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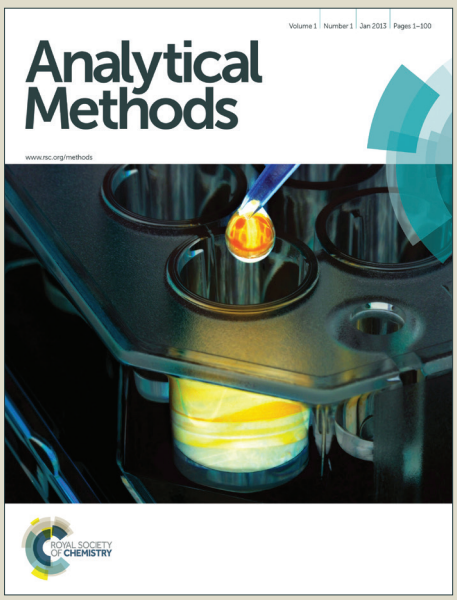

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A novel digital color analysis method for rapid glucose detection

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■ **Abstract**

Spectrophotometer is the most used analysis equipment in traditional colorimetric methods. However, the operation using spectrophotometer is time-consuming and labor-intensive, which presents practical difficulties in rapid detection. For this end, we presented a digital color analysis method with the typical 3, 5-Dinitrosalicylic acid (DNS) method for glucose detection as example. Primary colors from 3 color spaces (Red-Green-Blue, Hue-Saturation-Value, Hue-Saturation-Intensity) were studied as quantitative analytical parameter for glucose concentration and the Red color (from Red-Green-Blue colorspace) of the assay image provides superior prediction precision (>99.8%). Combine with color analysis, two calculation algorithms, nonlinear regression and artificial neural networks, were compared for the detection of high concentration glucose. Then a microtiter plate (48-well plate) platform based on color analysis was set up. Compared to existing methods using spectrophotometer, digital color analysis method owns large detection range (0-10 g/L), high accuracy (0.07 g/L) and fast detection rate (150 samples detected within about 15 min). It also shows great promise for use in a variety of reducing sugar measurements such as xylose, fructose and maltose. These aforementioned features render this newly developed method highly suitable for quick detection applications.

Key words: Spectrophotometer, Glucose detection, Microtiter plate platform, Digital color analysis, Rapid detection.

■ Introduction

Quantifying the concentration of glucose solutions is vital in medical diagnosis[1] , food analysis [2], processing optimization applications [3] . This put an extremely high demand on the detection accuracy and speed [4, 5]. Hence, development of cheap, fast, and robust standard analysis techniques are of great importance. Nowadays, the colorimetric methods (such as the Anthrone method, Phenol-Sulfuric Acid method and 3, 5-Dinitrosalicylic acid method) are still widely used because of simplicity and low cost. However, the measurement process was carried out in spectrophotometer, the low throughput of which presents practical difficulties of usage in quick detection.

Color is a 3-dimensional psychophysical phenomenon [6]. By defining a color space, color information can be transformed into numerical values, color library data, etc., that can be treated as analytical information [7]. By treating colors as digital information, not only the "colors" themselves can be made use of, but algorithms also can be applied to mine the numerical values converted from color information [8]. With the help of camera and data processing software (such as Matlab, R, etc.), the image of thousands of samples can be recorded within seconds and rapidly transform into measurement report on a personal computer. Until now the color digital analysis have found applications in the chemical medicine, biological, drug and food analysis fields[1, 9-12]. Compared to conventional methods, digital color analysis have many fundamental advantages, including (1) a higher throughput; (2) a shorter analysis time; (3) an improved performance and reliability; (4) the potential for in situ operation; (5) the potential for real-time analysis. Furthermore, the digitized color data are easily

integrated with automated algorithms to achieve the automated operation.

In the present paper, we attempted to set up a digital color analysis method to substitute the use of spectrophotometer in colorimetric glucose detection. The suitable calculation algorithm for glucose detection using color information was investigated. Experimental tests are also performed to compare the accuracy and efficiency of the proposed method with that of the traditional DNS and High Performance Liquid Chromatography (HPLC) method. The feasibility for other reducing sugar detection also was investigated. It is expected to open a new and effective way in the efforts for material measurement and biochemical analysis.

■ Experimental

Chemicals

3, 5-Dinitrosalicylic acid, NaCl, sodium potassium tartrate, Na_2SO_4, phenol and glucose were all of analytical reagent grade and pursued from Kepujia Experiment Reagent Co., Ltd (Beijing, China). Water was obtained from a Milli-Q purification system (Millipore). The DNS solvent was prepared as following: 6.3 g DNS and 262 mL of 2 mol / L NaCl were added into 500 mL hot water containing 182 g sodium potassium tartrate. And then 5 g phenol and 5 g Na_2SO_4 were supplemented into the solvent and solved by mixing. After cooled, the mixture was diluted with water to 1000 mL and kept in cool shielded from light for seven days before usage to keep phenol from photo oxidation and remove flocculent precipitate.

The traditional DNS method

In the traditional DNS method, 0.5 mL of the standard glucose solvent with the concentrations of 0 ~ 3 g/L was added into the colorimetric tubes and diluted with water to 2 m L. Then 2 mL DNS broth was added and kept in the boiling water bath for 2 minutes. After cooled in the running tap water, the solution was diluted to 10 mL using water and scanned for the absorption spectrum against a blank solution within 3 minutes using a spectrometer (UV2550, Shimadzu, Japan) at OD_{540}. The experimental procedure of the traditional DNS method was shown in Figure 1a.

Digital Image Processing method

Color development recording

The working procedure of digital image processing method for measurement was shown in Fig. 1b. In this method, glucose samples were detected in 48 - well microtiter plate (as shown in Fig. 2a). Each well of microtiter was loaded with 1 mL water in advance. 100 μL of samples and 100 μL DNS were transferred into 48 - well microtiter plate and mixed using pipette (A0632010, Eppendorf, Germany). The final volume of each well is 1.2 mL. Then the plate was placed in thermostat heater (MT70-2, Hangzhou Aipu Instrument & equipment Co.,LTD, China) at 100 °C for color development. The image of samples was taken by digital camera (TP614000B, Hangzhou ToupTek Photonics Co., Ltd, China) after or during color development. To avoid impact from the environment, the images were taken in a special box equipped with light and microwell plate heater (WS-350P/WS-350B, Shinetek Instruments

CO.LTD, Beijing, China).

