

# Optimization of Preparation Conditions of Carboxymethyl Potato Starch through Orthogonal Experimental Design

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**ABSTRACT:** To optimize the preparation conditions of carboxymethyl potato starch (CMPS), the effects of relevant factors on viscosity ( $\eta$ ) of 2% CMPS aqueous solution and degree of substitution (DS) were investigated. These condition parameters included etherification temperature, alkalinization and etherification time, water content in the mixed solvent, ratio of liquid volume to starch mass, molar ratio of sodium hydroxide to monochloroacetic acid, and molar ratio of monochloroacetic acid to anhydroglucose unit. After individual parameter influencing  $\eta$  and DS was researched one by one, an orthogonal experiment of  $L_{18}(2 \times 3^7)$  was designed to identify the main factors affecting them. In light of range analysis, the comparative importance of factors

impacting  $\eta$  and DS was obtained, separately. Results of variance analysis showed that the most effective factor to control DS was etherification temperature, whereas the influences of all factors on  $\eta$  were not significant. Meanwhile,  $\eta$  and DS of the optimized final product were found to be 12,000 mPa.s and 0.68, respectively. In addition, the structure of CMPS was characterized by Fourier transform infrared (FTIR) spectrophotometer. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 113: 24–33, 2009

**Key words:** carboxymethyl starch; degree of substitution; viscosity; orthogonal experimental design; chemical modification

## INTRODUCTION

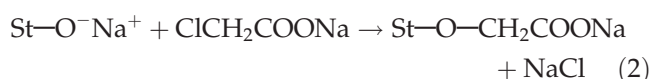
Starch is a natural abundant polysaccharide produced from many plants and is an environmentally friendly biodegradable material.<sup>1–7</sup> However, there are still some disadvantages to the application of starch, such as the lack of free-flowing properties and insolubility in cold water.<sup>8</sup> These shortcomings restrict the further use of starch; for example, in the textile, papermaking and pharmaceutical industries.<sup>8</sup> Therefore, chemical modification of starch is necessary to improve specific properties. Starch has become cold water soluble by substituting the hydroxyl groups with monochloroacetic acid (MCA) or sodium monochloroacetate (SMCA) to give carboxymethyl starch (CMS).<sup>9</sup>

CMS can be prepared by the reaction of starch and MCA or SMCA in the presence of an alkali. The first step of carboxymethylation is an alkalinization. In this step, the hydroxyl groups (–OH) on starch mol-

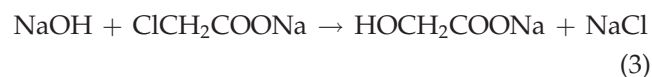
ecules are activated and transformed into the more active alkoxide form ( $\text{StO}^-$ ):



Here, St is the starch backbone. Etherification takes place in the second step:



The side reaction is the formation of sodium glycolate from SMCA and sodium hydroxide according to:



Sodium glycolate can react with itself or SMCA to give sodium diglycolate.<sup>10</sup>

The carboxymethylation can be performed in water as a solvent,<sup>11–14</sup> or in a water-miscible organic solvent containing small amounts of water.<sup>15–18</sup> From an economical point of view, it may be more advantageous to use ethanol-water. The amount of carboxymethyl groups formed is indicated by the degree of substitution (DS). The DS is defined as the average number of substituents per anhydroglucose unit (AGU) of starch. Each AGU contains three hydroxyl groups, so the DS lies between zero and

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three. Meanwhile, the viscosity of CMS aqueous solution is another important physical parameter of CMS and decides its applications. The CMS with low viscosity is used as textile sizing, paper coating, and water-based adhesives, whereas the CMS with high viscosity can be employed in the area of thickening of food, printing and coating materials.<sup>19</sup> The viscosity of CMS aqueous solution is influenced by various factors, such as temperature, low molecular weight electrolytes and concentration of CMS aqueous solution. For instance, Zhang and Wu<sup>20</sup> explored the dependence of the viscosity of CMS aqueous solution on pH value, NaCl concentration, DS, molecular weight and substituent distribution.

Orthogonal experimental design is a powerful design of experiments tool. It provides a simple, efficient, and systematic approach to optimize designs for performance, quality, and cost.<sup>21</sup> Actually, orthogonal experimental design is one of the factorial designs, which can enable all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them.<sup>22</sup> According to range analysis, orthogonal experimental design can ascertain the significance sequence of all the studied affecting factors and the optimal level of variables. It can be also deduced from variance analysis whether the effect of every factor on final response is significant. Although orthogonal experimental design has been successfully applied in many scientific domains, information on the application of orthogonal experimental design to the carboxymethylation of polysaccharides is still scarce. Moreover, few publications have reported on the simultaneous optimization of reaction conditions for DS and viscosity of CMS aqueous solution, especially using orthogonal experimental design.

On the basis of the above background, the aim of this work is to optimize the reaction conditions of carboxymethyl potato starch (CMPS) using an orthogonal experimental design. Through the statistical analysis, the optimal procedures for preparing CMPS with both higher DS and viscosity ( $\eta$ ) of 2% CMPS aqueous solution were obtained in the solvent of ethanol-water mixture.

