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Optimization of synthesizing glucose 1-phosphate by sodium tripolyphosphate as a phosphorus acylating agent using response surface methodology

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Abstract: With glucose as the starting material, sodium tripolyphosphate as the phosphorus acylating agent, and urea as the catalyst, glucose 1-phosphate was synthesized and its properties were analyzed. The synthesis conditions were selected according to the phosphorus content and optimized through the response surface methodology (RSM), based on a 3-level, 3-variable Box–Behnken experimental design. The results showed that the phosphorus content of glucose 1-phosphate was 8.12% using a reaction temperature of 70 °C, a molar ratio of glucose to sodium tripolyphosphate of 1.54:1, and catalyst amount of 3.7 g. It coincided well with the experimental value (8.34%). The structure of the product was confirmed by IR, UV, and ^{1}H NMR spectra; electrical conductivity; TG; and DTA. The proposed method has high phosphorus content and low toxicity.

Key words: Synthesis, sodium tripolyphosphate, glucose 1-phosphate, response surface methodology

1. Introduction

Carbohydrate phosphate ester, a kind of important active material, plays an important role in several life processes. Most studies on carbohydrate phosphate ester mainly use starch as the raw material for enzyme catalytic synthesis. However, due to the decomposition of starch in hot alkaline environments, the phosphorus acylation products obtained were usually complex and had low purity. Bismut and Plas discovered the antitumor, antiviral, antibacterial, and immunomodulator biological activities of glucose phosphate derivatives, as well as their clinical application as drugs.[1] Due to their limited supply in nature, the synthesis of glucose phosphate derivatives has been studied by many researchers.[2] Phosphorus oxychloride, phosphorous acid, phosphate, phosphate ester, amine phosphate, and ring phosphating reagent are commonly used as phosphorus acylating agents.[3] The phosphorus content of glucose phosphate is only 2.23% when phosphoric acid and phosphoric acid anhydride are used as phosphorus acylating agents.[4] Because of the hydrolysis in the acidic environment, the phosphorus content is only 0.25% when phosphate is used as phosphorus acylating agent; moreover, these agents have weak esterification abilities and require repeated operations.[5] Glucose can be selectively acylated into 6-a hydroxyl with a ring phosphorus acylating agent and without a 1-a phosphorus acylation product.[6,7] However, a widely used phosphorus acylating agent and simple synthesis method are not available. Furthermore, some shortcomings have been observed in the current synthesis procedures, such as low selectivity and product complexity. A synthetic method with nontoxic side effects is of great significance to achieve highly active selectivity. In view of these concerns, the glucose phosphate acylation reaction in

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the present study was carried out using sodium tripolyphosphate as the phosphorus acylating agent and urea as the catalyst. We optimized the preparation procedures using response surface methodology (RSM) based on a 3-level, 3-variable Box–Behnken experimental design, with the phosphorus content of the product as the standard. The procedure has low catalyst toxicity and high phosphorus acylating agent selectivity, does not need repeat operations, and can generate glucose 1-phosphate with high phosphorus content.

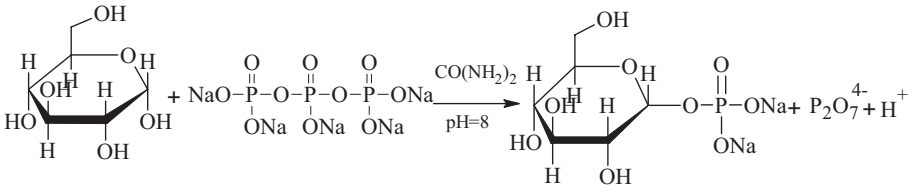
2. Experimental section

2.1. Materials

Phosphorus reagent (H_2SO_4 (3 mol/L):H_2O:ammonium molybdate (2.5%):ascorbic acid (10%) = 1:2:1:1), glucose, sodium hydroxide, 95% ethanol, phenolphthalein, sodium tripolyphosphate, and urea were used. The chemicals were of analytical reagent grade.

