Influence of oxygen saturation on the optical scattering properties of human red blood cells in the spectral range 250 to 2000 nm

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Abstract. The intrinsic optical parameters absorption coefficient μ_a, scattering coefficient μ_s, anisotropy factor g, and effective scattering coefficient μ_s' were determined for human red blood cell (RBC) suspensions of hematocrit 33.2% dependent on the oxygen saturation (SAT O_2) in the wavelength range 250 to 2000 nm, including the range above 1100 nm, about which there are no data available in the literature. Integrating sphere measurements of light transmittance and reflectance in combination with inverse Monte Carlo simulation were carried out for SAT O_2 levels of 100 and 0%. In the wavelength range up to 1200 nm, the absorption behavior is determined by the hemoglobin absorption. The spectral range above the cells' absorption shows no dependence on SAT O_2 and approximates the absorption of water with values 20 to 30% below the respective values for water. Parameters μ_s and g are significantly influenced by the SAT O_2-induced absorption changes. Above 600 nm, μ_s decreases continuously from values of 85 mm^{-1} to values of 30 mm^{-1} at 2000 nm. The anisotropy factor shows a slight decrease with wavelengths above 600 nm. In the spectral regions of 1450 and 1900 nm where water has local absorption maxima, g shows a significant decrease down to 0.85, whereas μ_s' increases. [DOI: 10.1117/1.3127200]

Keywords: human red blood cells (RBCs); oxygen saturation; absorption coefficient; scattering coefficient; anisotropy factor; Monte Carlo simulation; visible; near infrared.

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1 Introduction

The development of spectroscopic methods for blood analysis requires knowledge of the light-scattering and absorption properties of human red blood cells (RBCs). It is also important for many diagnostic and therapeutic applications in laser medicine, hematology, and medical routine diagnosis. Hemoglobin oxygenation, which has a significant influence on the optical behavior of red blood cells, is an especially important diagnostic parameter in the fields of heart surgery, intensive care, and neonatology. Possible applications are measurement of the blood oxygen saturation in cardiopulmonary systems or measurement of the brain's oxygen supply.

According to the radiation transport theory, the optical properties of red blood cells can be described by the intrinsic optical parameters: absorption coefficient μ_a, scattering coefficient μ_s, and anisotropy factor g, together with the appropriate phase function. Various approaches have been taken to determine the optical properties of blood cells.[1–8] In most cases, the investigated wavelength range did not extend to 1100 or 1200 nm. Due to the high optical density of blood, especially at physiological concentrations, it has not been possible, in most cases, to determine the parameters μ_s and g separately. Only the effective scattering coefficient $\mu_s'=\mu_s(1-g)$ could be determined for single wavelengths or for small spectral ranges where the absorption of hemoglobin is low. Using the double integrating sphere technique combined with an inverse Monte Carlo simulation (iMCS),[2,4,9] all three optical parameters for a flowing red blood cell suspension could be determined independently in the spectral range of 250 to 2000 nm, including the spectral areas of high hemoglobin absorption and the two distinct absorption peaks of water.

It is known that the optical behavior of blood depends on various physiological parameters,[10–12] one of the most important being the oxygen saturation of the hemoglobin. It is well known that a change in the oxygen saturation causes characteristic changes in the absorption behavior of hemoglobin, which determines the absorption of red blood cells. It is also known that changes in μ_a can influence the scattering properties, especially the anisotropy factor g.[4,13] With regard to the

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physiological importance of the oxygen saturation and the increasing application of optical methods in medicine, it is a matter of some interest as to whether the oxygen saturation can influence the scattering properties.

The aim of the present study is to determine the optical parameters μ_a, μ_s, and g of a flowing red blood cell suspension at physiological hematocrit (Hct) in the wavelength range above 1100 nm and to investigate the influence of oxygen saturation on the optical properties in the wavelength range 250 to 2000 nm. To do this, the optical parameters μ_a, μ_s, and g were determined for human red blood cells (RBCs) for both the fully oxygenated (SAT O_2 100%) and deoxygenated states (SAT O_2 0%). In order to facilitate comparison with data from the literature, μ_s' was also calculated. In all experiments, native RBCs were suspended in saline solution and kept flowing with a wall shear rate of 600 s^{-1} to avoid cell aggregation.[4]

