Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

THE DEVELOPMENT AND QUANTITATIVE PERFORMANCE TEST OF LOW-COST VISIBLE SPECTROPHOTOMETER AND ITS COMPARISON WITH COMMERCIAL SPECTROPHOTOMETER

(Pembangunan dan Ujian Prestasi Kuantitatif dari Spektrofotometer Nampak Kos Rendah dan Perbandingannya dengan Spektrofotometer Komersil)

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Received: 13 December 2022; Accepted: 1 April 2023; Published: 23 June 2023

Abstract

A simple, portable, visible spectrophotometer was constructed and used for analytical measurements. The spectrophotometer was built using consumer electronics components such as an LED as the light source and a web camera as the detector. Using parameters including linearity, limit of detection, quantification, and precision to analyze the methyl red and dichromate standards, the analytical performance of the webcam spectrophotometer was evaluated. Results showed that, for methyl red, the webcam spectrophotometer gave a linearity range of 2–10 ppm with a limit of detection and limit of quantification of 0.69 and 2.11 ppm, respectively, and precision as a relative standard deviation of approximately 5%. The dichromate standard gave a linearity range of 0.7–1.5 ppm with a limit of detection and limit of quantification of 0.12 and 0.41 ppm, respectively, and precision as a relative standard deviation under 5%. These analytical performances are comparable but not better than those of the two commercial spectrophotometers used as comparators. The application of a webcam spectrophotometer to quantify the rhodamine B content in a traditional food sample, *pacar cina* jelly, also gave an equivalent analytical performance, and the rhodamine B analyte quantification capability was not significantly different from that of commercial spectrophotometers.

Keywords: visible spectrophotometry, webcam spectrophotometer, analytical performance, quantitative analysis

Abstrak

Spektrofotometer nampak dan mudah alih telah dibina dan digunakan untuk pengukuran analitik. Spektrofotometer dibina menggunakan komponen elektronik pengguna seperti LED sebagai sumber cahaya dan kamera web sebagai pengesan. Menggunakan parameter termasuk kelinearan, had pengesanan, kuantifikasi dan ketepatan untuk menganalisis piawaian metil merah dan dikromat, prestasi analisis spektrofotometer kamera web telah dinilai. Keputusan menunjukkan bahawa, untuk metil merah, spektrofotometer kamera web memberikan julat lineariti 2-10 ppm dengan had pengesanan dan had kuantifikasi masing-

masing 0.69 dan 2.11 ppm, dan ketepatan sebagai sisihan piawai relatif kira-kira 5%. Piawaian dikromat memberikan julat lineariti 0.7–1.5 ppm dengan had pengesanan dan had kuantifikasi masing-masing 0.12 dan 0.41 ppm, dan ketepatan sebagai sisihan piawai relatif di bawah 5%. Prestasi analisis ini adalah setanding tetapi tidak lebih baik daripada dua spektrofotometer komersial yang digunakan sebagai pembanding. Penggunaan spektrofotometer kamera web untuk mengukur kandungan rhodamine B dalam sampel makanan tradisional, jeli *pacar cina*, juga memberikan prestasi analisis yang setara, dan keupayaan pengkuantifikasian rhodamine B tidak jauh berbeza daripada spektrofotometer komersial.

Kata kunci: spektrofotometri nampak, spektrofotometer kamera web, prestasi analisis, analisis kuantitatif

Introduction

Spectrophotometry is a qualified technical analysis method that is commonly used to provide qualitative and quantitative solutions to problems in food, agriculture, pharmacy, biology, and clinical chemistry. Spectrophotometry techniques typically used in the food sector include fluorescence, Fourier transform infrared (FTIR) spectroscopy, near infrared (NIR) spectroscopy, and ultraviolet–visible (UV–Vis) spectrophotometry. Specifically, the UV–Vis spectroscopy has been used to analyze different food matrices (such as meat, milk, coffee, wine, vegetables, fruits, drinks, and olive oil) in relation to their composition, authenticity, adulteration, and quality [1].