## EXPERIMENTAL

### Materials

Potato starch (moisture content between 13.0 and 17.2%) was kindly supplied by Gansu Tengsheng Agricultural Products Group, China. Glycolic acid (99%) was bought from J and K China Chemical. J acid (6-amino-1-naphthol-3-sulfonic acid) (90%) was purchased from Tokyo Kasei Chemical Industry, Japan. All the other chemicals used in this investiga-

tion were of analytical grade. MCA, glacial acetic acid, absolute methanol and absolute ethanol were purchased from Tianjin Guangfu Fine Chemical Research Institute, China. Sodium hydroxide was bought from Tianjin Chemical Reagent NO.6 Factory, China. Ammonium acetate was obtained from Beijing Chemical Works, China. Concentrated sulfuric acid was supplied by Baiyin Liangyou Chemical, China.

### Synthesis of carboxymethyl potato starch

A series of CMPS samples with different DS and  $\eta$  were prepared by the following procedure. Typically, potato starch (15.00 g) and ethanol-water mixture (54 mL) were added into a 250 mL three-necked round-bottom flask equipped with a mechanical stirrer. Subsequently, NaOH (5.05 g) was introduced into the flask and the mixture was constantly agitated for 30 min. Then, MCA (11.57 g) and NaOH (2.53 g) were added into the reaction mixture in turn. The reaction was allowed to proceed at 45°C for 240 min. The resulting product was neutralized with glacial acetic acid. The resulting CMPS was purified by filtration and washing with 80% aqueous methanol until the filtrate gave negative response to silver nitrate solution. The final product, CMPS, was then dried in a vacuum oven at 60°C. The effects of the reaction conditions on the carboxymethylation were studied by varying one parameter at a time whereas the others were maintained at certain conditions (given in Table I).

### Orthogonal experimental design for CMPS

Based on above tests, the statistic analysis of the effects of reaction conditions on DS and  $\eta$  were investigated through orthogonal design experiments.<sup>23</sup> Eight relevant factors were investigated: charging times of sodium hydroxide, mixing time of starch and solvent, etherification temperature, alkalinization time, water content in the mixed solvent, ratio of liquid volume to starch mass ( $V_L/m_{st}$ ), molar ratio of sodium hydroxide to MCA ( $n_{NaOH}/n_{MCA}$ ) and molar ratio of MCA to AGU ( $n_{MCA}/n_{AGU}$ ) (see Table II). The orthogonal table  $L_{18}(2 \times 3^7)$  was designed (see Table II), which suggested the optimal reaction conditions for carboxymethyl potato starch.

### Characterization of CMPS by FTIR

Potato starch and CMPS were characterized by a Fourier transform infrared (FTIR) spectrophotometer (American Nicolet Corp. Model 170-SX). The samples of potato starch and CMPS were ground with

**TABLE I**  
**Overview of Experimental Conditions**

Experiment	No.	Etherification temperature (°C)	Alkalization time (min)	Etherification time (min)	Water content in the mixed solvent (wt %)	$V_L/m_{st}$ (mL/g)	$n_{NaOH}/n_{MCA}$ (mol/mol)	$n_{MCA}/n_{AGU}$ (mol/mol)
A	1	25	60	180	0	3.60	1.94	1.54
	2	30						
	3	35						
	4	40						
	5	45						
	6	50						
	7	55						
B	1	45	30	180	0	3.60	1.94	1.54
	2		60					
	3		90					
	4		120					
	5		180					
	6		240					
	7		270					
C	1	45	30	60	0	3.60	1.94	1.54
	2			120				
	3			180				
	4			210				
	5			240				
	6			270				
	7			270				
D	1	45	30	240	9.89	3.60	1.94	1.54
	2				10.96			
	3				12.03			
	4				12.88			
	5				14.13			
	6				16.04			
	7				16.25			
E	1	45	30	240	14.13	1.80	1.94	1.54
	2					2.70		
	3					3.60		
	4					4.50		
	5					5.40		
	6					6.30		
	7					7.20		
F	1	45	30	240	14.13	3.60	0.75	1.54
	2						1.00	
	3						1.25	
	4						1.50	
	5						1.75	
	6						2.00	
	7						2.25	
G	1	45	30	240	14.13	3.60	1.50	0.75
	2							1.00
	3							1.25
	4							1.50
	5							1.75
	6							2.00
	7							2.25

dried KBr powder. The KBr disk was dried again and subjected to FTIR spectrophotometer.

