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Study for High Throughput Anthrone-Sulfuric Acid Method for Determination of Polysaccharides

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Abstract: A rapid, sensitive and high throughput micro-plate method was established to quantify polysaccharides in this study. Anthrone-sulfuric acid micro-plate assay in a certain reaction was tested. A single test and a response surface methodology was applied to design and optimize four factors including reaction time, incubation temperature, anthrone concentration and the amount of anthrone in order to maximize R^2 and minimize RSD in each standard curve which obtained in different conditions. It was demonstrated that the order of significant impacting R^2 is incubation temperature, anthrone concentration, reaction time and the quantity of anthrone add to while the order of significant impacting RSD is incubation temperature, anthrone concentration, the amount of anthrone and reaction time. Preferred reactive condition is be discovered and the best incubation temperature is 96°C, the best reaction time is 36 min, anthrone concentration is 1.1 mg mL^{-1} and the amount of anthrone is 150 µL. The detectable range of this method is 20-260 µg L^{-1} under the optimized condition. Recovery test showed high accuracy and practicability in this method by recovery rate ranged in 99.5-100.6%. The method was successfully applied to quantify polysaccharides of different source and the method has high resistance to interference of protein during measurement process.

Key words: Micro-plate, anthrone-sulfuric acid, polysaccharide, response surface methodology, recovery test

INTRODUCTION

There are many methods to measure the polysaccharides while the method mainly used is are visible absorption spectra based on reducibility of polysaccharides and reaction of furfural-condensation including 3,5-Dinitrosalicylic Acid Colorimetric Method (DNS Method), Nelson-Somogyi Method (Breuil and Saddler, 1985), Orcinol-Hydrochloric Acid (sulfuric acid) Method (Rouau and Surget, 1994), Phenol-Sulfuric Acid Method (Taylor, 1995) and Anthrone-Sulfuric Acid Method (Somani *et al*., 1987). But large amount of sulfuric acid, glucose solution and colour developing reagent are required, besides, the batch checks always takes a long time to do a few research and also the environment pollution is serious (Laurentin and Edwards, 2003).

Recently, the research on extraction, structure and activity of polysaccharides which include microbial polysaccharide, plant polysaccharide and animal polysaccharide grows increasingly (Ding *et al*., 2012). It is necessary to develop a more accurate, sensitive and high-throughput method to determinate polysaccharides for the rapid development of technology on extraction, structure and activity of polysaccharides, especially the method of gel retardation technology and ion-exchange technology's application in separation and purification of polysaccharide (Li *et al*., 2012; Dai *et al*., 2012; Dreywood, 1946). Recent years, Masuko *et al*. (2005) and Leyva *et al*. (2008) inquired the reaction conditions which influence the phenol-sulfuric acid method and anthrone-sulfuric acid method reaction proceeded in micro-plate (Masuko *et al*., 2005; Leyva *et al*., 2008).

Response surface design is an statistical approach which gets the data through experiments with the help of a reasonable experimental design method. By using the quadric multipleregression equation, the functional relationship between factors and response values can be fitted. The optimal parameter can be found through the analysis of the regression equation. And then, the multi-variable problem can be solved. It has been used widely in combinatorial optimization experiment in chemometry as one of the optimization methods (Khuri and Mukhopadhyay, 2010; Peng *et al*., 2013; Tarley *et al*., 2009).

For unlike, Masuko *et al*. (2005) and Leyva *et al*. (2008), this study optimized the determinative system and reactive condition of the Anthrone-Sulfuric Acid Method proceeded in micro-plate carried out by response surface methodology design and a high-through output method of determining polysaccharides was established. In the research, foreign substances which may impact on the method was studied and polysaccharides from different

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sources was determined to perfect the feasibility of the method. In the end, a quantitative detection method was established in polysaccharides researching with character of rapid sensitive accurate wide range of applied and anti-interference as well as less environmental pollution.

