Study of the Influence of Reaction Conditions on the Degree of Substitution, Intrinsic Viscosity, and Yield of Oxidized Cellulose Acetate by Factorial Experimental Design

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ABSTRACT: A half-fraction, two-level, four-factor factorial experimental design was used to study the effects of the acetic anhydride concentration, reaction temperature, reaction time, and sulfuric acid concentration on the degree of substitution, intrinsic viscosity, and yield of oxidized cellulose acetate (OCA). Oxidized cellulose containing 20% (w/w) carboxylic acid was used as the starting material. The data were fitted by multiple regression analysis with SAS software. The correlation coefficients obtained from plots of the predicted and observed values for the degree of substitution, intrinsic viscosity, and yield were 0.985, 0.993, and 0.991, respectively. Residual normal plots of the regression models showed a linear relationship. Lenth and main-factor-effect plots revealed an increase in the degree of substitution of OCA with an increasing concentration of acetic anhydride. The latter had no effect on the intrinsic viscosity and yield of OCA. An increase in the reaction temperature led to an increase in the degree of substitution and a decrease in the intrinsic viscosity and yield of OCA. The influence of the reaction time on the degree of substitution and intrinsic viscosity followed a trend similar to that observed with the reaction temperature, but the yield of OCA was unaffected. Increasing the concentration of sulfuric acid reduced the degree of substitution, intrinsic viscosity, and yield of OCA.
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Key words: oxidized cellulose esters; oxidized cellulose acetate; 6-carboxycellulose acetate; factorial experimental design

INTRODUCTION

Recently, considerable interest has been focused on the use of carboxyl-functionalized cellulose [6-carboxycellulose, commonly called oxidized cellulose (OC); Fig. 1] as a drug carrier[1–10] and as a biomaterial.[11,12] Ashton and Moser[13] reported that OC with a carboxylic acid group concentration as low as 3% was biocompatible and bioresorbable. Currently, OC with a 14–24% carboxyl concentration is commonly and widely used to stop bleeding during surgery and to prevent the (re)formation of adhesions after surgery.[11,12] Studies have shown that OC also possesses anti-inflammatory,[14] antitumor,[15] immunostimulant,[16] and wound-healing[17] properties. However, because OC is insoluble in water and common organic solvents, it presents limited formulation flexibility. To overcome this problem, we have recently transformed OC into oxidized cellulose acetate (OCA) by a treatment with a mixture of acetic anhydride (AC_2O) and acetic acid (HOAC) in the presence of sulfuric acid (H_2SO_4) as a catalyst.[18] The new material is soluble in a variety of organic solvents, including binary solvent systems such as methylene dichloride and methanol.

In this article, we report the results of a two-level, four-factor factorial experimental design used to study the effects of the AC_2O concentration (X_1), reaction temperature (X_2), reaction time (X_3), and H_2SO_4 concentration (X_4) on the degree of substitution (Y_1), intrinsic viscosity (Y_2), and yield of OCA (Y_3).

EXPERIMENTAL

Materials

OC containing 20% carboxylic acid groups (w/w) was prepared from cotton linters (grade 10-270; Southern Cellulose Products, Inc., Chattanooga, TN) by a treatment with a mixture of phosphoric acid, nitric acid, and sodium nitrite according to a procedure reported recently by Kumar and Yang.[18] All other chemicals were analytical-reagent-grade and were used as received.

Factorial experimental design

A two-level, four-factor factorial experimental design, with X_1, X_2, X_3, and X_4 as independent variables and

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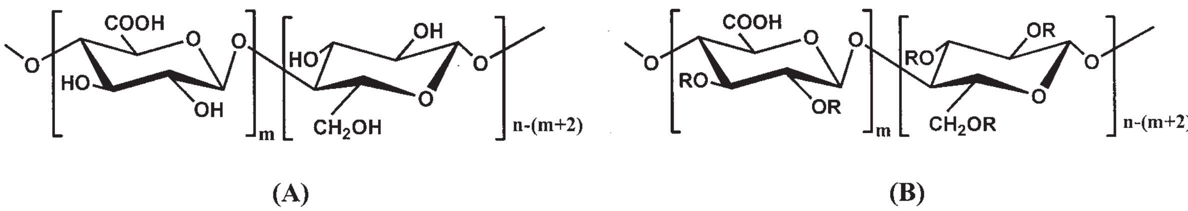

Figure 1 Structures of (A) OC and (B) OCA [R = H or $C(O)CH_3$].

Y_1, Y_2, and Y_3 as dependent variables, was employed. In this two-level, four-factor, half-fractional factorial experimental design, the confounding rule was 0 = 1234, 1 = 234, 2 = 134, 3 = 124, 4 = 123, 12 = 34, 13 = 24, and 14 = 23. The resolution was 4. The lower and upper levels of the independent variables are listed in Table I. These four independent variables were major factors that were expected to have pronounced effects on the dependent properties of OCA and control the reaction output. Lower order interactions of main factors were also studied, but contributions from higher order interactions were assumed to be insignificant. The regression expression (reduced model) for a four-factor analysis-of-variance model is

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_{12} (\text{or } X_{34}) + \beta_{13} X_{13} (or X_{24}) + \beta_{14} X_{14} (or X_{23}) + \varepsilon$$

where y is the response parameter of interest; X_1, X_3, and X_4 are independent variables; X_{12}, X_{13}, X_{14}, X_{23}, and X_{24} are second-order interactions of independent variables; β_0, β_1, β_2, β_3, β_4, β_{12}, β_{13}, and β_{14} are model coefficients determined by the multiple linear regression analysis; and ε is the residual error. The response data were fitted to the model with SAS software (SAS Institute, Cary, NC).