2 Material and Methods

2.1 *Blood Cell Preparation*

In order to measure native RBCs in saline solution, three samples of fresh human erythrocytes from a healthy blood donor were centrifuged three times (250 g) and washed with isotonic phosphate buffer (300 mosmol/L, pH 7.4) to remove the blood plasma and free hemoglobin. This procedure does not influence the biological function of the RBCs but inhibits the formation of aggregates. The hematocrit was determined using a red blood cell counter (Micros 60 OT 18, ABX Diagnostics, Montpellier, France). To investigate the influence of the oxygen saturation, the blood sample was adjusted to a Hct of 32.2% and the sample was divided in half. One part was equilibrated with a gas mixture of 96% O_2 and 4% CO_2 to keep the sample in the fully oxygenated state; the other part was equilibrated with a gas mixture of 96% N_2 and 4% CO_2, after addition of 0.3% sodium dithionite ($Na_2S_2O_4$) to ensure complete deoxygenation.[9] The oxygen saturation was determined with a blood gas analyzer (OPTI Care, AVL Medizintechnik GmbH, Bad Homburg). A miniaturized blood circulation setup was used with a roller pump (Sorin Group, Germany) and a blood cell reservoir, which was constantly aerated with the gas mixture. The temperature was kept constant at 20 °C. The red blood cell suspension was gently stirred to avoid uncontrolled sedimentation or cell aggregation within the reservoir. The cell suspension was kept flowing by a customized turbulence-free cuvette with laminar flow and a sample thickness of 116 μm. A cuvette thickness of approximately 100 μm ensures multiple scattering and significant light transmission as well as avoiding obstruction phenomena. The flow was adjusted for each sample to keep constant wall shear rates of 600 s^{-1} at the inner cuvette surfaces, avoiding aggregation or sedimentation at flow stop and shear-rate-induced deformation of the cells at high shear rates above 1000 s^{-1} (Refs. 11 and 14).

2.2 *Spectral Measurements*

The diffuse reflectance R_d, the total transmission T_t, and the diffuse transmission T_d (=T_t−transmission within an aperture angle of 5.3 deg) of all blood cell samples were measured using an integrating sphere spectrometer (Lambda 900, Perkin Elmer, Rodgau-Jügesheim, Germany) in the spectral range of 250 to 2000 nm at 5-nm intervals in the range 250 to 1200 nm and at 10-nm intervals above 1200 nm. The optical arrangement of a sample and a reference beam compensates for light intensity shifts and changes in the inner sphere reflectivity by positioning the glass cuvette in front of or behind the sphere. For the measurement of T_t, the reflectance port of the sphere is closed with a diffuse reflecting Spectralon standard. T_d is measured after the standard is removed so that nonscattered and scattered transmitted light leaves the sphere within an angle of 5.3 deg. R_d is measured relative to a certified reflectance standard, and the Fresnel reflectance of the cuvette glass leaves the sphere. The experimental setup allowed measurement of the macroscopic radiation distribution with an error of less than 0.1% and has already been described by Friebel et al.[4] A special inverse Monte Carlo simulation (iMCS) program was used, including forward Monte Carlo simulations to calculate the parameters μ_a, μ_s, and g iteratively on the basis of a given phase function and the measured values for reflection and transmission. It considers all kinds of radiation losses by simulating the exact geometry of the illuminating light beam and of the sample, the cuvette, and the integrating sphere, including all diaphragms and apertures. As starting values, the parameters μ_a, μ_s, and g were estimated from the Kubelka-Munk theory, and the approximation to the measured values was carried out by calculating a gradient matrix.[4] Hct-dependent effective phase functions for RBCs flowing with a shear rate of 600 s^{-1} were evaluated previously using the double integrating sphere technique.[4] The iMCS was carried out using the Reynolds-McCormick phase[15] function with α=1.7 for Hct 33.2%. An error threshold of 0.1%, i.e., the difference between measured and simulated macroscopic radiation distribution, was used for the calculation of the intrinsic optical parameters of the red blood cells. A total of three independent measurement series were carried out for all three samples, and independently simulated, using 10^7 photons for each simulation. Due to the strong interindividual differences of RBCs, standard data can be obtained only by taking the mean values of many different samples. In order to exclude the optical influence due to this biological variability of the RBCs, all measured changes in the optical properties induced by reducing the oxygen saturation in the wavelength range 250 to 1100 nm were calculated as relative values related to those determined at 100% oxygen saturation. The absolute values were obtained by multiplication with the averaged parameters of a standard RBC suspension with the same Hct 33.2% (Ref. 12). As standard data are available only in the spectral range 250 to 1100 nm, the optical parameters determined in this experiment for the wavelength range 1100 to 2000 nm were linearly adapted to the standardized data in the range 250 to 1100 nm to reach a smooth junction in the region of 1100 nm.