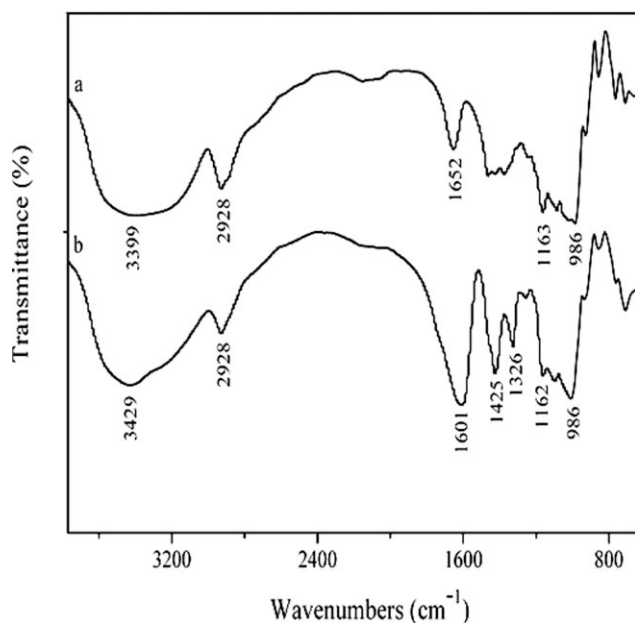
#### Determination of degree of substitution

The DS was determined by the J acid colorimetric method<sup>24</sup> using a literature procedure.<sup>20,25,26</sup> CMS sample (0.1 g) was dissolved in 0.25 mol/L NaOH solution and the mixture was transferred to a 250-mL volumetric flask to fix its volume. A series of the

standard solution of glycolic acid (with concentration between 15 and 100 µg/mL) was prepared. One milliliter standard solution and 1 mL solution of the above sample were added into each of the 25-mL colorimetric tubes by a pipette, respectively. 0.5 mL 1% J acid and 5 mL concentrated sulfuric acid were added into each of these tubes in turn. Then, these mixtures were heated in a boiling water bath for 1 h after shaking evenly. These tubes were cooled to room temperature and 30% NH<sub>4</sub>Ac solution was

TABLE II  
The Orthogonal Layout and the Results of Experiments

Experiment code	A	B	C	D	E	F	G	H	Test contents	
	Charging times of sodium hydroxide (times)	Mixing time of starch and solvent (min)	Etherification temperature (°C)	Alkalinization time (min)	Water content in the mixed solvent (wt %)	$V_L/m_{st}$ (mL/g)	$n_{NaOH}/n_{MCA}$ (mol/mol)	$n_{MCA}/n_{AGU}$ (mol/mol)	DS	$\eta$ (mPa.s)
1	1	0	40	0	15.53	3.20	1.40	1.48	0.54	6580
2	1	0	43	15	14.67	3.60	1.50	1.54	0.58	7000
3	1	0	46	30	14.15	4.00	1.60	1.60	0.66	2250
4	1	5	40	0	14.67	3.60	1.60	1.60	0.54	4300
5	1	5	43	15	14.15	4.00	1.40	1.48	0.46	2800
6	1	5	46	30	15.53	3.20	1.50	1.54	0.67	5600
7	1	10	40	15	15.53	4.00	1.50	1.60	0.54	3800
8	1	10	43	30	14.67	3.20	1.60	1.48	0.61	1520
9	1	10	46	0	14.15	3.60	1.40	1.54	0.50	9100
10	2	0	40	30	14.15	3.60	1.50	1.48	0.54	3700
11	2	0	43	0	15.53	4.00	1.60	1.54	0.56	3650
12	2	0	46	15	14.67	3.20	1.40	1.60	0.60	3900
13	2	5	40	15	14.15	3.20	1.60	1.54	0.54	7000
14	2	5	43	30	15.53	3.60	1.40	1.60	0.62	3500
15	2	5	46	0	14.67	4.00	1.50	1.48	0.55	12,500
16	2	10	40	30	14.67	4.00	1.40	1.54	0.52	6000
17	2	10	43	0	14.15	3.20	1.50	1.60	0.62	4100
18	2	10	46	15	15.53	3.60	1.60	1.48	0.64	900



**Figure 1** FTIR spectra of potato starch (a) and carboxymethyl potato starch (b).

added gradually to their 25 mL lines. The values of absorbance of these solutions were measured at 620 nm in a Lambda 35 UV/VIS spectrometer (Perkin-Elmer Corp., Norwalk, CT) on the basis of a sample comparison. The data were processed with computer to obtain the glycolic acid equivalent (B) of the CMPS sample. The DS value was then calculated according to the following equation<sup>20</sup>:

$$DS = \frac{162B}{76 - 58B} \quad (4)$$

### Measurement of viscosity of 2% CMPS aqueous solution

$\eta$  was measured by NDJ-1 viscometer (Shanghai Hengping Science Instruments, China) at room temperature.

## RESULTS AND DISCUSSION

### Characterization of CMPS by FTIR

Figure 1 illustrates that the FTIR spectra of potato starch and CMPS (DS = 0.54). Compared with potato starch [Fig. 1(a)], CMPS [Fig. 1(b)] has new and strong characteristic absorption peaks of carboxyl at 1601 and 1425  $\text{cm}^{-1}$ . These new bands indicate that the hydroxyl groups of potato starch molecules were carboxymethylated. Similar observations were reported for carboxymethyl potato starch.<sup>27</sup>

