Determination Of Concentrations Of Human Serum Albumin In Phosphate Buffer Solutions Using Near-Infrared Spectroscopy In The Region Of 750-2500 Nm

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Citation

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Abstract

Near-infrared spectroscopy of human serum albumin (HSA) in the region of 750-2500 nm was subjected for developing reagentless measurement of HSA. Near-infrared diffuse reflectance absorption spectra of powder state HSA and differential absorbance from a reference phosphate buffer solution were measured for phosphate buffer solutions (pH=7.4) of HSA in the wavelength range of 750-2500nm. In the diffuse reflectance absorption spectra of powder state HSA, positive peaks can be observed near 1650-1750 nm and 2150-2350 nm. In the differential spectra of HSA solutions from the reference, peaks that follow HSA concentration were able to be clearly observed in the 1650-1750 nm and 2150-2350 nm region, and the peaks on 2150-2350 nm region was relatively greater. The near-infrared spectra band of the 750-1350nm, 1550-1850nm and 2052-2500nm regions were subjected to partial least squares (PLS) regression to obtain calibration models predicting the concentrations of the HSA. The calibration in the concentration of 0-5g/dl has yielded a correlation coefficient of 0.999 and a standard error of prediction of 0.0292 g/dl with latent variable of seven. These result suggests that the near-infrared differential spectra of HSA from the reference HSA solution and its calibration model constructed by using PLS regression can extract up effectively the information about albumin contained in phosphate buffer solution samples. These results are considered as a base of reagentless measurement of HSA in human serum and also in vivo HSA measurement.

INTRODUCTION

Blood substances provide important information of physiological status. Then, there have been many attempts of those determinations. Though current blood analyzing is mainly done by chemical and/or biological reaction with using reagents because of those high precision, a blood constituents measurement without any reagents, also called reagentless measurement has been considered. Because reagentless monitoring has possibility of rapid response, cost-effectiveness, the possibility of performing real-time monitoring and simultaneous measurement of plural constituents [1-3], there have been many attempts to realize the monitoring. As an example of reagantless measurement of blood constituents, measurement of oxygenated/deoxygenated status of hemoglobin contained on red blood cell using light has long history and the measurement is called CO-oximetry [4, 5]. And the CO-oximetry has been expanded to the in vivo measurement named “pulse oximetry” to measure the oxygenation status of a patient's hemoglobin [6]. Besides of the success of those hemoglobin oxygenation measurements, the challenges of developing reagentless measurement of blood constituents other than oxygenation measurement are still developing.

For reagentless measurement of blood substances, electrical impedance measurement [2], [Farace] and optical measurement have been applied because those methods can measure electrical or optical properties of blood, plasma and/or serum nondestructively. Among them, optical measurement appears accumulating many attempts. In previous attempts of the measurements, main interests were focused on blood constituents such as total hemoglobin, glucose, protein and cholesterol that can be considered as important substances [3, 8-13]. Recent advances of blood substances reagentless measurement has closely connected with near-infrared spectroscopy and chemometrics that is one of the fields of chemistry to extract and pick-up information from a chemical system by using data-driven means such as statistics, applied mathematics and computing science.

Though there have been many results using near-infrared spectroscopy reported, almost of the studies were highly depended on chemometrics and statistical methods. The mechanism/origin of those measurements were still unclear in many cases. While many previous attempts have been especially focused on the statistical analyses for making calibration model, we have thought that the obtained data/parameter such as absorbance spectra should be discussed further for establishing reagentless monitoring ob blood substances. In this study, we focused on the human serum albumin (HSA), tried to observe the feature of HSA in the measured spectra and make a calibration model for determination of HSA concentration of its solution.

MATERIAL AND METHODS

SAMPLES

Powder state Human serum albumin (Albumin from Human Serum, 019-10503, WAKO Pure Chemical Industries Ltd., Japan) was used for experiments. Purity of the HSA sample was proved by electrophoresis to be higher than 95%.