3 Results

It is known that the optical parameters of blood samples from different donors with identical hematocrit can show considerable variability.[12] Therefore, in prior studies, Hct-dependent standard blood parameters were defined as averaged values of a number of individual blood samples measured in the spec-

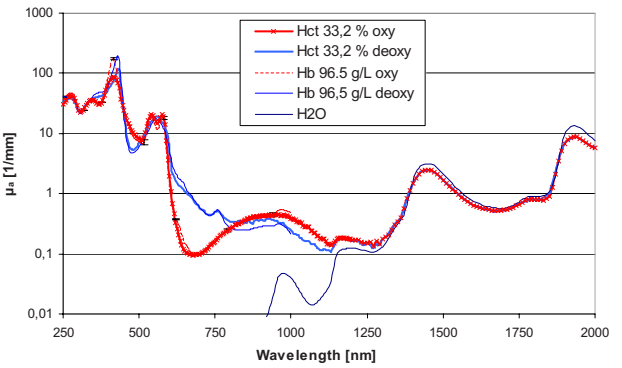

Fig. 1 μ_a of RBCs in saline solution with a Hct 33.2% dependent on wavelength with an oxygen saturation of 100% and 0% compared with a hemoglobin solution with a corresponding hemoglobin concentration of 96.5 g/dL.

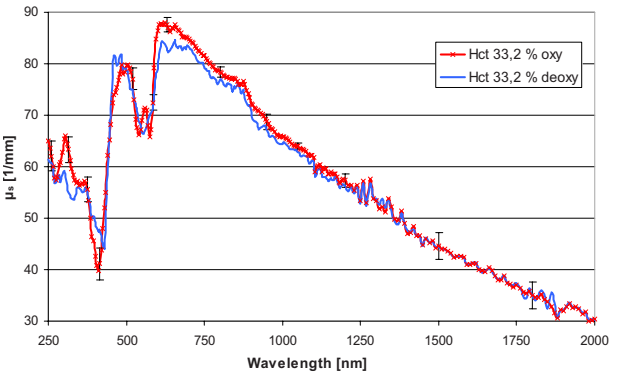

Fig. 2 μ_s of RBCs in saline solution with Hct 33.2% dependent on wavelength with an oxygen saturation of 100% and 0%.

tral range 250 to 1100 nm under standard conditions (wall shear rate 600 s^{-1}, oxygen saturation 98 to 100%, osmolarity 300 mosmol/l, pH 7.4, 20 °C).[12]

The resulting standard deviations of these standardized data, caused by the biological variability, averaged over all wavelengths are 4.9% for μ_a, 8.4% for μ_s, 12.8% for $(1-g)$, and 16.0% for μ_s' (Ref. 12) The standard deviations of three independent measurements made in this paper are for μ_a 2 to 4%, for μ_s and $(1-g)$ 3 to 8%, and for μ_s' 8-12%, as shown in the diagrams.

