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Smartphone Based 3D Printed Colorimeter for Biomedical Applications

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DEDICATION

I would like to dedicate this thesis to my beloved parents, V.R.Konnaivan and R.Rajeswari for making me be who I am today.
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ABSTRACT

Here we present a novel Smartphone-based colorimeter and demonstrate its application to the measurements of glucose and protein concentrations in biological samples. The key innovation of our approach was to combine powerful image processing encoded into a mobile phone application with a low cost 3D printed sample holder that allowed to control lighting conditions and significantly improved sensitivity. Different solutions with protein and glucose concentrations ranging from 0 to 2000 mg/dL were prepared and analyzed using our system. The Smartphone-based colorimeter always correctly classified the corresponding reagent strip pads, what confirms that it can be used as a low cost alternative for commercial test strip analyzers.
CHAPTER 1:
INTRODUCTION

1.1 Colorimetry Overview

Colorimeter is a device used for measuring the concentration of a colored substance in a solution. It works based on Beer-Lambert's law which states that the absorbance of collimated monochromatic beam in a homogeneous medium is related to the concentration of the absorbing species and the path length of the beam through the medium [1].

\[ A = \log \frac{I_0}{I_t} \]

where \( A \) denotes the absorbance of the sample, \( I_0 \) is intensity of incident beam and \( I_t \) is intensity of transmitted beam.

\[ A = kdC \]

where \( d \) denotes the distance through the absorbing substance, \( C \) is the concentration of absorbing substance and \( k \) is the absorptivity of the substance. Typically, \( k \) and \( d \) are constant for a particular experiment, therefore absorbance can be plotted with respect to the concentration of the absorptive substance [2].

Colorimeter has several important components as shown in Figure 1. It consists of light source, normally a low voltage filament lamp which emits a broadband light. A monochromatic filter is used to isolate the wavelength of the light that the substance absorbs more effectively. The monochromatic light beam is then passed through the cuvette consisting of the sample where a portion of light gets absorbed and rest are transmitted. The transmitted light is measured using
a photoelectric detector and the absorbance value can be displayed by a digital meter. In addition, a computer or data logger can be used to compute the concentration of the substance by the absorbance data and the calibration curve.

![Simplified diagram of colorimeter.](image)

**Figure 1.1.** Simplified diagram of colorimeter.

A calibration curve is used to determine the concentration of the substance in an unknown sample. To obtain a calibration curve, several samples with known concentration are prepared and tested using the colorimeter. These values are plotted with the absorbance values to obtain the calibration curve from which the concentration of unknown sample can be obtained. In order to build a calibration curve, multiple experiments can be conducted. Broadband light from the source initially goes through the monochromatic filter. For the control measurement, the power of monochromatic light is measured in the absence of sample as a control. A colored solution is then inserted into the colorimeter and the photodetector measures the intensity of the monochromatic light after going through the sample. The relative drop of power is then determined, and the concentration of the absorptive component is measured based on the intensity of light measured at the detector. The results of the measurement are plotted with respect to concentrations of the substance in solution. Every substance must have separate calibration curves.
Colorimeter has a wide range of applications in different fields. For example, in chemistry it is used for measurement of concentrations of chlorine, phosphorous, iron, cadmium, chromium, copper, lead, manganese, nickel and zinc [3-6]. Additionally, in medicine, colorimeter can determine the concentration of hemoglobin in the blood [7], can be applied to diagnoses conditions like dental caries and erythema based on analyzing the tooth color [8] and skin tone [9]. Colorimeters are also used in textile, printing, paint and food industries.

The most common types of colorimeter are color photometer and color densitometer. Color photometer measures the transmission and reflection of the color intensity at specific wavelength especially in visible region of electromagnetic spectrum, whereas color densitometer measures the density of colors like red, green and blue. Spectrophotometer is a similar kind of device like color photometer, but the spectral bandwidth ranges from near-infrared to near-ultraviolet including the visible spectrum.

1.2 Motivation for Using Smartphone as Biosensor

Recent breakthrough in Smartphone technology, especially the improvement in processing power, storage capability and wireless connectivity simplifies our day-to-day activities by cutting down many time consuming processes. Over time, the price and size of mobile phones have decreased making worldwide mobile-cellular telephone subscription to reach about 7 billion users [10]. In comparison with laptops and PCs, mobile phones are significantly more portable and have lower power consumption. Additionally, a number of innovative Smartphone applications were enabled by the development of built-in sensors, such as cameras, accelerometers, gyroscopes, GPS, microphones, etc. Some of the application of the mobile phones in the medical and biomedical field include weight management [11], lens-free
microscopy [12], hypertension monitoring system [13], label free immunoassays [14], monitoring system for Parkinson's disease patients [15], retinal disease diagnostic device [16], system for monitoring kidney metabolomics [17], flow cytometry [18] and other biosensing applications [19].

