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Polyaniline-based cadaverine sensor through digital image colorimetry

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Abstract: The detection of biogenic amines is important due to their correlation with spoilage and toxicity. Polyaniline (PANI) is a polymer capable of changing color from blue to green and *vice versa* through acid-base reactions. This study tested the potential of PANI as a sensing material for the presence of cadaverine. PANI was fabricated on nylon cloths through the redox reaction of aniline and ammonium persulfate. The PANI-coated nylon cloths were exposed to different amounts of cadaverine in a fixed reaction time of 5 min. Through digital image colorimetry, plots of the different color values vs. concentration were prepared and the curve with the highest linearity was obtained using the yellow value. Values for limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 0.8816 mM and 2.9386 mM, respectively. An Android application which analyzes the color values of the PANI image in relation to the amount of cadaverine was also developed.

Keywords: Android application; biogenic amines; cadaverine; digital image colorimetry; polyaniline (PANI).

1 Introduction

Biogenic amines (BAs) are basic, nitrogenous compounds with low molecular weights that are formed through the decarboxylation of specific free amino acids. Histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine and agmatine are the most common BAs found in foods such as fish, meat, cheese, vegetables and wines (1). The presence of these

amines has been correlated with the amino acid composition, spoilage and maturation time of foods. Thus, BAs have been analyzed due to their potential toxicity and their potential use as a food quality indicator (2).

Cadaverine is a biogenic amine that is derived from the amino acid lysine (2). It has harmful toxicological effects when consumed in large amounts. The chemical structure of cadaverine, $\text{NH}_2(\text{CH}_2)_5\text{NH}_2$, is also very similar to that of putrescine, $\text{NH}_2(\text{CH}_2)_4\text{NH}_2$, which is also a biogenic amine that is observed during spoilage. However, cadaverine is the principal BA observed to increase in concentration prior to spoilage, particularly of meat products (3).

The food industry, such as that of the meat industry, currently uses sophisticated machines and equipment, including chromatography, spectrometry, electrophoresis, mass balance, chemiluminescence and electrochemistry, to determine the acceptability of meat. However, the costs of these analyses are high and results are not obtained quickly (4). By utilizing the current knowledge about cadaverine, along with other volatile biogenic amines, and its correlation to food spoilage, inexpensive and time efficient sensors can be synthesized chemically.

Polyaniline (PANI) is a feasible option as a sensing material for basic BAs as it is a mixed oxidation state polymer with three oxidation states (5, 6). Different oxidation states can be attained through protonation and deprotonation of PANI using acids and bases (7, 8). Leuco-emeraldine is the fully reduced oxidation state of polyaniline which appears as white or colorless. Emeraldine is the intermediate between the fully reduced and fully oxidized state of polyaniline and may appear in two colors – blue or green. Pernigraniline is the fully oxidized state and appears as bluish violet in color. Observation is made easier as the shift in oxidation of PANI within the emeraldine state is accompanied by color changes – green when protonated and blue when deprotonated. Emeraldine can be utilized for colorimetric sensors dealing with weak bases such as biogenic amines being the transitional oxidation state (9).

Digital imaging colorimetry (DIC) is an innovative type of colorimetry method that is inexpensive, and convenient. This technique involves two processes: image acquisition and color readout (10). The alterations in the color of polyaniline after exposure to cadaverine can be captured in still

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images and processed through DIC by utilizing compact digital cameras, computers and suitable graphical software to quantify and analyze surface colors (11, 12). The data output of DIC is provided by the image software eliminating the influence of the naked eye and greatly improving the accuracy of the detection. The image analysis of DIC is principally based on RGB (red, green, blue) space which decomposes any color into RGB values ranging from 0 to 255 to be read out by the software (13).

Android is an emerging operation system, with all the dramatic increase in mobile phone usage worldwide over the last several years, that is owned by Google. As its entry in the mobile market, Google launched the Android mobile platform in 2007 that is suitable for the hardware of mobile devices such as smartphones, computers and net books. Its open source nature and architectural model enables Android to gain the interest of the field and industry (14). Thus, usage of Android to analyze colors in images will further add to the efficiency and accesibility of sensing methods.

This study presents polyaniline as a viable sensing material for the presence of cadaverine. This can also be used to quantify the amount of the biogenic amine through the analysis of color changes using digital image colorimetry. The development of an Android application will also add to the ease of analyzing the color change of PANI. The capacity and sensitivity of PANI as a sensing material for cadaverine through the synthesis of polyaniline and the fabrication of the tentative sensing device in the derivation of a calibration curve were done. Likewise, this study serves to test the potential of PANI as a future sensor for biogenic amines similar to cadaverine, thus an initial step for an inexpensive and time efficient sensor spoilage in food products.

