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ITJ

ISSN 1812-5638

INFORMATION TECHNOLOGY JOURNAL

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Information Technology Journal 12 (1): 192-197, 2013
ISSN 1812-5638 / DOI: 10.3923/itj.2013.192.197

A Novel HGB Concentration Measurement Method Based on Single-wavelength Spectrophotometry

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Abstract: Traditional HGB (hemoglobin) concentration measurement system hasn't been integrated with close-loop controlling, which always reduces the accuracy of HGB concentration. And the measurement method is also complex. Herein, a novel HGB concentration measurement system with closed-loop controlling is introduced. Utilizing Lambert-Beer Law and single-wavelength spectrophotometry, the calculation and controlling of PI (proportional-integral) are completed with C8051F020, which not only reduces the complexity of the hardware system, but also improves the reliability of the system. Furthermore, the software of the system was provided too. Experimental results show that this system has higher stability and accuracy compared to the traditional HGB concentration measurement system.

Key words: HGB, Lambert-beer, spectrophotometry, proportional-integral, closed-loop

INTRODUCTION

In clinical medicine, HGB concentration measurement instrument has been widely used in operation theater, emergency ward, neonatal intensive care units and so on, therefore, it is very important to measure the HGB concentration. Since the clinical examination is difficult to separate hemoglobin in the blood, the red blood cell should break up by hemolysin (Shimada *et al*., 2000) so that the internal hemoglobin is dissolved in the diluent samples and stable hemoglobin is formed. Now-a-days, the majority methods of measuring HGB concentration are single-wavelength spectrophotometry, dual-wavelength spectrophotometry and derivative spectrophotometry (Yufa *et al*., 2012; Adamczyk and Gebler, 1997).

At present, most of the blood analyzers measure the HGB concentration by calculating the frequency which is transformed from intensity strength, then, they obtain the HGB concentration via counting the number of pulses which is completed in complex processor FPGA or ARM and they are basically open-loop controlling and dual-wavelength spectrophotomety (Larsen *et al*., 1991). All of these will enhance the complexity and costs as well as reduce the accuracy of measurement.

In response to these deficiencies, this study proposed a new HGB concentration measurement system which is based on the microcontroller and closed-loop controller. Single-wavelength spectrophotometry is used in the system and the data directly from AD converter is calculated. Moreover, it can also make blank diluent stable to the reference value so that the influence of external factors is eliminated and meantime, the stability of the system is improved quite well.

BASIC PRINCIPLE OF SINGLE-WAVELENGTH SPECTROPHOTOMETRY

The principle of HGB concentration measurement of the system is using single-wavelength spectrophotometry (Oppenheimer, 1997). Figure 1 illustrates the relationship between wavelength of visible light and absorbent in cyanide methemoglobin solution (Susanto *et al*., 2006). It is clear from Fig. 1 that visible light reaches a maximum of absorbance near the wavelength of 540 nm (green light). Consequently, the accuracy of measurement concentration with this wavelength is higher than that at other wavelengths.

Steps of measuring HGB concentration by single-wavelength spectrophometry are described as followings: Green light with intensity I_{in} incidence into blank diluent (Fig. 2). Assuming the emission intensity is I_0, absorption of scattering and refraction is A_{S0}.

Same green light with intensity I_{in} incidence into blood sample (Fig. 3). Assuming the emission intensity is I_1, absorption of scattering and refraction is A_{S1}.

According to the Lambert-beer law (Cejnar *et al*., 1993), the equation can be expressed as:

$$KL[C_0] = \log\left(\frac{I_{in}}{I_0}\right) + A_{s0} \quad (1)$$

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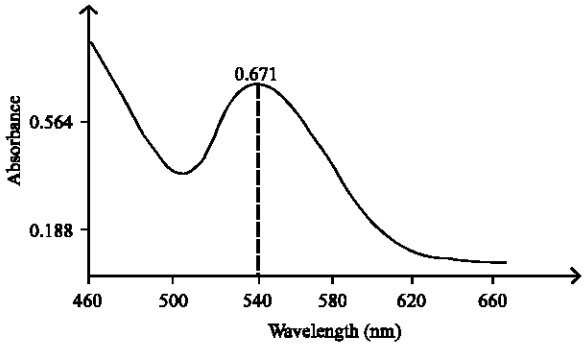