MATERIALS AND METHODS

Chemicals and pharmaceutical preparation and apparatus

Chemicals and pharmaceutical preparation: Glucose (2012.07.08, Tianjin Zhi Yuan Chemical Co., Ltd.), anthrone (2012.01.10, Tianjin Kemiou Chemicals Ltd.), sulfuric acid (2012.02.29, Lanzhou Three Dimensional Chemical Factory), Xylose (2011.04.16, Tianjin Zhi Yuan Chemical Co., Ltd.), fructose (2011.06.14, Tianjin Heaton Chemical Co., Ltd.), soluble starch (2011.08.09, Tianjin Zhi Yuan Chemical Co., Ltd.), sucrose (2011.06.17, Tianjin Fu Morning Chemical Co., Ltd.), Bovine Serum Albumin (BSA) (2012.09.08, Shanghai Snow Biotech Ltd.), Lysozyme (LZM) (2012.06.25, Global Science and Technology Co., Ltd.), GSH (2012.9.6, Tianjin Tian'an Pharmaceutical Co., Ltd.), Gly (2012.8.21, Tianjin Tian'an Pharmaceutical Co., Ltd.), Asp (2012.9.8, Tianjin Tian'an Pharmaceutical Co., Ltd.). All chemicals of analytical grade were used as supplied. Microbe Exocellular Polysaccharide (MEP), chitosan and Lycium Barbarum Polysaccharide (LBP) are all made in laboratory.

Apparatus: Electro-Thermostatic Water Bath (HH4, Changzhou GuoHua electric appliance limited company), Electronic balance (AB104-N, Mettle-Toledo Group), Micro-Plate Reader (Elx808, Gene-tech Ltd., USA) and Micro-Plates (Hi-tech laboratory equipment Ltd.,Beijing)

Experimental method

Operation of the method and evaluation index: Add glucose standard solution and anthrone-sulfuric acid to micro-plate and cover with lid, heat the micro-plate in electro-thermostatic water bath in certain temperature after cool it for 4 min at room temperature. At last put the micro-plate into micro-plate reader and run scan at 630 nm. The 5 replicates was read in each scan in this experiment, the values used in drawing standard curves are mean values of each five replicates. R^2 and Slope of standard curves are used to evaluate the result of the method, higher R^2 shows high correlation of the standard curves and high accuracy of the experiment, in contrast low R^2 means big errors in design of the method. Slope is sensitivity index, higher slope shows higher sensitivity of the corresponding method.

Single-factor experiment: Single-factor experiment reaction was carried out to search each best reactive condition which may have significant effect on accuracy of the method. Including reaction time (10, 15, 20, 25, 30, 35, 40 min), reaction temperature (88, 90, 92, 94, 96°C), concentration of anthron-sulfuric (0.5, 0.75, 1.0, 1.5, 2.0, 2.5 mg mL^{-1}) and addition of anthron-sulfuric (100, 125, 150, 175, 200 μL). R^2 and slope of each standard curve of assays are used as index to optimize the single factor.

Table 1: Levels and factors coding table of response surface method

	Levels		
Factors	-1	0	1
A: Reaction time (min)	30.00	35	40.00
B: Reaction temperature (°C)	92.00	94	96.00
C: Concentration of anthron-sulfuric (mg mL^{-1})	0.75	1	1.25
D: Addition of anthron-sulfuric (mL)	125.00	150	175.00

Response surface methodology design: According to the principle of Box-Behnken center combination design and results of single-factor experiment, a assay of 4 factors and 3 levels' response surface method was designed by choosing 4 factors which have significant effect on accuracy of the method including reaction time, reaction temperature, concentration of anthron-sulfuric and addition of anthron-sulfuric. Table 1 shows levels and factors coding table of response surface method.

The recovery experiment: The recovery experiment was carried out by justifying the accuracy of the optimized method. Fructose, xylose, sucrose and soluble starch were used in this part separately. The concentration of these four samples is 100 μg mL^{-1}, samples addition for 25 μL. The concentration of glucose standard solution is 200 μg mL^{-1}, sample addition for 25 μL and 5 groups parallel experiments. Reaction condition for heating 35 min under 96°C. The recovery rate results from the shown formula.

Determination of the content of polysaccharides of different source: Optimized method and traditional-anthrone method were used to determinate the contention of the micro-polysaccharides and plant-polysaccharides, respectively and also the detective results of their respective standard curve and Abs of both methods were compared. Exo-polysaccharides, chitosan and lycium barium solution were separated by the laboratory, diluted 10:1, detective addition for 50 μL (total 200 μL) by optimized method and 2.5 mL (total 10 mL) by Traditional-Anthrone Method.

