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Optimization of the Preparation of Aqueous Suspensions of Waxy Maize Starch Nanocrystals Using a Response Surface Methodology

Hélène Angellier,[†] Luc Choisnard,[‡] Sonia Molina-Boisseau,[†] Patrick Ozil,[§] and Alain Dufresne*[,||]

Centre de Recherches sur les Macromolécules Végétales (CERMAV−CNRS), Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 9, France, Département de Pharmacologie Moléculaire (DPM-UJF), Université Joseph Fourier, 38243 Meylan Cedex, France, Laboratoire d'Electrochimie et de Physico-chimie des Matériaux et des Interfaces (LEPMI−INPG), BP 75, 38402 St Martin d'Hères Cedex, France, and Ecole Française de Papeterie et des Industries Graphiques de Grenoble (EFPG−INPG), BP 65, 38402 St Martin d'Hères Cedex, France

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Response surface methodology was used to investigate the effect of five selected factors on the selective H_2SO_4 hydrolysis of waxy maize starch granules. These predictors were temperature, acid concentration, starch concentration, hydrolysis duration, and stirring speed. The goal of this study was to optimize the preparation of aqueous suspensions of starch nanocrystals, i.e., to determine the operative conditions leading to the smallest size of insoluble hydrolyzed residue within the shortest time and with the highest yield. Therefore empirical models were elaborated for the hydrolysis yield and the size of the insoluble residues using a central composite face design involving 31 trials. They allowed us to show that it was possible to obtain starch nanocrystals after only 5 days of H_2SO_4 hydrolysis with a yield of 15 wt % and having the same shape as those obtained from the classical procedure after 40 days of HCl treatment, with a yield of 0.5 wt %.

Introduction

There is currently a considerable interest in processing polymeric composite materials filled with nanosized rigid particles (essentially inorganic). This class of material attracting both scientific and industrial communities is called "nanocomposites". Because of the nanometric size effect, these composites have some unique outstanding properties with respect to their conventional microcomposite counterparts. Nowadays, the application of nanoparticles and then the development of new nanocomposite materials are restricted by both their limited availability and their strong tendency to aggregate preventing their homogeneous dispersion within a continuous matrix, which is the key step required for high mechanical performances.

Starch is a natural polymer available in large amounts from several renewable plant sources and it is produced in abundance beyond available markets. Starch is the cheapest biopolymer and is totally biodegradable. These two main reasons lead to the growing interest in the nonfood usage of starch-based products for applications in which synthetic polymers have traditionally been the materials of choice. It is well-known that native starch granules contain more or less concentric "growth rings" that are readily visible by optical or electron microscopy.[1] Acid treatment is needed to reveal the concentric lamellar structure of starch granules. It has been shown that these lamellae, around 5000 Å thick, have subspacing of a few hundred Å.[2] The purpose of this treatment using hydrochloric acid is to dissolve away regions of low lateral order so that the water-insoluble, highly crystalline residue may be converted into a stable suspension by subsequent vigorous mechanical shearing action.[3]

In previous works,[4,5] such starch nanocrystals obtained from potato starch granules were used as a reinforcing phase in a polymeric matrix and displayed substantially improved mechanical properties. The insoluble hydrolyzed residue obtained from waxy maize was found to be composed of crystalline nanoplatelets around 5−7 nm thick with a length of 20−40 nm and a width of 15−30 nm.[6] However, the main drawbacks for the more extensive use of such nanoplatelets in nanocomposite applications are the duration (40 days of treatment) and the yield (0.5 wt %) of the HCl hydrolysis step.[3]

Starch disruption by acid hydrolysis depends on many factors such as the botanic origin,[7−13] namely the crystalline type, the relative proportion of amylose and amylopectin, and the granules morphology. It also depends on the conditions of acid hydrolysis, namely, the acid type,[14,15] acid concentration,[7] temperature,[7] and hydrolysis duration.[7] No deep interest has been brought to the influence of stirring and starch concentration. Suspensions were usually stirred

* To whom correspondence should be addressed. E-mail: Alain.Dufresne@efpg.inpg.fr.
[†] CERMAV−CNRS.
[‡] DPM-UJF.
[§] LEPMI−INPG.
[||] EFPG−INPG.

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manually every day and a large panel of starch concentration has been used, for instance 1.5 wt%,[10] 1.67 wt%,[16,17] 2 wt%,[13] 2.5 wt%,[12] 5 wt%,[3,4,6,9] and 33 wt%.[18] If HCl hydrolysis has been largely studied,[7] equivalent works about sulfuric hydrolysis remain limited.