Fig. 1: Absorbance and wavelength

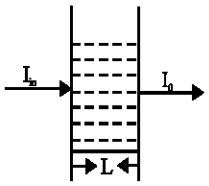

Fig. 2: Blank diluent, I_{in}: Input intensity of green light, I_0: Output intensity of blank diluent, L: Thickness colorimetric cell

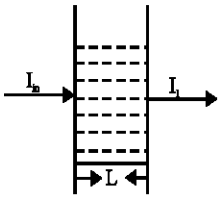

Fig. 3: Blood sample, I_{in}: Input intensity of green light, I_1: Output intensity of blood sample, L: Thickness colorimetric cell

$$KL[C_1] = \log\left(\frac{I_{in}}{I_0}\right) + A_{s1} \quad (2)$$

Where:
K = Absorptivity
L = Thickness colorimetric cell
C_0 = Blank diluent concentration (its value is 0)
C_1 = Blood sample concentration

Subtract Eq. 2 from Eq. 1, it can be formulated in the following way:

$$[C_1] = \frac{1}{KL}\log\frac{I_0}{I_1} + \frac{1}{KL}(A_{s1} - A_{s0}) \quad (3)$$

The A_{S0} and A_{S1} have the same value because of the same system, so C_1 can be rewritten as:

$$[C_1] = \frac{1}{KL}\log\frac{I_0}{I_1} \quad (4)$$

Equation 4 indicates that sample concentration is proportional to the logarithm ratio of light intensity.

THE NOVEL HGB MEASUREMENT SCHEME BASED ON SINGLE-WAVELENGTH SPECTROPHOTOMETRY

According to the principle mentioned above, if the values of I_0 and I_1 can be confirmed, the concentration of HGB is also specific. In this study a new method is put forward. That is to control the current of LED through the VCCS (Thompson, 2006). The light current received by photodiode will be covered to voltage through an amplifier and then through ADC it will be covered to a digital value to be calculated. Lastly, the feedback net, which is consists of PI controller, will stabilize blank diluent reference value.

Circuits are designed for the novel HGB concentration measurement. The system is consisted of a microcontroller as the core controller unit, VCCS (voltage-controlled constant current source), photovoltaic conversion and Human-machine Interface (HMI) subsystem. Figure 4 shows the specific frame for the system.

The AD and DA converter with external integrated chips will make the circuit complicated; instead, the C8051F020 which has an on-chip 12-bit SAR ADC and 12-bit DAC is used in the system. It is fully compatible with the MCS-51 (Mo, 2007) core and instruction set. The maximum speed is 25 MIPS. The main function module will be introduced as followings.

Voltage-controlled constant current source: Luminance is mainly controlled by LED driving current, so it is better to drive LED with a constant-current source. The system uses the DAC and the voltage-current circuit to facilitate the design of Voltage-controlled Constant Current Source (VCCS). The structure diagram is shown in Fig. 5.

The reference voltage of the DAC is 2.43 V. When the output voltage of DAC changes, the output current also changes accordingly, since a linear relationship exists between the current and voltage have. Therefore the LED will have different intensity.

Voltage-current converter: The mainly part of the voltage-current converter circuit is the operational amplifier LM358 (Fig. 6).

R_6 is the load resistance and I_{out} is output current. According to the circuit, the equations are expressed as:

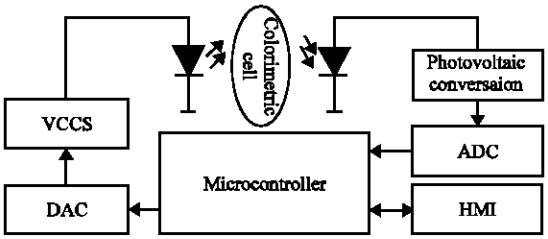

Fig. 4: The structure diagram of the hardware for the system, VCCS: Voltage-controlled constant current source, DAC: Digital-to-analog converter, HMI: Human-machine interface, ADC: Analog to digital converter

