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Feasibility for quantitative determination of deoxyribonucleic acid by using near-infrared diffuse reflectance spectroscopy

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ABSTRACT

A method for quantitative determination of fish sperm deoxyribonucleic acid (fsDNA) in solutions was developed by using adsorption preconcentration and near-infrared diffuse reflectance spectroscopy (NIRDRS). A high capacity adsorbent of amino-modified silica particle (AMSP) was prepared for preconcentration of fsDNA in solutions. Under the optimized conditions, the adsorption rate can be above 90% within 3 min. After adsorbing the DNA onto the adsorbent, near-infrared (NIR) spectra in diffuse reflectance mode were measured and partial least squares (PLS) model was established for fast quantitative prediction. The results show that the correlation coefficient (R) between the predicted and the reference concentration is 0.9894 and the recoveries are in the range of 92.9–123.4% for the validation samples in the concentration range of 3.00–29.38 mg L^{-1}. Therefore, the feasibility for quantitative analysis of DNA in solutions by NIRDRS is proved. This may provide an alternative way for fast determination of DNA in solutions.

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1. Introduction

The quantitative analysis of nucleic acids has become increasingly important in the fields of molecular biology, medical diagnostics and environment application, etc. Analytical methods for quantitative analysis of deoxyribonucleic acid (DNA) by using spectrophotometry [1,2], spectrofluorometry [3,4], resonance light scattering (RLS) [5–7], chemiluminescence (CL) [8,9] and electrochemistry [10–12], etc. have been well studied. Spectrophotometric method [1,2] takes the advantage of the reaction of DNA with various organic dyes to form colored derivatives, and spectrofluorometric and RLS methods are generally based on the enhancement or quenching effect on the probes such as dyes [3,4], metal complex [5] and even nanoparticles [6,7]. CL method [8,9] measures the light emission occurring in chemical processes, thus having excellent sensitivity, low detection limit and wide linear range. Electrochemical method is often used to study the interaction of small molecules such as metals [10], drugs [11] and organic dyes [12] with DNA, and quantitative determination can be achieved by the specific interactions.

Near-infrared (NIR) spectroscopy has been proved a convenient, fast and nondestructive technique for complex sample analysis, and has been broadly adopted in various fields [13,14]. However, NIR spectra are commonly composed of weak, broad and overlapping peaks. Chemometric methods must be used for the quantitative analysis [15–17]. Another drawback of NIR spectroscopic analysis is its high detection limit or low sensitivity, which makes the technique not suitable for micro or trace analysis. Therefore, efforts have been devoted to improve the detection limit or sensitivity. Adsorption in combination with near-infrared diffuse reflectance spectroscopy (NIRDRS) has been used for determination of low content components in solutions or real samples with complex matrix. Enrichment using silica-based monolithic material was combined with NIRDRS to improve the sensitivity for the determination of low concentration ethyl carbamate [18], carbaryl [19] and lead [20]. In our previous works, preconcentration technique combined with NIRDRS was used to simultaneously determine low content substances, such as organic acids [21], metal ions [22,23] and phenols [24], etc. The feasibility has been proved by these studies to quantitatively determine the analytes in dilute solutions using adsorption preconcentration and NIRDRS. On the other hand, indirect modeling of trace element contents and NIR spectra has been investigated for natural or environmental samples, such as food, soils and sediments, etc. [14]. In our recent work, trace tobacco specific N-nitrosamines (TSNAs) in tobacco samples were determined with the help of chemometric methods [25].

The purpose of this work is to investigate the feasibility for the quantitative determination of DNA in dilute solutions by using NIRDRS. By using a high efficient adsorbent, amino-modified silica particle (AMSP), fish sperm DNA (fsDNA) is preconcentrated from dilute solution, and then the adsorbent with the adsorbate is

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directly measured by NIRDRS. Due to the complexity of the spectra, which are composed of the weak spectral signal of the adsorbate and the strong spectral responses of the absorbent, partial least squares (PLS) regression is used as a calibration tool.