Interference: In order to measure protein effect on determination of polysaccharides, a small amount of lysozyme, bovine serum albumin, glutathione, glycine and aspartic acid were add into experimental group for determination of the samples of soluble starch, sucrose and fructose. Calculate the recovery rate of polysaccharide in the presence of proteins.

Concentration of soluble starch, sucrose and fructose all are 200 μg mL^{-1}, quantity for 40 mL. Concentration of proteins for 50 μg mL^{-1} and addition for 10 mL.

RESULTS AND DISCUSSION

Single-factor experiment

The effect of reaction time on the method: Trends of R^2 and RSD different reaction time (10-40 min) shows as Table 1, 2 and Fig. 1. As is shown, with the heating time increasing, R^2 shows a rising trend while RSD goes down during all different temperature, indicate that linearity of calibration curve increased gradually and the parallel error decreases with the heating reaction going on. When the reaction was carried out by 35 min, there is a slight decline of R^2 and a slight increase in RSD, it may be because that there is carbonization of glucose happened when the temperature is too high (96°C), so in the charring or carbonization reaction solution, make the difference between parallel larger, linear variation. So, the final optimal reaction time chose as for 35 min.

The effect of reaction temperature on the method: Trends of R^2 and RSD different reaction temperature (88-96°C) shows in Table 3, 4 and Fig. 1. As is shown, with the heating temperature increasing, R^2 shows a rising trend while RSD goes down with the heating time goes on, it indicate that linearity of calibration curve increase gradually and the parallel error decreases in certain heating time with the heating temperature elevate. The optimum reaction temperature is 96°C.

Table 2: Response surface design and results

A: time (min)	B: Tem. (°C)	C: Con. (mg mL^{-1})	D: Addition Qua (μL)	R^2	Slope
30	92	1.0	150	0.9908	0.00701
40	92	1.0	150	0.9921	0.00810
30	96	1.0	150	0.9941	0.01052
40	96	1.0	150	0.9982	0.01071
35	94	0.5	125	0.9910	0.00712
35	94	1.5	125	0.9932	0.00951
35	94	0.5	175	0.9928	0.00686
35	94	1.5	175	0.9951	0.01182
30	94	1.0	125	0.9931	0.00897
40	94	1.0	125	0.9938	0.00981
30	94	1.0	175	0.9951	0.01007
40	94	1.0	175	0.9945	0.01122
35	92	0.5	150	0.9892	0.00766
35	96	0.5	150	0.9949	0.00822
35	92	1.5	150	0.9913	0.00934
35	96	1.5	150	0.9980	0.01294
30	94	0.5	150	0.9928	0.00508
40	94	0.5	150	0.9931	0.00561
30	94	1.5	150	0.9960	0.00953
40	94	1.5	150	0.9956	0.01103
35	92	1.0	125	0.9891	0.00790
35	96	1.0	125	0.9945	0.00981
35	92	1.0	175	0.9911	0.01013
35	96	1.0	175	0.9956	0.01201
35	94	1.0	150	0.9962	0.01016
35	94	1.0	150	0.9965	0.01014
35	94	1.0	150	0.9976	0.01110
35	94	1.0	150	0.9978	0.01009
35	94	1.0	150	0.9963	0.01021

