NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF ANALYTICAL CHEMISTRY

DETERMINATION OF CALCIUM IN HUMAN SERUM BY SMARTPHONE DIGITAL IMAGE COLORIMETRY

M.Sc. THESIS

LAMIA ALJAJA

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Approval

We certify that we have read the thesis submitted by **Lamia Aljaja** titled "**determination of calcium in human serum by smartphone digital image colorimetry**" and in our combined opinion is fully adequate, in scope and quality, as a thesis for the degree of Master of Analytical Chemistry.

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Declaration

I forthwith declare that all information, documents, analysis, experimental, statistical analysis, and results in this thesis have been collected, done, and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also hereby declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

LAMIA ALJAJA

31/05/2023

Acknowledgments

First and foremost, praise and thanks to God, the Almighty, for his showers of blessings throughout my research work to successfully complete the research.

I would like to express my deep appreciation to my thesis supervisor, Dr. Jude Joshua Caleb, for his exceptional teaching, unwavering guidance, and constant inspiration. He has been not only a mentor but also a true friend throughout this journey.

I would also like to extend my gratitude to Dr. Selin Isik, the head of the department, and Dr. Mais Al-Nidawi from the Department of Analytical Chemistry.

I express my sincere gratitude to Asst. Prof. Dr. Usama Alshana for introducing me to the captivating realm of analytical chemistry. Your guidance and expertise have been priceless, and I am truly thankful for your mentorship.

I am filled with immense gratitude towards my parents for their love, prayers, and support they made to provide me with education and prepare me for the future. Their presence and guidance have been instrumental in my journey, and I am incredibly thankful to them.

I am thankful to my sister, brothers and friends support, encouragement, and prayers. Their presence and support have been a source of strength and inspiration throughout my academic pursuits. I am truly blessed to have such a loving and supportive network of family and friends, and I am grateful for every one of them.

Dedication

I dedicate this research paper to my beloved father, whose unwavering support has been a source of strength and motivation throughout the entirety of my research journey. His encouragement, guidance, and belief in my abilities have been invaluable, and I am forever grateful for his presence in my life.

I also dedicate this dissertation to my dear mother, whose unwavering love and constant encouragement have been a driving force behind my success. Her belief in me and her unwavering support have been instrumental in shaping me into the person I am today. I am deeply grateful for her sacrifices, understanding, and unwavering faith in my abilities.

Furthermore, I would like to extend my dedication to my circle of friends, whose support and encouragement have been a constant source of inspiration.

Abstract

Determination Calcium in Human Serum by Smartphone Digital Image Colorimetry

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JUNE 2023, 51 Pages

In this study, we employed a combination of smartphone digital image colorimetry (SDIC) to determine the calcium content in human serum. To capture images of the samples using a smartphone, we designed and constructed an in-house aluminium colorimetric box. This box served as a single-line light source and wavelength generator, emitting a specific path of electromagnetic energy for the analysis.

The concentration of the analyte was determined using the SDIC method, and the limit of detection (LOD) was found to be 0.02 mg dL^{-1}. This indicates that the method performed adequately, as evidenced by satisfactory linearity and a well-fitted regression model with a coefficient of determination (R^2) of 0.9991. The percentage relative standard deviation (%RSD) was calculated to assess precision, and good intraday and intraday precisions of 1.68% and 4.18%, respectively, were obtained. The result was compared with an independent study using flame atomic absorption spectrometry (FAAS).

Keywords: Smartphone digital image colorimetry, calcium, blood, flame atomic absorption spectrometry.

Öz

Akıllı Telefon Dijital Görüntü Kolorimetrisi ile Kandaki Kalsiyum Tayini

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Danışman: Dr. Jude Joshua Caleb

HAZİRAN 2023, 51 Sayfa

Bu çalışmada, kan numunelerindeki kalsiyum içeriğini analiz etmek için akıllı telefon dijital görüntü kolorimetrisi (SDIC) ve akıllı telefon alev atomik absorpsiyon spektroskopisi (FAAS) yöntemlerinin bir kombinasyonunu kullandık. Bir akıllı telefon kullanarak numunelerin görüntülerini yakalamak için şirket içi bir alüminyum kolorimetrik kutu tasarladık ve oluşturduk. Bu kutu, analiz için belirli bir elektromanyetik enerji yolu yayan tek hatlı bir ışık kaynağı ve dalga boyu oluşturucu görevi gördü.

Analitin konsantrasyonu SDIC yöntemi kullanılarak belirlendi ve tespit limiti (LOD) 0,16 µg g-1 olarak bulundu. Bu, tatmin edici doğrusallık ve 0,9991 belirleme katsayısına (R2) sahip iyi uyan bir regresyon modeli ile kanıtlandığı gibi, yöntemin yeterince performans gösterdiğini gösterir. Kesinliği değerlendirmek için yüzde göreli standart sapma (%RSD) hesaplandı ve sırasıyla %1,68 ve %4,18'lik iyi gün içi ve gün içi kesinlik elde edildi.

Anahtar Kelimeler: Akıllı telefon dijital görüntü kolorimetrisi (SDIC), Kalsiyum, Kan, resimler, Alev atomik absorpsiyon spektroskopisi (FAAS).

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List of Abbreviations

%RSD	Percentage Relative Standard Deviation
CA	Calcium
EBT	Eriochrome Black T
EDTA	Ethylenediaminetetraacetic acid
FAAS	Flame Atomic Absorption Spectrometry
LOD	Limit of Detection
LDR	Linear Dynamic Range
PC	Personal Computer
RDA	Recommended Daily Allowance
RGB	blue, Green, red
LOQ	Limit of Quantitation
ROI	Region of Interest
SD	Standard Deviation
DIC	digital image colorimetry
SDIC	Smartphone Digital Image Colorimetry
R2	Coefficient of Determination
PTH	parathyroid hormone
µmol/L	micromoles per litter
mg/dL	milligrams per decilitre
PH	potential of hydrogen
SREC	Scientific Research Ethical Committee
WHO	World Health Organization

CHAPTER I

Introduction

Calcium is commonly linked to maintaining strong bones and teeth, it also plays crucial roles in other bodily functions. These include its involvement in blood clotting, facilitating muscle contractions, regulating normal heart rhythms, and supporting proper nerve functions. Hence, calcium's significance extends beyond skeletal health to encompass various vital physiological processes within the body. ("Dietary Reference Intakes for Calcium and Vitamin D," 2011).

Most of the calcium in the body, approximately 99%, is stored in the bones, providing structural support and strength. The remaining 1% of calcium is distributed among the blood, muscles, and various other tissues, where it plays important roles in physiological processes beyond bone health. (Chau et al., 2017).

To perform these vital daily functions, the body maintains a steady amount of calcium in blood and tissues. If calcium levels drop too low in the blood, parathyroid hormone (PTH) signals to the bones to release calcium into the bloodstream.

This hormone may also activate vitamin D to improve the absorption of calcium in the intestine. Simultaneously, PTH signals the kidneys to release less calcium in the urine.

When the body has enough calcium, a different hormone called calcitonin works to do the opposite: it lowers calcium levels in the blood by stopping the release of calcium from bones and signalling the kidneys to rid more of it in the urine (Kopecky et al., 2016).

An acute deficiency of calcium in the blood (less than 8.5 mg/dL) can result in various symptoms and complications, including fainting, congestive heart failure, numbness and tingling sensations, muscle spasms, and tetany. It may also cause bronchospasm and wheezing, laryngospasm and difficulty swallowing, irritability, depression, fatigue, and even seizures. On the other hand, chronic calcium deficiency can lead to noticeable changes such as coarse hair, brittle nails, psoriasis, dry skin, itching, compromised dental health, and the development of cataracts (Vassallo et al. 2019).

Certain factors can increase the body's need for calcium by affecting its absorption and excretion. Nutrients and medications that can interfere with calcium absorption in the gut or increase its excretion in urine include corticosteroids (such as prednisone), a high intake of sodium, phosphoric acid present in dark cola sodas, excessive alcohol consumption, and foods high in oxalates.

These factors may disrupt the balance of calcium in the body, leading to a higher demand for dietary calcium to maintain optimal levels. It is important to consider these factors when assessing an individual's calcium requirements and overall nutritional needs. (Curhan et al., 1997).

Benefits of calcium

Prevention of Osteoporosis

Calcium is necessary for bone development and growth, especially during childhood and adolescence. It is essential for the mineralization of bone tissue, which ensures adequate bone density and strength (Weaver et al., 2016).

