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Development of a Method through Digital Image Analysis for Analyzing Polyaniline Based Sensors for Total Volatile Basic Nitrogen (TVBN) as Fish Freshness Indicators

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Food spoilage is a prevailing issue to society. This study addresses it by developing a freshness indicator using polyaniline through digital image analysis that monitors the condition of fish, which release amine vapors upon spoilage. The Polyaniline film prepared is analyzed through its digital image captured by a smartphone camera. The images' RGB values were measured through the use of ImageJ Software. The RGB values are dependent on the film's color, which reflects the effect of trimethylamine exposure. This study includes film preparation, analysis of the film's RGB values upon exposure to trimethylamine standard and headspace of a real sample, and the results' comparison with the UV-Vis Spectrophotometry's. Results are: Blue color value signal stability: 1.72% RSD, Repeatability test RSD: 35.68%, Blue value LOD: 0.01384M, Blue color value sensitivity: 248.87 color value change/molar concentration with linear coefficient of R^2=0.97391. Spectrophotometry's results were more accurate as expected. Nevertheless, determination through RGB values produced acceptable results. However, data acquisition for the ImageJ software application, the configuration of the optical system, and data treatment can still be improved. The method showed promising results for detecting volatile nitrogen samples such as amines in fish samples as they begin to spoil, using a smart phone.

Keywords: sensor, polyaniline, fish, digital image analysis, spoilage, trimethylamine

INTRODUCTION

During spoilage and autolysis of fish proteins, peptides and free amino acids are produced which alters the quality of fish. Trimethylamine N-oxide (TMA-O), protein found in fish, decomposes into trimethylamine (TMA). Its degree of spoilage can therefore be identified by determining the amount of TMA in the headspace of the fish (Connell 2006).

Polyaniline (PANI) is a reliable sensor in determining fish quality because of its sensitivity to TVB-N (Dhaouadi et al. 2007). This creates color response standard for fish freshness that is comprehensible for average consumers such that they can directly identify fish freshness from the sensor color (Kuswandi et al. 2012).

Digital Image Analysis uses RGB values that vary from one device to another (Schneider et al. 2012). This can be utilized to analyze the response of polyaniline film to trimethylamine. Hence, the study uses only one cellular phone in obtaining the changes of RGB values. To address these, a standard procedure for the analysis is established by identifying the necessary specifications of the camera and the positioning of the set-up using a given sample of fish.

The objectives of the study are: to develop a PANI-film sensor to evaluate the condition of blue marlin fish samples through visible color change upon exposure to TVBN; to determine the reliability of using digital image analysis over UV-Vis spectrophotometry which is used as reference.

EXPERIMENT

Materials

The Blue Marlin Fish sample was brought from the local market. Aniline, ammonium persulfate, TMA-HCl and NaOH purchased from Sigma-Aldrich (USA). Clear acetate sheets were bought from a local stationary store.

Preparation of Polyaniline-coated Films

The same concentration of 0.2 M aniline and ammonium persulfate solutions were prepared in 1.0 M hydrochloric acid and sonicated with acetate films for 15 minutes. After polymerization, excess polyaniline was removed by washing the films with 0.01M hydrochloric acid and stored in light tight containers. Readings were done with the film inside a plastic cuvette with Teflon lid.

Experimental Procedure for Using UV-Vis Spectrophotometer

The working wavelength was determined by scanning the blank film and the film exposed to TMA in the visible region in a lambda 25 Perkin Elmer UV-Vis spectrophotometer. The spectra was analysed to determine the wavelength at which their difference is highest

Signal stability test was done by reading the absorbance of the blank film at the working wavelength. The relative standard deviation, RSD was determined. Repeatability test was done by reading alternately the absorbance of the blank film and the same film exposed to 0.5M trimethylamine pipetted in a small cotton ball on the lid of the cuvette.

Sensitivity test was done by film exposure alternately as a blank and in a cuvette containing trimethylamine with concentrations 0.11, 0.088, 0.0704, 0.0563, 0.0450, and 0.03604 M prepared by serial dilution.

