Smartphone-Based Colorimetric Determination of Solution Concentration

Group 1: University of the West of England

Guy Anderson, Michael Kunov, Douglas Mallaband, Adam Potton and Shannon Widjaja

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School of Engineering Mathematics and Technology

University of Bristol

Declaration of AI use

AI was used to inspire some ideas for next steps for our project, but all code was written by us with help from internet resources. The report was written mostly without the help of AI, but was sometimes used to improve sentence structure and provide synonyms.

1 Introduction

In today's rapidly evolving technological landscape, smartphones have advanced beyond communication devices to become useful in a range of sectors. One area modern smartphones can have an impact is analytical chemistry. This report delves into a smartphone-based colorimetric determination of solution concentrations. The motivation for this report is hydrogen peroxide, where detection within solutions has many applications including peroxide-based explosives [1]; commercial blood glucose biosensor [2]; laboratory chemical reagents [3] and historically medical and dentistry antiseptics [4].

Integrating smartphones into chemistry has allowed for various analysis methods. Colorimetry provides a way to quantify colour [5] and can be used to measure colour intensity, to determine the concentration of the coloured chemicals in solution. This can be achieved by relying on captured spectral images, taken from the camera of the smartphone. This technique has gained prominence due to it's simplicity [6]. Colorimetry has many uses, such as measuring pH or urine colour via the use of sensors like litmus paper that change colour in the presence of the respective analyte [6]. There have been many smartphone applications made available for this purpose, however according to Procida and Honeychurch [7] when used in various lighting conditions it is difficult to obtain accurate results. Their report was able to accurately detect the concentration of a chemical within beer, which is both carbonated and coloured, despite a small sample of both beer and added chemical. This shows that their method was both precise and accurate. The report by Procida and Honeychurch uses a single point at the centre of a cross hair from which they obtain the colour data [7]. This is susceptible to human error or bias as well as lighting factors as it is they choose where they place the crosshair on the image of the solution. A way to improve this would be to take a mean of a rectangle that fits entirely inside the image of the solution and is placed automatically using edge detection software. This removes the human element of placing the point and alleviates any inconsistent lighting. Machine learning (ML) was used to accurately quantify colorimetric tests from smartphone images [8]. This was done by developing a spectrometer app for colorimetric detection and training ML classifiers using hydrogen peroxide strip images. They were able to get a classification accuracy of over 90% from their method. They use the grey-world algorithm to achieve constant colours regardless of the lighting of the image, which helps to make the training of the ML accurate by giving it consistent images to learn from. The type of image file used in their experiments was JPEGs, which undergoes preprocessing and compression making them undesirable for image processing. Despite this, Solmaz et al. found that JPEG files showed a similar performance to forms of unprocessed image types such as RAW files when applied to their ML analysis. This is a useful result as it can be difficult to get images in an unprocessed form [8]. Their paper used three different colour spaces to measure colour within an image, and therefore to train the ML, these being: RGB, HSV and LAB. Standardly, pixel information is stored in the form of RGB, so it was converted into the others in MATLAB. As stated by Paciornik et al., HSL, a colour space similar to HSV yielded the best results, and showed a linear relationship between the value for hue and mercury concentration using a commercial scanner [9]. By separating colour components and measuring the average pixel intensity, they found that the hue component of the HSL scale had the closest linear correlation to the concentration of mercury present in the fish samples. Their paper shows that HSL can be used to get the best results when measuring the colour of an object or solution at different concentrations [9]. Fan et al. review chemical image analysis using a smartphone and conclude that at present, improvements must be made for it to become viable for analytical processes [10]. They conclude that better lighting does not necessarily lead to a better quality image and that consistency of light intensity, roughness of the object surface and homogeneity (from the same angle, position and zoom of the lens) between photographs of the solution is important to achieve consistent results and minimise error [10]. The ideas proposed by Fan et al. to provide consistency when gathering images are logical and therefore should be followed when gathering ones own images using a smartphone camera. Martinez et al published a report in 2008 in which they used images from phone cameras, which were then transferred to a lab for off-site analysis [11]. They highlight that the focus of a phone camera is less reliable than with a scanner. Despite the significant advances in mobile phone cameras in the time since the paper was published, the focus of a camera is still something to be considered when gathering images. Many mobile phones, for example the Apple iPhone, have an auto-focus feature [12] which aim to focus on the assumed target of a photograph in the instant it is taken. This could lead out of focus images if photos are taken with other potential targets in the image, so additionally to the conditions in the paper by Fan et al, it would be best to have a photo with blank background. Martinez et al. were able to detect and graph the presence of clinically relevant levels of glucose and protein in urine with known concentrations of each [11]. They did this by dipping a paper device loaded with reagents into the sample and then photographing the device once the analyte had mixed with the various reagents used. When analysing the results they found that the cyan channel of the CMYK colour space gave the largest dynamic range of colour and so provided better precision than a grey-scale image for their specific chemical. Using least squares fitting they were able to obtain results with an R^2 value around 0.986 for the mobile phone camera, showing that their results were precise [11]. The variation in colour from the paper device before, and the device after it has been dipped in urine is significant. This would mean that the camera quality and small variations in the images would be less noticeable due to the large change. For a small change in colour, the smartphone camera would not be sensitive enough to detect this change.