The experimental procedure and characterization methods used in the study are described next.

Preparation of OCA

OC (10 g; activated by soaking in 50 mL of water for 30 min and then dehydrated with 200 mL of glacial HOAC) was added slowly to a $100 - (X_1 + X_4)/X_1/X_4$ (w/w/w) mixture of HOAC, AC_2O, and H_2SO_4 (Table II) with constant stirring at 0°C. The resulting mixture was allowed to react at 30 or 60°C for 1 or 4 h. The clear solution obtained was cooled in an ice–water bath for 30 min, and 200 mL of diethyl ether was then added to the reaction mixture. The white solid that precipitated was filtered, washed with distilled water to a constant pH, and then vacuum-dried.

TABLE I
Experimental Variables and Factor Levels

Independent variable[a]	Lowest level (−1)	Highest level (1)
X_1 (wt %)	30.0	80.0
X_2(°C)	30.0	60.0
X_3 (h)	1.0	4.0
X_4 (wt%)[a]	0.1	0.8

[a] The weight ratio of HOAC to AC_2O to H_2SO_4 in the reaction was $100 - (X_1 + X_4):X_1:X_4$.

Determination of the degree of substitution of acetyl groups (DS) (Y_1)

DS was determined by ^{1}H-NMR with the following relationship:

$$DS = \frac{\text{Peak area of methyl protons}(\delta 1.5-2.3\text{ppm})/3}{\text{Peak area of glucose/glucuronic ring protons}(\delta 3.5-5.0\text{ppm})/5.5}$$

The ^{1}H-NMR spectra were recorded in solutions of dimethyl sulfoxide-d_6 (DMSO-d_6) on a Bruker MSL-300 spectrometer (Bruker Biospin Co., Billerica, MA). DMSO-d_6 also served as an internal reference (^{1}H = 2.49 ppm).

TABLE II
Experiment Matrix and Results

Run	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3
1-1	−1	−1	−1	−1	1.19	0.488	68.0
1-2	−1	−1	−1	−1	1.04	0.477	67.5
2-1	1	−1	−1	1	1.61	0.377	66.9
2-2	1	−1	−1	1	1.67	0.355	67.4
3-1	−1	1	−1	1	1.67	0.217	61.0
3-2	−1	1	−1	1	1.76	0.206	61.9
4-1	1	1	−1	−1	2.08	0.366	60.8
4-2	1	1	−1	−1	2.09	0.387	61.5
5-1	−1	−1	1	1	1.39	0.316	65.2
5-2	−1	−1	1	1	1.32	0.322	65.7
6-1	1	−1	1	−1	2.02	0.426	70.0
6-2	1	−1	1	−1	2.01	0.435	71.0
7-1	−1	1	1	−1	1.96	0.342	63.0
7-2	−1	1	1	−1	2.09	0.336	62.8
8-1	1	1	1	1	2.00	0.192	56.3
8-1	1	1	1	1	2.01	0.200	59.1

