Refraction, fluorescence and Raman spectroscopy of normal and glycated hemoglobin

Ekaterina N. Lazareva[a,b,c], Andrey.Y. Zyubin[a,d], Ilya G. Samusev[a,d], Vasily A. Slezhkin[d,f], Vyacheslav I. Kochubey[b,c], Valery V. Tuchin[b,c,g]

[a]Center for Functionalized Magnetic Materials (FunMagMa), Immanuel Kant Baltic Federal University, 14 A, Nevskogo str., 236041, Kaliningrad, Russia; [b]Research Educational Institute of Optics & Biophotonics, Saratov State University, 83 Astrakhanskaya str., 410012, Saratov, Russia; [c]Interdisciplinary Laboratory of Biophotonics, Tomsk State University, 36 Lenin av., 634050, Tomsk, Russia; [d]Immanuel Kant Baltic Federal University, 14 A, Nevskogo str., 236041, Kaliningrad, Russia; [f]Department of Chemistry, Kaliningrad State Technical University, 1 Sovietsky prospect, 236022, Kaliningrad, Russia; [g]Laboratory of Laser Diagnostics of Technical and Living Systems, Institute of Precision Mechanics and Control RAS, 24 Rabochaya Str., 410028, Saratov, Russia;

ABSTRACT

In this study, the optical properties of glycated (HbA1c) and non-glycated (Hb) hemoglobin are compared using refractometry, fluorescence and Raman spectroscopy. The fluorescence measured at an excitation wavelength of 270 nm indicates differences in the molecular structure of hemoglobins. Analysis of the spectral shift of Raman spectra also showed variations indicating differences in their molecular structure. The refractive index measured in the visible and near IR regions for different temperatures allowed for quantification of mean values of temperature increment, which are quite different as $dn/dT = -(1.03 \pm 0.05)\times10^{-4}$ °C^{-1} for Hb and $-(1.37 \pm 0.07)\times10^{-4}$ °C^{-1}for HbA1c.The data obtained in the work can serve as a basis for further study of the optical properties of glycated hemoglobin and other glycated proteins.

Keywords: refraction, refractive index, temperature increment, fluorescence, Raman spectroscopy, hemoglobin**,** HbA1c, Hb

1. INTRODUCTION

Statistical data on the diabetes mellitus show that the number of cases increases every year several times. Among methods of diagnostics are methods based on the quantitative determination of glycated proteins, which are biological markers of the disease.[1-3] One of them that is often used as a marker isHbA1c which is formed by the nonenzymatic glycosylation (glycation) of hemoglobin exposed to blood glucose and therefore has a strong correlation with the average glucose concentration in the bloodstream in the preceding three-month period (life span of the erythrocytes). Because of this strong correlation, HbA1c levels have been regularly used to monitor long-term glucose control in established diabetics and has been recently approved for screening for diabetes (HbA1c $\geq$ 6.5%) and prediabetes (5.7% $\leq$ HbA1c $\leq$ 6.4%).[1-6]

Currently, there are more than 20 methods for the determination of glycated hemoglobin, such as cation exchange chromatography, electrophoresis, affinity chromatography and immunoassay, but each of them measures various fractions of glycated hemoglobin.[1-3] Most of the methods used are based on the chemical separation of the non-glycated and glycated hemoglobin fractions and the subsequent determination of the concentration of glycated hemoglobin by subtracting the unbound form from the total amount of hemoglobin. These methods are invasive and have significant drawbacks, for example, chromatography is very sensitive to changes in temperature and pH.[2]

At present, optical methods represent ample opportunities and are promising for the development of rapid and non-invasive methods of diagnosis and therapy of early stages of diseases such as diabetes, cancer and others.[7-9] Knowledge of the optical properties of blood, hemoglobin, glycated hemoglobin and its distinctive features will help in developing a quick, simple and noninvasive method for diagnosing these diseases. For example, *in vivo*