This report aims to verify known methods and explores the optimisation of a colorimetric method for the purpose of measuring the concentration of a diluted solution. Through the use of smartphones, this method offers a cost-effective and accessible solution. This paper uses white, grey, and black reference cards, and polynomial fitting to calibrate the images to make the results consistent, regardless of the conditions the image is taken in. MATLAB image processing is used to manipulate the images and detect the edges of the reference cards to automate the process. The solutions used in this paper are known concentrations of various flavours of squash as they provide variety in colour that was used to build and test the model. This paper details how the model has improved on prior methods and how it could be improved further.

2 Methods

2.1 Experimental Methods and Materials

Firstly, data was gathered with a simple experiment involving three different squash flavours: Robinson's Double-Strength Orange Squash, Robinson's Single-Strength Summer Fruits Squash, and Tesco's Quadruple-Strength Blackcurrant Squash. These squash flavours were chosen due to their accessibility and variety of colours. Squash was measured in grams using a digital pocket scale which had an error of ±0.005g, because sensitive volume measuring equipment was not available.

The quantity of squash was increased with regular intervals; approximately 2 gram intervals for Orange and Blackcurrant and 4 for Summer Fruits because of it's smaller change in colour. The measured amount is mixed thoroughly with 500ml of tap water in a transparent glass. This experiment was repeated for 12 recorded concentrations of the Orange squash, and 14 recorded concentrations of the Summer Fruits and Blackcurrant squash.

The three squashes have different densities, so this was measured using a measuring cylinder and recording the weight of the volume. After repeating this procedure three times and taking a mean value of the results, the densities are as follows in Table 1.

Squash Flavour	Mean density value (g/ml)
Orange	1.1967
Blackcurrant	0.8893
Summer Fruits	0.9027

Table 1: The densities of each squash flavour, measured in g/ml.

Images of the samples were obtained using an Apple iPhone 13 running Apple IOS version 16.1.1. The iPhone 13 has a 12MP (megapixel) main camera which has an aperture of $f/1.6$ [13]. The experiment was completed on a worktop where the iPhone was set with the camera 11cm from the sample, using $1\times$ zoom on the main camera. The camera settings were set to the iPhone 13 factory default, with the exposure setting and flash turned off. Uniform white light illumination was kept consistent throughout the experiment. All the resulting images were saved as a JPEG, as it is the default format in the tested phone. These choices were kept constant throughout to reduce instrumental errors at all stages. Alongside the sample were the reference cards.

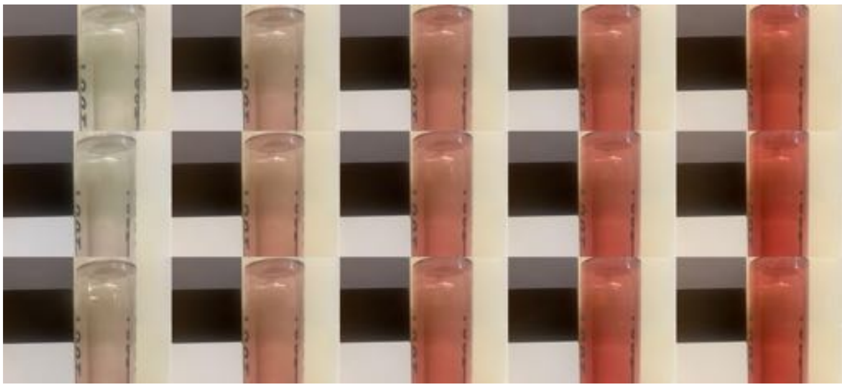


Figure 1: 15 samples of diluted Blackcurrant squash with increasing concentration with no image processing.

2.2 Image Processing

2.2.1 Colour model

A colour model is a system used to represent a colour. The red, green and blue (RGB) colour model, in which said colours are additively combined in various amounts to obtain any colour on the visible spectrum. RGB is the main colour model used to display an image in an electronic device in modern graphics.