Before delving into the work, we shall briefly describe the definitions and differences between the color models we considered.

RGB

Color in the RGB system is produced by any additive or subtractive mixture of the spectra of the three primary colors red (R), green (G) and blue (B). Their corresponding monochromatic primary stimuli occur at 700, 546 and 436 nm, respectively. On a 8-bit digital system color is quantified by numeric tristimulus R, G, B values that range from 0 (darkness) to 255 (whiteness)[13]. The three colors were 3 color matrixes[7]. In our research, the color value is the average value of the corresponding color matrix, which was analyzed by ***Equation 1***.

$$\mathrm{R} = \frac{\sum_0^j \sum_0^i R(x,y)\ from\ R\ color\ matrix\ in\ RGB\ color\ space}{j \times i} \qquad (1)$$

Where, ***i*** and ***j*** are the row and column numbers of the ***R*** color matrix. The G and B (from RGB color space) and primary colors from HSV and HSI were also calculated in the same way. The calculation processing was carried out in Matlab 2014a (Mathworks, USA) with the Function *mean2* from the toolbox of Image Processing Toolbox.

HSV

HSV (hue-saturation-value) and HSI (hue-saturation-intensive) are the two most common cylindrical-coordinate representations of points in an RGB color model. HSV describes colors (hue or tint) in terms of their hue, shade (saturation) and their brightness (value). It was the firstly used to describe colors by Alvy Ray Smith in

1978. Comparing with RGB system, HSV is a very intuitive way to represent colors in some applications, such as object tracking, human face detection etc. In the calculation processing, HSV are firstly converted from RGB followed the method of Ford and Adrian proposed [14], which is achieved in Matlab 2014a (Mathworks, USA) with the Function *rgb2hsv* from the toolbox of Image Processing Toolbox. Then the average values of the corresponding color matrix (HSV) were calculated with Function *mean2.*

HSI

The HSI model defines a color model in terms of its components, which refers to the hue, saturation and color intensive. This space has the ability to separate the intensity of the intrinsic information of color; therefor, it is suitable for processing images that present lighting changes. Hue and Saturation in HSI model are a bit different from those in HSV model because of the differences on conversion equations. In the calculation processing, HSI are firstly converted from RGB followed the method of Ford and Adrian proposed [14]. Then the average values of the corresponding color matrix (HSI) were calculated with Function *mean2.*

All the parameters used for color description are dimensionless. The scale of parameters are summarized as following: RGB model: R, 0-255; G, 0-255; B: 0-255; HSV model: H, 0.0-1.0; S, 0.0-1.0; V, 0.0-1.0; HSI model, H, 0.0-1.0; S, 0.0-1.0; V, 0.0-1.0;

Based on our research, The R^2 (correlation coefficient) between primary colors and glucose are: a) RGB color space: R, 99.8%; G,51.3%; B, 61.6%; b) HSV color space:

H, 56.9%; S, 92.3%; V, 82.3%; HSI color space: H, 60.1%; S, 93.4%; I, 67.9%.

The setup of calculation algorithms

Nonlinear fit method

Fig. 3 illustrates the colorimetric reaction between DNS reagent and glucose. As shown, it is the formation of 3-amino, 5-nitro salicylic acid that leads to the color development. According to ***Law of mass action***, the formation rate of 3-amino, 5-nitro salicylic acid was controlled by the concentration of glucose and DNS. Assuming the reaction obeys a second order reaction kinetics, the generation rate of 3-amino, 5-nitro salicylic acid can be determined in accordance with **Equation 2**.

$$\frac{\mathrm{dc}}{\mathrm{dt}} = k(x_0 - c)(c_0 - c) \qquad (2)$$

Where,

c is the concentration of 3-amino, 5-nitro salicylic acid produced (mol/L); x_0 is the initial concentration of glucose before reaction occurred（mol/L); c_0 is the initial concentration of DNS before reaction occurred(which is 0.0276 mol/L in our research). t is the reaction time. k is reaction rate constant calculated by ***Arrhenius formula*** [15] **(Equation 3*);***

$$\mathrm{k} = k_0 e^{-E_0/RT} (3)$$

Where, k is reaction rate constant, $\mathrm{k_0}$ is the frequency factor ($\mathrm{min^{-1}}$), E_0 is the activation energy (kJ/mol); R is the universal gas constant, R=8.314 J/(mol/K); T is the reaction temperature, which is set at 100 ºC throughout our study. It should be noted that k is a fixed value in a specified reaction under a stable temperature.

Therefore, by integrating Equation 3, the formation of 3-amino, 5-nitro salicylic

acid (also the glucose consumption kinetic equation) was achieved as **Equation 4.**

$$c = -\frac{c_0 - x_0 e^{(c_0 - x_0)(c_3 + kt)}}{e^{(c_0 - x_0)(c_3 + kt)} - 1} \quad (4)$$

Where,

$c_3 = \frac{Ln(\frac{c_0}{x_0})}{c_0 - x_0} (Calculated\ from\ C_{t=0} = 0)$;

Our experiments proved that this kinetics model also applies to other reducing sugars including xylose, fructose, and maltose except different values on k.

Artificial Neural Networks (ANNs)

Artificial Neural Networks was constructed followed the method of D. Ozyurek [16] as shown in Fig. 6a and Fig. 6b. The structure parameters were listed in Electronic Appendix 1. 100 samples used for ANNs construction were divided into training set and testing set. The training set (including input_train and output_train) contains 80% of the total data. Input_train consist of R values of the detected sample at 0.2， 0.4， 0.6， 1, 1.5, 2.0， 3.0, 4.0， 5.0， 6.0, and 9.0 minutes. The glucose concentration (output_train) is the output. The testing set (input_test and output_test) consists 20% of the rest data with the same set parameters as well.

All these were realized in Matlab 2014a (Mathworks, USA) with the toolbox of Neural Network Toolbox. Program code with brief explanatory note was given in Electronic Appendix 2.