Two groups of HAS samples were prepared. For group I, a powder state HSA sample was used. Group II consisted of the phosphate buffer solutions of HSA. The phosphate buffer solution (PBS) of pH 7.4 was prepared with using purchased PBS powder (phosphate buffer solution powder, 167-14491, WAKO Pure Chemical Industries Ltd., Japan) and pure water supplied with AUTOPURE (WT100, Yamato Corp. Japan). After sterilizing the PBS, its pH was adjusted to 7.4, a typical pH value of human blood, serum and plasma. The concentration of prepared HSA solution samples were in the range of 0.0 g/dl (PBS) to 5.0 g/dl, shown as Table 1. and each concentration contains three samples. For prepare HSA solutions, the weights of powder HSA were measured by a electronic balance and the volumes of solutions were made up by volumetric flasks.

Figure 1

Table 1 Concentration of human serum albumin in samples.

Concentration of human serum alubmin solutions of samples[g/dl]
0.0 (phosphate buffer solution), 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0

INSTRUMENTS

A FT-NIR spectrometer (Spectrum One NTS, PerkinElmer Japan Co., Ltd., Japan) was used for the absorbance measurements in the region of 750-2500 nm at a 1.6 nm resolution of wavelength. For group I, a diffuse reflectance cell was used for measurement. For group II, a flow cell of 1 mm optical length with using a quartz plate glass window (Quartz Glass Products, 6220-72011, GL Sciences Inc., Japan) was applied. For each sample of group II, absolute absorbance (background of the measurement was run with no sample) and differential absorbance from a reference PBS solution (pH: 7.4, concentration of HSA: 0.0 g/dl) were measured respectively. The temperature of the solution samples was kept at 27.0±0.3°C during the measurements. In the group II, the sequence of the measurement was randomized.

DATA PROCESSING AND CALIBRATION

The software “R” (version 2.9.2), its module “pls” (version 1.2-1) and “RTisean” (version 3.0.11) were used for spectral data processing [14]. All the data from group II were processed by the ‘Savitzky-Golay' filter for eliminate noise and artifacts on the spectral data. Multi variate regression analysis was performed for group II to buile calibration model estimating HSA concentration. Partial least square (PLS) regression was applied for the spectral data after ‘Savitzky-Golay' processing to analyze spectral data and develop a calibration model.

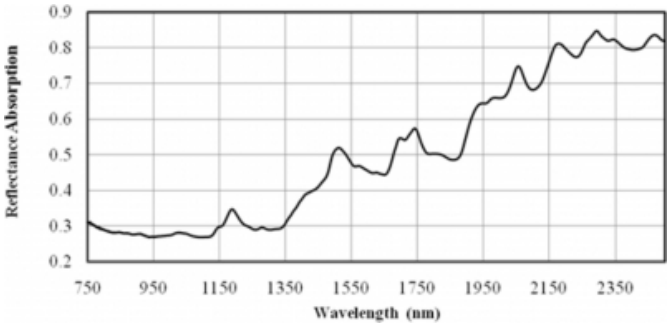
RESULTS

NIR SPECTRA OF SERUM ALBUMIN IN A POWDER STATE

The measurement of a powder HSA sample (Group I) using the FT-NIR spectrometer provided a diffuse reflectance absorption NIR spectra of the sample. Fig. 1 shows the diffuse reflectance absorption spectra of powder HSA in the 750–2500 nm region. From the spectra, several positive peaks (convex peaks) can be observed. The form of the spectra on the 1300–1850 nm region was quite similar with a previous report [Murayama] by using a conventional spectrometer with monochromator. As the previous paper suggested, peaks on spectra contained information of molecular feature of HSA.

Figure 2

Fig. 1 Near infrared diffuse reflectance near infrared spectra of human serum albumin in powder state.