Another colour model is hue, saturation and lightness (HSL). Saturation, also known as chroma, defines the purity of the colour on a scale of 0 to 100%, describing how much the colour is diluted with white light. The lightness dimension indicates the amount of black and white in the colour and it is measured linearly from 0 to 100% with black as the darkest (0) and white as the lightest (100). The lightness property is different to brightness, which is used in the HSB (hue, saturation, brightness) colour model (widely known as HSV where V stands for value). In this report, HSL is more suitable since lightness can be used to calculate subtractive colour intensity (for example mixing paints) compared to brightness, which is used for additive colour mixing (for example, combining visible light beams).

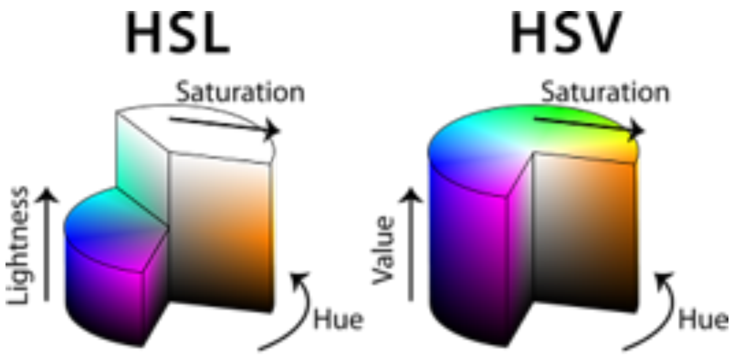


Figure 2: HSL and HSV colour model representation [14].

2.2.2 Different file formats

Image files can be stored in a range of different file types, with the most common and widely used type being JPG/JPEG (joint photographic experts group) 8-bit file . JPEGs contain 3 arrays with values from 0-255 (2^8), one for each red, green and blue (RGB). JPEGs have great potential for editing and manipulating due to their small file size and compatibility with nearly all modern software and editing tools. They have a smaller file size than other image file types due to the compression ratio that a JPEG uses, which allows for easier storing of images. This file format is used by modern smartphones. For this paper, JPEG files are used due to their easy manipulation, and ease of obtaining them on a modern smartphone.

A PNG is another frequently used image file type that does not use traditional lossy compression, which results in larger files [15]. It allows for more colour depth and contains a feature called alpha transparency, which is a matrix of information about the transparency of each pixel in the image. For the purposes of this report, a PNG would allow for greater image analysis, due to the transparency matrix making analysis of mixed colours in one sample simpler. PNGs cannot be used in this report however, due to the image capturing resources available being limited in modern smartphones, which do not return information in the alpha transparency channel.

2.2.3 Colour Calibration

Due to a number of factors, the colour displayed on a photo can be vastly different from the true colour. These factors include environment brightness, material reflection, incorrect colour data, camera settings, or file format conversion issues. In order to keep data consistent and ensure results are comparable, the RGB values for each data point must be calibrated. A method called 'White Balance' [16] is a commonly used technique in photography, graphics and image software such as Adobe Photoshop which balances out colours by using a single white reference point. In the method outlined by Olivier [17], professionally produced white, black and grey samples are used as reference points next to the sample being analysed.

- White: (255, 255, 255)
- Grey: (128, 128, 128)

- Black (0, 0, 0)

To achieve a high degree of accuracy in the reference samples, they must be printed with sensitive and expensive printing equipment. The samples used for this colour calibration were purchased on Amazon from a reliable supplier called Anwenk. The next step was to develop MATLAB code to calibrate the images. MATLAB was chosen due to it's powerful image processing toolbox and array manipulation capabilities. The premise of this MATLAB code is as follows:

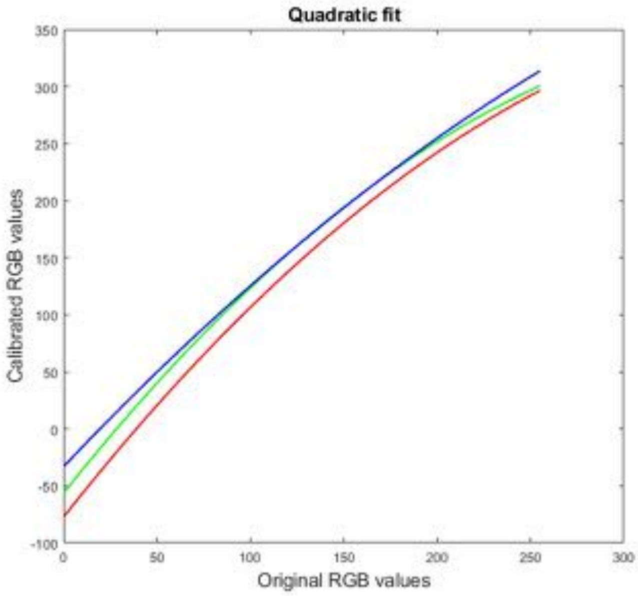


Figure 3: Quadratic from fitting the white, grey and black cards mapping all RGB values to their corrected values.