2. Experimental

2.1. Reagents and chemicals

All chemical reagents are of analytical grade. Cyclohexane, hexanol, triton X-100, ethyl orthosillicate, ethanol and ammonia were purchased from Guangfu Chemical Co., Ltd. (Tianjin, China). (3-Aminopropyl) triethoxysilanne was purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). FsDNA is high purity grade and was purchased from Biodee Biotechnology Co. Ltd (Beijing, China). Double distilled water was used to prepare the samples.

2.2. Preparation and characterization of AMSP

AMSP was synthesized by microemulsion method [26]. At first, a microemulsion was prepared with 40 mL cyclohexane, 10 mL triton X-100, 10 mL n-hexanol and 36 mL doubly distilled water. Then, a mixture of 10 mL ethyl orthosillicate and 10 mL (3-aminopropyl) triethoxysilanne was prepared and added into the microemulsion. Finally, 20 mL 25% ammonia solution was added to make the system synchronously hydrolyzed. After stirred 24 h, the particles of the product can be obtained by adding 20 mL acetone into the system and centrifugation. After washing with ethanol and doubly distilled water, drying 10 h in 100 °C, the ground particles can be used as the adsorbent. Characterization of the product was performed by Fourier transformation infrared spectroscopy (FT-IR) and elemental analysis. Fig. 1 shows the infrared spectrum of the adsorbent. The peaks are explained as follows: 3427.0 cm^{-1} ν (N–H or O–H), 2930.4 cm^{-1} ν (CH_2), 1638.3 cm^{-1} δ (NH), 1055.8 cm^{-1} ν_a (Si–O–Si), 452.7 cm^{-1} ν_s (Si–O–Si). The result of elemental analysis shows that the content of nitrogen element in the material is 5.5%, confirming that the amino group was bonded to the material.

2.3. Sample preparation

Stock solution (300 mg L^{-1}) was prepared by dissolving fsDNA in double-distilled water and stored at 0–4 °C for 48 h before use. As the matrix of the samples, a solution containing sodium chloride, potassium chloride, nonahydrous aluminum nitrate, anhydrous magnesium sulfate, copper sulfate, albumin bovine V and D-glucose was prepared. The concentration of matrix substances in the synthetic samples is listed in Table 1. 58 samples were prepared for the following studies, and the concentration of fsDNA ranges from 3.00 to 29.8 mg L^{-1}. In the 58 samples, 40 samples were prepared for building the calibration model, and 9 samples with a duplicate for each were prepared as validation set. The duplicates were used to investigate the reproducibility of the method.

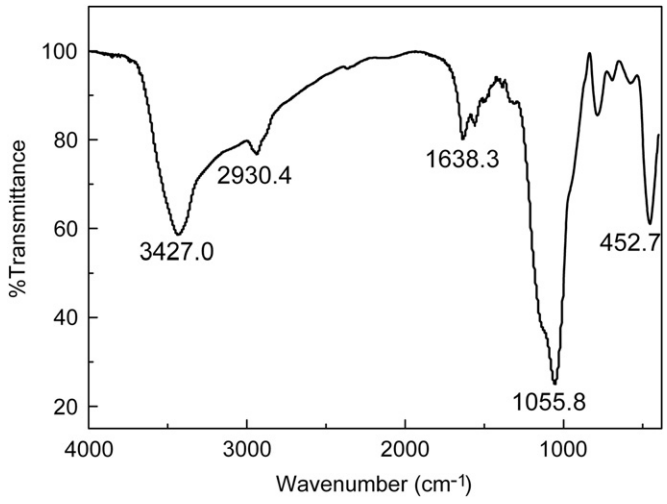

Fig. 1. FT-IR spectrum of the adsorbent.

2.4. Adsorption operation

The adsorption operation was performed at room temperature (ca. 25 °C). 100 mL of the sample solution and ca. 0.3 g adsorbent were added into a conical flask, and then the flask was shaken for 3 min. After the adsorption, the solution was filtered with the aid of vacuum pump and the solids were further dried with ethanol at the end of the filtration. Finally, the adsorbent with the analyte was used for the spectral measurement. A UV-3600 spectrophotometer (Shimadzu, Japan) was used to measure the remained DNA in the samples after adsorption.