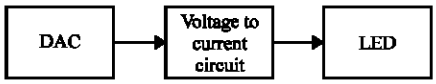

Fig. 5: Structure diagram of the VCCS, DAC: Digital-to-analog converter and LED: Light-emitting diode

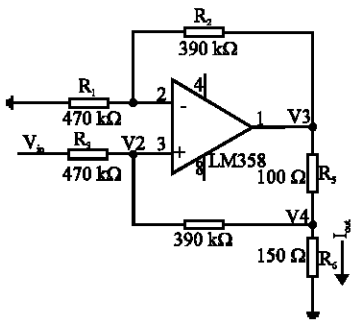

Fig. 6: Voltage-current converter circuit

$$\frac{V_2 - V_{in}}{R_1} = \frac{V_4 - V_2}{R_2} \quad (5)$$

$$V_2 = \frac{R_1}{R_1 + R_2} V_3 \quad (6)$$

Assuming R4>>R5, R4>>R6, that is to say, shunting effect of R4 to output current I_{out} can be ignored. Finally, an equation can be represented as:

$$V_4 = \frac{R_6}{R_5 + R_6} V_3 \quad (7)$$

Making R4/R3 = R2/R1 and put Eq. 6 and 7 into 5 it can be formulated in the following way:

$$I_{out} = \frac{R_4}{R_3 * R_5} V_{in} \quad (8)$$

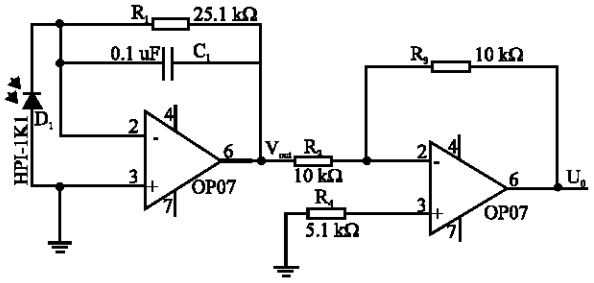

Fig. 7: Photoelectric conversion circuit

It is a simple circuit but has a high-precision voltage-current converter (Naishadharn, 2001). The output current range is 0 to 20 mA and it is not affected by the load resistance.

Photovoltaic conversion: As known, PN junction can work with photoconductive effect (Thompson and Schlecht, 1997). On the condition that the inverse bias of PN junction and the absence of light, high impedance and small reverse current is the case. When light is on, the electrons move to N area while the hole move to P area, so photocurrent is formed with direction same as reverse current. The light sensitivity of the photodiode at λ is $S_\lambda = I_{sc}/I_\lambda$ and Vout = $-I_{sc} \times R$ can be obtained in an amplifier circuit. Therefore, I_λ and V_{out} are proportional to each other. Due to the acquisition of the microcomputer voltage being 0 to 2.43 V, so the negative output voltage of the photoelectric conversion circuit should be converted to a positive U_0 through an inverter. U_0 was led into the ADC as input afterwards (Fig. 7).

Selection of blank diluent reference: The blank diluent reference value refers to the value (unit: mV) which goes through the photoelectric conversion circuit and is converted by AD when green light incidences into blank diluent. The ADC in the system has a precision error itself, so in the case of low voltage, the conversion error is relatively large. If I_0 is too small, not only the wasting of accuracy of the ADC is induced but also the accuracy and stability of the system will be affected in high concentrations of blood. As the ADC sampling voltage range is 0-2.43 V, a margin should be made to prevent the over range of sampling rang. For this purpose, the value of 2000 mV is set as the system's blank diluent reference.

