Sensors for Rapid Detection of Environmental Toxicity in Blood of Poisoned People

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Abstract Recently, the diagnosis and treatment of poisoned person can be done only in specialized centers. Furthermore, currently used clinical methods of intoxication diagnosis are not sufficient for early detection. Conventional laboratory tests based on urine and blood require professional, high skilled staff, high cost equipment as well as they are arduous and lasting analytical procedures. There is a need to elaborate relatively cheap and easy to use tests, which can simplify and shorten the process of diagnosis of intoxicated patients as well as simply monitoring of patients from high-risk groups (firemen's, miners, security, policemen, soldiers etc.) having contact with toxic gases. This chapter focuses on novel, early detection sensor for rapid diagnostics of environmental toxicity in blood of people intoxicated with carbon monoxide. Sect. 1 presents, a brief overview of physiological relevance of blood carbon monoxide levels on poisoning and overview of commercially available methods of CO detection in human blood. In Sect. 2 the optical properties of blood are presented. Section 3 shows optoelectronic systems, such as absorption spectroscopy and low-coherence interferometry designed to detect optical properties of blood, such as refractive index, absorption. In Sect. 4, the sensor for detection of environmental toxicity in blood is presented. The application of the sensor can shorten the time of analyses of poisoned patients. It will be dedicated to

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support diagnostics of all patients in bad clinical state, where anamnesis is difficult to obtain, intoxicated people or fire victims. We assume to use such method in the Emergency Departments, small clinics and doctors' offices. In the Sect. 5 the hollow core microstructured waveguide biosensors for applications in biomedical sensors are presented. This waveguide can be used for investigation of optical parameters (scattering, refractivity and other) of many biological liquids. The last section focuses on our conclusion about optoelectronic method which can be used for rapid detection of environmental toxicity in blood of poisoned people.

Keywords Biosensors · Environmental toxicity · Blood · Carbon monoxide · microstructured waveguide

1 Introduction

Carbon monoxide (CO) is a chemical compound naturally occurring in nature, and its presence is essential for the proper functioning of ecosystems (e.g. photosynthesis, which is the phenomenon of synthesis of organic compounds from inorganic compounds, with the participation of light). However, the excess of carbon monoxide leads to serious consequences for human health.

Carbon monoxide poisoning is the most common cause of accidental poisoning deaths in developed countries. The formation of carbon monoxide is due to incomplete combustion of hydrocarbon-containing products. Most of the carbon monoxide occurs in the exhaust gases manufactured by, among others, motor vehicles and industry. Particular high accumulation of this gas can be observed in the tunnels, garages and other poorly ventilated areas. Moreover, large amounts of carbon monoxide are released as a result of fires. Poisoning can also occur through contact with the solvents (as a result of intoxication by vapors), and by inhalation of tobacco smoke [1–3].

According to the decision of the U.S. Federal Register [4], for exposure of a human adult to 8 h of lasting harmful conditions, the concentration of carbon monoxide in the external environment should not be higher than 8 ppm, while for 1 h exposure should not exceed 25–50 ppm. Because of this, it is essential to ensure the control and monitoring of carbon monoxide levels in the environment in order to protect the public health. Environmental Protection Agency (EPA) specifically underlines that, it is necessary to design sensors with sensitivity allowing measurements of carbon monoxide concentrations lower than 1 ppm [4].

1.1 Physiological Relevance of Blood CO Levels/Poisoning

Carbon monoxide is a colorless, odorless and tasteless gas. These properties make it particularly dangerous, because people exposed to poisoning are not able to

recognize the looming danger. Symptoms of carbon monoxide poisoning are nonspecific and can occur in different forms—from headache, confusion, loss of consciousness to respiratory depression and cardiac disorders. A milder form of intoxication is often confused with flu symptoms or, particularly in children (with symptoms such as headaches, dizziness and vomiting), viral infection. Severe carbon monoxide poisoning can cause extensive damage to internal organs or even death. Moreover, carbon monoxide poisoning is often not recognized by the lack of specific medical signature of this substance. All above reasons make carbon monoxide often called "the silent killer" [5, 6].

The most common way of CO poisoning is inhalation. Then, when CO gets into the bloodstream, it displaces oxygen from binding to hemoglobin to form carboxyhemoglobin (COHb). The affinity of hemoglobin to CO is considerably larger (200–300 times) than oxygen and therefore this reaction proceeds rapidly until equilibrium is reached between the concentration of CO in the ambient air and the concentration of CO in the blood of victims of poisoning. When the hemoglobin binds with carbon monoxide, it is no longer able to transport oxygen. As a consequence, victim's body undergoes hypoxia, which can lead to death [3, 7].