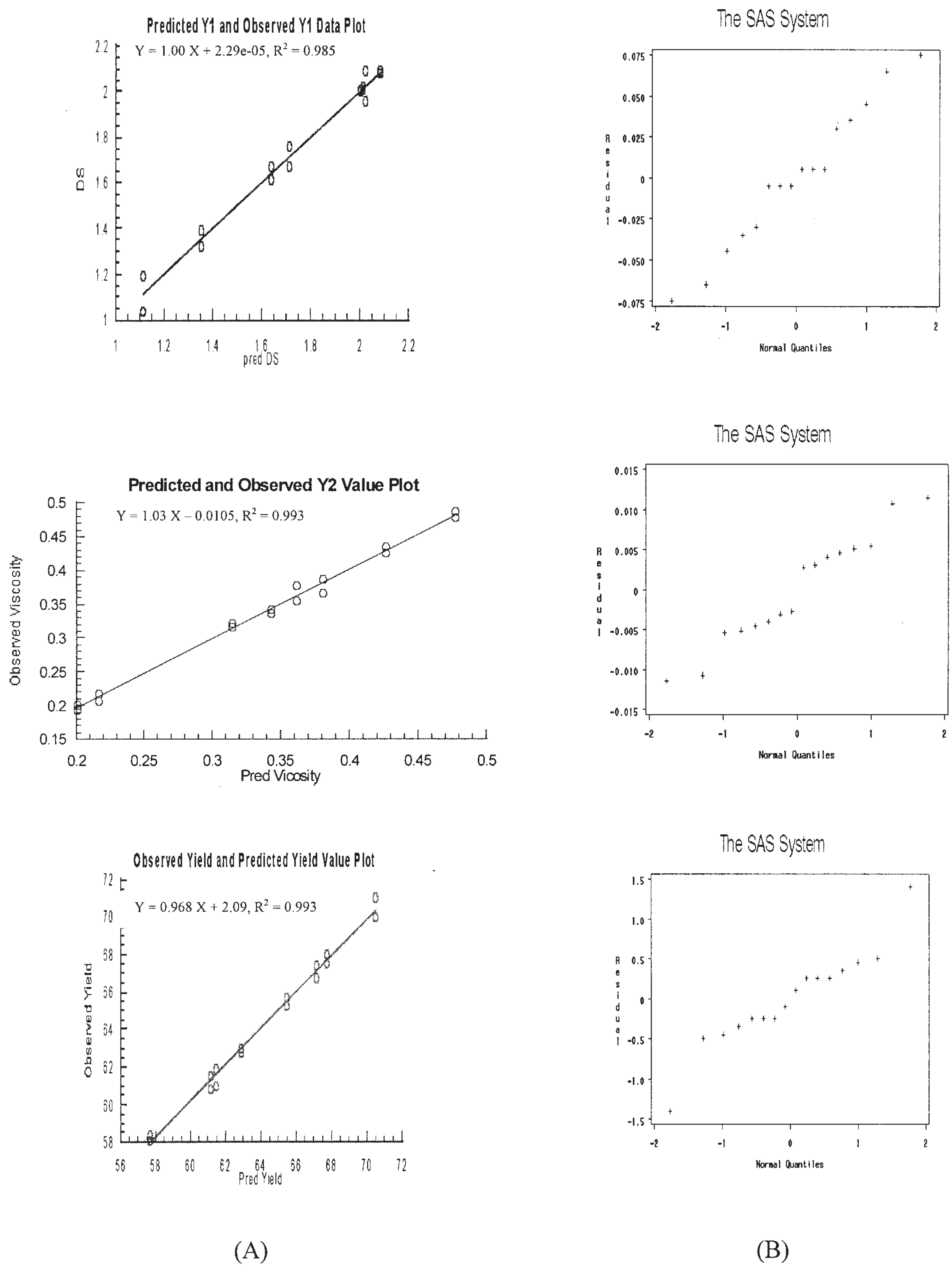

Figure 2 (A) Relationship between the predicted and observed values of Y_1, Y_2, and Y_3 and (B) normal residue plots.

TABLE III
Overall F Tests of the Coefficients of the Models (from SAS Output)

Model	Source	Degrees of freedom	Sum of squares	Mean squares	F value	p value
Y_1	Model	7	1.79	0.256	72.7	<0.0001
	error	8	0.03	0.004		
Y_2	Model	7	0.136	0.0200	218	<0.0001
	error	8	0.001	0.0001		
Y_3	Model	7	247	35.2	51.6	<0.0001
	error	8	6	0.7		

Determination of intrinsic viscosity (Y_2)

Y_2 was determined in a 10:1 (v/v) mixture of acetone and water at 20.0 ± 0.1°C with a Canon-Fenske capillary viscometer (size 100). Ten milliliters of the OCA solution in acetone/water (0.2–0.8% w/v) was transferred to the lower bulb of the viscometer and then equilibrated to 20.0 ± 0.1°C in a precisely controlled water bath for 5 min. The equilibrated solution was drawn into the upper bulb (above the upper mark) by suction and then allowed to flow freely. The time required for the fluid meniscus to flow from the upper mark of the bulb to the lower mark was recorded and used to calculate the relative viscosity (η_{rel}) as follows: $\eta_{rel} = t/t_0$, where t and t_0 are the efflux times for the sample solution and blank solvent (10:1 acetone/water), respectively. The specific viscosity (η_{sp}) was calculated by the subtraction of 1 from η_{rel}. Y_2 was determined from a plot of η_{sp}/C versus C, where C is the concentration of an OC solution (g/dL).

Determination of yield (Y_3)

Y_3 was calculated according to the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of the product}}{\text{Theoretical weight of the product}} \times 100$$

TABLE IV
Coefficient Estimates of the Y_1 Model and t Test (α = 0.05)

	Degrees of freedom	Estimate	t value	p value
Intercept	1	1.74	118	<0.0001
X_1	1	0.192	12.9	<0.0001
X_2	1	0.213	14.4	<0.0001
X_3	1	0.106	7.12	<0.0001
X_4	1	−0.0656	−4.43	0.0022
X_{12}/X_{34}	1	−0.104	−7.04	0.0001
X_{13}/X_{24}	1	−0.0319	−2.15	0.0638
X_{14}/X_{23}	1	−0.0481	−3.25	0.0118

TABLE V
Coefficient Estimates of the Y_2 Model and t Test (α = 0.05)

	Degrees of freedom	Estimate	t value	p value
Intercept	1	0.340	144	<0.0001
X_1	1	0.00213	0.900	0.3930
X_2	1	−0.0594	−25.2	<0.0001
X_3	1	−0.0189	−8.02	<0.0001
X_4	1	−0.0669	−28.4	<0.0001
X_{12}/X_{34}	1	0.00331	1.40	0.1990
X_{13}/X_{24}	1	−0.00999	−4.19	0.0030
X_{14}/X_{23}	1	0.00564	2.39	0.0444

The theoretical weight of the product was calculated as follows: (weight of OC/173.0)(173.0 + DS × 42), where 173.0 is the average molecular weight of the anyhydroglucose ring in OC and 42 is the molecular weight of the carboxylic acid groups.

RESULTS AND DISCUSSION

The values of the different levels of the independent variables (X_1, X_2, X_3, and X_4) used in this study are presented in Table I. Table II lists the values of the response factors (Y_1, Y_2, and Y_3). The regression expressions obtained for these response factors are as follows:

$$Y_1 = 1.74 + 0.192X_1 + 0.213X_2 + 0.106X_3 - 0.066X_4 - 0.104X_{12}(\text{or } X_{34}) - 0.032X_{13}(\text{or } X_{24}) - 0.048X_{14}(\text{or } X_{23})$$

$$Y_2 = 0.340 + 0.002X_1 - 0.059X_2 - 0.019X_3 - 0.067X_4 + 0.003X_{12}(\text{or } X_{34}) - 0.010X_{13}(\text{or } X_{24}) + 0.006X_{14}(\text{or } X_{23})$$

TABLE VI
Coefficient Estimates of the Y_3 Model and t Test (α = 0.05)

