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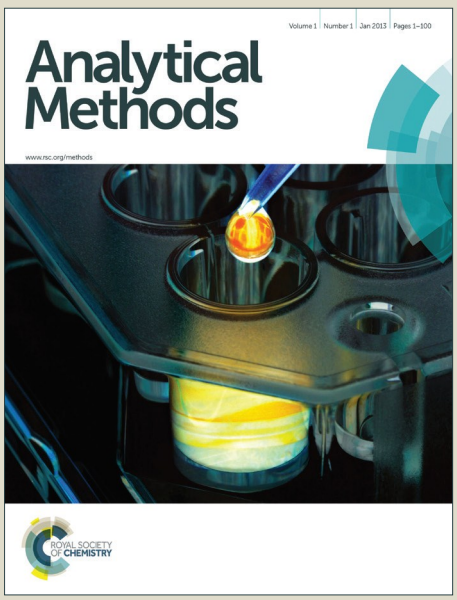

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Detection of free hemoglobin in blood products using transmission spectra and fluorescence spectra for quality assurance

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As the level of free hemoglobin is an indicator for hemolysis resulting from destroyed red blood cells, it is an important parameter to assess the quality and usability before transfusion. In order to ensure the quality of red blood cells before transfusion, sensitive and reliable pre-testing of blood products is required to avoid the errors due to hemolysis. In this paper, we presented a method to detect the free hemoglobin (fHb) concentration with the transmission spectra and the fluorescence spectra. It laid the reliable foundation for the further study of fHb concentration measurement in blood bags non-destructively. In this study, whole blood and plasma with different fHb concentration varying from 0.140 to 2.865g/L were prepared by professionals. All samples including fifty two whole blood samples and forty nine plasma samples. The fluorescence spectra and transmission spectra were then acquired respectively. Partial least squares model was then established to predict the fHb concentrations respectively. The concentration of fHb in whole blood or plasma can be detected well with transmission spectrum within a short time. And the fluorescence spectrum can only measure the concentration of fHb in plasma well. In addition, the prediction correlation coefficient has reached more than 0.98. The results further demonstrate a great potential of the transmission spectrum and the fluorescence spectrum for fHb concentration non-destructive measurement before transfusion.

1. Introduction

As the level of fHb is an indicator for hemolysis resulting from destroyed red blood cells, it is an important parameter to assess the quality and usability before transfusion.[1] During the collection, processing, preservation and transportation of blood or blood products. The rupture of the red blood cell is caused by many reasons, especially the improper preservation conditions in the process of the cold-chain logistic.[2-3] As a result, hemoglobin will drift away outside the cell, leading the fHb concentration increasing obviously and hemolysis. Therefore fHb content can reflect the extent of the damage of blood cell.[4] Previous researches have shown that fHb is an important new predictor of survival in severe sepsis and a novel diagnostic test for assessment of the depth of burn injury, etc.[5-6]

Through the decomposition of adenosine triphosphate (ATP) with glycolysis in the body, blood cells gain energy to maintain its morphology and function. As time goes by, the consumption of energy within cells and ATP results in the changes of blood cells morphology and function. These changes in the structure of membrane and cationic active transport function are hindered. Then hemoglobin drifted away from the cell. Infusing high content fHb will cause adverse reactions which affect the function of the nervous system and circulatory system and normal kidney function especially.[7-8] In addition, transfused blood contains fHb, as well as fragile erythrocytes that produce additional fHb.[9] Therefore, clinical inspection work should be taken all possible measures to avoid the specimen with hemolysis.[10] Studies on the fHb concentration non-destructive detection in blood bags need to be attached more attention in the future. In this paper, we presented the transmission spectra and the fluorescence spectra method to measure the fHb concentration non-destructively in quartz colorimetric utensil preliminarily.