1.3 State of Art Mobile Phone Based Colorimeter

Mobile phone based colorimetry uses mobile camera as a detector. There are a number of systems that are demonstrated based on this principle. For example, mobile phone colorimeters with designated app to process the color information are excellent low cost alternative for expensive commercial colorimetric readers for quantifying the concentrations of pH, glucose and protein in artificial urine [20]. Running a colorimetric test using this app just requires commercially available reagent test strips and does not require external housing, LED light sources or batteries which make it a stand-alone application. The main limitation of this approach is that the user needs to calibrate with a set of reference images whenever there is a change in ambient light conditions.

Furthermore, another type of Smartphone based colorimetric reader was developed for model assays like direct enzyme-linked immunosorbent assay (ELISA) for horse radish peroxidase (HRP), rapid sandwich ELISA for human C-reactive protein (CRP) and commercially available BCA protein estimation assay [21]. This system uses a custom made hood, base holder and a gadget's screensaver that act as a light based bottom assembly to illuminate in the corresponding region of the bottom of microtiter plate's well. Despite the fact that it is a cost effective point of care diagnostic device for different ELISA tests, it requires careful positioning
of the microtiter plate over the designated illuminated area of the gadget's screensaver which may lead to possible error if there is any position mismatch.

Finally an android Smartphone based colorimetric analyzer was developed to test the concentration of phosphorus in the agricultural field [22]. This setup requires an analyzer box made out of polystyrene material covered with a black plastic board to block the environmental light, a LED operated by battery power and an android Smartphone with fixed focus, single image mode, automatic ISO and white balance control. Developing a customized analyzer box with appropriate dimension of black plastic board for diverse variety of mobile phone can be a limitation of this type of colorimeter. In addition, different smart phones have their own unique camera configuration that can add error to the measurement without a calibration algorithm during interphone repeatability.

There are numerous mobile phone based colorimeters which work on the same principle and they are differentiated by their field of application. Smartphone based colorimeters are broadly classified into two categories 1. App with hardware components and 2. Stand-alone app. Each category has its own advantages and limitations. Colorimetric app with a dark hood and external lighting setup works great in controlling the ambient lighting condition which is one of the important parameter in colorimetry. This setup also provides a constant position for loading the sample which eliminates the auto recognition function of the app and decreases the computation load. This hood setup can be an ideal choice for holding the sample at constant position for continuous tracking of color change at particular location on the sample. Building a hood for the colorimetric application requires accurate dimensions of the mobile phone and specific design based on the position of the camera. In addition to this, it requires battery powered light source like LED array to keep the illumination constant. Assembling the setup is
one of the main limitations with this method that led to development of stand-alone app for colorimetric measurements. These apps have features of auto recognition of the sample holder or sample, color correction algorithm for normalizing the varying light conditions, conversion of RGB (Red, Green, Blue) values to specific model like tristimulus color space values (CIE XYZ) for improving the accuracy [20]. In spite of all these advantages, these methods have some limitations like regular calibration of the app whenever there is a change in environmental lighting conditions and computational load. Therefore it cannot be used for continuous monitoring of color change in the sample.

1.4 Thesis Organization

In this thesis, we propose a development of a cost effective hybrid point-of-care mobile phone based colorimeter. This device overcomes the limitation of both types of mobile phone based colorimeters described in the previous section and demonstrates great performance, high reproducibility, accuracy and stability under varying lighting conditions.

The thesis is composed of six chapters. Chapter one describes the overview of the colorimetric measurements and a role of mobile phone in developing low cost point-of-care devices like colorimeter. In addition, it also discusses motivation and challenges behind building a Smartphone based colorimeter. Chapter two is focused on the development of different components of mobile phone based colorimeter and testing the performance of the device under different lighting conditions. In chapter three, we describe the software components in the mobile app and its process flow. Chapter four provides the information about calibration of the device. In chapter five, results of mobile phone based colorimeter are presented, as well as an
analysis and discussion of the results. Chapter six gives a summary of our mobile phone based colorimeter and the future work.
CHAPTER 2:
HARDWARE COMPONENTS OF OUR SMARTPHONE-BASED COLORIMETER

2.1 Introduction

In order to make a precise and reproducible mobile phone based colorimeters, we need to fix a number of hardware parameters. This includes distance between a mobile camera and an object, multiple camera settings and stable built-in illumination source [23]. In order to keep a sample at constant distance from the camera, we introduce a 3D printed sample holder (Chroma-dock). The Chroma-dock consists of a main piece attached to a mobile phone and a removable cassette serving as a sample holder. This way it not only allows keeping the sample at constant distance from the camera, but also creates a controlled-light environment. This allows to capture very reproducible images and eliminates the background noise.

Another important set of hardware parameters that have to be fixed is such camera settings as exposure rate, ISO (International Organization of Standardization) or APA (American Standards Association) setting, white balance, sharpness, hue, saturation and gamma. In the case of a stand-alone application-based colorimeter that does not include any additional hardware, these settings have to be dynamically changed, according to varying environmental conditions, what introduces additional noise.