	Degrees of freedom	Estimate	t value	p value
Intercept	1	64.3	311	<0.0001
X_1	1	−0.131	−0.640	0.5430
X_2	1	−3.46	−16.7	<0.0001
X_3	1	−0.119	−0.570	0.5813
X_4	1	−1.32	−6.38	0.0002
X_{12}/X_{34}	1	−1.24	−6.02	0.0003
X_{13}/X_{24}	1	0.0938	0.450	0.6621
X_{14}/X_{23}	1	−0.381	−1.85	0.1022

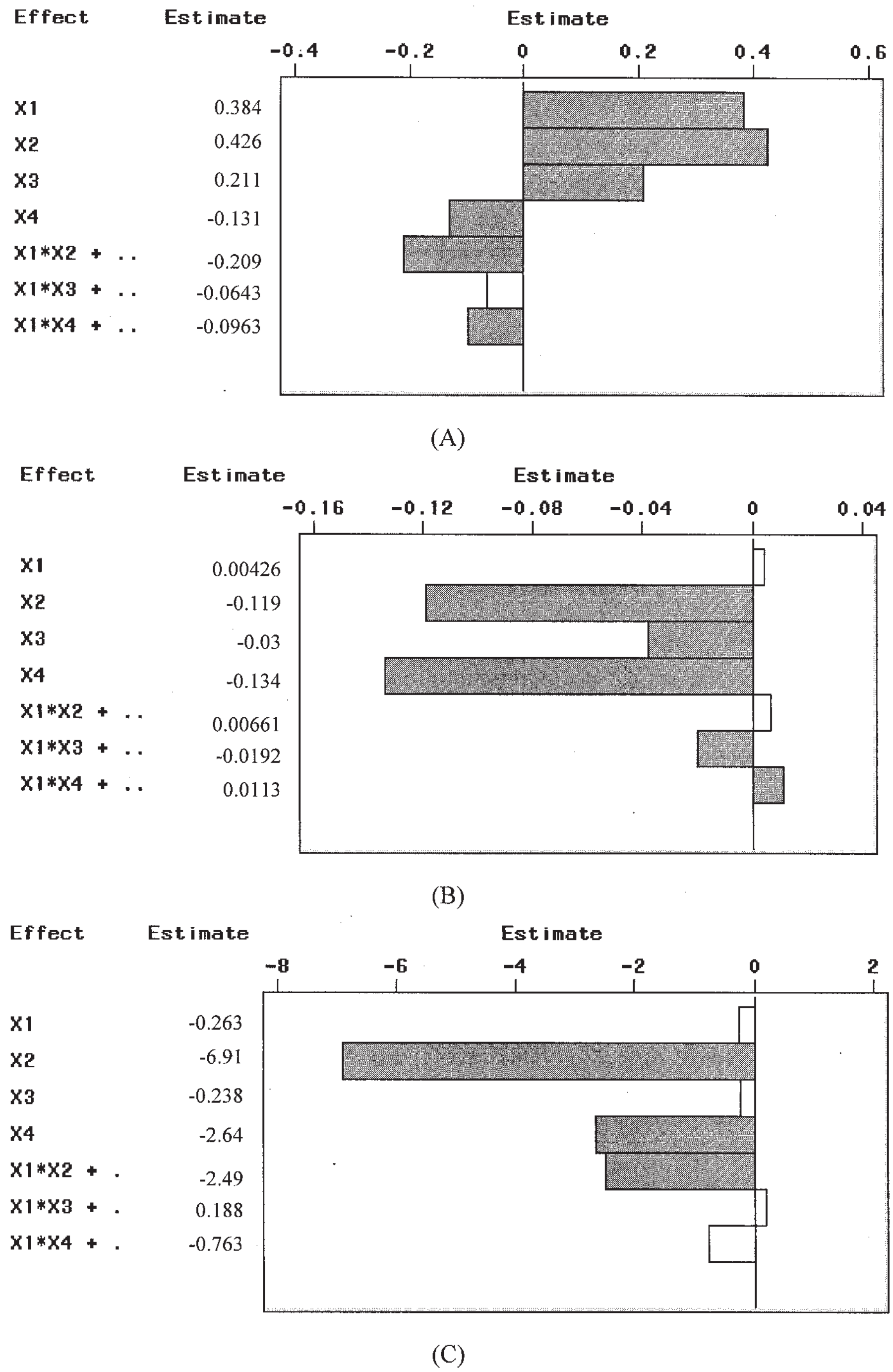

Figure 3 Lenth plots of (A) Y_1, (B) Y_2, and (C) Y_3 models.

$$Y_3 = 64.3 - 0.131X_1 - 3.46X_2 - 0.119X_3 - 1.32X_4 - 1.24X_{12}(\text{or } X_{34}) + 0.009X_{13}(\text{or } X_{24}) - 0.381X_{14}(\text{or } X_{23})$$

where X_{12} (or X_{34}), X_{13} (or X_{24}), and X_{14} (or X_{23}) represent the second-order interactions between X_1 and X_2 (or X_3 and X_4), X_1 and X_3 (or X_2 and X_4), and X_1 and X_4 (or X_2 and X_3) factors, respectively. The interaction terms X_{12}, X_{13}, and X_{14} confound with X_{34}, X_{24}, and X_{23}, respectively. Interaction measures the extent to which the effect of one factor changes for different values of other (one or more) factors. Interactions are mathematically independent of their constituent main effects; that is, the presence of a significant interaction does not require or preclude significant main effects.