2.2. Synthesis of glucose 1-phosphate

The reaction of sodium tripolyphosphate, glucose, and urea was carried out at a specified temperature for 8 h in an oven. It was a nucleophilic substitution reaction, in which glucose was the nucleophilic reagent, and was easier in an alkaline environment. Before the reaction, the chemicals were completely ground in a mortar. Glucose 1-phosphate was formed resulting from the reaction in the Scheme. The procedure has low catalyst toxicity and high phosphorus acylating agent selectivity, and can generate glucose 1-phosphate with high phosphorus content.



Scheme. Synthesis of glucose-1-phosphate ester with glucose and sodium tripolyphosphate.

The mixture was dissolved in water (20 mL). Alcohol (40 mL, 95%) was added to the mixture, and the pH was adjusted to 4 with H_3PO_4 in ice water. The resulting mixture was stored for 30 min and then vacuum filtrated. The sediment was discarded, and the filtrate was adjusted to pH 8 by addition of saturated sodium hydroxide solution. The filtrate was stored for 4 h, and the resulting white precipitate was filtered to collect the crude product.

The crude product was dissolved in distilled water (10 mL). Alcohol (20 mL, 95%) was added to the solution, and the pH was adjusted to 4 with HCl (10 mol/L). The resulting white precipitate was filtered, and saturated sodium hydroxide solution was added to adjust the pH to 8. The filtrate was stored for 4 h and then vacuum filtrated to obtain the pure product.

2.3. Determination of the phosphorus content of the product

According to the molybdenum blue standard curve method, the standard phosphorus content (μg) is the horizontal ordinate and the light absorption value is the vertical coordinate.[8] The phosphorus content standard curve equation is $Y = 0.04859X - 0.00043$ and the R^2 value is 0.99967.

A certain amount of the product was diluted at predetermined times. A solution of the product (0.2 mL) was poured into 2 test tubes. In one test tube, HCl (1 mL, 2 mol/L) was added, and the solution was heated

to 100 °C for 10 min of hydrolyzation. When the mixture was cooled to room temperature, 2 to 3 drops of phenolphthalein indicator were added to the tube, and the mixture was adjusted to red with NaOH solution. In the other test tube, the HCl was replaced with water and no heating was performed in order to remove the nonreacted residues of the inorganic phosphates in the sample. The mixture was diluted to 3 mL with distilled water.

Phosphorus reagent (3 mL) was added to both mixtures. The reaction mixture was kept in water at 45 °C for 25 min. The absorption maximum was found to be 660 nm, as measured by a UV-9100 spectrophotometer. The phosphorus contents of the sample cell and the control tube were determined from the standard curve. The phosphorus content was calculated using Eq. (1):

$$\text{Phosphorus content}/\% = \frac{(m_1 - m_2) \times n}{m \times 10^6} \times 100 \quad (1)$$

where m_1 is the phosphorus content of the sample cell (μg), m_2 is the phosphorus content of the control tube (μg), m is the mass of the sample, and n is the number of dilutions.

2.4. Experiment design by response surface methodology

RSM may be summarized as a compilation of statistical tools for constructing and exploring an estimated functional relationship between a response variable and a set of design variables.[9] It is a collection of mathematical and statistical techniques that are useful for modeling and analyzing problems with numerous variables that influence the response and objective to optimize the response.[10,11] This process involves 3 steps: performing the statistically designed experiments, estimating of coefficients in the proposed model and predicting the response of process, and checking the validity of the model. Experimental data were analyzed using RSM. Because the conditions of the reaction, such as temperature, the ratio of reactants, and the amount of catalyst, play a key role in the synthesis of glucose 1-phosphate, these parameters were extensively investigated. A 3-variable, 3-level Box–Behnken design was used to optimize the preparation conditions to obtain high phosphorus content. The 3 independent variables were temperature (factor X_1), molar ratio of glucose to sodium tripolyphosphate (factor X_2), and catalyst (factor X_3). Each variable set had 3 levels, all of which are listed in Table 1. A total of 15 experiments were designed, with 12 factorial points and 3 center points.[12–14] The center points were used to evaluate the experimental errors.