Adequate calcium intake throughout life reduces the risk of osteoporosis, a disorder marked by decreased bone density and increased fracture susceptibility. Maintaining enough calcium levels can benefit bone health and reduce bone mass loss associated with aging (Weaver et al., 2016).

Supports Dental Health

Calcium is essential for maintaining healthy teeth and preventing dental issues It is an important component of tooth structure and supports the strength of the jawbone (Krall & Dawsonhughes, 1993).

Saliva contains calcium ions, which play a role in the remineralization of dental enamel. Demineralization occurs when the enamel is exposed to acids from bacteria or dietary causes, resulting in tooth decay. Calcium-containing saliva replenishes lost minerals, assisting in the repair of early-stage enamel defects (Featherstone, 2000).

Calcium is also important in the maintenance of healthy gums. It helps to maintain the strength and integrity of periodontal tissues, hence preventing gum disease (Ritchie et al., 2002).

Support Blood

Calcium concentration in the blood serum, commonly referred to as serum calcium, is typically measured in milligrams per deciliter (mg/dL) or millimoles per liter (mmol/L).

Serum calcium levels within the normal range can vary slightly depending on the laboratory and reference values used. These are generally accepted as the normal ranges for total serum calcium (Hoda & Hoda, 2020).

8.5 to 10.5 mg/dL (2.12 to 2.62 mmol/L)

8.6 to 10.3 mg/dL (2.15 to 2.57 mmol/L)

Calcium is required for blood clotting, promotes clotting factor activation, ensures appropriate coagulation, and prevents excessive bleeding (Allgrove, 2015).

Calcium is involved in platelet activation, which is necessary for the production of blood clots. Platelets attach to the damaged site and become activated when the blood vessels are injured.

Calcium ions promote platelet aggregation, and the creation of platelet plugs by facilitating the release of chemical messengers from platelets (Brass & Diamond, 2016).

Pregnancy

Significant transplacental calcium transfer occurs when calcium is required for the correct development of the fatal skeleton, including the creation of bones and teeth. Adequate calcium intake during pregnancy improves bone growth and mineralization.

Pregnancy places additional demands on the mother's body, and if calcium intake is insufficient, the developing fetus may suck calcium from the mother's bones. Sufficient calcium intake helps prevent maternal calcium deficiency, which lowers the risk of osteoporosis and other calcium-related disorders later in life (Melnik, 2011).

Adequate calcium intake has been associated with a lower risk of developing pregnant women with high blood pressure diseases, such as preeclampsia. Calcium supplementation may help reduce blood pressure and the risk of these problems (Hofmeyr et al., 2019).

Muscle function

Calcium is necessary for proper muscle contraction and relaxation, including contraction of heart muscles. It aids in maintaining a regular heartbeat, and normal functioning of the skeletal muscles is required for effective muscular coordination and control (Prefumo et al., 2016). It allows nerves and muscles to communicate, allowing signals to be transmitted from the neurological system to muscles.

This coordination ensures smooth and controlled movements (Rios and Pizarro, 1991). Maintaining the muscle tone requires adequate calcium levels. Muscle tone is the small tension present in muscles while at rest, which permits them to respond quickly and efficiently to external stimuli. Calcium aids in the regulation of muscle tone and ensures proper muscle function (Shetty, 2013).

Thyroid and parathyroid

Calcium homeostasis is primarily regulated by parathyroid glands, which are located near the thyroid gland. The parathyroid glands produce (PTH), which helps regulate calcium levels in the blood.

parathyroid hormone acts on various target organs, including the kidneys, bones, and intestines, to raise calcium levels. (Kapoor, 2000).

Calcium toxicity and hypercalcemia

Hypercalcemia is defined as increased calcium levels in the blood, which can result in calcium toxicity. While calcium is necessary for a variety of physiological activities, excessive amounts can be harmful to the body. Hypercalcemia can be caused by a variety of factors, including excessive calcium intake, certain medical conditions, or drug use (Hong et al., 2008).

and hypercalcemia symptoms might vary based on severity and underlying cause. Excessive thirst, frequent urination, abdominal pain, nausea, constipation, fatigue, muscle weakness, confusion, and even cardiac abnormalities are common symptoms (Schipani et al., 2009).

Sources of calcium

• Dairy Products: cheese, Milk and yogurt These meals include calcium and other critical nutrients, such as protein and vitamin D, which aid in calcium absorption (Lesher et al., 2008).

• Leafy Green Vegetables: Calcium is abundant in spinach, kale, and broccoli. Calcium bioavailability from plant-based sources, on the other hand, may be decreased by the presence of chemicals such as oxalates and phytates, which can block calcium absorption (Heaney & Weaver, 1990).

• Fortified Foods: Calcium is added to certain foods to increase their nutritional value. Fortified orange juice, plant-based milk replacements (such as soymilk and almond milk), and breakfast cereals are examples (Holick, 2007).

• Seafood: Some types of seafood, particularly canned fish with bones such as sardines and salmon, are high in calcium. Consuming these fish with their edible bones provides an extra calcium boost (Niu et al., 2022).

• Nuts and seed: Calcium is found in almonds, sesame seeds, and chia seeds, and these can help with overall calcium intake. doi:10.5005/jp-journals-10005-1539

Recommended intake of calcium

1.	The Infants:
•	0 to 6 months: 200 mg/day
•	7 to 12 months: 260 mg/day
2.	Children:
•	1 to 3 years: 700 mg/day
•	4 to 8 years: 1,000 mg/day
3.	Adolescents and Adults:
•	Males 9 to 18 years: 1,300 mg/day
•	Females 9 to 18 years: 1,300 mg/day
•	Males 19 to 50 years: 1,000 mg/day
•	Females 19 to 50 years: 1,000 mg/day
•	Males 51 to 70 years: 1,000 mg/day
•	Females 51 to 70 years: 1,200 mg/day
•	Males and females 71 years and older: 1,200 mg/day
4.	Pregnant and Lactating Women:
•	Pregnant and lactating women 14 to 18 years: 1,300 mg/day
•	Pregnant and lactating women 19 to 50 years: 1,000 mg/day

("Dietary Reference Intakes for Calcium and Vitamin D," 2011)

Calcium Deficiency

Lack of Calcium can reduce bone strength and cause osteoporosis, which is characterized by calcium deficiency, also known as hypocalcaemia, which occurs when there is an inadequate supply of calcium in the body or when calcium is not properly absorbed or utilized.

It can have a substantial impact on a variety of physiological systems as well as overall health (Holick, 2007).

Muscle cramps, numbness, tingling sensations, brittle nails, weak bones (osteoporosis), dental difficulties, weariness, and irregular pulse are all symptoms of calcium shortage. Calcium deficiency can increase the risk of osteoporosis, impede muscular function and nerve transmission, affect cardiovascular health, and contribute to dental problems (Weaver et al. 2016).

Impact of High calcium Intake

High calcium levels can be caused by excessive calcium supplementation, overactive parathyroid glands, certain medical disorders, or drugs (Bestepe et al., 2022).

It is characterized by digestive problems (nausea, vomiting, and abdominal pain), excessive thirst, frequent urination, fatigue, muscle pain, kidney problems (kidney stone formation), and mental confusion (Weaver & Peacock, 2019).

Highlight of the Problem

The misuse of calcium can lead to diseases associated with dysfunctional calcium regulation. Furthermore, calcium is present in food and supplements.

On one hand, consuming an adequate amount of calcium can help lower the risk of several diseases. However, it is important to note that excessive intake of calcium can have negative consequences. Increased calcium intake beyond recommended levels can potentially lead to acute gastrointestinal events, the formation of kidney stones, and an elevated risk of cardiovascular diseases. It is essential to maintain a balanced and appropriate intake of calcium to avoid these potential health risks.

Calcium consumption and supplementation should be based on the health status of an individual.

Objective of the Study

The study's goal is to create a low-cost method for determining calcium in blood using a simple home-made colorimetric box for detection using (SDIC) as a detection technique.

Significance of the Study

The significance of this study lies in the introduction of smartphone digital image colorimetry as a simple and cost-effective method for determining calcium in human serum. Despite its simplicity, it provides results and performances that are on par with high-end complex instrumental methods, but at a lower cost and with less reliance on electrical power. The method's benefits extend to various settings, making it a valuable tool for enhancing calcium analysis capabilities.

