Advanced Bilirubin Measurement by a Photometric Method

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***Abstract*—This paper describes the design of an electronic instrument for measuring bilirubin by the optical method of light transmission through the skin. There was the knowledge of light transmission and absorption on a specific tissue compartment applied. The relevant skin photo-diagnostics handle 450nm - green and 575nm- blue monochromatic light. The registration of transmitted light of different frequency combinations presents the bilirubin quantity in human body by a non-invasive way. This used device was successfully tested and relevantly confronted with accurate laboratory instruments used for bilirubin measurements. The proposed device is more than ten times cheaper and easy to use. That provides reliable care after newborns within the postnatal care.**

***Index Terms*—Electronics, measurements, bilirubin, non-invasive.**

I. INTRODUCTION

Current technologies allow us to determine the value of bilirubin using several methods, both invasive and non-invasive. Invasive bilirubin measurement is carried in the umbilical cord and venous blood, amniotic fluid and cerebrospinal fluid.

Unconjugated bilirubin is bound to albumin by reaction with reagents and is known as indirect bilirubin. To make it respond, it must be released from the albumin binding by an accelerator (caffeine, sodium benzoate). Conjugated or direct bilirubin reacts directly with the agent [1].

Most clinically used methods for the determination of bilirubin and its conjugates (in the form of esters of mono- and diglucuronide of bilirubin and albumin covalently bound) are based on its reaction to diazotised sulphanilic acid. Ehrlich described it as early as in 1883. In 1916, it was applied by Van den Bergh and Müller for the quantitative determination. It is based on the creation of azo dyes, the so-called azobilirubin that acts as an acid-basic indicator. In a weak acidic solution and at neutral pH, azobilirubin is red, while in a strong acidic and alkaline area bilirubin is of the blue colour. Later, there were various forms and conjugates of bilirubin found.

An interesting and progressive method determining the amount of both total and direct bilirubin (yellow) was described by Doumas. It is based on oxidation with oxygen to the green biliverdin catalysed by bilirubin oxidase (BOX). The direct bilirubin is determined at pH 4.5 [2].

Decreases in absorbency of the reaction mixture of bilirubin are measured in the band 424 - 465nm in a kinetic way within the time interval of about 5 minutes. The total bilirubin is determined at pH 8.5 at the presence of accelerating detergents.

One of the other options is to determine bilirubin via biliverdin after oxidation by vanadic acid. This reaction is again based on the known oxidation of yellow bilirubin to green biliverdin. The oxidising agent - vanadic acid, and the two-point method measuring the absorbency before and after the oxidation are new. This allows for the determination of the total and the direct bilirubin. The total bilirubin is determined by a cation-active detergent and conjugated accelerator (cetyl-trimethyl-ammonium bromide). Oxidation is completed within about 3 minutes.

II. TRANSCUTANEOUS BILIRUBINOMETRY

Transcutaneous bilirubin bilirubinometry measures the amount of bilirubin transferring from serum to the skin tissue. In newborns, melanin, maturity of skin tissue, haemoglobin, and bilirubin participate in the skin reflectance. The measurement taking place also reflects in the quality of the results. Measurements taken on foreheads and sternum have proved the best correlation with serum bilirubin [2].

The first attempts of non-invasive bilirubin measurements took place in 1960. Gosset described the use icterometer at that time. This device, based on reflectance measurements, was unsuitably sensitive and specific and provided reproducible results at the rate of 20 to 40% [2].

The first sophisticated device for non-invasive measurements of bilirubin - Minolta / Air Shields Jaundice Meter - was launched in 1980. This instrument and the following model JM-102 provided a numerical index requiring the correlation of serum bilirubin. The device ColorMate III (Chromatisc Color Sciences International

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Inc.) operating within the wavelength range 460 - 550nm, required basal measurements of serum bilirubin. The current devices do not require any measurements of basal values and allow corrections by the skin colour and maturity [2].