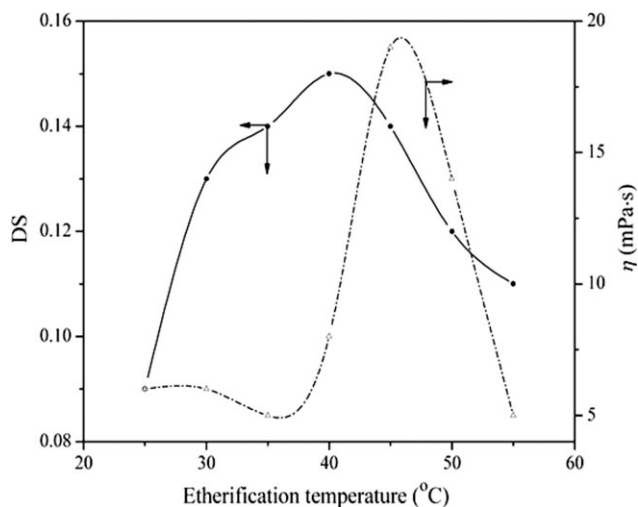
### Influence of individual factors on DS and $\eta$

#### Etherification temperature

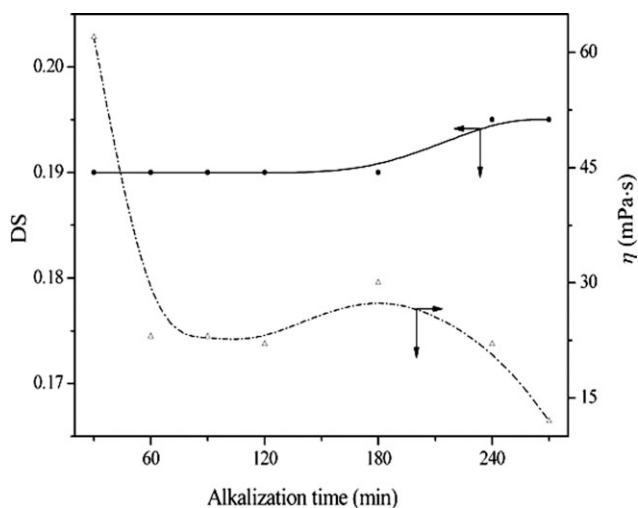
It can be seen from Figure 2 that the effect of etherification temperature on DS and  $\eta$ . DS increased with etherification temperature until it reached a maximum at 40°C. The  $\eta$  changed in a similar way as the DS (Fig. 2). Two factors have noticeable effects in this process. Firstly, with the rising of the etherification temperature, the swelling would destroy the crystalline regions of starch granules, which would become amorphous.<sup>28</sup> At the same time, active reaction centers would be formed, thus increasing reaction rates.<sup>28</sup> In contrast, because the eq. (1) is an exothermic reaction, the higher temperature resulted in a backward shift of the chemical equilibrium, which decreased DS. Furthermore, at higher temperature, the molecular chains may undergo degradation and starch particles may break down,<sup>29</sup> resulting in the reduction of  $\eta$ . Figure 2 also displays that the right temperature for higher DS and  $\eta$  were 40 and 46°C, respectively. It is reported that the viscosity of CMS aqueous solution was affected by many factors like DS, molecular weigh and distribution of the substituent group.<sup>20</sup> Therefore, the  $\eta$  was not quite high though DS of product was high. To prepare CMPS with both higher DS and  $\eta$ , a temperature of 45°C between two high peaks (40–46°C) was selected as the proper temperature.

#### Alkalization time

Figure 3 reveals that the curve of DS almost leveled off at alkalization duration from 30 to 180 min and then increased slightly. This is because ethanol with strong polarity restrained the swelling of starch and



**Figure 2** Dependence of DS and  $\eta$  on etherification temperature. Other reaction conditions are shown in Table I (A).