It is now accepted that the partial crystallinity of native starch granules is due to a clustered organization of amylopectin side chains[16] and that an increasing amylose content results in a decrease of the susceptibility of starch granule to acidic degradation.[19] Therefore, amylopectin-rich starch, i.e., waxy maize, was chosen for the present study. Furthermore, a previous work[20] has shown that H_2SO_4 acid hydrolysis resulted in more stable suspensions than hydrochloric ones, which is an important characteristic for composite materials processing. Thereby, we chose to focus this work on optimizing the H_2SO_4 hydrolysis of waxy maize starch granules taking into account the following parameters: hydrolysis duration, temperature, acid concentration, starch concentration, and stirring speed.

Response surface methodology was carried out to investigate the effect of these selected factors. This classical method is largely used and well adapted to process optimization in the macromolecular science area.[21−23] Laser granulometry, yield calculation, and transmission electron microscopy were used to characterize the insoluble hydrolysis residues.

Experimental Section

Acid Hydrolysis. A given weight of native waxy maize starch granules (Waxylis, Roquette S. A.) was mixed with 250 mL of H_2SO_4 solution at a known concentration in a 500 mL Erlenmeyer flask. The suspensions were then put on a platform in a thermo-stated atmosphere and continuously stirred at a selected speed with an orbital shaking action. After various durations of hydrolysis, the suspensions were washed by successive centrifugations in distilled water until neutrality. They were stored at 4 °C with several drops of chloroform.

The hydrolysis yield (wt %) was calculated as the ratio between the weight of freeze-dried hydrolyzed particles and the initial weight of native granules for an aliquot of 50 mL taken in the 250 mL of hydrolyzed suspensions. It was verified that these aliquots were representative of the entire volume of 250 mL.

Characterization. Transmission electron microscopy (TEM) observations were performed using a Philips CM200 microscope with a 80 kV accelerating voltage.

Laser granulometry measurements were carried out with a Malvern Mastersizer. The suspensions were characterized from the median particle size d_{50}, which divides the population into two equal halves.

Design of Experiments. The five following parameters were varied: hydrolysis temperature u_1, acid concentration u_2, initial starch concentration u_3, hydrolysis duration u_4, and speed of stirring u_5.

Parameters u_3 and u_5 do not influence hydrolysis kinetic because of its catalytic nature. These two parameters have been however taken into account in the present work because they were varying in a large range in the literature.

Table 1. Setting Levels of Parameters u_i

parameter	unit	low level $x_i = -1$	medium level $x_i = 0$	high level $x_i = +1$
u_1, temperature	°C	35	37.5	40
u_2, acid concentration	mol/L	2.2	2.8	3.4
u_3, starch concentration	g/100 mL	5	10	15
u_4, time	day	1	5	9
u_5, stirring	rpm	0	50	100

The settings of the different parameters were determined for all of the predictors from our preliminary experiments[20] and keeping in mind the two following intuitive rules. On one hand, the optimal conditions have a lot of chance to be outside of the region of interest if selecting a too narrow variation range. On the other hand, the predictive power of the model risks to be poor if choosing a too large range. Table 1 shows the minimal ($u_{i,\min}$), the midrange ($u_{i,\mathrm{mid}}$), and the maximal ($u_{i,\max}$) values used for each parameter, which respectively correspond to −1, 0, and +1 levels in terms of orthogonal variable x_i defined as

$$x_i = \frac{2(u_i - u_{i,\min})}{(u_{i,\max} - u_{i,\min})}$$

Here the two responses under study were (1) the hydrolysis yield as previously defined, y_{yield} (wt %), and (2) the median size, y_{size} (μm), of hydrolyzed particles treated by Ultra Turrax (13 000 rpm, concentration of starch of 1 wt %, treated volume of 60 mL) during 5 min measured by laser granulometry.

Since both response non linearities and interactions between factors were expected, the response model was postulated to be a quadratic one for each response η_k. This model may be expressed in terms of orthogonal variables as

$$\eta_k = b_0 + \sum_{i=1}^{5} b_i x_i + \sum_{\substack{i,j=1 \\ i \neq j}}^{5} b_{ij} x_i x_j + \sum_{i=1}^{5} b_{ii} x_i^2$$

This postulated model takes into account the linear effects ($b_i x_i$) and the quadratic ones ($b_{ii} x_i^2$) as well as first-order interactions ($b_{ij} x_i x_j$) and its knowledge requires to estimate the coefficients b_i, b_{ii}, and b_{ij}.