The BiliChek unit (Philips Respironics) measures within the wavelength range from 380 to 760nm and uses more than 100 reading points. This allows corrections by interfering factors such as haemoglobin, melanin and the skin thickness. The advantage of the device lays in the possibility of its connection with a bar code reader or LIS. The disadvantage relates to the need to use a clean removable tip for each measurement and that increases the cost of measuring significantly. The measuring principle is shown in Fig. 1 [2].

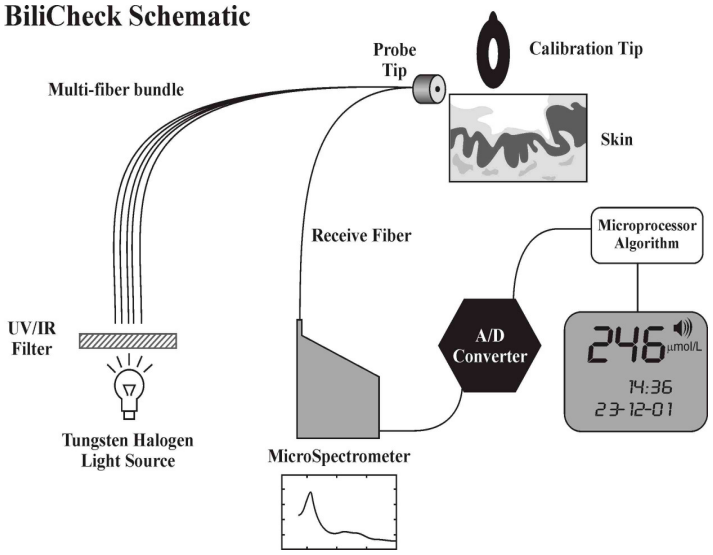

Fig. 1. Principle of BiliCheck measurement.

The great advantage of the transcutaneous bilirubinometry is its non-invasive character. Therefore, we expect that the measuring of transcutaneous bilirubin should result in a reduction in complications such as infections or osteomyelitis [2].

III. Direct Photometry Measurements

Direct photometric measurements are based on direct measurements of suitably solved serum at the wavelength of 455nm, which is the absorption maximum of bilirubin. Direct photometry can be used only in newborns. In the case of most children and adults, the serum includes many other pigments of similar colours and reactions as bilirubin.

Direct measurements may be also interfered by opalescence that results from the serum dilution or at the presence of oxyhaemoglobin in the neonatal serum, which is often hemolytic and absorbs light at the wavelength of 455nm.

These interferences might be suppressed by a proper adjustment of the working process, by measurements taken at two wavelengths of 455 and 575nm. The bilirubin concentrations are found from the absorbency differences. The first one corresponds mainly to the bilirubin content and the second one to the oxyhaemoglobin content [3].

A high quality device with a narrow definition of the monochromatic light must be used when measuring bilirubin by the direct spectral photometry. When calculating the concentration of bilirubin, we use the value of the molar bilirubin absorption coefficient. The molar absorption bilirubin coefficient ε is numerically equal to the bilirubin solution absorbency value having the concentration 1mol/L at a defined wavelength, temperature and layer width of 1cm [3].

The method is simple, but less sensitive. It is mainly used for obtaining fast information on the bilirubin concentration in neonatal hyper bilirubinemia.

Methods for the spectrophotometric determination of bilirubin in whole blood were developed at the end of the 1990s. The analyses utilise parameter analysers such as ions or metabolites. These analysers are equipped with a co-oximeter, which enables determination of bilirubin and various forms of haemoglobin (the total, oxy-, carboxy-, sulpho-, and methaemoglobin). Although the absorption spectra of bilirubin and haemoglobin differ, a large difference in concentrations of both parameters requires a complex measurement process. The analysers must be also able to quantify individual forms of bilirubin, which differ in their absorption maxima [2] as follows:

1) 459nm not conjugated bilirubin;
2) 422nm conjugated bilirubin;
3) Delta-bilirubin 433nm.