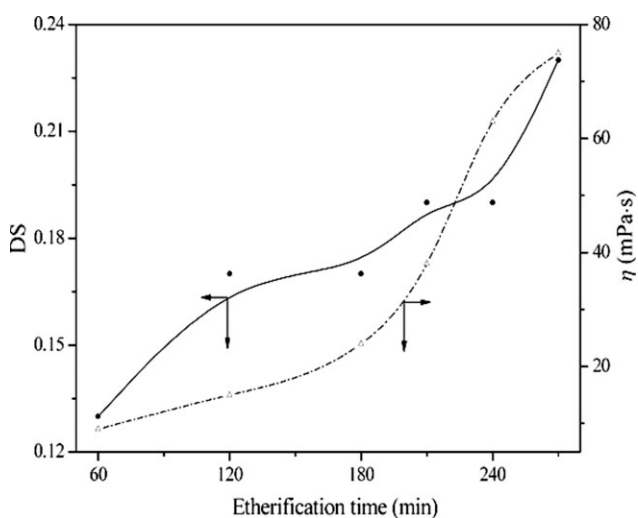


**Figure 3** Dependence of DS and  $\eta$  on alkalinization time. Other reaction conditions are shown in Table I (B).

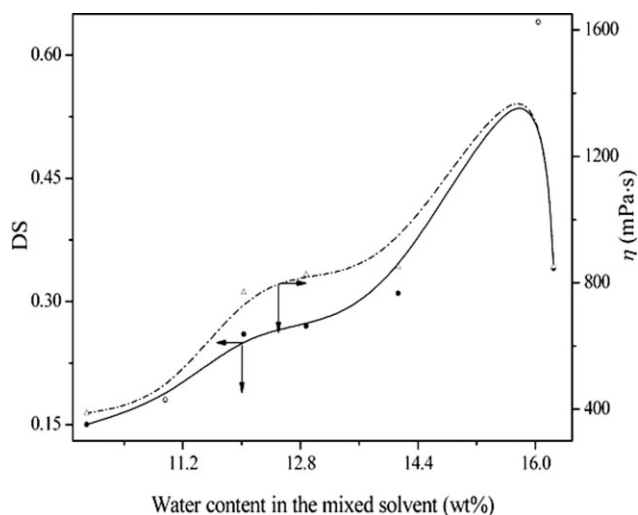
the raise of the DS of products.<sup>29</sup> However, the plot of  $\eta$  versus alkalinization time was different from that of DS (see Fig. 3). As is known, the alkaline condition might cause the degradation of the polysaccharides.<sup>30</sup> Rombouts and Thibault<sup>30</sup> reported that the average molecular weight and intrinsic viscosity of alkaline extracted pectin from sugar beet were lower than those of water extracted pectin. They ascribed this to the alkaline degradation of pectin molecules. Therefore, the alkalinization time should not be too long. In general, the proper time was 30 min.

#### Etherification time

Figure 4 presents that both DS and  $\eta$  increase monotonously with increasing etherification time. Increase in etherification time benefited the swelling of starch and the diffusion and adsorption of the



**Figure 4** Dependence of DS and  $\eta$  on etherification time. Other reaction conditions are shown in Table I (C).



**Figure 5** Dependence of DS and  $\eta$  on water content in the mixed solvent. Other reaction conditions are shown in Table I (D).

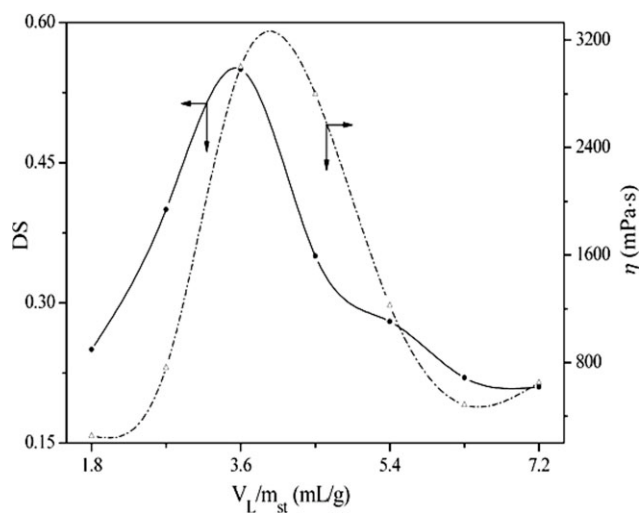
reactants.<sup>26</sup> However, 240 min was selected as the etherification time in the following experiments in view of reducing cost.

#### Water content in the mixed solvent

Water content in the mixed solvent directly influences the swelling of starch in the process of carboxymethylation, consequently affecting the utilization of MCA and the viscosity of aqueous solutions of products.<sup>29,31,32</sup> Figure 5 depicts plots of the DS and  $\eta$  against water content in the mixed solvent. When water content in the mixed solvent was less than 15.80 wt %, DS and  $\eta$  of product increased as the water content increased. But, this did not mean that the larger the content of water, the better the result of reaction. There was an acceptable explanation. If the content of water was larger, the polarity of the solvent increased and the solvation of solvent to  $\text{Na}^+$  strengthened, thus hindering the small molecules from penetrating into the starch particles.<sup>29</sup> A further increase in the water content led to more  $\text{HOCH}_2\text{COONa}$  hydrolyzed by  $\text{ClCH}_2\text{COONa}$ . Because of the conglomeration of CMS causing by large amount of water, the reactants were prevented to permeate through starch particles. Thus, the contact area was reduced and even the main reaction stopped.<sup>29</sup> Moreover, when water content was more than 16.04 wt %, the sample produced was very difficult to grind. Based on these considerations, the water content in the mixed solvent (14.13 wt %) was used in the test following.

#### Ratio of liquid volume to starch mass ( $V_L/m_{st}$ )

The ratio of liquid volume to starch mass has a significant effect on the DS and viscosity of CMS