3.1 *Absorption*

Figure 1 shows the absorption spectrum of red blood cells of Hct 33.2% with an oxygen saturation of 100% and 0% in the wavelength range 250 to 2000 nm. For comparison with data from the literature, the absorption spectra of pure water and of hemoglobin solutions extrapolated to a hemoglobin concentration of 96.5 g/L (Ref. 16), are also depicted in the wavelength range 250 to 1100 nm.[17] At complete oxygenation, the absorption spectrum shows the characteristic peaks at 415 nm (87 mm^{-1}) and the double peak at 540/575 nm with values of 20.8 mm^{-1}. The extrapolated hemoglobin curve is between 250 and 1000 nm, within the error tolerance identical to the RBC absorption spectrum, with the exception of the maximum of 174 mm^{-1} at 415 nm. This difference results from the phenomenon of absorption flattening and can be explained by the Sieve effect.[18] Above 1100 nm, the μ_a curve increasingly approximates that of water absorption. Above 1360 nm, the RBC absorption follows exactly the shape of the water curve but with values 20 to 30% below the water absorption values. This can be explained by the absence of significant hemoglobin absorption in this spectral area leading to a μ_a decrease due to the reduced water content in the cells as a result of the replacement by the hemoglobin molecules. The curve of the deoxygenated sample shows characteristic changes in the absorption behavior with a wavelength shift of the maximum peak of 117 mm^{-1} to 430nm and a unique peak at 550 nm (19.2 mm^{-1}). This result also corresponds to the absorption curve of deoxygenated hemoglobin from the literature, taking into account that the Sieve effect induces a lower absorption of the red blood cell suspension compared to the hemoglobin solution with 195 mm^{-1} at 430 nm. In the spectral range above 1150 nm, μ_a is independent of the hemoglobin oxygen saturation.

3.2 *Scattering*

Figure 2 shows the spectrum of the scattering coefficient μ_s of red blood cells of Hct 33.2% with an oxygen saturation of 100% and 0% in the wavelength range 250 to 2000 nm. In the spectral range up to 600 nm, the μ_s spectrum shows the characteristic decreases correlated with the absorption peaks at 415 nm and 540/575 nm as described earlier.[4] According to the phenomenon of the absorption-induced decrease in μ_s, the curve of completely deoxygenated RBCs shows the corresponding changes. The wavelength of the local minimum shifts from 415 nm to 430 nm, and the double peak at 540/575 is replaced by the single peak at 550 nm. After reaching the maximum in the region of 600 nm, with μ_s values between 83 and 88 mm^{-1}, μ_s decreases continuously with increasing wavelength to values of about 30 mm^{-1} at 2000 nm, independent of the oxygen saturation. This decrease is with $\lambda^{-0.93}$ and near to the theoretically expected value of λ^{-1} according to Mie theory when the sphere size is large compared to the wavelength.[19]

3.3 *Anisotropy*

Figure 3 shows the values of the anisotropy factor g of RBCs

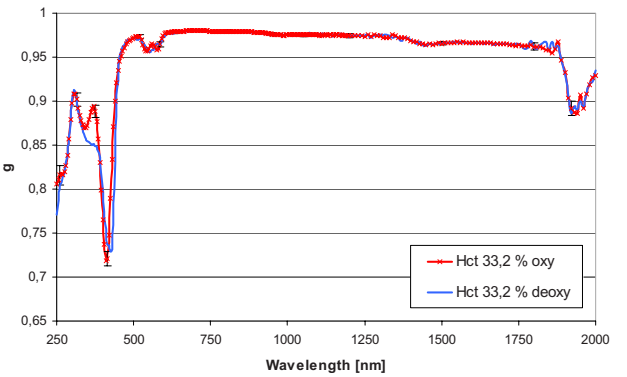

Fig. 3 g of RBCs in saline solution with Hct 33.2% dependent on wavelength with an oxygen saturation of 100% and 0%.

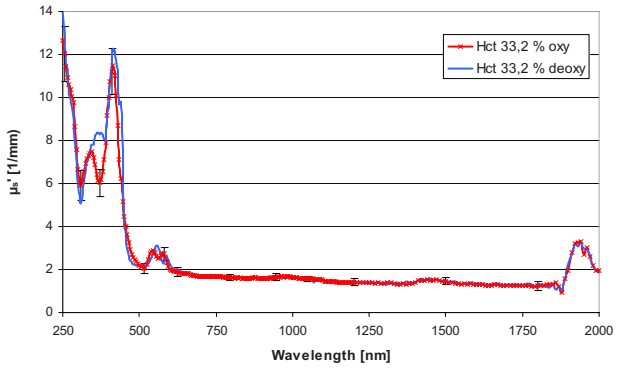

Fig. 4 μ_s' of RBCs in saline solution with Hct 33.2% dependent on wavelength with an oxygen saturation of 100% and 0%.