Co-oximeter modules measure at multiple wavelengths, the number and range of which differ with varied devices (Table I). Measurements can be taken from a non-haemolysed sample or after the chemical or ultrasonic haemolysis. The total bilirubin is calculated using a complex computational algorithm expressed in equivalents of the serum concentration [4]–[6].

TABLE I. CO-OXYMETER MODULES MEASURE AT MULTIPLE WAVELENGTHS, THE NUMBER AND RANGE OF WHICH DIFFER BY DEVICES.

Manufacturer	Device	Range λ	Number λ
Radiometer	ABL 835	478 - 672	128
Siemens	RapidLab 1265	500 – 680	256
Roche	OMNI S	460 – 660	512
Nova	Stat Profile CCX	557 – 650	7
IL	GEM 4000	480 - 650	about 1000

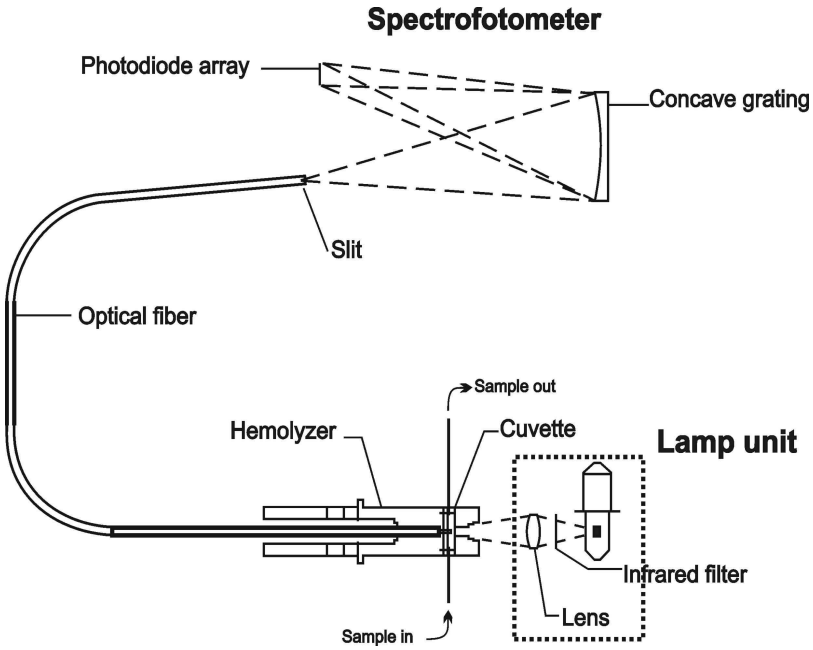

Fig. 2. Principle of spectrophotometer Radiometer ABL 800 FLEX analysis.

IV. Possible Errors in the Bilirubin Determinations

The colouring of serum itself may interfere with results of analyses at the interface of physiological and pathological values. That is why the bilirubin determination should be accompanied by the serum own blind test.

Blood sampling does not require any special conditions, but an improper storage of samples is a common cause of errors, mostly lower results. Bilirubin is very sensitive to light and the blood, and especially the blood plasma or the serum, must not be exposed to active light. Decomposition is also helped by an increased temperature and it is thus necessary to process specimens as soon as possible, or store them in a cool and dark place, no longer than for 12 hours [3].

V. Design and Realization of a Bilirubin Meter

The basis of an electronic bilirubin meter is a dual-channel circuit with an optoelectronic sensor that evaluates changes in the luminous flux of the corresponding wavelengths. Each channel consists of an optical sensor, analogue circuits for signal conditioning, and the A/D converter. The sensitivity of sensors - phototransistors - corresponding to the wavelength of incident light plays an important role in the selection of components. The mechanical design of the sensing adapter with a light source is another important factor for accurate measurement. After digitising the signal in the DAU unit, data are fed via the USB interface to PC, where they are processed by the LabView program.