The concentration of carboxyhemoglobin in the blood of healthy, non-smoker patient should not exceed 3, and 10 % in the smoker's blood. First symptoms of poisoning such as dizziness, nausea, vomiting or headaches, appear when the level of carboxyhemoglobin exceeds the range of 10–20 %. As the concentration increases to more than 20 %, disorders of the cardiovascular and nervous systems begin: tachycardia, confusion, weakness, chest pain and shortness of breath. It should be emphasized that heart and brain are most sensitive to the toxic effect of carbon monoxide. Severe monoxide poisoning is characterized by the concentration of carboxyhemoglobin in blood above 60 %. This condition may lead to irreversible changes in the brain caused by hypoxia, and consequently to fatal outcome [8]. Regardless of the results of laboratory tests, assessment of the level of CO poisoning requires taking into consideration the duration of exposure to harmful conditions. This is necessary because there is no explicit correlation between the measured concentration of carboxyhemoglobin in the blood and the patient's clinical status.

1.2 Conventional Blood CO Monitoring Methods

At the present, an accurate diagnosis and treatment of poisoned people can be done only in a specialized medical center. Conventional laboratory tests based on blood or urine require professional, high skilled staff, high cost equipment as well as they are arduous and lasting analytical procedures. The measurements take usually about 15 min and the results are available in 24 h. Such long time period is a serious drawback, as a late diagnosis poses risks to patient health state.

Diagnosis of carboxyhemoglobin level is mainly based on an examination of the patient's blood in analytical laboratory. This requires collecting venous blood from

the patient, certainly without air bubbles. It is also important to use an appropriate anticoagulant. In this case, it is possible to use lithium heparin. Collected sample must be delivered to toxicology laboratory in 20 min, when the sample is stored at room temperature or in 2 h if the sample is placed in ice-water (about 5 °C). The blood test is performed with a spectrophotometer using two wavelengths of monochromatic light. The spectrophotometer measures the absorption spectra, and discerns total concentrations of carboxyhemoglobin (HbCO), oxyhemoglobin (HbO2), deoxyhemoglobin (Hb) and methemoglobin (metHb). Determination of HbCO is performed by taking the absorbance ratio of HbCO maximum at the wavelength 539 nm, to the Hb and HbCO isobestic point at 578 nm. Because spectra of carboxyhemoglobin and oxyhemoglobin are very similar, in order to correctly determine level of carbon monoxide in the blood, it is necessary to numerically remove the measurement data related to oxyhemoglobin. Valid blood examination requires collection of blood from patient in the morning, preferably on an empty stomach. The test result is available for the patient on the next day, however the laboratory procedure itself takes about 20 min. The biggest disadvantage of this method is first and foremost the necessity of collecting blood samples and then carrying them to a specialized laboratory. This means it is not possible to obtain immediate information on the level of CO poisoning of the patient during medical diagnosis. Moreover, blood test result does not indicate the current level of poisoning of the patient, but gives information about his condition from a few hours earlier. Furthermore, this method is helpful in detection of CO poisoning only when the ratio of HbCO to Hb is higher than usual 5 %, or about 9 % for heavy smokers. This is a significant barrier to implementation of appropriate treatment.

State-of-the-art sensors for carbon monoxide poisoning measurement are based on optical spectroscopy, which detects changes of the absorption spectra of hemoglobin derivatives (Masimo rainbow® Pulse CO-Oximetry™, Masimo, USA). Existing commercially available hand-held devices for CO measurement (piCO+™ Smokerlyzer™, coVita LLC, USA) are based on breath CO analysis measuring its concentration by electrochemistry. While breath detection method is useful in some simple cases, where CO exposure is certain (firemen during an action), it lacks in versatility for other usages (patient monitoring). Moreover, the toxic CO levels in blood are the clinical standard to confirm CO poisoning, while breath concentrations may vary substantially.

Devices allowing immediate, noninvasive measurement of carboxyhemoglobin are not generally available due to their high cost and the need to be operated by qualified personnel. It should be emphasized that the most popular non-invasive method to measure blood oxygenation, which is pulse oximetry, is not applicable in the diagnosis of CO poisoning because this method does not distinguish between carboxyhemoglobin and oxyhemoglobin.

On the other hand, optoelectronic and optical fiber sensors are powerful, low cost and precise devices for monitoring in liquid environment. Equipped with sensitive layers, resistive to aggressive environment, they can be used for detection of low quantities of analytes. Combining multimode optical fibers and nanotechnology for the development of sensitive elements are actual and innovative topics. The main

expected approach is to build up optical fiber sensor with high surface-to-volume aspect ratio sensitive layer.

2 Materials/Methods

Blood is a bodily fluid which is supplied to all the cells of the body by means of the circulatory system. The role of the blood in the body is to transport oxygen and carbon dioxide, the supply of nutrients and disposal of end products of metabolism. In addition, the blood provides thermal regulation of the organism and self-healing mechanisms for preventing blood leakage from injured blood vessels. Blood is a heterogeneous solution, which consists of formed elements (cells) suspended in plasma, all of which have different characteristics and fulfill different functions in the organism. There are three types of the cellular components: red blood cells (RBC), white blood cells (WBC), and platelets (PLT), which in total account for about 35–50 % of the volume of whole blood, while the remainder is plasma [9–12].