Tem. = Temperature; Con. = Concentration

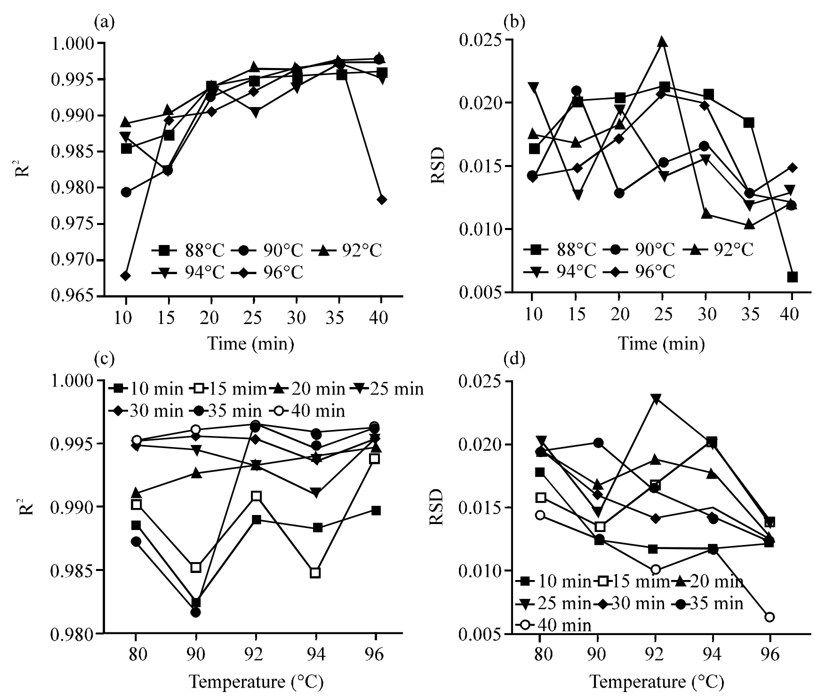

Fig. 1: a-d) R^2 in different temperature and reaction time comparison

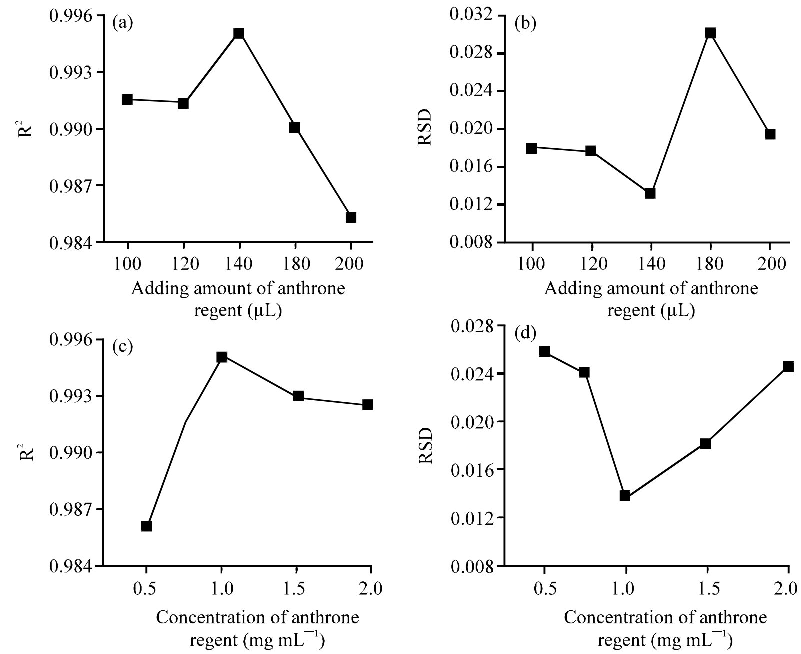

Fig. 2: a-d) R^2 in different addition and concentration of anthrone-sulfuric acid comparison

Table 3: R^2 variance analysis of regression model

Source	Sum of squares	df	Mean square	F-value	p-values	Prob.
Model	0.000171	14	1.22E-05	18.53342	<0.0001	**
A	2.43E-06	1	2.43E-06	3.678302	0.0758	-
B	8.37E-05	1	8.37E-05	126.7589	<0.0001	**
C	1.98E-05	1	1.98E-05	29.91585	<0.0001	**
D	7.52E-06	1	7.52E-06	11.38432	0.0045	*
AB	1.96E-06	1	1.96E-06	2.966861	0.1070	-
AC	1.22E-07	1	1.22E-07	0.185429	0.6733	-
AD	4.22E-07	1	4.22E-07	0.639540	0.4372	-
BC	2.5E-070	1	2.5E-07	0.378426	0.5483	-
BD	2.02E-07	1	2.02E-07	0.306525	0.5886	-
CD	2.5E-090	1	2.5E-09	0.003784	0.9518	-
A^2	4.49E-06	1	4.49E-06	6.791242	0.0207	*
B^2	2.91E-05	1	2.91E-05	44.09414	<0.0001	**
C^2	1.67E-05	1	1.67E-05	25.34558	0.0002	**
D^2	2.91E-05	1	2.91E-05	44.09414	<0.0001	**
Residual	9.25E-06	14	6.61E-07	-	-	-
Lack of fit	6.94E-06	10	6.94E-07	1.202917	0.4650	
Pure error	2.31E-06	4	5.77E-07	-	-	-
Cor total	0.000181	28	-	-	-	-