Concerns about food quality and safety have grown in recent years among the public, apparently due to changes in eating habits, consumer behavior, development, and improvements in food supply chain industrialization. The high quality and safety requirements of food products require high standards for quality control and processing, which in turn require appropriate analytical tools to investigate the food [2]. In line with advances in consumer electronics technology, some studies have attempted to use smartphones as food diagnostic tools, such as for food allergen determination, functional food active compound detection, and foodborne pathogen identification [3]. Other studies have attempted to develop web camera-based spectrophotometers for detecting food oils or food colorings [4]. The idea behind developing this consumer electronics-based instrument is to make it available to a wide range of users, including the possibility of citizen-based food safety control [5].

Generally, a spectrophotometer may be assembled from several components, such as a light source from a white LED, a diffraction grating using a DVD, a detector from a CCD/CMOS webcam, and an image-based data acquisition/processing software or a direct spectrum recorder [6]. As a food diagnostic tool, a low-cost multifunctional webcam spectrophotometer was developed and applied to measure molecular absorption and fluorometry. The results of the determination of the same compound are comparable with other commercial spectrophotometers [7]. Moreover, a spectrophotometer constructed with LED components as the light source and a webcam as the detector was successfully built and was capable of providing a representative spectrum from various fruits such as apples, cherries, and pears [8].

This study attempted to build a portable absorption spectrophotometer that can be used to analyze food and other related materials using consumer electronic components. The reproducibility of performance from device to device can be a major challenge when using consumer electronics in portable systems [9]. Moreover, each portable spectrophotometer must provide results with verified precision and accuracy [10]. As an initial step towards developing a portable spectrophotometer-based food analysis system, this study, in addition to presenting the constructed portable spectrophotometer configuration, also shows a performance test of the spectrophotometer using organic and inorganic standard compounds. Furthermore, a performance comparison with commercial spectrophotometers is performed. In addition, a spectrophotometer for detecting hazardous materials contained in food was successfully developed.

Materials and Methods

The main materials used in this research were purchased from Merck: methyl red, $K_2Cr_2O_7$, rhodamine B, ethanol (96%), 1,5-Diphenylcarbazide, and other reagents. A sample of *pacar cina* jelly was obtained from a local market in Bogor. The main component of the webcam

spectrophotometer is the web camera used as the detector, which is a Logitech HD c270. The commercial spectrophotometers used for comparison was the Spectronic 20D+ and Genesys 10UV (Thermo Fisher Scientific).

Webcam absorption spectroscopy development

The spectrophotometer was constructed based on the scheme shown in Figure 1(a). A white LED lamp (HPL Epistar) powered by a 9-volt battery was used as the light source. The optical layer of a DVD (GT-Pro) was used as the diffraction grating, and a webcam was used as the detector. Spectrum data acquisition was performed using Spectral Workbench, which was provided by Publiclab.org as a web instrument with a feature to capture the spectrum using a webcam and other features to visualize, manipulate, and store the spectrum from the web browser page [11]. Figure 1(b) shows the spectrophotometer built using all the components. The light source, cuvette holder, grating, and webcam were located in a black box. The grating is attached to the front of the webcam lens. The positions of the components, particularly the webcam and light source, were then determined experimentally. The webcam was placed opposite to the 45° incident light, and the distance was set to minimize the probability of saturation due to excessive light. In this spectrophotometer, the distance between the webcam used as a sensor and the light source was 25 cm (S1), and the distance between the webcam and slit was 7 cm. The slit width was set to 1 mm, and a 1 mL cuvette was placed directly behind the slit.

