Carbohydrate Polymers 88 (2012) 917–925

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effect of enzymatic pretreatment on the synthesis and properties of phosphorylated amphoteric starch

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ARTICLE INFO

Article history:
Received 11 December 2011
Received in revised form 3 January 2012
Accepted 13 January 2012
Available online 21 January 2012

Keywords:
Enzymatic pretreatment
Amphoteric starch
Response surface methodology
Properties

ABSTRACT

Phosphorylated amphoteric starch was prepared from enzyme-modified tapioca starch in the aqueous alcoholic–alkaline medium. Optimization of the reaction parameters for maximum degree of substitution (DS) of phosphorylated amphoteric starch were carried out using response surface methodology (RSM). Optimum modification conditions were shown as following: NaOH concentration 3.71 g/100 g starch (dry basis), CHPTAC concentration 6.34 mL/100 g starch (dry basis), STP concentration 6.13 g/100 g starch (dry basis), water/ethanol ratio 1.16, reaction temperature 50 °C, reaction time 3 h, and the starch concentration 30%. Under the optimal condition, the maximum anion degree of substitution (DS_A) and cation degree of substitution (DS_C) were 0.0274 and 0.0408, respectively. Compared with the DS of the amphoteric starch prepared from native tapioca starch (NPTAS), the DS of the amphoteric starch from enzyme-pretreated tapioca starch (EPTAS) was higher at the same reaction conditions, suggesting enzymatic pretreatment improved the reaction processes significantly. Scanning electron microscopy and X-ray diffraction data showed that the granular shape and crystalline structure of EPTAS did not change. Compared with NPTAS, the pasting stability of EPTAS with the same DS was also significantly improved.

1. Introduction

In the paper industry, starch is one of the most common paper chemicals and is used in different applications, such as a strength-increasing agent, surface sizing agent, retention agent and as a binder in paper coatings (Whistler, BeMiller, & Paschall, 1984). However, native starches have limited uses in papermaking industry due to their nonionic properties. To extend their applications, chemical modification, introducing functional groups into the macromolecules to substitute the free hydroxyl groups, is often used to alter physicochemical properties of starch. Cationic starch, a cationic polyelectrolyte with amino, imino, ammonium, sulfonium, or phosphonium groups, is a large-scale commercial product widely used in papermaking (Yang, Zhu, Sun, Zhao, & Liu, 2009). Cationic starch has numerous applications in the paper industry, especially to increase the tensile fold and bursting strength of the paper (Howard & Jowsey, 1989; Lindström & Florén, 1984). It adsorbs the negatively charged cellulose fibers because of its cationic charge, which can improve the adhesive interactions between the fibers. Howard and Jowsey (1989) suggested that cationic starch increased the specific joint strength and the molecular contact area.

To enhance the functional use of cationic starch in papermaking industry, it is further modified by introducing anionic or nonionic groups into the same starch molecules. Phosphorylated amphoteric starch is widely used in papermaking which is produced by reaction of cationic starch with anionic phosphate salts or phosphate etherifying reagents (Solarek, 1986). Numerous studies have shown variations in the cationization and phosphorylation procedures employed by industry (Carr & Ill, 1978; Jarowenko & Hernandez, 1977; Lim & Seib, 1993; Tessler & Edison, 1978). However, the costly drying and heating process, the long reaction time, as well as residual reagents in the final product limited the development of amphoteric starches. Kweon, Bhirud, and Sosulski (1996), Kweon, Sosulski, and Bhirud (1997a) and Kweon, Sosulski, and Bhirud (1997b) developed the aqueous alcoholic–alkaline solvent for combined cationization and phosphorylation in a simultaneous process in the production of amphoteric starch, and compared simultaneous and sequential methods. The results showed that both methods had no need for drying, catalysts or high temperatures, and residual reagents were removed with other effluents. However, the previous research mostly focused on the effect of the single factor such as NaOH concentration, 3-chloro-2-hydroxypropyltrimethy-lammonium chloride (CHPTAC) concentration, sodium tripolyphosphate (STP) concentration or water/ethanol ratio on degree of substitution (DS) of amphoteric

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doi:10.1016/j.carbpol.2012.01.034

starches. The integrative factors and their interaction affecting DS were seldom reported.

Huber and BeMiller (2000) reported that pores on the surface of starch granules play a very important role in reacting with chemicals. Generally, pores in the starch granules will remarkably increase the surface area of the starch particles and make chemical reagent easily enter starch granules, which can enhance the reaction rate and change the distribution of substitution groups. Enzymatic hydrolysis has been used to produce porous starch (Fitt & Snyber, 1984; Luo & Fu, 2010; Yao & Yao, 2002; Zhao, Madson, & Whistler, 1996). However, relatively few studies had been reported on the effect of enzymatic pretreatment on the synthesis and properties of starch derivative.

In this work, tapioca starch was pretreated with α-amylase and glucoamylase. Subsequently, response surface methodology (RSM) was conducted to optimize the levels of the preparation variables (NaOH concentration, CHPTAC concentration, STP concentration and water/ethanol ratio) for maximum DS of amphoteric starch. We further investigated the effect of enzymatic pretreatment on DS, morphology, crystalline and pasting property of amphoteric starch.

