ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal
www.orientjchem.org
Est. 1984
ISSN: 0970-020 X
CODEN: OJCHEG
2014, Vol. 30, No. (2):
Pg. 445-449

Chlorophyll as a Simple, Inexpensive and Environment-friendly Colorimetric Indicator for NO_2 Gas

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http://dx.doi.org/10.13005/ojc/300206

(Received: April 15, 2014; Accepted: May 20, 2014)

ABSTRACT

Chlorophyll is utilized as a simple, inexpensive and environment-friendly ("green") colorimetric indicator for nitrogen dioxide (NO_2) gas. A drastic color change from green to yellow was observed when chlorophyll, either dissolved in CH_2Cl_2 solution or absorbed into paper, was exposed to NO_2 gas. Other gases such as CO_2 and SO_2 did not exhibit any color change with chlorophyll. Spectroscopic analysis showed nitration of chlorophyll as possible cause for the color change.

Key words: Chlorophyll, Colorimetric, Indicator, Sensing, Nitrogen dioxide, Test strips.

INTRODUCTION

Nitrogen dioxide (NO_2) is one of the prevalent oxide species of nitrogen in the atmosphere collectively known as NO_x. It is characterized by a reddish-brown color with a pungent irritating odor. This pollutant gas is produced from combustion of fossil fuels and industrial processes. It is also a component of photochemical smog and responsible for production of acid rain. Its accumulation poses a threat to the environment because NO_2 is highly reactive, corrosive and oxidative, causing discoloration and degradation of plant species.[1] Its presence in the troposphere can also lead to various health issues. At low concentrations, NO_2 can induce cough, nasal and eye irritations. At higher concentrations, it can lead to serious respiratory infections.[2] These environmental and health concerns due to extensive circulation of NO_2/NO_x in the atmosphere necessitates development of novel approaches for its detection and monitoring.

In the last decade, many studies have been devoted to selectively monitor the levels of NO_2 in the atmosphere. These include metallic oxides,[3–6] organic semiconductors,[7–9] metal complexes,[10,11] and cage-like molecules.[12–17] These approaches however require expensive materials and lengthy procedures for its synthesis. Here, we report a very simple and cheap approach towards sensing of

NO_2 gas by using a common pigment naturally occurring in plants: chlorophyll.

Chlorophyll is a metalloporphyrin pigment responsible for the green color of plants. It plays a major role in photosynthesis. However, chlorophyll is greatly affected by pollutant gases. Chapados and co-workers have observed that chlorophyll reacts with gases such as SO_2, H_2S, NO and NO_2,[18] while Guidi et al noted that it can interact with ozone.[19] Bevilaqua and co-workers also suggested that chlorophyll can potentially bind CO_2 gas based on density functional calculations.[20] These studies provide possibilities for chlorophyll as gas sensor. However, application of this natural pigment for naked-eye detection of pollutant gases has not been explored.

EXPERIMENTAL

All reagents and chemicals used were analytical grade and purchased from commercial sources. Solvents were used without any purification.

Extraction of chlorophyll

100 g of cogon grass (*Imperata cylindrica*) was grinded and macerated for 3-5 min. in ethyl acetate. The crude sample was filtered, placed in a shaker for 5 min then left overnight at room temperature. The extract was rotavaped to a final volume of 200 mL and carefully decanted into an evaporated dish to exclude any undissolved matter. The extract was then heated to dryness in a water bath at 40°C. The residue was purified using column chromatography (30 g of silica gel with ethyl acetate). The first eluate which was yellow in color was discarded. Methanol was then used to elute the purified extract. The eluate was air dried, resulting to a dark green solid product (0.0395 g). The green product was dissolved with CH_2Cl_2 and used throughout the experiment.

Production of gases

Sulfur dioxide (SO_2) was prepared by adding 10 drops of 2 M HCl to 0.1 g $NaHSO_3$ in a test tube. Nitrogen dioxide (NO_2) was prepared by reacting a small piece of copper metal with concentrated HNO_3. Carbon dioxide (CO_2) was produced by reacting 0.1 g $CaCO_3$ with 10 drops of 5M HCl. The gases were introduced into 3.0 mL chlorophyll solutions via a 5-mL syringe.