The final important consideration for a stable mobilephone colorimeter is a presence of a high quality light source. While an external LED is a great can light source for mobile colorimetric measurements, it requires external power supply and wiring, what adds complexity
to the system. Fortunately, contemporary cellphones have high quality flash based on LED light integrated with the camera. This LED can be directly controlled using the same application. Detailed descriptions of all the hardware components of our mobile phone based colorimeter are explained in the following sections.

2.2 Design and Fabrication of the Chroma-dock

We designed the Chroma-dock, our mobile phone attachment, to control the influence of external light and to increase the accuracy of the colorimetric measurements. The dimensions of the box were defined by the size of the mobile phone - Motorola's Moto X. The sample holder was 3D-printed using a 3D model created in Autodesk Maya that was exported as STL (STereoLithography) file format.

2.2.1 3D Modeling

The 3D model of the Chroma-dock is shown in Figure 2.1. It consists of a holder and a cassette. The holder contains two insertion ports at front and back of the box that allow to attach the phone and the cassette in a fixed position. The dimensions of the sample holder and the cassette can be altered based on the dimensions of a needed mobile phone. Additionally, an opening for the mobile phone camera and the flash light were built into the model. Tolerance of $\pm 0.5$ mm were added to the dimensions of the 3D-model. Stereolithography (STL) file format of the model was then exported from the Maya and 3D-printed.
Figure 2.1. 3D model of the Chroma-dock with the mobilephone (a) Front and (b) back view, section (1) shows a holder for the mobile phone, (2) is a cassette to hold the test strip, (3) the test strip and (4) the mobile phone. Panels (c) and (d) show the front and back view of the assembled system.

2.2.2 3D Printing

Currently, 3D Printers are widely available. Makerbot Replicator 3D printer was used to print the Chroma-dock. Figure 2.2 illustrates the 3D printing of the model. The total time to print the sample holder and the cassette was approximately 15 hours. The printed model attached to the mobile phone is shown in the figure 2.3.
Figure 2.2. 3D printing of the Chroma-dock prototype. (white material is used for better visibility).

Figure 2.3. (a) Mobile phone based colorimeter with a cassette and a holder, (b) the cassette is inserted.
Black PLA (PolyLactic Acid) material was selected for 3D printing, since it can eliminate the background light and minimize the backscattered light. The total cost for printing the holder was less than 15 USD in which the cost of printing the cassette was almost less than a dollar. This makes it a much lower cost alternative for a colorimeter. Additionally 3D printing adds lot of flexibility for design modification. For example, to perform other types of colorimetric measurements, just the cassette and the mobile app can be modified instead of fabricating the entire mobile housing unit.

2.3 Smartphone Camera Parameters

Smartphone camera acts as a detector measuring color characteristics of the sample. Because of that, the camera parameters such as exposure rate, white balance, sharpness and ISO, play a vital role in error free colorimetric measurement.

Exposure rate defines the amount of light per unit area that reaches the camera sensor. Our colorimetric measurements were performed inside a dark box where an auto settings can cause overexposure and loss of color details [24]. To avoid this, the exposure compensation value was set to the minimum.

A white balance setting adjusts the color of the captured image based on the light source used while shooting the picture. Color reproducibility for images captured under different light conditions can be achieved by using the automatic white balance setting [25]. Since our system is using a stable light source, the white balance was set to the constant mode. Specifically, the white balance was programmed in the mobile application to use the parameters of the 'daylight' mode that allows to normalize the color values based on standard daylight illuminant source D65.
Autofocus is a critical feature for stand-alone mobile phone colorimeters that use automatic focus at a specific region of interest (ROI). The movement of the camera might cause a blur in the image that can add error to the measurements [26]. Constant distance between the object and the camera eliminates the need for autofocus. Therefore, the mobile application was programmed to use 'fixed focus' mode.

ISO number of a digital camera measures the sensitivity of the image sensor. The larger the ISO number, the worse is the signal to noise ratio (SNR) [27], so a lower ISO value (ISO 400) was programmed to yield a better SNR. Results of the colorimetric measurements using the app with customized camera parameter values will be discussed in the following section.

2.4 Optimization Test

Color stability of the pictures captured using Chroma-dock under different environmental lighting conditions were analyzed. This analysis was crucial to ensure the stability of the device performance and to evaluate the role played by the sample holder for controlling the variables during measurement.

2.4.1 Performance Testing with Different Background Colors

One of the challenges for stand-alone colorimetric apps is to control the white balance of camera during the measurements. Automatic white balance feature detects the white or neutral tone in the scene and automatically calibrates the rest of the image with respect to the neutral color temperature. This feature is frequently used in stand-alone colorimetric apps to counterplay the changes caused by the varying environmental conditions. Occasional errors in automatic white balance settings are inevitable [25] even when specialized techniques are used to minimize
the influence of such errors. For example, 25% of an image can be covered by using commercially available reference white card [28].