Table 1. Variables and levels of the 3-variable, 3-level Box–Behnken design.

Factor / Level	X_1(temperature)/°C	X_2(molar ratio)	X_3(catalyst)/g
1	80	2:1	4.8
0	70	1.5:1	3.6
−1	60	1:1	2.4

2.5. Analysis of product

2.5.1. Ultraviolet spectroscopy

The room-temperature UV absorption spectra of the product and glucose were determined using a UV-visible spectrophotometer (UV-9100 LabTech, Columbia, MO, USA) in the range from 190 to 400 nm wavelength respectively.[15]

2.5.2. Infrared spectroscopy

Infrared spectroscopies measure the fundamental molecule vibrations.[16] Spectra were recorded in the range 4000–400 cm^{-1} with a Nicolet 6700 (Thermo Fisher, Waltham, MA, USA) spectrophotometer using samples dispersed in spectroscopically pure KBr pellets.

2.5.3. NMR spectroscopy

NMR spectra were recorded with a Bruker FT-NMR (Bruker, Germany) spectrometer (Broad Band 5 mm probe, inverse detection). Nominal frequencies were 600.13 MHz for ^1H. An internal lock on the deuterium of D_2O was used for spectra.

2.5.4. Electrical conductivity measurements

Research on electrical conductivity can help in studies of structure. In this study, the DDS-11A (China) conductivity meter was used to determine the electrical conductivity. To measure the electrical conductivity, different molarities of the product and glucose were added in water to 25 mL.

2.5.5. Thermal behavior

TG–DTA analysis involves a continuous and simultaneous measurement of weight loss and energy change during heating of the sample. The mass loss can be used to compare the relative abundance of more or less labile C while the position of DTA peaks can be related to the structure and chemical composition of the sample.[17] Thermal properties of carbohydrate materials such α-D-glucose have often been investigated and much information is available in the literature.[18] Thermogravimetry (TG) and differential thermal analysis (DTA) measurements were performed with a simultaneous thermal analyzer model Netzsch/STA409C (Bruker, Germany) instruments. Nonisothermal experiments were performed in the temperature range from 25 to 450 °C and at a heating rate of 20 °C/min.

3. Results and discussion

3.1. Response surface analysis and optimization

According to the scheme designed by Minitab14 software, a series of experiments were performed. The phosphorus contents of the products are listed in Table 2.

Multivariable linear regression was used to calculate the coefficients of the second-order polynomial equation, and the regression coefficients were obtained. Quadratic models were built to fit the results, of which the coefficients were calculated by multiple regression analysis. The functions of Y only with significant terms were obtained in the following Eq. (2) in terms of coded factors:

$$Y = 8.07667 + 0.22X_1 + 0.41375X_2 + 0.34625X_3 - 3.31833X_1^2 - 3.43583X_2^2$$

$$-2.04083X_3^2 + 0.0925X_1X_2 - 1.1225X_1X_3 + 0.61X_2X_3, \quad (2)$$

where Y is the response value (phosphorus content), and X_1, X_2, and X_3 are the coded levels of the 3 variables.

Analysis of variance for the model was employed. The model P-value is 0.002, which is less than 0.01, indicating that model terms are statistically significant. The coefficient of determination (R^2) was 0.973, implying that the accuracy of the polynomial model is adequate. The model F-value (20.25) was higher than

Table 2. Experimental 3-level, 3-variable design and phosphorus content obtained by experiments.