Biophotonics: Photonic Solutions for Better Health Care VI, edited by Jürgen Popp, Valery V. Tuchin, Francesco Saverio Pavone, Proc. of SPIE Vol. 10685, 1068540
 · doi: 10.1117/12.2307102

flow cytometry of diabetic patients with melanoma may have additional background signal from glycated erythrocytes.[7]

In this paper, a comparative analysis of the optical properties of glycated and normal hemoglobin was performed by using refractometry, Raman and fluorescence spectroscopy. Optical methods chosen in this study are most often used as diagnostic techniques and allow for detection of substances by differences in their molecular structure.[10-12] Raman spectroscopy (RS) is widely used as a powerful diagnostic tool in biomedical research due to possibility to provide a fingerprint information of the molecular composition and structure of a sample, such as proteins, lipids and so on.[13] For diabetes diagnosis, Han et al.[14] used RS to analyze the serum of diabetic patients as Barman et al.[15] used it to detect the glycated hemoglobin in hemolysate model formed by two component mixture (Hb and HbA1c).[14,15] As an alternate method for HbA1c detection, Ishikawa and co-workers have recently reported the application of surface-enhanced resonance Raman spectroscopy (SERRS).[16] Although promising in approach, precise quantification of the analyte of interest (HbA1c) using SERSS is difficult (as also noted by the authors), because of poor spectral reproducibility and the generation of spurious background signals.[17] The use of Raman spectroscopy allows for a qualitative analysis of the samples and clarifies the results obtained by refractometric and fluorescent methods. It has been shown the possibility of Raman spectroscopy to identify the distinctive features of glycated forms of proteins (albumin, hemoglobin) from non-glycated.[12, 18, 19] Native fluorescence spectroscopy of blood plasma in rats with experimental diabetes was used to identify the characteristic features of the fluorescence spectrum associated with glucose metabolism. The incubation of albumin with glucose *in vitro* showed that there is a new fluorescence band, which is caused by the formation of cross-links giving fluorescence in the range 400-430 nm upon excitation at 320-350 nm. The fluorescent properties of this band are similar for different proteins (for example, for albumin and collagen), since one of the main glycation products that causes this fluorescence is pentosidine.[12] The refractive index is one of the most important optical characteristics of tissues and the knowledge of its dispersion and temperature dependences for various forms of hemoglobin is important for understanding the changes in the optical properties of blood and biological tissues.[11, 20-23] Since the structure and composition of tissues and correspondingly their optical propertiesare changing in the course of pathology development, then several research groups have suggested the refractive index as a marker for differentiation of normal and pathological tissue, including experimental diabetes in animals.[11, 20, 21]

For the studies, glycated and normal hemoglobin solutions of 3 g/l concentration were used. A comparative fluorescence analysis of hemoglobins was done for excitation wavelengths of 260, 270 and 280 nm. We applied Surface-Enhanced Raman Scattering (SERS) to detect spectra from the dried drops of Hb and HbA1c. The refractive indices of two forms of hemoglobin were measured for 12 visible and near infrared wavelengths (480-1550 nm) at temperature25°C and for wavelengths 546, 589 and 644 nm at temperatures from 23 to 43°C.

2. METHODS AND MATERIALS

The optical properties of solutions of glycated and non-glycated forms of hemoglobin were investigated by using a multimodal approach with three independent optical methods, which allows us to make a detailed comparison of optical properties and to reveal specific features of hemoglobins for their discrimination.

Samples

As samples of the study, solutions of pure Hb and HbA1c were taken. Lyophilized powder of human Hemoglobin (Hb) and hemolysate of glycated hemoglobin (HbA1c) (Sigma-Aldrich) were used. The dilution was performed with a physiological solution of 0.9% NaCl.

Fluorescence spectroscopy

The studies were carried out on a fluorescence spectrometer Cary Eclipse (Varian, Belgium). Excitation wavelengths were selected as 260, 270 and 280 nm. The solutions for measurements were highly diluted to a

concentration at which the optical density is of 0.1 in the spectral region of excitation. The optical density was measured on a spectrometer UV-3600 (Shimadzu, Japan).