The correlation coefficients of these models were tested by plots of the predicted and observed values and by residual normal plots [Figs. 2(A,B)]. The coef-

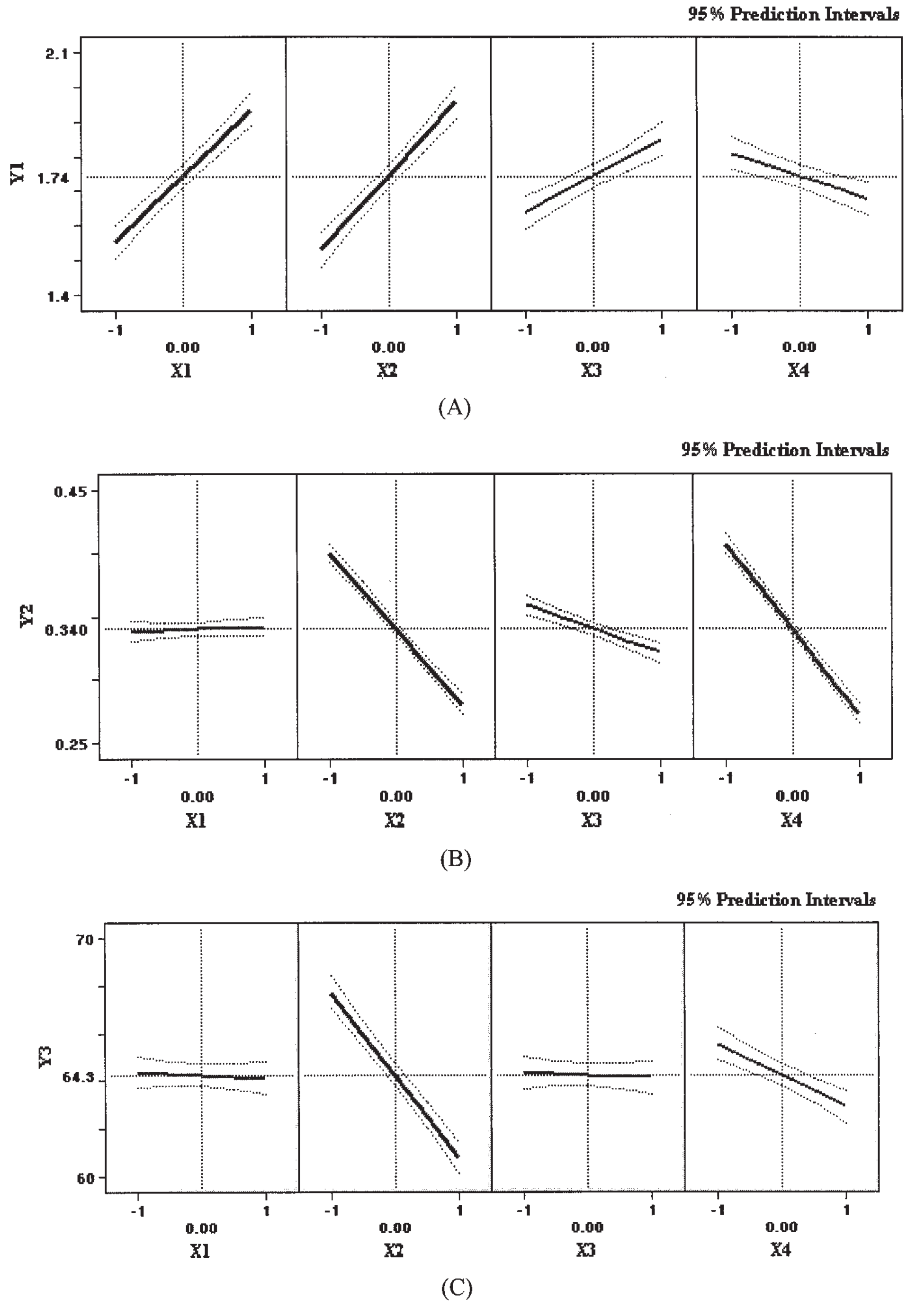

Figure 4 Main-effect plots of (A) Y_1, (B) Y_2, and (C) Y_3 models.

ficient of determination (R^2) values were 0.985, 0.995, and 0.978 for the Y_1, Y_2, and Y_3 models, respectively. These results suggest that there is a reasonably good linear relationship between the predicted and observed correlation coefficient values. A nearly linear normal plot [Fig. 2(B)] shows a normal distribution of residuals for these models, indicating that the models obtained are good predictors of the effects of the main factors on Y_1, Y_2, and Y_3.

Estimation and examination of the coefficients

The coefficient of an experimental factor represents the effect of that factor on the output of responses. A positive sign indicates a synergistic effect, whereas a negative number indicates an antagonistic effect on the response. The overall F test was used to test whether or not a regression relation existed between the Y response variable and the set of X variables