Red blood cells (erythrocytes) are the most frequently occurring blood cells. There are approx. 5×10^6 µl ^{1} red blood cells (RBCs) in normal human blood. They have a round shape of a biconcave disk, with an average diameter of 7.2–7.8 µm, thickness 1.4–2.1 µm, and an area of 130 μm^2 and a volume of 90 μm^3. Their index of refraction is 1.4 in the wavelength range 600–1100 nm [9]. They are very flexible and elastic, which allows them to squeeze through narrow blood vessels and protects against mechanical damage. Their task is to deliver oxygen from the lungs to all cells of the body and discharge carbon dioxide from them back to the lungs, where the gas exchange occurs. The ability to transport oxygen is enabled by the hemoglobin contained in them. Erythrocytes have no nucleus or mitochondria, they cannot divide, and cannot recover from damage. Thus they are formed by the body itself, which takes place in the bone marrow. Their average life is approx. 120 days [10–12].

White blood cells (leukocytes) area part of the body's immune system. Their key role is to protect the body against viruses, bacteria and other foreign bodies and remove dead erythrocytes. There are several types, which differ in structure and purpose: neutrophils, eosinophils, basophils, monocytes, macrophages and lymphocytes. They contain nuclei and have the ability to move. They neutralize the threat by phagocytosis (devouring) of pathogens. Their number is roughly 4×10^3–10×10^3 μl^{-1} [10]. They are formed like spheres with diameter of 8–22 µm [9].

Platelets (thrombocytes) are devoid of the cell nucleus are broken away portions of specific bone marrow cells of irregular shape. Platelets in the blood stream are biconvex, disk-like particles with diameter ranging between 2–4 µm. There are approx. $(2–4) \times 10^5$ μl^{-1} cells in human blood [9–12]. They are used to maintain hemostasis, or the prevention of blood leakage in the event of vascular injury. They contain clotting factors, which are released during platelet aggregation. Within

seconds after the injury, platelets adhere to collagen fibrils to form a platelet plug which is sustained by the fibrin fibers, which prevents further the bleeding [10].

Blood plasma—is an extracellular scaffolding, which is mainly used to transport morphotic elements, nutrients and metabolic products. Plasma water constitutes approx. 90 % of plasma, where the rest is composed of proteins, lipids and other components in smaller quantities, including glucose, albumin, globulin, cholesterol, triglycerides, lipoproteins, hormones, minerals, electrolytes [10–12].

Hematocrit (Hct) is one of the basic parameters of the blood. It is defined as the ratio of the volume of RBCs to the total blood volume [9–12]:

$$\text{Hct } [\%] = V_{RBC}/V_{Blood}, \tag{1}$$

where V_{RBC} is the volume of red blood cells, and V_{Blood} is the total volume of blood. This parameter is extremely important, because it provides the information on the amount of RBCs in the blood sample, which dominates all optical properties of blood samples. Elevated levels of Hct may be the result of overproduction of RBCs, which is a sign of the circulatory system diseases. This can cause blockages, which increases the risk of stroke and myocardial infarction [9–11].

The mean corpuscular volume of red blood cells (MCV) is defined as the ratio of the hematocrit to the total amount of RBCs, the Red Blood Cell Count (RBCC):

$$\text{MCV}[fl] = Hct[\%]/RBCC\left[10^6/\mu l\right] \tag{2}$$

MCV determines the size of blood cells. The smaller sizes of blood cells can be caused by iron deficiency, while larger sizes are the evidence of deficiency of vitamin B12 or folic acid.

The mean corpuscular hemoglobin (MCH)—the average weight of hemoglobin in a RBC is defined as the ratio of total hemoglobin in a sample to the amount of RBC's:

$$\text{MCH}[pg] = Hb[g/l]/RBCC[10^6/\mu l]. \tag{3}$$

The average weight of hemoglobin carried by the cell is determined by this parameter. Various disease states, and particularly of anemia caused by iron deficiency, reduce MCH.

The mean corpuscular hemoglobin concentration (MCHC) is an average concentration of hemoglobin in RBCs and is defined as the ratio of hemoglobin to hematocrit:

$$\text{MCHC}[g/l] = Hb[g/l]/HCT[10^6/\mu l]. \tag{4}$$

The Hb saturation of RBCs increases in the states of overhydration, due to penetration of water into the cells. In contrast, dehydration is reflected in artificially low MCHC. The drop in MCHC is also typical for microcytic anemias.

The main parameters relating to **white blood cells** is that their total volume (WBC White Blood Cell Count), and given percentage content of the various types of leukocytes: neutrophils (NEUT%), lymphocytes (Lymph%), monocytes (MONO %), eosinophil (EO%) and basophils (BASO%).

