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Exploring Smartphone-based Spectrophotometry for Vitamin B12 Quantification

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Abstract

Imbalanced nutrition is a global health issue with significant downstream effects. Existing nutrient assessment methods face several limitations, with accessibility being a major concern. In this study, we step towards developing technology for measuring nutrient status in an accessible way. We prototyped a smartphone-based spectrophotometer and tested its feasibility for measuring the absorbance spectra of vitamin B12 in a solution. We investigated the effects of various light sources and reference spectra calculation methods on smartphone-based spectrophotometry. To further validate our prototype, we compared the device to a benchtop laboratory spectrophotometer. Leveraging the Beer-Lambert Law, our prototype quantified the amount of vitamin B12 in a solution with an accuracy of up to 91.3%. Our work provides initial evidence for the utility of smartphone-based spectrophotometry as an accessible method to identify and quantify nutrients, paving the way for future developments aimed at other nutrients or in-body assessment.

CCS Concepts

- Human-centered computing → Ubiquitous and mobile computing systems and tools; Smartphones; Laboratory experiments;
- Applied computing → Consumer health; Health informatics.

Keywords

mobile health, nutrition assessment, spectrophotometry

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1 Introduction

Imbalanced nutrition can impair growth and hinder the proper functioning of the human body [10], underscoring the importance of assessing nutritional status. However, nutrient assessment often

requires invasive, expensive, and in-person clinic visits, making awareness of nutritional status largely inaccessible [1]. Current methods involve indirect, infrequent, in-person clinical examinations and complex in-lab analyses on blood.

Developing accessible, affordable, and low-burden methods for routine nutrient status assessment is essential for enabling timely nutritional interventions. This study explores the use of spectroscopic techniques, which can accurately identify and quantify chemical compounds, including nutrients. With the rapid advancement of smartphone technology and their widespread global adoption, smartphones offer a promising platform for ubiquitous nutritional assessment. For example, smartphone cameras are capable of capturing and analyzing spectral density and wavelengths of materials and chemical solutions [3, 4, 15]. It remains unclear how well smartphones can capture the spectrophotometric signatures of nutrients like vitamins and minerals. While our long-term goal is *in vivo* nutritional assessment, this study takes a necessary first step by evaluating whether nutrient profiles can be reliably detected in simple liquid solutions. This foundation must be established before advancing to more complex biological settings.

With the ultimate goal of measuring nutrient levels within the body, this work sets the stage by investigating smartphone-based spectrophotometry to quantify and identify nutrients in a solution. We utilized low-cost, DIY (do-it-yourself) materials (e.g., smartphone, 3D-printed components) to prototype a spectrophotometer for analyzing vitamin B12 solutions (selected due to its distinct pink-red coloration and its critical role in supporting health and well-being [9]). We also formalized an open-source signal analysis pipeline using ImageJ [12] and Python to extract absorbance spectra from smartphone images over the visible range (400 to 700 nm). Lastly, we compared and analyzed four distinct light sources and three reference spectra calculation methods to assess the parameters that most impact spectrophotometric analysis. The experimental results compared to a benchtop spectrophotometer demonstrate that smartphone-based spectrophotometry holds potential as a method for non-invasive micronutrient status assessment.

2 Background and Related Work

Spectrophotometry Overview. Spectrophotometry measures how much light a substance absorbs or transmits, revealing its chemical properties and enabling identification or quantification [6]. In visible spectrophotometry, used in this work, a light source passes through a sample, and a photodetector captures the transmitted light. Components such as a diffraction grating, light slit,



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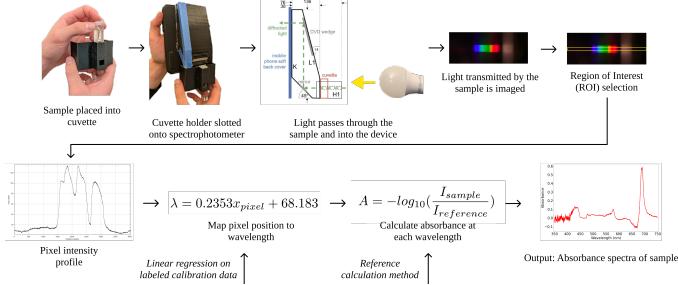