Measurement of xylose, fructose, and maltose by HPLC

Sugars were determined by HPLC was according to the method described by Jahanbin[17] with small modification: the high performance liquid chromatography

(HPLC) (Agilent 1200, American) was equipped with a RI detector (G1362A) and Aminex HPX-87H column. 5 mM H_2SO_4 was set as the mobile phase at 65 °C at the rate of 0.5 mL/min. Sugars were identified according to their retention times by comparing with sugar standards. The sugar concentration was calculated by using the calibration curve of each sugar.

Result and Discussion

The relationship between primary colors and glucose concentration in DNS method

Color is commonly measured using spectrophotometer in the colorimetric methods. However, color can be broken up into different primary colors in different color spaces. Different color models are suitable for different purposes, including descriptive purpose, analysis purpose and so on [13]. The best suited color model(s) can provide best result. Therefore, to determine the best suited model for quantitative description of DNS method, the images of reacted broth with different glucose concentrations (0,0.5,1,...2.5 g/L) was analyzed in different color models (RGB, HSV, HSI). The correlationship analysis between primary color values and glucose was given in Fig. 2.

The correlation coefficients range from 51.3% to 99.8% and the best result was achieved by R from the RGB color space (99.8%). A.S. Raja etc. also found that RGB data of color image of the assay is suitable for blood glucose measurement [1]. As the comparison group, R^2 (correlation coefficient) of the traditional method was 99.6% (data not shown). It means that the accuracy of the concentration measurements

obtained using R (RGB color space) is comparable to that of the measurements obtained using the traditional method. Furthermore, this parameter is stable, simple to calculate, easily obtained from commercial devices such as scanners and digital cameras.

Fit process is conducted on R value and glucose concentration. The result was given in Equation 2.

$$R = 150 - 60 * Glu \quad (5)$$

Where R is the R (or red) value (from RGB color space) of the reacted broth image and Glu is the glucose concentration of the samples for detection (g/L).

By converting Glu into the true molar concentration in the mixture, Equation 2 can be written as following:

$$R=150-60*180 *c \quad (6)$$

Where; c is molar concentration of glucose sample for measurement (mol/L); 180 is the molecular weight of glucose (g/mol).

The calculation algorithm for direct calculation of high concentration glucose by color analysis

Obviously, samples with glucose less than 2.5 g/L can be directly calculated by digital color analysis method (Equation 3). However, R value of any sample with glucose more than 2.5 g/L fall to zero if the reaction proceed completely. In other word, the digital color analysis method can only measure the samples with glucose less than 2.5 g/L in this case. However, how to expand the measurement range seems worthy studying. It is because that large detection range can not only liberate the operators

from the time-consuming dilution operations, but also avoid some possible human bias in measuring. Large detection range will bring convenience to many bio-analysis applications, such as strain screening [18], fast sugar quantification in food [2], process monitoring [19, 20], etc.

To extend the detection range of color analysis, there are two protocols in the toolbox: nonlinear fit based on color development dynamics and artificial neural network trained with the experiment data of R values and glucose. Therefore, the goal of the following part was to find the suitable strategy for direct detection of high concentration glucose.

Nonlinear fit based on color development dynamics for direct detection of high concentration glucose

Color development dynamics models are widely employed for chemical measurement[7, 21]. Conventionally, to obtain a sufficiently accurate and robust mathematical model for color development process is a time-consuming and harsh task due to the exceeding complexity in the accurate recording of the colors and glucose concentration. However, with the help of digital camera and Equation 6, this can easily be achieved by: 1) recording the color development process; 2) extracting the color values from the images; 3) fitting the data to the kinetic equation.

The kinetic equation for the color development was deduced in ***Experimental***. The color development kinetics (Equation 7) can be achieved finally by combining Equation 4 and Equation 6.

$$R = 150 + 60 * 180 * n * \frac{c_0 - x_0 e^{(c_0 - x_0)(c_3 + kt)}}{e^{(c_0 - x_0)(c_3 + kt)} - 1} \quad (7)$$

Where,

n is the dilute factor, which is 12 in our study. $c_3 = \frac{Ln(\frac{c_0}{x_0})}{c_0 - x_0} (Calculated\ from\ C_{t=0} = 0)$; k was calculated according to ***Arrhenius Equation*** [15] (Equation 3). Because the operation is set at 100 °C throughout our study, k is a fixed value.

To determine the parameter k, we recorded the dynamic process of color development of 2.5 g/L glucose sample at 100 ºC (as shown in Fig. 4). The values of k was determined to be 18.49 $(mol \cdot min)^{-1}$ by fitting the experimental data to Equation (7) in Matlab 2014a (Mathworks, USA) with the toolbox of non-linear regression toolbox. Therefore, the kinetic curves for different concentration of glucose were given in Fig. 5 (shown by the red lines).

For verifying the accuracy of the calculation result, comparison between the measured data of difference concentration glucose samples and the predicted data by kinetic equation above were compared in Fig. 5. All the correlation coefficients between the measured and predicted are above 93%, validating the high accuracy and validity of our proposed model.

So, basing on the color development dynamics achieved, calculation of glucose by nonlinear fit method can be achieved by: (1) recoding the image at several time points; (2) fitting the data of R values (R>0) from different time point to Equation 7. With the help of commercial digital and data analysis software (Matlab, R, and Origin), the image recording and nonlinear fit process can be carried out automatically.

To determine the accuracy of nonlinear fit method, 20 samples were used for evaluation. R data from night time points (0.5， 1, 1.5, 2.0， 3.0, 4.0， 5.0， 6.0, and

9.0) of each sample were used for nonlinear fit. The results were given in Electronic Appendix 3. R^2 of the measured values and true values was 91.3%. Measurement error gets larger with the increase of glucose concentration detected, and the maximum error (10.9%) was observed in the detection of samples containing 20 g/L glucose.

Artificial neural networks for direct detection of high concentration glucose

Calculation tasks can be handled with artificial neural network (ANN) techniques without any a priori knowledge requirements on the interdependencies of the process variables and, thus, offering a direct approach[22]. Artificial neural networks have the capability to learn from known input/output vector pairs through iterative training, and to handle highly non-linear problems[23]. By using ANNs trained with experimental R data at specified given time point, it is possible to obtain the calculation results with high accuracy.

