Optical densitometry method for liver function assessment using indocyanine green

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Abstract. Indocyanine green is a tricarbocyanine dye, that is stable in human blood plasma and can be completely eliminated by liver in several days. Due to its properties it can be used to assess the metabolic liver function. We present optical densitometry device and theory for taking measurements of concentration of indocyanine green in patient’s blood in vivo.

1. Introduction
Dynamic assessment of liver function can be critically important for patients before and after liver surgery. There is a number of methods to diagnose and predict course of liver condition. Analysis of blood samples for special contents incudes blood sampling from a patient which is not always available option, and also it takes a lot of time to get results. Ultrasound scan, MRI and computer tomography scan can show liver form, structure and appearance, but they are not capable to measure liver function [1-5]. Currently, optical methods are widely used for medical purposes [6-8] and for other fields of science and technology [9-14]. In this paper, it is proposed to use the method of optical densitometry to liver function assessment [5].

There is a method based on measurement of disappearance rate of a dye injected to patient intravenously. Indocyanine green is popular solution by reason of it can be easily detected in patient’s body by optical methods, because it has notable peak of absorption spectrum in near infrared section. Bounded with plasma indocyanine green has absorption peak on 805 nm [5, 15] and in water solution on 790 nm.

![Indocyanine green absorption spectra](image)

Figure 1. Indocyanine green absorption spectra in relative intensity units
Indocyanine green is biologically inert, which means it does not participate in the biochemical reactions in the body and does not permeate through the cell membrane. Indocyanine green is not toxic and can be completely removed from the human body by the liver.

Measurement of plasma disappearance rate of indocyanine green with light sensor is approved method for assessment of liver function [5,15,16], but existing commercial realization – LiMON device (Pulsion, Germany) – does not give complete data set of measurement process and also is quite expensive. Our sensor and processing system design is aimed to be cheaper and to give the full access to the data collected.

Liver function assessment is carried out by measurement of decreasing rate of dye concentration in blood. Decreasing rate is called PDR for Plasma Disappearance Rate which is measured in percent per minute and commonly located between 2 and 30 % / min.

2. Theory

Optical densitometry method is based on the Beer–Lambert–Bouguer law which describes intensity of light transmitted through the solution as negative exponential function of concentration and path length in the absorbing medium.

\[ I(c,z) = I_0 10^{-\varepsilon c z} \]  

where \( I_0 \) – reference light intensity, \( z \) – light path length through solution, \( I(z) \) – intensity of light traveled distance \( z \), \( c \) – solute concentration, \( \varepsilon \) – absorptivity, coefficient describing light absorption by one particle depending on wavelengths.

To calculate the concentration of solution first we have to determine \( I_0 \) value which is equal to intensity of transmitted light when solute concentration \( c \) is zero. Also we need to determine coefficient \( X \), taking into account diode emission spectrum, dye absorption spectrum and photodiode spectral sensitivity. Alternatively \( X \) can be calculated in serial experiments with known concentration.

\[ c = -\frac{\log \left( \frac{I(c,z)}{I_0} \right)}{\varepsilon z} \]  

The value \( \frac{I(c,z)}{I_0} \) is called transmittance, and value \( \varepsilon c z \) is called absorbance.

\[ T = \frac{I}{I_0}; A = \varepsilon c z; A = \log \left( \frac{1}{T} \right) \]  

In practice, the exponential dependence between the absorbance and transmitted light is observed only in a certain concentration range. If the solution is too diluted, deviations can be observed due to dissociation, hydrolysis, solvation, etc. With too high concentration of the solution deviations appear as a result of the processes of association, polarization, etc. Thus, outside a definite concentration range of the solution, the number of particles involved in light absorption does not correspond to the initial concentration.

Furthermore, a deviation from the main law of light absorption can be caused by chemical reactions that also change the light-absorbing properties of the medium under study. In addition, in case of using non-monochromatic radiation sources a mismatch with the basic law of light absorption can be observed if the radiation spectrum and the absorption spectrum of the test substance are correlated incorrect.

Eventually for measurements outside the determined concentration range application of a comparative analysis method is inevitable. There are methods of a calibration curve and direct comparison with a reference solution. As far as our method is intended to be applied for measurements of dye concentration dynamic inside human body it is impossible to apply comparison with reference sample. Therefore, we take measurements to describe the calibration curve.

3. Experimental setup

To test optical densitometry method before applying it to measure the concentration of indocyanine green in patient’s blood, we use the following scheme (see Figure 1). Light from the emitting diode with peak intensity wavelength 808 nm passes through cuvette with the studied solution, alternatively with finger (to calibrate setup for finger-clip sensor design) and is received by photo detector [17]. The
microcontroller is applied to operate light-emitting diode, to control [18] and collect data from photodetector (I²C protocol) and to transmit data by USB interface.

![Diagram](image)

**Figure 2.** The experimental setup: 1 — cuvette; 2 — emitting diode; 3 — photodetector; 4 — microcontroller; 5 — USB interface.

Measurements proceed by the following method. A number of solutions of different concentrations are prepared. Then we take measures of transmitted light intensity for every concentration.

4. **Results and conclusions**

Measurements with water solutions of indocyanine green are taken.

Samples are prepared using frozen droplets of water solution with a volume of 25 μl and a concentration of 1 μg / μl. To prepare the sample a single droplet is being unfrozen for a minute and then it is diluted to access required concentration. A concentration of solution in droplets is determined by the concentrations used in the medical measurements during liver function assessment. Initial concentrations in blood that allow measurements in vivo are close to 25 μg / ml. During the measurements concentration decreases by tens of percents a minute. Thereby we take measurements for low concentrations mostly between 2 and 4 μg / ml

All samples are examined in standard cuvettes with a base area of 10 × 10 mm and a height of several tens of millimeters. The volume of samples involved in the measurements varies from fractions of milliliters to several milliliters.
Figure 3. Observed transmitted light intensity dependence of solution concentration.

Figure 3 shows experimental transmitted light intensity dependence of solution concentration and exponential approximation of the curve. Curve contains deviation from the expectations corresponding to the light absorption law due to the use of non-chromatic light source with peak wavelength relevant for indocyanine green bounded with plasma proteins such as albumen.

In this work optical densitometry device and theory for taking measurements of concentration of indocyanine green are presented. We demonstrated the basic principles of light absorption in solution. The theory we suggested allow concentration measurements by referent method.

In further works we plan to use finger-piece design of setup to take measurements with patients during medical tests.

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