Parameters related to **blood platelets** are formulated in the same way as the parameters describing the RBCs. There are number of platelets (PLT) trombocrit (PCT), which is measured in a manner analogous to the hematocrit, mean platelet volume (MPV) and platelet distribution width (PDW). These parameters are determined in the same manner as for the measurement of RBCs [9–12].

2.1 Optical Properties of Blood

Optical properties of blood can be consider in the micro- and macroscopic way. As a microscopic object, blood can be treated as a medium having scattering centers. It is a heterogeneous medium consisting of plasma and blood cells. Blood plasma contains almost 90 % of water and 10 % of protein. Blood cells consist primarily of erythrocytes (almost 99 %), leukocytes (1 %) and platelets. RBC's geometrical dimensions are typically: 6.2–8.2 μm. The WBCs differ in size depending on their group; as follows: neutrophils 10–14 μm, lymphocytes 8–12 μm, monocytes 12–18 μm, eosinophils 10–14 μm, basophils 8–14 μm [9–12].

The microscopic approach of optical parameters of blood depends mainly on its components' optical properties: the scattering and absorbing capacity of optical radiation, but also on their size and shape.

On the other hand, treating blood as a macroscopic object, its optical properties become those of a homogeneous scattering medium. In this case, optical properties of the investigated object can be described by: scattering coefficient, absorption coefficient and refractive index. In this case, the complex refractive index can be introduced as [13]:

$$\hat{n} = n(1 + j\kappa) \tag{5}$$

where: n is the real part of complex refractive index: $n = c/v$; κ is the attenuation index (also described as attenuation coefficient, μ_t that is the sum of absorption (μ_a) and scattering (μ_s) coefficients, $\mu_t = \mu_a + \mu_s$).

$$\mu_t = \frac{4\pi}{\lambda}\kappa \tag{6}$$

where: λ is the wavelength in the medium.

Equations (5) and (6) are correct if the wave equation is shown as:

$$\hat{E} = E_0 \exp(kz - \omega t) \tag{7}$$

where k is the wave vector: $k = \frac{2\pi}{\lambda}$; ωt is the phase.

The complex refractive index described by Eq. (5) is an adequate parameter to characterize blood as a homogeneous dispersion medium.

The most important optical parameters of blood which define the total extinction (attenuation) coefficient of a blood sample, are the absorption and scattering coefficients [14]. They are closely related to the standard clinical measures of OD (optical density), or A (absorbance). The macroscopic optical properties of a blood sample are dominated by its morphology, specifically the parameters related to RBC's, such as: mean corpuscular volume of a RBC (MCV), and a hematocrit (Hct), the volume fraction of cells to the total volume of blood. It was experimentally established, that the following equations for absorption and scattering coefficient are generally a valid approximations [15]:

$$\mu_a = \frac{Hct}{MCV}\sigma_a \tag{8}$$

$$\mu_s = \frac{Hct}{MCV}\sigma_s, \quad for\, Hct \to 0 \tag{9}$$

$$\mu_s = \frac{Hct(1 - Hct)}{MCV}\sigma_s, \quad for\, Hct \to 1 \tag{10}$$

In the case of scattering, the first formula is most accurate for low values of Hct, below the clinically important region of 0.3–0.5. On the other hand, the second approximation works best for the unnaturally high values of Hct > 0.8. Thus, it is of interest of many groups to establish that relationship more precisely, considering other parameters as well. Therefore, the scattering and absorption properties of blood vary heavily with the patient- specific blood morphologic differences. It should be noted, that the binding state of hemoglobin changes only the spectral characteristics of absorption, while the scattering remains independent from the hemoglobin derivative.

2.2 Optical Blood Measurement Techniques

The basic law in absorption spectroscopy is the Beer-Lambert law, which describes the absorption of radiation passing through the media with absorbing centers. This law results from the combination of two previous laws: Lambert, which determines the exponential decrease in radiation intensity with the thickness of material

through which the light passes, and Beer's law which relates the absorbance to the concentration of the substance. The beam gradually transmits part of the energy during propagation through the material composed of absorbing molecules. The decrease in the intensity of radiation through the sample volume contribution is proportional to the intensity of the incident light and depends on the path length through the sample and the concentration of particles, which can be written as equation [16, 17]:

$$\frac{\mathrm{dI}}{\mathrm{dL}} = -\varepsilon \mathrm{C}\ \mathrm{I}_0, \tag{11}$$

where I is the intensity of radiation after passing through the sample, I_0 is the intensity of incident radiation, L is the path length, C is the particle concentration (molar or mass), ε is the extinction coefficient. Solving the previous equation gives the Lambert equation [16, 17]:

$$\mathrm{I(L)} = \mathrm{I}_0 e^{-\varepsilon L}. \tag{12}$$

To determine the ratio of the I_0 intensity of light incident on the sample to I the intensity of light that has passed through the sample, we use the equation determining the transmission T, where in practice instead of the natural logarithm a decimal logarithm is used. More commonly, the absorbance values are used in practice, so the transformed equation for absorbance A, is needed [15, 16]:

$$\mathrm{T} = \frac{\mathrm{I}}{\mathrm{I}_0} \cdot 10^{-A} \rightarrow \mathrm{A} = \log_{10} \frac{I_0}{I}. \tag{13}$$