THE DESIGN OF SOFTWARE

When the system initialization is completed and the first measurement command is sent, the system will detect whether blank diluent reach to the reference value. If not,

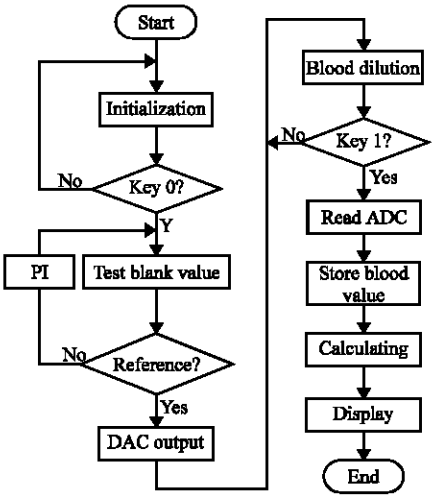

Fig. 8: Main flowchart of software

it is adjusted to the reference value of PI and records the voltage. When the blood dilution is completed, the system samples the blood voltage. In this way, the blank value and blood value are stored and calculated by the microcontroller. The flow chart of the software is shown in Fig. 8.

Digital PI controller: PID control is a second-order linear controller. Good closed-loop performance is available in most of the control systems by setting the coefficient of proportion, integral and differential. In this system, the PI controller (Hagglund and Astrom, 1995) is also used to get a good closed-loop performance. The equation of PI controller is written as followings:

$$u(k) = K_p e(k) + K_i \sum_{j=0}^{k} e(j) \quad (9)$$

Here, u(k) indicates the current output. K_p reflects the error signal e(k). If the deviation comes, K_p will work immediately in order to reduce the deviation. K_i is mainly used to eliminate the static error. The structure diagram of PI controller is shown in Fig. 9.

The program design of PI controller: The reference value of the system will have some offsets because of the aging of LED, the damaging of optical receiver, changing of amplifier and so on. Taking these into consideration, it should be adjusted by PI controller (Qian and Song, 1983). The PI controller will start to work when the current of blank diluent is not equal to the reference value. It will continue working until the current gets to the reference value. For example, the system will increase the incident

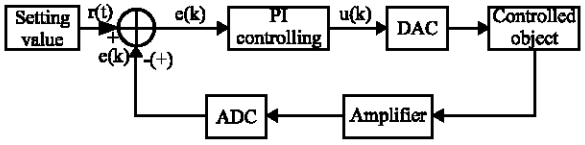

Fig. 9: Structure diagram of the PI controller, PI controlling: Proportional-integral controlling, DAC: Digital-to-analog converter, ADC: Analog to digital converter

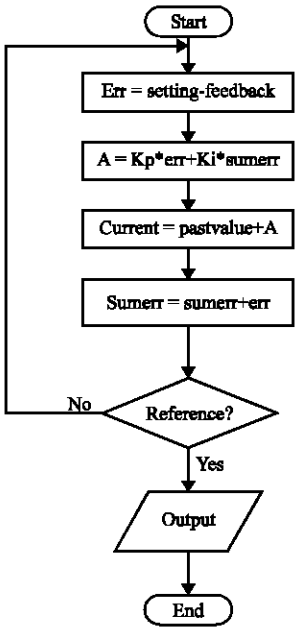

Fig. 10: Flowchart of PI controller

intensity of the LED when magnification decreases, in order to enhance the value of voltage. The chart of PI controller is shown in Fig. 10.

EXPERIMENTAL RESULTS

VCCS: When the range of input voltage is 0-2.43 V, a curve can be drawn up to display the relationship between input voltage and output current, as shown in Fig. 11.

The current-voltage curve were determined and compared to the theoretical one. The ΔI_{max} = 0.97 mA, so its nonlinearity error is 4.85% FS.

Stability of blank diluent value: The stability of blank diluent value is an important indicator of measurement of HGB concentration. With the blank reference as 2000 mV, the blank value is measured for 10 times. The measurement data are shown in Table 1.

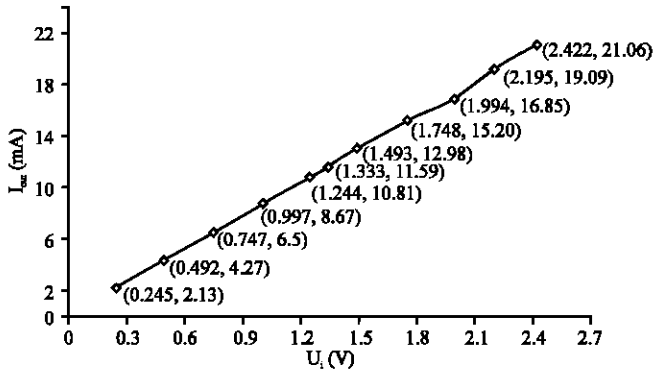

Fig. 11: The curve of VCCS

Table 1: Stability testing of blank diluent

Second value (mV)	Error (%)
2010	0.50
2006	0.30
2003	0.15
1998	0.10
1994	0.30
2001	0.05
1999	0.05
2001	0.05
2003	0.15
1999	0.05