Raman spectroscopy (SERS)

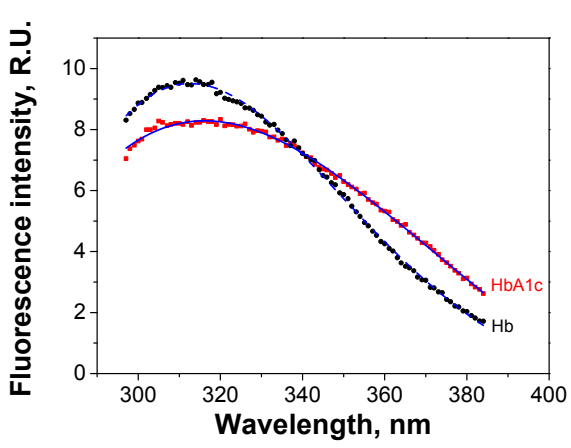
Raman spectra were obtained by CENTAUR U (Nanoscantechnology LLC, Russia and ZAO SolarLS, Republic of Belarus) unit with diode-pumped solid-state laser (DPSS) excitation (λ = 632 nm, radiation power of 50 mW). Raman spectra were recorded using a CCD detector matrix with 5 s signal accumulation. Substrates of anodically-dissolved silver, deposited electrochemically on a polished copper base with an anodic dissolution depth of silver of 500 nm, were used. Solutions of hemoglobin and glycated hemoglobin, concentrations of 3 g/l, were deposited as drops on substrate. Samples were dried on air, as a result a ring with a higher thickness of the sample was formed at the edges of dried drops. The diameter of the rings was not more than 3 mm. Measurements were carried out for 5 samples of each solution.

Refractometric measurement

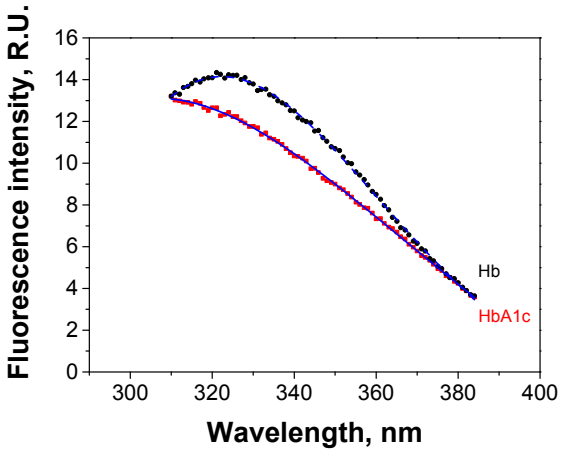
The commercial Multi-wavelength refractometer Abbe DR-M2/1550 (Atago, Japan) was used to measure the refractive index of solutions of Hb and HbA1c at concentration of 3 g/l. In the setup, the source of radiation was a high-power candescent lamp. To select the wavelength we used narrow-band interference filters for 480, 486, 546, 589, 644, 656, 680, 800, 930, 1100, 1300, and 1500 nm. The measurement error introduced by the instrument amounts to ±0.0002.In the beginning of every measurement, the calibration of the instrument using the known tabulated value of the refractive index of distilled water was executed. The sample temperature during the measurements was kept by water circulation in the refractometer provided by a thermostat. The temperature was 25°C when measuring the refractive index for 12 wavelengths. The refractive index was measured at the wavelengths of 546, 589, and 644 nm at temperatures from 23 to 43°C.

