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Colorimetric Sensing of Ascorbic Acid Using Cu-Phen MOFs and Subsequent Digital Image Analysis with Smartphone

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Abstract
Vitamin C, also known as ascorbic acid, is a crucial nutrient involved in a number of enzymatic processes for tissue healing. Additionally serving as an antioxidant, ascorbic acid is crucial for keeping the immune system strong. It is essential to create a quantitative analytical approach to ascertain ascorbic acid concentration in many samples since its consumption from the daily food should be in the proper quantity. Here, we present a colorimetric probe made of synthetic metal-organic frameworks (MOFs) that changes its color only when samples containing ascorbic acid are present. In this study, phenanthroline served as an organic ligand or linker while copper served as the core metal that formed bonds with it. Cu-Phen MOF color fluctuations at 410 nm (from 408 nm to 412 nm) are linearly related to variations in light absorption. Moreover, as a substitute for spectrophotometry UV-visible, we have created a digital image-based colorimetry. The Cu-Phen MOFs' color change has the maximum slope and linearity when the blue color intensity is used. The detection limit with high precision of 4.2% under ideal circumstances was 0.1 ppm. The established approach allowed for the exact and accurate assessment of ascorbic acid in genuine samples of star fruit (*Averrhoa carambola* L.).

Keywords
Ascorbic Acid, MOFs, Biosensor, Digital Image Colorimetry, Smartphone

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1. INTRODUCTION

MOFs, also known as metal organic frameworks, are artificially created crystalline substances with a high porosity and large surface area (Wang, 2017). These characteristics make MOFs applications appealing in a variety of fields, as does the extreme degree of changeability of the organic and inorganic components in their structure (Rahmidar and Syarif, 2022). MOFs demonstrate the elegance of chemical structure and the ability to blend inorganic and organic chemistry at the molecular level. Generally speaking, these two areas of chemistry are seen as distinct fields of study. MOFs have been used as a storage medium for gases such as hydrogen and methane as well as an adsorbent to eliminate harmful gases (Abdel-Gawad and Abdelhameed, 2021). Additional uses, like catalysis (Xu et al., 2020), membranes (Tan et al., 2019), and biomedical and imaging sensors (Du et al., 2021) are also growing. The uses of MOFs in the field of bio-analytical chemistry are still limited that warrant further studies. Previously, MOFs have been used as a probe to detect various biomolecules colorimetrically such as exosomes (Li et al., 2023), glucose (Chen et al., 2017) and uric acid (Kou et al., 2020), and Cu ions (Khalil et al., 2016).

A number of foods contain ascorbic acid, also known as vitamin C, which is also available as a dietary supplement that has a wide use in medical practices. According to Lim et al. (2007), ascorbic acid is a necessary nutrient that is involved in collagen formation, tissue repair, and the synthesis of specific neurotransmitter enzymes. It is required for the immune system to function properly as well as for the appropriate operation of various enzymes. Additionally, ascorbic acid has antioxidant properties. Ascorbic acid can be found in a variety of foods, such as bell peppers, strawberries, broccoli, Brussels sprouts, guava, and citrus fruits. Foods with longer shelf lives or higher cooking temperatures may contain less ascorbic acid. The human body can generally tolerate ascorbic acid. A lack of ascorbic acid can cause a number of illnesses as well as lowered immunity. Excessive dosages may result in headache, skin redness, gastrointestinal distress, and trouble sleeping (Arya et al., 2000). Pregnancy is safe when taking normal dosages. Physicians advise against using high dosages. This dose needs to be monitored, so developing analytical techniques is essential. Ascorbic acid has always been analyzed using chromatographic techniques, especially high-performance liquid chromatography (HPLC) (Iwase, 2000), fluorimetric methods (Arya et al., 2000), UV-visible spectrophotometry (Vermeir et al., 2008), Voltammetry (Pisoschi et al., 2011), etc. It is well known that instrumental analysis required many resources and is expensive, not to mention that it should be run by high-skill staff and require many chemicals. Therefore, an alternative method that offers simple, low-cost, easy to use and portable sensing methods is highly demanding.

DIC or Digital image colorimetry using a smartphone is a rapidly growing analytical method that received large attention among the analytical chemists due to their simplicity, easy on use, low cost and portable (de Carvalho Oliveira et al., 2022; Firdaus et al., 2023). DIC has been used to determine heavy metals (Firdaus et al., 2014; Firdaus et al., 2019; Masawat et al., 2022; Meelapsom et al., 2022), biomolecules (Firdaus et al., 2022), chemicals in food and textiles industries (Eddy et al., 2023; Masawat et al., 2015; Permana et al., 2023; Yadav et al., 2022), etc. Furthermore, the DIC method can be combined with chemometrics analysis for further application in various fields and samples (Gaião Calixto et al., 2023; Gusti et al., 2021). We report for the first time, in this paper, a solvothermal synthesis of Cu-Phen MOFs that was employed as colorimetric agents to identify ascorbic acid using a smartphone as a detector or instrument, and were evaluated using UV-vis spectrophotometry.