suspended in saline solution for two different oxygen saturation states, dependent on wavelength. The anisotropy factor follows the absorption spectrum inversely with the distinct minimum of 0.72 at 415 nm at an oxygen saturation of 100%, as described earlier.[4] In the completely deoxygenated state, the minimum shifts to 430 nm. The local absorption minimum in the oxygenated state at 370 nm results in a relative g maximum, whereas this g maximum disappears as expected in the deoxygenated state. The double minimum at 540/575 nm in the oxygenated state turns into a single minimum at 550 nm with complete deoxygenation. Above 600 nm, where g is at the maximum level of 0.977, the g spectrum of the oxygenated RBCs and the deoxygenated RBCs are equal. The value of g decreases continuously to about 0.96 at 1800 nm, with the exception of a slight minimum at 1480 nm and a distinct local minimum at 1900 nm with a value of 0.87. In contrast to the scattering parameter μ_s, g appears to be influenced not only by the hemoglobin absorption in the wavelength range below 600 nm, i.e., absorption within the scattering cells, but also by the absorption maxima of water in the region of 1480 nm and 1900 nm, i.e., absorption within the medium and a reduced absorption within the scatterer.

3.4 *Effective Scattering Coefficient*

Figure 4 gives the calculated values of the effective scattering coefficient $\mu_s'=\mu_s(1-g)$ for both oxygen saturation states in the wavelength range 250 to 2000 nm. If compared to the anisotropy factor, μ_s' shows inverse spectra for both 100% and 0% oxygen saturation, which reflects the absorption spectrum. The maximum for the deoxygenated state is 13.9 mm^{-1} at 250 nm in the observed spectral range. The other distinct maximum is 8.3 mm^{-1} at 380 nm. At 530 nm, the characteristic relative single maximum is about 3 mm^{-1}. At complete oxygen saturation, the respective relative maxima are 12.5 mm^{-1} at 250 nm, 11.5 mm^{-1} at 360 nm, and the double peak at 530/575 nm with values in the region of 3 mm^{-1}. Above 600 nm, μ_s' is in the range of 1.8 mm^{-1} and decreases continuously and independently of oxygen saturation to values of about 1.0 mm^{-1} at 1880 nm. At 1950 nm, μ_s' shows the water-absorption-induced maximum with values of 3.3 mm^{-1}. Corresponding to the minimum value of g at 1480 nm, a local maximum is visible but not significant.

4 Discussions

4.1 *Oxygenation-Induced Effects*

It could be shown that differences in the oxygen saturation induce changes not only in absorption but also in the optical scattering parameters μ_s, g and μ_s'. This is due to the characteristic change in the absorption behavior of hemoglobin. As the hemoglobin absorption is invariant against the oxygen saturation between 1300 and 2000 nm but exhibits strong saturation dependence in the range 250 to 600 nm, the induced changes of the scattering parameter μ_s occur mainly between 250 and 1100 nm, whereas the saturation-induced changes of g and μ_s' occur only between 250 and 600 nm.

The strongest oxygen saturation dependence of g, in relation to the oxygenated state, is at wavelengths 250 nm and 375 nm, with a decrease of 4.5%, and in the range 410 to 430 nm, with a maximal decrease of 12%. Due to changes in saturation, the maximum change in μ_s is 15% at 400 nm and 5% at 570 nm. In the range 700 to 850 nm, μ_s decreases by about 2%. Above 750 to 800 nm, the induced changes in μ_s are not significant. Apart from absorption, μ_s' shows the strongest oxygen dependence at the same wavelength regions as g. The maximum change in μ_s' of about 35% is at 375 nm, up to 25% in the range 410 to 430 nm and 15% at 575 nm At 250 nm, μ_s' increases by 10%, which means that significant changes have to be considered when the scattering behaviour of RBCs is investigated and there are changes in the oxygen saturation.

The results partly contradict earlier results published by our group[9] because the scattering has now been shown to be influenced by the absorption properties. This has been shown using a commercially available spectrometer, which offers a much higher degree of accuracy and considerably shorter measurement times, leading to a reduction in the influence of hemolysis-induced optical effects.