2.5. NIR spectral measurement

A Vertex 70 spectrometer (Bruker Optics, Ettlingen, Germany) with an NIR integrating sphere diffuse reflection accessory (Bruker Optics, Ettlingen, Germany) was used for measuring the spectra. The spectra are obtained at resolution 4 cm^{-1} from 4000 to 10,000 cm^{-1} and digitalized with ca. 2 cm^{-1} interval in Fourier transform. Therefore, each spectrum is composed of 3111 data points. Before the measurement, a reference spectrum was taken with the gold-coated background provided with the instrument. To increase signal to noise ratio, both reference and sample spectra were measured with scan number 64, and each spectrum was averaged from three parallel measurements. The spectrometer was kept balance at 25 °C ambient for 60 min before use. Fig. 2 shows the measured spectra of the 58 samples. Due to the low concentration, it is hard to see the spectral response of fsDNA in the spectra. Therefore, in the calculations, the wavenumber region of 5154.9–4258.2 cm^{-1} was used considering that the two peaks of DNA arisen from the N–H and C–H are located around 5059.9 and 4414.9 cm^{-1}.

2.6. PLS modeling

PLS regression was used for modeling and prediction. The calculation was performed in a computing environment of Matlab (MathWorks, USA). The performance of the calibration model was evaluated by the correlation coefficient (R) and root mean square error of cross validation (RMSECV), which was determined by leave-one-out cross validation (LOO-CV). Residual predictive deviation (RPD), the ratio of standard deviation (SD) to

Table 1
Concentration of matrix substances in the prepared samples.

Substance	Concentration (mol L^{-1})
Na^+ (Cl^-)	5.1×10^{-4}
K^+ (Cl^-)	2.0×10^{-4}
Mg^{2+} (Cl^-)	4.0×10^{-4}
Al^{3+} (NO_3^-)	2.0×10^{-6}
Cu^{2+} (SO_4^{2-})	1.1×10^{-6}
D-Glucose	2.5×10^{-5}
Albumin bovine V	3.0×10^{-8}

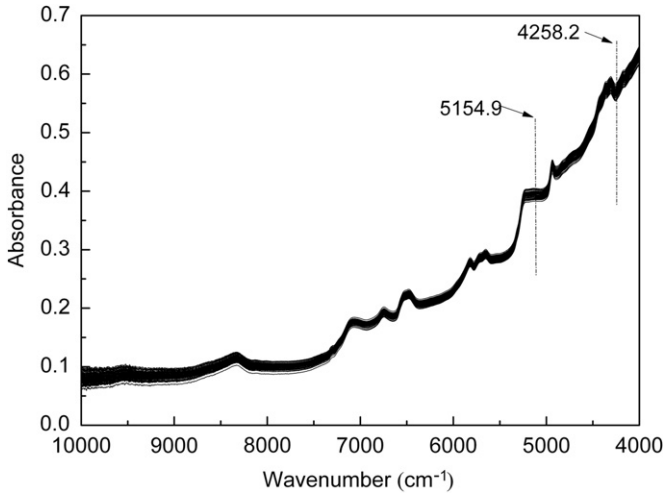

Fig. 2. NIR spectra of the 58 samples.

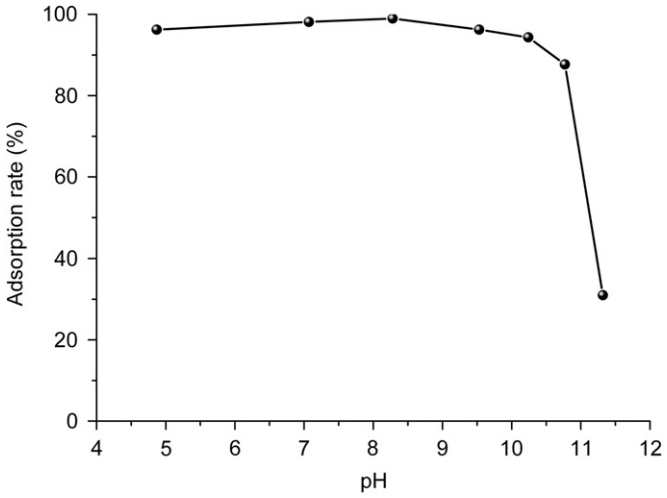

Fig. 3. Effect of pH on the adsorption rate.