2. EXPERIMENTAL SECTION

2.1 Chemicals and instrumentation

Every substance was of analytical quality obtained from Merck Ltd. (Germany). An instrument of SEM (Scanning electron microscopy) from Hitachi SU3500 (Japan) was utilized to know the morphology of Cu-Phen MOFs. The X-ray powder diffractometer (XRD) analysis was done using X-Ray PW1835 XRD instrument from Phillips Analytical (UK) with Cu Kα radiation. The spectra of infrared absorption were obtained by a FTIR (Fourier Transform Infrared) spectrometer Nicolet-iS10, Thermo Scientific (USA). A UV-visible Spectrophotometer Genesys 20 from Thermo Fisher Scientific (USA) was used to obtain the spectra starting from 350 nm up to 800 nm, with a cuvette of quartz that has path length 1 cm (Gusti et al., 2021). This UV-visible Spectrometer was also used to validate the digital image from the smartphone Vivo V5 (China) which acts as colorimetric instrument.

2.2 The Synthesis of MOFs

The solvothermal method was used to create the MOFs of Cu-Phen. We used dimethylformamide (DMF) as the solvent in this study. Cu-Phen MOFs are so named because copper was utilized as a central metal to form a coordination bond with phenanthroline, an organic ligand. After dissolving 0.54 grams of phenanthroline in 10 milliliters of DMF, the mixture was stirred for 20 minutes. $CuSO_4$ weighing 0.249 grams was dissolved in DMF and stirred for 30 minutes. After combining the solutions, they were transferred to a 100 mL Teflon autoclave and heated for 24 hours at 140°C in an oven. After being separated by centrifugation, the synthesized MOFs were ethanol-washed three times. The MOF precipitate was dried in an oven set to 110°C for twenty-four hours (Rahmidar and Syarif, 2022).

2.3 Quantification of Ascorbic Acid

Ascorbic acid was analyzed with accuracy and precision following preliminary protocols on the sensitivity and selectivity experiments (Firdaus et al., 2019). A variety of chemical compounds were used for the selectivity test, and different ascorbic acid concentrations were used for the sensitivity test. A smartphone was used to monitor the color change that corresponds to the ascorbic acid concentration, serving as a model for digital image colorimetry. Samples were placed inside cuvettes, and the smartphone was used to take pictures while the lighting was controlled in a box studio. The Lambert-Beer equation was used to compute the Red, Green, and Blue (RGB) color amounts to a log scale (Firdaus et al., 2014; Firdaus et al., 2019). The UV-vis spectrometer was used as the reference method. The developed method was applied for ascorbic acid determination of star fruit (*Averrhoa carambola* L.). A 10 g of the star fruit was pounded to pieces and mixed with 10 mL water. The mixture was then filtered and ready for the ascorbic acid analysis (Firdaus et al., 2017).

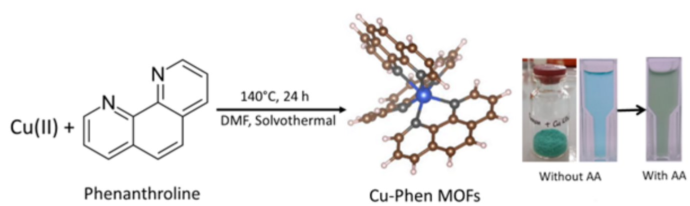

Figure 1. The Chemical Reaction for Synthesis of Cu-Phen MOFs. On the right, a picture of the MOFs is displayed together with its solution in a cuvette, with and without ascorbic acid (AA)

3. RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of Cu-Phen MOFs

The MOFs of Cu-Phen were made from complexes of $CuSO_4$ with phenanthroline that were mixed under pressure using Teflon container in autoclave for 24 hrs at 140°C. As shown in Figure 1, the synthesized Cu-Phen MOFs are stable and have a crystalline structure that only slightly dissolves in water. When the Cu-Phen MOFs come into contact with ascorbic acid as an analyte, their light blue color changes to a turquoise green. The color of Cu-Phen was not changed when mixed with other biomolecules and chemicals such as glucose, sucrose, fructose,