Our experiments demonstrated that reagent test strip images taken on different background produced completely different outcomes (Figure 2.4), when the automatic white balance feature was used.

![Images of the same test strips taken on six different backgrounds.](image)

**Figure 2.4.** Images of the same test strips taken on six different backgrounds.

CIE L*a*b* color parameters were measured without using the Chroma-dock box on six different colored backgrounds. The need for selecting CIE L*a*b* color model in our model will be discussed in following chapter. Figure 2.6, 2.7 and 2.8 provides the mean L*, a* and b* values for the same reagent pad of the test strip placed on six different backgrounds. Therefore, it is beneficial to choose the same background color for all the colorimetric measurements. This justifies the use of the Chroma-dock to ensure the control over the background color.
Figure 2.5. CIE L* values for six colors of background.
Figure 2.6. CIE a* values for six colors of background.
Figure 2.7. CIE b* values for six colors of background.
2.4.2 Performance Testing Under Different Lighting Conditions

One of the important requirements for a colorimeter is to conduct reproducible measurement independently of changing lighting conditions. Stand-alone mobile phone colorimeters require regular calibration or a color correction method every time before conducting a measurement [20]. During a field test, the lighting condition can be significantly different from the ones used for calibration. This may result in a completely different measurement outcome. On the other hand, mobile colorimeters with an attached box can completely eliminate the errors related to the changes in the lighting conditions. Here we demonstrate our experiments of stability testing under different lighting conditions with and without the Chroma-dock (Figure 2.8 and 2.9). The test was conducted under bright light illumination, dim light and in a very low light intensity. Figure 2.8 demonstrates the same object has completely different colors under three different lighting conditions.

Later on CIE L*a*b* color parameters were determined for the measurements conducted with and without the Chroma-dock. Figure 2.10, 2.11 and 2.12 provide the mean L*, a* and b* values for corresponding two images (Figure 2.8 and 2.9). It can be noticed that the use of Chroma-dock resulted in stable measurements with a minimum standard errors. While measurements without the box resulted in large standard errors and highly non-reproducible measurements. This illustrates the importance of using the Chroma-dock.

![Figure 2.8. Test strip captured by Smartphone without Chroma-dock at different locations with different lighting conditions.](image)

Location 1  Location 2  Location 3
Figure 2.9. Test strip captured by Smartphone with Chroma-dock at different locations with different lighting conditions.

Figure 2.10. CIE L* values under different lighting conditions.

Figure 2.11. CIE a* values under different lighting conditions.
Figure 2.12. CIE b* values under different lighting conditions.
CHAPTER 3:
MOBILE APP FOR COLOROMETRIC MEASUREMENT

3.1 Introduction

Mobile app plays a crucial role in converting Smartphone into a portable colorimeter. Mobile apps extend the functionality of the device by controlling the hardware components of the mobile phone. Worldwide 70% of mobile phones use Android operating systems, and thus it is a most popular platform for development of mobile apps [29]. Therefore we choose Moto X mobile phone for building our application using Android Studio which is one of the integrated development environment (IDE) developed by Google. A detailed description on various components of the app is explained in the following sections.

3.2 Mobile Application Development

Mobile application development for colorimetric measurements involves capturing, storing and processing the image of the sample. Color information from the image has to be extracted and matched with the values from the calibration curve to determine the concentration of the substance. Figure 3.1 shows the algorithm used for the colorimetric measurement.

Some of the important features of the app, as shown in Figure 3.2, are selection of the region of interest (ROI), changing of the measurement interval, color space conversion and computing the concentration value of the substance based on the L*a*b* values.
**Figure 3.1.** Flow chart of Smartphone based colorimetric algorithm.
3.2.1 Constructing the ROI

Customized camera settings were used to control the camera hardware using the framework Application Program Interface (API). This provides the ability to add custom views to the camera preview screen. Overlay of ROI over the camera preview was used to indicate the boundary as well as the area on reagent pad that was considered for the color measurements. This overlay presents a reference area where the test strip can be appropriately positioned. In addition, this overlay includes a small square box, centered over the reagent pad of test strip covering 8x8 pixels where the color information was measured. Figure 3.3 displays the screenshot of the camera preview with ROI overlay which was represented in green line.