3. RESULTS AND DISCUSSION

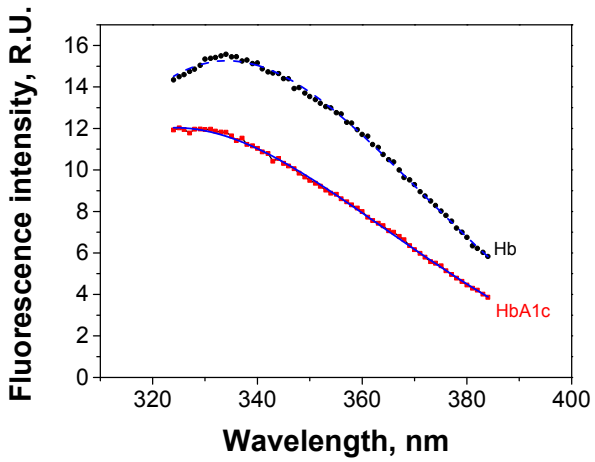
Figure 1 shows fluorescence spectra of solutions of Hb and HbA1c at excitation wavelengths of 260, 270 and 280 nm. These excitation wavelengths were chosen because of the published data where the peak of the hemoglobin fluorescence spectrum observed at 320 nm corresponds to the fluorescence of tryptophan that absorbs UV radiation with maxima at 220 nm and 280 nm and fluoresces in proteins at 328 ÷ 350 nm.[18,19] The obtained spectral dependences were processed by Gaussian analysis in the program "OriginLab Pro". The main parameters of fluorescence spectra of hemoglobin and glycated hemoglobin are given in Table 1.



A



B



C

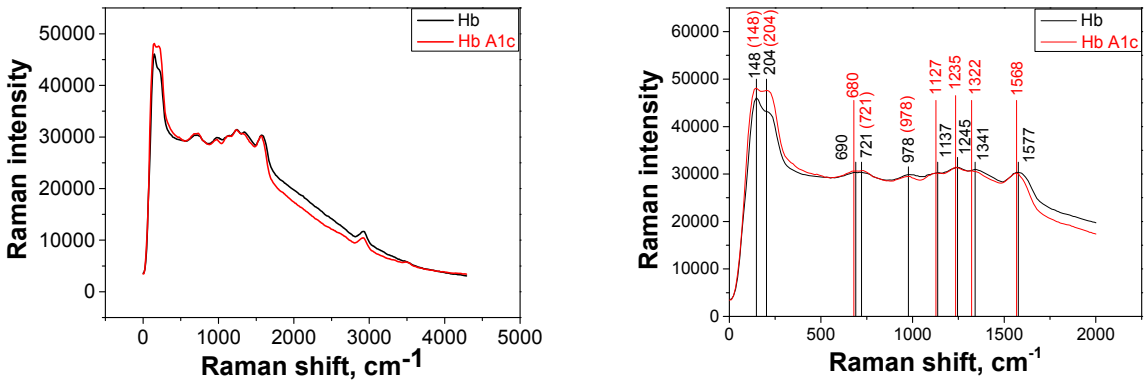
Figure 1. Fluorescence spectra of Hb and HbA1c solutions with optical density of 0.1 at excitation wavelengths: A - 260 nm; B – 270 nm; C - 280 nm. (Hb – ■ (Black squares), HbA1c – ● (Red circles), Gaussian analysis – Blue lines)

Table 1.Parameters of fluorescence spectra of Hb and HbA1c: FWHM is the full width on half-maximum, FI is the fluorescence intensity (R.U.), and R^2 is the correlation coefficient.

	Excitation wavelength 260nm nm				**Excitation wavelength 270 nm**				**Excitation wavelength 280 nm**			
	λ, nm	**FWHM**	**FI, R.U.**	**R^2**	**λ, nm**	**FWHM**	**FI, R.U.**	**R^2**	**λ, nm**	**FWHM**	**FI, R.U.**	**R^2**
Hb	312	86	9.10	0.998	322	87	13.98	0.999	334	82	14.58	0.999
HbA1c	317	112	8.75	0.999	303	145	16.58	0.999	322	98	12.23	0.998
$\Delta_{HbA1c-Hb}$	5	26	0.35	-	-19	58	2.6	-	-12	16	-2.35	-

The best result for the fluorescence spectrum was obtained at an excitation wavelength of 270 nm as can be seen from Table 1. The position of the fluorescence peak of Hb at an excitation wavelength of 270 nm is observed at 322 nm, and its FWHM is 87 nm. At the same excitation wavelength for HbA1c, the fluorescence peak is observed at a wavelength of 303 nm, and FWHM is145 nm. The change in the position of the maximum of the band and the simultaneous change in its half-width indicate the presence of at least two types of fluorescence molecules.