Figure 1: The processing pipeline for generating absorbance spectra of a sample from a smartphone-captured image. The design schematic in top-center is reprinted from [3].

and sample holder help isolate and direct specific wavelengths. The transmitted light intensity is used to calculate transmittance as $T = \frac{I_{sample}}{I_{reference}}$, and absorbance is derived as $A = -\log_{10}(T)$ [14]. When absorbance is linearly related to concentration, the Beer-Lambert Law applies as follows: $A = \epsilon lc$, where ϵ is molar absorptivity, l is path length, and c is concentration. This study leverages visible spectrophotometry using smartphones for chemical analysis.

Smartphone-based Spectrophotometry. There is growing interest in bringing spectrophotometry out of the lab and into everyday settings using portable and accessible tools. Smartphones are increasingly used in this space due to their built-in cameras, LED flash, and processing power. A wide range of smartphone-based spectrophotometers have been developed for applications such as measuring creatine [5], detecting glucose and cardiac markers [16], analyzing water quality [13], and reading colorimetric assays for vitamin C [17] and vitamin B12 [8]. However, most of these approaches rely on specialized components or complex colorimetric reactions, which reduce accessibility. Some studies have explored spectrophotometry using smartphones for educational purposes, proposing simple, do it yourself designs made from low cost materials [2, 3, 7], but these designs have not been validated against laboratory instruments or applied to nutrition. Our work addresses this gap by developing a simple, accessible, and low cost smartphone-based spectrophotometer for measuring vitamin B12, and we evaluate its performance by comparing it directly to laboratory-grade equipment. This demonstrates the potential for more inclusive, practical tools to support nutritional assessment without relying on reagents or specialized chips.

3 Prototype Development

We implemented a design by Bruininks et al. [3], which is comprised of a 3D-printed, periscope-like assembly (Fig. 1). Two razor blades are used to create a narrow, straight slit for light to pass through. Internally, the light from the slit bounces off a mirror and into the diffraction grating, for which we used the polycarbonate substrate of a DVD-R. The incident (reflected) light from the diffraction grating is directed into the smartphone camera sensor (Fig. 1). The housing is attached to a smartphone case so the camera is reliably positioned in the center of the spectrometer's opening.

We developed a custom, 3D-printed cuvette holder that attached directly to the main assembly to ensure precision, reproducibility, and versatility (Fig. 1). The holder accommodated larger, more intense light sources (e.g., an LED bulb) by allowing light to easily pass through two relatively large, square openings on either side of the cuvette. For this prototype to be viable as a mobile tool and to facilitate experimentation at a greater scale, a streamlined process of extracting quantitative information from the obtained smartphone images was necessary. Thus, we implemented a semi-automatic, Python-based signal analysis pipeline (Fig. 1) to analyze hundreds of samples at scale and tune several parameters involved in the signal analysis process. Given a horizontal image of the diffracted light, a consistent ROI is selected. This is the integration region over which the intensity profile is determined (a function of the average y-axis gray value over the pixels in the x-axis, where the gray value is a measure of intensity). The software ImageJ was used to calculate this profile [12]. Using a calibration image of a CFL bulb and a blank sample, we fit a linear equation to labeled pixel positions of the known emission peaks of a CFL (436.6, 487.7, 546.5, and 611.6 nm). The result is a linear equation that maps the x-axis location of pixels in an image to wavelengths.

4 Experimental Evaluation

4.1 Experimental Setup

4.1.1 Nutrient Preparation. To ensure the robustness of our experiments, we tested two common brands of vitamin B12 (cyanocobalamin) supplements: Walgreen's (WG) and Nature's Bounty (NB). Multiple concentrations of vitamin B12 were prepared for each brand as follows: 1. Supplements were dosed, ground, and dissolved into distilled water at a concentration of 20 $\mu\text{g}/\text{mL}$, creating stock solutions; 2. The stock solutions were filtered through a paper filter to remove any insoluble material that remained from the supplement; 3. The filtered stock solutions were diluted to four lower concentrations (10, 4, 2, and 1 $\mu\text{g}/\text{mL}$).