Experiments were conducted adopting a central composite face design (CCFD). This type of design was suitable for our objective, which was the optimization of a potential complex process. The CCFD (Table 2) involves a fractional factorial design 2^{5-1} (trials 1−16), the face centers (trials 17−26, all coordinates equal to zero except one equal to +1 or −1), and five replications at the central point (trials 27−31, all coordinates equal to zero). The run order of trials was randomized in order to prevent systematic errors.

The determination of the optimal conditions proposed by the MODDE software is based on the maximization of a desirability coefficient D defined as a weighted average of

Table 2. Results Obtained for the Complete Set of 31 Experimental Points

trial	X_1	X_2	X_3	X_4	X_5	Y_{yield} (%)	Y_{size} (μm)
1	−1	−1	−1	−1	+1	76.3	13.40
2	+1	−1	−1	−1	−1	68.1	13.00
3	−1	+1	−1	−1	−1	47.6	12.44
4	+1	+1	−1	−1	+1	26.3	9.23
5	−1	−1	+1	−1	−1	70.7	13.18
6	+1	−1	+1	−1	+1	54.8	13.22
7	−1	+1	+1	−1	+1	57.8	12.66
8	+1	+1	+1	−1	−1	35.9	10.93
9	−1	−1	−1	+1	−1	43.9	11.91
10	+1	−1	−1	+1	+1	20.3	6.29
11	−1	+1	−1	+1	+1	5.4	5.84
12	+1	+1	−1	+1	−1	2.8	5.21
13	−1	−1	+1	+1	+1	44.8	8.00
14	+1	−1	+1	+1	−1	29.3	7.61
15	−1	+1	+1	+1	−1	16.7	5.90
16	+1	+1	+1	+1	+1	2.1	5.08
17	−1	0	0	0	0	42.3	11.81
18	+1	0	0	0	0	26.5	6.74
19	0	−1	0	0	0	30.4	11.56
20	0	+1	0	0	0	21.3	6.45
21	0	0	−1	0	0	24.1	9.43
22	0	0	+1	0	0	34.9	8.33
23	0	0	0	−1	0	56.4	12.85
24	0	0	0	+1	0	20.6	6.75
25	0	0	0	0	−1	36.0	9.65
26	0	0	0	0	+1	20.3	4.64
27	0	0	0	0	0	37.6	7.56
28	0	0	0	0	0	31.8	8.28
29	0	0	0	0	0	28.3	8.43
30	0	0	0	0	0	29.7	7.52
31	0	0	0	0	0	28.9	7.79

the individual response desirabilities d_i[24]

$$D = \frac{1}{M}\sum_{i=1}^{M} d_i = \frac{1}{M}\sum_{i=1}^{M} w_i \left(\frac{y_i - T_i}{T_i - L_i}\right)^2$$

M being the number of responses and the experimenter having to provide the following data for each response y_i: the weight w_i (here, $w_i = 1$ for all the responses), the desired response target T_i, and its worst acceptable value L_i.

Results and Discussion

Models and Analysis. The responses measured for each trial are reported in Table 2.

Plotting the N probability versus deleted studentized residuals has shown that there were no deviating experiments also called outliers, considering that the action limit was ±4 standard deviations (not shown in this paper). Thereby, no experiment was performed again nor excluded from the analysis. Moreover, the analysis of raw data through the Box-Cox transformation showed that no response transformation was useful to improve the models, considering a 0.95 confidence interval.

The responses were fitted owing to a multi-linear regression method (MLR) as quadratic models expressed in terms of orthogonal variables x_i as said above and refined using a backward step by step technique based on a 0.95 confidence level.[25,26]

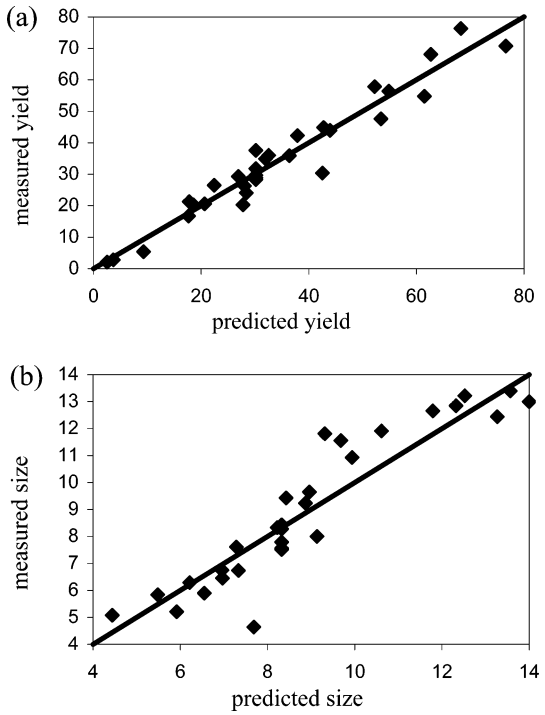

Figure 1. Plot of measured responses versus predicted responses for (a) η_{yield} and (b) η_{size}.