2 Methodology

2.1 Sensing material preparation

An aniline solution (Sigma-Aldrich) was prepared by dissolving enough amounts of the monomer in 0.1 M HCl to produce 0.1 M aniline solution. The solution was then sonicated for 15 min before 1 by 1 cm nylon cloths which were obtained from the local market in Angeles, Pampanga, Philippines were added in the solution. The cloths were left in the solution for 30 min to allow adsorption of the monomer on the surface of the fiber. Thirty milliliters of 0.2 M ammonium persulfate (Sigma-Aldrich) were then added dropwise into the mixture, without any mechanical agitation. The solution was then left to polymerize until a prominent metallic sheet was formed. The cloths were

collected and rinsed with 0.2 M HCl to avoid deprotonation of polyaniline (15). Acetone was also used, afterwards, to remove any leftover stains from the aniline solution (16). Prior to polymerization, the nylon cloths were washed with distilled water and soaked in acetone to degrease the material and eliminate any contaminant. The fabricated polyaniline-coated nylon cloths were analyzed through Fourier-transform infra red (FTIR) spectroscopy using the Agilent Cary 630 FTIR spectrometer to confirm the success of the polymerization of the aniline.

Aniline and ammonium persulfate were obtained from Sigma Aldrich and distributed by Chemline Scientific Corporation, Quezon City, Philippines. HCl and acetone were acquired from Yana Chemodities, Philippines. The FTIR used was from the Agilent Technologies, USA.

Nylon cloth was used due to its durability in comparison to other materials such as paper and plastic considering that the material undergoes several intensive processes including polymerization of aniline solution in acidic medium and exposure basic compound. This also adds to the possibility of reusing the material after usage by protonating it through an acid solution (15). Likewise, a high yield is expected from polymerization on fabrics due to the high surface area of the cloth (17).

2.2 Measurement procedure

The protonated PANI cloth can be converted to its emeraldine base (EB) form through deprotonation. This is done by subjecting the polymer to an alkaline solution (7). Cadaverine is an aliphatic alkaline compound that is capable of deprotonating polyaniline (18). Two hundred microliters of 0.5 M NaOH were added to different amounts of cadaverine hydrochloride from Sigma Aldrich and distributed by Chemline Scientific Corporation, Quezon City, Philippines in 10-ml vials with caps. The polymer was attached at the bottom side of the vial cap. Although no direct contact is done between the PANI cloth and cadaverine, deprotonation still occurred due to the nature of cadaverine to vaporize at room temperature (19) and this was observed in the color change that occurred. Likewise, the NaOH in the solution of cadaverine did not contribute to the deprotonation of PANI as the vapor pressure of NaOH at room temperature is only 1.82×10^{-21} mm Hg (20).

The DIC set-up follows the diagram in Figure 1. PANI cloths were placed inside the light box with dimensions of $5 \times 4 \times 6$ inches in length, width and height, respectively. A backlight was installed at the base of the lightbox. Pictures of the PANI cloths were taken using an Iphone SE 12-megapixel camera.

Figure 1: Diagram of the set-up for digital image colorimetry.

After capturing the images of the PANI cloths that were reacted with cadaverine, the color values of the images of PANI were analyzed using Adobe Photoshop CS6. A 101×101 pixel radius average color sampler in Photoshop was used to better analyze the color change in 10 sample points in the image. Then, an equation for the calibration curve for each color space was derived using Microsoft Excel by plotting the average percentage of the color component vs. $\ln$ of the concentration of cadaverine. The calibration curve that will produce the highest linearity was chosen to represent the concentration of cadaverine in terms of color change.

2.3 Statistical analyses

The change in color through the percentage of yellow values was analyzed using one-way analysis of variance (ANOVA) with a confidence interval (CI) of 95%. For multiple comparisons of the set-ups, Tukey's test was used with 95% CI.

2.4 Coding of the android application

Android Studio was utilized to produce an Android application that is able to automate the process of obtaining the concentration of cadaverine through digital image colorimetry. Due to the limitations of Android Studio and the Android platform itself, RGB color space and color components were used in the application. The calibration curve with respect to the mean red value was used due to it exhibiting the highest linearity with the $\ln(M)$ of cadaverine.

The code includes a method for importing the photo from an image gallery application with permission from the user, a cropping function to select the specific area of interest, and an algorithm that returns average values for each component of the RGB color space. The calculated

calibration curve was also incorporated into the code to calculate and return the value for the concentration of cadaverine.

2.5 Comparison of obtained concentrations

To verify the accuracy of the RGB color space in the detection of the concentration of cadaverine, a paired t-test was done using the GraphPad prism to analyze the results produced from the coded application and the known concentrations for each image. The photos taken of the sensor reacted with fixed concentrations and masses of cadaverine were subjected to analysis using the produced Android application and the RGB histogram function of ImageJ, which functions as the standard for acquiring the average color components of the picture. The results for the obtained concentration of cadaverine from the two methods were statistically compared using a paired t-test at $\alpha = 0.05$ in GraphPad Prism 7 to determine significant differences which evaluates the capacity and accuracy of the application as a more accessible replacement of a desktop application.