Titration of chlorophyll solution

NO_2-CH_2Cl_2 solution was prepared by carefully bubbling NO_2 gas to dry CH_2Cl_2. Concentration of NO_2 was determined according to published procedure.[21] 0-900 mL of NO_2-CH_2Cl_2 solution was used in the titration.

Gas detection using test strips

Filter paper strips (5 cm x 1.5 cm) were soaked in a 5-ml chlorophyll-CH_2Cl_2 solution until dark green strips were formed. Strips were air dried, transferred in a vial and covered. Gas was introduced into each vial using a syringe.

Spectral analysis

Spectral scans of untreated and gas-treated chlorophyll solutions in CH_2Cl_2 were recorded on a Shimadzu 1700 spectrometer from 300-800 nm. Infrared spectra were recorded on a KBr pellet and analyzed using a Perkin-Elmer 1600 series Fourier Transform Spectrophotometer.

RESULTS AND DISCUSSION

Chlorophyll, a green pigment in plants, was investigated for its potential use as colorimetric indicator for NO_2. Chlorophyll was extracted from cogon grass using ethyl acetate, purified using column chromatography, and then dried. The CH_2Cl_2 solution of chlorophyll was placed in a sealed vial and bubbled with NO_2 gas. This resulted to a drastic color change of the chlorophyll solution from green to yellowish-brown (Fig. 1). Other gases such as CO_2 and SO_2 did not exhibit any color change. Previous studies have shown that CO_2 has no effect on the structure of chlorophyll, while SO_2 gas does not react with chlorophyll in the absence of water.[18,22]

Visible spectra of chlorophyll exposed to NO_2 show prominent bands that are distinct from the bands observed in chlorophyll alone (Fig. 2A). Absorption bands with λ_{max} at 414 nm and 666 nm shifted to 424 nm and 679 nm, respectively. The band around 470-480 nm also disappeared upon addition of NO_2. These spectral changes are consistent with a study conducted by Chapados et

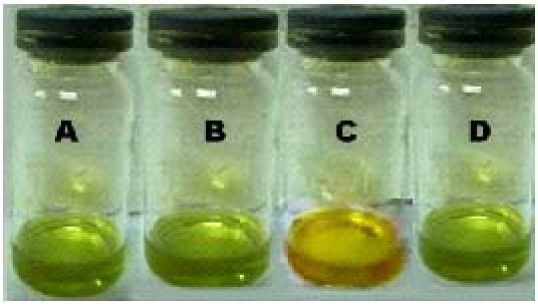

Fig. 1: Visual detection of gas samples in solution. A) chlorophyll solution in CH_2Cl_2, B) chlorophyll solution after exposure to CO_2, C) chlorophyll solution after exposure to NO_2, D) chlorophyll solution after exposure to SO_2

al. showing the effect of NO_2 gas on chlorophyll *a* multilayer.[18]

The same spectral change was recorded when chlorophyll was titrated slowly with NO_2-CH_2Cl_2 solution (Fig. 2B). It was also noted that the chlorophyll solution was able to detect NO_2 with concentrations greater than 2 mM in CH_2Cl_2 solution (Fig. 2B insert). Similar titration experiments have been conducted by Rudkevich et al. on calixarenes with NO_2-CH_2Cl_2 solution to estimate limit of detection for NO_2.[16]