2. Materials and methods

2.1. Materials

Tapioca starch was supplied by Ming Yang Co. (Guangxi, China). An aqueous solution of 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) was purchased from the Dow Chemical Co. (America). Sodium tripolyphosphate (STP) were purchased from Tianjin Kermel Chemical Reagent Co. (Tianjing, China). Both α-amylase (activity 4000 unit/g) and glucoamylase (activity 100,000 unit/g) were supplied by Xuemei Enzyme Technology Co. (Wuxi, China). One unit of α-amylase activity corresponds to the amount of enzyme which liberates 1 g of soluble starch per hour at pH 6.0 at 60 °C. One unit of glucoamylase activity is defined as the amount of enzyme which liberates 1 mg of glucose from soluble starch per hour at pH 4.6 at 40 °C. All other chemicals were of analytical grade.

2.2. Enzymatic pretreatment

Tapioca starch (50 g, dry basis) was suspended in 100 mL of sodium acetate buffer solution (pH 5.5) with stirring. 100 mL of α-amylase and glucoamylase solution (125 mg α-amylase and 125 mg glucoamylase dissolved in 100 mL of pH 5.5 sodium acetate buffer) was added and then the mixture was incubated at 55 °C for 16 h. Samples were washed in distilled water and dried in a convection oven at 45 °C over night and then ground using a mortar and pestle. The hydrolysis percent reducing value of all samples was determined using the method of Bruner (1964).

2.3. Preparation of amphoteric starch

According to the methods reported by Kweon et al. (1997b), amphoteric starches were prepared by combining cationization and phosphorylation simultaneously. A weighed quantity of STP (3.0, 4.0, 5.0, 6.0, 7.0 g/100 g dry basis starch) and NaOH (2.5, 3.0, 3.5, 4.0, 4.5 g/100 g dry basis starch) were dissolved in sufficient distilled water/ethanol solution with the volume ratio of water/ethanol at 0.4, 0.6, 1.0, 2.0, 4.0, respectively, and then added to enzymatic hydrolysis starch slurry (30%, w/w) for 10 min at 50 °C. Weighed CHPTAC (3.0, 5.0, 7.0, 9.0, 11.0 mL/100 g dry basis starch) was added to the starch slurry. The reaction mixture was incubated at 50 °C for 3 h with constant stirring. After the reaction, the mixture was neutralized with 3 mol/L HCl to pH 6.5 and centrifuged at 8000 × *g* for 20 min. The sample pellet was washed twice with distilled water and two times with 95% ethanol. The phosphorylated amphoteric starch was oven-dried at 40 °C for 24 h, and then passed through a 100 mesh sieve.

2.4. Determination of the degree of substitution

Nitrogen content was measured according to the micro-Kjeldahl method (Kweon et al., 1996) for the cationic groups. The phosphorous content was measured by modified colorimetry for anionic groups (Pollman, 1991). DS values of cationic and anionic groups were calculated from the nitrogen and phosphorous contents by the following equations, respectively:

$$DS_A = \frac{162X_P}{3100 - 259X_N} \quad (1)$$

$$DS_C = \frac{162X_N}{1400 - 117X_N} \quad (2)$$

where DS_A is the anion degree of substitution (%) and DS_C is the cation degree of substitution (%). X_P is the phosphorous content (%) and X_N is the nitrogen content (%).

2.5. Response surface methodology

Response surface methodology consists of a group of empirical techniques devoted to the evaluation of relations existing between a cluster of controlled experimental factors and measured responses, according to one or more selected criteria. On the basis of the single factor experimental results, the ranges of independent variables including NaOH concentration, CHPTAC concentration, STP concentration and water/ethanol ratio were confirmed. The preparation conditions were optimized by RSM. The range and center point value of four independent variables are summarized in Table 1. To apply Box–Behnken central composite design, 24 experimental runs were carried out and the zero experiment was repeated five times (Table 1). The Design Expert 7.0 software package was used to establish the mathematical model and obtain the optimum conditions of technological progress. In developing the regression equation, the test factors were coded according to the equation:

$$x_i = \frac{X_i - X_i^x}{\Delta X_i} \quad (3)$$

where x_i is the coded value of the ith independent variable, X_i is the natural value of the ith independent variable, X_i^x is the nature value of the ith independent variable at the center point and ΔX_i is the step change value.

$$Y = b_0 + \sum_i b_i x_i + \sum_i \sum_j b_{ij} x_i x_j + \sum_i b_{ii} x_i^2 \quad (4)$$

where Y is the measured response, b_0 is the intercept term, b_i is the first-order model coefficient, b_{ii} is the quadratic coefficient for the factor i, b_{ij} is the linear model coefficient for the interaction between factors i and j. The variable $x_i x_j$ represents the first-order interactions between x_i and x_j ($i < j$).

2.6. Scanning electron microcopy (SEM)

The surface structure of the starch granules was observed by scanning electron microscopy. Starch samples were mounted on circular aluminum stubs with double adhesive tape, coated with 20 nm of gold and examined and photographed in a scanning electron microscope (LEO, Oberkochen, Germany, model 1530VP) at an accelerating potential of 20 kV.