Raman spectra of Hb and HbA1c are shown in Fig. 2A and 2B. Determination of the position of the peaks was carried out using Gaussian analysis in the program "OriginLab Pro". Differences in the spectra for Hb and HbA1c are observed in the region 0-2000 cm^{-1}. In this region, the position of the maxima of 9 peaks is established, the position of 4 peaks of which coincide for Hb and HbA1c, and the position of 5 peaks is different. Data for the position of the maxima of Hb and HbA1c are given in Table 2.



A B

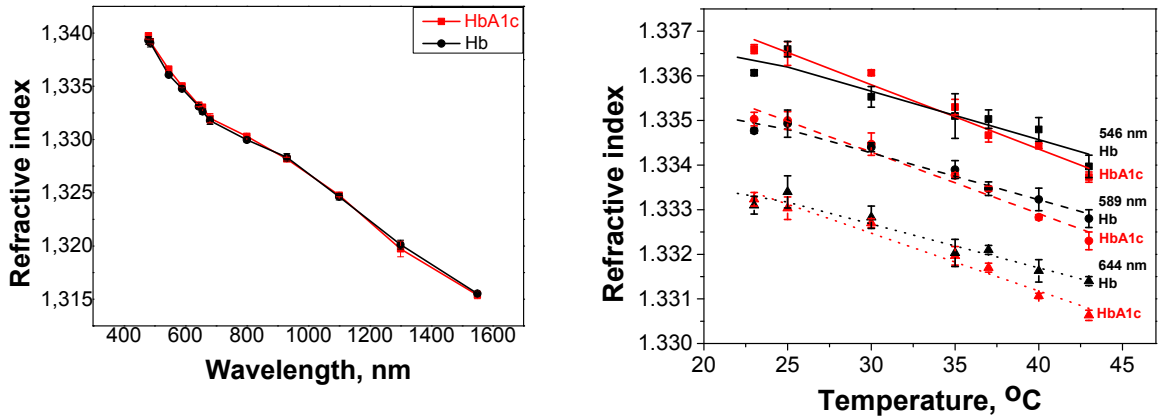
Figure 2. Raman spectra acquired from dry drops of Hb and HbA1c: A – full spectral range; B – spectral range 0-2000 cm^{-1}

Table 2. Raman peak positions of Hb and HbA1c (cm^{-1}) and tentative assignments.

Hb	148	204	690	721	978	1137	1245	1341	1577
HbA1c	148	204	680	721	978	1127	1235	1322	1568
$\Delta_{HbA1c-Hb}$	0	0	-10	0	0	-10	-10	-19	-9

The bands 680, 1127, 1235 obtained for the glycated hemoglobin are shifted relative to the normal hemoglobin extraction by 10 cm^{-1}. The bands 1322 and 1568 are also shifted to the left for HbA1c with respect to the bands for Hb at 19 and 9 cm^{-1}, respectively. A slight shift in the position of some peaks can be explained by differences in the molecular structure. Barman et al. showed by using Raman spectroscopy that a high level of glucose in the blood not only influence on protein function, but also changes the structure of the heme. Importantly, the presence of subtle but distinct features in the difference spectra between Hb and HbA1c deposits highlight the sensitivity of the proposed approach to very small structural variations in the protein molecules. The variations in the two protein spectra can then be attributed to structural changes in hemoglobin molecule related to the binding of a glucose moiety. The structure and molecular differences of Hb and HbA1c are well studied and described in the literature. [15,24,28]

For the first time in this paper, we have studied HbA1c dispersion and refractive index temperature increments in a wide spectral range using Abbe prism refractometry. Figure 3A shows the dispersion of the refractive index of glycated and non-glycated hemoglobin solutions at 25 °C. The refractive index was measured for a temperature range of 23-43°C for three selected wavelengths, 546, 589, and 644 nm. The temperature dependence of the refractive index is shown in Fig. 3 B.



A B

Figure 3.Dispersion measured at 25 °C (A) and temperature (B) dependencies of refractive index of Hb and HbA1c solutions at concentration of 3 g/l.