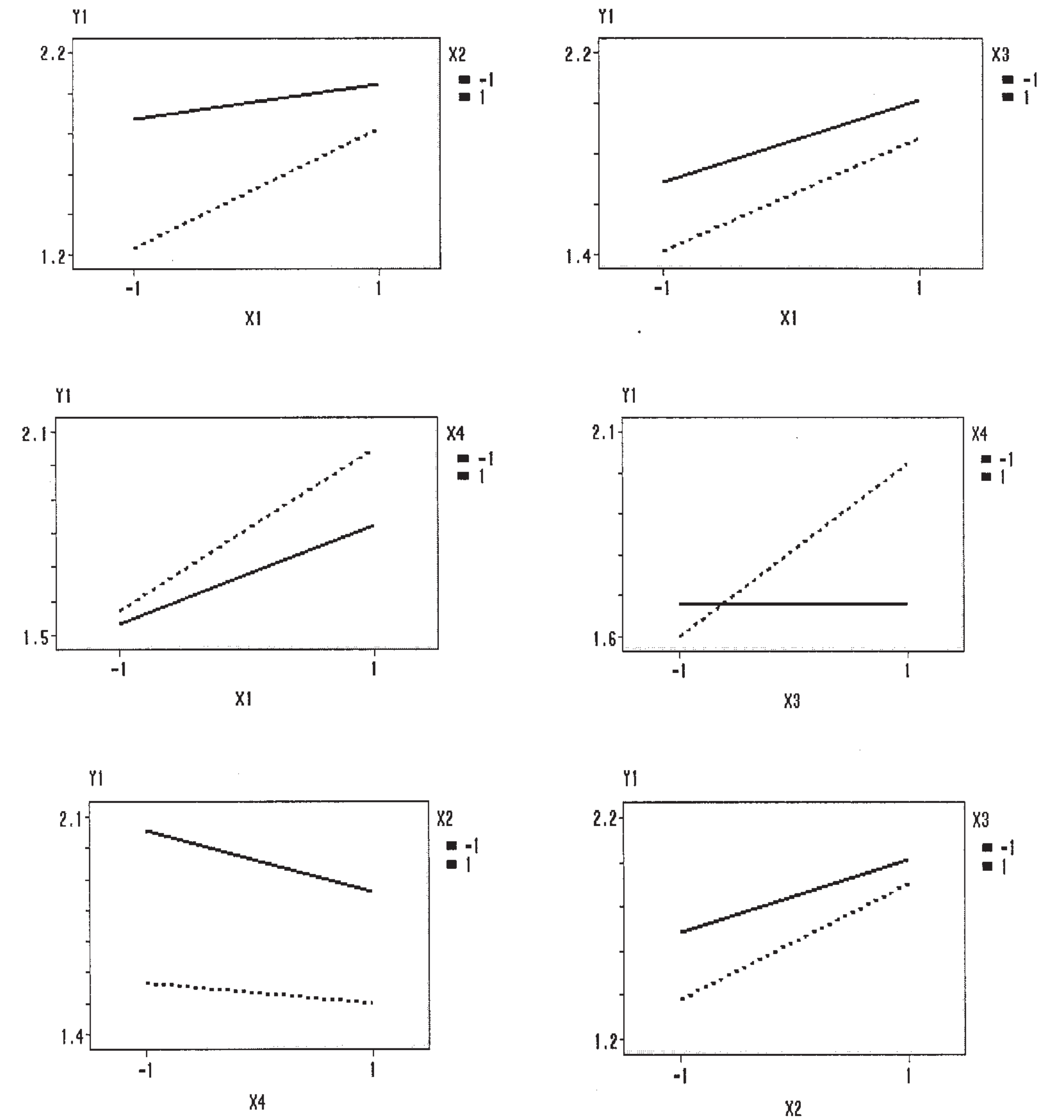

Figure 5 Two-way interaction plots for the Y_1 model.

(Table III). The alternatives were as follows: H_0, $\beta_0 = \beta_1 = \beta_2 = \ldots \beta_{14}$ (or β_{23}) = 0, and H_a, $\beta \neq 0$. The p value of this entire test was 0.000. Thus, for the model of each response, there must be a coefficient not equal to zero. The coefficient values estimated for the Y_1, Y_2, and Y_3 models are listed in Tables IV–VI.

Analysis of the factorial design output

The main-effect and partial second-order interaction coefficients obtained for the three models are listed in Tables IV–VI. The coefficient values for the three factors (X_1, X_2, and X_3) in the Y_1 model were 0.192, 0.213, and 0.106, respectively, and the p values for all the interaction coefficients were 0.0001; this suggested that Y_1 was significantly affected by X_1, X_2, and X_3. The positive coefficient values meant that Y_1 increased with increasing X_1, X_2, and X_3. The estimated coefficient and the p value for X_4 were −0.066 and 0.002, respectively, and this meant that Y_1 was also affected by X_4 of the reaction system. However, in comparison with the influence of the other three factors, it decreased with increasing X_4. Lenth and main-effect plots showing these effects are depicted in Figures 3 and 4, respectively.

In Table IV, X_{12}/X_{34}, X_{13}/X_{24}, and X_{14}/X_{23} represent second-order interactions between X_1 and X_2 (or X_3 and X_4), X_1 and X_3 (or X_2 and X_4), and X_1 and X_4 (or X_2 and X_3), respectively. The estimated coefficient values of −0.104, −0.032, and −0.048 and the p values of 0.0001, 0.064, and 0.012 for X_{12}/X_{34}, X_{13}/X_{24}, and X_{14}/X_{23}, respectively, indicate significant interactions between X_1 and X_2 (or X_3 and X_4) and X_1 and X_4 (or X_2 and X_3) pairs only. The negative sign indicates an