Thus, the known Lambert–Beer equation assumes the form for absorbance:

$$\mathrm{A} = \varepsilon CL, \tag{14}$$

Due to the linear dependence of absorbance on the sample thickness, it is typically used instead of the transmission. Beer-Lambert law is satisfied for low concentrations of substances, while at high concentrations deviates from linearity due to the effects associated with multiple light scattering in the sample. Especially important parameter of this equation is molar extinction coefficient ε, because it determines the degree of loss of radiation through the mechanisms of absorption and scattering, and can be described as follows [15]:

$$\varepsilon \equiv \mu_\mathrm{t} = \mu_\mathrm{a} + \mu_s \tag{15}$$

where ε is the extinction or attenuation coefficient [mm^{-1}], μ_a is the absorption coefficient [mm^{-1}], μ_s is the scattering coefficient [mm^{-1}].

An important law is the additivity of absorbances, which allows the summation of the effects of the absorption of many substances contained in the solution. This can be described by the equation [16, 17]:

$$A_N = L \cdot (\varepsilon_1 C_1 + \varepsilon_2 C_2 + \cdots + \varepsilon_N C_N) \tag{16}$$

or as a sum:

$$A_N = L \cdot \sum_{i=1}^{N} \varepsilon_i C_i \tag{17}$$

where A_N is the resulting absorbance of N substances in a solution, ε_i is the extinction coefficient of ith substance, C_i is the concentration of ith substance, L is the probing path length, and N is the total number of substances in solution.

For blood carboxyhemoglobin determination through optical spectroscopy, a CCD-based spectrometer measurement system was developed. First consideration for the spectrometer is the wavelength range in which the spectra should be collected. In the UV region up to over 400 nm, strong absorption bands of various plasma proteins are present, as well as the Soret band of other hemoglobin derivatives. Therefore it was concluded to avoid this range, so that the measured spectra would not be dominated by the patient-specific differences. In the near infra-red (NIR) region, a known isobestic point of oxy- and deoxygenated hemoglobin is present at roughly 805 nm which serves as a good reference value for spectra normalization [18, 19]. The changes in oxygenation state of blood between patients should be then easier to remove, as the prohibited from direct measures of COHb in a manner similar to pulse oximetry. The region of most significant differences in the spectra of hemoglobin derivatives is the Q-band region spanning from approx. 500 to 600 nm [18, 19].

The measurement system Fig. 1a comprises of the spectrometer (SP), a broadband light source (LS), delivery and collection fibers and an optical probe (OP) and sample holder with horizontal geometry working in the transmission mode. A commercial miniature diffractive (DG) spectrometer (OceanOptics USB4000, USA) with CCD detector working in VIS-NIR (400–900 nm) was used. It is important to note that the absorption and scattering coefficients of blood are very high, especially in the Q-band region [20, 21]. Use of cuvettes or capillaries with 100 μm or smaller light path is recommended to avoid problems with low light levels. The data processing protocol can be either a straightforward ratiometric approach, where absorbance at specific peaks corresponding to each hemoglobin derivative on use, along with isobestic points, or more elaborate multivariate methods. The spectra of normal whole human blood and blood exposed to a threshold value of 30 ppm CO in air are presented on Fig. 1c. Due to the convoluted nature of hemoglobin derivatives spectra, it is advised to use the multivariate calibration methods [21, 22], and utilize more information from the spectra than only the peak wavelengths.