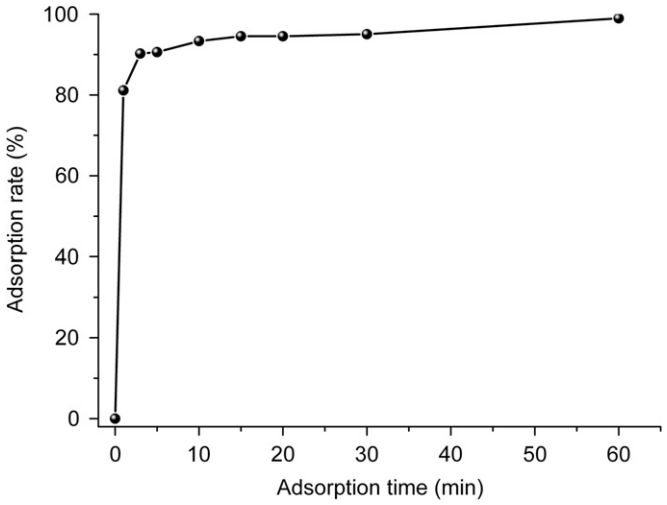

Fig. 4. Effect of adsorption time on the adsorption rate.

standard error of prediction (SEP) in cross validation, was also used to indicate the quality of the models. Generally, a model with RPD over 3.0 is considered suitable for screening and process control and over 5.0 for accurate quantitative analysis [27]. Root mean square error of prediction (RMSEP) and correlation coefficient (R) between the reference and prediction concentration were used to evaluate the prediction of the developed model by using the validation set. In addition, Monte Carlo cross validation (MCCV) combined with adjusted Wold's R criterion [28] was utilized to determine the latent variable (LV) number in the modeling.

In order to obtain an optimized PLS model, signal processing techniques such as multiplicative scattering correction (MSC) [29], standard normal variate (SNV) [30], derivative [31,32] and continuous wavelet transform (CWT) [33–35] were investigated. Derivative was performed using Savitzky–Golay algorithm [32] with the aim of reducing the fluctuating noise and removing baseline variations. SNV and MSC were used for baseline or background correction, and CWT serves to eliminate both the varying background and the noise.

3. Results and discussion

3.1. Effect of adsorption condition

In order to investigate the effect of pH on the adsorption rate, experiment was conducted in the pH rang from 4.87 to 11.32, which were adjusted with diluted hydrochloric acid and diluted sodium hydroxide. In the experiment, 0.3 g AMSP, 1 h adsorption time and 100 mL 30 mg L^{-1} fsDNA solutions were used. Fig. 3 shows the variation of the adsorption rate under different pH. It can be seen that, in the neutral condition, an adsorption rate above 90% can be obtained with the maximum 99.0% at pH 8.28. When pH is beyond 10, however, there is a sharp descending for the adsorption rate. The result is in accordance with the literature [27]. Therefore, pH around 7 was used for the adsorption, because the adjusting operation is not needed.

For understanding the effect of adsorption time on the adsorption rate, the variation of the adsorption rate in different adsorption time was investigated at pH 7.07. In the experiment, 0.3 g adsorbent and 100 mL 30 mg L^{-1} DNA solutions were used. Fig. 4 shows the variation of the adsorption rate in adsorption time from 1 to 60 min. It is clear that the adsorption rate ascends significantly with the increase of adsorption time before 3 min, and then reaches an almost constant with a very slight increase. The adsorption rate at 3 min is 90.6%. Therefore, 3 min was used in this study for speeding up the experiment.

Table 2
PLS models and the results of cross validation.

Preprocessing	nLV[a]	R	RMSECV	RPD
No preprocessing	8	0.9893	1.30	6.86
MSC	7	0.9901	1.27	6.99
SNV	7	0.9901	1.27	7.00
First derivative	6	0.9821	1.66	5.37
CWT	6	0.9849	1.53	5.83

[a] nLV = Number of latent variables.