The temperature dependence for the interval 23-43°C was approximated by a linear function:

$$n(T) = n_0 + (\mathrm{d}n/\mathrm{d}T)T, \quad (1),$$

where n_0 is the refractive index for $T = 0$°C; $\mathrm{d}n/\mathrm{d}T$ is the refractive index derivative with respect to temperature or the temperature increment of the refractive index. The estimated values of n_0 and $\mathrm{d}n/\mathrm{d}T$ for glycated and non-glycated hemoglobin forms are given in Table 3.

Table 3. Refractive index for $T = 0$°C (n_0) and temperature increment ($\mathrm{d}n/\mathrm{d}T$) from the equation (1).

	546 nm		589 nm		644 nm	
	n_0	$-\mathrm{d}n/\mathrm{d}T$, $\times10^{-4}$ °C^{-1}	n_0	$-\mathrm{d}n/\mathrm{d}T$, $\times10^{-4}$ °C^{-1}	n_0	$-\mathrm{d}n/\mathrm{d}T$, $\times10^{-4}$ °C^{-1}
Hb	1.3389 (±0.0005)	1.08 (±0.16)	1.3374 (±0.0003)	1.05 (±0.09)	1.3356 (±0.0004)	0.98 (±0.10)
HbA1c	1.3401 (±0.0005)	1.44 (±0.11)	1.3384 (±0.0003)	1.38 (±0.10)	1.3364 (±0.0003)	1.30 (±0.10)
$\Delta_{\mathrm{HbA1c\text{-}Hb}}$	0.0012	0.36	0.0010	0.33	0.0008	0.32

Based on the results obtained, we can note an increase in the temperature incremental modulus for the glycated form of hemoglobin by an average of (0.34 ± 0.02) for the wavelengths 546, 589, and 644 nm. The total derivative of the refractive index with respect to temperature is related to the effect of thermal expansion and the temperature dependence of the molecular polarizability.[29, 30] In connection with this, the differences obtained in this study for Hb and HbA1c can be explained by their different molecular structure. The molecular complex of hemoglobin with glucose has a large polarizability. The dependence of the refractive index of dry erythrocytes from diabetic patients on pH (pH = 2-13) and, consequently, on the charge of the protein R-group was shown by Mazarevica et al. by using polarization-sensitive interference microscopy for the wavelength 550 nm.[31]

Thus, all the optical methods used in the work demonstrate the possibility of using them for the selection of HbA1c from Hb and HbA1c mixture without the use of additional chemical methods. High sensitivity, as shown by us, has the refractometric method applied for the first time to measure temperature increments for different wavelengths. In the future, we plan to use the refractometric method to study the dependence of the temperature increment of the refractive index on the degree of glycosylation of hemoglobin.

4. CONCLUSION

Analysis of three different optical methods revealed the presence of characteristic differences for HbA1c from Hb for each of them. Fluorescence analysis showed the presence of characteristic distinctive features for glycated hemoglobin at an excitation wavelength of 270 nm. Raman spectroscopy showed differences of Raman bands for Hb/HbA1c: 690/670, 1137/1127, 1245/1235, 1341/1322 and 1577/1568 cm^{-1}.The mean increase in the temperature increment modulus of the refractive index of HbA1c in comparison with Hb in the visible spectral region is (0.34 ± 0.02).

The data obtained in the work confirm and supplement those that are available in the literature, and can also serve as a basis for further study of the optical properties of glycated hemoglobin and other glycated proteins. Data received are important for the development of optical diagnostic and therapeutic methods, in particular for *in vivo* fluorescent and photoacoustic cytometry of melanoma cells in the blood stream of diabetic patients.

ACKNOWLEDGMENT

E.N. Lazareva, A.Y. Zubin and I.G. Samusev acknowledge support from the 5 top 100 Russian Academic Excellence Project at the Immanuel Kant Baltic Federal University of SERS measurements and providing hemoglobins.

E.N. Lazareva, V.I. Kochubey and V.V. Tuchin acknowledge support by grant of the Russian Federation Government № 14.Z50.31.0044 of fluorescence and refraction measurements.