R^2 = 0.9846; R^2_{Adj} = 0.9281; **For very significant ($p<0.01$); *For significant ($0.01<p<0.05$)

The effect of addition of anthrone-sulfuric acid on the method: When R^2 and RSD of the curve get the maximum and minimum, respectively the addition of anthrone-sulfuric acid is 15 μL as reaction time for 35 min and reaction temperature for 96°C. Select the optimum amount of anthrone-sulfuric acid for 150 μL.

The effect of concentration of anthrone-sulfuric acid on the method: The higher the concentration of concentration of anthrone-sulfuric acid, the greater of Abs for detective result is, because more anthrone which as chromogenic agent in reaction result in deeper color results on reaction. But as is shown in Table 3, 4 and Fig. 2, R^2 of standard curves are lower and RSD are higher than the concentration of anthrone-sulfuric acid for 1 mg mL^{-1} when the concentration of anthrone-sulfuric acid is too low or too high. May be due to reaction is incomplete or reaction out of sync, resulting in the curves and poor stability When the concentration of anthrone is too low. While anthrone remained in reaction solution which affect the absorption of light by shielding effect when the concentration of anthrone is much higher. There with high R^2 as well as RSD in the group of high concentration of anthrone, so select the optimum concentration of anthrone-sulfuric acid for 1 mg mL^{-1} by comprehensive consideration.

Response surface optimization

Design and analysis of response surface: Using design expert 7.0 designs response surface experiment, reaction time (A), reaction temperature (B), concentration of anthrone-sulfuric acid (C) and Addition of anthrone-sulfuric acid (D) for the response variable, R^2 and Slope for response index. The 29 groups tests were designed table, of which the first 24 group is a factorial experiment and 5 group are 5 group are center trial. The 29 test points divided into factorial points and zero points, the value of factorial points that as variables in a vertex of three dimensional constructed by A-D; take zero point for the region's central point, repeat zero point test for 5 times in order to estimate experimental errors. Regression

Table 4: Slope variance analysis of regression model

Source	Sum of squares	df	Mean square	F-values	p-values	Prob.
Model	9.16E-05	14	6.54E-06	11.53749	<0.0001	**
A	2.34E-06	1	2.34E-06	4.126439	0.0617	-
B	1.65E-05	1	1.65E-05	29.08117	<0.0001	**
C	4.65E-05	1	4.65E-05	81.95652	<0.0001	**
D	6.74E-06	1	6.74E-06	11.87253	0.0039	*
AB	2.02E-07	1	2.02E-07	0.356969	0.5597	-
AC	2.35E-07	1	2.35E-07	0.414656	0.5300	-
AD	2.4E-08	1	2.4E-08	0.042351	0.8399	-
BC	2.31E-06	1	2.31E-06	4.072791	0.0632	-
BD	2.25E-10	1	2.25E-10	0.000397	0.9844	-
CD	1.65E-06	1	1.65E-06	2.910792	0.1101	-
A^2	5.49E-06	1	5.49E-06	9.678101	0.0077	*
B^2	4.55E-08	1	4.55E-08	0.080202	0.7812	-
C^2	1.08E-05	1	1.08E-05	18.95435	0.0007	**
D^2	4.47E-09	1	4.47E-09	0.007879	0.9305	-
Residual	7.94E-06	14	5.67E-07	-	-	-
Lack of fit	7.21E-06	10	7.21E-07	3.955292	0.0986	-
Pure error	7.29E-07	4	1.82E-07	-	-	
Cor total	9.96E-05	28	-	-	-	-