Run	X_1	X_2	X_3	P Content /%
1	0	0	0	8.09
2	–1	1	0	1.75
3	1	–1	0	0.71
4	0	–1	–1	2.04
5	1	0	1	2.42
6	–1	0	–1	0.77
7	0	1	1	4.38
8	1	1	0	1.38
9	0	0	0	8.09
10	0	–1	1	1.99
11	1	0	–1	4.45
12	–1	0	1	3.23
13	–1	–1	0	1.45
14	0	1	–1	1.99
15	0	0	0	8.05

the value of $F_{0.05}$ (9, 2) = 10.38, implying that the model is significant.[19] Therefore, the model is suitable for this experiment to synthesize glucose 1-phosphate.

The 3-dimensional response surface plot and its corresponding contour plot were obtained using the response surface model (Eq. (2)) to examine the effects of the variables and interactions on the phosphorus content of the product and to optimize each variable for maximum responses. These plots are shown in Figures 1 to 3. The effect of temperature and amount of catalyst used on the phosphorus content when the molar ratio of glucose to sodium tripolyphosphate is 1.5:1 is shown in Figure 1. The effect of the molar ratio of glucose to sodium tripolyphosphate and amount of catalyst used on the phosphorus content when the temperature is 70 °C is shown in Figure 2. Finally, the effect of temperature and molar ratio of glucose to sodium tripolyphosphate on the phosphorus content when the amount of catalyst used is 3.6 g is shown in Figure 3.

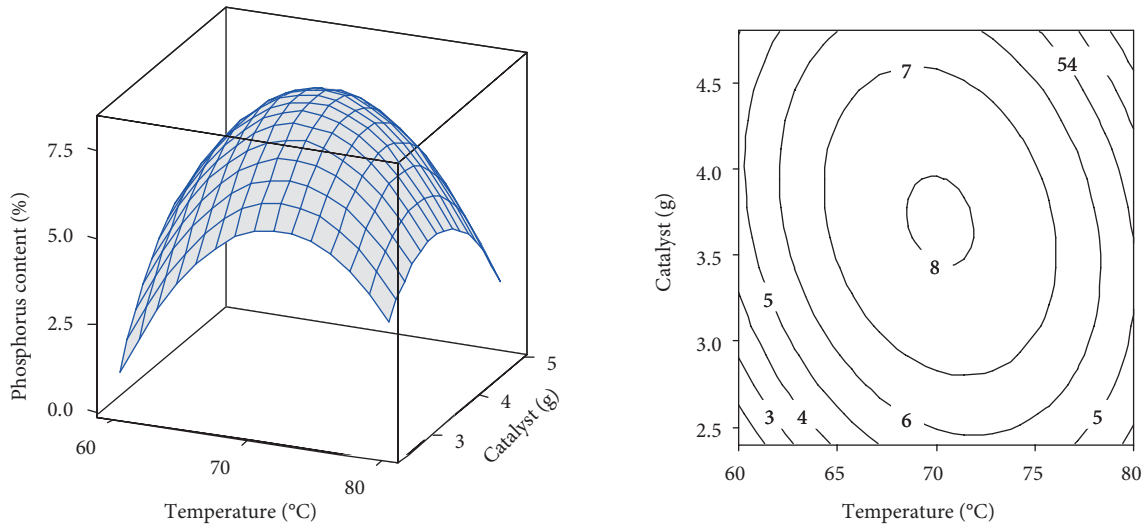


Figure 1. Response surface and contour plot of the effect of temperature and catalyst on phosphorus content.