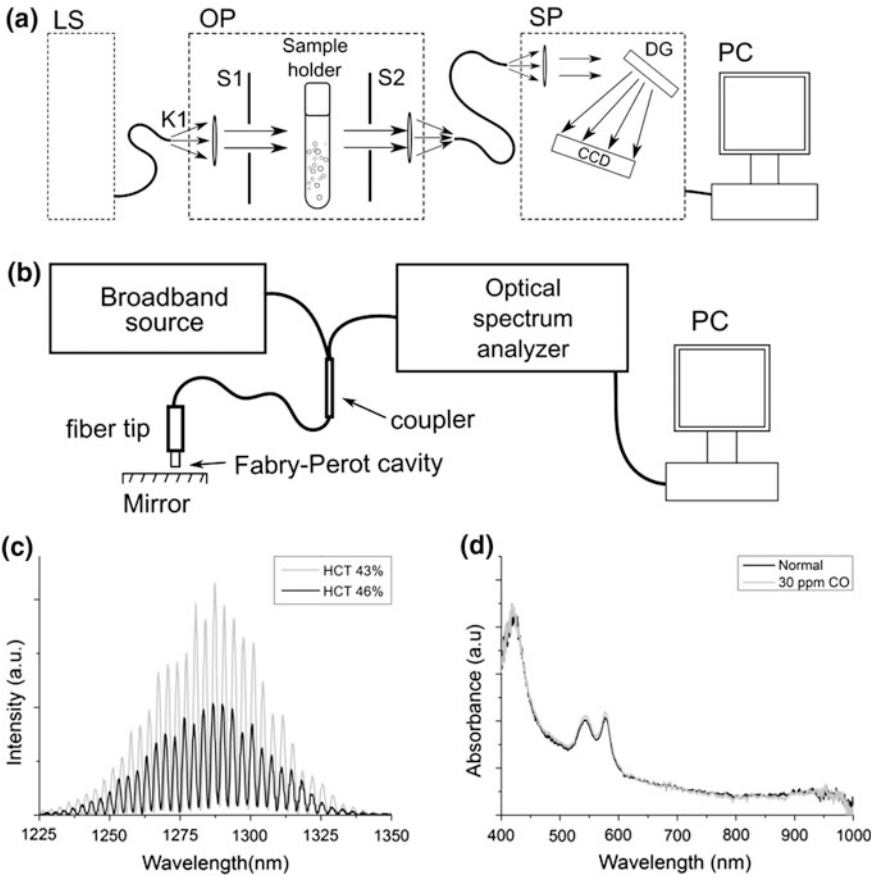

Fig. 1 Measurements systems and spectra: **a** absorption spectrometer set-up; **b** low-coherence interferometry set-up; **c** spectra of human blood samples with different Hct level measured by the low-coherence interferometry set-up; **d** absorption spectra of normal and CO-exposed whole blood samples

One of the methods allowing measurements of blood parameters, such as complex refractive index, is low-coherence interferometry. The light from the broadband source is transmitted to the sensing interferometer by the coupler and an optical fiber. At the sensing interferometer the amplitude of light is divided into two components and an optical path difference (OPD), which depends on the instantaneous value of the measurand, is introduced between them. The sensing interferometer is designed in such a way that a defined relationship exists between the optical path difference and the measurand. The signal from the sensing interferometer is transmitted back by the fiber to the optical processor. The optical processor consists of a second optical system, the output of which is a function of OPD generated at the sensing interferometer. The sensing interferometer is located inside the measurand field whilst the

optical processor is placed in a controlled environment. The optical processor is either a second interferometer (when the phase processing of the measured signal is used) or a spectrometer (when the spectral processing of the measured signal is used) [23]. The measurement system with the phase processing of the measured signal possesses very high measurement sensitivity and resolution, much higher than that of the system with spectral processing. However, the system with spectral processing of the measured signal has two important advantages. It does not need movable mechanical elements for precise adjustment as well as it is immune to any change of the optical system transmission. This is possible because in such a system the information about the measurand is encoded in the spectra of the measurement signal. Optical intensity at the output of such an interferometer can be expressed as [24]:

$$I_{out} = \left\langle E_1 E_2^* \right\rangle, \tag{18}$$

where $E = E_1 + E_2$, E_1 and E_2 are the amplitudes of the electric vector of the light wave reflected from the first and the second reflective surfaces inside the sensing interferometer respectively, brackets $\langle\rangle$ denote time averages; asterisk * denotes the complex conjugation.

When the spectral signal processing is used, the recorded signal can be expressed as [25]:

$$I_{out}(v) = S(v)[1 + V_0 \cos(\Delta\phi(v))] \tag{19}$$

where S(ν) is the spectral distribution of the light source; V_0 is the visibility of the measured signal, Δϕ(ν) is the phase difference between interfering beams.

The phase difference between interfering beams can be calculated from a following equation [26]:

$$\phi(v) = \frac{2\pi\, v\, \delta}{c} \tag{20}$$

where: δ is the optical path difference, c is the velocity of the light in vacuum.

If the light source exhibits a Gaussian spectrum, the normalized spectra pattern is predicted to be a cosine function modified by the Gaussian visibility profile. In the spectral domain signal processing the modulation frequency of the measurement signal gives information about the measurand. It can be noted that for Δϕ = 0 there is no spectral modulation. If the phase difference between the interfering beams varies from zero, the function takes the form of the cosine curve.

Low-coherence measurement system with spectral signal processing is not sensitive for any change of a transmission of the optical system. This is possible because in the system information about the measurand is encoded in the spectra of the measured signal. Therefore such a setup is the most convenient for the low-coherence blood samples measurements.