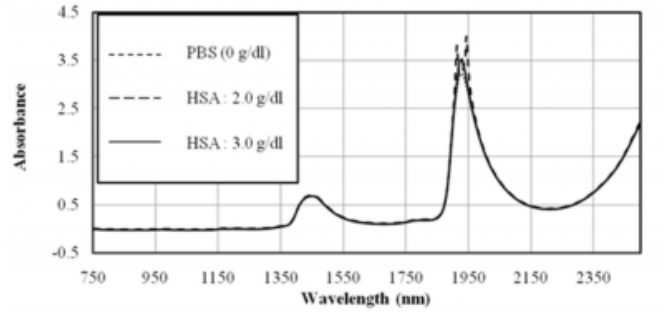


ABSOLUTE ABSORPTION OF HSA SAMPLES

Examples of absolute absorption spectra in the 750–2550 nm region of PBS and HSA solutions are shown in Fig. 2. The baseline shift on the measured spectra was removed by subtracting absorbance at 1200 nm. As shown in Fig. 2, strong absorption peaks near 1450 nm and 1900 nm were observed. Those peaks were from water absorption [15, 16]. Those strong water absorption mask the absorption of HSA, then it is too difficult to observe any features of dissolved HSA from the absolute absorption spectra.

Figure 3

Fig. 2 Examples of near infrared absolute absorbance spectra of phosphate buffer solutions of human serum albumin and PBS.

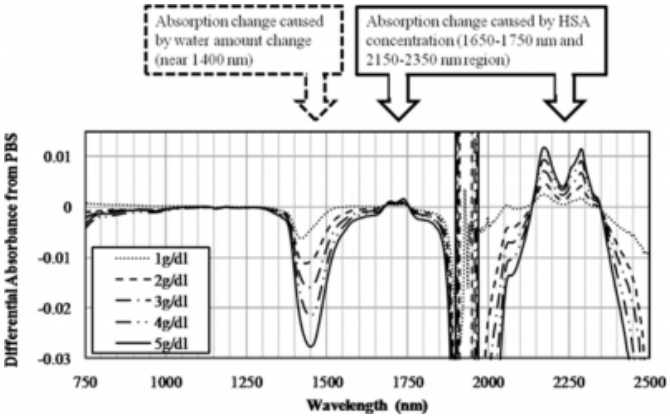


DIFFERENTIAL ABSORPTION OF HSA SOLUTIONS FROM A REFERENCE SOLUTION

Examples of differential absorption spectra HSA solutions from a reference PBS in the region of 750–2550 nm are shown in Fig. 3. The baseline shift on the measured differential spectra was also removed by subtracting absorbance at 1200 nm. As shown in Fig. 3, the band near 1950 nm cannot be measured because of the strong water absorption in the band. The observed peak near 1450 nm is also considered as caused by water amount change on the sample. Because the higher HSA concentration sample contains smaller water amount, therefore the sample of higher HSA concentration must have smaller absorption in the water dominant region such as near 1450 mm. In contrast, the peaks on the region of 1650-1750 nm and 2150-2350 nm should be considered as corresponding with HSA concentration of samples themselves. In those regions, the sample of higher HSA concentration had bigger absorbance peaks and the peaks on 2150-2350 nm were greater than the peaks on 1650-1750 nm. Additionally, the position of the differential absorption peaks on those regions coincided well with the position of the peaks on the diffuse reflectance spectra of the powder state HSA.

Figure 4

Fig. 3 Examples of near infrared differential absorbance spectra of phosphate buffer solutions of human serum albumin. Differential absorbencies were measured with the reference phosphate buffer solutions of human serum albumin whose concentration was 2.0 mg/dl.