Table 1
Box–Behnken central composite design for independent variables and their response.[a]

Runs	A NaOH (g/100 g)	B CHPTAC (mL/100 g)	C STP (g/100 g)	D Water/ethanol ratio (mL/mL)	DS_A	DS_C
1	−1(2.5)	0(7)	1(7)	0(1.0)	0.0194	0.0297
2	−1	0	0(5)	1(1.5)	0.0115	0.0211
3	0(3.5)	1(11)	−1(3)	0	0.0117	0.0159
4	0	0	0	0	0.0253	0.0413
5	1(4.5)	0	0	1	0.0202	0.0312
6	0	0	1	−1(0.5)	0.0140	0.0358
7	−1	0	−1	0	0.0124	0.0246
8	0	−1(3)	0	1	0.0135	0.0269
9	−1	1	0	0	0.0164	0.0323
10	0	0	0	0	0.0242	0.0406
11	0	−1	1	0	0.0188	0.0287
12	0	0	0	0	0.0260	0.0409
13	0	1	1	0	0.0095	0.0410
14	0	0	0	0	0.0248	0.0420
15	1	0	0	−1	0.0206	0.0318
16	1	1	0	0	0.0075	0.0408
17	0	0	−1	−1	0.0108	0.0115
18	0	0	−1	1	0.0110	0.0324
19	0	1	0	−1	0.0226	0.0401
20	0	1	0	1	0.0231	0.0404
21	−1	0	0	−1	0.0098	0.0204
22	0	−1	−1	0	0.0102	0.0109
23	1	0	−1	0	0.0133	0.0294
24	0	0	1	1	0.0213	0.0377
25	−1	−1	0	0	0.0101	0.0268
26	1	−1	0	0	0.0125	0.0238
27	1	0	1	0	0.0145	0.0366
28	0	−1	0	−1	0.0128	0.0243
29	0	0	0	0	0.0244	0.0411

[a] Other reaction conditions: reaction temperate 50 °C, reaction time 3 h, starch concentration 30%.

2.7. X-ray diffraction

X-ray diffractograms were obtained with a RU200R X-ray diffractometer (Rigaku, Tokyo, Japan) with a chart speed of 20 mm/min. The starch powder was scanned through the 2θ range of 4–50°. Traces were obtained using a Cu Kα radiation detector with a nickel filter and scintillation counter operating under the following conditions: 40 kV, 50 mA, 1°/1° divergence slit/scattering slit, 0.30 mm receiving slit, 1 s time constant, and scanning rate of 3°/min.

The relative crystallinity (RC) was determined according to Herman's methods, as described by Fujita, Yamamoto, Sugimoto, Morita, and Yamamori (1998), using a peak-fitting software (Origin-version 6.0, Microcal, Inc., Northampton, MA, USA).

2.8. Pasting curves of starches

The starch gelatinization curve was obtained with the Brabender Viscoamylograph (C.W. Brabender Instruments Inc., Hackensack, NJ, Germany). A starch slurry underwent a controlled heating and cooling cycle under constant shear where it was heated from 25 to 95 °C at 1.5 °C/min, held at 95 °C for 30 min, cooled to 50 °C at 1.5 °C/min, and held at 50 °C for 30 min. A 6% suspension of starches was studied.

2.9. Statistical analysis

All determinations were replicated three times and mean values and standard deviations were reported. Analyses of variance (ANOVA) were performed and the mean separations were performed by Tukey's HSD test ($p < 0.05$) using SigmaStat Version 2.0 (Jandel Scientific/SPSS Science, Chicago, IL, USA).

3. Results and discussion

3.1. Single factor experiment

A single factor experiment is called one-variable-at-a-time technique which is a parameter change in the general practice of determining the optimal operating conditions while keeping the others at a constant level.

The alkaline reaction condition causes hydroxyl groups of starch to produce negative oxygen ion and CHPTAC to form strongly active epoxy structures. When the epoxy structure of CHPTAC is broken, one of its ends has the nucleophilic substitution reaction with hydroxyl groups. Simultaneously, the other end reacts with STP. The effect of NaOH concentrations on DS_A and DS_C of amphoteric starch is shown in Fig. 1a. Both DS_A and DS_C increased when the concentration of NaOH increased from 2.5 to 3.5 g/100 g starch (dry basis), and then decreased after the concentration of NaOH was more than 3.5 g/100 g starch (dry basis).

CHPTAC could affect DS of amphoteric starch significantly as well. To study the effect of different CHPTAC concentrations on DS of amphoteric starch, different concentrations of CHPTAC were employed. The results are shown in Fig. 1b. It is easy to find that when CHPTAC concentration increased from 3.0 to 11.0 mL/100 g starch (dry basis), DS_C of amphoteric starch increased significantly, while DS_A of amphoteric starch decreased when CHPTAC concentration was more than 7.0 mL/100 g starch (dry basis).

As shown in Fig. 1c, DS_A of amphoteric starch increased with STP concentration increasing, while DS_C of amphoteric starch decreased when STP concentration was more than 5.0 g/100 g starch (dry basis). The result was on the contrary of the effect of CHPTAC on DS.

The effect of water/ethanol ratios on DS_C and DS_A is presented in Fig. 1d. When water/ethanol ratio varied from 0.4 to 1.0, both DS_C and DS_A increased. DS_C and DS_A dropped sharply when water/ethanol ratio was more than 1.0. Hence, the water–ethanol