The fHb is cracked into dimer combining with haptoglobin when hemolysis produced, and then it removed by the reticuloendothelial system. Once beyond the combining ability of haptoglobin, patients will get haemoglobinaemia in transfusions.[11] Therefore, hemolysis of different degrees is an important parameter to evaluate the quality of blood cells. At present, we evaluate the degree of hemolysis based on the concentration of fHb generally in clinic. Using no more than 0.8% or 1% hemolysis (the corresponding fHb concentration is at least no larger than 2 g/L) as the standard to evaluate the quality of blood products which transfused.[12]

At present, fHb quantitative analysis method includes spectrometry and chemical method. However, most of the analytical methods and testing equipment are limited to department or laboratory. The orthotolidine method and the three-wavelength method always have been used in detecting fHb concentration in clinical. The former has obvious disadvantages: tedious operation steps, many matters needing attention and poor

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stability. In addition, RSD (relative standard deviation) of the orthotolidine method is about 4%. And the latter can only measure the fHb concentration in plasma with complicated calculation, through detecting fHb standard concentration which are known, the deviation of this method is about 6%.[13-14] In addition, most methods depended on reagent additives to achieve the desired effect.[15] Although domestic and international transfusion units have sound security in the aspect of hemolysis, the hemolysis degree of red cell still can't be detected well.[16-17] Zhao et al. designed the portable fHb detector using spectrophotometry, which determines the concentration of free hemoglobin with adding benzidine.[18] And this method is appropriate for the fHb standard liquids instead of blood products, even though its correlation coefficient of prediction reaches up to 0.9998.

In order to ensure the quality of red blood cell before transfusing whole blood or blood products in pre-hospital, and avoid reperfusion injury induced by hemolysis, we presented the transmission spectra and the fluorescence spectra method to verify the reliability of fHb concentration measurement in this paper. It consequently ensures that we can detect the fHb concentration non-destructively, efficiently and accurately in pre-hospital.

2. Materials and methods

2.1 Samples

Fifty-two different fHb concentration of red blood cell suspensions and forty-nine different fHb concentration of plasma were configured in the Institute of Transfusion Medicine of the Academy of Military Medical Sciences. The fHb concentration ranged 0.140g/L from 2.865g/L, as is shown in the table 1. All experiments performed were in compliance with relevant laws, as well as with the guidelines of Chinese Academy of Sciences, Institute of Transfusion Medicine of the Academy of Military Medical Sciences and State Key Laboratory of Precision Measurement Technology and Instruments, Tianjin University. All the above mentioned institutes have approved the experiments. The volunteers had given their consents for the experiments. The work described in this paper has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Then anticoagulants were immediately added into the blood samples after they were acquired. Each transmission spectrum was measured for one hundred times with the integration time of fifty milliseconds and each fluorescence spectrum was measured for ten times with the integration time of two seconds.

2.2 Sample treatment methods

2.2.1 Blood routine test

After getting the blood samples, we took out one milliliter in a clean bench after shaking well. Then red blood cell (RBC), hemoglobin (Hb) and hematocrit (HCT) were detected with a hematology analyzer (BECKMAN COULTER Ac·T diff).

2.2.2 Free hemoglobin solution preparation

We took out five to ten milliliter red blood cell suspensions and made them all hemolysis after repeated freezing and thawing. Then supernatant was fHb solution after high speed centrifugation. We used colorimetric method to measure the fHb concentration. Kit used: the plasma free hemoglobin assay kit (Beijing Ruierda Bio-Technique Co. Ltd.).

2.2.3 Blood samples of different degree hemolysis preparation

The blood samples were divided into thirteen tubes which capacity was ten milliliter. Then we calculated in fHb concentration order which was 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2g/L respectively. The corresponding amount of fHb solution was added into the corresponding sample tube. And the samples were turned upside down several times for standby application.

2.2.4 Blood routine test of different hemolysis degree blood sample

One milliliter blood sample prepared was taken out to measure RBC, Hb and HCT in hematology analyzer.

2.2.5 Instrumentations and data collection

The spectra data acquiring system consisted of three major components – a test bench with a light source and an optical bundle, a spectrometer and a PC for controlling and saving data. As illustrated in Fig. 1. The broadband light source to measure transmission spectra was a constant-current tungsten lamp (C. F. technology (Beijing) Co., Ltd., 0-20W, 350-2600nm). The excitation light source to measure fluorescence spectra was a 365nm UV LED. The optical fiber bundle which contains several fibers with a numerical aperture (NA) of 0.22. The fiber optic spectrometer (AvaSpec-HS1024x58TEC-USB2, manufactured by Avantes, Holland) which has an adjustable spectral resolution (1.5-20nm), continuously scanned the transmission spectra or fluorescence spectra of blood samples over visible and NIR wavebands (200-1160nm) for a certain amount of time and the scans were stored in internal memory in real time. The spectrometer was controlled by the PC, and after the last measurement had been made the spectrometer sent the data through the USB to the PC. The quartz colorimetric utensil was used as a vessel. In order to obtain the best signal-to-noise ratio, the integration time of transmission spectra and fluorescence spectra was fifty milliseconds and two seconds respectively. To avoid the interference of excitation light source when measure the fluorescence spectra, the optical fiber bundle should be placed at the vertical side of the excitation light source, and this position can prevent the fluorescence from being absorbed in the blood sample again hopefully. The principle diagram of the specific position is shown in Fig. 2.