4.1.2 Light Source. An ideal light source emits light radiation at a uniformly strong intensity over the visible spectrum. Considering device accessibility, we evaluated four consumer-available light sources: a CFL bulb, an LED bulb, a single LED diode, and an LED diode matrix. LEDs are well represented by this sample because of their ability to generate light at a more even intensity.

4.1.3 Reference Spectra Calculation. Next, we experimented with different ways of defining the reference spectra, $I_{reference}$. Since a smartphone-based spectrophotometer is highly susceptible to inter-trial variance, a reliable method of "zeroing out" the device is needed. This can be achieved by taking multiple images of the blank sample and combining information from each to inform the reference spectra. Three different strategies were employed to define the reference spectra of the blank sample: 1) averaging the intensity across each blank sample, $I_{average} = \{I_\lambda : \frac{1}{n} \sum_{i=0}^n I_{\lambda,i}\}$ 2) selecting the spectra with the sum largest intensity (maxcurve), $I_{maxcurve} = \arg \max_i \sum^{\forall \lambda} I_{\lambda,i}$ and 3) taking the maximum of the three intensities at each wavelength, $I_{max} = \{I_\lambda : \max I_{\lambda,i}\}$.

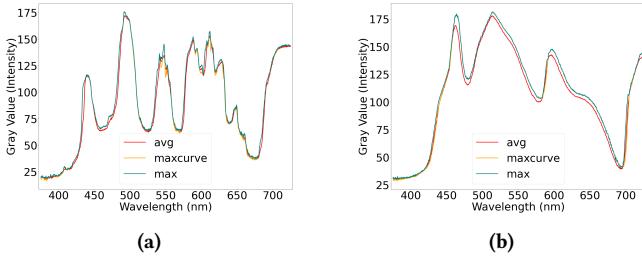


Figure 2: Intensity profiles across three reference spectra calculation methods. (a) The CFL bulb shows strong overlap among methods. (b) The LED matrix illustrates a worst-case scenario, with differences in peak amplitude but not location.

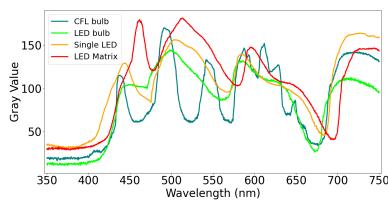


Figure 3: Prototype-derived reference spectra for each light source.

4.2 Experimental Procedure

The same smartphone (iPhone XR) captured the images of the diffracted light transmitted through the sample, and all samples were analyzed in a quartz cuvette designed for UV-visible spectrophotometry. We took several steps to control for variance induced by the smartphone's imaging system. First, we chose to take images in JPEG (instead of Apple's HEIC). We also turned off "Live Photos", "Prioritize Faster Shooting", and "Smart HDR". To account for auto-focus and auto-exposure, we utilized a feature in the iOS camera app that allows users to lock focus and exposure by pressing and holding the focus area. All four light sources and the three strategies of reference spectra calculation were implemented and compared. Three images of each sample were captured using the prototype. Each image was taken immediately after the previous one, and their intensity profiles were averaged. Additionally, three images of the blank sample (distilled water) were captured under the four different light source conditions. These images were combined using the three different reference spectra calculation methods.

4.3 Evaluation Criteria

Qualitatively, ideal results would produce absorbance spectra with relatively low noise, well-defined absorbance peaks or valleys, high inter-trial reliability (low variance), and a clear ordering of concentrations. Quantitatively, the results should follow the Beer-Lambert Law, which demonstrates that absorbance at a given wavelength is directly associated with concentration (Sec. 2). Hence, a result that adheres to Beer's law will display a distinct ordering of concentrations at a peak, as absorbance is expected to show a linear relationship with the sample concentration.