In our experiments, the specified point-in-time was set at 0.2， 0.4，0.6， 1, 1.5, 2.0， 3.0, 4.0， 5.0， 6.0, and 9.0 min respectively (Fig. 6a). The neural network model architecture was shown in Fig. 6b. The data of 100 samples were given in Electronic Appendix 4. 80 samples were used for training and the rest 20 were used for testing. Less than 200 iterations were needed for a proper learning. The detection result was given in Fig. 6c and Fig. 6d. R^2 was 99.71% and the largest error was 4.7%, implying that empirical models derived from ANN can be used adequately to detect the high concentration glucose (>2.5 g/L).

The comparison of the three calculation algorithms in this paper was summarized in Table 1. (1) Linear fit algorithm is the easiest one, which is also the widely employed one in the tradition colorimetric methods. However, it can only be used for the measurement of low concentration glucose (0 ~ 2.5 g/L). (2) Nonlinear fit method can apply to higher concentration glucose (0~ 10 g/L) with higher accuracy. However, it must base on the knowledge of color development dynamics. Before application, it is required to do sufficient preliminary experiments to set up the suitable kinetic models. This disadvantage hinders its application in other reducing sugar detection. (3) ANNs exhibits obvious advantages on practicality, accuracy and calculation robustness. The training of ANNs is easy and does not require *priori* process knowledge, making ANNs process great potential for other sugar detection.

Rapid detection of glucose on 48-well plate by color analysis

Based on the studies above, we designed the quick and high-throughput measurement method shown in Fig. 1. The method includes 3 main steps: 1). Mix the glucose solution sample with DNS in the 48 - well microtiter plate and get them to react for the corresponding optimal reaction time under appropriate temperature (usually 100 ℃). 2). Extract the R value of the reacted broth image by image processing .3) Calculate the glucose concentration by pre-trained ANNs model (Readers also can used linear relationship between glucose and R value as shown in Equation 5 or 6 for glucose detection. In this case, the dilution process is needed for samples with high concentration glucose).

Experimental tests were performed to compare the accuracy of the digital color analysis method with the traditional DNS method and HPLC method. Results were given in Table 2. The detection range of the traditional DNS method was only 0 - 3 g/L. When the glucose concentration surpasses 3 g/L, the detection error increases sharply. Meanwhile, the colorimetric method was determined by a spectrophotometer, and samples should be treated one by one, which was time-consuming and labor intensive. HPLC was the most accurate and robust method. Both the maximum error and the variable coefficient are within 5% in the range of 0-10 g/L. However, the measurement of one sample needs at least 15 minutes, which hinder its application in high-throughput screening. Thought not as exact as HPLC, the digital color analysis method can still well meet the requirement of daily analytical work: the maximum measurements errors of 150 samples were less than 9%. Most importantly, our proposed method owns significant advantage on throughput and speed. The speed of the digital color analysis method is 150- and 10- fold of the HPLC and DNS method (shown in Fig. 7). This advanced feature renders this newly developed assay method highly suitable for applications in glucose-related research.

Expanding the application of the digital color analysis method for other reducing sugar detection

In order to expand the application of digital color analysis method for other reducing sugar/materials detection, samples of xylose, fructose, maltose and ascorbic acid with known concentrations were used for evaluation. The experiment results were shown in Table 3 and Table 4. The measurement process of ascorbic acid was

given in Appendix 5.

Validation experiments showed that the digital color analysis method also apply well to xylose, fructose, maltose and ascorbic acid. The maximum error of all the experiments was less than 10%, and detection results for 150 samples are available within 15 minutes. Most importantly, there is no requirement on color development dynamics knowledge of the sugars detected, making the proposed method easy to be implemented. Comparing with the existing methods, its rapidity and simplicity makes it a very promising alternative for the quick detection of reducing sugars.

In order to make sure whether the method is suitable for measurement of sugar mixture, we carried out the validation experiments of glucose-xylose mixture in Appendix 6. Results showed that when used for the measurement of sugar mixture, its measurement accuracy decreases (the measurement error arise from 6% for the single sugar to 10% for mixed sugar), but is still more accurate than the traditional OD method (measurement error was 15%). In summary, the detection power of the digital color analysis method is superior the traditional OD method both on single sugar or sugar mixture.

The detection limit and rang of digital color analysis method is compared to that of other prevailing method including bio-sensor, electrochemical sensor and surface-enhanced raman spectroscopy (SERS) sensor (as shown in Table 4). With regard to operability, our proposed method exhibits obvious advantages. It does not require specialized equipment or professional knowledge. Common commercial imaging devices such as digital cameras or desktop scanners are completely satisfied

for present experiment. The color analysis can easily be achieved using common commercial software, such as Matlab (MathWorks, USA), R (Development Core Team, Austria, http://www.R-project.org), Photoshop (Adobe Systems, USA). An untrained person can carry out the measurements easily. However, on the contrary, most of these electrodes and surface-enhanced Raman spectroscopy glucose sensor are not available in the common libraries, which hinders its application in common experiments.

In the near future, more work will be done to expand our proposed method in the filed where colorimetric methods are being employed, such as detection of ascorbic acid, erythromycin, enzyme activities and so on.

■ Conclusion

This study has presented a sensitive and quick glucose detection method based on color analysis. The Red color provides superior precision (99.8%), which is comparable to absorbance which is used in the traditional typical 3, 5-Dinitrosalicylic acid (DNS) method. Combined with ANNs algorithm, it can be used for the detection of high concentration glucose with high accuracy. Based on the study above, a microtiter plate (48-well plate) platform based on color analysis was set up, which owns high measurement throughput and speed. The measurement of 150 samples only needs 150 minutes. It also has wide potential application in reducing sugar measurements including xylose, fructose and maltose. The method proposed does not require specialized equipment or professional knowledge. Its rapidity and simplicity makes it a very promising alternative for the quantification of other reducing

substances.