Calibration and mode of measurement of webcam spectrophotometer

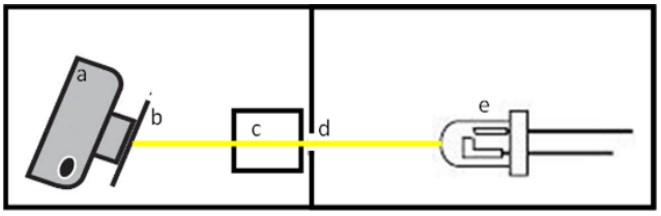
The spectrophotometer produces a sample spectrum with the aid of the tools from Spectral Workbench. Sample measurements began with a spectrophotometer connected to a computer using an I/O cable from the webcam. Using the profile that has been registered in the Spectral Workbench, the 'Capture Spectra' facility in the Spectral Workbench is used to recognize the webcam from the spectrophotometer and capture the spectra from the sample. The sample spectrum was calibrated using a CFL calibration spectrum to obtain a line spectrum with an intensity value at a certain wavelength. When the spectrophotometer was turned on (turning the white LED on), the light passed through the gap into the cuvette and towards the webcam. The grating attached in front of the webcam diffracts white light into the color components that the webcam captures, functioning as the sensor. The color spectrum of the diffraction pattern was visualized in real time on a Spectral Workbench (Figure 2). The Spectral Workbench simultaneously shows the transmission spectrum, with the y-axis representing the percentage of intensity (%I) and the x-axis representing the wavelength. To obtain the correct scale of the wavelength in the spectrum, calibration was first performed using a CFL lamp as the light source. A CFL lamp produces a well-known spectrum characterized by sharp and identifiable peaks. The Spectral Workbench performed the calibration process by adjusting the scale of the wavelength on the spectrum based on the peaks of the specified CFL emission, that is, at 436 and 546 nm.

The Spectral Workbench acquired the spectrum of the sample by transforming the color spectrum image into data. The resulting data can be exported in xls format by tabulating the wavelength and approximating the light intensity as the average values of RGB divided by 257 (interpretation of the Spectral Workbench algorithm to a single 8-bit byte pixel) (I') (S2 and S3). A percentage of approximate intensity on the y-axis of the transmission spectrum was calculated using Eq. (1):

$$\%\mathrm{I}' = \frac{\mathrm{I}'}{257} \times 100\% \,. \quad (1)$$

The webcam spectrophotometer was constructed as a single-beam spectrophotometer. To obtain the absorbance (A) of the sample, the approximate intensities of a blank and sample or standard were compared using Eq. (2):

$$\mathrm{A} = log \frac{\%I'_{blank}}{\%I'_{standard}} \,. \quad (2)$$

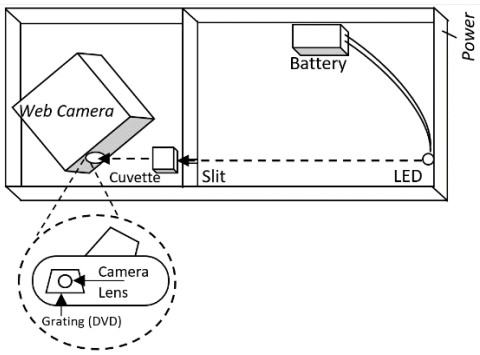


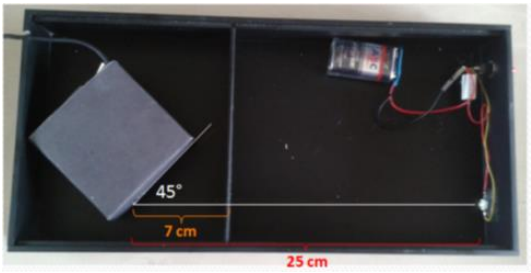
a: web camera

b: DVD grating

c: cuvette

(a)





(b)

Figure 1. Diagram illustrating general components for the webcam-based spectrophotometer (a) and scheme and view of the device from above with all of its components (ii)