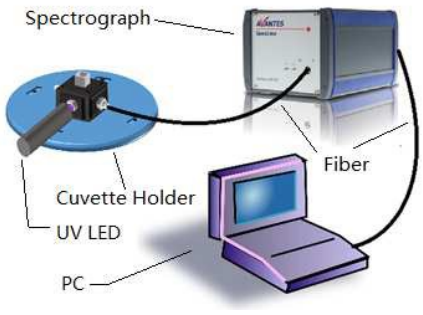


Fig. 1 Schematic diagram of the spectral data acquiring system.

Table 1 Fifty-two whole blood of different fHb concentration, and forty-nine plasma of different fHb concentration, except number 17, 31, 32 which were highlighted in italics

No.	fHb (g/L)	No.	fHb (g/L)	No.	fHb (g/L)	No.	fHb (g/L)
#1	0.1400	#14	0.7460	#27	1.2450	#40	1.7580
#2	0.2044	#15	0.8190	#28	1.2850	#41	1.7780
#3	0.2440	#16	0.8510	#29	1.3170	#42	1.9210
#4	0.2630	***#17***	***0.8870***	#30	1.3440	#43	2.0450
#5	0.3390	#18	0.9290	***#31***	***1.3660***	#44	2.0580
#6	0.4030	#19	1.0050	***#32***	***1.4140***	#45	2.0960
#7	0.4730	#20	1.0450	#33	1.4170	#46	2.2060
#8	0.5010	#21	1.1010	#34	1.4570	#47	2.2610
#9	0.5660	#22	1.1130	#35	1.6020	#48	2.4700
#10	0.5780	#23	1.1150	#36	1.6300	#49	2.5020
#11	0.6120	#24	1.2060	#37	1.6650	#50	2.5810
#12	0.7180	#25	1.2280	#38	1.7360	#51	2.5350
#13	0.7310	#26	1.2440	#39	1.7450	#52	2.8650

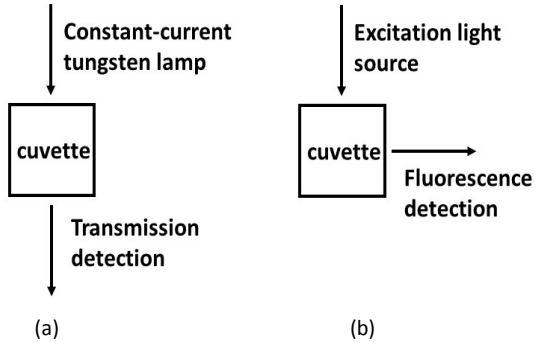


Fig. 2 The position of transmission spectra detection (a) and fluorescence spectra detection (b).

2.3 Data processing

All the data preparation and construction of statistical models were performed using MATLAB R2014a. For each transmission spectrum and fluorescence spectrum, one hundred spectra and ten spectra were collected respectively. These spectra were then averaged to form a representative single spectrum. Data standardization was used before constructing the model. The PLS method was used to predict free hemoglobin concentration.[19-20] The transmission spectra of whole blood, the fluorescence spectra of plasma and the transmission spectra of plasma are shown in Fig. 3. Generally, fluorescent peak was wide spectrum, rather than a sharp peak. Moreover, the sharp spectral peak should be part of the light source. As shown in Fig. 4. The 365nm UV LED we used in this experiment has several spectral peak or spectral band, especially at the wavelength of 740nm approximately. However, these sharp peaks did not affect the result of the modeling analysis because these wavelengths were out of band for modeling.

2.4 Partial least squares regression

Partial least-squares regression (PLSR) is a new multivariate statistical data analysis method. It was first proposed by S. Wold and C. Albano in the early 1980s for analyzing NIR spectra. Analyze the data which contains multiple dependent variable and multiple independent variable through extracting latent variable (that is the principal component) is the key. In order to ensure that extracted variables and output have a linear correction with significant level. PLS method relates the measured object information (such as composition concentration) to the extraction of principal component in the process of extracting principal component. PLS method holds the strong ability of identification and analysis, and it is suitable for quantitative regression analysis of complicated blood products.