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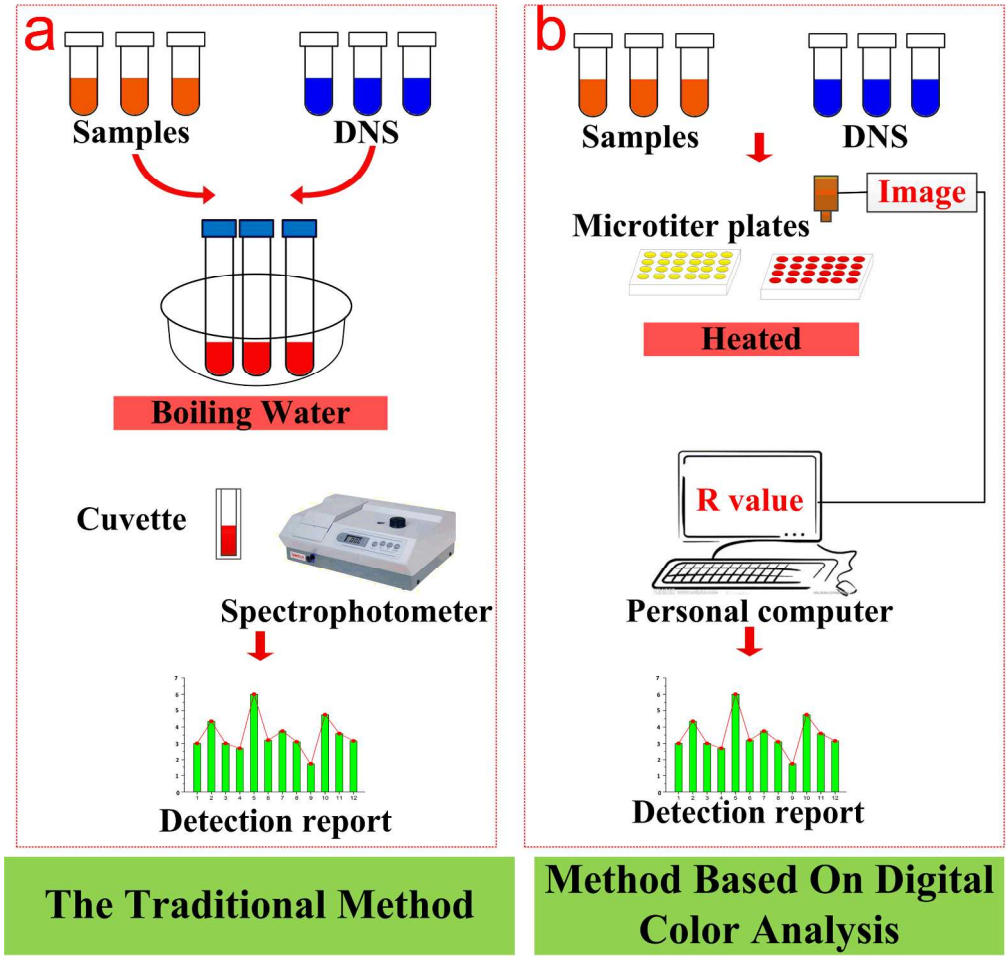


Fig. 1 The procedure comparison between the traditional method and digital color analysis method. Fig. 1a shows the working procedure of the traditional OD method and Fig. 1b shows the working procedure of digital color analysis method.
511x496mm (300 x 300 DPI)