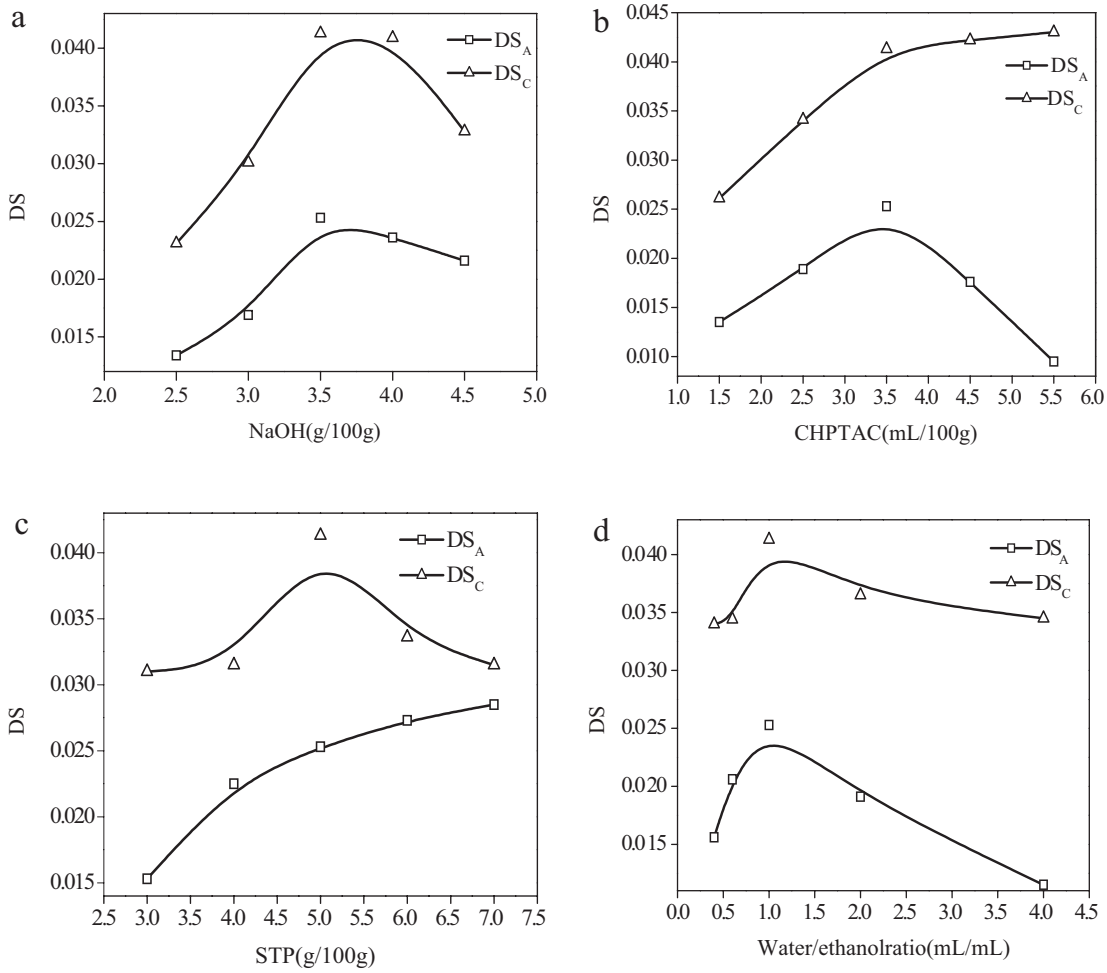

Fig. 1. Effects of different reaction conditions on DS_A and DS_C. (a) NaOH concentrations, (b) CHPTAC concentrations, (c) STP concentrations, and (d) water–ethanol ratios.

medium plays an important role in the preparation of amphoteric starch which can effectively protect STP from hydrolyzing. To know the interactions among NaOH, CHPTAC, STP and water/ethanol ratio, further studies were needed with Box–Behnken central composite design (CCD).

3.2. Establishment of quadratic regression equation

Box–Behnken central composite design was performed to determine the optimum conditions. It was selected with three-level-four-factors (concentrations of NaOH, concentrations of CHPTAC, concentrations of STP and water/ethanol ratio). Table 1 lists the experimental factor codes and results on the basis of experimental design. All 29 designed experiments were conducted and the results were evaluated via regression analysis and tested for significance. Design Expert 7.0 (Myers, Douglasc, Montgomery, Christime & Anderson, 2008) software was used to analyze the experimental data (Table S1 and Table 2). The following regression equation in terms of coded factors was fitted to the response resulted from CCD:

$$\begin{aligned} DS_A = {} & 0.025 + 0.0018A + 0.00066B + 0.0053C + 0.00015D \\ & -0.0028AB + 0.0017AC - 0.00053AD - 0.0040BC \\ & -0.000050BD - 0.00028CD - 0.0060A^2 - 0.0069B^2 \\ & -0.0033C^2 - 0.0025D^2 \end{aligned} \quad (5)$$

$$\begin{aligned} DS_C = {} & 0.041 + 0.0033A + 0.0080B + 0.0048C + 0.0022D \\ & +0.0029AB + 0.0053AC - 0.0033AD - 0.0050BC \\ & -0.00058BD - 0.0048CD - 0.0071A^2 - 0.0033B^2 \\ & -0.0055C^2 - 0.0065D^2 \end{aligned} \quad (6)$$

The analysis of variance (ANOVA) is used to evaluate the quality of the model fitted. It is designed to compare the variation due to the treatment (change in the combination of variable levels) with the variation due to random errors inherent to the measurements of the generated responses. From this comparison, it is possible to evaluate the significance of the regression equation used to foresee responses considering the sources of the experimental variance. A model will be well fitted to the experimental data if it presents a significant regression and a non-significant lack of fit (Bezerraa et al., 2008). The ANOVA for the response surface model is provided in Table S1 and Table 2. The coefficients of the response surface model as provided by Eqs. (5) and (6) were also evaluated.