We make an assumption that there are p independent variables $\{x_1, x_2, \cdots\cdots, x_p\}$, q dependent variables $\{y_1, y_2, \cdots\cdots, y_p\}$ and n samples. Then, corresponding matrix of the spectral data is $X=[x_1, x_2, \cdots\cdots, x_p]_{n\times p}$ and the matrix of multicomponent concentration data is $Y=[y_1, y_2, \cdots\cdots, y_q]_{n\times q}$, PLSR procedure is as follows:

(1) First, spectra data matrix X and Y should be standardized and they are marked E_0 and F_0 respectively;

(2) The first principal component t_1 and u_1 which represent the information of E_0 and F_0 as far as possible are extracted from E_0 and F_0 respectively, and the variables have the highest degree relate to (t_1, u_1), so the covariance between t_1 and u_1 reaches the maximum. Unit feature vector w_1 corresponding to the maximum eigenvalue of the matrix $F_0^T E_0 E_0^T F_0$ has been solved, and we can obtain the first principal component $t_1 = E_0 w_1$ of independent variable. In the same way, we can obtain the first principal component $u_1 = F_0 c_1$ of dependent variable.

(3) The residual matrix E_1 and F_1 of the first principal component are calculated respectively: $E_1 = E_0 - t_1 p_1^T$, $F_1 = F_0 - u_1 r_1^T$, which regression coefficient are $p_1 = E_0^T t_1 / \|t_1\|^2$, $r_1 = F_0^T u_1 / \|u_1\|^2$;

(4) Make $E_0 = E_1$, $F_0 = F_1$, repeat step (2) and step (3). Then extract principal component again until the optimal number of principal components is obtained;

(5) In order to minimize the RMSEP, the optimal number of principal components with a certain criterion like leave-one-out cross-validation methods should be choosed.

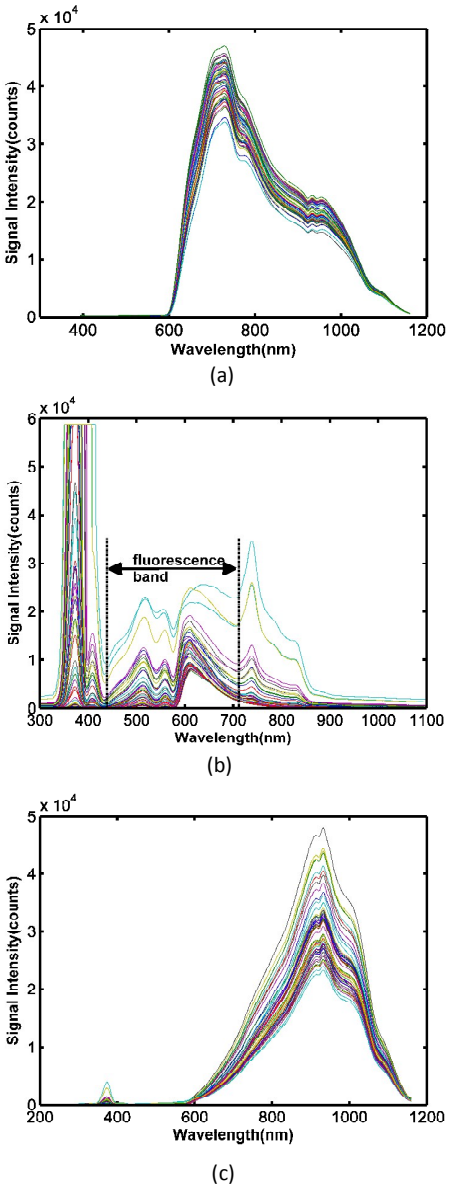


Fig. 3 (a) The transmission spectra of whole blood. (b) The fluorescence spectra of plasma. (c) The transmission spectra of plasma.