3.2.2 Capturing the Image

To start the test the user needs to press the button from the user interface (UI) screen. Upon receiving the initiate command, the app captures and stores the images every 5 seconds over a 60 seconds period. An internal timer function was used to perform this task. The color information was extracted from the sequence of images.
3.2.3 Color Processing

Color information in the ROI of the captured images was extracted using a built-in function. Red, green and blue (RGB) values of the corresponding pixels inside the ROI were obtained. RGB is a non-absolute color space as the color values depend on external factors like illumination, sensitivity of camera sensor, etc [30-32]. CIE L*a*b* color model provides more accurate and uniform color representation [33]. L* value indicates lightness and it ranges from 0 to 100 (black to white). a* value indicates red/green color components (positive value represents red region and negative value represents green region). b* value indicates yellow/blue color components (positive value represents yellow region and negative value represents blue region). There is no direct standard formula to convert RGB to L*a*b* values. Mobile app is
programmed to convert obtained RGB values to CIE L*a*b* values indirectly, by calculating XYZ tristimulus values. Standard illuminant D65 was considered for the XYZ to L*a*b* color space conversion.

3.2.4 Measurement Technique

Color values obtained in the previous step were used to compute the concentration value of the substance. Equations fitting the calibration curves (chapter 4) were built into the mobile app. Some substances change color non linearly with linear change of concentrations, what adds complexity to the computation procedure. In this case, calibration equation of particular color component from L*,a* and b* is used to determine the concentration of the substance in the sample and the values obtained from the calibration equations of other color components are used in further decision making process. Detailed information on building the calibration curve and how it was used to measure the concentration of the substance will be discussed in chapter four.

3.3 Algorithm for Interphone Repeatability

Different mobile phones working on android platform can be used for colorimetric measurements. Smartphone can be transformed into portable colorimeter, provided customized Chroma-dock module and a mobile app. Color response varies from camera to camera, what can introduce a significant error [34] in interpreting the concentration of the substance. Constant color response over different types of Smartphones can be achieved by calibrating the device before the test. Stand-alone apps without any housing unit requires regular calibration whenever there is a change in ambient light conditions [20]. Our colorimetric module requires one-time
calibration with a set of control samples before starting with the actual samples. Regular
calibration is not required for our module as the change in environmental light does not have
influence over the light conditions inside the Chroma-dock hood structure. Calibration button
was added into the user interface screen of the mobile app that the user must select before
performing the first sample test. In response to the input from the user the color values of the
control samples are recorded and compared with the standard control values in the program.
Difference in those values will be added to the predefined calibration equations extracted from
the calibration curves. This algorithm provides a degree of freedom for transforming any
Smartphone into colorimetric reader by producing constant color response over the different
cameras.

3.4 Conclusion

Here we described the core components of the mobile app. In future it can be
implemented not only on android platform but also on other platforms like iOS, Windows and
BlackBerry OS. Additionally, built-in calibration allows seamless transition between different
mobile phones.
CHAPTER 4:
CALIBRATION OF THE DEVICE

4.1 Need for a Calibration Curve

"A calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration" [35]. Colorimeter utilizes it for the instrument calibration, mobile phone based colorimeters compare the measured color components to the values on three different calibration curves (one for each component). Different types of substances have different calibration curves.

In our mobile phone based calorimeter we analyze Urinalysis Reagent Strips. These multi-analyte test strips have different reagent pads, that produce characteristic color change upon reaction with a particular substances present in the sample [36]. Mobile app simultaneously monitors these color changes and calculates the concentration values based on the in-built calibration curves. This way the concentrations of different substances present in a sample can be measured independently.

We generate our own experimental calibration curve for each substance. Generally, a reference color chart is used to visually determine the approximate concentrations. However, since the lighting conditions can vary significantly, the perception of the colors from the reference charts (visual or automatic) is going to change. Therefore, without additional control over lighting conditions the charts from the manufacturer cannot be used for automatic calibration. Figure 4.1 and 4.5 illustrates the color difference between the reference color chart
provided by the manufacture and the one that we experimentally obtained by using standard concentrations of glucose and protein. In order to use the most precise calibration parameters we need to conduct careful experimental calibration for all needed substances. Additionally, users are recommended to calibrate their device before the first test to increase the accuracy of the system.

4.2 Materials and Methods

Our Smartphone based colorimeter was programmed to measure the concentrations of two different types of analyte - glucose and protein, in which glucose produces a nonlinear color change and protein produces a linear color change in response to increase in concentration. In order to build two calibration curves the test strips were immersed into the samples with predefined concentrations and the color responses were measured. L*, a*, b* values were plotted with respect to the concentrations.

4.2.1 Glucose Sample Preparation

D-(+)-Glucose solution (45%) from Sigma-Aldrich was used as a source of glucose. It was then added to the artificial urine solution from Flinn scientific inc. Six samples (10ml) with different concentrations of glucose were prepared according to the Table 4.1.