5 Results

5.1 Effect of Different Light Sources

We found that the single LED and LED matrix light sources performed poorly when comparing absorption spectra within the same concentration, brand, and prototype conditions. These sources showed high inter-trial variability in most cases. Any peaks or dips that could be determined were of low quality, with concentrations out of order and/or at similar absorbance values. The exception was the LED matrix samples, likely because of the methodological decision to select the single clearest diffraction pattern as the ROI out of the 2-3 visible from the matrix of LEDs. When evaluated on test samples, the CFL and LED bulbs produced clear peaks and minimal noise. The LED bulb showed more uniform emission spectra, with fewer emission peaks and valleys, when evaluated on a blank (Fig. 3). The bulbs likely performed best because of the magnitude of the light they emit, whereas the individual LEDs are weaker, with a narrower degree of illumination. Therefore, an LED bulb is the most ideal light source for our device out of those tested.

5.2 Effect of Reference Spectra Calculation

Reference spectra calculation methods were compared by plotting their intensity spectra together and auditing the peaks, valleys, and general shape (Fig. 2). In each case, the shape of the spectra (peak and valley locations) are nearly identical for all methods (Fig. 2a). As expected, I_{max} resulted in the highest intensity and was often made up of the $I_{maxcurve}$ sample (Fig. 2b). The $I_{average}$ reference was generally lower in intensity. Because only the amplitude (absorbance) was periodically affected, we found that no particular approach was distinct when comparing the various reference spectra strategies.

5.3 Laboratory Spectrophotometer Benchmark

We evaluated the performance of the smartphone-based spectrophotometer device in identifying the spectral profile of vitamin B12 against a laboratory UV-visible spectrophotometer (Hach DR6000). For this comparison, we selected the most optimal design for the described smartphone-based spectrophotometer: LED bulb light source and averaged reference calculation. The procedure was identical in all other aspects.

First, the laboratory device revealed no obvious differences between WG and NB. Each had a prominent peak at 361 nm, just into the UV spectrum, and a flatter peak at 551 nm (see Fig. 4a for WG, and c for NB). These results agree with previously published data on the absorption spectra of cyanocobalamin [11]. Overall, the inter-trial variance was negligible. Comparing this to results from the smartphone device, we see that both supplements (WG and NB) have identical peak locations (~577 nm) and similarly-shaped absorbance spectra (Fig. 4b and d). However, there is also considerable variance in the peak absorbance of each sample concentration. Noise is especially evident towards the boundaries of the visible spectrum (<450 and >650 nm), where the prototype device is unable to accurately measure transmittance (Fig. 4b and d).

Importantly, however, the peak at ~577 nm is only ~20 nm red-shifted from the laboratory device results, demonstrating agreement. Quantitative analysis confirms this for the WG samples, with

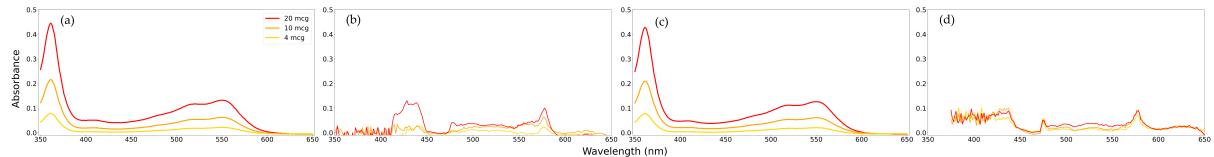


Figure 4: Absorbance peaks in the visible spectrum for different concentrations of vitamin B12 as determined by the laboratory and prototype device. Although the noise level varies, the prototype agrees with the laboratory results on a peak within 550 to 575 nm for both WG and NB. (a) Laboratory device exhibits peaks at 361 and 551 nm for the WG samples. (b) Prototype (LED bulb) exhibits peaks at 577 for the WG samples. (c) Laboratory device exhibits peaks at 361 and 551 nm for the NB samples. (d) Prototype (LED bulb) exhibits peaks at 577 nm for the NB samples. Concentrations are $\mu\text{g/mL}$.