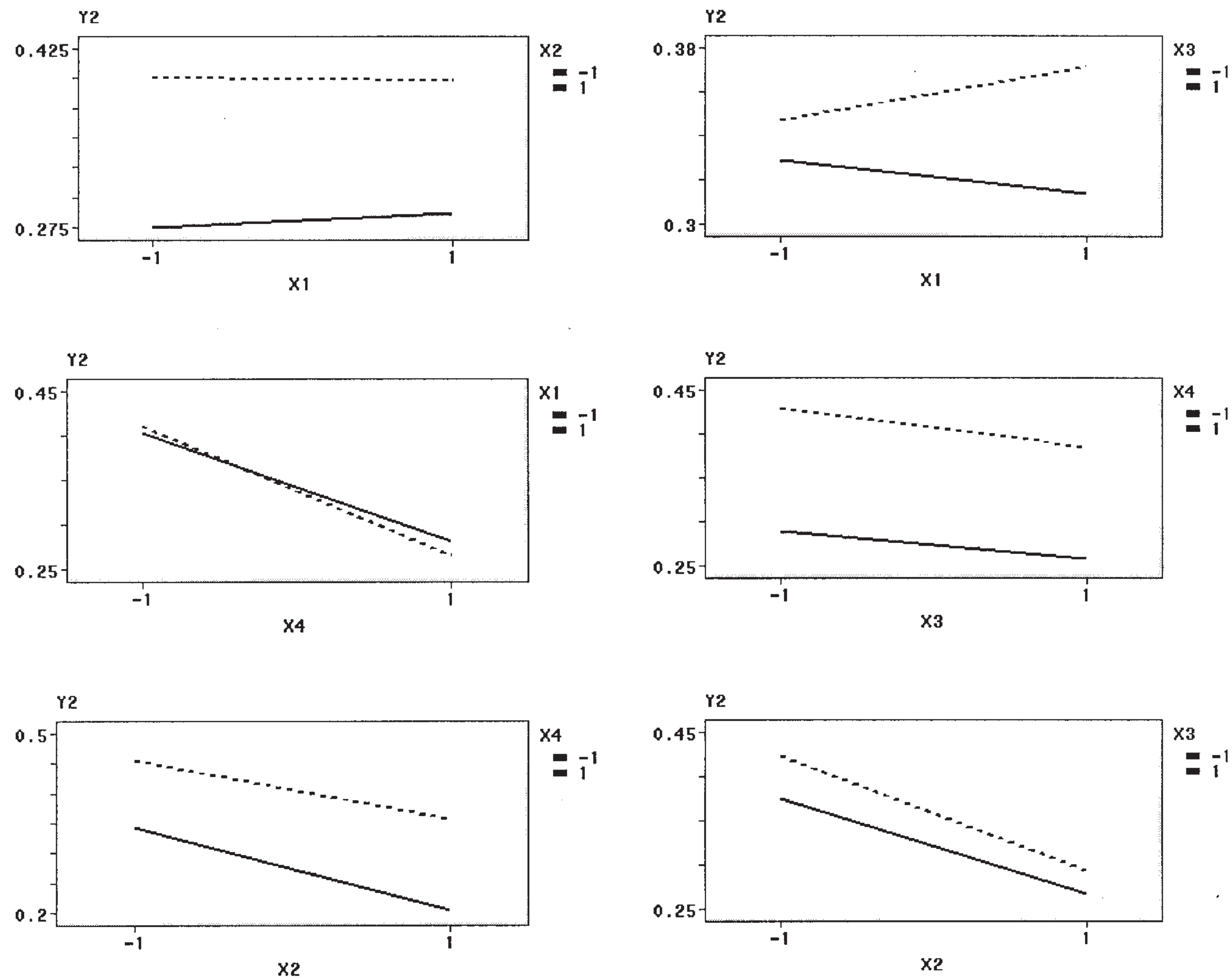

Figure 6 Two-way interaction plots for the Y_2 model.

antagonistic effect of each pair. Because X_{12} was equal to X_{34}, X_{13} was equal to X_{24}, and X_{14} was equal to X_{23}, two-way interaction plots were used to identify which interaction effect dominated the term. Figure 5 shows two-way interaction plots for the Y_1 model. Separate curves were drawn for each of the second factor levels (-1 and $+1$). For example, in the X_1–X_2 plot, the dotted line stands for the Y_1 value change when X_2 is at its lowest level (-1), and the dark line stands for the Y_1 value change when X_2 is at its highest level ($+1$). Both dotted and dark lines are almost parallel in the X_1–X_2 plot, whereas they cross each other in the X_3–X_4 interaction plot. These results indicate a strong interaction between X_3 and X_4 and virtually no interaction between X_1 and X_2.

In the X_1–X_3 and X_2–X_4 interaction plots (Fig. 5), the dotted and dark lines are all parallel, and this indicates that there is no significant interaction between X_1 and X_3 or between X_2 and X_4. In comparison, the dotted and dark lines in the X_1–X_4 and X_2–X_3 interaction plots are nonparallel, and this mean that both pairs display interaction.

Except for X_1, which had a p value of 0.393, all other reaction variables (X_2, X_3, and X_4) were found to significantly affect Y_2 ($p < 0.05$; Table V). The estimated coefficient values of -0.059, -0.019, and -0.067 for X_2, X_3, and X_4, respectively, indicated that increases in X_2, X_3, and X_4 led to a decrease in Y_2. The main-effect and Lenth plots depicting this trend are shown in Figures 3 and 4.