3 Results and discussion

3.1 Characterization of PANI

The nylon cloths were observed to change in color from plain white to blue after the polymerization process and then to a green color after protonation through washing with hydrochloric acid. The change in color of the nylon cloth is attributed to the polymerization of PANI on the surface of the cloth. The initial fabricated PANI was in its non-protonated and non-conductive form or EB which is denoted by the blue color of the cloth. After washing with hydrochloric acid, a color change from blue to green is observed in the PANI indicating protonation and conversion of the PANI to its conductive form or emeraldine salt (ES) (21, 22). Due to the acidic nature of hydrochloric acid, it was able to protonate the PANI.

The FTIR spectrum of the fabricated PANI as seen in Figure 2 showed significant peaks at around 1550, 1465, 1290, 1232, 1120, 1025, 864, 789 and 690 cm^{-1} . The functional groups of the identified significant peaks in the FTIR spectrum of PANI-ES denoted the stretching of quinoid ring ($\text{N}=\text{Q}=\text{N}$) at 1550 cm^{-1} , stretching of benzene ring at 1465 cm^{-1} , and N-H out-of-plane bending at 789 cm^{-1} which are structures evident in PANI-ES shown in Figure 2. Other

Figure 2: FTIR spectrum of fabricated PANI (ES) with resolution of 4 cm^{-1} , 400 scans, and structure of PANI-ES (red for the quinoid ring, blue for the benzene rings, and green for the N-H out of plane bending).

peaks at 1290 , 864 , 690 , and 1232 , 1120 , 1025 cm^{-1} signifies the presence of C-N in secondary amines, C-H out-of-plane bending of 1,2,4-ring, C-H out-of-plane bending of 1,2-ring, and C-H in plane bending of 1,4-ring, respectively. The chemical structures and functional groups of the peaks were identified in line with the findings of Molina et al. (15).

3.2 Analysis of the digital image

The typical histogram of the images captured are presented in Figure 3. The histograms show a normal image with high white component seen as the spike in the right side of the histogram. This refers to the white spaces in the background of the PANI cloths. The images were converted from the usual RGB color scheme to the CMYK color scheme to better quantify the yellow value which is an important color that relates green and blue color in the CMYK model. CMYK is a subtractive color model which indicates that addition of color X to Y subtracts the effect

of color Y, creating a new color Z. Thus, in the case of PANI cloth A which is observed to be green in color, the yellow value is assumed to be high and is able to mask the color blue. However, in the case of PANI cloth B which is observed to be blue in color, the yellow value is assumed to be low and is not able to mask the color blue and create the color green (23). In addition to the CMYK color space, the RGB color space was also analyzed for more comparison and range of color spaces which may show a trend. Point sampling was done in order to exclude the other values that are not within the area of interest in the image such as the white spaces. Multiple points were also chosen for better accuracy of the analysis of the color values.

3.3 Optimization of the reaction time between polyaniline and cadaverine

The optimal amount of time for a reaction to occur between polyaniline and cadaverine was tested. The fabricated PANI cloths were exposed to 1 M concentration of cadaverine solution with $200\text{ }\mu\text{l}$ of 0.5 M sodium hydroxide for different times: 10, 5, and 3 min. Both PANI cloths exposed at times 10 and 5 min showed a clear and noticeable color change from green to blue while PANI cloths exposed for 3 min did not show clear color changes. Tukey's multiple comparison test showed that there was no significant difference in the color change of the PANI cloth exposed for 10 and 5 min. For subsequent testing, 5 min exposure time was employed for faster analysis.

3.4 Calibration of polyaniline with cadaverine

For the preparation of the solutions for the calibration of PANI with cadaverine, the following concentrations of

Figure 3: Image of PANI cloth A (unexposed to cadaverine) and PANI cloth B (exposed to 1 M of cadaverine for 5 min) with the CMYK histogram from Adobe Photoshop CS6.

cadaverine were used: 0, 30, 45, 90, 150, 300, 500, 700 and 1000 mM.

To measure the response of the PANI cloth to cadaverine, PANI cloths were exposed to different amount of the amine. The graph of the CMYK and RGB color values vs. the amount of cadaverine created a decreasing logarithmic trend. A calibration curve was then constructed by applying $\ln$ to the amounts of cadaverine in order to attain a linear trend shown in Figures 4 and 5.