Research Questions and Hypothesis

- How effective is (SDIC) for determining the concentration of calcium in blood samples?
- What is the sensitivity of SDIC in detecting low concentrations of calcium in blood?
- How does the performance of SDIC compare to other analytical methods, such as flame atomic absorption spectroscopy (FAAS), in terms of accuracy and precision?
- The SDIC method will provide accurate and precise measurements of calcium concentration in blood samples.
- sensitivity of SDIC will enable the detection of low concentrations of calcium in blood.
- SDIC will exhibit comparable or superior performance to FAAS in terms of accuracy and precision for calcium determination in blood samples.

CHAPTER II

Literature Review

Role of Optical Instruments in (SDIC) and Their Major Components

Smartphones and mobile devices have been increasingly used as scientific research tools in recent years. Digital image colorimetry refers to the process of capturing, analysing, and reproducing accurate colours in digital images using the camera inside the box and display technology of the smartphone.

Colorimetry is the science of measuring and describing colours, and it is critical to ensure that the colours captured by a smartphone camera are accurately displayed on a device's display. Colorimetry, the scientific explanation and quantification of human vision, is scientifically classified in two main categories: visual colorimetry, which deals with the optical aspects of human vision, and photoelectric colorimetry, which focuses on the optical properties of light and its interactions with substances. (Tanil & Yong, 2020).

We can simply detect the concentration by using the naked eye to observe colour changes, whereas the latter uses measurement systems such as spectroscopy, which is also efficient and selective in measurement, making it more efficient in selectivity and quantification (Zou et al., 2018).

In colorimetric quantification, colorimetric techniques are employed to convert colour information from images into numerical data This process is carried out using photoelectric colorimetry. DIC is the subdividing of colorimetry that digitalizes images captured by smartphones or camera and it play vital roles in understanding and manipulating colour in digital images, enabling better control and utilization of colour in several domains. (Balaur et al., 2021).

DIC on smartphones is regarded as a powerful, fast, and low-cost analysis method for measuring target analytes with colour changes in digital images obtained by a built-in camera.

Digital image colorimetry is a method used to measure and analyse the colour properties of digital images, and has gained tremendous momentum in recent years. It is used in industries such as graphic design, photography, printing, quality control, and many applications in various fields.

Digital camera and smartphone are the most widely employed devices for capturing images in this case, because of their movability, light weight, and advancements in applications and systems in digital camera. DIC aims to provide objective and standardized measurements of colour in digital images (Fan et al., 2021).

The term "SDIC" refers to the application of digital image colorimetry techniques using smartphones as the major capturing devices, this approach combines the capabilities of smartphones, such as their built-in cameras and computational power, with colorimetric analysis to enable efficient and convenient colour measurement and quantification.

The employment of a smartphone's camera as a capturing tool within a digital image colorimetric system constitutes a significant aspect that warrants further exploration and analysis. smartphone based on digital image colorimetry is refer as smart digital image colorimetry.

The SDIC involves two main steps:

- Using a smartphone camera to capture the Image
- Using an image processing tool to analyse the captured image such as ImageJ

A homemade colorimetric aluminium box with black paint in order to rid of light interference to ensure the captured images are consistent and reproducible.

Optical instruments are devices that uses the principles of optics to manipulate and analyse light for various purposes. They are used in a wide range of fields such as physics, biology, medicine, and photography. These instruments allowing us to see objects clearly, magnify small details, analyse spectral properties, and capture images.

Some common types of optical instruments such as microscopes, telescope and cameras. Each instrument has its own specific design and function for its intended application.

SDIC refers to the process of measuring and analysing colours in digital images captured by smartphones using the visible portion of the spectrum which is approximately from 400 to 700 nanometres (nm) because it is visible to the human eye.

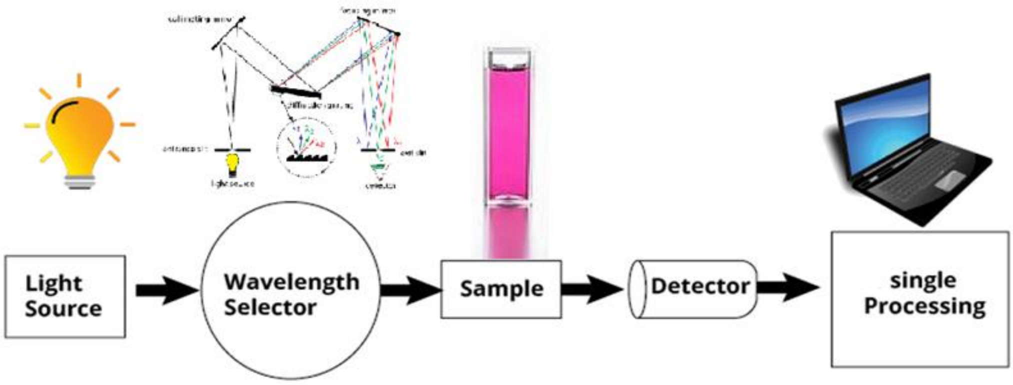
the visible portion of the spectrum is utilized to determine the colour properties of objects or scenes captured in the images (Fan et al., 2021).

Optical instruments main parts (Skoog et al., 2017):

- The light source is one of the key components of an optical instrument.
- Wavelength selectors are important instrumental components used to obtain portion of electromagnetic radiation spectrum for quantitative analysis and selecting a wavelength range that is suitable for accurately measuring and quantifying of a sample.
- Detection systems are employed to convert light energy into electrical signals for communication purposes.
- A device that transforms data into a visual representation presented on a scale called signal processor.

Figure 1

Optical System Configuration

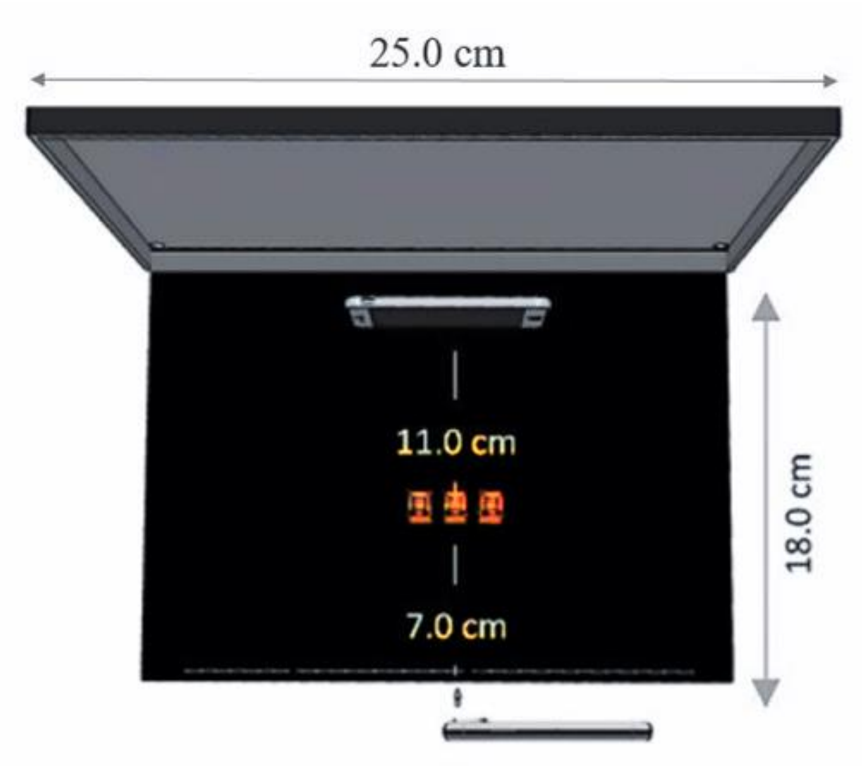


Design and Construction of a Colorimetric Box for SDIC

This colorimetric box manufactured from aluminium, with dimensions of 25 cm × 18 cm × 9 cm, was designed to have the appearance of a basic optical instrument (Caleb et al., 2023). To reduce light scattering, the interior of the box was coated with black paint and for capturing the images of sample solutions a small aperture was created on the side of the box through drilling a hole. the smartphone was positioned inside the box with a full screen unicoloured image that illuminated the sample with a 590 nm light. the box was closed for image capture by another smartphone through the hole.

Figure 2

Design of Colorimetric Box



Optimizing Performance in SDIC

- In order to enhance the performance of SDIC (Smartphone Digital Image Colorimetry), specific crucial elements, like the wavelength of the single-line, light source, need to be adjusted.
- Select the suitable channel (green, blue, or red) of captured image.
- The optimum space between the detection camera and the sample solution can vary depending on factors such as the camera specifications, desired image quality, and the characteristics of the sample.
- Region of interest (ROI)

Optimizing Single-Line Light Source Wavelength in SDIC

The wavelength of a single-line light source refers to the specific wavelength of light emitted by the source and is an important parameter in various scientific and technological applications, such as optical measurements and other fields.