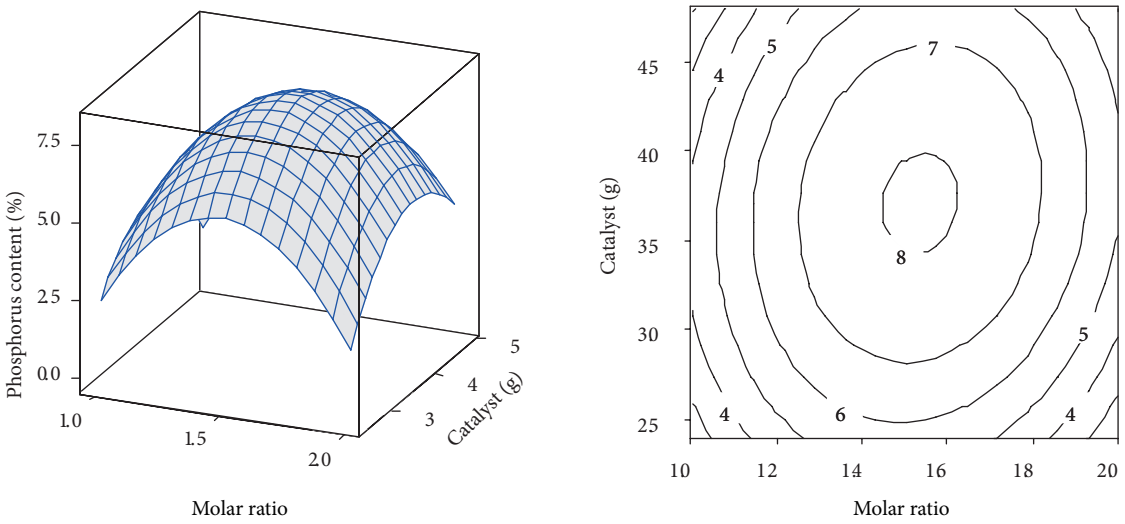


Figure 2. Response surface and contour plot of the effect of molar ratio and catalyst on phosphorus content.

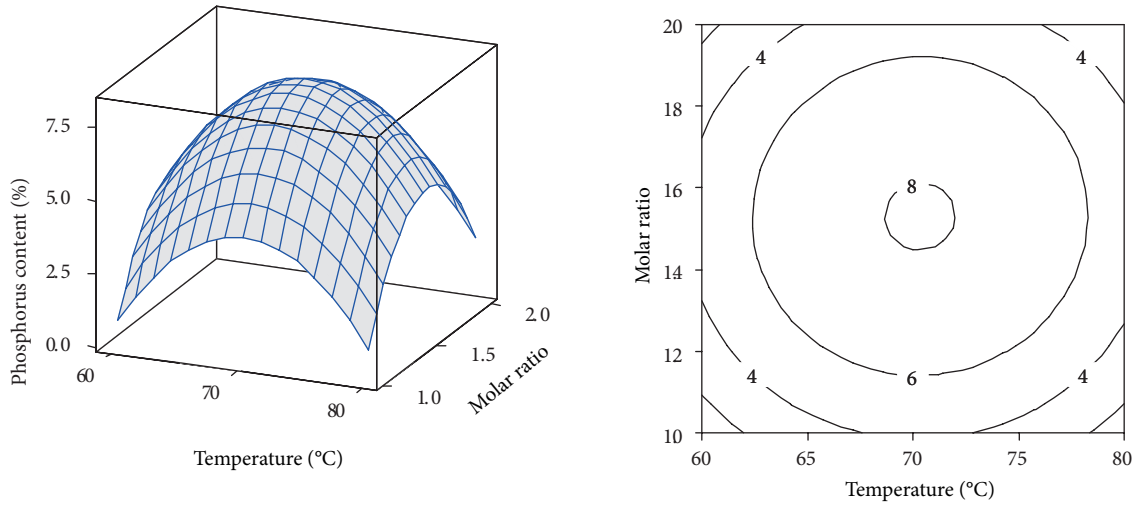


Figure 3. Response surface and contour plot of the effect of temperature and molar ratio on phosphorus content.

Figure 1 shows that the phosphorus content increases within the range of 60 °C to 70 °C and 2.4 g to 3.6 g of catalyst. The phosphorus content then decreases with further increases in temperature and amount of catalyst. The contour is an ellipse, which indicates that the interaction among the factors is significant; otherwise, the contours would be circular (i.e. the interaction is insignificant).[20] Apparently, the interaction between the temperature and catalyst is significant to the response. This result is in agreement with that predicted from the regression results.