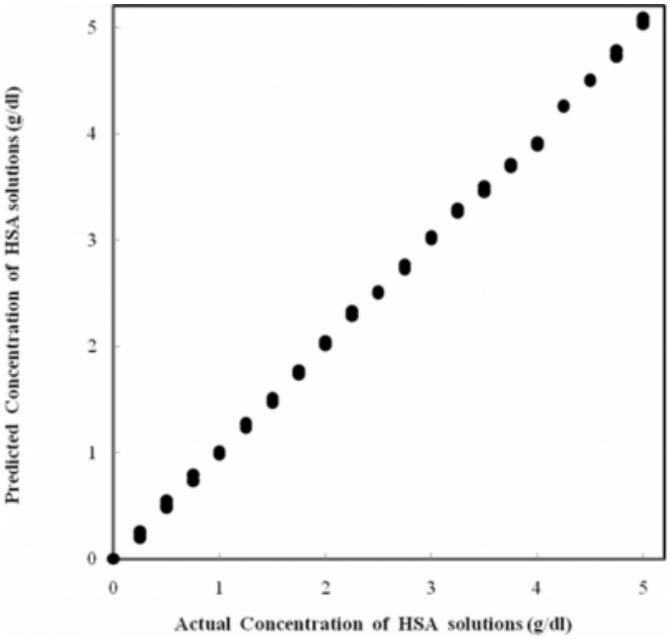


CALIBRATION MODELS FOR DETERMINATION OF THE HSA CONCENTRATION

The PLS regression analysis was applied to correlate the NIR differential spectrum from samples with the HSA concentrations of samples. The absorbance spectrum of 750-1350nm, 1550-1850nm and 2052-2500nm were applied for PLS analysis. The absorbance bands of 1351-1549 nm and 1851-2051 nm were rejected in advance, because water absorbance should be considered dominant in those bands. The leave-one-out cross validation was applied to evaluate regression model. As the result, the latent variable of PLS eight was obtained as the optimum parameter and the PLS model gave the standard error of prediction (SEP) of 0.0347 g/dl. Fig. 4 shows the result of the leave-one-out cross validation and the correlation coefficient of actual HSA concentration and predicted concentration is 0.999. This result strongly suggested that the differential absorbance involved information of HSA concentration.

Figure 5

Fig. 4 Calibration plot of predicted concentration of human serum albumin (HSA) solutions against actual concentration by leave one out cross validation.



Secondly, the samples were divided into two data set, one of which was used as a training data set and the other one as a test data set that was not used for constructing the calibration model using PLS regression. Concentrations of samples in each data set are shown respectively in the Table 2. The absorbance spectrum of 750-1350nm, 1550-1850nm and 2052-2500nm were also applied to make a calibration model. A PLS calibration model was made by the training data set in the latent variable of PLS seven and SEP for validation data set were of 0.0292 g/dl. Fig. 5 shows the HSA concentration prediction of validation data set and the correlation coefficient of actual HSA concentration and predicted concentration is 0.999.

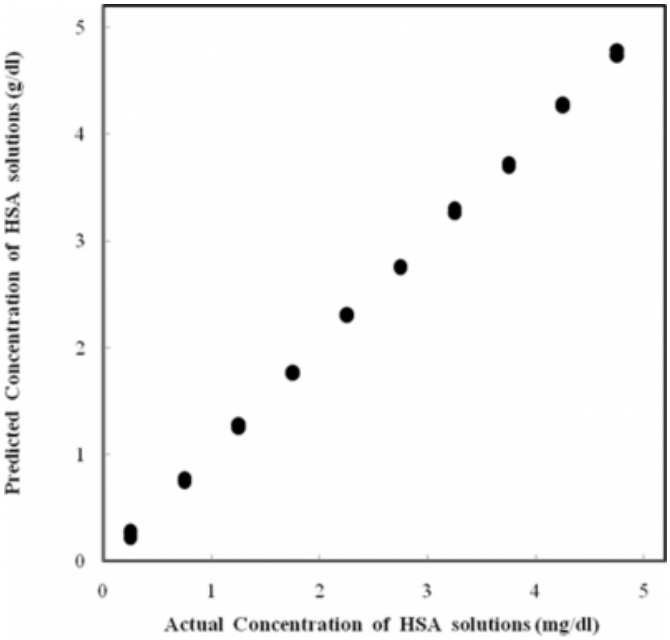
Figure 6

Table 2 Concentration of human serum albumin in each data set; training data set and validation data set.

Data set	Concentration of human serum albumin solutions of samples [g/dl]
Training data set	0.0 (phosphate buffer solution), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0
Validation data set	0.25, 0.75, 1.25, 1.75, 2.25, 2.75, 3.25, 3.75, 4.25, 4.75

Figure 7

Fig. 5 Calibration plot of predicted concentration of human serum albumin (HSA) solutions against actual concentration. The regression calibration model was made by training data set and the predicted values were obtained from the model and spectrum of validation data set.