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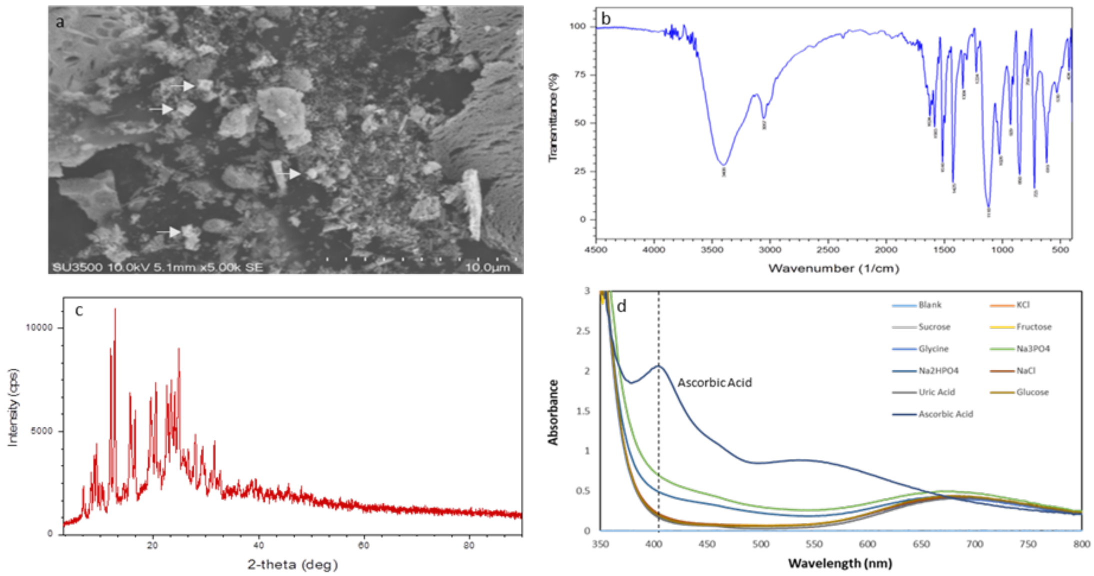

Figure 2. Results of the SEM Morphology (a), the FTIR (b), and XRD Spectra (c). The Spectrogram of UV-Vis spectrometer (d) and the Selectivity of MOFs Cu-Phen to Ascorbic Acid Appears at Wavelength of 410 nm

glycine, uric acid, Na2HPO4, Na3PO4, NaCl and KCl. Thus, we can use the Cu-Phen MOFs as colorimetric agents that were selective only to ascorbic acid.

Characterization was done using several instruments as shown in Figure 2. The morphology, structure, and size of Cu-Phen MOFs is shown in Figure 2(a). In the SEM image, Cu-Phen MOFs appear as a cubic morphology with size 400 nm. Crystals having a cubic structure can have an octahedral shape with eight equivalent facets, a cubic morphology produced by six equivalent facets, etc. Further confirmation of the crystal structure is shown by the XRD pattern in Figure 2(c). The peaks located at 14.7, 21.8, and 32.5 can be assigned to the (111), (200), and (220) planes of face-centered cubic of Cu-Phen MOFs. These results are in accordance with other published research (Zhang et al., 2019). The vibration stretching peaks using FTIR for C=N, C=C double bonds become visible at 1624 cm^{-1}, 1583 cm^{-1} for Cu-Phen MOFs (Figure 2(b)). The vibration peak of skeleton appears at 1516 cm^{-1}, and the vibration peak for bending of C-H bond shows at 723 cm^{-1}. The stretching vibration of Cu-N bond was shown on the band absorption at 424 cm^{-1} in the complex and exhibits that the nitrogen in the ligand participate in coordination (Wang et al., 2015). Figure 2(d) shows the UV-Visible spectra of the system. A new strong peak absorbance at 410 nm is produced by the mixture of Cu-Phen MOFs with ascorbic acid, while the mixture of Cu-Phen MOFs and other compounds (glucose, sucrose, fructose, glycine, uric acid, Na_2HPO_4, Na_3PO_4, NaCl, and KCl) produces a weak peak absorbance at 680 nm (and in the UV ranges) (Figure 1 and Figure 2). There is no peak at 410 nm for the other chemicals. These findings demonstrate the strong ascorbic acid selectivity of Cu-Phen MOFs, which opens up new applications for the colorimetric probe in ascorbic acid quantification. The color changes were likewise linearly proportional to the ascorbic acid content because the absorbance changes are within observable ranges (Firdaus et al., 2017). As a result, we can utilize the system with digital image colorimetry.

