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Relationship between polarization characteristics and hemolysis rate and its potential application

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ABSTRACT

Mueller polarimetry has been widely investigated in biomedical field. However the application in hemolysis monitoring is unavailable. This study deals with the backscattering polarimetric characterization of erythrocyte suspensions in low-osmolarity-induced hemolysis condition. Scattering and absorption are observed to decrease with increasing hemolysis. Increasing degree of polarization (DOP) corresponds to a decrease of scattering; increasing diattenuation corresponds to a decrease of scattering. However the decreasing absorption increases first and then decreases DOP in erythrocyte suspensions which are typical of Mie scatterers; reducing diattenuation corresponds to a decrease of absorption. Hence it is demonstrated that higher DOP is preserved in more serious hemolysis condition. In addition, DOP increasing trends are different in the conditions of below/above 6% hemolysis rate. Diatteunation shows an increasing trend at increase of blood hemolysis percentage with an exception when the hemolysis rate ranges from 5% to 6%. These results may be helpful for monitoring of hemolysis by polarimetric optical method.

1. Introduction

In recent years various optical methods have been proposed to monitor blood diseases. Near-infrared spectroscopy was investigated to monitor bacterial contamination of blood products [1]. Raman spectroscopy has been applied to analyze blood components [2]. Optical methods have also exhibited potential application in noninvasive blood glucose monitoring [3,4]. Hemolysis is another common blood disease which will take place in vivo or in blood transfusion process. It is a phenomenon that a cell loses its hemoglobin into the plasma due to the rupture of its erythrocyte membrane [5], which may be caused by immune system defects or external impacts originated from mechanical, chemical and biological factors. Hemolysis may lead to the diseases such as anemia and jaundice. Serious hemolysis is life-threatening and may be unrecognized because of lacking for specific symptoms. Identifying hemolysis and treating it promptly are essential for the survival of patient. Severe hemolysis can be detected by quantifying the hemoglobin amount in the urine. Generally, hemolysis can be measured by checking the half-life period of erythrocytes. However this method needs a radioactive nuclide which is harmful to the human body. Hence noninvasive or minimally invasive monitoring of slight hemolysis is of paramount importance.

Presently, polarimetry, as a promising developing optical method, has generated continuous interest because of its potential use in monitoring many diseases [6–8]. Mueller matrix represents the transfer function of an optical system in its interactions with polarized light. It encodes properties such as depolarization, diattenuation, linear retardance and optical activity. These properties embrace rich morphological and functional information of tissue and its underlying structure [9–11]. The polarization effects extracted from Mueller matrix have shown great potential in the biological field [12]. However the application in hemolysis monitoring is unavailable.

Here, we report the results of the backscattering polarization characteristics of erythrocyte suspensions in hemolysis condition given that the backward detection geometry is of clinical value [13]. It is found that on the whole both DOP and diattenuation show increasing trends with the increase of blood hemolysis percentage. However diattenuation drops when the hemolysis rate ranges from 5% to 6%. In addition, DOP increasing slope is larger in the condition below the hemolysis rate around 6% than the case when hemolysis rate is larger than 6%. When hemolysis rate is less than 6%, DOP is almost linearly proportional to it. This value is very close to the threshold of hemolytic reaction, 5% [14]. These results will find potential application to measure hemolysis rate by polarimetric method, especially in the case of slight hemolysis. Meanwhile the results shown in this paper are meaningful for minimally invasive hemolysis monitoring. Given that a little amount of blood is required in this study, it's possible to use this

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method to achieve minimally invasive monitoring of hemolysis, which can be achieved by puncturing the finger to draw a drop of blood. In addition, a recent investigation has shown the feasibility to combine spectral intensity and polarization signals to determine blood glucose noninvasively in turbid medium [15]. The results presented in this paper will supply as a significant study to achieve non-invasive hemolysis monitoring by the method.

2. Materials and methods

2.1. Preparation of erythrocyte suspensions in various osmolarities

Erythrocyte suspensions in various hemolysis conditions were prepared by the following procedures. Fresh blood was withdrawn from apparently healthy New Zealand rabbits by venipuncture and collected in centrifuge tubes with CPDA (anticoagulant: Citrate Phosphate Dextrose Adenine). The blood was kept at 4 °C for no more than 1 day and then centrifuged at 1500 rpm for 5 min three times with isotonic saline solution to remove blood plasma. Fig. 1 shows a sample image after the blood was centrifuged three times. It is seen that erythrocytes exists on the bottom. After separating erythrocytes from the whole blood, erythrocyte suspensions were then prepared by mixing erythrocytes and saline solution.

The hematocrit (hct) was adjusted to be 4% by dilution with saline solution buffers in various osmolarities. Distinct hypotonic buffers were formed by adding different amount of distilled water into physiological saline solution. The relationship between osmolarity and concentration of saline solution can be expressed as

$$osm = \frac{\text{Solute grams per kilogram of solvent}(g/kg)}{\text{Molecular weight of solute}(g)} \times n \times 1000\,\text{mOsmol/kg} \quad (1)$$

Here n is the number of particles formed by dissolution of a solute molecule. For sodium chloride, n is equal to 2 and the molecular weight is 58.5. Accordingly the isotonic osmolarity is calculated as 307.7 mOsmol/kg. The other osmolarites can be obtained according to Eq. (1).