3.2. Quantitative model

PLS model was developed with both raw and pretreated spectra. Spectral preprocessing methods were optimized by using LOO-CV. However, as mentioned above, the number of LV was determined by using MCCV. For improving the PLS model, four spectral processing methods were investigated. The results of the models using different preprocessing methods were summarized in Table 2. Clearly, the LV numbers of these models are reasonable because the responses from adsorbent, albumin bovine V, D-glucose and metal ions besides fsDNA. The decrease of the LV number after preprocessing can be

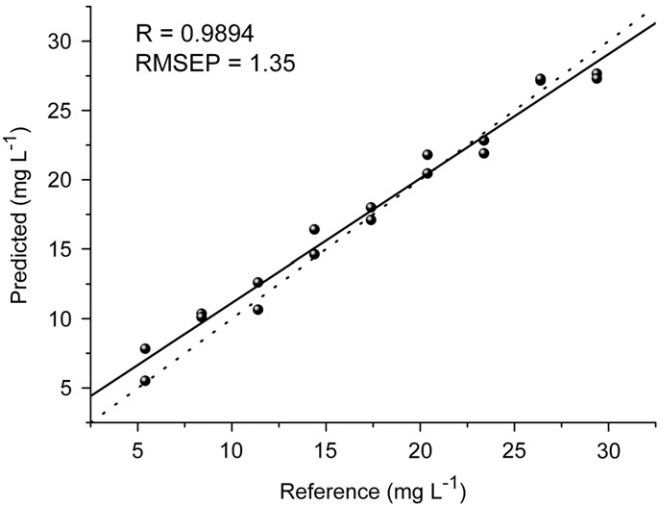

Fig. 5. Relationship of predicted concentration and the reference values. The straight line is obtained by least squares fitting, and the dot line shows the diagonal of plot.

accounted for by the correction and the removal of the variant background. From the correlation coefficient (R) in the table, it can be seen that all the models are acceptable, and the background correction (MSC and SNV) can further improve the model, although the improvement is not so significant. From the RMSECV, however, the statistical test (F-test) shows that the background removal (first derivative and CWT) make the model slightly worse. The result may be explained by the fact that the variance of the background is not large as shown in Fig. 2. The same result can be shown by RPD. All the values of RPD are above 5, indicating that all the models are accurate enough for quantitative analysis. For obtaining a minimal error, SNV was used for signal preprocessing in this study.

3.3. Validation of the PLS model

In order to test the practicability of the PLS model, external validation was done with the 18 validation samples. In the validation, the same conditions were used for the adsorption and the spectral measurements, and the optimized PLS model was used for prediction. Fig. 5 shows the relationship between the predicted and reference concentrations of the analyte. The straight line is obtained by least squares fitting of the points and a dot line is drawn to show the diagonal of the plot. It can be seen that the predicted and the reference concentration are in a good linearity, although there is a slight deviation between the two lines. The correlation coefficient (R) obtained by least squares fitting is 0.9894, and the RMSEP is 1.35. The results obviously indicate that the predicted concentrations are acceptable. For further investigation of the results, the recoveries are calculated. It was found that the recoveries range from 92.9 to 123.4% except for one sample of the lowest concentration (5.40 mg L^{-1}). On the other hand, the reproducibility of the method can be seen from the two predicted results of the samples with same concentration. Except for the one of the lowest concentration, the differences between the two predicted values are less than 2.0 mg L^{-1} and the relative errors are less than 20%.

4. Conclusion

A method for determination of fsDNA in solutions was developed by using NIRDRS and a high efficient adsorbent (AMSP). The absorbent was proved to capture more than 90% of the analyte within 3 min. In the method, DNA is concentrated onto the adsorbent for enhancing the sensitivity. NIRDRS and PLS regression are used for a fast measurement and prediction. With the samples in a concentration range from 3.00 to 29.38 mg L^{-1}, it was demonstrated that the recoveries of the quantitative determination are from 92.9 to 123.4% with an acceptable reproducibility, even when interferences of albumin bovine V, D-glucose and metal ions are included in the samples. Therefore, the method may provide an alternative for fast determination of DNA in solutions. Although further works on improving the detection limit of the method is still needed, the method may be helpful for fast or even online analysis.

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