Firstly, the image is imported into MATLAB and turned into a 3-dimensional array with a matrix for each value of RGB for each pixel in the image. The image is then segmented into 3 parts (black, white, grey), and each of these three segments is averaged and put into three vectors of their RGB values. The method of segmentation will be explored in more detail in a subsequent section. A function is required to map from the RGB values of the cards in the photos to the actual RGB values of the cards. A second-order polynomial (quadratic) is used because the relationship is defined by three points (RGB values 0, 128, and 255) Figure 3. These three RGB set values represent the global minimum, midpoint, and global maximum of the quadratic fit. The area of interest (the chemical solution) is cropped and the mean RGB value is colour-corrected with the quadratic to return an accurate RGB of the chemical solution.

2.2.4 Beer-Lambert Law

The Beer-Lambert Law can be used to measure the absorbance of a solution when light is passed through it. This is useful way of measuring how much of a solute is in a solution if the amount of solute added changes the absorption of the solution. Usually, this experiment is done using a spectrophotometer, although it can be replicated using only a smartphone camera as outlined above, obtaining comparably accurate results [18]. The equation used for the Beer-Lambert law is,

$$A = -\ln\left(\frac{I}{I_0}\right) = \epsilon b C, \tag{1}$$

where A is the absorbance, I is the intensity of light transmitted through the sample, I_0 is the intensity of light transmitted through a blank sample (in the case of the experiments, this would be a clear glass of water), ϵ is the molar absorptivity and b is the length of the sample through which the light travels [19]. This equation demonstrates that absorption and concentration have a linear relationship for a fixed sample length and molar absorptivity. The intensity is calculated from the RGB value using the equation,

$$I = 0.2989R + 0.5870G + 0.1140B, \tag{2}$$

which is the formula for calculating lightness when converting from the RGB colour space to the HSL colour space. There are other formulas for calculating intensity, for example the mean of the RGB values, but in practice, Equation 2 is more perceptually relevant by utilising weighted averages based on their contribution to perceived lightness. This equation is used in MATLAB's 'im2gray' function which converts the specified true colour image RGB to a grey-scale intensity image. In a grey-scale image, each pixel takes a value from 0 to 255, which represents the lightness of that pixel.

2.2.5 Colour Card Detection

The method for colour calibration required the user to manually select the white, grey and black reference samples from the images in the experiments. In this context, this was a faster way to extract the data because the position of the cards was identical for all the photos so the images could be cropped identically. However, this would be difficult to adapt into an app for wider use, because the code requires cropped smaller images of four parts: the black, white and grey references, and the coloured sample. Automating the process of card detection would be more user-friendly in the context of an app on a smartphone and overall would take less time.

A method for detecting the cards is to split the image up into a number of smaller, equally sized sub-images, and find the median colour by averaging the RGB values. The number of sub-images to split the image into is a parameter that can be tuned depending on image size. By splitting up the image in this way, areas of high contrast are created, that will show where the white-black border is on the cards. By not segmenting the image or using too many segments, the desired sharp change in contrast will not be as explicit. A new image of these smaller images is recompiled and turned into greyscale can then be used to find the area with the biggest change in greyscale value. This change can be visualised on a 3-dimensional plot, where the 2^{nd} derivative [20] of the image shows a clear high point representing the biggest change of greyscale values in the entire image, which will always be the border between the black and white cards. Having determined the coordinates of the white-black border in the image, the three crops of the white, black and grey can be extracted, having already measured the dimensions of the cards. Figure 4 shows an example of finding where the white black border between the colour cards is located in a sample image.

This method almost completely automates the process of colour calibration, leaving just the crop of the sample down to the user. This can be automated on MATLAB too, given that the images from the experiments are all from the same place and angle, such that the sample area will be the same coordinates each time.