Experimental Procedure Using Digital Image Analysis

For the signal stability of the digital image analysis, a photo of the unexposed film was taken for 45 min at 20 second intervals. The RSD for the red, green and blue color values were determined.

Repeatability, response time and sensitivity test was determined in a similar way with the UV-Vis. A video of the film's response to the addition of trimethylamine was recorded. The change in the red, green and blue color values of the film was reflected in the plot and the response time was determined. The RSD was determined for the alternate reading of the blank and the exposed film.

The RGB color values of each image are analyzed using an image editing software, ImageJ.

Real Sample

Three blank films in cuvettes samples were configured to the setup separately and an image of the film was captured for 1 minute. A sample of the blue marlin fish was cut and fitted into the cap of the cuvette. The cuvettes are then sealed and again, configured into the setup. An image of the film with the fish inside the cuvette is taken for 10 minutes.

RESULTS AND DISCUSSION

The absorbance spectra of the blank and exposed polyaniline (PANI) films were plotted in Figure 1. The difference between the plotted absorbance of the exposed and unexposed PANI film were calculated to identify the wavelength where absorbance was at a maximum. The largest difference was observed at 589 nm.

The change of absorbance can be attributed to the different forms of polyaniline depending on the pH of the environment. Upon exposure to bases such as ammonia, the polyaniline chains are oxidized and deprotonated, losing hydrogen atoms among the nitrogen group. This will result to change its form from the green-colored Emeraldine form to the blue-colored Pernigraniline form. As its structure changes, so will its spectral properties. And this results to its change in color, as well as its absorbance value (Cao et al. 2005).

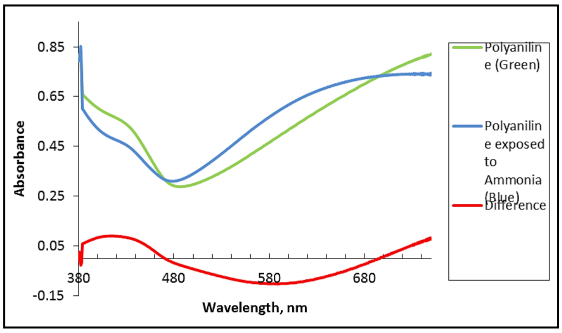


Fig. 1: Spectra of polyaniline in blank cell and in basic medium (in NH_3)

UV-Vis Spectrophotometry Film Figures of Merit

The film did not manifest significant changes in its absorbance throughout the recording and a relative standard deviation of 0.13% was calculated. This shows a fairly stable signal.

The response of the film to 0.5M of Trimethylamine (TMA) exhibited a significant increase in absorbance 685 seconds after exposure to the TMA solution. (t_{∞}= 685 s). For a more practical measure of response time, a 95% change in absorbance is used instead. This has a response time of 294 seconds upon exposure to the sample (t_{95} = 294 s).

In Figure 2, analysis of the polyaniline film using UV-VIS Spectrophotometer was used as a reference to observe using Digital Image Analysis. Higher absorbance for films exposed to trimethylamine and lower absorbance for unexposed films was observed. The RSD value is 8%. The low repeatability could be caused by the abrasions accumulating in the film as it is used in experiments for multiple times.

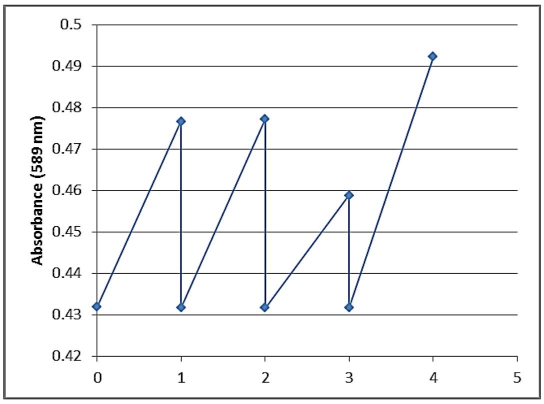


Fig. 2: Repeatability test using UV-Vis Spectrophotometer

The results of the sensitivity test illustrated a direct relationship between concentration and absorbance. However, the linearity was not high with an R^2 of 0.87 as shown in Figure 3.