R^2 = 0.9202; R^2_{Adj} = 0.8405; **For very significant ($p<0.01$); *For significant ($0.01<p<0.05$)

analysis data obtained by Design-Expert Software and the results are shown in Table 3 and 4. Using software to perform fitting of nonlinear regression quadratic polynomial, the forecasting model are as follows:

$$R^2 = 1.00+4.500E\text{-}004\times A+2.642E\text{-}003\times B+1.283E\text{-}003\times C+7.917E\text{-}004\times D+7.000E\text{-}004\times AB-1.750E\text{-}004\times AC-3.250E\text{-}004\times AD+2.500E\text{-}004\times BC-2.250E\text{-}004\times BD+2.500E\text{-}005\times CD-8.317E\text{-}004\times A^2-2.119E\text{-}003\times B^2-1.607E\text{-}003\times C^2-2.119E\text{-}003\times D^2 \quad (1)$$

$$Slope = 0.010+4.975E\text{-}004\times A+9.475E\text{-}004\times B+1.838E\text{-}003\times C+5.125E\text{-}004\times D-2.250E\text{-}004\times AB+2.425E\text{-}004\times AC+7.750E\text{-}005\times AD+7.600E\text{-}004\times BC-7.500E\text{-}006\times BD+6.425E\text{-}004\times CD-9.200E\text{-}004\times A^2-8.375E\text{-}005\times B^2-1.288E\text{-}003\times C^2+2.625E\text{-}005\times D^2+5.825E\text{-}004\times A^2B+6.300E\text{-}004\times A^2C+1.150E\text{-}004\times A^2\times D-1.775E\text{-}004\times AB^2+1.000E\text{-}005\times A\times C^2-2.375E\text{-}004\times B^2C+5.950E\text{-}004\times B^2D+9.250E\text{-}005\times BC^2 \quad (2)$$

Variance results and the analysis of R^2 return model shows in Table 3, significance analysis of return variance suggests that the regression model is significant ($p<0.0001$) and lack of fit out of significance, in the model R^2 = 0.9846 and R^2Adj = 0.9281 which indicated that the model fit better with actual experiment and linear relationships are significant between variables and response values, the model can be used to predict linearity standard curve of the method.

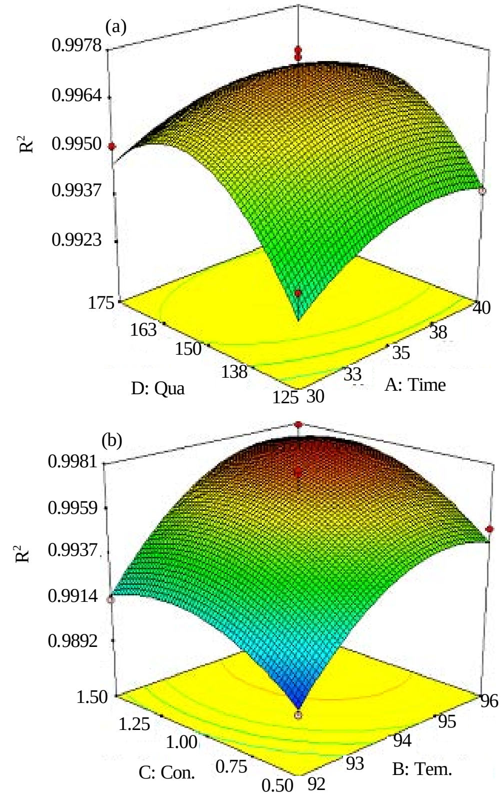

Fig. 3: Interaction among various factors effect on the linearity of standard curve; a) representing A and D interaction of R^2; b) represent B and C interaction of R^2

Variance results and the analysis of SLOPE return model shows in Table 4, significance analysis of return variance suggests that the regression model is significant ($p<0.0001$) and lack of fit out of significance, in the model R^2 = 0.9266 and R^2Adj = 0.8532 which indicated that the model fit better with actual experiment and linear relationships are significant between variables and response values, the model can be used to predict linearity standard curve of the method.