4.2.2 Protein Sample Preparation

Bovine serum albumin from Sigma-Aldrich was used as a protein source that was added to artificial urine solutions. Six samples (10ml) with different concentrations of protein were prepared according to the Table 4.2.
Table 4.1. Preparation of glucose samples.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Glucose solution (ml)</th>
<th>Artificial urine (ml)</th>
<th>Glucose concentration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.0222</td>
<td>9.9778</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>0.0555</td>
<td>9.9445</td>
<td>250</td>
</tr>
<tr>
<td>4</td>
<td>0.111</td>
<td>9.889</td>
<td>500</td>
</tr>
<tr>
<td>5</td>
<td>0.222</td>
<td>9.778</td>
<td>1000</td>
</tr>
<tr>
<td>6</td>
<td>0.444</td>
<td>9.556</td>
<td>2000</td>
</tr>
</tbody>
</table>

Table 4.2. Preparation of protein samples.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Protein (g)</th>
<th>Artificial urine (ml)</th>
<th>Protein concentration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.0015</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>0.0030</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>0.0100</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>0.0300</td>
<td>10</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>0.2000</td>
<td>10</td>
<td>2000</td>
</tr>
</tbody>
</table>

4.3 Results

Multi-analyte test strips were briefly dipped into each samples and placed in the cassette. Then the cassette was inserted into the holder module and imaged. Mobile app was used to extract the color intensity values (L*, a*, b*) from these images. These values were plotted with respect to the concentration to define the calibration curve for glucose and protein. Calibration
equations were extracted from the curve fitting and incorporated into the mobile app for calculating the intermediate values.

4.3.1 Calibration Curve for Glucose

Three multi-analyte test strips were used for each glucose concentration. Figure 4.1 compares the reference chart to the color variation observed for the test strips dipped into different concentrations of glucose.

![Figure 4.1. Comparison between (a) reference chart from the manufacturer and (b) experimental color change for different concentrations of glucose.](image)

In order to minimize variation of the measurements we conducted a number of experiments. Mobile app was programmed to capture the image of the test strip for every 5 seconds interval over 60 seconds period. 12 images were captured and stored for each test strips. A total of 36 (3 test strips x 12 images) L*, a* and b* values were measured for each concentration of glucose. Table 4.3 provides the average and standard deviation of the obtained values. Standard deviations were less than 2 units which indicate the good precision of the device in measuring different glucose concentrations.
Table 4.3. CIE L*a*b* color response for different concentrations of glucose.

<table>
<thead>
<tr>
<th>Glucose concentration (mg/dL)</th>
<th>L Value</th>
<th>A Value</th>
<th>B Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Standard deviation</td>
<td>Average</td>
</tr>
<tr>
<td>0</td>
<td>91.0</td>
<td>1.4</td>
<td>-33.2</td>
</tr>
<tr>
<td>100</td>
<td>84.7</td>
<td>0.5</td>
<td>-38.7</td>
</tr>
<tr>
<td>250</td>
<td>78.9</td>
<td>0.8</td>
<td>-35.9</td>
</tr>
<tr>
<td>500</td>
<td>67.8</td>
<td>0.9</td>
<td>-20.6</td>
</tr>
<tr>
<td>1000</td>
<td>46.3</td>
<td>1.4</td>
<td>4.9</td>
</tr>
<tr>
<td>2000</td>
<td>37.6</td>
<td>2.1</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Figure 4.2, 4.3 and 4.4 show the graph with average values of L*, a* and b* plotted against different glucose concentrations. The obtained plots were non linear and a generalized calibration equations cannot be extracted from these plots. The calibration equations were obtained by dividing the curves into multiple sections. Plots and the corresponding equations of these sections are provided in the appendix A.

Figure 4.2. CIE L* value for different concentrations of glucose.
4.3.2 Calibration Curve for Protein

Three multi-analyte test strips were used for each protein concentration. Figure 4.5 compares the reference chart to the color variation observed over test strips dipped in different concentrations of protein.

Mobile app was programmed to capture the image of the test strip for every 5 seconds interval over 60 seconds time period. 12 images were captured and stored for each test strips. A total of 36 (3 test strips x 12 images) L*, a* and b* values were measured for each concentration.

Figure 4.3. CIE a* value for different concentrations of glucose.

Figure 4.4. CIE b* value for different concentrations of glucose.
of protein. Table 4.4 provides the average and standard deviation of the obtained values. Standard deviations were less than 2 units which indicate the good precision of the device.

Table 4.4. CIE L*a*b* color response for different concentrations of protein.

<table>
<thead>
<tr>
<th>Protein concentration (mg/dL)</th>
<th>L value</th>
<th>A value</th>
<th>B value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Standard deviation</td>
<td>Average</td>
</tr>
<tr>
<td>0</td>
<td>83.1</td>
<td>1.9</td>
<td>-14.5</td>
</tr>
<tr>
<td>15</td>
<td>81.3</td>
<td>1.1</td>
<td>-18.4</td>
</tr>
<tr>
<td>30</td>
<td>74.6</td>
<td>0.8</td>
<td>-25.9</td>
</tr>
<tr>
<td>100</td>
<td>69.9</td>
<td>0.7</td>
<td>-30.47</td>
</tr>
<tr>
<td>300</td>
<td>67.7</td>
<td>0.7</td>
<td>-31.4</td>
</tr>
<tr>
<td>2000</td>
<td>63.5</td>
<td>1.0</td>
<td>-31.7</td>
</tr>
</tbody>
</table>

Figure 4.5. Comparison between (a) reference chart from the manufacturer and (b) experimental color response for different concentrations of protein.
Figure 4.6, 4.7 and 4.8 show the graph with average values of L*, a* and b* plotted against different protein concentrations. The obtained plots were non-linear and a generalized calibration equations cannot be extracted from these plots. The calibration equations were obtained by dividing the curves into multiple sections. Plots and the corresponding equations of these sections are provided in the appendix A.