The statistics test, F, is defined as MSR/MSE, where MSR is the mean square of regression, obtained by dividing the sum of squares of regression by the degree of freedom. MSE is the mean square of error from the analysis of variance. If the calculated value of F exceeds that in F table at a specified probability level [i.e., $F(P-1, \nu, 1-\nu)$], then a "statistically significant" regression model is obtained, where ν is the degree of freedom of error and P is the number of parameters. $F(P-1, \nu, 1-\nu)$ is the F value

Table 2
ANOVA for response surface quadratic model of DS_C.

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	Prob > *F*	Significant[a]
Model	0.0020	14	0.00015	8.19	0.0002	Significant
A-NaOH	0.00013	1	0.00013	7.00	0.0192	**
B-CHPTAC	0.00077	1	0.00077	43.26	<0.0001	**
C-STP	0.00028	1	0.00028	15.56	0.0015	**
D-water/ethanol ratio	0.000056	1	0.000056	3.11	0.0995	*
AB	0.000033	1	0.000033	1.85	0.1948	
AC	0.0000011	1	0.0000011	0.06	0.8072	
AD	0.00000042	1	0.00000042	0.02	0.8798	
BC	0.000098	1	0.000098	5.50	0.0343	**
BD	0.0000013	1	0.0000013	0.07	0.7893	
CD	0.000090	1	0.000090	5.06	0.0411	**
A^2	0.00033	1	0.00033	18.54	0.0007	**
B^2	0.000072	1	0.000072	4.02	0.0646	*
C^2	0.00020	1	0.00020			
D^2	0.00027	1	0.00027			
Residual	0.00025	14	0.000018			
Lack of fit	0.00025	10	0.000025			
Pure error	0.0020	4	0			
Cor total	0.0023	28				

[a] *Significant ($p < 0.05$); **Extremely significant ($p < 0.01$).

at the v probability level. The Model *F*-value of 3.00 and 8.19 for DS_A and DS_C implied that both models were significant, and there was only a 2.42% and 0.02% chance that a "Model *F*-value" could occur due to noise, respectively, with a very low probability value [(Prob > *F*) = 0.0242] for DS_A and [(Prob > *F*) = 0.0002] for DS_C. The goodness of the fit model was checked by determination coefficient (R^2). In this case, correlation coefficient for DS_A ($R^2 = 0.8501$) and for DS_C ($R^2 = 0.8912$) indicated that the regression equation fit very well with the actual situation, reflecting the relationship between the DS of amphoteric starch and preparation conditions (NaOH concentration, CHPTAC concentration, STP concentration, water/ethanol ratio). Table S1 summarizes the items including C, A^2, B^2 have significance effect on DS_A. Table 2 demonstrates A, B, C, BC, CD, A^2, C^2, D^2 are significant model terms for DS_C.

3.3. The analysis of RSM

The graph of RSM reflected the interaction between the independent variables on the DS of amphoteric starch. The visualization of the predicted model equation can be obtained by the surface response plot (Bezerraa et al., 2008). These types of plots can reflect the effects of two variables on the response value at a time. In all the presented figures, the other factors were kept at level zero. The three-dimensional responses and two-dimensional contour plots are generally the graphical representations of the regression equation. The values of DS_A and DS_C for different concentration of variables are shown in Fig. S1 and Fig. 2, respectively.

Response surface plot represents the magnitude of response value. If the slope of response surface plot is relatively flat, it indicates that the response value has little effect with the change of preparation conditions. On the contrary, if the slope of response surface is relatively abrupt, it indicates that the response value has great effect with the change of preparation conditions (Bezerraa et al., 2008). The maximum predicted value is indicated by the surface confined by the smallest ellipse in the contour diagram (Kshirsagar & Singhal, 2007). As shown in Fig. S1 and Fig. 2, by the center of the four influence factors (zero level), and the maximum DS_A and DS_C of amphoteric starch can be obtained being located inside the experimental region.

Contour plot representing the response value is the same within an ellipse-shaped region. The maximum response value is obtained in the center of ellipse-shaped region and gradually decreasing from center to edge. The ellipses are arranged densely, indicating that the response values have great effect on DS value of amphoteric with the change of reaction conditions. The shape of contour plots can reflect the extent of interaction effect; ellipse shows a significant interaction effect between factors whereas circular shows no significant impacts. From Table 2 and Fig. 2, *p*-value and contour plot of *BC* and *CD* indicated that it had extremely significant interaction effect on DS_C between CHPTAC concentration and STP concentration, STP concentration and water/ethanol ratio, respectively. It had non-significant interaction effect between other factors. From Table S1 and Fig. S1, it had non-significant interaction effect on DS_A between four factors.

3.4. Attaining optimum condition

The optimal values of the variables were obtained using Design Expert software. The optimum reaction conditions for DS (NaOH concentration 3.71 g/100 g starch (dry basis), CHPTAC concentration 6.34 mL/100 g starch (dry basis), STP concentration 6.13 g/100 g starch (dry basis), water/ethanol ratio 1.16) were obtained from the regression equation (Eq. (4)). The maximum predicted response values of DS_A and DS_C under the above conditions were 0.0274 and 0.0408, respectively.

The adequacy of the predicted model here was examined by an additional independent experiment at the suggested optimal synthesis conditions. The actual experimental values were DS_A 0.0278 and DS_C 0.0406, indicating that experimental values for DS were significantly the same as the predicted values. Thus, modeling and optimization of the synthesis of amphoteric starch from enzyme-pretreated starch were successfully developed by RSM.

