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Digital Image Processing Technique for Blood Glucose Measurement

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Abstract: Colorimeters have been used in the Clinical Laboratories for estimating the concentration of Blood Glucose of the Diabetic patients for the past 5 decades. The complex substances appearing in Blood serum develop color following the reaction with a reagent. Colorimeter measures the concentration of the Blood substance based on the amount of absorption of a monochromatic light in that solution. Error of 5-20% is not uncommon because those measurements require a highly monochromatic source. The spectral bandwidth of the filters used for this purpose and the operational wavelength of the photo detector are also behind the inaccuracy in the result. This paper addresses Digital Image Processing technique as a complementary and alternative method of Glucose measurement. The performance of measurement of Blood Glucose based on the RGB data of Colour Image of the assay is compared with that of the Colorimeter. The results show the consistent performance of Digital Image Processing even in the non-linear region of the assay.

Key words: Colorimetry, absorption, colour image, RGB data, tristimulus colorimeter, colour measurement

INTRODUCTION

Blood Chemical tests are done at the Clinical Laboratories to help Doctors for making a prognosis. Hence error in the measurement of concentration of Blood Chemical would misguide the Physicians and that would result severely in the treatment of patients. In the case of diabetic patients, error in the measurement of Blood Glucose (Often called Blood sugar) might result in the perennial complications such as Heart attack, Brain attack, Strokes, Blindness and Kidney failure, etc.

Blood Glucose test is done as per *Glucose Oxidase* method[1,2] in the Clinical laboratories with the help of instruments such as Colorimeter. The complex substances appearing in Blood serum develop colour following the reaction with a reagent. Colorimeter measures the concentration of the Blood substance based on the amount of *absorption* of a monochromatic light in that solution. Errors of 5-20% are not uncommon because those measurements require a highly monochromatic source[3,4]. The spectral bandwidth of the filters used for this purpose and the operational wavelength of the photo detector are also behind the inaccuracy in the result. Colorimeter exhibits more measurement complexity especially in the non-linear region of the reagent solution. For example, only up to the Blood Glucose concentration of 400 mg dL^{-1}, the reagent used for Blood Glucose measurement offers linearity. Whereas, measurement of sugar level in this non-linear region is very crucial as far as the diabetic patients are concerned.

As the Blood chemical tests are basically Colour measurement, it is evident that *Digital Image Processing* technique using a Computer with Digital Camera can replace the Colorimeters with a great deal of accuracy. A 24-bit RGB data can distinguish 2^{24} (1,67,77,216) numbers of colours. Hence it is evident that the *Digital Image Processinging* technique would be far superior for the Colour measurement based applications.

Characterizing the Digital Camera as an absolute Tristimulus Colorimeter[5] has paved way for the use of Digital Still Cameras for Colour measurement with the help of the RGB data of the image. This Digital Imaging technique has been successfully applied for Colour measurement related applications such as Automatic visual inspection of Colours in an industrial production process[6], Estimating the quality from the colour of Red wines in Refineries[7], measuring and analyzing the textured dye material and printed fabric[8] and detecting skin cancer in Dermatology[9]. This research paper aims to adopt this Digital Camera based *Tristimulus Colorimeter* technique to replace the Colorimeter in Clinical labs.

Colorimeter measurement: Blood sample is collected under aseptic conditions. The serum or plasma is separated from this sample as soon as possible and this serum is kept in the refrigerator until the measurement is taken place. This serum consists of all Blood chemicals.

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The estimation of Blood Glucose is carried out in many methods. The popularly used *Glucose Oxidase method* is given here as an example. In this method, Glucose Oxidase oxidizes the Glucose in serum to Gluconic acid with the Gluconolactone as intermediate. The Hydrogen Peroxide, which is also formed, is broken down to water and Oxygen by a peroxidase in presence of an Oxygen acceptor, which is converted to a coloured compound.

The intensity of the coloured complex thus produced is directly proportional to the concentration of Glucose in serum. After adding the serum to the reagent, the entire solution should be kept in the incubator at the temperature of 37°C (i.e., Body temperature) and the colorimetric measurement should be done exactly after 10 min by passing the monochromatic light with a wavelength of 505 nm in the Colorimeter (Fig. 1).

In order to obtain purity in measurement, optical colour filters are used to select a narrow wavelength spread (Bandwidth) of light that shines on the Photo detectors. As most of the photo detectors offer their optimum performance near the wavelength of 520 nm (green), most of the Colorimeters prefer this wavelength only. Green's complementary colour of pink or purple would be the colour developed in the assay.