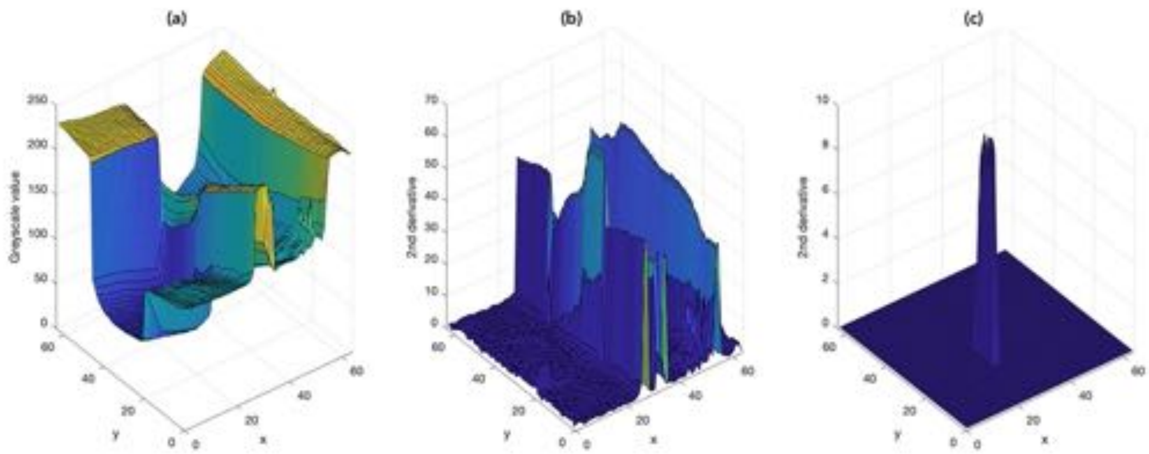
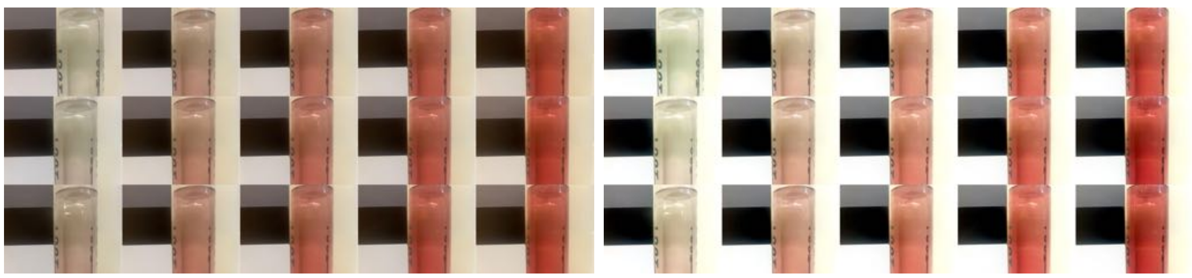


Figure 4: An example of edge detection shown using MATLAB surf plots. (a) shows a greyscale image plotted in 3d, where the x and y axis represent the pixels in the photo and the z-axis shows the greyscale value. (b) shows the second derivative of plot a, which highlights the points of highest contrast in the image. (c) shows the isolated point of highest contrast, which is the border between the black and white cards.

3 Results

3.1 Colour Calibration



(a) No image processing. (b) Colour corrected.

Figure 5: 15 samples of diluted Blackcurrant squash with increasing concentration.

Figure 5a shows the 14 samples of Blackcurrant squash with increasing concentrations next to the black, white and grey cards. Each image is individually colour corrected, shown in Figure 5b. This process is repeated for a further 14 samples of Summer Fruits squash and 12 samples of Orange squash. The mean of each RGB value is taken from an area of the diluted squash, from which the intensity and absorption can be calculated.