DISCUSSION

Almost of previous report of protein concentration measurement including HSA measurement using NIR absorbance did not discussed about the measured spectral of protein itself. Among them, Hall et al. attempted to measure of albumin and gamma-goblin concentration in serum [Hall]. They found absorption change following albumin concentration in near-infrared region, however, correlation coefficient of absorption change and albumin concentration was negative. As Beer–Lambert law showing, the correlation coefficient of absorption change and the concentration of absorbing species in the material should be positive. Therefore, the result of Hall et al. is dubitable and further discussion is required to be accepted. From another group, Murayama et al. focused on the diffuse reflectance absorption spectra of powder state HSA and found the feature peak of absorption at 1650-1750 nm region [Murayama]. They also tried to determination of HSA concentration of phosphate buffer solution (pH: 7.0) using absolute absorbance spectra. Although they cannot find clear peaks on the spectra, the PLS calibration model employing second derivatives of the spectra provided good result. They found a positive peak on the regression coefficient for calibration and the position of the peak of regression

coefficient was well matched with the position of the peak of reflectance absorption spectra. Then, they concluded their calibration reflected the concentration of HSA. Their result can be considered as a good example of chemometrics that can find hidden chemical information from absorbance spectra. In contrast, our study tried to observe the clear peak on absorbance spectra of HSA phosphate buffer solution (pH: 7.4) and used differential absorbance from the reference solution to avoid strong water absorption, and then found clearly peaks corresponding with HSA concentration in the regions of 1650-1750 nm and 2150-2350 nm and the peaks on 2150-2350 nm were greater than the peaks on 1650-1750 nm shown as Fig. 3. The position of the position of the peaks of differential absorbance spectra of solutions was well matched with the position of the peak of reflectance absorption spectra. With comparison of differential absorbance spectra of solutions and reflectance absorption spectra of powder state HSA, it is clear that differential absorbance spectra of HSA consists information of its concentration that is hidden in absolute absorbance spectra. And the information of concentration can be picked up effectively by PLS regression. This is the first result of HSA concentration determination with using clearly observed spectral feature.

Additionally, preparation of the HSA solution sample was improved in this study compared to the previous Murayama's protocol. While the sample pH was controlled as 7.0 in Murayama's experiment, the sample pH of 7.4 was selected in our experiment, because blood, serum and plasma pH are regulated within the narrow range of 7.35 to 7.45 [for example, Kettel]. Blood pH of 7.0 means respiratory acidosis and the sample pH of 7.0 is too low as a simulated blood, serum and plasma.

Recently, we have proposed non-invasive in vivo measurement of blood glucose level using near infrared multi wavelength photoplethysmogram (PPG) named "pulseglucometry." [17-19] The "pulseglucometry" is based on the facts that glucose has absorption bands in near infrared region and PPG is derived from volume change of the arterial blood vessel of measurement site. We have attempted a spectral analysis of PPG and obtained good prediction of blood glucose level. This study shows that HSA has absorption bands in near infrared region, especially the regions of 2150-2350 nm. Therefore, PPG in the region could involve the information of albumin in arterial blood. As the next step, we plan in vivo measurement of albumin in blood using PPG with near infrared radiation.

CONCLUSION

Near-infrared diffuse reflectance absorption spectra of powder state human serum albumin (HSA) and differential absorbance from a reference phosphate buffer solution were measured for phosphate buffer solutions of HSA in the wavelength range of 750-2500nm. In the diffuse reflectance absorbance spectra of powder state HSA, positive peaks can be observed. In the differential spectra of HSA solutions from the reference, peaks that follow HSA concentration were able to be clearly observed especially in the region of 2150-2350 nm. The near-infrared spectra were subjected to partial least squares regression and obtain a good prediction of the concentrations of the HSA. These results are considered as a base of reagentless measurement of HSA in human serum and in vivo HSA measurement.

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