Spectroscopic systems operating in the ultraviolet and visible regions of the electromagnetic spectrum exhibit a higher absorbance range that is specific to various analytes. This highlights the importance of using a monochromatic light source that can emit a specific wavelength of light in such systems (Caleb et al., 2023).

This response is often characterized by the appearance of a distinct colour. To achieve an optimal response, the single-line light source is adjusted in the (red, green, blue) colour space. This adjustment is facilitated by a program capable of converting a given wavelength to its corresponding colour. For instance, 405nm.com is a freely available online converter that can perform this task. By inputting a desired wavelength within the visible spectrum, the converter can accurately determine and display its corresponding colour.

Once the desired colour has been obtained from the (450nm.com) website, it can be copied and pasted in Microsoft Paint then can be saved and transferred to the chosen device for the backdrop display, this transfer can be done through telegram or WhatsApp.

Once the desired colour has been generated using the online converter, it's essential to evaluate its response across a range of wavelengths and select the one with the strongest signal.

Optimizing Channel Selection in SDIC

The RGB model consists of three channels red, green, and blue. Each channel has a range of values from 0 to 255, where 0 represents no intensity and 255 represents maximum intensity for that channel. By adjusting the values for each channel, you can create different colours and achieve the desired colour balance. For example, setting the green channel to 255 and the red and blue channels to 0 would result in a pure green colour. (Fan et al., 2021).

Within the RGB colour model, each channel can deliver an optimal signal specific to a particular analyte, distinguished by a unique coloration compared with the other channels. To identify the optimal channel, it is necessary to split the captured image into individual RGB channels. This enabled the selection of a channel that exhibited the most pronounced signal for the desired analyte. By isolating and analysing each channel separately, the optimum channel can be determined for accurate and precise analysis (Abughrin et al., 2022).

ImageJ software was used for this process to convert image signals into numerical data.

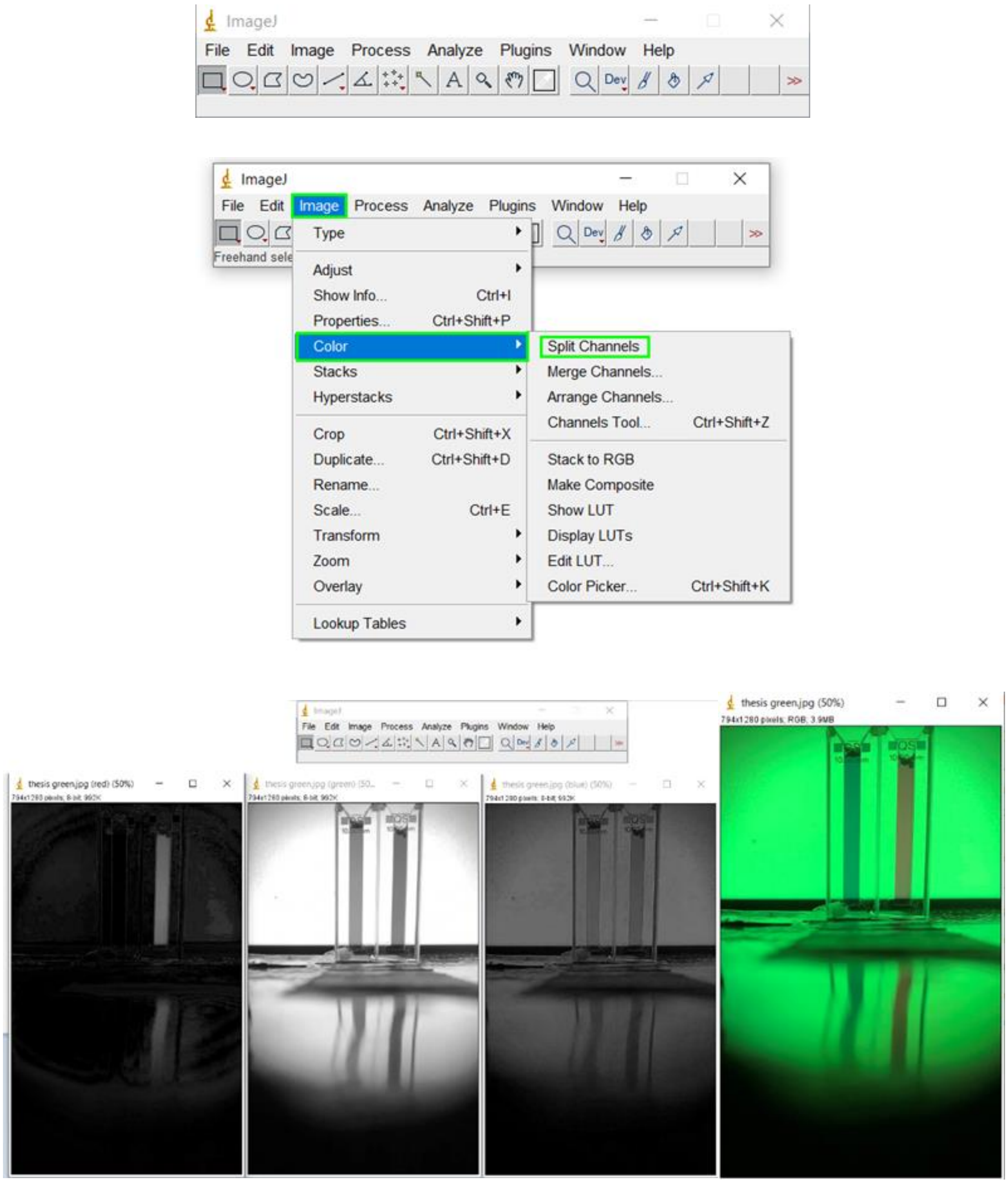
steps to using ImageJ:

- Open ImageJ software click ctrl + F Choose the required image
- Select Image then Colour and then Split Channels.
- This action will create three separate RGB channels for each image.
- Analyse the intensity of each channel and select the channel with the highest intensity
- Based on observation, the GREEN channel appears to be the most vivid so it will be chosen for analysis.

By performing these steps, you can use ImageJ to split images into (green, blue, red) channels then select the most intense channel, such as the GREEN channel, for further analysis (Caleb, 2022).

Figure 3

Select of optimal Channel in SDIC



Optimal distance Between Detection Camera and Sample Solution.

Is a critical factor in various analytical techniques and experiments. It is important to find the appropriate distance because if it becomes too close or too long, it can result in blurry or unclear images.

Blurriness in images can result in over-pixelation, causing individual pixels to lose their distinctness and affecting the overall image quality. Over-pixelated images are not suitable for accurate data processing and analysis purposes (Venn, 2023).

Therefore, it is crucial to determine the optimal distance between the sample solution and analyte. This optimal distance ensures that the captured images are clear and of high quality, facilitating effective data processing and analysis. By maintaining the appropriate distance, one can obtain reliable and accurate results from the imaging process.

Selection of Region of Interest (ROI)

The program designates a specific region, known as the region of interest, which is highlighted and used to convert the pixel intensity into a value that can be correlated with the concentration of the analyte for quantitative analysis (Caleb, 2022).

The ROI is a defined area within the image that is selected based on its relevance to the analyte of interest. By focusing on this region, the program can accurately measure and analyse the pixel values, allowing for the quantification of the analyte concentration (Caleb, 2022).