Figure 2 shows that, regardless of whether the molar ratio and the catalyst were at low or high levels, the change in phosphorus content declined after an initial increase. Apparently, the interaction between the molar ratio and the catalyst is significant to the response. This result is in agreement with that predicted from the regression results.

Figure 3 shows that the phosphorus content increased within the range of 60 °C to 70 °C and molar ratio of glucose to sodium tripolyphosphate was 1 to 1.5. The phosphorus content then decreased as the temperature

and molar ratio continued to increase. Apparently, the interaction between temperature and molar ratio is insignificant to the response. This result is in agreement with that predicted from the regression results.

The optimum preparation conditions (reaction temperature = 70 °C, molar ratio of glucose to sodium tripolyphosphate = 1.54:1, and catalyst amount = 3.7 g) to yield the maximum phosphorus content in the product were estimated using the model equation by solving the regression equation and analyzing the response surface contour plots. The predicted value from the fitted equations was 8.12% under the above conditions. The optimum extraction conditions were used to confirm the prediction based on the model. A mean value of phosphorus content in the product (8.34%) was obtained from 3 independent actual experiments, which confirmed that the response model was adequate for the optimization.

3.2. Ultraviolet spectroscopy analysis

A certain amount of the product and glucose were added in water to 100 mL, which made the concentration 1 g/L. UV-spectra of the product and glucose with distilled water as the blank solution are given in Figure 4. Figure 4 shows that the spectrum of the product exhibits obvious absorption in the 190 to 250 nm wavelength section with a maximum absorption peak at 193.21 nm, but there is no ultraviolet absorption for glucose. This suggests that something different from the material was formed.

3.3. Infrared spectroscopy analysis

The IR spectra of the product in the range of 400 cm^{-1} to 4000 cm^{-1} are given in Figure 5. The absorption band in the range of 3200 cm^{-1} to 3650 cm^{-1} is attributed to –OH. The spectra show the presence of hydroxyl groups, indicating that the hydroxyls of glucose have not been completely replaced. The peak at 1635.6 cm^{-1} is attributed to P–OH groups. An intense peak at 1043.5 cm^{-1} is attributed to P=O (1300 cm^{-1} to 960 cm^{-1}). Absorption peaks in the range of 940 cm^{-1} to 1100 cm^{-1} are attributed to the stretching vibration of P–O–C. These results suggest that glucose is phosphorylated, which coincides with the report by Li.[3]

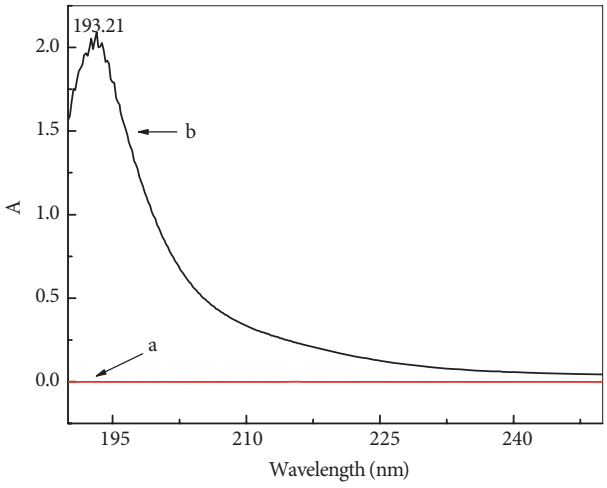


Figure 4. UV spectra of glucose and product a. glucose; b. product.

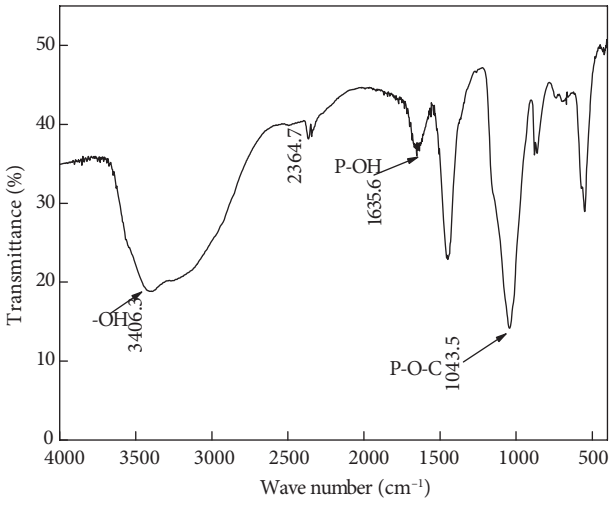


Figure 5. Infrared spectra of the product.