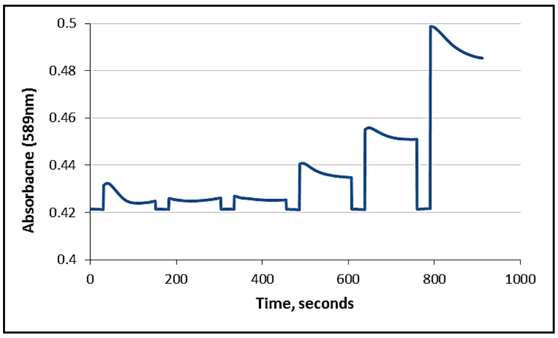


Fig. 3: Sensitivity test using UV-Vis Spectrophotometer

The lower limit of detection was calculated using Eq. 1.

$$Lower\ limit\ of\ detection = \frac{3\ x\ SD}{slope} \quad (1)$$

"SD" is the standard deviation from the stability test. Slope is from the linear regression of the sensitivity test. The lower and upper limit of detection acquired for the UV-Vis Spectrophotometer was 0.0025 and 0.0167 M/change in absorbance respectively.

Digital Image Analysis

The corresponding RGB vs time as shown in Figure 4 gave an RSD of 2.27% for Red, 1.35% for Green and 1.72% for blue, resulting to a fairly stable signal.

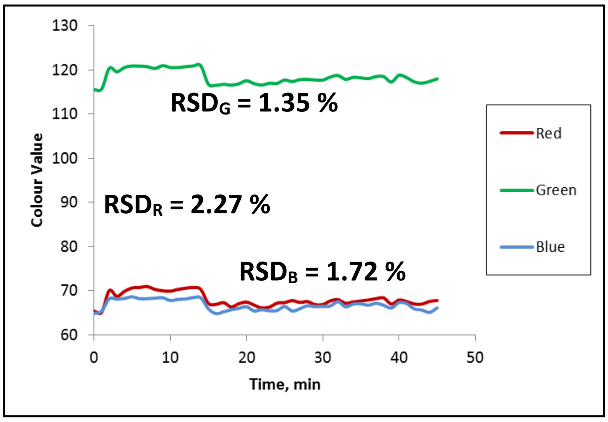


Fig. 4: Stability of the film using Digital Image Analysis

The response time was at 36 seconds. Inside the cuvette, the trimethylamine molecules are left to saturate the headspace gas on their own. Hence, a longer response time is expected.

Repeatability test was done to the polyaniline film sensor using digital image. In comparison with the plot for the UV-Vis spectrophotometer, a similar trend was observed for the color values. Lower RGB values for unexposed film and higher RGB values for films exposed to trimethylamine. The combined RGB values of the unexposed film showed "green" as having the highest color value. The exposed film, on the other hand, has higher RGB values than the unexposed film's values. The calculated RSD values for red, green, and blue are 13.51717, 18.45889, and 35.67783 respectively showed lesser repeatability of the image, however, a similar trend was observed. Sensitivity analysis showed that the color value was directly proportional to the TMA concentration to which it was exposed shown in Figure 5. For the blue color value, the data showed better linearity with an R^2 of 0.91 as shown in Figure 5.

Limit of detection for the Digital Image Analysis was 0.014 M for the blue color value. The Blue color value, while having the most reliable trend line, also showed the lowest limit of detection among the three color values showing the reliability of using the blue color value. The limit of detection was 0.0933 M.

Real Sample

Three trials of real fish samples were used in the real sample test as shown in Figure 6, the concentration of volatile basic gases in the fish sample was 0.0733 M. This was above the limit of detection which was 0.014 M. Therefore, the concentration of TMA in the given fish sample is way before the spoiled stage.