The interaction of factors: As is shown in Table 2, B and C in the model have very significant response to the value of R^2 ($p<0.01$), followed by D ($p<0.05$). R^2 are affected with quadratic B^2, C^2 and D^2 significantly and ($p<0.01$); A^2 for sinnificant ($p<0.05$). A number of more significant interaction effects on R^2 were shows in Fig. 3, combined with the data in Table 2, researchers can see that interaction effects on the R^2 all are not significant. Larger F-value represent more significant influence the factor have to the response value, so according to F-value in Table 2, the order of factor which significantly influence

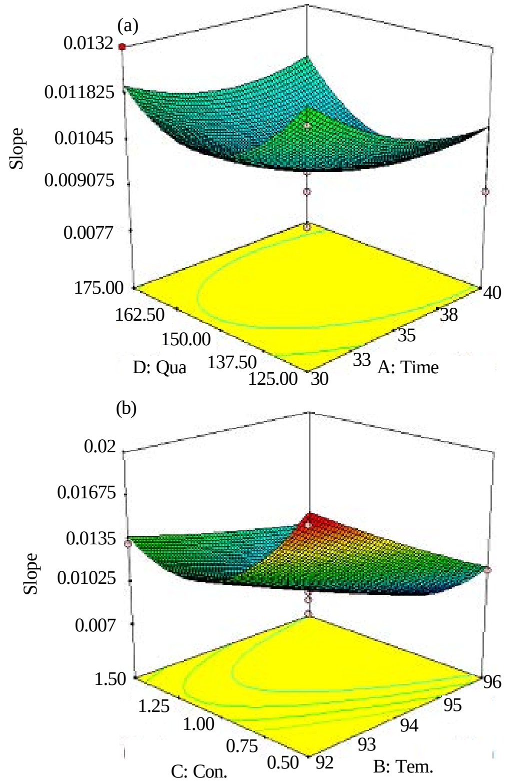

Fig. 4: Interaction among various factors effect on the linearity of standard curve; a) representing A and D interaction of slope; b) represent B and C interaction of slope

linearity of standard curve in the method is reaction temperature (B), concentration of anthrone-sulfuric acid (C), addition of anthrone-sulfuric acid (D) and reaction time (A).

As is shown in Table 3, B and C in the model have very significant response to the value of R^2 ($p<0.01$), followed by D ($p<0.05$). Slope are affected with quadratic C^2 significantly and ($p<0.01$); A^2 for significant ($p<0.05$). A number of more significant interaction effects on Slope were shows in Fig. 4, combined with the data in Table 3, researchers can see that interaction effects on the slope all are not significant. Larger F-value represent more significant influence the factor have to the response value, so according to F-value in Table 3, the order of factor which significantly influence sensitivity of standard curve in the method is concentration of anthrone-sulfuric acid (C), reaction temperature (B), addition of anthrone-sulfuric acid (D) and reaction time (A).

Determination of optimum reaction conditions: To further determine the optimum, choose a starting point within the range in concentration of the model use rapidly rising way

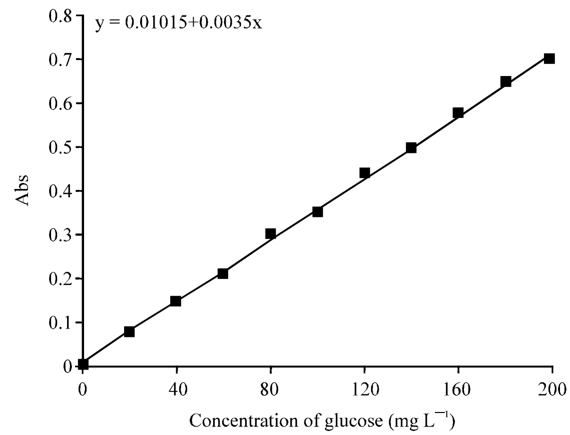

Fig. 5: Standard curve of glucose

to optimize best reaction conditions for determining the content of polysaccharides by anthrone-sulfuric acid method. When the R^2 reached maximum in ①, the reaction temperature is 95.36°C, the reaction time was 36.32 min, concentration of anthrone-sulfuric acid is 1.16 mg mL^{-1} and the addition amount of anthrone-sulfuric acid is 152.64 μL, R^2 of the standard curve got in the condition is 0.9986.

When the RSD reached maximum in ②, the reaction temperature is 95.66°C, the reaction time was 37.12 min, concentration of anthrone-sulfuric acid is 1.06 mg mL^{-1} and the addition amount of anthrone-sulfuric acid is 149.87 μL, RSD of the standard curve got in the condition is 0.00747395.