3.4. ^{1}H NMR spectroscopy analysis

The ^{1}H NMR spectrum of the product in D_2O (δ 4.70 ppm) is shown in Figure 6. The H-1, H-2, H-3, H-4, H-5, H-6, and H-6' signals of glucose 1-phosphate resonate at δ 5.08 ppm, 3.56 ppm, 3.72 ppm, 3.38 ppm, 3.84 ppm, 3.74 ppm, and 3.66 ppm, respectively. The spectra of the product showed a spectral pattern very similar to that of α-D-glucose-1-phosphate dipotassium salt in the Spectral Database for Organic Compounds (SDBS).

3.5. Electrical conductivity measurements

Figure 7 shows the effective electrical conductivity of glucose and glucose 1-phosphate at different molarities. It is seen that the electrical conductivity of glucose 1-phosphate increases with increasing molarities and it is more than 30 times that of glucose. The electricity conductivity is further improved, which shows the presence of glucose 1-phosphate anion on sodium glucose 1-phosphate. After the dissociation of glucose 1-phosphate anion bond, the electrical conductivity of glucose 1-phosphate in aqueous solution increases by a large margin.

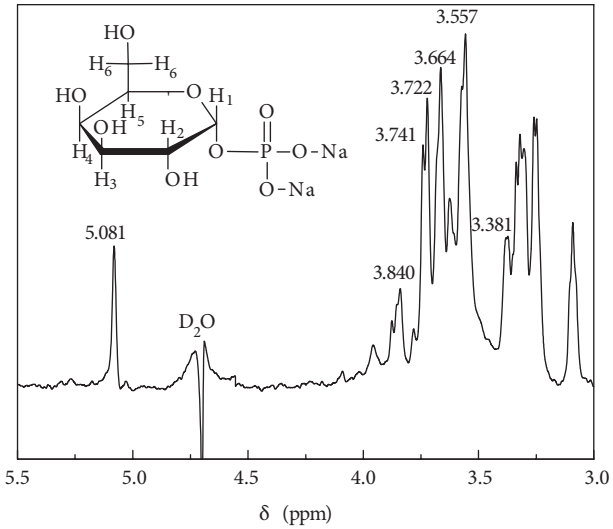


Figure 6. ^{1}H NMR spectrum of the product.

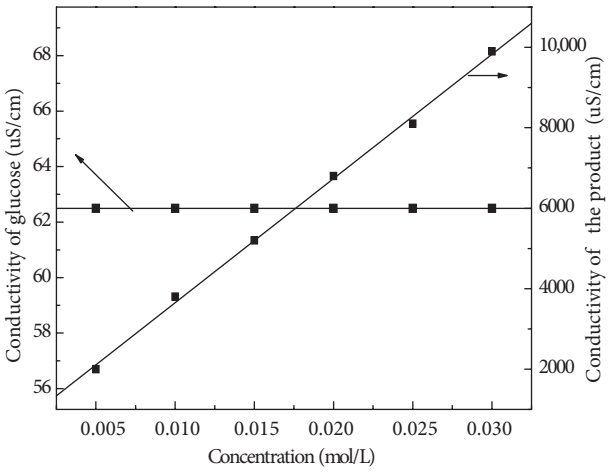


Figure 7. The conductivity of glucose and the product.