![Graph of L Value vs. Protein concentration](image)

**Figure 4.6.** CIE L* value for different concentrations of protein.

![Graph of A Value vs. Protein concentration](image)

**Figure 4.7.** CIE a* value for different concentrations of protein.
4.4 Conclusion

Calibration equations for glucose and protein samples were experimentally obtained and then programmed into the mobile application. Color values of the multi-analyte test strips dipped in the known sample were measured and used in the calibration equations for L*, a* and b*. Concentration value obtained from the calibration curve with a larger slope was considered more sensitive and used for concentration approximations, while the other two color components were used to ensure the accuracy of the measurement. Our Smartphone based colorimeter with the built-in calibration equations can be used to detect the presence of glucose and protein in an unknown sample and to measure their concentrations.
CHAPTER 5:
EXPERIMENTAL RESULTS FOR THE SMARTPHONE-BASED COLORIMETER

5.1 Introduction

Clinical utility of the Smartphone based colorimeter was demonstrated using Urinalysis Reagent Strips. Twelve samples with different concentrations of glucose and protein were prepared. Test strips were briefly dipped into the artificial urine samples. After ensuring all the reagent pads on the test strip were moistened, excess of the sample were removed by wiping the edge of the test strip. After the needed reaction time the test strips were placed on the cassette and loaded into the holder module. Mobile app programmed with the calibration equations for the respective substances was started. It captured and stored the images of the test strip and extracted the color values from the images. The calibration equations were used to calculate the concentrations of substances present in the samples. After that the concentration values were displayed in a message box. The accuracy of the measurements is going to be discussed in the following section.

5.2 Results Obtained Using Smartphone-based Colorimeter

5.2.1 Glucose Measurement

Six samples with different glucose concentrations were prepared according to Table 5.1. Two test strips were used for each concentration. The colorimetric analysis was conducted using
our system. The concentration values for each sample were averaged for the two test strips. Mean concentration values and their standard deviations were added to the table.

Table 5.1. Standard glucose concentrations and app estimated glucose concentrations.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Standard glucose concentration (mg/dL)</th>
<th>Estimated glucose concentration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>17.2 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>90.9 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>261.3 ± 29.9</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>566.8 ± 8.9</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>1002.3 ± 5.9</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>1840.1 ± 31.6</td>
</tr>
</tbody>
</table>

Correlation graph between the standard glucose concentrations in the sample and the measured concentration values by Smartphone based colorimeter is shown in the figure 5.1. These values can be used to detect medical conditions related to glucosuria - excretion of glucose in the urine, that may be due to untreated diabetes mellitus or due to renal glucosuria [37]. Coefficient of determination (R^2) from the correlation graph is almost 1, what indicates good fit between the observed and the modeled values.
5.2.2 Protein Measurement

Six samples with different protein concentrations were prepared according to the Table 5.2. Two test strips were used for each sample and they were analyzed using our Smartphone based colorimeter. Obtained concentration values for each sample were averaged. Mean concentration values and their standard deviations were tabulated as shown below. Correlation graph between the standard protein concentrations in the sample and the measured concentration values by Smartphone based colorimeter is shown in the figure 5.2.

These values can be used to detect medical conditions related to proteinuria - excretion of protein in the urine, that may be due to nephrotic syndrome, pre-eclampsia, sickle cell disease, glomerular disease, diabetes mellitus, dehydrations, toxic lesions of kidneys, HELLP syndrome,
etc [38]. Coefficient of determination ($R^2$) from the correlation graph is almost 1 what indicates good agreement between observed and modeled values.

**Table 5.2.** Standard protein concentrations and app estimated protein concentrations.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Standard protein concentration(mg/dL)</th>
<th>Estimated protein concentration(mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.4 ± 3.3</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>19.1 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>32.3 ± 5.8</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>81.8 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>235.9 ± 15.6</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>2082.2 ± 159.7</td>
</tr>
</tbody>
</table>

**Figure 5.2.** Correlation graph for the protein concentrations in the samples and the values measured by the app. Graph with lower concentrations of protein is displayed in figure 5.3.

\[
y = 1.0336x + 2.4 \\
R^2 = 0.9981
\]
Figure 5.3. Correlation graph of the protein concentrations in the samples (up to 300mg/dL) and the values measured by the app.