3.2 Beer Lambert Law

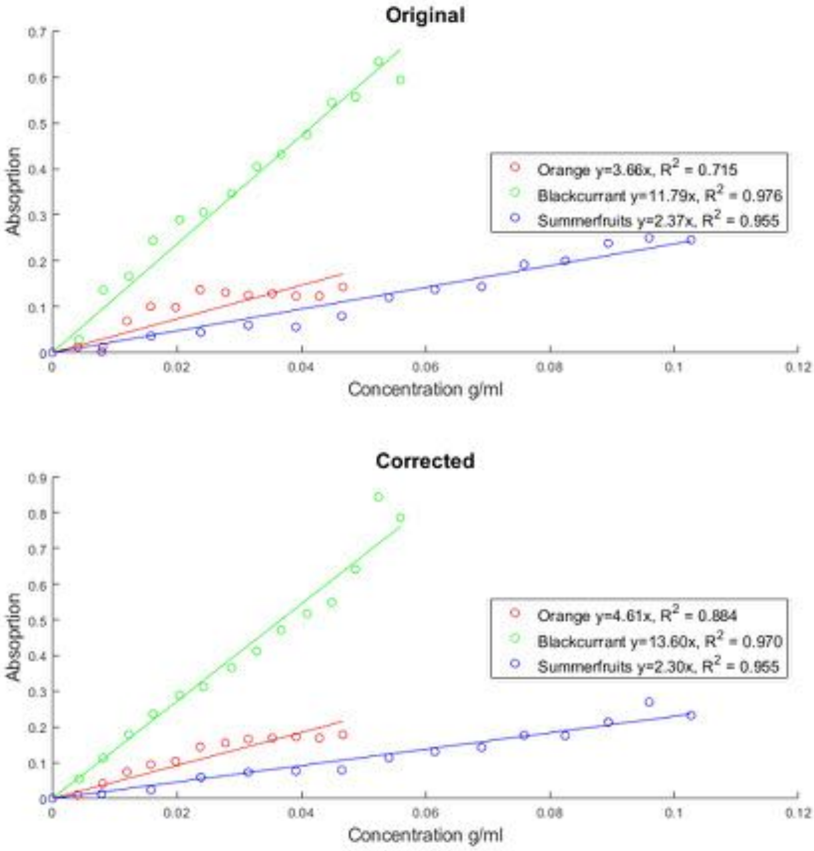


Figure 6: Absorption of increasing squash concentration for the original and colour corrected images.

Figure 6 demonstrates the linear relationship between concentration and absorption with the test demonstration on three different squashes by calculating intensity based on colour correction RGB values. A regression line that is forced to pass through the origin is calculated for each with it's respective r-squared score. The r-squared values here represent how well the linear regression has performed, taking values between 0 and 1, with a number closer to 1 representing better performance.

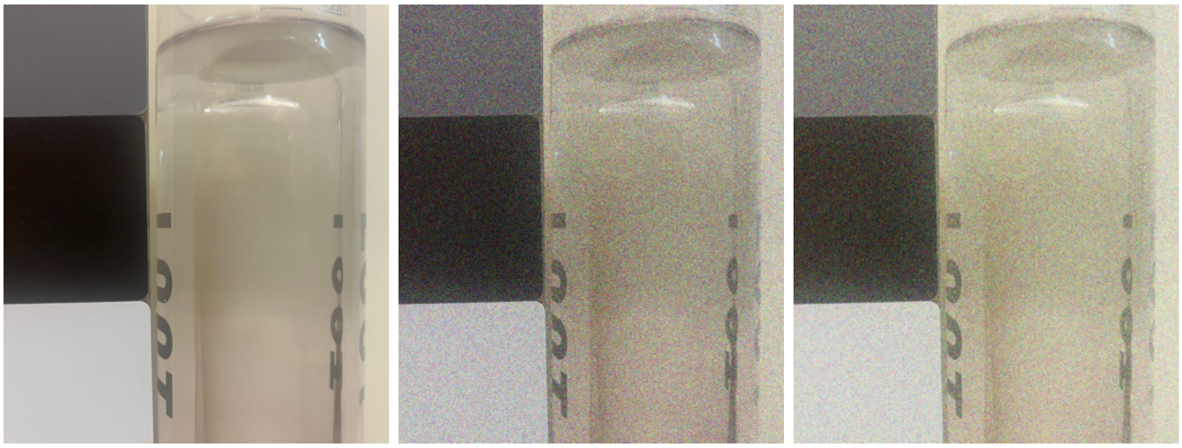
4 Discussion

4.1 Analysis of results

The mean r-squared score for concentration against absorption for the unprocessed images of the three squash varieties is 0.882. The method of colour correction yields a mean r-squared score of 0.936. This demonstrates that, for this experiment using squash, the methods used within this report have improved the accuracy of smartphone-based colorimetry.

4.2 Effect of Noisy Data

In real-world scenarios, the conditions images are taken in will vary. Environmental factors such as noise and uneven lighting will introduce unexpected variations in RGB data. To assess the robustness of the model, filters and noise can be applied to the data to analyse how it impacts the RGB values. In this context, noise represents incorrect image pixels.



(a) Original image.

(b) Original image with 0.05 Gaussian noise applied.

(c) Corrected image with Gaussian noise applied.

Figure 7: Process of adding artificial noise to an image and then colour correcting the image.

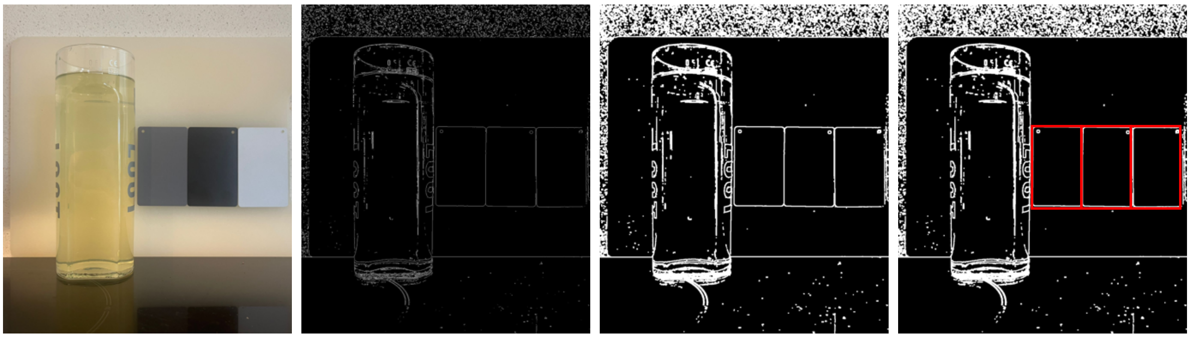
Figure 7 shows the process for artificially adding noise to the original images and then using the colour correction method to adjust it. Simple filters such as a Gaussian filter can be used to create a uniform image, the downside of which is it creates a blurry image. This however suits the purposes of this method since the blurriness of the image does not affect the RGB values greatly, although this may make the edge detection methods to locate the sample cards more inconsistent.