Basic Colorimeter analysis involves the precise measurement of light intensity. Transmittance (T) is defined as:

$$T = (I_o/I_{in}) \times 100\ \% \quad (1)$$

Where I_o is the attenuated light intensity and I_{in} is the initial light intensity.
Absorbance (A) [Optical density] is defined as

$$A = \log (1/T) \quad (2)$$

If the path length or concentration increases, the Transmittance decreases and the Absorbance increases. Essentially, Beer-Lambert Law can express this phenomenon, by

$$A = a\,C\,L \quad (3)$$

Where A is Absorbance, L is Cuvette path length, C is concentration of absorbing solution and a is absorbtivity or Molar Extinction Coefficient related to the nature of the absorbing substance and optical wavelength.

If *Absorbance* $A_{Standard}$ is known for the Standard Glucose solution with Glucose concentration of $C_{Standard}$ then the Glucose concentration in the unknown assay can be found from the following relationship:

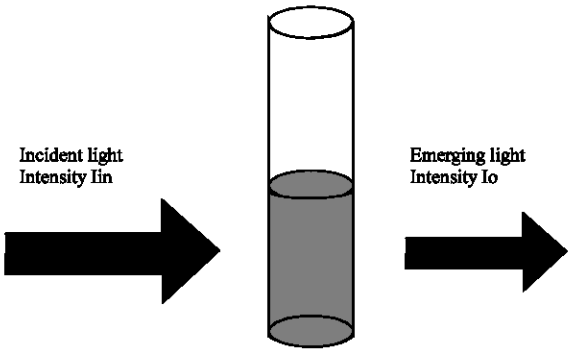

Fig. 1: Colorimetry

$$C_{Unknown} = C_{Standard} \times \frac{A_{Unknown}}{A_{Standard}} \quad (4)$$

Where Absorbance of the assay $A_{Unknown}$ is obtained from the Colorimeter.

Colour image theory: A Digital Camera can be used to capture the Colour Image of the assay. This Colour image is represented in the RGB model that represents each pixel with three primary spectral components such as Red, Green and Blue. Each RGB colour pixel {that is, a triplet of values (R, G, B)} is said to have a depth of 24 bits and each primary colour is represented in 8 bits in it. The term full colour image is often used to denote a 24 bit RGB colour image.

Any colour picture or image has three characteristics to specify its visual information. These are (i) Luminance or Brightness (ii) Hue or Tint and (iii) Saturation. Luminance is the amount of light intensity as perceived by the eye regardless of the colour. Hue is the predominant spectral colour of the pixel and Saturation is the spectral purity of the colour.

As the concentration of the colour developed in the assay changes in accordance with the change of Glucose concentration, it is clear that the Hue or Tint of the Colour of the assay will not change for any variation in the concentration of Blood Glucose whereas the change can be in either Luminance or Saturation. Less concentrated coloured solution will possess more brightness and high concentrated coloured solution will possess less brightness. Hence, like *Absorbance* in Colorimeter, this *Luminance* of the colour pixel in the colour image can be used as a parameter to estimate the Blood glucose concentration in the assay.

Brightness or Luminance of the colour in a particular pixel is given by

$$Y = 0.3\,R + 0.59\,G + 0.11\,B \quad (5)$$

For example, a White image with its RGB data of $FFFFFF_H$ (equivalent to $255\ 255\ 255_D$) will have a brightness equal to 255_D as per Eq. (5) and it is equivalent to 100%. Similarly a pixel with RGB data of $230\ 200\ 200_D$ will have a brightness of 210_D which is equivalent to 82.35% only.

MATERIALS AND METHODS

The methodology followed to measure the concentration of the Blood Glucose experimentally in the Laboratory from the Colorimeter and the Digital Colour Images (Fig. 2) is as follows:

Standard Glucose solutions are used for conducting experiments and the reagent used for the measurement of Glucose concentration is from AUTOSPAN. The basic assay parameters of this reagent solution are listed in Table 1.

Step 1: 1 mL of the Blank reagent solution is taken in the test tube whose path length is 1 cm. This test tube is inserted into the Colorimeter. Colorimeter's wavelength is set to 520 nm and the Colorimeter's gain is adjusted so as to get the null Absorbance in the meter. At the same time, 1ml of the Blank reagent is taken in a specially designed cuvette and its colour image is captured and stored in the Computer. Its RGB data of the assay can be obtained from any pixel of the colour image. *Luminance* (Y) is calculated from this image using Eq. 5.