REFERENCE

[1] Yazdanpanah, S., Rabiee, M., Tahriri, M., Abdolrahim, M., Rajab, A., Jazayeri, H. E. and Tayebi, L., "Evaluation of glycated albumin (GA) and GA/HbA1c ratio for diagnosis of diabetes and glycemic control: A comprehensive review," Journal Critical Reviews in Clinical Laboratory Sciences 54 (4), 3 (2017).

[2] Negre-Salvayre, A., Salvayre, R., Auge´, N., Pamplona R. and Portero-Otı´n, M. "Hyperglycemia and Glycation in Diabetic Complications," Antioxidants& Redox signaling 11(12), 3071-109 (2009).

[3] Selvin, E., Steffes, M. W., Zhu, H., Matsushita, K., Wagenknecht, L., Pankow, J., Coresh, J., and Brancati, F. L., "Glycated Hemoglobin, Diabetes, and Cardiovascular Risk in Nondiabetic Adults," N Engl J Med 362, 800-811 (2010)

[4] Pan, T., Li, M., Chen, J., and Xue, H.,"Quantification of glycated hemoglobin indicator HbA1c through near-infrared spectroscopy," Journal of Innovative Optical Health Sciences 7(4), 1350060 (2014).

[5] Mortensen, H. B., Christophersen, C., "Glucosylation of human haemoglobin a in red blood cells studied in vitro. Kinetics of the formation and dissociation of haemoglobin A1c," Clin. Chim. Acta 134, 317−326 (1983).

[6] Executive Summary: Standards of Medical Care in Diabetes—2010, Diabetes Care 33 (Supplement 1), S4-S10 (2010).

[7] Tuchin, V.V., Tárnok, A., and Zharov, V.P., "In Vivo Flow Cytometry: A Horizon of Opportunities," *Cytometry A* 79A(10), 737–745 (2011).

[8] Lin, J. Q., Chen, R., Feng, S. Y., Pan, J. J., Li, Y. Z., Chen, G. N., Cheng, M., Huang, Z. F., Yu, Y., Zeng, H. S., " A novel blood plasma analysis technique combining membrane electrophoresis with silver nanoparticle based SERS spectroscopy for potential applications in non-invasive cancer detection," Nanomed Nanotechnol. Biol. Med. 7(5), 655–663 (2011).

[9] Lin, J., Lin, J., Huang, Z., Lu, P., Wang, J., Wang, X., Chen, R., "Raman spectroscopy of human hemoglobin for diabetes detection", Journal of Innovative Optical Health Sciences 7(1), 1350051 (2014).

[10] Barman, I., Dingari, N. Ch., Kang, J. W., Horowitz, G. L., Dasari, R. R., and Feld, M. S., "Raman Spectroscopy-Based Sensitive and Specific Detection of Glycated Hemoglobin," Anal. Chem. 84, 2474−2482 (2012).

[11] Zhernovaya, O. S., Tuchin, V. V., Meglinski, I. V., "Monitoring of blood proteins glycation by refractive index and spectral measurements," Laser Phys. Lett. 6, 460–464 (2008).