The final refined models (η_{yield} and η_{size}) and their corresponding statistics (coefficient of regression R^2, coefficient of determination R^2_{adj}, and coefficient of prediction Q^2) are as follows:

$$\eta_{yield} = 30.1615 - 7.7444x_1 - 12.3722x_2 + 1.7889x_3 - 17.1111x_4 - 2.3833x_5 + 2.5625x_3x_5 + 7.6051x_4^2$$

$$R^2 = 0.940, \quad R^2_{adj} = 0.922, \quad Q^2 = 0.884$$

$$\eta_{size} = 8.3223 - 0.9905x_1 - 1.3572x_2 - 0.1022x_3 - 2.6844x_4 - 0.6372x_5 + 0.5712x_3x_5 + 1.3166x_4^2$$

$$R^2 = 0.859, \quad R^2_{adj} = 0.817, \quad Q^2 = 0.751$$

For both models, R^2, R^2_{adj}, and Q^2 statistics have quite high values close to unity with a difference R^2 and Q^2 lower than 0.2, which is an indication of suitable models. Here the adjusted R^2 statistics are greater than 0.85, so proving a good descriptive power of the models. This power can be illustrated besides by the plot of experimental responses versus the predicted ones (Figure 1) which should be ideally the diagonal line and is here characterized by an acceptable dispersion. Moreover, the predictive power, as evidenced by Q^2 values greater than 0.5, is excellent for η_{yield} and not so good for η_{size} ($Q^2 = 0.751$) but still acceptable.[21]

Moreover, a classical analysis of variance (ANOVA) using F-tests allows us to analyze the total response variation by identifying the parts corresponding to the sources of variation (regression model, pure experimental error) and to analyze the residuals in order to point out the possible lack of fit of the postulated model when replicates are available. Here we could conclude that there is no lack of fit for the model concerning the hydrolysis yield, whereas some lack of fit

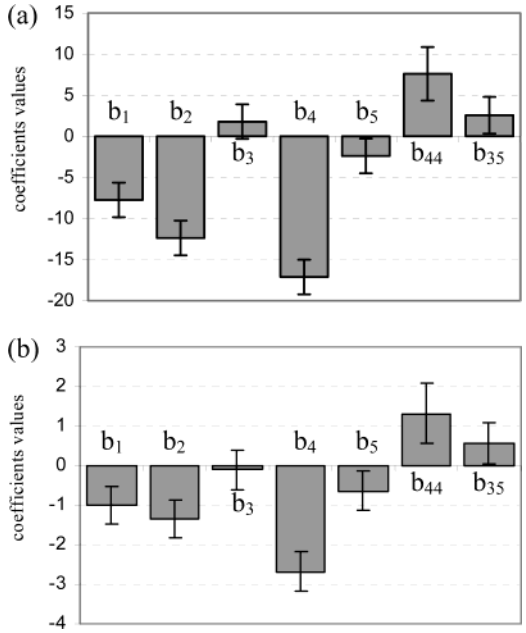

Figure 2. Main effects for (a) the yield (η_{yield}) and (b) the median size (η_{size}) for a confidence level of 0.90.

exists for the median size. That is however not critical because of a Q^2 statistic greater than 0.5 ($Q^2 = 0.751$).

The stirring speed (x_5) and the overall starch concentration (x_3) do not have any significant influence on the yield and the median size (Figure 2). Effectively, the probabilities of significance (p) of estimated coefficients b_3 and b_5 were superior to the critical limit of 0.05. They were respectively $p = 0.1598$ and $p = 0.0653$ for η_{yield} and $p = 0.7230$ and $p = 0.0356$ for η_{size}. However, these terms were kept in models according to the hierarchy principle. Starch concentration did not have an effect on the yield of hydrolysis and the median size of insoluble residues. We could have thought that a decrease of starch concentration would have favored the action of the catalyst. This result proves that, in the chosen ranges, acid is largely in excess in the reactor.

Only one interaction between starch concentration and stirring has been detected.

The response surfaces and the corresponding contour plots were drawn for both responses. They allow the impact of two selected parameters to be illustrated by keeping the other three parameters at constant values (Figures 3−6).