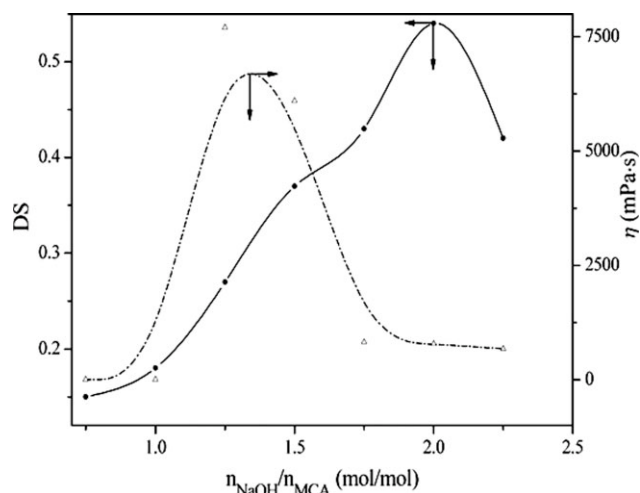


**Figure 6** Dependence of DS and  $\eta$  on  $V_L/m_{st}$ . Other reaction conditions are shown in Table I (E).

aqueous solution.<sup>26,32</sup> Liquid was considered as the total volume of solvent including water. Figure 6 illustrates that the influence of  $V_L/m_{st}$  on the DS and  $\eta$ . Initially, the DS and  $\eta$  gradually increased with increasing ratio of liquid volume to starch mass; then both decreased with a further increase in  $V_L/m_{st}$  (see Fig. 6). In the step of carboxymethylation, the destruction of the crystalline regions did not take place completely and it was difficult to form the active center of reaction if the volume of liquid is too small.<sup>28</sup> So, the DS and  $\eta$  increased with the increment of the  $V_L/m_{st}$  ratio, which can be seen in Figure 6 in the left-hand parts of two curves. However, when the volume of liquid was raised continuously, the concentration of the active centers declined which hampered the reaction.<sup>28</sup> The proper  $V_L/m_{st}$  ratio was about 3.60 mL/g, where DS and  $\eta$  were obtained 0.55 and 3000 mPa.s, respectively.

#### Sodium hydroxide to monochloroacetic acid molar ratio ( $n_{NaOH}/n_{MCA}$ )

At a constant value of  $n_{MCA}/n_{AGU}$ , the  $n_{NaOH}/n_{MCA}$  ratio was varied by changing the amount of NaOH. Figure 7 displays that the effect of  $n_{NaOH}/n_{MCA}$  on DS is presented by a curve which goes through a maximum. A similar change of the DS was also obtained by Khalil et al.<sup>31</sup> They explained the obtained result by assuming that the side reaction between NaOH and SMCA [eq. (3)] became more significant with increasing NaOH content, decreasing in that way the concentration of SMCA in the reaction mixture. Consequently, these were responsible for lower DS. Figure 7 also reveals that the highest value for the DS was obtained for a  $n_{NaOH}/n_{MCA}$  ratio of 2.0 and for the highest value of  $\eta$  this ratio was 1.4. Stojanović et al.<sup>32</sup> also reported a mimetic

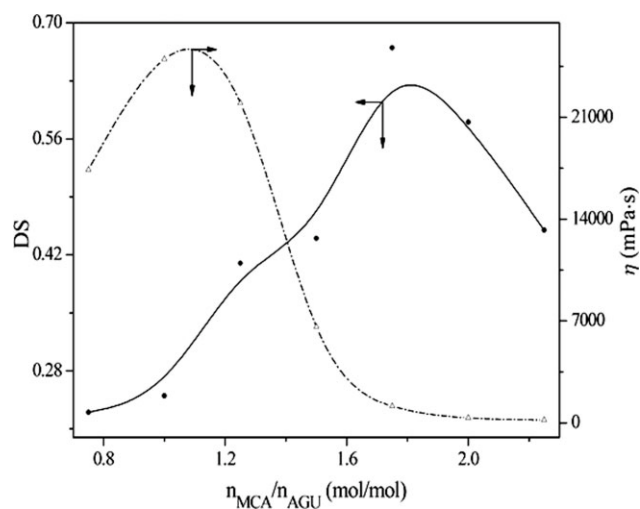


**Figure 7** Dependence of DS and  $\eta$  on  $n_{NaOH}/n_{MCA}$ . Other reaction conditions are shown in Table I (F).

result. These results indicated that with increasing  $n_{NaOH}/n_{MCA}$  ratio, a significant degradation of the starch molecules occurred, and even intramolecular or intermolecular cross-linking might take place,<sup>32</sup> which led to the decrease of  $\eta$ .

#### Molar ratio of MCA to AGU ( $n_{MCA}/n_{AGU}$ )

Figure 8 depicts the relationship between DS and  $n_{MCA}/n_{AGU}$ . On the one hand, an increment in the amount of MCA helped etherification reaction shift to target product, which brought about the higher DS.<sup>27</sup> On the other hand, an increase in MCA concentration also caused a higher reaction rate of the side reaction and more sodium glycolate presented [see eq. (3)], thus causing the lower DS.<sup>27</sup> Figure 8 also displays that 1.80 is the right  $n_{MCA}/n_{AGU}$  for preparing CMPS with high DS, whereas 1.10 is most favorable for CMPS with high  $\eta$ . The reason is the