Results and discussion

3.1 Calibration set and Prediction set

The preprocessed spectra are shown in Fig. 3. These spectra are the total averaged data for their respective classed after preprocessing. All samples were divided into calibration set and prediction set. To get stable prediction results, calibration set and prediction set were selected by a ratio 2-to-1, and the fHb concentration of calibration set and prediction set covered the entire range of fHb concentration.[21-22]

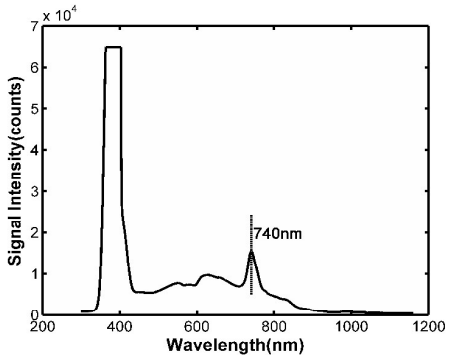


Fig. 4 The intrinsic spectrum of 365nm UV LED.

3.2 Construction of a model

The leave-one-out cross-validation method was used to choose the best number of factors.[23-24] The plots of the root mean square error of cross-validation (RMSECV) value versus the number of PLS factors are shown in Fig. 5 (a), Fig. 6 (a) and Fig. 7(a). The result of PLS modeling analysis is shown in Table. 2.

The determination of the optimal main factors is the committed step of PLS modeling. When PLS method is used to establish calibration model, the number of main factors is related to the predictive power of model. The spectral reconstruction fitting is insufficient if the main factor is too little. Meanwhile conversely, the spectral reconstruction fitting is excessive if the main factor is too much. In this paper, the performances of the developed calibration models were evaluated by the correction coefficient R, the root-mean-square error of cross-validation (RMSECV) and the root-mean-square error of prediction (RMSEP), and we choose the optimal main factors with leave-one-out cross-validation methods. The number of main factor is the optimal main factors when RMSECV reaches the minimum. Three spectroscopic method are analyzed as follows:

(1) Analysis of the transmission spectra of whole blood is shown in Fig. 5. When the number of factors was ten, the RMSECV value was the smallest. Therefore, ten factors were used to build the PLS-DA model. The correlation coefficient of calibration set is 0.9917 and the correlation coefficient of prediction set is 0.9524. The RMSEP is 0.2966.

(2) Analysis of the fluorescence spectra of plasma is shown in Fig. 5. When the number of factors was three, the RMSECV value was the smallest. Therefore, three factors were used to build the PLS-DA model. The correlation coefficient of calibration set is 0.9817 and the correlation coefficient of prediction set is 0.9774. The RMSEP is 0.1726.

(3) Analysis of the transmission spectra of plasma is shown in Fig. 5. When the number of factors was ten, the RMSECV value was the smallest. Therefore, ten factors were used to build the PLS-DA model. The correlation coefficient of calibration set is 0.9939 and

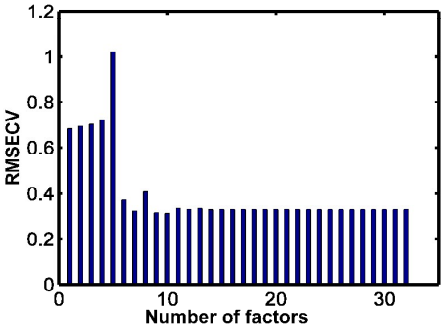
the correlation coefficient of prediction set is 0.9729. The RMSEP is 0.1640.

Table 2 The result of PLS modeling analysis

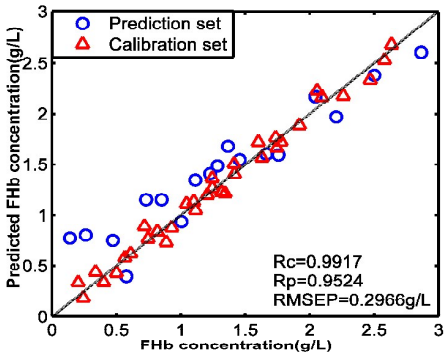
No.	Calibration Set	Prediction Set	N	R_m	R_t	RMSEP
1	34	18	10	0.9917	0.9524	0.2966
2	33	16	3	0.9817	0.9774	0.1726
3	33	16	10	0.9939	0.9729	0.1640

*No. 1 represents the transmission spectra of whole blood, No. 2 represents the fluorescence spectra of plasma and No. 3 represents the transmission spectra of plasma. In addition, N represents the number of main factors.