[12] Shirshin, E., Cherkasova, O., Tikhonova, T., Berlovskaya, E., Priezzhev, A. and Fadeeva, V., "Native fluorescence spectroscopy of blood plasma of rats with experimental diabetes: identifying fingerprints of glucose-related metabolic pathways," Journal of Biomedical Optics 20(5), 051033 (May 2015)
[13] Feng, S. Y., Chen, R., Lin, J. Q., Pan, J. J., Chen, G. N., Li, Y. Z., Cheng, M., Huang, Z. F., Chen, J. S., Zeng, H. S., "Nasopharyngeal cancer detection based on blood plasma surface-enhanced Raman spectroscopy and multivariate analysis," Biosens. Bioelectron. 25(11), 2414–2419 (2010).
[14] Han, H. W., Yan, X. L., Dong, R. X., Ban, G., Li, K., "Analysis of serum from type II diabetes mellitus and diabetic complication using surface-enhanced Raman spectra (SERS)," Appl. Phys. B 94(4), 667– 672 (2009).
[15] Barman, I., Dingari, N. C., Kang, J. W., Horowitz, G., Dasari, R. R., Feld, M. S., "Raman spectroscopy based sensitive and specific detection of glycated hemoglobin," Anal. Chem. 84(5), 2474–2482 (2012).
[16] Kiran, M. S., Itoh, T., Yoshida, K., Kawashima, N., Biju, V., Ishikawa, M., "Selective Detection of HbA1c Using Surface Enhanced Resonance Raman Spectroscopy," Anal. Chem. 82(2), 1342−1348 (2010).
[17] Sackmann, M., Bom, S., Balster, T., Materny, A., "Nanostructured gold surfaces as reproducible substrates for surface-enhanced Raman spectroscopy," J. Raman Spectrosc. 38(3), 277−282 (2007).
[18] Liu, J. T., Chen, L. Y., Shih, M. C., Chan, Yu., Chen, W. Y., "The investigation of recognition interaction between phenylboronate monolayer and glycated hemoglobin using surface plasmon resonance," Analytical Biochemistry 375(1), 90-96 (2008).
[19] Li, J., Chang, K. W., Wang, C. H., Yang, C. H., Shiesh S.C., Lee G. B., "On-chip, aptamer-based sandwich assay for detection of glycated hemoglobins via magnetic beads," Biosensors and Bioelectronics 79, 887-893 (2016).
[20] Giannios, P., Koutsoumpos, S., Toutouzas, K. G., Matiatou, M., Zografos, G. C., and Moutzouris, K., "Complex refractive index of normal and malignant human colorectal tissue in the visible and near-infrared," J. Biophotonics 10 (2), 303–310 (2017).
[21] Carvalho, S., Gueiral, N., Nogueira, E., Henrique, R., Oliveira, L., Tuchin, V. V., "Wavelength dependence of the refractive index of human colorectal tissues: comparison between healthy mucosa and cancer," J of Biomedical Photonics & Eng, 2(4), 040307-9 (2016).
[22] Zirk, K., Poetzschke, H., "On the suitability of refractometry for the analysis of glucose in blood-derived fluids," Medical Engineering & Physics 26, 473–481 (2004).
[23] Zirk, K., Poetzschke, H., "A refractometry-based glucose analysis of body fluids," Medical Engineering & Physics 29, 449–458 (2007).
[24] Wood, B. R., Caspers, P., Puppels, G. J., Pandiancherri, S., McNaughton, D., "Resonance Raman spectroscopy of red blood cells using near-infrared laser excitation," Anal. Bioanal. Chem. 387(5), 1691–1703 (2007).
[25] Premasiri, W. R., Lee, J. C., Ziegler, L. D., "Surface enhanced Raman scattering of whole human blood, blood plasma, and red blood cells: Cellular processes and bioanalytical sensing," J. Phys. Chem. B 116 (31), 9376–9386 (2012).
[26] Gelder, J. D., Gussem, K. D., Vandenabeele, P., M'oens, L., "Reference database of Raman spectra of biological molecules," J. Raman Spectrosc. 38(9), 1133–1147 (2007).
[27] Sen, S., Kar, M., Roy, A., Chakraborti, A. S., "Effect of nonenzymatic glycation on functional and structural properties of hemoglobin," Biophys. Chem. 113, 289−298 (2005).
[28] GhoshMoulick, R., Bhattacharya, J., Roy, S., Basak, S., Dasgupta, A. K., "Compensatory secondary structure alterations in protein glycation," Biochim. Biophys. Acta 1774, 233−242 (2007).
[29] Erokhin, A.I., Morachevskii, N.V. and Faizullov, F.S., "Temperature dependence of the refractive index in condensed media," Zh. Eksp. Teor. Fiz. 74, 1336-1341 (1978).
[30] Volkenshtein, M. V., Molecular Optics, Gosteskhizdat, Moscow (1951).
[31] Mazarevica, G., Freivalds, T., Jurka, A., "Properties of erythrocyte light refraction in diabetic patients," Journal of Biomedical Optics 7(2), 244–247 (2002).