Mean Noise Added	Avg. RGB Correlation Coefficient	Avg. Root Mean Square Error
0.01	0.9843	0.0087
0.05	0.9838	0.0081
0.25	0.9804	0.0089
1.0	0.9814	0.0096

Table 2: Noisy data colour corrected data correlation coefficients and error with original data.

Table 2 shows the results from experimenting with the colour correction method on images with added noise of varying density and intensity. At higher noise intensity, the average correlation to the original data was lower, and the error was larger. However, even in intense noise, the data collected was still accurate. This is due to the method used to take samples of the cards and the solution, which was to take a mean, rather than an individual value. This negated most of the impact of the noise, and highlights the robustness of the colour correction method and the potential of using it in real-world conditions.

4.3 Edge Detection



(a) Original image. (b) Edge-detected image. (c) Dilated image. (d) Cards located.

Figure 8: Edge detection followed by dilation and locating the card reference samples.

To automate the colour calibration method, another edge detection method was tested. The image was changed to a binary image to find large changes in colour. Since the reference samples vary compared to their background, a high threshold can be set to define their boundaries accurately. Although the edge detection identified the reference samples, the edges are thin and disconnected in lots of places. To solve this, the binary image is dilated to connect gaps in edges and thicken the boundaries of the samples. Finally, the reference samples can be found; since the samples are of known shape the image can be searched for rectangles within a small threshold of their ratio $\frac{length}{width}$. Figure 8 shows the process for finding the reference samples automatically.

This method, however, ran into a range of issues when being tested for the purpose of this report. This was due to the limitations of MATLAB's edge detection function and not being able to automatically adjust the functions required parameters. This defeated the purpose of the edge detection using MATLAB's function, which is why the first method summarised in 2.2.5 was used. Using machine learning, a method could be developed to completely automate this unused method,

4.4 Multiple squash

In theory, the concentration of two different squashes could be calculated from it's RGB values (as well as the transparency from the alpha channel from a PNG file). The RGB values can be expressed as an approximation of a function of concentration in Figure 9 (in this case for squash a regression line would be sufficient).