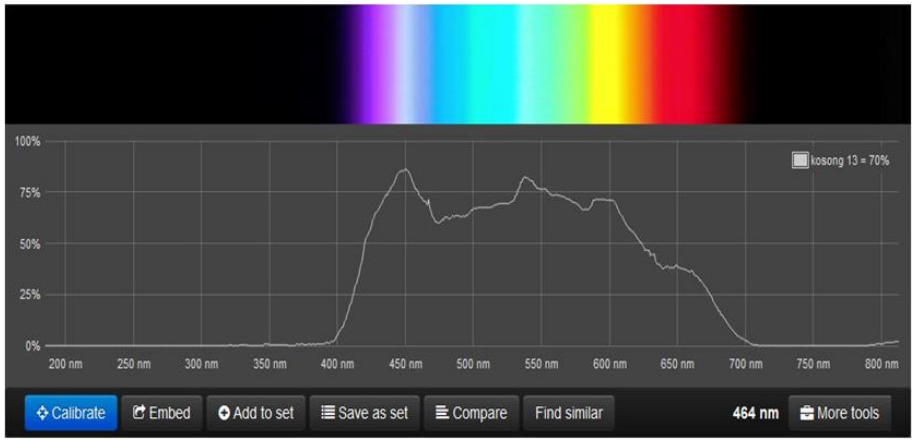


Figure 2. Example of a color spectrum and a spectrum with calibrated scale of wavelength from the webcam-based spectrophotometer

Webcam spectrophotometer performance and performance comparison with commercial spectrophotometers

The analytical performance of the webcam spectrophotometer was evaluated by determining the linearity, limit of detection, limit of quantification, and precision of methyl red and dichromate standard concentrations. The measurement results of the webcam spectrophotometer were then compared with those of the commercial spectrophotometers, Spectronic 20D+ and

Genesys 10UV (Thermo Fisher).

Linearity test

This test was performed by preparing calibration curves for five concentrations of methyl red and dichromate standard solutions. Ten milligrams of methyl red were dissolved in ethanol in a 100-mL volumetric flask. This standard solution was then diluted to yield a series of methyl red standard solutions with concentrations of 2, 4, 6, 8, and 10 ppm in a 25-mL volumetric flask. The concentrations of the methyl red standard solutions were measured directly using a spectrophotometer. Dichromate solutions were prepared using the $K_2Cr_2O_7$ standard at five different concentrations of 0.7, 0.9, 1.1, 1.3, and 1.5 ppm. The dichromate standards were measured after the first reaction with diphenylcarbazide (DPC). Each of the dichromate standard solutions (10 mL) was added to a reaction tube, followed by the addition of 0.4 mL of 0.25% DPC. After shaking the solution, 1 mL of a phosphoric acid-water mixture (1:1) was added. The solution obtained was shaken again, allowed to sit for 15 min, and finally measured with a spectrophotometer. Absorbance was measured in six replicates. Calibration curves were constructed by connecting the concentration (x-axis) and absorbance (y-axis). Linearity can be deduced from the correlation coefficient (r).

Limit of detection (LoD) and limit of quantification (LoQ)

The LoD and LoQ of the webcam spectrophotometer were determined by measuring the blank absorbance in ten replicates, and the standard deviation of the blank absorbance was calculated. The LoD and LoQ values were calculated using Eq. (3):

$$LOD = \frac{3.3 \times SD}{S}, \quad LOQ = \frac{10 \times SD}{S} \qquad (3)$$

where SD is the standard deviation of the blank absorbance, and S is the sensitivity (slope) of the calibration curve.

Precision

Precision was assessed using the relative standard deviation (RSD) of seven standard solutions with the same concentration. Methyl red was used at a concentration of 5 ppm, and dichromate was used at a concentration of 1 ppm.

Analysis of Rhodamine B in traditional food pacar cina jelly

To demonstrate the application of the spectrophotometer in sample analysis, a webcam spectrophotometer was used to determine the level of Rhodamine B in the traditional food, *pacar cina* Jelly. The results were compared to those obtained using commercial spectrophotometers. A 5 g sample of *pacar cina* jelly sample was weighed and immersed in 2% ammonia in 70% ethanol for 24 h to dissolve all the dyes. The colored solution was filtered, evaporated, and concentrated on a hot plate at 65 °C for 4 h. The concentrated solution was added to 30 mL of an aqueous solution and stirred to homogeneity. After adding the homogenous solution to a separatory funnel, 6 mL of 10% NaOH was added and shaken, followed by 30 mL of diethyl ether, which was shaken again until two layers were formed. The water layer was discarded, and the ether layer was washed with 5 mL of 5% NaOH and shaken. The ether layer was extracted three times with 10 mL of 0.1 N HCl. The HCl extracts were then collected in a 50-mL volumetric flask and diluted with 10 mL of 0.1 N HCl to the mark. The samples were then ready for measurement. The concentration of rhodamine B was measured by using a calibration curve of rhodamine B at concentration series of 1, 2, 3, 4, and 5 ppm and 0.1 mL HCl as the blank.