To study the reliability of the models and validate them, several experimental conditions were tested. We succeeded in producing controlled yields with a respective confidence level of 0.95. On the other hand, the experimental value of the median size was outside the predicted interval (difference between the measured sizes and the interval of prediction and the predicted values of 6.1% and 24%, respectively) so confirming a light lack of fit for the model. Nevertheless, the model will be used for optimizing the median size because of the suitable value of the Q^2 statistic greater than 0.5.

Optimization. The multivariable models obtained from the statical design of experiments were used for predicting the optimal conditions of H_2SO_4 hydrolysis that should allow aqueous suspensions of waxy maize starch nanocrystals to be obtained in the shortest time, with the smallest median size particle and the highest yield.

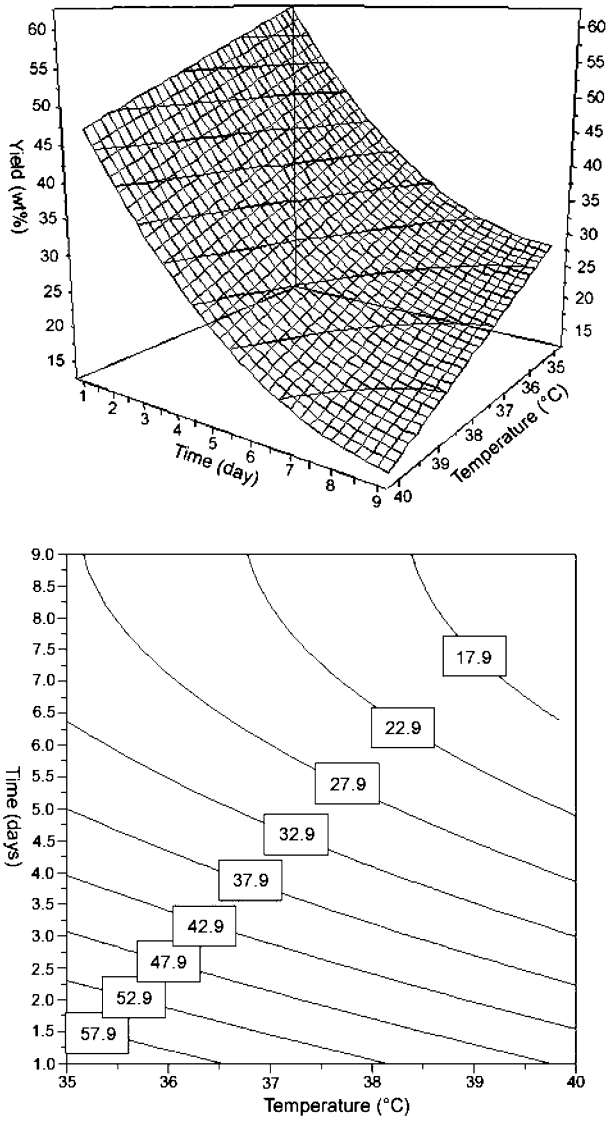

Figure 3. Response-surface plot (upper) and its contour plot of yield (η_{yield}): temperature versus duration of hydrolysis with constant level of acid concentration (2.8 M), starch concentration (10 wt %), and stirring (50 rpm).

We assumed that the highest yield for the preparation of starch nanocrystal suspensions that could be reached was about 30−40% which is the relative crystallinity of native waxy maize starch granules.[27] Preliminary studies[20] have shown that the morphology of nanocrystals began to be observed after 7 days of acid hydrolysis with a corresponding yield of 26.9 wt % and that 40 days of HCl hydrolysis were needed to obtain a suspension of insoluble residues all having the shape of nanoplatelets, with a corresponding yield of 0.5 wt %. These results showed that selective acid hydrolysis takes time. Thereby, a reasonable goal was to obtain such suspensions in less than 7 days, with a yield of 20 wt %.

For optimization, the yield and the median size were considered as targets, with a constrained time value and all other factors varying freely in the considered experimental domain (Table 3). First, a target of 20 wt % for the yield (η_{yield}) and of 5 μm for the median size (η_{size}) have been chosen, with a duration of hydrolysis of 7 days. These criteria corresponded to "optimization 1". Second, a duration of hydrolysis of 5 days has been chosen, with the same target for the yield but a median size of 6 μm, considering that we