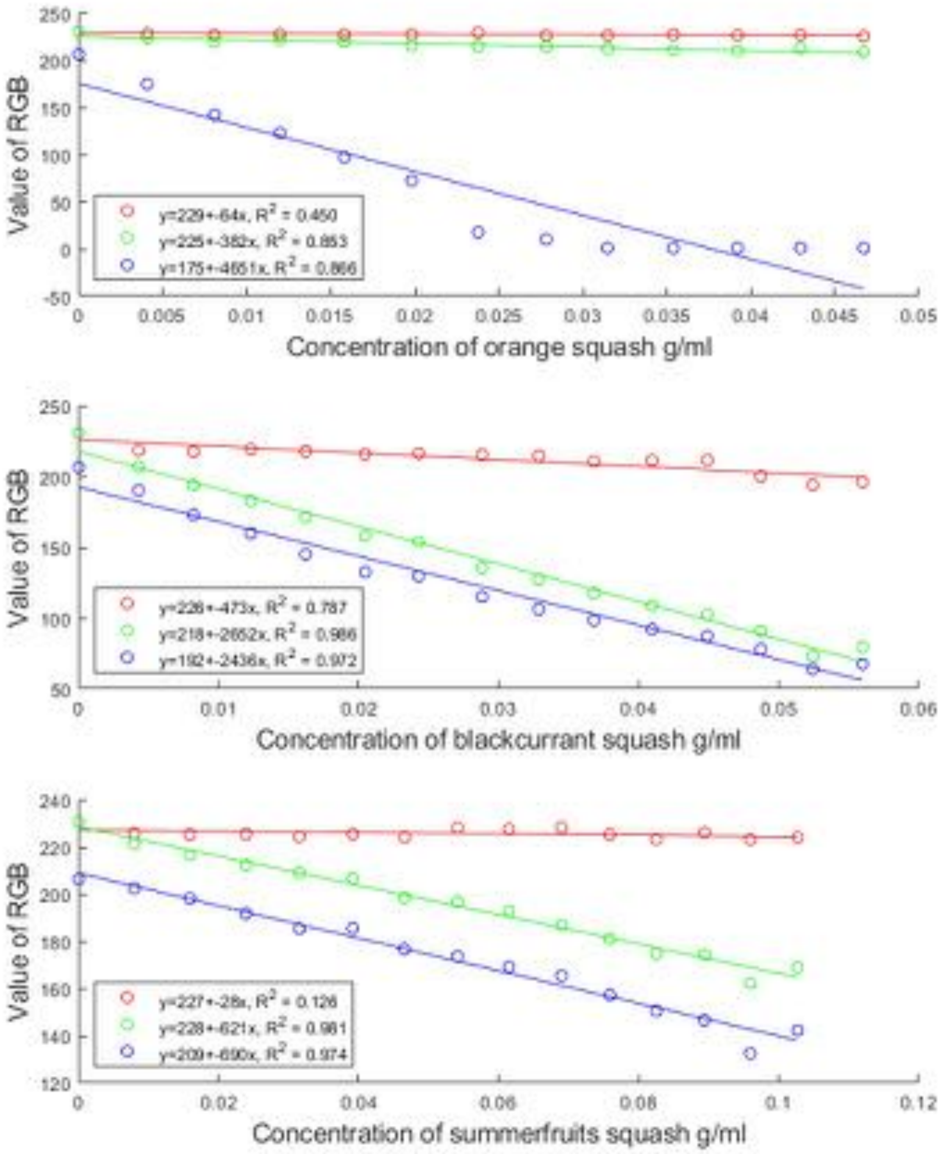


Figure 9: Approximation of concentration against RGB values for each Orange, Blackcurrant and Summer Fruits squash.

Such equations can be solved simultaneously along with,

$$\alpha_o = \alpha_a + \alpha_b(1 - \alpha_a), \tag{3}$$

$$C_o = \frac{C_a \alpha_a + C_b \alpha_b (1 - \alpha_a)}{\alpha_o}, \tag{4}$$

to find the contribution to the RGB value from each squash to then find the concentration of each squash [21]. Where C represents the colour component in each pixel (RGB), α represents the alpha value in the respective pixel and o represents the result of combining images a and b.

This method can only work for a solution made of three or less known compounds (squash flavours), each with a known approximation formula for the RGB value based on concentration. For more than three compounds, the system of equations would become degenerate since there would be more unknowns than equations, leading to no unique solution.

4.5 Shortcomings

The process of colour correction is undoing any function that has changed the image from how to object appears in real life. Such function is essentially arbitrary and it represents any computations within the camera via the compression when putting the image in JPEG form; auto-white balance computed by the smartphone and any environmental factors which impacts how the image file appears on a screen compared to how it looks in real-life, such as shadows and non-white lighting. The method approximates its inverse with a quadratic that is fitted using RGB values of the reference cards in the image. Ideally, a higher order polynomial would be more accurate. As in professional photography, a card with more colours (66) is used correct an image [22]. Furthermore, in a professional laboratory setup a spectrometer could be used to identify the exact compound. Such spectrometers could be used to detect more than three compounds present in a solution [23].

Moreover, the three colour cards (white, black and grey), despite being more accurate to their intended RGB values than other sources (e.g. printed images), could have been produced in a higher quality. For example, the white card could have been smoother, eliminating tiny shadows on the surface, and the black could have been less reflective. Another issue faced was being unable to completely eliminate shadows when taking the images.

5 Conclusion

A smartphone-based colorimetric procedure has been successfully developed to measure the concentration of a diluted solution. The method used previously in other papers was verified, and then improved upon. The model processes images taken from a simple squash experiment, involving three different squash flavours. By using a colour calibration method developed through MATLAB using colour cards, the conditions changing true colour were reversed to return an accurate RGB value for a given image. Results were made consistent by accounting for image noise using mean value of an image area. The method was optimised through using edge detection techniques, and by processing a large set of images all in one go. From the results obtained from this method, the absorption of the sample can be calculated, which is linear to concentration. Therefore, a regression line can be calculated to then be used to find the concentration of a given solution. The r-squared values obtained from the colour correction method were higher than from the original images. This demonstrates that, based on the experiments completed in this report that the methods used improved the accuracy of smartphone-based colorimetry.

The method outlined in this report could be improved upon through a range of methods including using more reference colours instead of just black, white and grey, and using more data points to span, which could be used to train a predictive model for solution concentration. Better lighting conditions could improve results. Using a PNG file as opposed to JPEG would allow for analysis of mixed squash samples, which would have a wider range of real-world applications.

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