3.5. Effect of enzymatic pretreatment on DS of amphoteric starch

Alpha-amylase, endoenzyme, is capable of hydrolyzing internal α-1,4-glycoside bonds in a long-chain polymers and acts as a contributor of newly formed non-reducing ends of starch molecules to glucoamylase by splitting the original starch molecules (Cheol & John, 2002; Fujii & Kawamura, 1985). Glucoamylase is exoenzyme which cuts off a monomer at the non-reducing end of the substrate molecule (Karakatsanis & Liakopoulou, 1997). A mixed α-amylase and glucoamylase system hydrolyzes starch granules efficiently, which makes the surface of starch granule rough. The same results were also confirmed by Ma, Cai, Wang, and Sun (2006).

In this study, α-amylase and glucoamylase were combined to hydrolyze native starch. DS values of the amphoteric starch from enzyme-pretreated tapioca starch (EPTAS) and the amphoteric

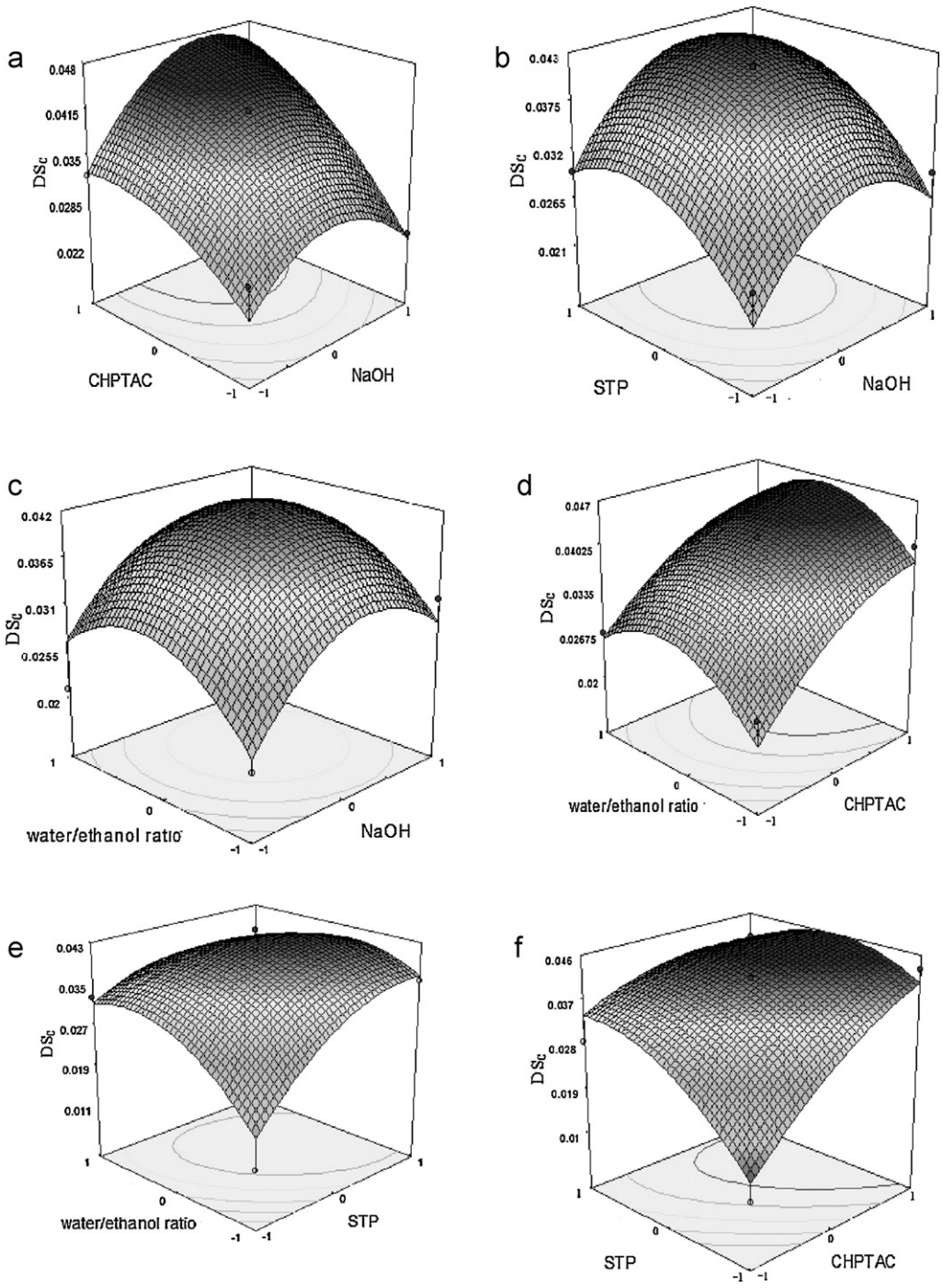

Fig. 2. Response surface plot showing the effect of the interaction: (a) Between NaOH concentration and CHPTAC concentration, (b) between NaOH concentration and STP concentration, (c) between NaOH concentration and water/ethanol ratio, (d) between CHPTAC concentration and water/ethanol ratio, (e) between STP concentration and water/ethanol ratio, and (f) between CHPTAC concentration and STP concentration on the DS_C.