Step 2: 10 μL of the standard Glucose solution with the concentration of 100 mg dL^{-1} is added to the blank reagent solution in the test tube and it is kept inside the incubator at the temperature of 37°C. After 10 min, Purple Colour would have developed in the assay. This test tube is inserted into the Colorimeter and the Absorbance shown on the meter of the Colorimeter is noted as $A_{Standard}$. This assay is now taken in the cuvette and its colour image is captured as another colour image and stored in the Computer. As in Step 1, the Luminance (Y) of this image is also calculated and tabulated. This step is repeated for the known concentrations from 150 to 800 mg dL^{-1} at the Glucose concentration gap of 50 mg dL^{-1} (Fig. 2). The corresponding RGB data and their respective *Luminance* values are calculated.

Table 1 : Assay parameters

Mode	End point
Wavelength (nm)	505 (49550 nm)
Sample volume (μL)	10
Reagent volume (μL)	1000
Incubation time (min)	10 at 37°C
Linearity high (mg dL^{-1})	500
Stability of colour	1 h
Concentration of standard	100 mg dL^{-1}
Blanking	Reagent Blank
Absorbance limit (max)	2.000
Optical path length (cm)	1
Units	mg dL^{-1}

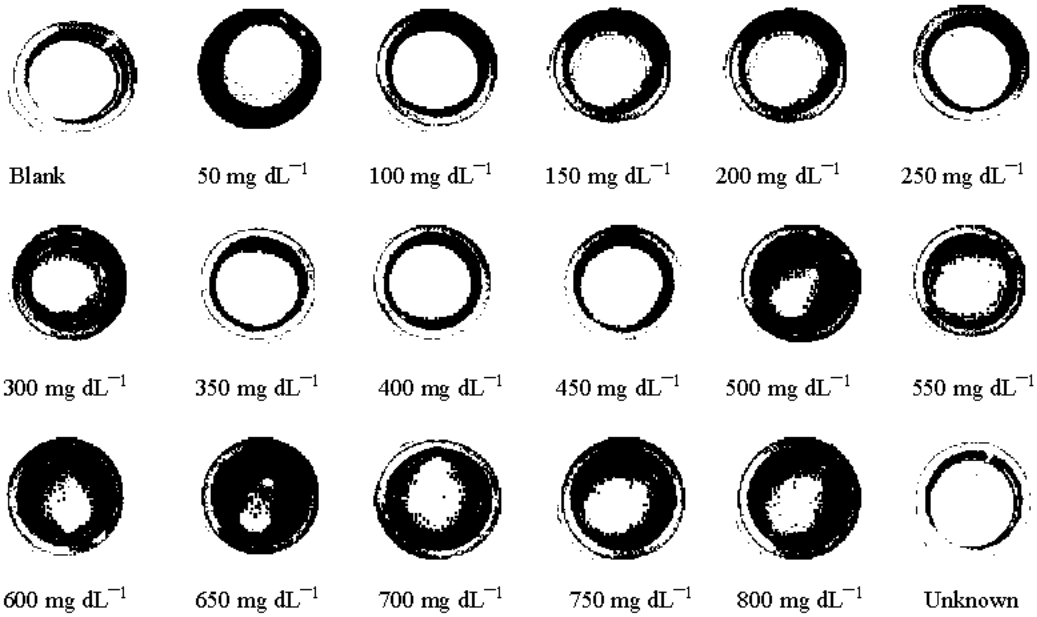

Fig. 2: Assay with different glucose concentrations

Step 3: Now, 10 μL of the unknown concentration of Blood Glucose is added to another 1 mL of Blank reagent and kept at 37°C for 10 min. Step 2 is repeated and Absorbance from the Colorimeter is taken as $A_{Unknown}$. This is substituted in Eq. (4) to find out the unknown Blood Glucose concentration. The assay is taken in the cuvette and the colour image of the assay is captured and stored in the Computer.

Step 4: For measuring the concentration of Blood Glucose using the RGB data, the *Luminance* (Y) values obtained in Step 2 for various known concentrations of Glucose (say X) are extrapolated to fit into an m^{th} order polynomial equation and this equation will characterize the behaviour of reagent used for Blood Glucose measurement.

In general for any order say 'm', the polynomial equation will be,

$$X = a_m Y^m + a_{m-1} Y^m + \ldots\ldots + a_2 Y^2 + a_1 Y + a_0 \quad (6)$$

Where a_m, a_{m-1}, ……., a_1, a_0 are the coefficients to be found.