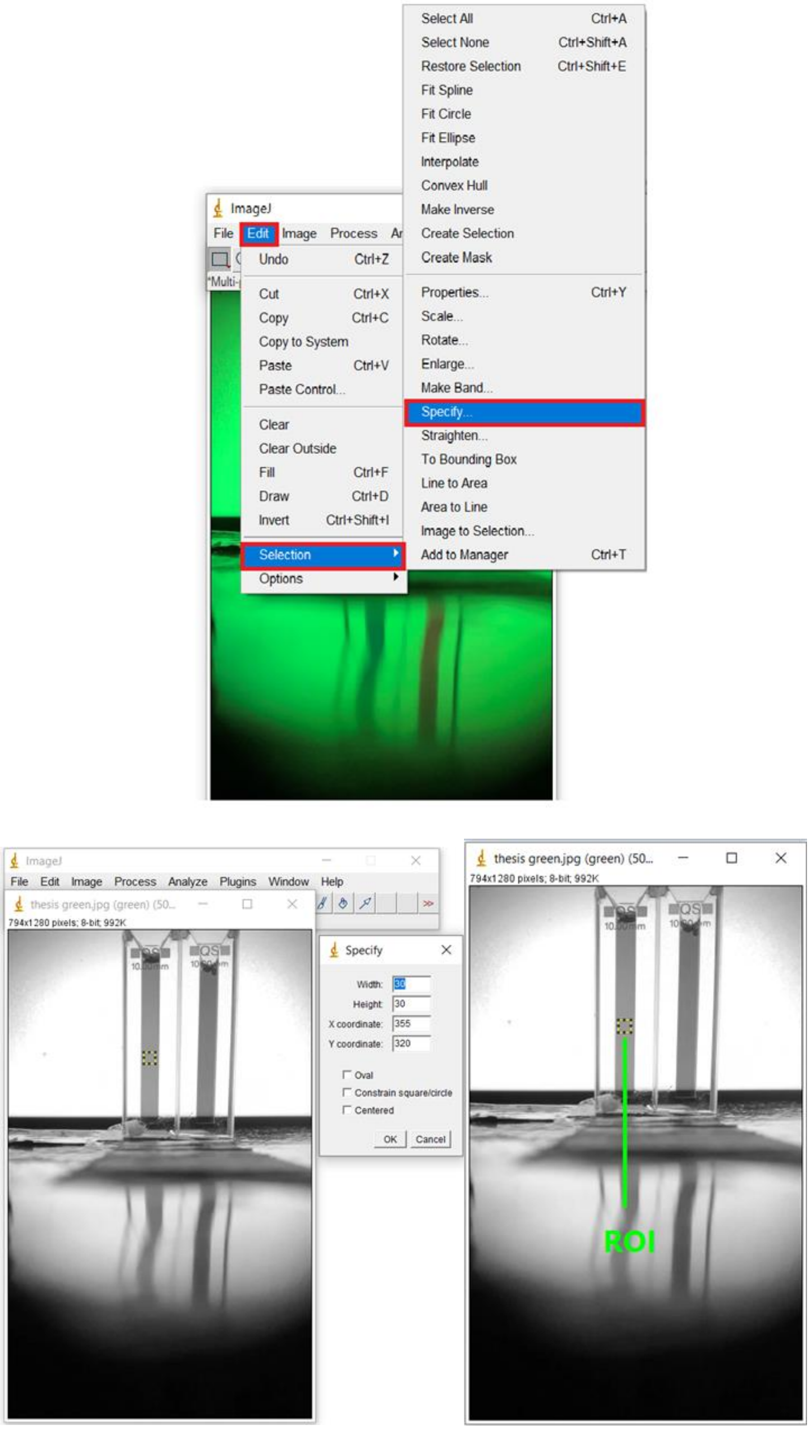
To select the region of interest (ROI) follow these steps:

- Open ImageJ software click on 'Edit' then menu.
- Select specify.
- Choose Selection.
- You will be presented with a drop-down menu that provides options for selecting the shape and alignment of the region of interest
- Choose the appropriate shape, such as a rectangle, circle, or polygon, based on the desired ROI area.
- Align the ROI as needed within the image.
- Once the ROI is defined and aligned, it can be used to analyse and quantify the relevant data within that specific region.

In this study, a centrally placed square was selected as the ROI. To modify X and Y coordinates of a sample solution, as well as the width and height of the ROI, the respective values could be adjusted (Caleb, 2022).

Figure 4

Selection of (ROI)

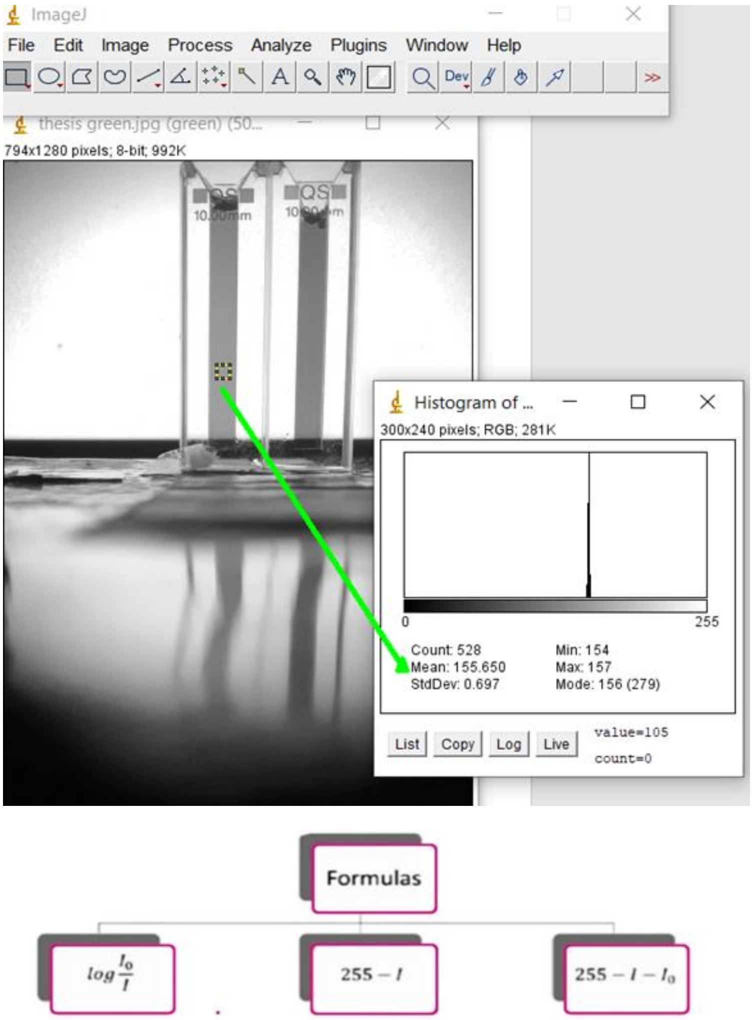


Signal Conversion.

The responses of sample solution and blank solution (represented as I_0) and sample solution (represented as I) are depicted by histograms in the specified channel, with each response provided a numerical value. Typically, the optimal blank is represented by the number 255, while the maximum value for the analyte is 0.

Figure 5

Signal Conversion



Quantification of Analyte Concentration through Histogram Analysis in Smartphone Digital Image Colorimetry

To generate a histogram from the region of interest (ROI) in the highest-intensity channel follow these steps:

- Open ImageJ Click on the 'Analyse' menu then from the dropdown menu, select the 'Histogram' option.
- This action will generate a histogram based on the pixel intensities within the region of interest in the highest intensity channel.
- The histogram provides a visual representation of the distribution of pixel intensities in the ROI.

In terms of quantification, the mean value is commonly used by calculating the mean, researchers can obtain a numerical value that reflects the average pixel intensity within the ROI. It is important to note that in smartphone digital image colorimetry, the relationship between the signal and analyte concentration is often inverse, unlike certain optical systems.

This reverse relation means that as the analyte concentration increases, the signal intensity decreases, and vice versa. Understanding this relationship is crucial when interpreting the results obtained from smartphone digital image colorimetry. (Fan et al., 2021).

CHAPTER III

Materials and Methods

Consumables

All the reagents and chemicals employed in this thesis for research and investigation were of analytical grade, unless specifically stated otherwise. This ensures that the highest quality and purity of chemicals were employed to ensure accurate and reliable results. Calcium (Ca) from merk (USA), ammonium chloride (NH_4Cl) and ammonium hydroxide (NH_4OH) from Sigma-Aldrich (Germany), Eriochrome black T from (Germany) were purchased.

Apparatus

In this investigation, consistent and repeatable photos were taken with a 5th generation iPad mini equipped with an 8 MP back camera featuring a f/2.4 aperture. The device had a permanent memory capacity of 65GB and a RAM of 3GB. It was powered by an Apple A12 Bionic chip processor, which provided efficient performance for image processing tasks.

The iPad mini also featured a touch display screen with a resolution of 1536 × 2048 pixels, offering clear and detailed visuals. The screen size measured 7.40 inches diagonally, providing a convenient and portable device for capturing and viewing images during the investigation.

A Samsung Galaxy Note 10 Plus smartphone was used as the source of the single-line radiation. The smartphone featured a large 6.8-inch touchscreen display that provided a spacious and interactive user interface.

The display has a resolution of 1440 × 3040 pixels, ensuring high-quality visuals with sharp details. The device is equipped with a 12-megapixel camera capable of capturing clear and detailed images. It offers a storage capacity of 128 GB, allowing ample data storage with 12GB RAM, ensuring a smooth and efficient performance. A Samsung Exynos 9825 processor was used, operating at a speed of 1x2.84 GHz.