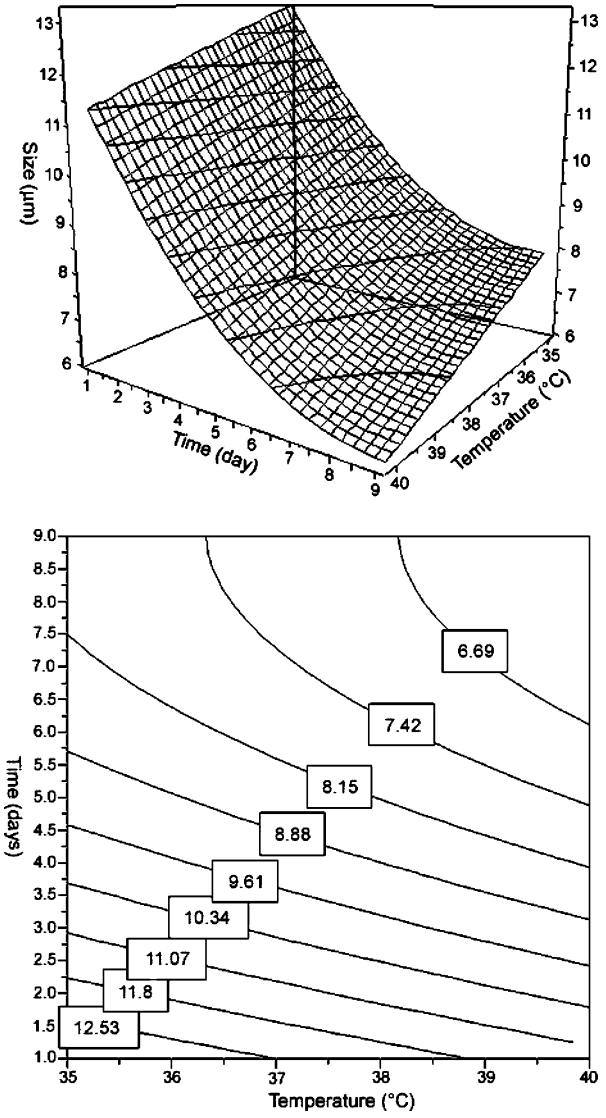

Figure 4. Response-surface plot (upper) and its contour plot of median size (η_{size}): temperature versus duration of hydrolysis with constant level of acid concentration (2.8 M), starch concentration (10 wt %), and stirring (50 rpm).

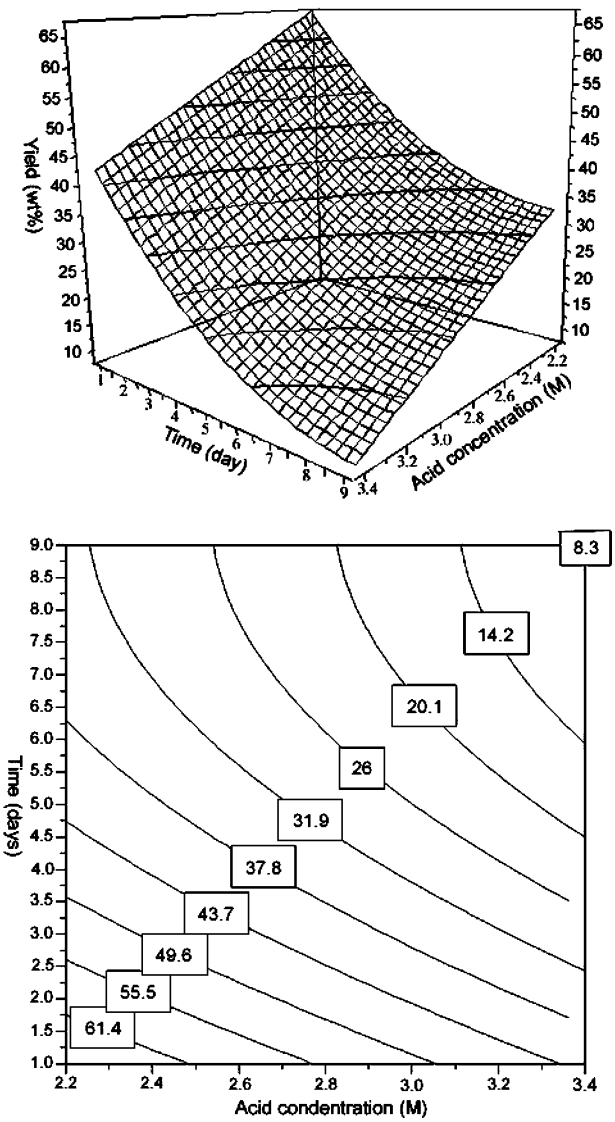

Figure 5. Response-surface plot (upper) and its contour plot of yield (η_{yield}): acid concentration versus duration of hydrolysis with constant level of temperature (37.5 °C), starch concentration (10 wt %), and stirring (50 rpm).

would not have been able to obtain the same median size than in 7 days. These criteria corresponded to "optimization 2".