Results and Discussion

The analytical performance of the webcam spectrophotometer was evaluated based on the linearity, limit of detection, quantification, and precision of methyl red and dichromate standard concentrations. All measurements were performed at wavelengths of 495 and 543 nm, which are the maximum wavelengths for methyl red and dichromate, respectively. Tables 1 and 2 show the analytical performance compared with that of Spectronic 20D+ and Genesys 10UV.

Table 1. Analytical performance of spectrophotometers in determining methyl red standard concentration

Parameter	Spect. Webcam	Spectronic 20D+	Genesys10UV
Linearity(R^2)	0.9638	0.9991	0.9996
LoD (ppm)	0.7	0.1	0.1
LoQ (ppm)	2.1	0.3	0.2
Precision (%RSD)	5.2	1.6	1.7

Table 2. Analytical performance of spectrophotometers in determining dichromate standard concentration

Parameter	Spect. Webcam	Spectronic 20D+	Genesys10UV
Linearity (R^2)	0.9991	0.9983	0.9998
LoD (ppm)	0.1	$2x10^{-3}$	$4x10^{-3}$
LoQ (ppm)	0.4	$8x10^{-3}$	$1x10^{-2}$
Precision (%RSD)	2.6	0.6	0.3

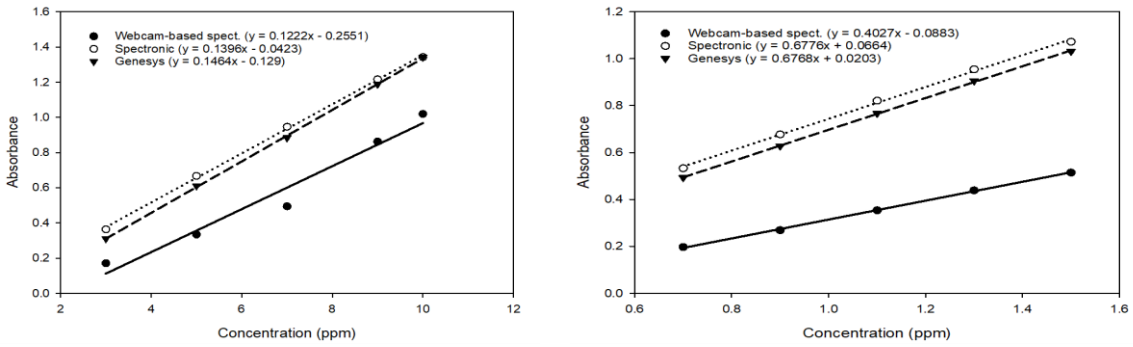
Generally, webcam spectrophotometers have not outperformed commercial spectrophotometers in terms of analytical performance. For the two standards used, the LoD, LoQ, and %RSD values of the webcam spectrophotometer were higher than those of the commercial spectrophotometers. In this study, the LoD and LoQ were calculated according to IUPAC definitions. Considering that the spectrophotometric method is used to measure standards using a simple matrix, the assumption of using blanks to determine LoD and LoQ can be fulfilled [12]. However, the linearities of commercial spectrophotometers were better than those of webcam spectrophotometers.

As shown in Figure 3, when compared to commercial instruments, the use of consumer electronics components (e.g., LED as the light source) as components of the webcam spectrophotometer results in a lower absorbance signal and a webcam as the detector results in lower calibration sensitivity. The webcam system for the detector typically undergoes signal deviation owing to the default image processing system and therefore offers limited quantitative data acquisition capability [13].