Table 2: Relationship between concentration and logarithmic

Blood concentration (μL)	HGB voltage value (mV)	log I_0/I_1
7	1388	0.158
9	1249	0.203
11	1130	0.247
13	1010	0.298
15	926	0.333
17	856	0.368

It can be drawn from the table that the maximum error of blank diluent is 0.5%. So it meets the criteria of the original design.

Linearity of blood concentration: The previous analysis shows that the equation of hemoglobin concentration is:

$$[C_1] = \frac{1}{KL}\log\frac{I_0}{I_1}$$

where, 1/KL is a constant, so the concentration and log I_0/I_1 have a linear relationship with each other. In actual measurement, the concentration is divided into 6 groups with 2 μL intervals and measured 5 times in each group. The data in Table 2 is obtained afterwards.

Here, Iv is the average of HGB concentration in the blood sample. The linear relationship can be presented with a curve (Fig. 12) based on the table above.

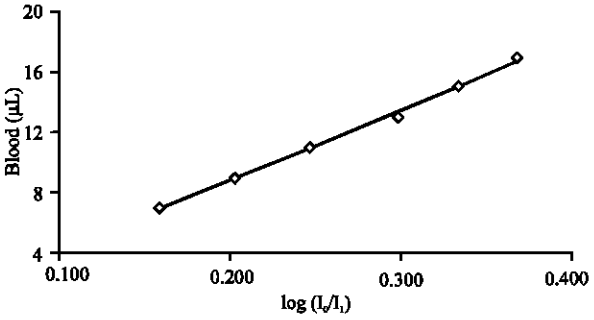

Fig. 12: The linearity of concentration

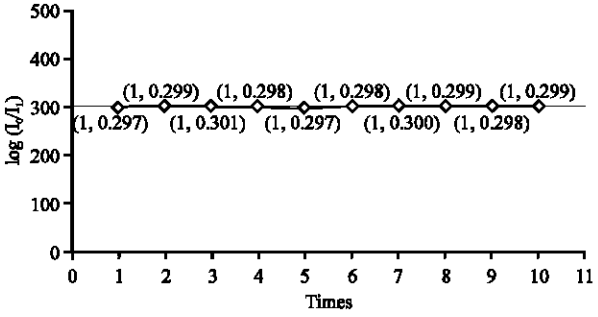

Fig. 13: Stability of blood

The maximum deviation between the measured concentration and fitting curve is 0.412 μL. Consequently, its nonlinearity error is 2.42% FS.

Stability of HGB concentration value: According to the relationship mentioned above, the stability of log I_0/I_1 is same as HGB concentration's stability. In the case of 13 μL of blood, it was measured 10 times and the value of log I_0/I_1 is recorded simultaneously to draw up the following Fig. 13.

It can be concluded that in the 10 measurements, the mean of log I_0/I_1 is 0.299 and the variance is 1.6×10^{-6}.

CONCLUSION

A novel HGB concentration measurement method based on single-wavelength spectrophotometry is proposed in the study. The system is mainly built up with VCCS, photovoltaic conversion and HMI subsystem. Both calculation and adjustment are performed by the microcontroller. The test results show that the maximum error of blank diluent is 0.5% and nonlinearity error of the concentration is 2.42%. The performance of this system meets the requirement for practical application and is similar to the performance of traditional HGB concentration measurement system. Therefore, a wide application prospect is expected for the system, since it can be transplanted into the blood analyzer easily and makes the instruments with portable HGB concentration measurement fairly possible.

ACKNOWLEDGMENT

This study was supported by Faculty of Information Science and Engineering of Ningbo University, Zhejiang, China and Brocade Medical Equipment Company.

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