Low-coherence interferometer measurement set-up has been assembled from commercially available components Fig. 1b. Super luminescent diode Superlum Broadlighter S1300-G-I-20 with following optical parameters: λ_0 = 1290 nm, $\Delta\lambda$ = 50 nm, was used as a broadband light source. Signal was propagated through standard telecommunication fiber SMF-28. Optical Spectrum Analyzer Ando AQ6319 (wavelength resolution of 1 nm, wavelength accuracy of ±50 pm) was used as an optical processor. Measurements were carried out in a highly controlled conditions (especially temperature in laboratory room). In order to achieve satisfactory measurement precision for blood parameters monitoring, a large number of in vitro experiments with whole human blood were carried out. Blood samples with various parameters were collected from heathy volunteers via Gdansk Blood Donor Centre. Therefore, measurement range has been limited to blood parameters of healthy persons. Examination of numerous samples using low-coherence interferometer measurement set-up, allowed to determine relationship between registered signals and optical parameters. With the use of the described measurement system, the complex refractive index of numerous samples have been measured [27]. Changes in the real part of complex refractive index of measured sample have changed the modulation frequency of measured spectra, whilst the imaginary part influences the value of visibility of measured signal, as shown on Fig. 1c.

3 Blood CO Sensor Design

The main purpose of the new sensor is to shorten the process of diagnosis and continuous monitoring of intoxicated patients. Developed sensors will be dedicated to support diagnostics of critically ill patients in the emergency room at the hospital, small clinics and doctors' offices as well as fire engines and ambulances on the scene of the accidents. That will enable early and appropriate diagnosis, adequate treatment recommendations and specific antidotes selection.

The lab-on-a-chip will be realized in polymer, by employing state-of-the-art technologies such as soft lithography using the elastomer polydimethylsiloxane (PDMS), or by injection moulding of a thermoplastic material such as e.g. cyclic olefin copolymer (COC) or cyclic olefin polymer (COP). The latter solution will permit rapid production of a high number of chips. A solution where a thermoplastic and a soft polymer are combined will also be investigated, as well as possibilities for direct integration onto a Low Temperature Co-fired Ceramic package. Signal readout will be realized using commercial available data sampling hardware and software and carefully adapted for sensors needs.

A possible realization of the microfluidic package is shown in Fig. 2. The package will provide a housing for the silicon electrode chip (i.e. the Lab-on-a-chip component itself) with microfluidic channels (∼50 µm × 400 µm: channel depth × width) for delivery and control of the sample liquid, thermal control, and electrical access to the electrode system of the silicon chip, as well as adequate optical access to the biosensing area.

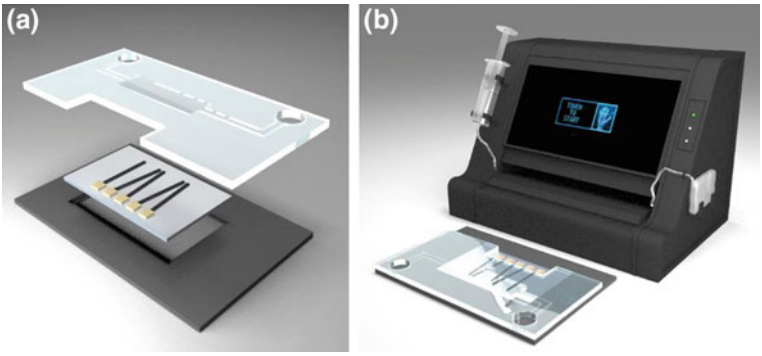

Fig. 2 Schematics of the proposed Lab-on-a-chip blood CO sensor: **a** disposable microfuidic chip; **b** a workstation housing optical and electronic parts of the system

The microfluidic sample delivery channel will ensure laminar flow in the sensing area of the silicon chip. Flow control will be achieved by an external syringe pump.

A particular challenge of the Lab-on-a-chip system is efficient integration of the electrical connection from the sensor chip to the external read-out electronics. Theoretical investigation of light interaction with blood in microfluidic channel of the sensor by Monte Carlo simulations will provide optimal parameters for the implementation of the optical fiber to the sensor. Nanotechnology will be used for the deposition of sensitive layers on the optical fibers surface. Reliability of the nano-sensor will be assessed by conducting a series of in vitro experiments on whole human blood with CO toxic levels measured in a reference analytical laboratory. Biostatistical analysis of measurement data will be used for validation of the sensor performance. Clinical trials will be conducted with the use of nano-sensor in development of approaches for diagnosis of "toxic effect of blood" on the brain microcirculation, which will contribute to the emergence of new knowledge about the resistance of cerebral vessels to hypoxia as a major factor, e.g. complicating stroke, post-birth brain injury, head trauma, brain edema.

