Chinese Chemical Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Quantification of albumin in urine using preconcentration and near-infrared diffuse reflectance spectroscopy

Xiao-Xu Ma [a], Cui-Cui Wang [a], Wen-Sheng Cai [a], Xue-Guang Shao [a,b,c,d,*]

[a] Research Center for Analytical Sciences, College of Chemistry, Nankai University, Tianjin 300071, China
[b] Tianjin Key Laboratory of Biosensing and Molecular Recognition, Tianjin 300071, China
[c] State Key Laboratory of Medicinal Chemical Biology, Tianjin 300071, China
[d] Collaborative Innovation Center of Chemical Science and Engineering, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form 1 March 2016
Accepted 4 March 2016
Available online xxx

Keywords:
Near-infrared diffuse reflectance spectroscopy
Quantitative determination
Multivariate calibration
Urinary albumin
Thiourea-functionalized silica nanoparticles

ABSTRACT

Urinary albumin is an important diagnostic and prognostic marker for cardiorenal disease. Recent studies have shown that elevation of albumin excretion even in normal concentration range is associated with increased cardiorenal risk. Therefore, accurate measurement of urinary albumin in normal concentration range is necessary for clinical diagnosis. In this work, thiourea-functionalized silica nanoparticles are prepared and used for preconcentration of albumin in urine. The adsorbent with the analyte was then used for near-infrared diffuse reflectance spectroscopy measurement directly and partial least squares model was established for quantitative prediction. Forty samples were taken as calibration set for establishing PLS model and 17 samples were used for validation of the method. The correlation coefficient and the root mean squared error of cross validation is 0.9986 and 0.43, respectively. Residual predictive deviation value of the model is as high as 18.8. The recoveries of the 17 validation samples in the concentration range of 3.39–24.39 mg/L are between 95.9–113.1%. Therefore, the method may provide a candidate method to quantify albumin excretion in urine.

1. Introduction

Chronic kidney disease is now recognized as a worldwide public health problem with an increasing prevalence [1,2]. In the early stage of the disease process, patients frequently experience no symptoms at all. However, even in the absence of symptoms, chronic kidney disease negatively affects various organs, raises the risk of cardiovascular events, and increases the risk of hospitalization and death [3]. Fortunately, current evidence has proved that some of the adverse outcomes can be delayed or even prevented by early detection and treatment [4,5]. A common clinical practice to identify and monitor the patients with kidney damage is testing the urine for albumin. Urine dipstick test is widely used as an initial screening tool for detecting proteinuria because of its low cost and wide availability [6]. However, the detection limit of urine dipstick is about 250 mg/L, which is much higher than the normal limit [7]. The decision of normal limit for urine albumin reported by laboratories varied from 15 to 30 mg/L [8], and recent studies have found that both renal and cardiovascular risks begin to increase at levels currently considered to be in the normal range [9]. Therefore, accurate and precise measurement of urinary albumin is essential in clinical medicine for kidney diseases.

Routine laboratory methods to determine albumin in urine are based on albumin-specific assays [10,11]. Since they are based on highly specific interactions between the protein of interest and a targeted antibody, some immunoassays allow detection of very low amounts of albumin in urine. However, immunochemical methods are usually subjected to weak antibody affinity, cross-reactivity, or denatured epitopes, and thus potentially bias the total albumin measurement [12]. Recently, the combination of internal standards and mass spectrometry (MS) has emerged as a powerful means for quantitative proteomics [13–16]. MS-based approaches do not require the generation of antibodies and can provide the level of molecular specificity and breadth of unbiased proteome coverage. Among the many types and configurations of mass spectrometer, liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) is perhaps the most commonly used method for MS-based proteomics studies. However, optimizing the performance of an LC–MS/MS system for quantitative proteomics measurements can be a challenging work, and absolute

* Corresponding author.
E-mail address: xshao@nankai.edu.cn (X.-G. Shao).

http://dx.doi.org/10.1016/j.cclet.2016.03.008

quantification methods for LC–MS/MS-based proteomics require internal standards, such as stable isotope-labeled proteins or peptides with known concentrations [17].

For routine operation, rapid analysis and inexpensive disposables (or no disposables) are desirable. While the method should be reliable, easily automated, the results should be accurate and precise [18]. Near-infrared diffuse reflectance spectroscopy (NIRDRS) has been proved to be a fast, convenient and non-destructive analytical method. Combining with chemometrics, accurate determination can be achieved [19–21]. Each NIRDRS measurement spend only less than 1 min. For protein analysis, quantification by NIRDRS does not need antibodies or internal standards. Therefore, NIRDRS in combination with chemometrics has the potential to be an effective analytical method for clinical laboratory measurements. However, drawback of near-infrared (NIR) spectroscopic analysis is its high detection limit. Much effort has been done for improving the detection limit. Among various trials, enrichment technique in combination with NIRDRS has been proved to be an efficient way [22]. In our previous works, low content substances, such as metal ions [23,24], organic acids [25], phenolic compounds [26], and deoxyribonucleic acid [27], etc. in complex matrix have been detected by NIRDRS combined with preconcentration. The detection limit of deoxyribonucleic acid in dilute solutions with interferences can be as low as 3 mg/L [27], and the measurement was directly performed with the absorbent without desorption. Therefore, an NIR-based method for the accurate and precise quantitation of urinary albumin in urine may be developed as a candidate for quantitative determination of urinary albumin.