Table 3
DS_A and DS_C of NPTAS and EPTAS prepared at the same conditions.[c]

Experiments	Starch samples	DS_A	DS_C	Increase in DS_A (%)	Increase in DS_C (%)
1[a]	EPTAS	0.0176a	0.0402a	39.7	28.8
	NPTAS	0.0126b	0.0312b	–	–
2[b]	EPTAS	0.0238a	0.0399a	31.7	23.1
	NPTAS	0.0183b	0.0324b	–	–

[a] Reaction conditions: starch concentration 30% (w/w), NaOH concentration 3.5 g/100 g starch (dry basis), STP concentration 5.0 g/100 g starch (dry basis), CHPTAC concentration 9.0 mL/100 g starch (dry basis), water/ethanol ratio 1.0 (v/v), reaction temperature 50 °C, reaction time 3 h.

[b] Reaction conditions: starch concentration 30% (w/w), NaOH concentration 4.0 g/100 g starch (dry basis), STP concentration 5.0 g/100 g starch (dry basis), CHPTAC concentration 7.0 mL/100 g starch (dry basis), water/ethanol ratio 1.0 (v/v), reaction temperature 50 °C, reaction time 3 h.

[c] Values in the same column with different letters (a, b) are significantly different ($p < 0.05$).

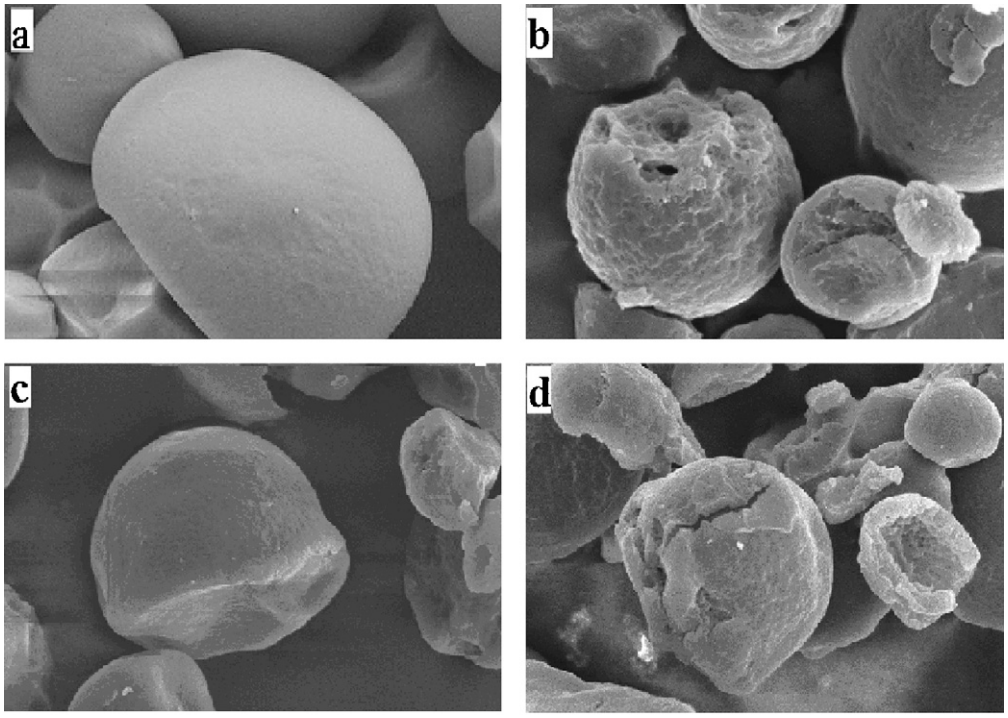

Fig. 3. Scanning electron micrographs of starches. (a) Native starch, (b) enzyme-pretreated starch, (c) NPTAS(DS_A0.0183, DS_C0.0324), and (d) EPTAS(DS_A0.0181, DS_C0.0325).

starch prepared from native tapioca starch (NPTAS) are illustrated in Table 3. It can be seen that both DS_A and DS_C of EPTAS were higher than those of NPTAS. In addition, the surface area of native tapioca starch granules was 0.38 m^2/g, while that of the enzyme-pretreated starch (hydrolysis rate 50.5%) was 3.45 m^2/g. On the one hand, the increase in surface area could provide more hydroxyl groups to react with reagents. Moreover, the pores or cracks in the surface (Fig. 3) caused reagents such as STP and HPTAC to infiltrate into the inner parts of the starch easily, which also caused more reagents to react with hydroxyl groups of enzyme-pretreated starch granules at the same amount of reagents added. Therefore, enzyme treatment increased the DS of the amphoteric starch significantly.

3.6. Scanning electron microcopy (SEM)

Scanning electron micrographs of starches are shown in Fig. 3. The granule surface of native starch was smooth and had no obvious fissures or cavities (Fig. 3a). After hydrolysis, starch granules have pits and become rough, and small holes extended into the interior of the granular structure, while granular size almost had no change (Fig. 3b). Similar results were found from starch granules hydrolyzed by amylase only (Li, Gao, Wang, Jiang, & Huang, 2011; Mélo et al., 1996).

Chemical modification did not change the shape of NPTAS(DS_A0.0183, DS_C0.0324) and EPTAS(DS_A0.0181, DS_C0.0325). NPTAS had the similar shape to native starch, while EPTAS remained the similar shape to enzyme-pretreated starch. However, a little change could be found on the surfaces of both starch granules. The surfaces of NPTAS and EPTAS were somewhat rougher than those of native and enzyme-pretreated starches, respectively. That is due to the reaction of starch with STP and CHPTAC during the preparation of amphoteric starch.