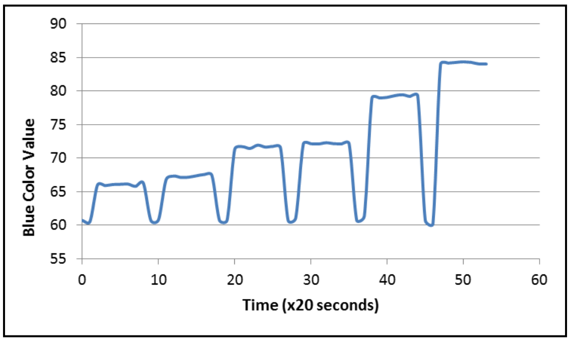


Fig. 5: Sensitivity test for blue color values

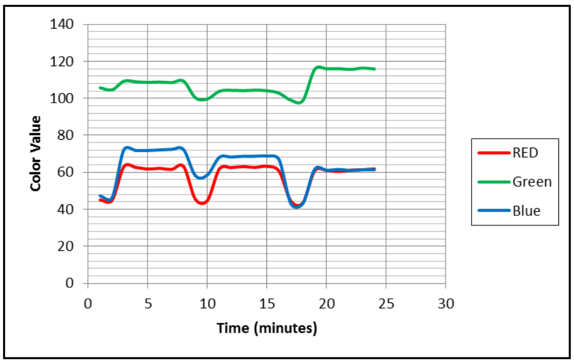
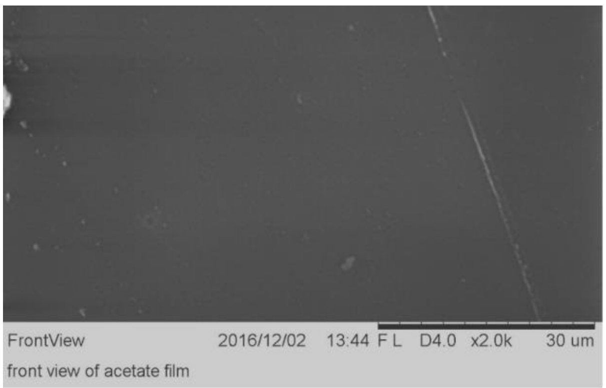
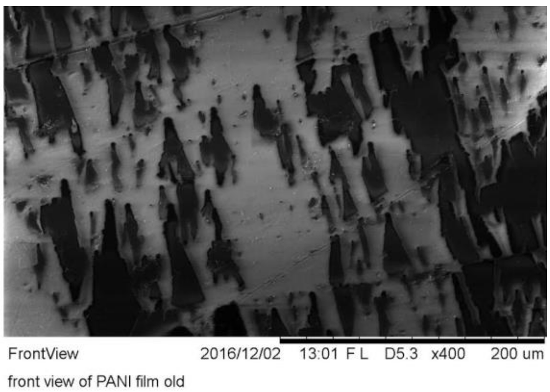


Fig. 6: Image readings for real sample



a. Unexposed film



b. Exposed film

Fig. 7: SEM images of front polyaniline-coated film

Morphology

An exposed and unexposed polyaniline-coated acetate film, was analyzed using a scanning electron microscope. The SEM images for the unexposed polyaniline film as shown in Figures 7a show a uniform surface. This indicates that on the surface level, the polyaniline had been coated evenly throughout the surface. For the exposed polyaniline film, as shown in Figure 7b, the surfaces show abrasions, which may have caused a poor repeatability.

Summary of Results

Table 1. Results Using the Two Methods

Figures of Merit	Parameter	UV-Vis	RGB
Stability	RSD	0.13%	1.72%
Response Time	Time (seconds)	294	36
Repeatability	RSD	8%	35.68%
Sensitivity	Coefficient of Det.	0.8683	0.9796
Limit of Detection	Molar Concentration (M)	0.0025	0.014

CONCLUSION AND RECOMMENDATIONS

A working polyaniline-coated acetate film sensor was developed. Analysis using the two methods showed that the film manifests a predictable color change when exposed to trimethylamine thus; the film can be used as a sensor for fish freshness.

Digital Image Analysis method was shown to be an alternative method to the UV-Vis Spectrophotometer in the analysis of the polyaniline film sensor because of the stability, response time, limit of detection and sensitivity. However, further studies are necessary for a more repeatable system.

Using the ImageJ software, the images were interpreted and blue color became the most reliable value among the three. A light tight box can improve the repeatability of the digital image analysis. Other models such as HSV can be also used. Other softwares may come in handy.

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