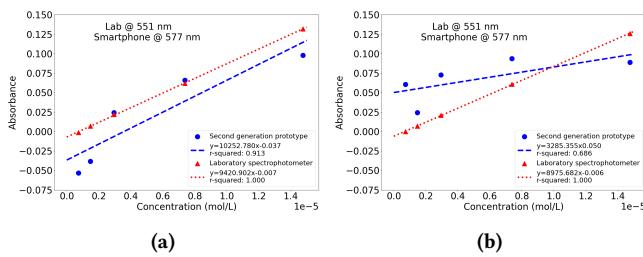


Figure 5: Absorbance-concentration relationship for the lab spectrophotometer and the prototype (LED light source, averaged reference, assessed at comparable absorbance peaks). (a) WG supplement (b) NB supplement.

regression analysis on the second generation smartphone device at 577 nm yielding an R^2 of 0.913 versus 1.0 for the laboratory device (Fig. 5a). The results from the prototype for the NB samples are less accurate, with an R^2 of 0.686 (versus 1.0 for the laboratory device; Fig. 5b). This analysis indicates that spectrophotometric results from our experiments follow Beer's Law, with some degree of variance (sec. 2). If provided with a vitamin B12 sample of unknown concentration, the prototype described herein can feasibly use the absorbance measured at 577 nm to determine the true concentration of the sample with an accuracy of 68.6% - 91.3%.

6 Discussion

We evaluated the feasibility of using smartphone-based spectroscopy for measuring nutrients via a prototype. Experimentation found that the device performed most reliably with a the LED bulb light source, and that all tested reference spectra strategies were similar. Comparisons to a laboratory spectrophotometer demonstrate comparable results, indicating the potential of smartphone-based spectrophotometry for nutritional applications. While these initial experiments with our prototype showed a performance comparable to the lab-based spectrophotometer, they also revealed several challenges, including more signal noise. Overall, the device offered ease of use through its compact, portable design and a tailored semi-automated pipeline for signal analysis.

This work takes several meaningful steps to inform future developments in accessible and non-invasive assessment of nutrient status, but limitations should be addressed. The most pressing of these is the high variance exhibited by the prototype relative to the

laboratory device. This could be caused by outside light interfering with the analysis, a fixed and non-adjustable light slit design, and/or flaws and artifacts in the diffraction grating or the smartphone camera. Associated with these issues, we also found that the smartphone-based device had limited sensitivity at the edges of the visible spectrum of light (e.g. Figure 4). We hypothesize that the diffraction grating likely causes the reduction in the spectral range of the prototype. If the intense red, green, and blue regions of the LED bulb (Fig. 3) appear a few pixels astray from where they exist in the reference image, then the sample data may produce erroneous peaks and valleys. This issue may be resolved by enlarging the diffraction grating, positioning it more directly in the light path, or substituting it with a commercial grating, although this could make the device less accessible.

Our experiments are also limited by using only one smartphone model and relying on nutrient supplements, which may contain additional compounds besides vitamin B12 itself. While the impact of smartphone image post-processing on spectrophotometric data is a legitimate concern, we believe our results in comparison to the laboratory spectrophotometer illustrate the relative effectiveness of our processing pipeline in mitigating this source of error. At the same time, we recognize the importance of conducting further research on how different smartphones perform in this context.

Future work should focus on improving device reliability, methodically building up to realistic scenarios and samples, ensuring that results hold across smartphone models, and expanding to assess other micronutrients. We envision future iterations that can detect vitamin B12 status through spectrophotometric blood analysis via the epidermis or through less invasive biosamples, such as saliva or urine, without the added cost and complexity of an assay.

7 Conclusion

In this work, we investigated smartphone-based spectrophotometry for quantifying nutrients in aqueous solutions. We developed a prototype and validated it against a laboratory spectrophotometer. We also examined the impact of four light sources and three reference spectra methods. Using the Beer-Lambert Law, our results show that vitamin B12 can be quantified with 68.6% to 91.3% accuracy. We highlight both the strengths and limitations of our design and argue that smartphone-based spectrophotometry holds promise as an accessible, noninvasive tool for nutrient assessment. By leveraging widely available mobile technologies, such tools could help individuals better monitor and manage their nutritional health.

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