The sets of conditions proposed by the MODDE software are given in Table 4. The given log *D* value is negative or near zero indicating that we are doing rather well.

Achieving an accuracy of 4 decimals for experimental conditions settings was of course impossible. Run 1 was performed using 35.35 g of starch (14.14 wt %) mixed in 250 mL of 2.87 M H_2SO_4 solution (161.8 mL of H_2SO_4 95% and 838.2 mL of distilled water), at 40 °C, 100 rpm and during 7 days. For run 2, 36.725 g of starch (14.69 wt %) were mixed in 250 mL of 3.16 M H_2SO_4 solution (178.1 mL of H_2SO_4 95% and 821.9 mL of distilled water), at 40 °C, 100 rpm and during 5 days.

The predictions calculated with a confidence interval of 0.95 and the measured responses corresponding to these sets of conditions are given Table 5.

Measured yields were inside the predictive intervals but not the median sizes, which were smaller than the lower limits. Whereas it confirmed the lack of prediction of the model η_{size}, this result is pleasantly surprising and interesting for our goal which was to prepare small residues, among others. Furthermore, contrary to all expectations, a smaller median size was obtained with the same yield after 5 days than after 7 days of acid treatment. Observations by transmission electron microscopy (TEM) were performed to verify the shape of the insoluble residues.

Characterization of Optimized Suspension of Nanocrystals. Observations by TEM clearly show that the insoluble residue obtained after 5 days of optimized H_2SO_4 hydrolysis (Table 5) have the shape of parallelepiped nanoplatelets (Figure 7a). Nanoplatelets were generally observed in aggregates of 1−5 μm (Figure 7a) or at best in barrets of several platelets (Figure 7b). Even if the parallelepipedic shape was the general shape that we observed, a lot of varying organizations (Figure 7b−d) were distinguished. Few stacks of nanoplatelets oriented edge-on were observed in a very little proportion (not shown here), which let us assume that the platelets were well separated.

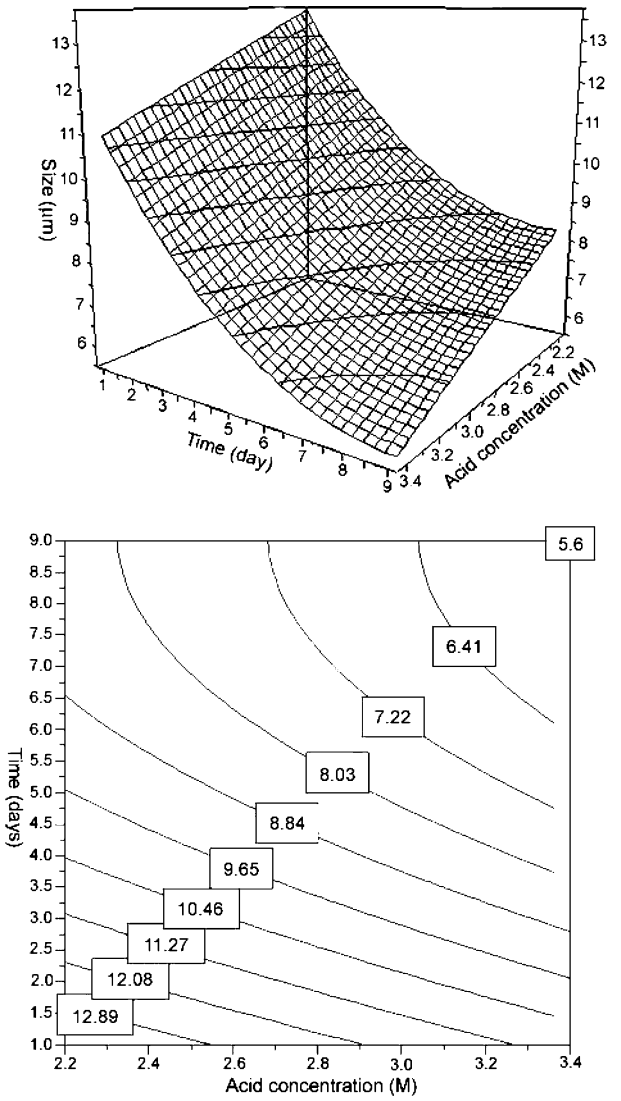

Figure 6. Response-surface plot (upper) and its contour plot of median size (η_{size}): acid concentration versus duration of hydrolysis with constant level of temperature (37.5 °C), starch concentration (10 wt %), and stirring (50 rpm).