The equation of the line is:

\[ y = 0.7826x + 2.4 \]

\[ R^2 = 0.9981 \]
CHAPTER 6:
CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1 In Summary

Advancements in Smartphone technology paved the way for development of innovative point-of-care diagnostic devices. Here we demonstrated a mobile phone-based low cost portable colorimeter that can determine the concentrations of protein and glucose in a biological sample. Our device does not require regular calibration, as it is independent of external lighting conditions. The same app can be programmed to measure concentration of other urine components, like ketone, bilirubin, haemoglobin, nitrite, leukocytes, urobilinogen, and other, by adding corresponding calibration equations.

Additional benefit of this convenient system is that the user does not require extensive training to operate our Smartphone based colorimeter. The app was programmed with an animation video explaining the procedure of device operation. In addition, the app was also programmed to display an augmented reality animation that provides a 3D operator manual for the device (Appendix B).

The total cost for manufacturing our Smartphone based colorimeter was less than 15 USD in addition to the existing mobile phone. This makes our device, a low cost alternative for expensive commercial test strip analyzers that cost several hundred dollars. Our mobile app provides the optional feature of sharing these results to the registered members. In addition, this app can store the concentration values in the phone memory along with the user identity
information and optionally the app can use cloud storage which makes it a potential product for telemedicine applications.

6.2 Future Work

Our Smartphone based colorimeter provides the concentration values of glucose and protein in the biological sample. List of symptoms related to glucose and protein concentrations can be programmed to the mobile app. These values can be combined with the existing symptoms for classifying the physiological conditions. For example, in addition to proteinuria detection, our app can also be programmed to predict the cause of occurrence by providing a set of questionnaires in an user interaction screen. Based on the user response, the app can predict the possible cause of proteinuria that can be due to nephrotic syndrome, hemoglobinuria, myoglobinuria or intaking of certain types of drugs like nicotine or aspirin. In telemetric application, the app can be programmed to contact the health professional in case of severe proteinuria condition.

Furthermore, since calibrating the system is a complex task, in future the user should be able to download existing calibration files for all the needed substances. This will allow to operate this device without any need for a programming background. Additionally, different colorimetric applications require to load variety of samples and mobile phones with different dimensions will require customized Chroma-dock unit. These models can be fabricated by designing or modifying an existing model based on the given dimensions. This process requires a graphics designer. By creating a cloud based automated development environment, where the user just needs to select the phone model and its make, to create a compatible Chroma-dock
model. This feature will provides a new way to fabrication of the model using a 3D printer without having any designing experience.

Finally, further development of Smartphone technologies will probably result in many other healthcare related applications.
REFERENCES


29. McCracken, H., *Who’s Winning, iOS or Android? All the Numbers, All in One Place*, in *Time*.


APPENDIX A:

CALIBRATION EQUATIONS FOR GLUCOSE AND PROTEIN DETECTION

A.1 Calibration Equations for Glucose

Figure A1. The CIE L* values for 0 to 1000 mg glucose concentration.

\[ y = -22.735x + 2046.7 \]

\[ R^2 = 0.9984 \]

\[ \text{Glucose concentration (mg/dL)} \]

\[ \text{L value (arb. unit)} \]
Figure A2. The CIE L* values for 1000 to 2000 mg glucose concentration.

Figure A3. The CIE a* values for 0 to 100 mg glucose concentration.
Figure A4. The CIE a* values for 100 to 250 mg glucose concentration.

Figure A5. The CIE a* values for 250 to 1000 mg glucose concentration.
Figure A6. The CIE $a^*$ values for 1000 to 2000 mg glucose concentration.

Figure A7. The CIE $b^*$ values for 0 to 250 mg glucose concentration.
Figure A8. The CIE b* values for 250 to 500 mg glucose concentration.

Figure A9. The CIE b* values for 500 to 2000 mg glucose concentration.
A.2 Calibration Equations for Protein

Figure A10. The CIE L* values for 0 to 15 mg protein concentration.

Figure A11. The CIE L* values for 15 to 30 mg protein concentration.
Figure A12. The CIE L* values for 100 to 2000 mg protein concentration.

Figure A13. The CIE a* values for 0 to 15 mg protein concentration.
Figure A14. The CIE a* values for 15 to 30 mg protein concentration.

Figure A15. The CIE a* values for 30 to 100 mg protein concentration.
Figure A16. The CIE a* values for 100 to 300 mg protein concentration.

Figure A17. The CIE a* values for 300 to 2000 mg protein concentration.
Figure A18. The CIE b* values for 0 to 15 mg protein concentration.

Figure A19. The CIE b* values for 15 to 100 mg protein concentration.
Figure A20. The CIE b* values for 100 to 2000 mg protein concentration.
APPENDIX B:

USER INSTRUCTION SCREENSHOTS

Figure B1. (a) App layout with animation video that illustrates the instruction to operate the device, (b) and (c) augmented reality based user guide.