4 Hollow Core Microstructured Waveguide Biosensors

Hollow core microstructured waveguides (MSWs) are novel unique materials for building highly sensitive sensors for a variety of biomedical applications including point-of-care medicine [28–30]. In addition to high sensitivity, basic advantages of MSW-based sensors are short response time and small size. The MSWs belong to photonic crystal waveguide family. Their hollow core diameter is a few times larger than the lattice period of the surrounding photonic crystal structures. The cross section of typical hollow MSW used for sensing is shown in Fig. 3a. The structure of the MSW, made from flint glass, consists of the hollow core of 150 μm in

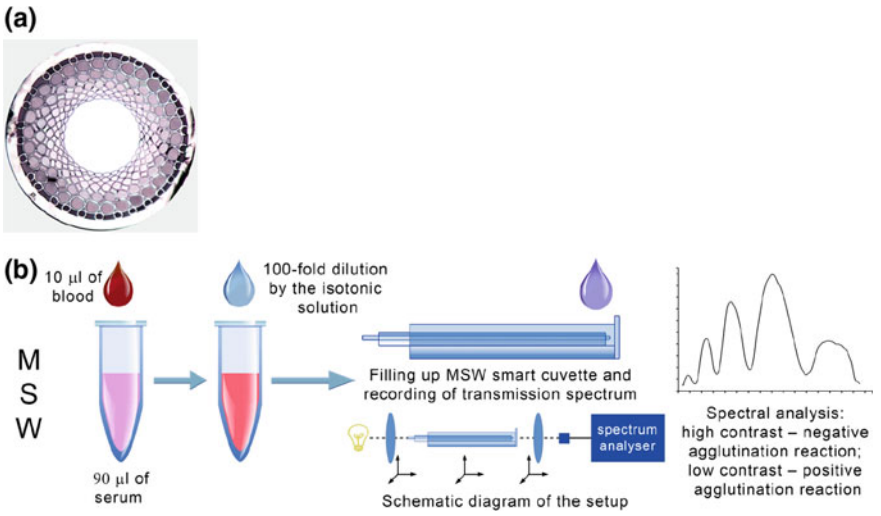

Fig. 3 MSW method for blood testing [33]: **a** electronic micrograph of the cross-section of hollow MSW (designed and manufactured by SPE "Nanostructured Glass Technology", Saratov, Russia); **b** protocol and equipment for blood testing using MSW smart cuvette and a portable spectrometer

diameter and five functional concentric layers of capillaries and an external buffer layer. Its outer diameter is around 1 mm.

The MSW possesses a few narrow and smooth transmission bands in the visible/NIR wavelength range (Fig. 3b, right). The location and number of transmission bands depend on the diameter of the MSW's core [28–33]. The unique features of MSW specify a high sensitivity to alterations of scattering, absorption, and refractivity of solutions filling up the MSW's core and channels in cladding. Usually, a short piece of MSW (a few centimeters in length) serves as a smart micro-cuvette filled up by a biological liquid sample tested in a portable fiber-optic spectrometer for providing spectral transmittance measurements.

For example, the 6 cm MSW with a hollow core diameter of 150 µm was utilized as a smart cuvette for blood typing [33]. Transmission spectra were measured using a setup containing a broadband light source (halogen lamp), fiber-optical elements for launching and collecting light, adjustable opto-mechanics providing a positional resolution of 1.25 µm, a spectrometer, Ocean Optics HR4000, operating in the visible/NIR range, and a PC (schematic diagram of the setup is present in Fig. 3b). An MSW-cuvette serves as a basic replaceable element of a biosensor for blood typing using a standard agglutinating serum technique. The protocol of the MSW technique for blood typing is present in Fig. 3b. A blood type (or group) is one of the most important characteristics of blood evaluated by the presence or absence of agglutinogens (antigens) on the surface of RBCs and antibodies in the blood plasma.

The new technique of blood typing based on hollow core MSW potentially allows one to decrease the percentage of mistakes [33]. After mixing the serum with

a drop of blood, in the case of a positive agglutination reaction, the majority of RBCs are clumped together. Thus, the number of RBCs suspended in the solution is reduced, which results in the reduction of the scattering coefficient of the solution and leads to transformation of its transmittance signature in the visible/NIR. Therefore, from the spectral transmission measurements using an MSW smart micro-cuvette, we can exactly distinguish positive and negative agglutination reactions. MSWs are prospective for blood biosensing, including such an important area of application as distinguishing the positive/negative agglutination reactions of RBCs. This method requires only a 10 μl of blood for one analysis. It is fast and simple enough to be used as a point-of-care modality.

5 Conclusion

In this chapter, we have presented a brief overview of physiological relevance of blood carbon monoxide levels on poisoning, as well as the optical properties of blood. Low-coherence interferometer and absorption spectroscopy set-up which we designed and elaborated are described. Those systems enable to obtain the optical parameters of blood such as complex refractive index and absorption spectrum. This gives us opportunity to find relationship between optical and hematological parameters of blood, such as complex refractive index and hematocrit level. We show the concept of optoelectronic sensors for rapid detection of environmental toxicity in blood of poisoned people. The application of the sensor can shorten the time of diagnosis of poisoned patients. It will be dedicated to support diagnostics of all patients in severe clinical state, such as fire victims. We plan to use such method in the Emergency Departments, small clinics and doctors' offices. The final section focuses on the hollow core microstructured waveguide biosensors for applications in biomedical sensors. Such waveguide can be used for investigation of optical parameters (scattering, refractivity, etc.) of many biological liquids, such as blood.

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