These results are partly in agreement with the data presented by Faber et al.,[10] who calculated the optical parameters in the wavelength range 250 to 1000 nm via Mie theory from the complex refractive indices of oxygenated and deoxygenated hemoglobin solutions taken from porcine blood. In that paper, μ_s shows similar characteristic differences between deoxygenated RBCs and fully oxygenated RBCs. The difference in the absolute values are of minor importance since Faber's values are linearly calculated from the scattering cross sections for a hematocrit of 50%, assuming independent scattering of the cells, which is not obvious at such high cell concentrations. The minimum value at 410 nm shifts to 430 nm, and the maximum value in the area of 500 nm shifts to a more pronounced peak at 470 nm. The double minimum at 540/575 nm changes into a single minimum at 560 nm, and in the range 600 to 1000 nm, the curve of the deoxygenated RBCs runs below the curve of oxygenated RBCs. It is only in the spectral range 250 to 400 nm that the data do not fit with the data presented here. This is possibly due to differences in the calculated complex refractive index of hemoglobin by Faber et al., which is quite different from other measured index values in this spectral range.[16] The anisotropy factor of oxygenated RBCs shows also a minimum near 420 nm, which shifts to longer wavelengths when deoxygenated. In the same way, the double minimum at 540/575 nm

vanishes. The constant higher level of the deoxygenation curve compared to the oxygenation curve above 500 nm together with the behavior of g in the wavelength range 250 to 400 nm could not be observed in the experiments presented here. Also, the absolute g values of Faber et al. are higher than the ones presented here, which can also be explained by assuming independent scattering within the calculation.

Even though Mie theory fails to predict the absolute values of the scattering parameters μ_s and g at physiological hematocrit values,[4] special phenomena can be recognized in the spectra that could be calculated according to Mie theory. For example, the relative minima of μ_s at 540 and 575 nm induced by the relative absorption maxima at the same wavelengths can also be seen in Mie calculations. With this in mind, the influence of the absorption on μ_s is also explainable in principle by the Mie theory.[4] However, the phenomenon of the significant decrease of the anisotropy factor g when μ_a is increased is more difficult. This behavior shows Mie theory only if μ_a were to be 100 times greater. Since the decrease of g tended to become smaller when the RBCs were changed to a more spherical shape, it can be assumed that the nonspherical shape is responsible for this discrepancy.[4] Independent of this, it seems to be feasible that an increase in the complex refractive index results in an increase in the reflection and a decrease in transmittance, resulting in increased backscattering of the photons, which means a smaller g.

There are further indications in the literature that absorption peaks in light scattering media may lead to a reduction of μ_s and g and an increase in μ_s' (Refs. 8, 10, and 13).

It is of considerable interest that the changes in water absorption at 1450 and 1900 nm induce changes in g in the same way as the strong changes in hemoglobin absorption do. In both cases, absorption is increased within the scatterer. In the case of water absorption, there is an additional and stronger absorption increase within the surrounding medium. μ_s shows no comparable change at the spectral regions of high water absorption, whereas g and μ_s' are influenced by the kind of absorption peaks described earlier. Classical Mie theory does not describe the scattering behavior of spheres in an absorbing medium. However, by using a modified Mie theory, it could be shown that under special conditions, similar to the blood situation for spheres and cylinders absorption within the surrounding medium, a decrease in μ_s and g, could be induced.[20,21] These results at least partly correspond to the data presented in this study and the results of other previous studies.[4,11,18]

5 Conclusions

This work presents the optical parameters μ_a, μ_s, and g of oxygenated and deoxygenated human RBCs with hematocrit 33.2% in the wavelength range 250 to 2000 nm. It shows that the oxygen saturation of hemoglobin has an influence not only on the absorption coefficient μ_a, but also on the scattering parameters μ_s, g and μ_s' of human RBCs, which are induced by changes in the absorption behavior of hemoglobin.

In addition, as the investigated wavelength range includes the spectral region above 1100 nm, where the absorption of hemoglobin tends to be negligible and the absorption of water has increasing values, it is possible to compare the physically interesting optical effect of absorption within the blood cell and the surrounding medium. It could be shown that in both cases, an increase in absorption can lead to a decrease in g and an increase in μ_s'. Therefore, it follows that the oxygen saturation has to be taken into account in order to estimate the scattering properties of blood. Moreover, not only the hemoglobin absorption within the blood cell but also the water absorption within the cell and the medium can influence the scattering properties of RBCs.

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