(1) The transmission spectra of whole blood



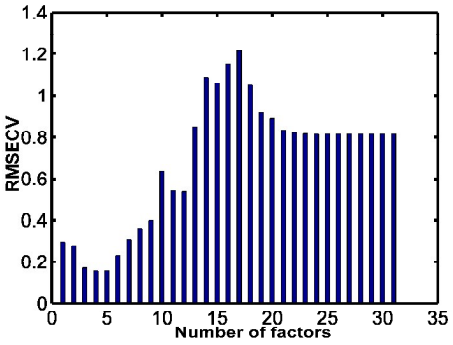
(a)



(b)

Fig. 5 Analysis of the transmission spectra of whole blood. (a) RMSECV versus different PLS factors. (b)Prediction of fHb concentration compared to reference fHb values obtained by hematology analyzer

(2) The fluorescence spectra of plasma



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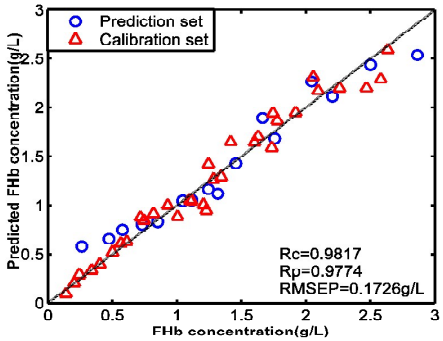
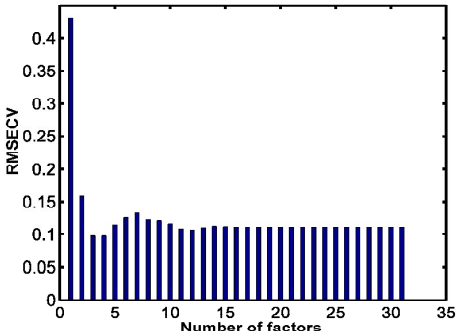


(b)

Fig. 6 Analysis of the fluorescence spectrum of plasma. (a) RMSECV versus different PLS factors. (b)Prediction of fHb concentration compared to reference fHb values obtained by hematology analyzer.

(3) The transmission spectrum of plasma



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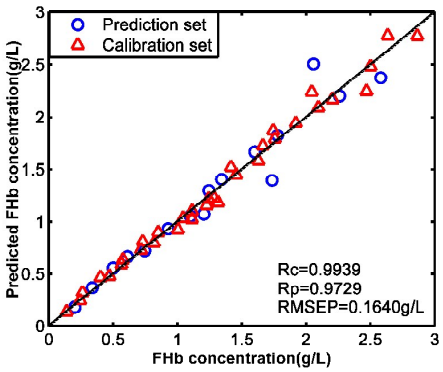

(b)

Fig. 7 Analysis of the transmission spectra of plasma. (a) RMSECV versus different PLS factors. (b)Prediction of fHb concentration compared to reference fHb values obtained by hematology analyzer.

Thus, we can come to the conclusion that the prediction ability of the method to identify the fHb concentration in whole blood or plasma with different spectrum. The remarkable prediction performance and the non-destructive characteristic of transmission spectroscopy or fluorescence spectroscopy make this approach well-suited for fHb concentration measurement. It consequently ensures that we can detect the fHb concentration non-destructively, efficiently and accurately in pre-hospital.

Conclusions

On the basis of whole blood and blood component standard quality, the fHb concentration is explicitly stipulated in the whole blood and red blood cells of cryopreserving and thawing besides red blood cell suspensions. It is particularly important to measure the fHb concentration of transfused blood when whole blood or blood products is hemolyzed. However, medical workers may not detect the fHb concentration and the degree of hemolysis accurately when the fHb concentration is greater than fifty mg/dl. Chemical measurement has some limitations, causing that it is difficult to operate outdoors when blood transfusion is badly in need.

In this study, we measured the whole blood and plasma of different fHb concentration, and the correlation coefficient (R_p) of whole blood transmission spectra, plasma fluorescence spectra and plasma transmission spectra is up to 0.9524, 0.9774 and 0.9729 respectively. Due to the high-concentration of whole blood, the serious reabsorption of fluorescence spectra lead to the weak signal. However, the three existing measuring results have been well shown in clinical fHb concentration detection. The transmission spectrum or fluorescence spectrum with the PLS method was demonstrated to be a powerful tool for fHb concentration measurement with a high degree of accuracy.

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The transmission spectrum and the fluorescence spectrum method for fHb concentration non-destructive measurement has been verified well.