This work is devoted to investigate the feasibility of the quantification for albumin in urine by NIRDRS combined with preconcentration and chemometric modeling. Thiourea-functionalized silica (TF-SiO_2) nanoparticles were used for preconcentration of albumin from urine solution. Then NIRDRS was applied to measure the adsorbent with the adsorbate directly without desorption. Partial least squares (PLS) regression was used as a calibration tool for modeling the weak spectral signal of albumin in the complex spectra of the absorbent, including the strong spectral responses of TF-SiO_2 nanoparticles. Signal processing techniques were used to optimize the model. The NIR-based method offers a new idea for urinary albumin detection method and could be rather helpful for early diagnosis of kidney disease.

2. Experimental

Chemical reagents, including ethanol, toluene and acetone, are of analytical purity grade. Thiourea (99%), silicon dioxide (SiO_2, 99.5%, particle size: 50 nm), and cyanuric chloride (99%) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). (3-Aminopropyl)triethoxysilanne (APTES, 98%) was provided by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China) and methanesulfonic acid (70% aq. soln.) was provided by Alfa Aesar Chemistry Co., Ltd. (Tianjin, China). Human albumin serum (HSA, 96–99%) was obtained from Takara Bio Co., Ltd (Tianjin, China). Pure water was obtained from Tianjin Wahaha Co., (Tianjin, China). Urine samples were collected anonymously from laboratory healthy voluntary donors. The supernatant of the urine was stored at –20 °C after centrifuged for 15 min at 3000 rpm using a TDL-5-A centrifuge (Anting Scientific Instrument Factory, Shanghai, China). The urinary albumin concentration was measured by a human microalbuminuria enzyme linked immunosorbent assay (ELISA) kit produced by Cusabio Biotech Co., Ltd. (Wuhan, China), and the absorbance was read on a Synergy 2 Multi-mode Microplate Reader (Bio-Tek, Vermont, USA). The urinary albumin concentration of the mixed urine sample was calculated to be 1.18 mg/L according to its absorbance. A 5.00 g/L stock solution of HSA was prepared in water, and all the urine samples were prepared by adding HSA solution in 3-fold diluted urine to reach a series of final concentrations ranging from 1.89 mg/L to 30.39 mg/L. 40 samples were prepared to establish the calibration model, and 17 samples were prepared for validation of the method. Among the 17 samples, there were 7 different concentrations of urine solutions with duplicates for each to check the reproducibility of the model, and one concentration with triplicates was prepared to calculate the relative standard deviation (RSD) value besides the reproducibility.

The reaction scheme for the preparation of TF-SiO_2 nanoparticles is shown in Fig. 1. Firstly, amine-functionalized silica (AF-SiO_2) nanoparticles were prepared through two steps of hydroxylation and condensation according to the published method [28]. SiO_2 (40 g), methanesulfonic acid (21.5 mL) and pure water (278.5 mL) were added into flask. After stirred and refluxed for 6 h, the suspension solution was centrifuged, washed with pure water to neutral, and dried. The product was added into flask with APTES and toluene. AF-SiO_2 nanoparticles were obtained after stirred and refluxed for 6 h, and were washed by ethanol before dried in vacuum. Then, cyanuric-functionalized silica (CF-SiO_2) nanoparticles were obtained according to the procedure described in a previous article with some modifications [29,30]. Cyanuric chloride and AF-SiO_2 nanoparticles in molar ratio of 1:1 were added into acetone that cooled in ice bath. The reaction was under continuously stirring and the temperature was kept at 0–5 °C. Aqueous solution of $NaHCO_3$ was then simultaneously added dropwise. After centrifugation and washing, CF-SiO_2 nanoparticles were dried under vacuum. Finally, TF-SiO_2 nanoparticles were prepared by condensation of thiourea and triazine ring of CF-SiO_2 nanoparticles [30]. Thiourea and CF-SiO_2 nanoparticles in molar ratio of 2:1 were added into water. After stirring at 50 °C for 12 h, the temperature was raised to 80 °C and kept for 6 h. The product was then centrifuged and washed for 5 times before dried under vacuum.

The adsorption operation was carried out by adding 0.30 g TF-SiO_2 nanoparticles and 100 mL of urine samples into a conical flask. After shaken for 3 min at room temperature (25 °C), the solution was filtered by vacuum pump. The solids were further air-dried under room temperature. The adsorbent with analyte was then directly measured to obtain NIR spectra. Before the measurement, Vertex 70 spectrometer (Bruker Optics, Ettlingen, Germany) was balanced at 25 °C for 2 h. The reference spectrum was obtained by the gold-coated background auxiliary provided with the instrument. NIR spectra of the samples were measured on the spectrometer with near infrared integrating sphere diffuse reflection accessory (Bruker Optics, Ettlingen, Germany). The wavenumber range of the measurement is from 10,000 to 4000 cm^{-1}, and the spectra were digitalized with ca. 2 cm^{-1} interval in Fourier transform. Scan number of both reference and sample spectra was set as 64 to increase signal to noise ratio. For each sample, three parallel measurements were performed to obtain an average spectrum. Fig. 2 shows the averaged spectra of the 57 samples.

Owing to the low content, it is difficult to recognize the information of albumin in the spectra. Therefore, PLS modeling was used for the quantitative analysis. To select informative wavenumber region, the software package OPUS 6.0 (Bruker, Ettlingen, Germany) was used initially. The principle of the method is the combination of different signal processing methods and wavenumber regions. The wavenumber regions that produced the best results in cross validation were selected. The calculation of PLS regression was then completed on Matlab (MathWorks, USA) platform. 40 calibration samples were used to establish the quantitative model. The latent variable (LV) number used during the construction of the model was determined by Monte Carlo cross validation (MCCV) [31]. The performance of the model was

Please cite this article in press as: X.-X. Ma, et al., Quantification of albumin in urine using preconcentration and near-infrared diffuse reflectance spectroscopy, Chin. Chem. Lett. (2016), http://dx.doi.org/10.1016/j.cclet.2016.03.008