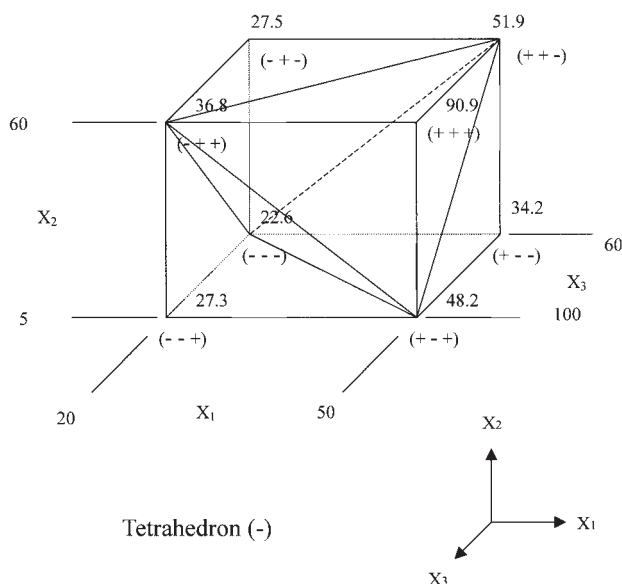


Figure 10 Determination of the three-factor-interaction effect of X_1 versus X_2 versus X_3 on the DD values of deacetylated products [(-)-tetrahedron].

TABLE IV
Summary of Main, Two-Factor-interaction, and Three-Factor-Interaction Effects of the DD Values of the Deacetylated Products

Main effect	Two-factor-interaction effect	Three-factor-interaction effect
$X_1 = 27.8$	X_1 vs $X_2 = 11.5$	X_1 vs X_2 vs $X_3 = 5.1$
$X_2 = 18.7$	X_1 vs $X_3 = 9.8$	
$X_3 = 16.8$	X_2 vs $X_3 = 7.4$	

ucts is X_3 (16.8) < X_2 (18.7) < X_1 (27.8). This result implies that X_1 is the most significant factor affecting the DD values of deacetylated products. That is, without NaOH in the reaction solution, even with high X_3 or X_2 values, the deacetylation of β -chitin cannot be conducted. In addition, X_2 also affects the deacetylation of β -chitin directly. Therefore, X_2 affects the DD values of deacetylated products more than X_3 . However, the main-effect difference between X_2 and X_3 is not significant.

The sequence of the two-factor-interaction effects on the DD values of deacetylated products is X_2 versus X_3 (7.4) < X_1 versus X_3 (9.8) < X_1 versus X_2 (11.5). As mentioned previously, X_1 is the most important individual factor. Furthermore, X_2 is the second most important individual factor. Therefore, the interaction effect between X_1 and X_2 is the highest in determining the DD values of deacetylated products. Similarly, the interaction effect between X_1 and X_3 is the second in order in determining the DD values of deacetylated products. The interaction effect between X_2 and X_3 is the third in order. Because the DD values of deacetylated products increase as X_1 , X_2 , or X_3 increases, the three-factor-interaction effect is significantly related to DD. Moreover, because of the positive effect on the DD values of deacetylated products for these three major deacetylation conditions, the main, two-factor-interaction, and three-factor-interaction effects are positive. From the results listed in Table IV, we can obtain a prediction equation:¹⁷

$$\hat{Y} = \bar{Y} + 13.9X_1 + 9.4X_2 + 8.4X_3 + 5.8X_1X_2 + 4.9X_1X_3 + 3.7X_2X_3 + 2.6X_1X_2X_3$$

where \hat{Y} is the predicted response; \bar{Y} , the average of all response values from the experimental data, is 42.4; and X_1 , X_2 , and X_3 are plus;1 (if high-level) or -1 (if low-level)

CONCLUSIONS

The DD values of deacetylated products prepared from β -chitin increase significantly with X_1 , X_2 , and X_3 . We have successfully applied a 2^3 -factorial experimental design to study the main, two-factor-interac-

tion, and three-factor-interaction effects of X_1 , X_2 , and X_3 on the DD values of deacetylated products. The sequence of the main effects on the DD values of deacetylated products is X_3 (16.8) < X_2 (18.7) < X_1 (27.8). The sequence of the two-factor-interaction effects on the DD values of deacetylated products is X_2 versus X_3 (7.4) < X_1 versus X_3 (9.8) < X_1 versus X_2 (11.5). The three-factor-interaction effect (5.1) is significantly related to the DD values of deacetylated products. The prediction equation is

$$\hat{Y} = 42.4 + 13.9X_1 + 9.4X_2 + 8.4X_3 + 5.8X_1X_2 + 4.9X_1X_3 + 3.7X_2X_3 + 2.6X_1X_2X_3$$

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