3.7. X-ray diffraction

The X-ray diffraction patterns of native starch, NPTAS(DS_A0.0183, DS_C0.0324) and EPTAS(DS_A0.0181, DS_C0.0325) are presented in Fig. 4. The A-type X-ray diffraction pattern was characterized by peaks at diffraction angles 2θ of 14.9°, 17.0°, 18.0°, and 23.0° (Zobel, 1988). The X-ray spectrums of native starch, NPTAS, enzyme-pretreated starch and EPTAS displayed the characteristic A-type crystalline patterns. RC (41.8%) of NPTAS was similar to native starch (42.3%), whereas RC (32.5%) of EPTAS decreased. Starch crystallinity has been shown to be influenced by: (1) crystallite size, (2) number of crystallites that are arranged in a crystalline array, (3) moisture content, (4) polymorphic content (Hoover, Hughes, Chung, & Liu, 2010). Decrease in RC of EPTAS could be attributed to disruption of crystalline region, which was hydrolyzed by α-amylase and glucoamylase and/or caused by the reaction of infiltrated reagents with starch molecules.

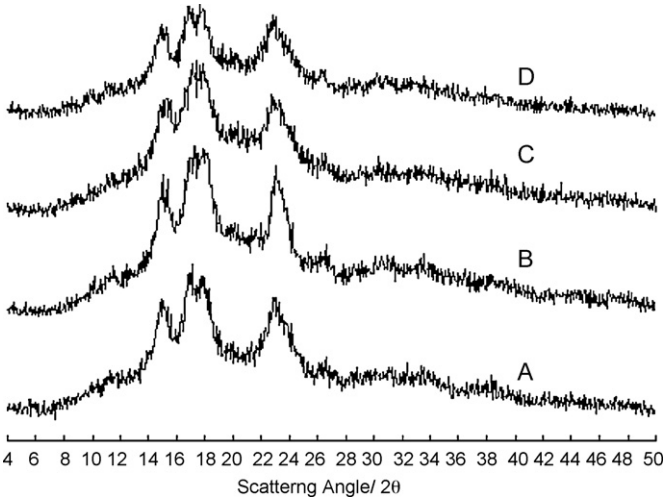

Fig. 4. X-ray diffraction of native starch (A), NPTAS(DS_A0.0183, DS_C0.0324) (B), enzyme-pretreated starch (C) and EPTAS(DS_A0.0181, DS_C0.0325) (D).

Table 4
Pasting properties of native starch, NPTAS and EPTAS.[a]

Starch samples	Gelatinization temperature (°C)	Peak viscosity (BU)	Breakdown (BU)	Setback (BU)
Native starch	62.1a	962.7a	712.7a	270.6a
NPTAS(DS_A0.0183, DS_C0.0324)	62.2a	821.1b	696.0b	35.5b
EPTAS(DS_A0.0181, DS_C0.0325)	62.0a	667.0c	542.0c	25.2c

[a] Values in the same column with different letters (a, b, c) are significantly different ($p < 0.05$).

3.8. Pasting properties of starches

The pasting characteristics of native starch, NPTAS(DS_A0.0183, DS_C0.0324) and EPTAS(DS_A0.0181, DS_C0.0325) are shown in Table 4. Compared with native starch and NPTAS, EPTAS with the same DS had the lowest peak viscosity, which might be due to the degradation of starch molecular chain. During the viscosity test, the starch slurries are subjected to high temperatures and mechanical shear stress which further disrupts starch granules, resulting in the alignment and leaching of amylose. This period is commonly associated with a breakdown in viscosity. Starch granules with lower breakdown values reflect higher stability when exposed to heat treatment at high temperature and mechanical stirring (Ragaee & Abdel-Aal, 2006). The ability of starches to withstand heating at high temperature and shear stress is an important factor in the papermaking processes. The breakdown of native and modified starches was in the order of native starch > NPTAS > EPTAS, indicating that EPTAS had the highest pasting stability. That was due to enzymatic pretreatment which not only increased the DS, but also improved the homogeneous distribution of substitution groups in amphoteric starch molecules.

Re-association between starch molecules, especially amylose, will result in the formation of a gel structure during cooling. This phase is commonly described as the setback (SB) region and is related to retrogradation and reordering of starch molecules. The low SB values indicate low rate of starch retrogradation (Miles, Morris, Orford, & Ring, 1985; Ragaee & Abdel-Aal, 2006). EPTAS had much lower setback value than native starch. Hence, EPTAS paste showed the lowest syneresis when cooling.

4. Conclusions

Preparation of phosphorylated amphoteric starch from enzyme-modified tapioca starch was investigated by RSM. The optimum reaction conditions obtained were as follows: NaOH concentration 3.71 g/100 g starch (dry basis), CHPTAC concentration 6.34 mL/100 g starch (dry basis), STP concentration 6.13 g/100 g starch (dry basis), water/ethanol ratio 1.16, reaction temperature 50 °C, time 3 h, and starch concentration 30%. Under the optimal condition, the maximum DS_A and DS_C of Phosphorylated amphoteric starch were 0.0278 and 0.0406, respectively. Enzymatic pretreatment increased the surface area of starch granules and improved the reaction process of amphoteric starch. Brabender pasting data indicated that the pasting stability of enzyme-pretreated amphoteric starch was significantly improved. The reason may be the homogeneous distribution of substitution groups in starch molecules. Future research will focus on the effect of the substitution pattern at the molecular level on the functional properties of amphoteric starch.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (21004023), the Key Project of Science and Technology of Guangdong Province (2009B090300272, 2009B020312006), the Fundamental Research Funds for the Central Universities, SCUT (2009ZM0124).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2012.01.034.

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