The estimated coefficients and p values calculated for X_{12}/X_{34}, X_{13}/X_{24}, and X_{14}/X_{23} (Table V) show a strong antagonistic interaction between X_1 and X_3 (or X_2 or X_4) and X_1 and X_4 (or X_2 and X_3) pairs ($p < 0.05$). The two-way interaction plots (Fig. 6), used to identify the dominant interaction effect, showed no significant interaction between the pairs of each factor. The zero slope obtained for the curves in the X_1–X_2 plot indicates no significant effect of X_1. In X_1–X_3 and X_1–X_4 interaction plots, the dotted and dark lines are non-

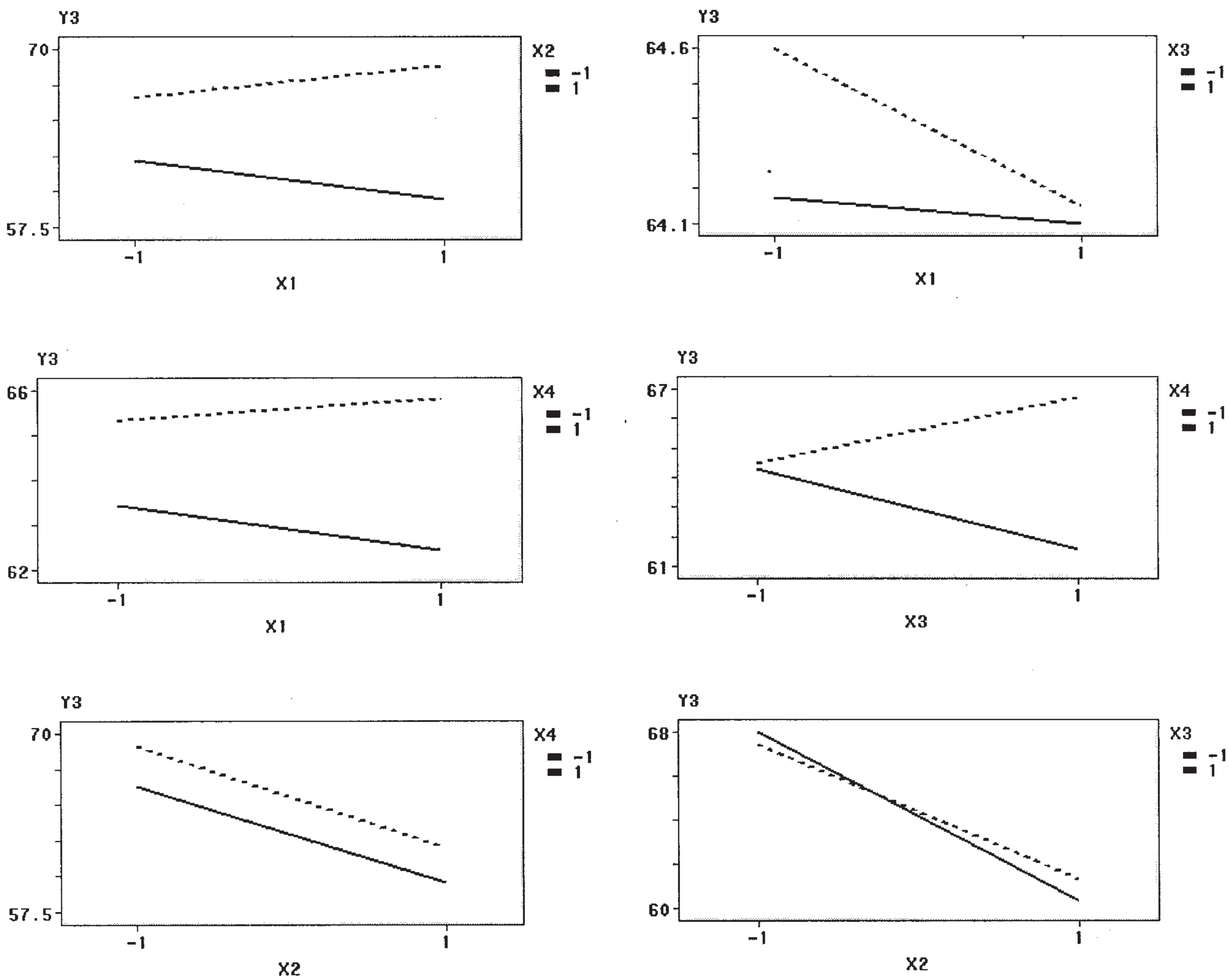

Figure 7 Two-way interaction plots for the Y_3 model.

parallel, whereas in the X_2–X_4 and X_2–X_3 plots, the two lines are parallel; this suggests that X_1–X_3 and X_1–X_4 interactions dominate the X_{13}/X_{24} and X_{14}/X_{23} terms, respectively.

The coefficient estimates and the p values obtained for the main effects in the Y_3 model (Table VI) show that Y_3 is independent of X_1 and X_2 but decreases with an increase in X_3 and X_4. The corresponding values for the X_{12}/X_{34}, X_{13}/X_{24} and X_{14}/X_{23} interaction terms suggest no significant interaction between X_1 and X_3 (or X_2 and X_4) and X_1 and X_4 (or X_2 and X_3) pairs but a strong antagonistic interaction between X_{12}/X_{34}. The two-way interaction plots shown in Figure 7 indicate significant interactions between pairs of X_1/X_2, X_3/X_4, X_1/X_4, and X_2/X_3 factors.

CONCLUSIONS

A half-fraction, two-level, four-factor factorial experimental design was used to study the effects of X_1, X_2, X_3, and X_4 on Y_1, Y_2, and Y_3 for OCA, a new class of biodegradable and bioresorbable polymers. The results showed an increase in Y_1 and no effects on Y_2 and Y_3 with an increase in X_1 from 30 to 80%. The increase in X_2 from 30 to 60°C significantly increased Y_1 but had adverse effects on Y_2 and Y_3. X_3 caused a synergistic effect on Y_1, an antagonistic effect on Y_2, and no effect on Y_3. X_4 adversely affected Y_1, Y_2, and Y_3. These results suggest that to prepare a product with a higher Y_1 value, a higher Y_2 value, and a good Y_3 value, a higher X_1 value, a lower X_2 value, a lower X_4 value, and a higher X_3 value are needed.

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