Table 3. Criteria for Optimizations 1 and 2

	criteria	target	min	max
optimization 1				
yield (η_{yield})	target	20	15	30
median size (η_{size})	target	5	4.5	5.8
u_1	free		35	40
u_2	free		2.2	3.4
u_3	free		5	15
u_4	constant	7		
u_5	free		0	100
optimization 2				
yield (η_{yield})	target	20	15	30
median size (η_{size})	target	6	5.5	6.5
u_1	free		35	40
u_2	free		2.2	3.4
u_3	free		5	15
u_4	constant	5		
u_5	free		0	100

These observations lead us to conclude that the optimization process was successful. We have shown that it was possible to obtain starch nanocrystals after 5 days of H_2SO_4 hydrolysis with a yield of 15 wt % having the same shape as those obtained after 40 days of HCl treatment, with a yield of 0.5 wt %.

Table 4. Sets of Conditions Proposed for Optimizations 1 and 2

optimization	u_1	u_2	u_3	u_4	u_5	log(D)
1	39.9993	2.8728	14.1457	7	99.9942	0.0205
2	39.9914	3.1636	14.6946	5	99.3155	−0.3706

Table 5. Predicted and Measured Responses for Sets of Conditions 1 and 2

	predicted	low limit	high limit	measured
yield				
optimization 1	15.4881	10.0454	20.9315	16.63
optimization 2	16.6481	10.6395	22.6568	15.7
size				
optimization 1	5.9059	4.6442	7.1677	4.64
optimization 2	6.3172	4.9243	7.7101	4.40

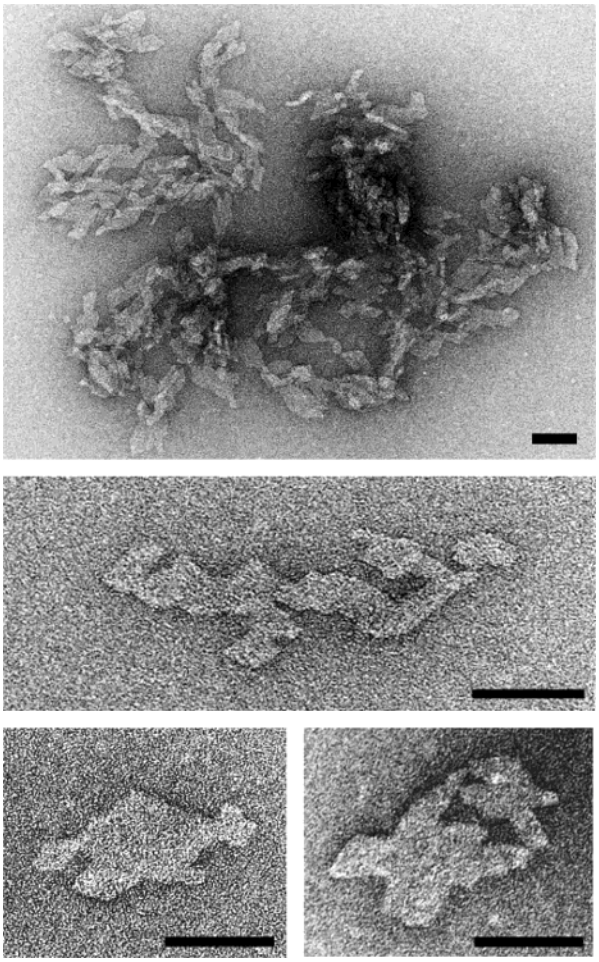

Figure 7. TEM of negatively stained nanocrystals obtained after 3.16 M H_2SO_4 hydrolysis of waxy maize starch granules during 5 days, at 40 °C, 100 rpm and with a starch concentration of 14.69 wt %. (a) Aggregates of nanocrystals and (b−d) organizations of nanoplatelets. Scale bar: 50 nm.

Conclusion

The statistical experimental design and the multi-linear regression analysis used in this study have proven to be very useful for establishing predictive models for both the yield of H_2SO_4 hydrolysis of waxy maize starch granules and the

median size of insoluble residues after acid treatment. We achieved the production aqueous suspensions of starch nanocrystals after 5 days of 3.16 M H_2SO_4 hydrolysis at 40°C, 100 rpm and with a starch concentration of 14.69 wt % with a yield of 15.7 wt % and the same shape than those obtained after 40 days of HCl hydrolysis. It should allow the preparation of starch nanocrystals for nanocomposite applications to be considered.

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