Overall, this smartphone served as an effective tool for generating the single-line radiation required for this study.

A homemade colorimetric box made of aluminium, with dimensions of 25 cm × 18 cm × 9 cm, was designed to have the appearance of a basic optical instrument. To reduce light scattering, inside the box was coated with black paint and for capturing the images of sample solutions a small aperture was created on the side of the box through drilling a hole.

The acquired images were processed and analysed using the ImageJ software which provided the necessary tools and features to convert the images into analytical data.

In FAAS a Thermo scientific ICE 3000 series (USA) atomic absorption spectrometer was used that was equipped with deuterium lamp background correction. A lead hollow cathode lamp monitored at 422.7 nm and an air-acetylene flame as an atomizer operated at a flow rate of 0.8 L min^{1}, were applied in all the measurements.

Sample Preparation

The blood samples used in this study were obtained from the leftover samples of patients collected at Near East Hospital. The collection of these samples was carried out according to the ethical guidelines and regulations set by (SREC) of Near East University. The study protocol and procedures were approved by the SREC, with the approval number NEU/2023/106-1605. The samples were processed promptly after collection and handled following standard procedures to ensure their integrity and reliability for analysis.

Preparation of Solutions

Stock Solution

To prepare a stock solution of 1000 mg L^{-1} of Ca, the required amount of calcium chloride was dissolved in DI water. The solution was thoroughly mixed until all the calcium compound was dissolved, resulting in a homogeneous solution in a 50 mL volumetric flask The flask was then filled with distilled water up to the mark on the flask, and the solution was mixed well to ensure homogeneity.

Buffer Preparation

A buffer of pH 10 was prepared by mixing appropriate amount of the mixture of ammonium hydroxide s and ammonium chloride to prepare a buffer of 1.0 $mmolL^{-1}$.

The pH meter was used to ensure that the desired pH was obtained and the solution was prepared with DI water.

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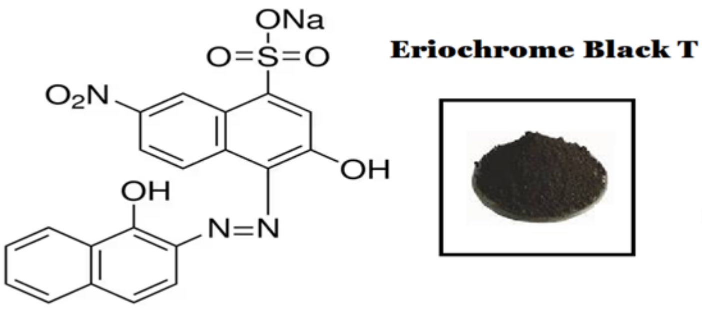
Preparation of the Eriochrome Black T (EBT) Solution

A 0.25 g of Eriochrome Black T indicator was weighed into a 25.0 mL volumetric flask, and 18.0 mL of concentrated ammonia solution was added. The flask was filled to the mark with DI water.

Figure 6

Structure of the EBT



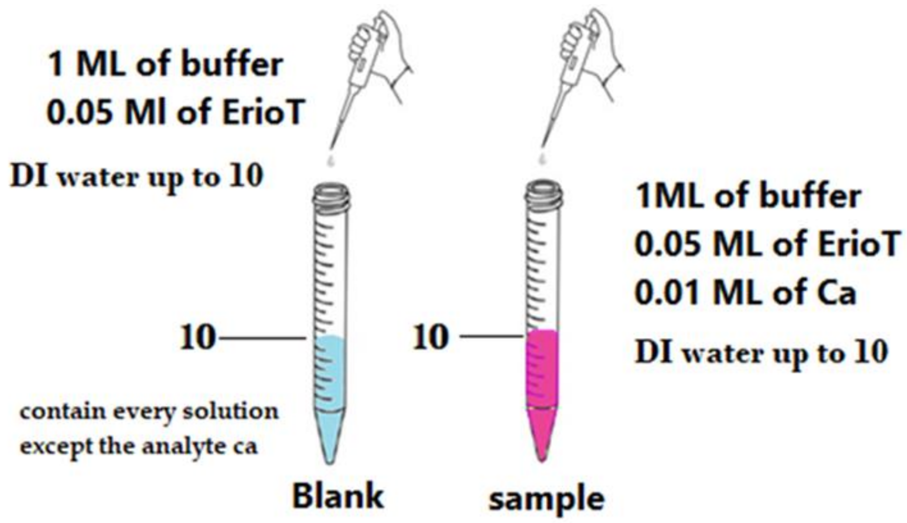
Sodium 1-[1-Hydroxynaphthylazo]-6-nitro-2-naphthol-4-sulfonate

Complexation of Ca with ErioT

A 100 mL of the serum was transferred into a 15 mL graduated centrifuge tube, 1 mL of the buffer and 50.0 µL of the ErioT were added. A complex reaction is formed between ErioT and Ca which is indicated by a color change of ErioT from deep blue to violet. A blank solution was prepared in like manner with DI water. The blank and sample solution were transferred into a UV/Vis microcuvette for detection in the colorimetric box. The complexation reaction is shown in figure 8.

Figured 7

Simplified Diagram of the Reaction



Smartphone Digital Image Colorimetry

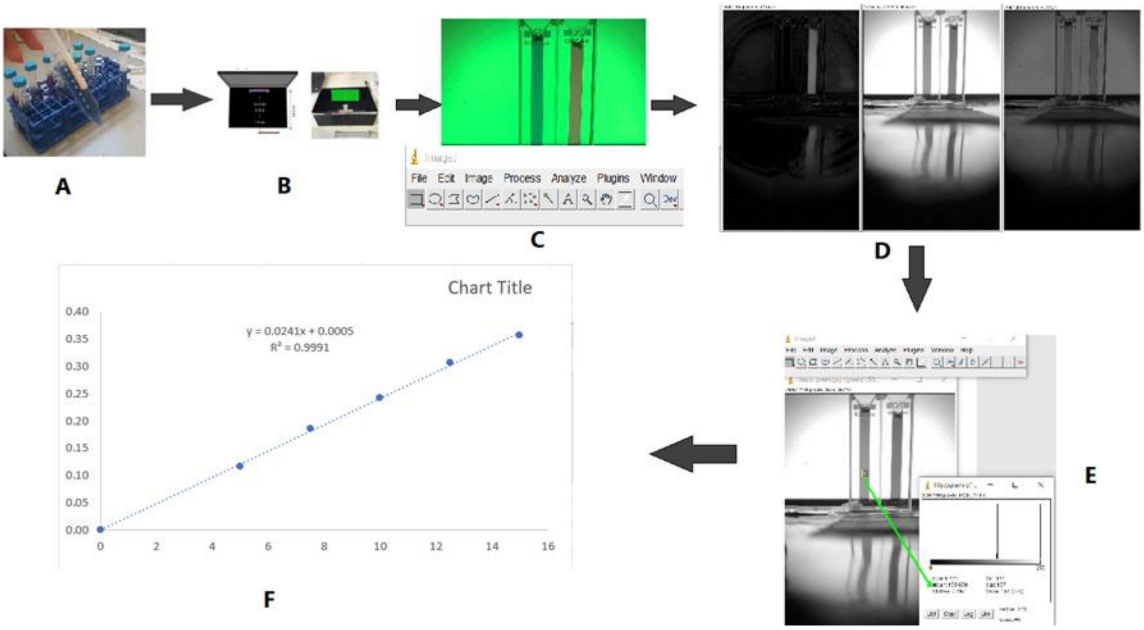
The first step was capturing the images and sent through Telegram application to a PC for processing with ImageJ. The images were split into their respective green, blue, and red channels, the green channel provided the best response among all the channels. Therefore, the green channel was used for all analysis. The proposed SDIC system is illustrated in figure 9. The following equation was used for quantification.

$$R_G = log\frac{S_b}{S_s}$$

Where R_G is the response obtained from the green channel, S_b is the signal obtained from the blank and S_s is the response obtained from the sample.