A similar phenomenon related to this analytical performance was found in another study by Danchana et al. [14], who developed a webcam-based molecular absorption spectrophotometer to measure iron levels, and in a study by Kotchabhakdi and Grudpan [15], who developed a digital camera-based atomic absorption spectrophotometer to measure metal elements in various types of food, water, and fertilizer samples. Nevertheless, the performance of this spectrophotometer was still reasonable and within the acceptable limit, as seen by the precision value that gave a lower %RSD than 2/3x CV Horwitz [16].

In this study, a webcam spectrophotometer was used to quantify the rhodamine B content in the traditional food *pacar cina* jelly. Rhodamine B is an organic chloride salt commonly used as a dye in plastics, textiles, and printing products. Rhodamine B was once used as a food additive, but its use in food is now prohibited [17]. However, some studies have found that Rhodamine B is still used illegally as a food additive, which could be harmful to consumer health [18].

Rhodamine B quantification by spectrophotometry began with a multi-stage extraction. Five samples of *pacar cina* jelly were purchased from local markets in Bogor. The samples were macerated and partitioned with various solvents to obtain rhodamine B in an HCl solution. The absorbance of the extracts was measured using a webcam spectrophotometer and subsequently compared with that of standard solutions to determine the level of rhodamine B. Measurements were performed using commercial spectrophotometers (Spectronic 20D + and Genesys 10UV). Table 3 shows the results of the rhodamine B content using the three types of spectrophotometers in conjunction with the statistical parameters of the method.



(a) (b)

Figure 3. Calibration curve for methyl red (a) and dichromate (b) standards

The webcam spectrophotometer used to quantify rhodamine B demonstrated the same pattern of analytical performance as the methyl red and dichromate determinations when compared with the two commercial spectrophotometers. The webcam spectrophotometer gave the largest relative standard deviation value compared to the two other spectrophotometers, but still maintained the precision within the acceptable limit of %RSD, which was lower than 2/3x CV Horwitz. The %CV Horwitz = $2^{1-0,5 \log C}$ acceptance limit can also be used as a starting point when the repeatability limits have not been determined through the control chart. Regarding the rhodamine B content in the samples, the three instruments yielded values that were not significantly different. Food producers used rhodamine B at a sufficiently high level, almost three times the 7.06 µg/g found in cassava chips [19]. The low F-value indicates that the model does not substantially differ in the mean of rhodamine B obtained from the three spectrophotometric methods.

Table 3. Rhodamine B content in samples and statistical parameters of method

Parameter	Spect. Webcam	Spectronic 20D+	Genesys10UV
[Rhodamin B] ($\bar{x} \pm sd$ µg/g)	21.65 ± 0.63	21.46 ± 0.34	21.83 ± 0.13
RSD (%)	2.91	1.56	0.61
2/3 CVHorwitz	6.7146	6.7236	6.7063

Note: ANOVA showed no significant difference (average of the three analyses).
($F_{calculation} = 0.9792 < F_{table}$, 0.05;2,12 = 3.8852, p = 0.4036)

Conclusion

A spectrophotometer model ("Webcam Spectrophotometer") has successfully been built utilizing consumer electronics components such as a LED as the light source and a webcam as the detector. The analytical performance was evaluated to determine the methyl red and dichromate standard solution concentrations, and the performance was compared with that of two commercial spectrophotometers. The analytical performance parameters of the webcam spectrophotometer (linearity, detection limit, quantification limit, and precision) were all within acceptable values of performance parameters and were comparable to those of commercial spectrophotometers. Further application of the spectrophotometer model to quantify the rhodamine B content in food samples demonstrated equivalent analytical performance with no significantly different analyte quantification capability compared to the other two commercial spectrophotometers. Based on the above, webcam spectrophotometers can potentially be used as an alternative instrument for food, related food materials, and product quality control.

Acknowledgement

This work was part of a research project, SP DIPA-023.04.2.189772, supported by the Ministry of Research, Technology, and Higher Education, Indonesia, and IPB University, Indonesia.

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