Incorporating the R^2 and RSD, the final selection criteria for the reaction temperature is 96°C, the reaction time is 36 min, concentration of anthrone-sulfuric acid is 1.1 mg mL^{-1}, addition of anthrone-sulfuric acid is 150 μL, the result of the above conditions was verified by 3 times repeat test, measured R^2 is 0.9991, 0.9994 and 0.9987, respectively, mean value is 0.9991; RSD is 0.00821, 0.00792 and 0.00735, respectively. The relative error of R^2 and RSD is 0.05 and 0.47%, respectively compare mean value with the theoretical prediction. Indicate that the regression equation which optimized by response surface methodology has a certain practical guiding significance. Standard curve obtained in new conditions as shown in Fig. 5. The R^2 is 0.9988, RSD is 0.00802.

Standard recovery experiment: Recovery experiment of monosaccharides and polysaccharides was carried out by the optimized method, the experimental results shows as Table 5.

The recovery rate was calculate between 99-101% that show the standard curve is more accurate after optimized by response surface methodology, the method

Table 5: Standard recovery experiment and result

Standard sample	Fructose	Xylose	Sucrose	Soluble starch
Amount of addition (μg)	2.5	2.5	2.5	2.5
Content of glucose (μg)	5	5	5	5
Abs	0.5359	0.5346	0.5361	0.53625
Measured content (μg)	7.533	7.476	7.542	7.547
Recovery rate (%)	100.44	99.68	100.56	100.63

Table 6: Determination of different sources polysaccharide content

Sample under test	MEP	Chitosan	LBP
In micro-plate (μg)	99.68	80.26	121.35
In colorimetric tube (μg)	98.56	79.89	122.52

Table 7: Protein interference to the determination of polysaccharides

Sample under test (8 μg)	Protein (0.5 μg)	Abs	Measured content (μg)	Response rate (%)
Xylose	BSA	0.56735	7.96	99.50
	LZM	0.57225	8.03	100.38
	GSH	0.56875	7.98	99.75
	Gly	0.56735	7.96	99.50
	Asp	0.57155	8.02	100.25
Sucrose	BSA	0.56875	7.98	99.75
	LZM	0.57225	8.03	100.38
	GSH	0.57575	8.08	101.00
	Gly	0.57505	8.07	100.88
	Asp	0.56735	7.96	99.50
Soluble starch	BSA	0.56595	7.94	99.25
	LZM	0.56735	7.96	99.50
	GSH	0.57085	8.01	100.13
	Gly	0.57225	8.03	100.38
	Asp	0.56945	7.99	99.88

can be used to determination the content of polysaccharides rapidly in high yield and it has a certain significance on practical guiding.

The determination of polysaccharide from different sources: The result of determination different sources of polysaccharides, they include Microbe Exocellular Polysaccharide (MEP), chitosan and Lycium Barbarum Polysaccharide (LBP) by method of anthrone-sulfuric acid proceed in micro-plate to compare with in colorimetric tube is shown in Table 6. It can be seen that there is little difference by determination with two methods and the former is more accurate than the latter.

Protein interference to the determination of method: The result of Bovine Serum Albumin (BSA), Lysozyme (LZM), Glutathione (GSH), Glycine (Gly) and Aspartic Acid (Asp) produced interference to determinate content of xylose, sucrose and soluble starch are shown in Table 7. It is observed that sugar recovery rate is very high with the presence of protein which betweens in 99-101% on average. It shows that proteins do not produce interference on determination of sugar content in this method.

CONCLUSION

By single factor experiment design and response surface analysis, optimum reaction conditions of determining polysaccharides in micro-plate were chose as: reaction time is 36 min, reaction temperature is 96°C, concentration of anthrone-sulfuric acid is 1.1 mg mL^{-1} and addition of anthrone-sulfuric acid is 150 μL.

The determined method has extensive applicability to determinate content of polysaccharides from different sources. Meanwhile, the method is also feasible for determination of polysaccharides which containing protein samples, the protein in the sample are almost not influence the determination of polysaccharides content by anthrone-sulfuric method proceed in micro-plate.

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