Figured 8

Proposed smartphone digital image colorimetry System



CHAPTER IV

Results and Discussion

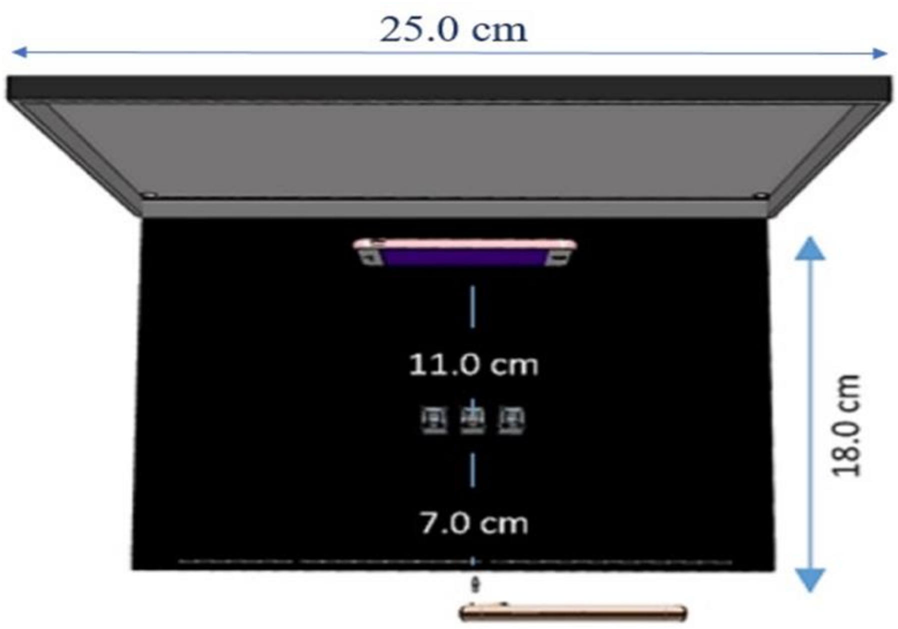
SDIC System Optimization Parameters

Design and Setup of the Colorimetric Box

This colorimetric box was manufactured from aluminium, with dimensions of 25 cm × 18 cm × 9 cm. It was designed to have the appearance of a basic optical instrument. To reduce light scattering, the interior of the box was coated with black paint and for capturing the images of sample solutions a tiny aperture was created on the side of the box through drilling a hole, to avoid excessive saturation the lens of the camera, the intensity of the monochromatic light source was carefully controlled and kept at a consistent level. Additionally, a backdrop was positioned to provide a suitable background for the sample. To capture the sample, the back-camera of the device was used to ensure that any unwanted glare or flare was eliminated from the captured images.

Figure 9

Design and Setup of the Colorimetric Box

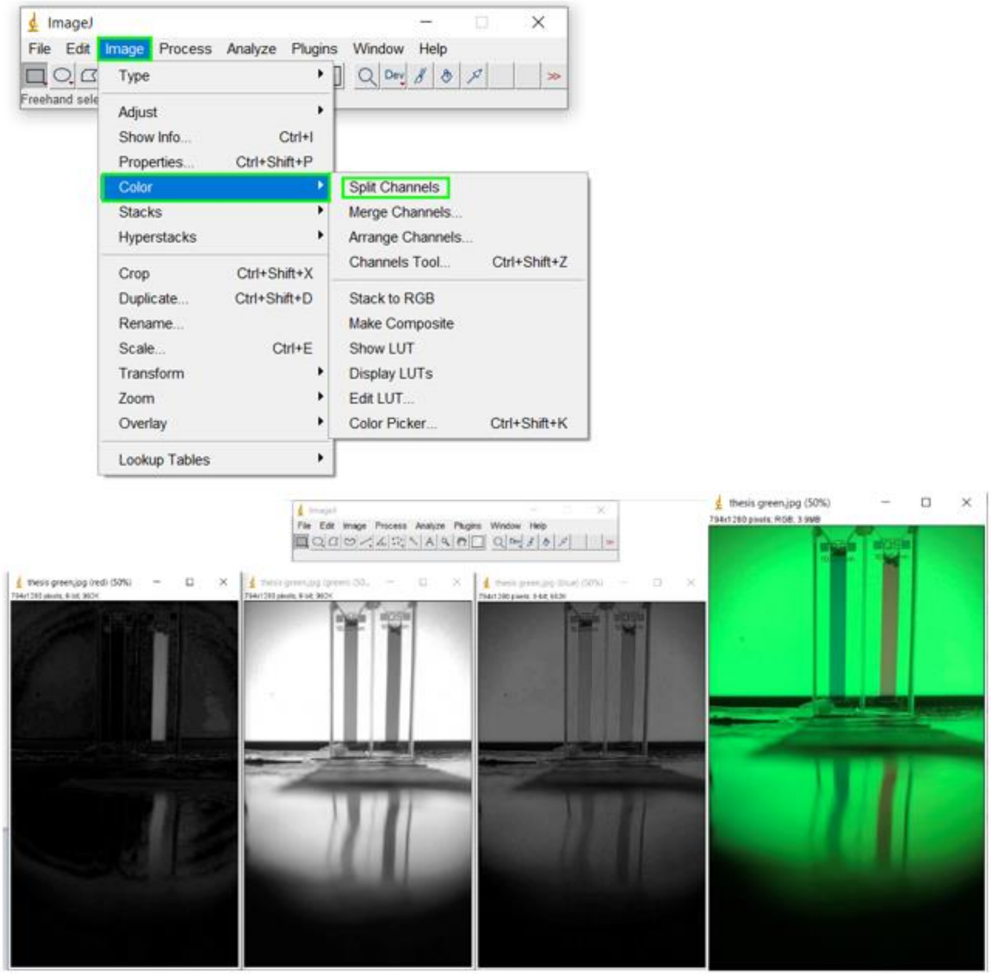


Selection of Optimal Channel for Analysis in SDIC

As mentioned previously, after splitting the image utilizing the ImageJ software, a specific channel was chosen to represent the sample solution. This selection is based on analysing the mean value of the histogram associated with each channel. In this study, the green channel exhibited a lower mean value of the histogram, indicating its suitability for analysis. Hence, the green channel was considered the optimal channel for obtaining accurate and reliable results.

Figure 10

Selection of Optimal Channel for Analysis in SDIC

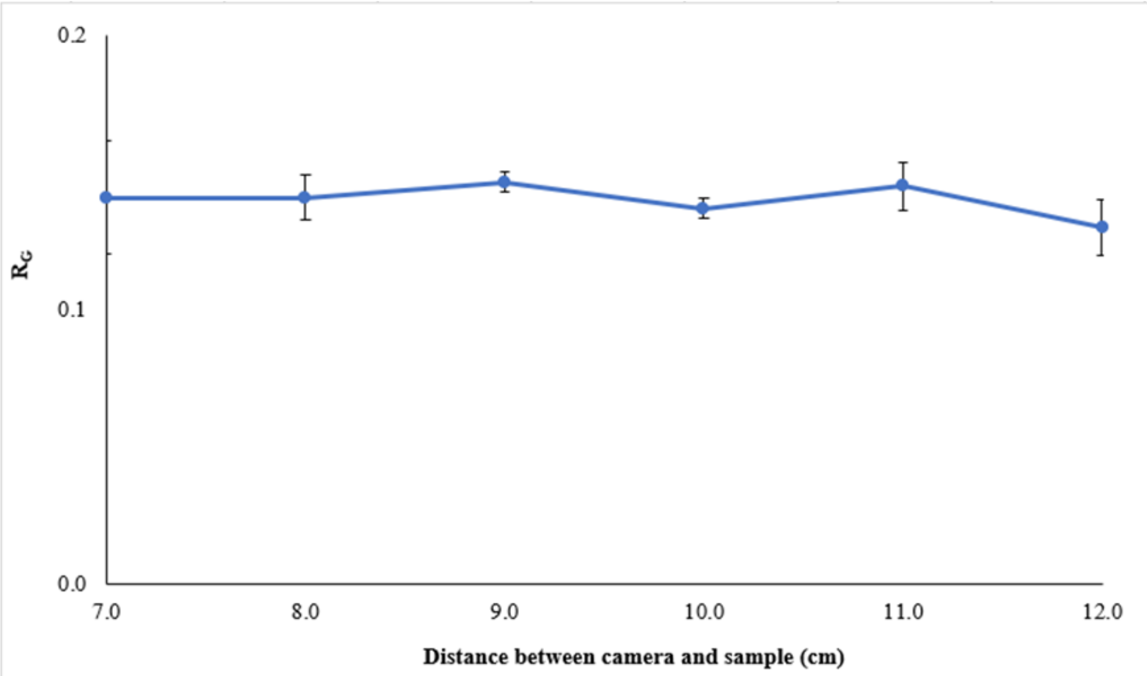


Optimization of Camera to Sample Distance for SDIC

To assess the impact of the distance between the detection camera and sample, measurements were conducted within a range of 7.0 to 12.0 cm. When the distance was set below 7.0 cm, the camera's focus was inadequate, resulting in a blurry image, and the image of the sample cuvette appeared to be grainy. However, at 9.0 cm, the image clarity improved significantly, and sharper images were obtained within the range of 9.0 to 11.0 cm. Considering the trade-off between sensitivity and repeatability, it was determined that a distance of 9.0 cm provided the optimal response.