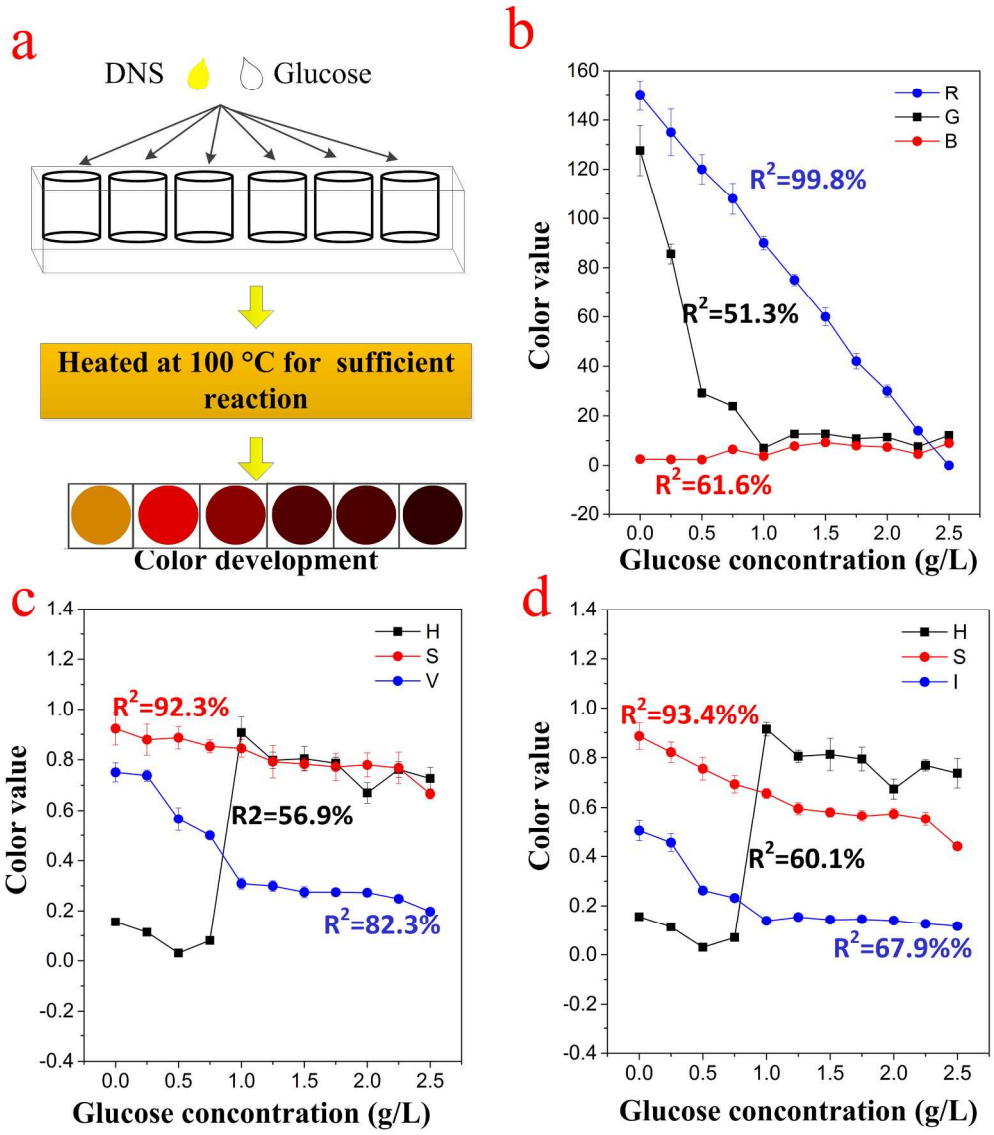
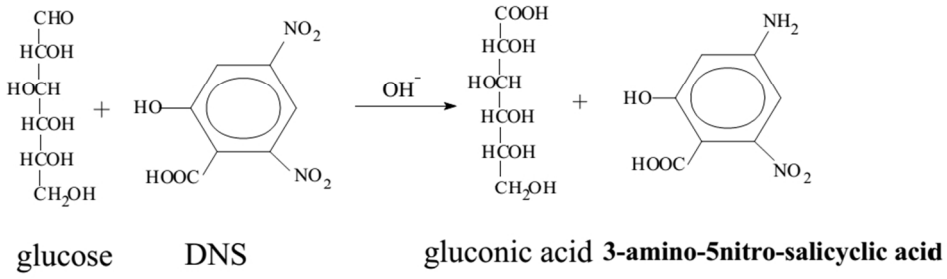
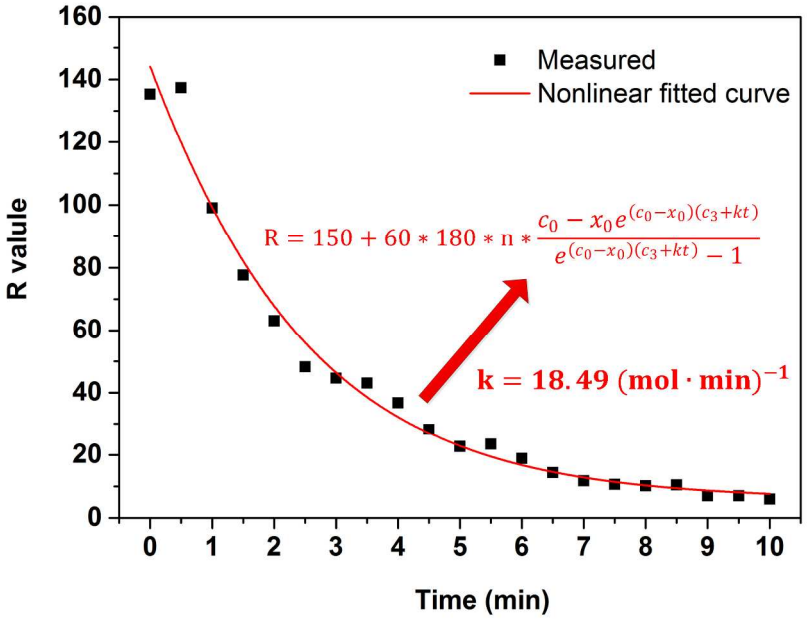


Fig. 2. The quantitative relation between primary colors and glucose concentration in DNS method. Figure 2a shows the color development procedure in 48-well microplate. Figure 1 b, c, and d stand for the quantitative relation between glucose concentration and primary colors from RGB, HSV, HSI color spaces respectively. 211x242mm (300 x 300 DPI)



Colorimetric reaction between DNS reagent and glucose
283x87mm (96 x 96 DPI)



Calculation of k (reaction rate constant) for the color development dynamics model. In our color development kinetic model, R is the color value; x0 is the glucose concentration of sample used for detection, which is 0.0138 mol/L (2.5 g/L) here; c0 is the initial concentration of DNS before reaction, which is 0.0276 mol/L in our research); t is the reaction time. The reaction temperature was set at 100 ºC.
289x202mm (300 x 300 DPI)

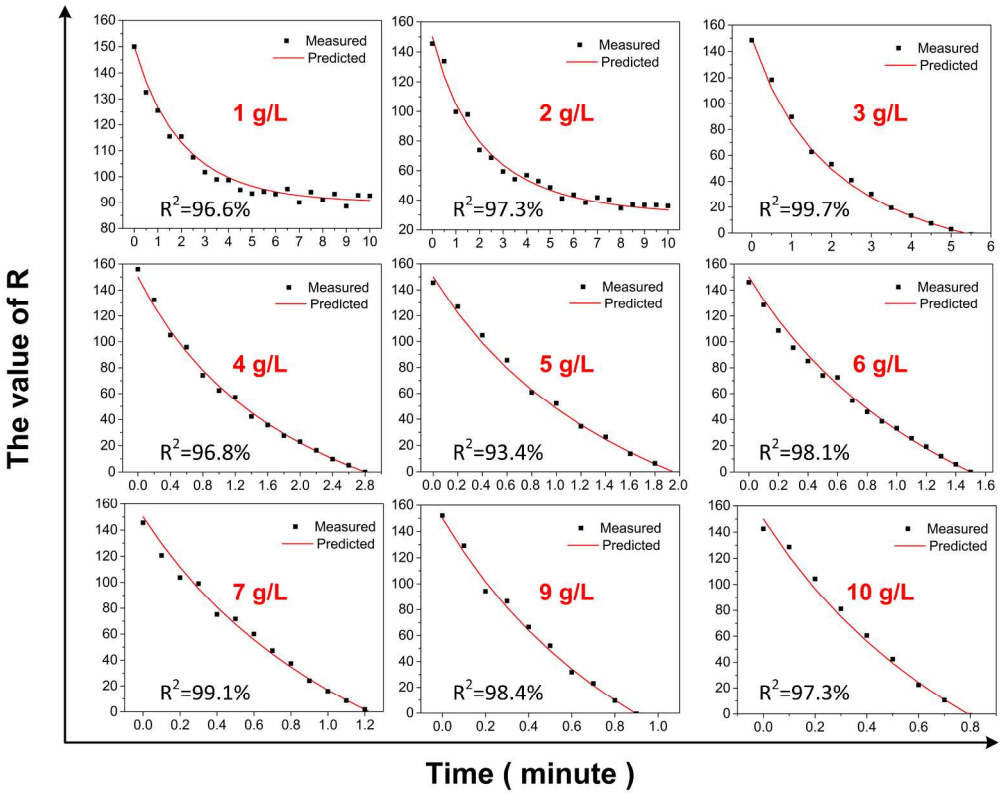


Fig. 5. The measured and predicted color development processes of samples with different glucose concentrations at 100 °C. The glucose concentrations were from 1 g/L to 10 g/L. The dots stand for the experiment data and the red lines stand for the predicted value by kinetics model of color development. 574x452mm (300 x 300 DPI)