The calibration curves for the red and yellow color spaces had a relatively higher linearity with the yellow color space producing the most linear trend with an R^2 value of 0.9989. The decreasing trend in both the calibration and the logarithmic curve of the yellow color supports the lowering of the subtractive effect of yellow to the blue color as discussed beforehand. While the decreasing trend in the red color made way for its blueish green complementary color to be more noticeable. Between the two color spaces, the red color space with the equation $y = -9.4025x + 110.48$ produced a calibration curve with a higher absolute value for the slope which indicates its higher sensitivity. As the color value y is assumed to be

linearly related to the concentration of cadaverine x , the limit of detection (LOD) and limit of quantification (LOQ) were computed based on the equation of the red calibration curve using equations [1] and [2] as described by Shrivastava and Gupta (24).

$$\text{LOD} = \frac{\pm 3S_a}{b} \quad [1]$$

$$\text{LOQ} = \frac{\pm 10S_a}{b} \quad [2]$$

S_a is the standard deviation of the color value, and b is the slope of the calibration curve. The standard deviation S_a of the y -residuals of the yellow calibration curve was computed through the $Sy \cdot x$ function in GraphPad Prism 7. This gave the value of 2.763. The slope b of the red calibration curve is -9.4025 . However, as the slope is negative, due to the decreasing trend, the factors used were also negative in order to attain a positive LOD and LOQ. The final LOD is found to be 0.8816 mM while the final LOQ is 2.9386 mM. Given these values, it is observed that PANI has a relatively low detection limit despite having a simple detection mechanism. It is comparable to other techniques that also employs DIC such as that for tetracycline detection which obtained an LOD and LOQ of 1.125 mM and 3.375 mM, respectively (11). This also indicates that PANI may be used as a possible meat spoilage sensor given that meat spoilage occurs when the amount of cadaverine increases past 50 mg/kg (25).

Statistical analysis through one-way ANOVA and Tukey's analysis showed that there are significant changes between the individual points in the red and yellow calibration curve. Further analysis of the points in the curves indicates that in each of the points there was a significant change in color as the cadaverine increased. Thus, PANI showed sensitivity to changes in the amount of cadaverine. Using Tukey's statistical analysis, it was shown that there were significant change in red color between all possible pairs of data. This further supports the higher sensitivity of the red color space for the change in color as compared to the yellow color space which showed no significant change in color between 500 and 700 mM, and 700 and 1000 mM, but showed a significant change in color between 500 and 1000 mM.

Using the Brown-Forsythe test, it was known that there were no significant differences between the standard deviations of each trial per set-up. This means that the use of digital image colorimetry to identify the change in the color value of the cloth produced a constant reading.

Figure 4: Calibration curve of cadaverine for the CMYK color space.

Figure 5: Calibration curves of cadaverine for the RGB color space.

Figure 6: Screenshots of Android application interface.

3.5 Android application suitability

Screenshots of the Android application developed is seen in Figure 6. Results from the paired t-test with 95% CI validate the accuracy and capability of the Android application in analyzing surface colors. The statistical analysis of data in GraphPad Prism 7 produced a p value of 0.0555 which translates as having no significant difference between the obtained cadaverine concentration from ImageJ histogram function and the Android application. Despite the proven capacity of the Android application, the existing versions of Android does not support photos to be taken with embedded CMYK ICC Profile. Due to this limitation, the examined photos are in the RGB color space. Moreover, Android Studio does not support the color longs for the CMYK color space to store the four color components in the said color model as data. This omits the use of the CMYK color model in the Android application. Instead, RGB color space was used in the application. Specifically, the red color space was used given its relatively high R^2 of 0.9653 in the calibration curve. The paired t-test verifies the validity of the use of RGB color space in the application as no significant difference was found in comparison to the known concentrations of cadaverine. The test resulted to a two-tailed p value of 0.1312 at $\alpha=0.05$ with 95% CI. However, there were no observable trends in the value that the application outputs with respect to the known concentrations. This inconclusiveness can be accounted for by the white spots present in the fabric due to the backlighting that skew the result as the application analyzes the color components of the image per pixel.

4 Conclusions

In this research, PANI was utilized as a material for sensing vaporized cadaverine. Measurement of cadaverine relied on the color change of PANI cloth from green to blue. Images of the PANI cloth captured using a smartphone were analyzed using digital image colorimetry and the role of the red color space in the relation between the change in color with the concentration of cadaverine was identified. PANI was able to detect cadaverine at an optimal time of 5 min. The calibration curve using the yellow value showed the highest linearity while the red calibration curve showed a high linearity and sensitivity. The equation of the red calibration curve was used to compute for the LOD and LOQ which were found to be 0.8816 and 2.9386 mM, respectively. PANI also demonstrated higher sensitivity in detecting cadaverine through the red color space. The coded Android application was proven to be able to detect the concentration of cadaverine that reacted with the sensor through digital image colorimetry in an automated process by utilizing the obtained calibration curve using the red color space.

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