Analogue electronics of each channel consists of three stages as shown in Fig. 4. The Stage 1 works primarily as I/U converter for the phototransistor. The Stages 2 and 3 amplify the signal and perform frequency filtering.

The output voltage signal U_2 enters the DAU unit in which it is sampled at a frequency 2kSPS and 12 bit resolution. For detection of bilirubin, the LabView uses mathematical functions primarily as a difference, averaging and frequency filtering.

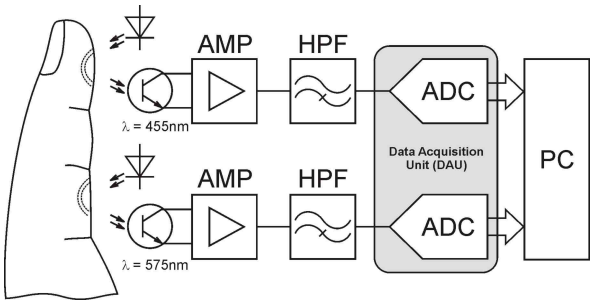

Fig. 3. Principle of the electronic circuit of bilirubin measurements.

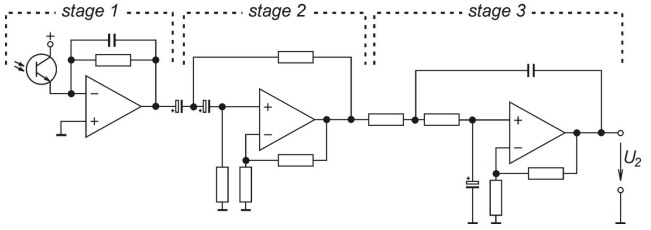

Fig. 4. Simplified diagram of the analogue channel for measurements of the pulse curve.

VI. Measurements and Tests

Functions of the complete implemented measurement chain were tested and verified in exact laboratory tests. The Radiometer ABL 835 system was used for bilirubin checking as a reference.

Concerning the laboratory reference values, there are the following ranges: newborns 0 – 1 day old: 0.0 – 38.0 µmol/L, 1 – 2 days: 0.0 – 85.0 µmol/L, 2 – 4 days old: 0.0 – 171.0 µmol/L, infants 3 weeks old – 1 year: 0.0 – 29.0 µmol/L man, woman 1 – 100 years old: 2.0 – 17.0 µmol/L.

There were tests of seven subjects 3 days to 42 years of age organised. The wide spectrum of testing subjects was chosen for the reason of variable bilirubin values.

TABLE II. BILIRUBIN MEASUREMENT RESULTS ACHIEVED WITH THE USE OF THE IMPLEMENTED BILIRUBIN PHOTOMETER IN CONFRONTATION WITH THE RESULTS GAINED BY THE RADIOMETER ABL835.

Test No.	Age	Radiometer ABL 835 [µmol/l]	Implemented device [µmol/l]	Relative error in %
1.	24y	11.7	11.6	-0.85
2.	3d	16.4	16.5	0.61
3.	12d	23.4	23.3	-0.43
4.	25d	19.3	18.8	-2.59
5.	8y	7.6	7.3	-3.95
6.	42y	6.2	6.2	0.00
7.	32y	31.5	31.8	0.95

The results of the tests have shown the principal measuring accuracy. The error level in measurements reached 3.95%. The relative error in all measurements was lower than 0.9%.

VII. CONCLUSIONS

The objective of this work was to design and implement a medical diagnostic measurement system for the bilirubin assessment. The system was implemented as a photometric method utilising measurement system with two specific wavelengths for the non invasive bilirubin value assessment. The electronic device was implemented and connected to an analogue digital unit for computer visualisation and recording the values and their time trends in a database. The relevant test was done in the Teaching Hospital in Ostrava with very good results related to both clinical and at home taken measurements. The maximum error in seven subjects reached less than 4% and the relative error was 0.9%.

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