Equation 4 used with Colorimeter is suitable only for linear responses whereas this polynomial equation of the form of Eq. 6 is suitable even for non-linear responses. This polynomial equation should be the characteristic equation of the reagent used for Blood Glucose measurement. Once this polynomial equation is ready then the Brightness (Y) of the Unknown Glucose concentrated assay can be substituted in this Eq. 6 and the Unknown Glucose concentration (X) can be found.

RESULTS AND DISCUSSION

Glucose standard solutions are used to conduct experiments in the laboratory. Glucose standard solutions of concentrations from 50 to 800 mg dL^{-1} at the Glucose concentration gap of 50 mg dL^{-1} are taken and 12 tests in each category are conducted. Averages of the readings observed in those experiments are tabulated in Table 2. This shows the response characteristic of the reagent used for the measurement of concentration of Blood Glucose.

The graph shown in Fig. 3 depicts the responses of Glucose concentration Vs Absorbance measured in the Colorimeter and Glucose concentration Vs Luminance measured from the images captured with Digital Camera.

This graph clearly shows the nonlinearity in the response of reagent used for the measurement of Glucose. Non-linearity prevails both in *Absorbance* and *Luminance* when the Glucose concentration is over 400 mg dL^{-1}. As

Table 2: Characteristic response of reagent

Glucose concentration (X) (mg dL^{-1})	Average colorimeter reading (Absorbance)	Average data from image R	G	B	Average luminance (Y) (%)
Blank (0)	0.000	224	215	208	84.96
100	0.306	234	171	17	74.99
150	0.460	226	146	161	67.39
200	0.617	230	12	14	63.33
250	0.790	227	115	141	59.41
300	0.896	227	101	131	55.66
350	1.010	223	83	118	50.41
400	1.144	216	66	105	45.33
450	1.255	211	56	4	41.70
500	1.31	201	45	81	37.44
550	1.36	200	40	72	35.81
600	1.38	195	35	65	33.94
650	1.406	184	32	62	31.89
700	1.453	183	30	54	30.76
750	1.48	178	26	50	29.17
800	1.57	177	23	45	28.08

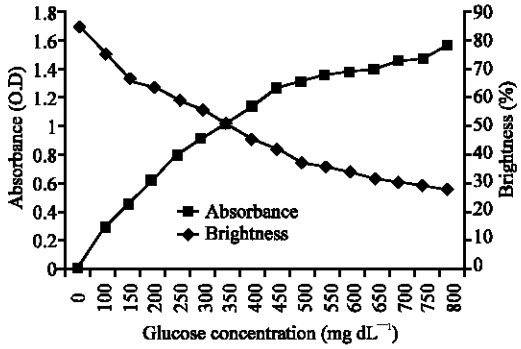

Fig. 3: Response curve of Reagent used for Glucose measurement

per the proposed RGB Data based Blood Glucose measurement method, this response curve of Glucose concentration Vs Luminance is fit into a polynomial equation of degree 6. This Eq. 7 is used for the actual measurement of Blood Glucose using RGB Data method.

$$X = -0.0007Y^6 + 0.0392Y^5 - 0.8664Y^4 + 9.5612Y^3 - 54.715Y^2 + 200.99Y - 153.13 \quad (7)$$

and its Correlation coefficient $R^2 = 0.9999$.

Since the Correlation coefficient of Eq. 7 is 0.9999, the error in the measurement of Blood Glucose can be minimized if the Colorimeter is made to adopt the method of Eq. 7 instead of Eq. 4. But, the error in measurement due to the requirement of monochromatic source will still persist in Colorimeter and this does not arise in the Digital Image Processing method. However, clinical measurements with the help of Eq. 4 can be done in this Digital Image Processing technique also for the measurement of other Blood Chemical Components.

CONCLUSION

In the above experiment, the colour pixel RGB data value differed marginally from pixel to pixel. In order to overcome this problem, an average of 13×13 pixels has been considered for measurement. Hence when the image of the assay is captured, identical surface structures and identical gloss characteristics should be maintained. i.e., the test tubes or the cuvettes used should be uniform in shape and size and the light applied over the image should be uniform.

From the preliminary experiments conducted in the laboratory, it is evident that analysis of Blood chemical substances by *Digital Image Processing* is more accurate. Hence, a new Clinical Instrument that uses a Digital Camera and Computer can be introduced in lieu of Colorimeter to measure the Blood Chemical components.

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