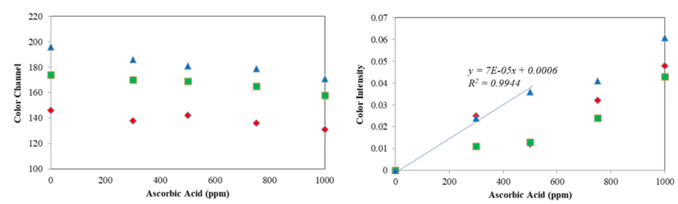

Figure 3. The Outcomes of Colorimetry Using Digital Images to Verify Sensitivity. The blue color intensity produced the highest linearity and slope. For this reason, we chose the color blue for all of the studies

3.2 Quantification of Ascorbic Acid

Next, the synthesized MOFs was used as a colorimetric probe for the detection of ascorbic acid. A standard calibration curve that was created prior to the analysis was used to quantify ascorbic acid in the samples. Table 1 displays the findings of digital image colorimetry with a smartphone acting as a detector. Using ImageJ software, the color channel (RGB) data were extracted from digital photos (Saputra and Firdaus, 2021). The Lambert-Beer equation was used to compute the color intensity, which was obtained from related color channels (Firdaus et al., 2014; Shen et al., 2012). The color intensities were then

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Table 1. Digital Image Colorimetry Employs RGB Color Channel

Ascorbic Acid (ppm)	Color Channel			Color Intensity		
	Red	Green	Blue	Red	Green	Blue
0	146	174	196	0	0	0
300	138	170	186	0.025	0.011	0.024
500	142	169	181	0.012	0.013	0.036
750	136	165	179	0.032	0.024	0.041
1000	131	158	171	0.048	0.043	0.061

Table 2. Comparison with Other Analytical Methods of Ascorbic Acid Analysis

Analytical method	LOD	Detection range	Reference
HPLC	0.1 ng	0.1 – 50 ng (/5 μL)	(Iwase, 2000)
HPLC - Electrochemical	90 nM	0.5 – 20 mM	(Gazdik et al., 2008)
LC-MS	10 μg/L	0.1 – 1 mg/L	(Frenich et al., 2005)
Voltammetry	0.087 mM	0.31 – 20 mM	(Pisoschi et al., 2011)
Fluorescence	500 nM	0.0001 - 0.1 mM	(Krishnan et al., 2016)
Spectrophotometry	0.08 μM	0.5 – 60 μM	(Zarei and Moghaddary, 2015)
Spectrometric - Smartphone	5 μg/mL	20 – 80 μg/mL	(Aguirre et al., 2019)
Digital Image Colorimetry	0.09 ppm	0 – 500 ppm	Present work

obtained by applying the Lambert-Beer rule to the RGB values and converting them to a logarithmic scale (Firdaus et al., 2014) with Equations (1-3) as follow:

$$I_R = \log\frac{R_0}{R_S} \quad (1)$$

$$I_G = \log\frac{G_0}{G_S} \quad (2)$$

$$I_B = \log\frac{B_0}{B_S} \quad (3)$$

The intensities of color for Red, Green, and Blue are written as I_R, I_G, and I_B, respectively. The letter of R_0R_S, G_0G_S, and B_0B_s are the numbers of color of RGB for blank and sample, respectively. The logarithmic conversion to RGB intensity is equivalent to the Vitamin C concentration. The resulted color intensity calibration curves were then used to calculate the concentration of Vitamin C in the samples.

As can be seen in Figure 3, the Blue color intensity had the maximum slope and linearity, with a coefficient of determination of R^2 = 0.9944. Between 0 and 500 ppm, the detection range that was obtained from the calibration curve was linear. Blue color intensity was thus employed in all of the studies. These findings indicate that the digital image colorimetry will use the reflected color of a sample's photograph. Unlike in spectrophotometry, there is no transmittance, absorbance, or complementary color theory. The detection limit (LOD) under the optimum conditions was 0.1 ppm, known as LOD = 3 s/m, where s is the standard deviation for 10 determination of the blank and m represents the slope of the calibration curve. The relative standard deviation (RSD) for 500 ppm ascorbic acid concentration replicate determination was 4.2% which shows a good precision of the developed method. These analytical performances are comparable to the other instrumental and modern analytical methods, as shown in Table 2.

Eventually, the ascorbic acid content of star fruit (*Averrhoa carambola* L.) was determined using the suggested procedure. The use of the method for real sample analysis was examined using the usual addition methodology (Firdaus et al., 2018). A satisfactory recovery yield of 109% was achieved. According to our research, each 100 grams of star fruit has 31 mg of ascorbic acid. These findings are consistent with earlier research (Muthu et al., 2016). Additionally, this number indicates that star fruit has one of the highest ascorbic acid contents in the fruit group, which is more than 41% w/w.

4. CONCLUSION

Through the use of a solvothermal technique, we have effectively synthesized Cu-Phen MOFs in this study. The MOFs are light blue, but when ascorbic acid is present in the samples, they turn turquoise green. The ascorbic acid sensing results obtained with the developed digital image colorimetry showed good agreement with other instrumental techniques including UV-visible spectrophotometry, LC-MS, voltammetry, and HPLC. When combined with a smartphone camera acting as the detector, this technology offers a low-cost, straightforward, accurate, precise, and portable way to measure ascorbic acid.

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