Figure 11

Optimization of Camera to Sample Distance for SDIC



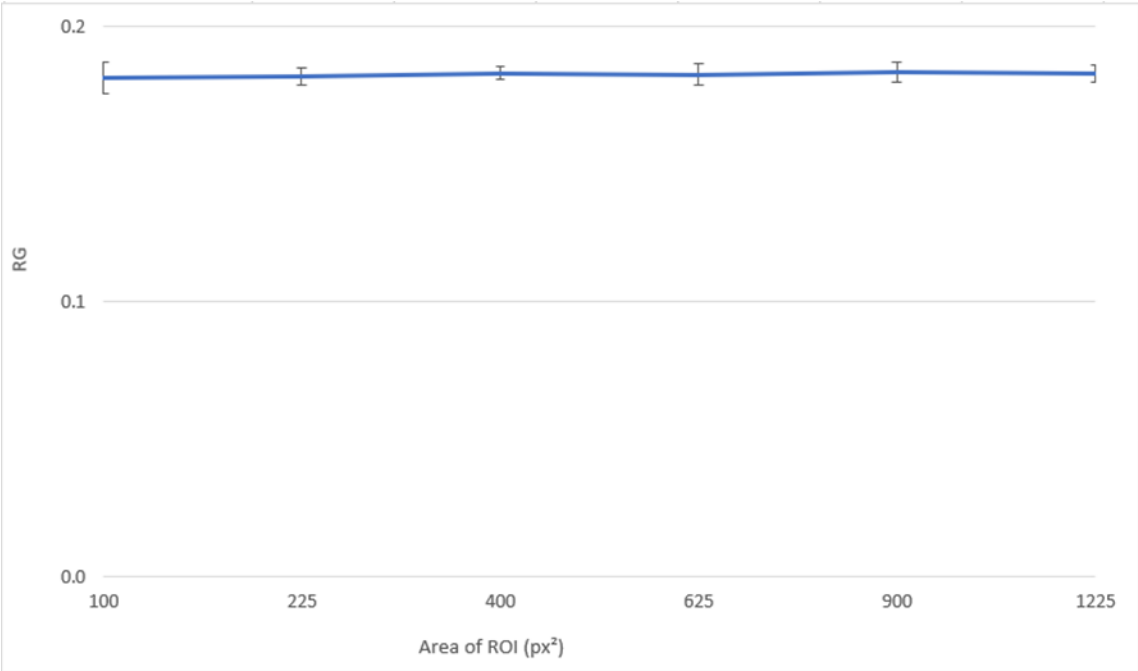
Optimizing the Region of Interest

The program identifies a specific region in the image, referred to as the region of interest, where the pixel strength can be converted into a value associated with the analyte concentration for quantification. It is possible to modify the X and Y coordinates of the sample solution, as well as specify the dimensions of the ROI by adjusting its width and height. The selected figure, represented by px^2, corresponds to the size of the ROI.

In this study, the Region of Interest was analysed by varying targeted area from 100 to 1225 px^2. Since the sample solution was homogeneous, there was no significant effect observed from any specific Region of Interest, as revealed by the consistent reaction. Therefore, any ROI within the image can be selected for measurement. After evaluation, an ROI size of 400px^2 was determined to be the optimal choice for the analysis.

Figure 12

Optimal Region of Interest (ROI)



Calibration and Analytical Performance Evaluation of SDIC and FAAS Methods for Calcium Analysis in Blood Samples

Calibration graphs were constructed using aqueous solutions of calcium standard with concentrations ranging from 5 to 15 µg mL^{-1}. The calibration graphs were generated using both SDIC and Flame Atomic Absorption Spectroscopy (FAAS) methods to assess the analytical effectiveness of the suggested methods.

The collected data demonstrates satisfactory linearity with coefficient of determination (R^2) value of 0.9991 for SDIC. The method showed good repeatability, as indicated by the percent relative standard deviation (%RSD) values for inter-day and intra-day precision, which were 1.68% and 4.18%, respectively. The limits of detection (LOD) and limits of quantification (LOQ), calculated using 3Sb/m and 10Sb/m, where Sb is the standard deviation of the intercept and m is the slope of the regression equation, were found to be 0.02 and 0.05 mg dL^{-1}, respectively. These outcomes shown in table 1 indicates the robustness and reliability of the method for the analysis of calcium concentrations.

Table 1. Analytical performance of FAAS and SDIC

Method	Sample	Regression equation[a]	R^2	LOD[b]	LOQ[c]	LDR[d]	%RSD[e]	
							Intraday	Interday
FAAS	Aq. Standards	$y = 0.0108\ (\pm 9.06 \times 10^{-5})\ x + 0.0052\ (\pm 2.7 \times 10^{-3})$	0.9987	0.08	0.25	0.005-0.002	2.90	5.40
SDIC	Aq. Standards	$y = 0.0246\ (\pm 1.5 \times 10^{-4})\ x - 0.015\ (\pm 1.3 \times 10^{-3})$	0.9991	0.02	0.05	0.001-0.015	1.68	4.18

[a]Response $(R)_G =$ Slope $(\pm SD) \times$ [Concentration of Ca (μg mL − 1)] + intercept $(\pm SD)$.
[b] Limit of detection (mg dL^{-1}).
[c] Limit of quantitation (mg dL^{-1})
[d] Linear dynamic range (μg mL^{-1})
[e] Percentage relative standard deviation, $n = 3$.

Determined calcium Concentrations in blood samples.

The calcium concentration in the human serum were determined by using the proposed SDIC method and FAAS. Similar analytical figures of merit were obtained with both techniques as shown in Table 1. The results are shown in mg dL^{-1}, which is the usual unit of reporting the serum calcium. Both methods gave concentration of Ca within the normal level. However, there is a statistically significant difference from the concentration of Ca obtained for both methods. This could be because of matrix effect for SDIC which can be eliminated by using a standard addition calibration curve. However, the SDIC method offers advantages in terms of simplicity, speed, and cost-effectiveness in comparison to the FAAS method.

Table 2.

Determined calcium Concentration in blood.

	B1	B2	B3	B4	B5
SDIC	3.06 ± 0.06	2.39± 0.04	2.59 ± 0.33	2.83 ± 0.56	4.95 ± 0.69
FAAS	11.85 ± 0.13	11.22 ± 0.13	11.47 ± 0.13	15.68 ± 0.06	12.95 ± 0.13

B1 to B5 =Blood one to blood Sample five (mg dL^{-1})

CHAPTER V

CONCLUSIONS

This study successfully employed smartphone digital image colorimetry (SDIC) as a detection technique for the analysis of calcium in blood samples. The results demonstrated the effectiveness and suitability of the SDIC method for quantifying calcium levels.

The study concludes that the use of straightforward and distinctive detection systems, such as SDIC, eliminates the need for additional hardware and significantly reduces costs. This cost-effectiveness makes SDIC an attractive option for determination techniques in various fields, offering high levels of precision and accuracy. Calibration methods and image-processing techniques can be employed to enhance measurement reliability and ensure consistent and reproducible results.

The findings of this study emphasize that smartphone digital image colorimetry is a viable option for advanced and complex systems, particularly in resource-limited settings, laboratories, and industrial sectors. Its main advantages, including rapid analysis, user-friendliness, portability, simplicity, and independence from electricity, make it an appealing choice.

In summary, the SDIC method presents a viable alternative for calcium analysis in blood samples, offering comparable accuracy and precision to the established FAAS method but at a lower cost and with greater ease of use.

Nevertheless, it is significant to note that the FAAS method remains a reliable technique for confirming the accuracy of results obtained using the SDIC method.

Overall, this study highlights the potential and value of SDIC as an effective and cost-efficient method for quantitative analysis, with implications for various applications in research, healthcare, and industry.

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NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :29.09.2022

Meeting Number :2022/106

Project number :1605

The project entitled **"Smartphone Digital Image Colorimetry for Quantification of Serum Proteins, Glucose, and Essential Elements"** (Project no: NEU/2022/106-1605) has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision* *Approved (✓) / Rejected (X)*	*Meeting Attendance* *Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. Mehmet Özmenoğlu	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	✓	✓
Doç. Dr. Emil Mammadov	✓	✓
Doç. Dr. Ali Cenk Özay	X	X

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