The erythrocyte suspensions were put into a laboratory beaker with 5 cm diameter. The sample thickness is set as 2.5 cm so that the volume for one sample is about 50 ml. This means that 4 ml whole blood is needed for one portion of erythrocyte suspension. Less amount of blood is also possible by reducing the container's diameter if the method is applied on clinical occasion. During experiment, the suspensions were gently stirred to avoid uncontrolled sedimentation or cell aggregation.

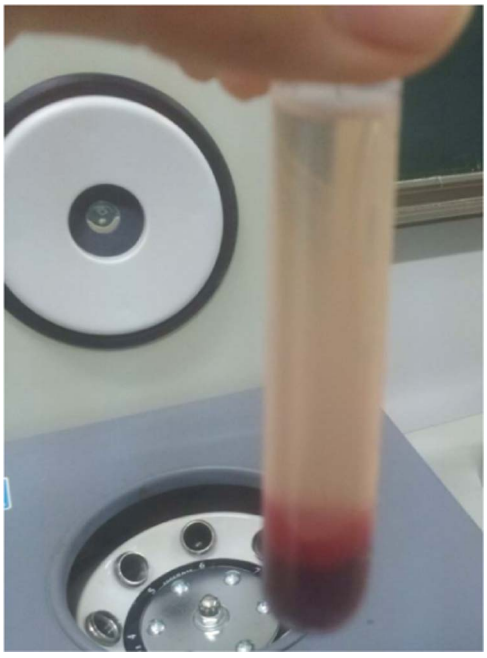

Fig. 1. Blood after centrifugation.

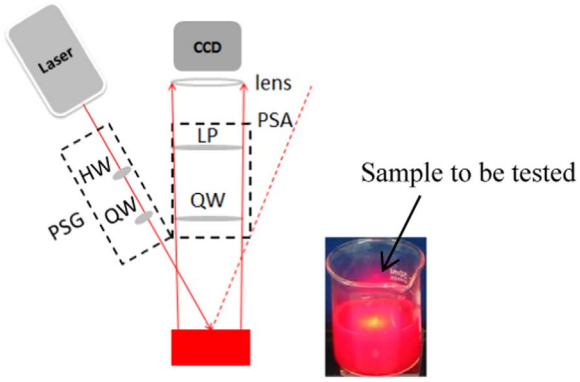

Fig. 2. Schematic diagram of the experimental setup. HW denotes a half-wave plate, QW denotes a quarter-wave plate, LP is a linear polarizer.

2.2. Imaging method

Backscattering Mueller matrices for erythrocyte suspensions in various low osmolarites were performed by a retarder/polarizer rotating polarimeter which has been investigated in many works [16–18]. The schematic diagram is shown in Fig. 2. The linearly polarized light at the wavelength of 632.8 nm is incident on the sample through a half-wave plate and a quarter-wave plate. The emergent light from the backscattering surface of the sample will go through a quarter-wave plate and a linear polarizer before it gets to the charge-couple device (CCD), which has no polarization sensitivity. A sample to be tested is also shown in Fig. 2. We calculated the backscattering Mueller matrix by Eq. (2) after acquiring 6×6 images with 6 different input and output polarization states which are horizontal (H), vertical (V), linear 45 deg (P), linear −45 deg (Q), circular right-handed (R) and circular left-handed (L) polarizations respectively. The two capital letters in Eq. (2) denote the polarization state of PSG (polarization state generator) and PSA (polarization state analyzer) respectively.

$$M = \begin{bmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{bmatrix} = \frac{1}{4} \begin{bmatrix} HH+HV+VH+VV & HH+HV-VH-VV & PH+PV-QH-QV & RH+RV-LH-LV \\ HH-HV+VH-VV & HH-HV-VH+VV & PH+QV-QH-PV & RH+LV-LH-RV \\ HP-HQ+VP-VQ & HP+VQ-VP-HQ & PP+QQ-QP-PQ & RP+LQ-LP-RQ \\ HR-HL+VR-VL & VL+HR-HL-VR & QL+PR-PL-QR & LL+RR-RL-LR \end{bmatrix} \quad (2)$$

It is necessary to address the problems originated from the experimental setup configuration. The first one is the speckle concern. The amplitude of the speckle corresponds to a white Gaussian noise from a signal processing point of view. In addition, the Brownian motion process is the time integration of the white Gaussian noise [19]. In our investigation, the exposure time of the CCD is adjusted to be 10 ms, which is far longer than the characteristic time of Brownian motion. Therefore the noise stemming from the speckle effect becomes uniform. As shown in Eq. (2), all the elements in Mueller matrix, except m_{11}, are obtained via the operations of two additions and two subtractions; hence the impact from the background noise, including speckle effect due to the optical instruments, can be minimized. Secondly, it's