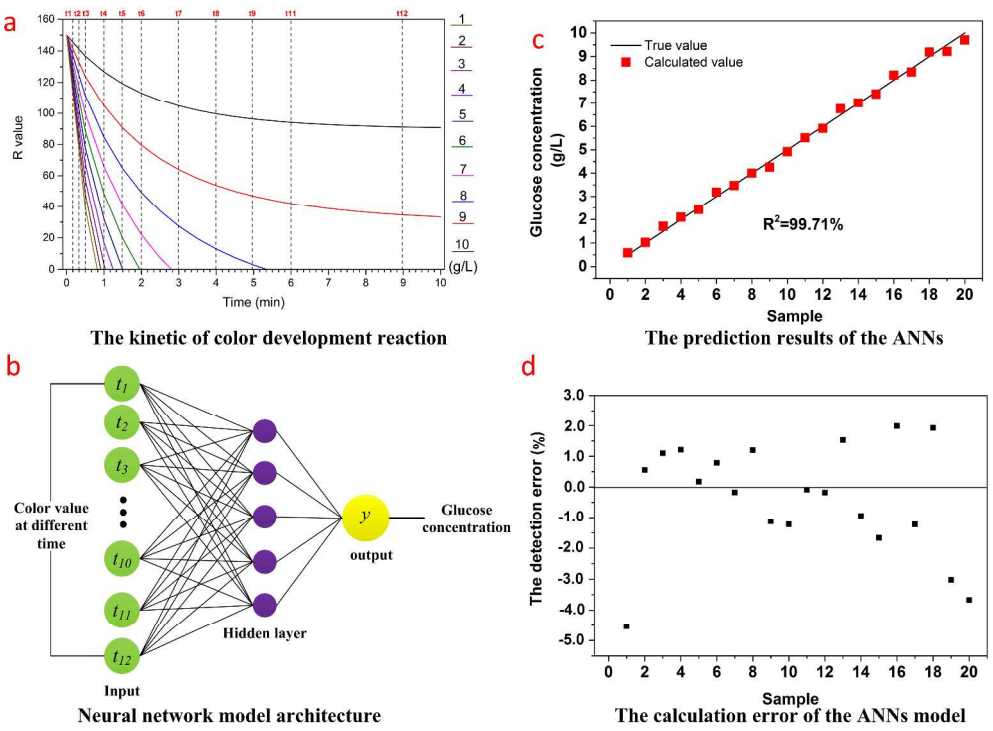


Fig. 6. The ANNs model for detection of high concentration glucose. Fig. 6a showed the kinetics of color development reactions with different glucose concentrations (1 - 10 g/L); Fig. 6b showed the architecture of the neural network model; Fig. 6c showed the prediction results of the ANNs (only 20 samples were given). The dots stand for the calculated values by ANNs; Fig. 6d showed the calculation error of the ANNs model.
420x306mm (300 x 300 DPI)

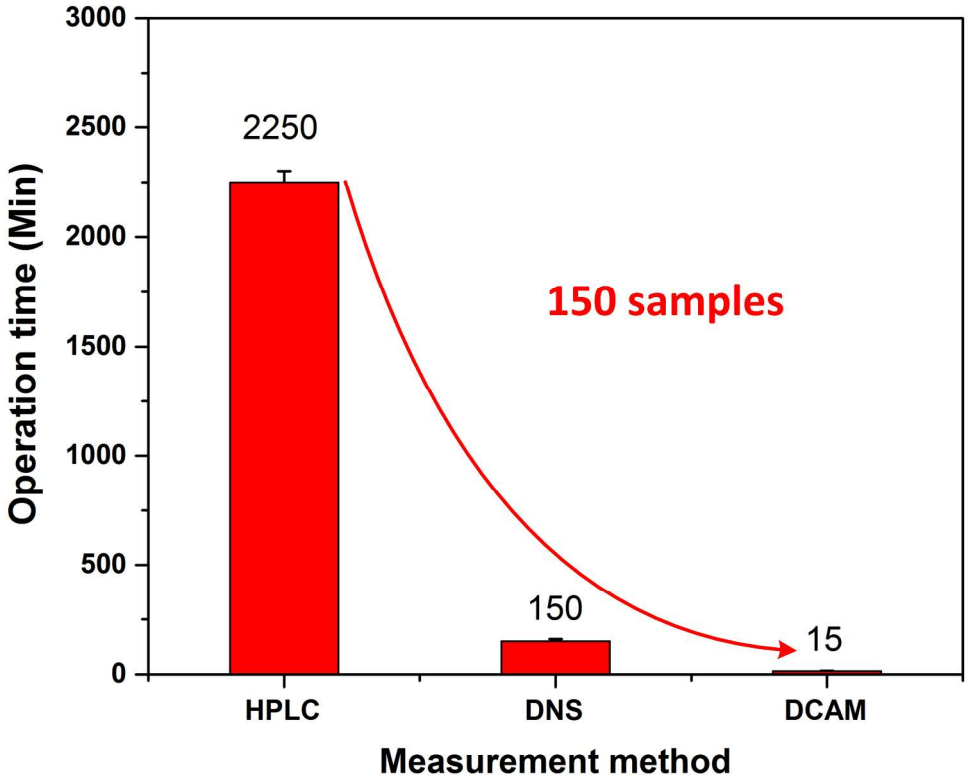


Fig. 7 The comparison of operation time of 150 samples with different measurement methods
228x184mm (300 x 300 DPI)

Table 1 Comparison of different calculation algorithms for glucose detection by color analysis