**Figure 8** Dependence of DS and  $\eta$  on  $n_{MCA}/n_{AGU}$ . Other reaction conditions are shown in Table I (G).

TABLE III  
Statistics Analysis for DS and  $\eta$

Factor	DS				$\eta$			
	$K_1$	$K_2$	$K_3$	$R_1$	$K'_1$	$K'_2$	$K'_3$	$R_2$
A	5.10	5.19		0.09	42,950	45,250		2300
B	3.48	3.38	3.43	0.10	27,080	35,700	25,420	10,280
C	3.22	3.45	3.62	0.40	31,380	22,570	34,250	11,680
D	3.31	3.36	3.62	0.31	40,230	25,400	22,570	17,660
E	3.57	3.40	3.32	0.25	24,030	35,220	28,950	11,190
F	3.58	3.42	3.29	0.29	28,700	28,500	31,000	2500
G	3.24	3.50	3.55	0.31	31,880	36,700	19,620	17,080
H	3.34	3.37	3.58	0.24	28,000	38,350	21,850	16,500

same as that for the effect of  $n_{\text{NaOH}}/n_{\text{MCA}}$  on  $\eta$ . At constant values of both  $n_{\text{AGU}}$  and  $n_{\text{NaOH}}/n_{\text{MCA}}$ , NaOH concentration increased with the increment of MCA in the reaction system. Higher NaOH concentration led to the remarkable degradation of CMPS and potato starch,<sup>27</sup> bringing on a reduction in  $\eta$ .

### Optimization of the factors by orthogonal experimental design

#### Range analysis

The range analysis was aimed to clarify the significance levels of different influencing factors on the DS and  $\eta$ . In addition, those most significant factors could be disclosed basing on the result of range analysis.<sup>23</sup> Table III summarizes the statistics analysis of the effect of different factors on the DS and  $\eta$ . The K value for each level of a parameter was the sum of nine or six values shown in Table III, and the optimal level of variables can be obtained by comparing with K value. The range value (R) for each factor was the difference between the maximal and minimal value of the two or three levels and R scales the effect of variables on the response. High R

of variable means that this variable has strong effect on the response.<sup>33</sup> Based on the results of range analysis, the significance sequence of all the investigated influencing factors was lined. The influence order of each factor was C > D = G > F > E > H > B > A for DS. The optimal levels for each factor should be C<sub>(46°C)</sub> D<sub>(30 min)</sub> G<sub>(1.60)</sub> F<sub>(3.20 mL/g)</sub> E<sub>(15.53 wt %)</sub> H<sub>(1.60)</sub> B<sub>(0 min)</sub> A<sub>(2 times)</sub> for DS, which was not presented in Table II. Supplemental experiments revealed the highest value of DS = 0.71, which was in agreement with the above orthogonal analysis. Table III displays the influence order of each factor was D > G > H > C > E > B > F > A for  $\eta$ , and the optimal levels for each factor should be D<sub>(0 min)</sub> G<sub>(1.50)</sub> H<sub>(1.54)</sub> C<sub>(46°C)</sub> E<sub>(14.67 wt %)</sub> B<sub>(5 min)</sub> F<sub>(4.00 mL/g)</sub> A<sub>(2 times)</sub>, which was also not depicted in Table II. The supplemental experiment indicated the best results for  $\eta$  (12,700 mPa.s), which was also in accord with the above orthogonal analysis.

#### Variance analysis

In our experiments, a blank column was set in the orthogonal table for error estimate. Thus, it was

TABLE IV  
Analysis of Variance for DS and  $\eta$

Factor	DS				$\eta$			
	$SS \times 10^3$	DF	$MS \times 10^3$	$F^a$	SS	DF	MS	$F^a$
A	0.45	1			293889	1		
B	0.83	2			10152133	2	5076067	1.07 <sup>c</sup>
C	13.43	2	6.72	6.72 <sup>b</sup>	12348633	2	6174317	1.30 <sup>c</sup>
D	9.23	2	4.62	4.62 <sup>c</sup>	29989633	2	14994817	3.15 <sup>c</sup>
E	5.43	2	2.72	2.72 <sup>c</sup>	10485300	2	5242650	1.10 <sup>c</sup>
F	7.03	2	3.52	3.52 <sup>c</sup>	643333	2		
G	9.23	2	4.62	4.62 <sup>c</sup>	25848133	2	12924067	2.72 <sup>c</sup>
H	5.70	2	2.85	2.85 <sup>c</sup>	23177500	2	11588750	2.44 <sup>c</sup>
Error	3.72	2			22833246	2		
Error*	5.00 <sup>d</sup>	5 <sup>d</sup>	1.00		23770468 <sup>e</sup>	5 <sup>e</sup>	4754094	

<sup>a</sup>  $F_{0.01}(2,5) = 13.72$ ;  $F_{0.05}(2,5) = 5.79$ .

<sup>b</sup>  $F_{0.05}(2,5) < F < F_{0.01}(2,5)$ .

<sup>c</sup>  $F < F_{0.05}(2,5)$ .

<sup>d</sup>  $SS_{\text{Error}^*} = SS_{\text{Error}} + SS_A + SS_B$ ;  $DF_{\text{Error}^*} = DF_{\text{Error}} + DF_A + DF_B$ .