3.6. Thermal behavior

The TG thermograms of glucose and the product with a heating rate of 20 °C min^{-1} from 20 °C to 550 °C under air are presented in Figure 8. As can be seen, there was apparent weightlessness in the heating process of the samples. Glucose decomposes in a one-stage process, which starts at 192.3 °C, while the product starts to decompose at 211.09 °C. It shows that the product has higher decomposition temperature and better thermal stability than glucose.

Figure 9 represents the DTA curves of glucose and the product. The curve of glucose shows an endothermic peak in the range from 192.3 to 272.19 °C. There was an apparent weightlessness in the range from 192.3 to 272.19 °C. It corresponds to the decomposition of glucose. The endothermic peak of the product was in the range from 211 to 308 °C. It shows that the product has higher decomposition temperature and better thermal stability than glucose. It is consistent with the result of thermogravimetric analysis. Moreover, the exothermic peak appeared in the curve of glucose, probably because of crystal transformation. There was no exothermic peak in the curve of the product, indicating that the crystalline structure of glucose is destroyed completely.

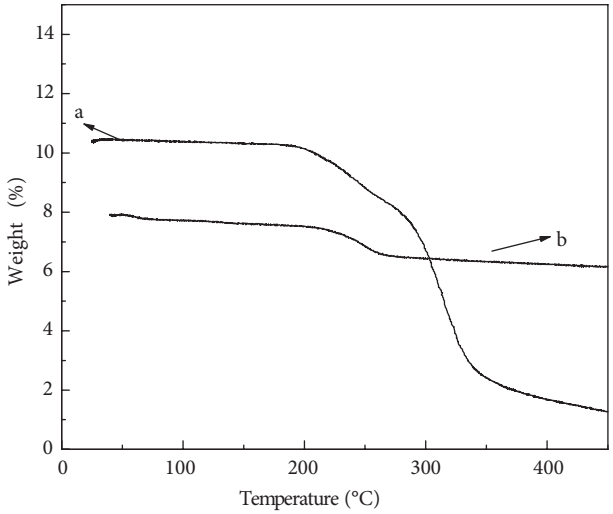


Figure 8. The TG thermograms of glucose and the product a. glucose; b. product.

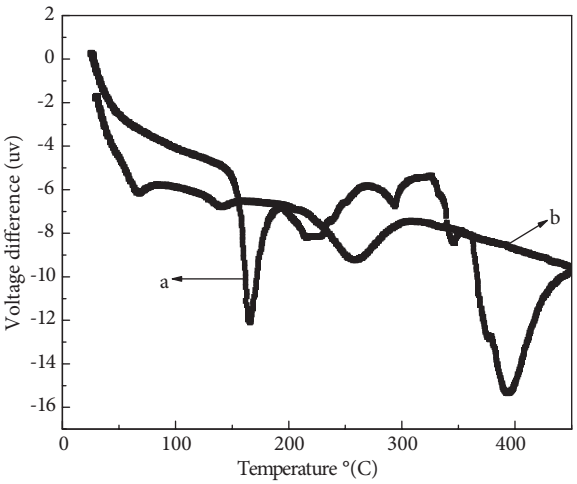


Figure 9. Differential thermal analysis curves of glucose and the product a. glucose; b. product.

4. Conclusions

Using Minitab14 software, we obtained the regression model of the relationship between phosphorus content and temperature, molar ratio, and catalyst. The model proved to be reasonably reliable. The optimized conditions include a reaction temperature of 70 °C, a molar ratio of glucose to sodium tripolyphosphate of 1.54:1, and a catalyst amount of 3.7 g. The phosphorus content of glucose 1-phosphate was 8.12%, coinciding well with the experimental value (8.34%) under optimized conditions. By analyzing the results of IR, UV, and ^{1}H NMR spectra; electrical conductivity; TG; and DTA, the structure of the product was conformed, and we discovered that glucose was phosphorylated during synthesis of glucose 1-phosphate. The proposed method provides a new approach for the synthesis of glucose 1-phosphate. The results obtained provide a reference for synthetic research on glucose 1-phosphate.

Acknowledgments

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