Model name	Detection range	Detection precision	Formula for data fitting	Number of image needed for calculation	Advantage	Disadvantage
Linear fit	0-2.5 g/L	0.07 g/L	*$R = 150 - 60 * Glu$ (Equation 5)	1	Easy to operate;	Low detection range; Dilution pretreatment is needed for high concentration glucose detection.
Nonlinear fit	0-10 g/L	0.20 g/L	#$R = 150 + 60 * 180 * n * \frac{c_0 - x_0 e^{(c_0 - x_0)(c_3 + kt)}}{e^{(c_0 - x_0)(c_3 + kt)} - 1}$ (Equation 7)	≥3	Precise;	Requirement of knowledge on color development dynamics
ANNs	0-10 g/L	0.10 g/L	None	≥3	Highly precise;	

Note: In Equation 5, R is the R (or red) value (from RGB color space) of the reacted broth image and Glu is the glucose concentration of the samples for detection (g/L); In Equation 7, n is the dilute factor, which is 12 in our study. $c_3 = \frac{Ln(\frac{c_0}{x_0})}{c_0 - x_0} (Calculated\ from\ C_{t=0} = 0)$； k was calculated according to ***Arrhenius Equation*** [14] (Equation 3);

Table 2 comparison of the detection results using traditional DNS, HPLC and digital color analysis method for glucose measurement

Method	Evaluation Criterion	Standard samples 0.5 g/L	1 g/L	2 g/L	4 g/L	6 g/L	8 g/L	10 g/L
DNS method	Mean	0.49	0.98	2.15	3.55	3.42	3.64	3.71
	Maximum error	2.62%	3.12%	4.6%	12.29%	55.36%	67.33%	82.38%
	*V-Coefficient	3.42%	4.65%	6.98%	38.96%	44.91%	38.97%	43.63%
HPLC	Mean	0.50	1.02	1.98	3.99	5.96	8.05	9.96
	Maximum error	1.13%	1.56%	4.65%	3.26%	4.36%	4.23%	4.32%
	*V-Coefficient	3.61%	3.64%	4.12%	3.78%	4.12%	3.89%	4.12%
Color analysis method	Mean	0.52	0.98	2.11	4.32	5.81	8.43	9.84
	Maximum error	5.13%	6.23%	8.96%	6.72%	8.88%	8.04%	7.75%
	*V-Coefficient	4.51%	5.92%	6.88%	5.78%	4.97%	7.59%	8.41%

*V-Coefficient stands for the variable coefficient. The variable coefficient=standard deviation/mean. The variable coefficient is used to evaluate the stability of the measurement results. The variable coefficient closer to zeroes stands for the higher stability. HPLC stands for High Performance Liquid Chromatography.

Table 3 Measurement results of xylose, fructose and maltose by the digital color analysis method

sugar	Evaluation Criterion	Standard samples						
		0.5 g/L	1 g/L	2 g/L	4 g/L	6 g/L	8 g/L	10 g/L
Xylose	Mean (g/L)	0.42	0.94	2.07	3.94	6.06	8.14	10.16
	Maximum error	5.23%	5.63%	7.66%	6.56%	6.91%	7.63%	5.97%
	*V-Coefficient	6.96%	7.65%	8.05%	7.03%	6.01%	8.82%	5.49%
Fructose	Mean (g/L)	0.38	1.12	2.16	4.03	5.87	7.85	9.79
	Maximum error	5.46%	7.65%	6.56%	4.78%	7.86%	5.01%	8.60%
	*V-Coefficient	3.09%	3.67%	5.99%	4.92%	6.83%	6.73%	6.27%
Maltose	Mean (g/L)	0.41	1.16	1.92	4.22	6.07	7.85	9.76
	Maximum error	5.61%	5.90%	5.71%	4.66%	5.98%	6.56%	8.12%
	*V-Coefficient	4.06%	5.83%	4.81%	6.71%	5.26%	5.42%	7.04%

*V-Coefficient stands for the variable coefficient. The variable coefficient=standard deviation/mean of the values. The variable coefficient is used to evaluate the stability of the measurement results. The variable coefficient closer to zeroes stands for the higher stability.

Table 4 Measurement results of ascorbic acid by the digital color analysis method

Material	Evaluation Criterion	Standard samples (g/L)						
		0.02	0.05	0.1	0.2	0.3	0.4	0.5
Xylose	Mean (g/L)	0.020	0.048	0.011	0.197	0.304	0.402	0.496
	Maximum error	2.11%	3.6%	4.41%	4.32%	4.24%	4.88%	6.42%
	*V-Coefficient	2.64%	4.1%	4.31%	5.34%	4.67%	5.21%	7.66%

Table 5 Comparison of bio-sensor, electrochemical sensor and Surface-Enhanced Raman Spectroscopy sensor for glucose detection

Method	Detection limit	Sensitivity	Linear range	Refs
Graphene–glucose oxidase biocomposite biosensor	0.1 mM	1.85 μA/(mM×cm^2)	0.1–27 mM	B. Unnikrishnan et.al[24]
TiO2–Graphene composite biosensor	Not given	6.2 mA/(mM×cm^2)	0 - 8mM	DJ. Hee et.al[25]
Zinc oxide nanocomb biosensor	0.02 mM	15.33 A/(cm^2×mM)	0.02 - 4.5 mM	J. Wang et. al[26]
Mesoporous Platinum electrochemical sensor	0.1 mM	9.6 μA/(cm^2 ×mM)	0.0 - 10.0 mM	S. Park et. al[27]
Nickel ion implanted-modified indium tin oxide electrode	0.5 μM	0.1895 mA/(mM×cm^2)	1-350 μM	H Tian et. al[28]
RGO-Ni(OH)$_2$/GCE	0.6 μM	0.01143 mA/(mM×cm^2)	2–3100 μM	Y Zhang et. al [29]
Surface-Enhanced Raman Spectroscopy Glucose Sensor	0.56 mM	Not given	0-25 mM	O Lyandres et.al [30]
Near-Infrared Surface-Enhanced Raman Spectroscopy	0.5 mM	Not given	0.5-44 mM	DA Stuart et. al[31]
Digital color analysis	0.38 mM	-	0-55.6 mM	This study