<sup>e</sup>  $SS_{\text{Error}^*} = SS_{\text{Error}} + SS_A + SS_F$ ;  $DF_{\text{Error}^*} = DF_{\text{Error}} + DF_A + DF_F$ .



unnecessary to repeat the experiments for each protocol.<sup>23</sup> Sum of squares of deviation (SS), degree of freedom (DF), and mean square deviation (MS) of DS and  $\eta$  were determined<sup>23</sup> and summarized in Table IV. Because of lower SS values of some factors, these values and that of error line combine into that of error\* line. The F value of every factor is the ratio of the MS value of the factor to that of error\* line in this article. Through comparing the obtained F value with the theoretical one of specific level and DF, the significance level can be determined for each factor. For DS, the value of error line and lower SS ones of A and B incorporate into that of error\* line (Table IV). As shown in Table IV, the influence of etherification temperature on DS is significant [ $F_{0.05}(2,5) < F < F_{0.01}(2,5)$ ]. For  $\eta$ , the value of error line and lower SS ones of A and F combine into that of error\* line. Table IV displays that the influences of eight studied factors on  $\eta$  are not significant [ $F < F_{0.05}(2,5)$ ] in the researched range.

#### Optimal reaction conditions

The preparation conditions of CMPS with higher DS and  $\eta$  could be obtained by systematically analyzing each factor that influenced DS and  $\eta$  (Tables III and IV). Comparing the influencing degree of each factor on DS and  $\eta$  (see *R* value in Table III and *F* value in Table IV), we concluded that the optimal levels for each factor should exist in one of the following:

1. C<sub>(46°C)</sub> D<sub>(0 min)</sub> G<sub>(1.50)</sub> H<sub>(1.54)</sub> E<sub>(15.53 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
2. C<sub>(46°C)</sub> D<sub>(0 min)</sub> G<sub>(1.50)</sub> H<sub>(1.54)</sub> E<sub>(14.67 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
3. C<sub>(46°C)</sub> D<sub>(0 min)</sub> G<sub>(1.60)</sub> H<sub>(1.54)</sub> E<sub>(15.53 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
4. C<sub>(46°C)</sub> D<sub>(0 min)</sub> G<sub>(1.60)</sub> H<sub>(1.54)</sub> E<sub>(14.67 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
5. C<sub>(46°C)</sub> D<sub>(30 min)</sub> G<sub>(1.50)</sub> H<sub>(1.54)</sub> E<sub>(15.53 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
6. C<sub>(46°C)</sub> D<sub>(30 min)</sub> G<sub>(1.50)</sub> H<sub>(1.54)</sub> E<sub>(14.67 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
7. C<sub>(46°C)</sub> D<sub>(30 min)</sub> G<sub>(1.60)</sub> H<sub>(1.54)</sub> E<sub>(15.53 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>;
8. C<sub>(46°C)</sub> D<sub>(30 min)</sub> G<sub>(1.60)</sub> H<sub>(1.54)</sub> E<sub>(14.67 wt %)</sub> F<sub>(3.20 mL/g)</sub>  
B<sub>(0 min)</sub> A<sub>(2 times)</sub>.

Supplemental experiments were conducted according to the above conditions and the optimal levels for each factor were.<sup>5</sup> The CMPS prepared under these conditions showed the highest DS and  $\eta$ , which were 0.68 and 12000 mPa.s, respectively.

#### CONCLUSIONS

An orthogonal design L<sub>18</sub> (2 × 3<sup>7</sup>) was introduced in the current study to optimize the carboxymethyla-

tion conditions for potato starch based on the results of single factor optimization. From range analysis, the important sequence of effective factors for DS was etherification temperature > alkalization time =  $n_{\text{NaOH}}/n_{\text{MCA}} > V_L/m_{\text{st}} > \text{water content in the mixed solvent} > n_{\text{MCA}}/n_{\text{AGU}} > \text{mixing time of starch and solvent} > \text{charging times of sodium hydroxide}$ , and the sequence of factors for  $\eta$  was alkalization time >  $n_{\text{NaOH}}/n_{\text{MCA}} > n_{\text{MCA}}/n_{\text{AGU}} > \text{etherification temperature} > \text{water content in the mixed solvent} > \text{mixing time of starch and solvent} > V_L/m_{\text{st}} > \text{charging times of sodium hydroxide}$ . In view to variance analysis, it was concluded that the most significant factor influencing DS was etherification temperature while all eight factors did not impact on  $\eta$  significantly. From the results of orthogonal analysis and supplemental experiments, the optimal conditions are as follows: etherification temperature 46°C, alkalization duration 30 min, etherification time 240 min, water content in the mixed solvent 15.53 wt %,  $V_L/m_{\text{st}}$  3.20 mL/g,  $n_{\text{NaOH}}/n_{\text{MCA}}$  1.50, and  $n_{\text{MCA}}/n_{\text{AGU}}$  1.54. Moreover, the amount of NaOH was divided into two parts, introducing into the reaction system before and after MCA was added, respectively. The CMPS prepared under optimal conditions indicated the higher DS and  $\eta$ , which were 0.68 and 12,000 mPa.s, respectively. In this work, we have laid the foundation of the process optimization of CMS in industry, and orthogonal experimental design is proved to be an effective tool to obtain the optimum conditions, which could be also used to investigate other various starches.

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