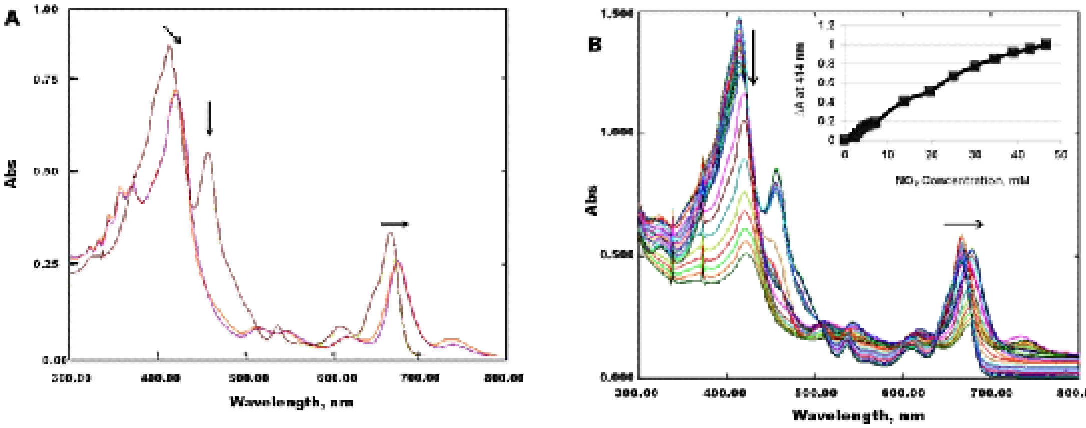

Fig. 2(A): UV-Vis spectra of chlorophyll bubbled with NO_2 gas. (B) UV-Vis titration of chlorophyll with NO_2-CH_2Cl_2 solution. Insert: Plot of change in absorbance at 414 nm versus NO_2 concentration

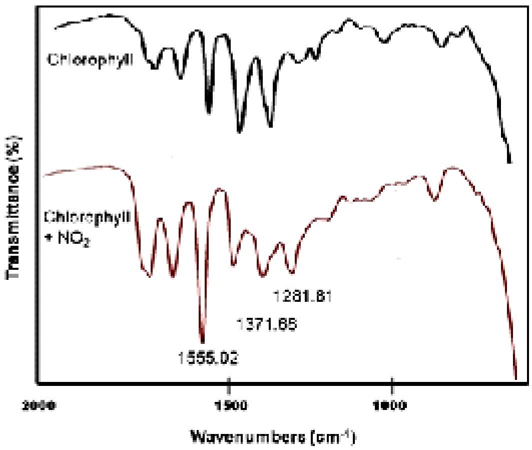

Fig. 3: Portion of FTIR spectra of chlorophyll before and after NO_2 exposure

Fig. 4: Gas detection using test strips. A) Test strip with chlorophyll alone, B) Test strip after exposure to CO_2, C) Test strip after exposure to NO_2, D) Test strip after exposure SO_2

Additionally, bubbling oxygen gas into the NO_2-treated chlorophyll solution did not change the spectra. This suggests that there is a strong interaction or irreversible reaction that took place between chlorophyll and NO_2 gas. Thin layer chromatography of the product formed from the reaction of chlorophyll and NO_2 suggests a complete transformation of chlorophyll into a new compound, possibly a nitrated product. This was confirmed by FTIR spectroscopy where distinct bands were detected at 1555 cm^{-1} and 1372 cm^{-1} characteristic of an $-NO_2$ group (Fig. 3). Another band at 1282 cm^{-1} which was not present at the spectrum of chlorophyll solution may be due to formation of an N-NO_2 group.[23] This result suggests that nitration occurred when chlorophyll was exposed to NO_2. Nitration reactions are known to occur in porphyrin complexes caused by NO_2 gas.[24]

Interaction between gases and chlorophyll was also studied in solid state using test strips. Figure 4 shows green coloration of filter paper after soaking it in chlorophyll solution. When the dried test strip was exposed to NO_2, the color turned from green to yellow-brown, similar to the color change observed in solution. No color change was noted when test strips were exposed to CO_2 and SO_2. This observation clearly indicates the selectivity of chlorophyll towards NO_2 gas. The study also demonstrates as proof-of-principle the potential use of chlorophyll-based sensors for naked-eye detection of NO_2 gas.

CONCLUSION

In conclusion, a simple and cheap colorimetric indicator for NO_2 gas based on chlorophyll was developed. This pigment can be obtained easily from plant materials and be used either in solution or as test strips.

Moreover, the method presented here is inexpensive since gases can be produced from common laboratory reagents, and the set-up requires minimal use of equipment. Thus, this method can be performed in the classroom to demonstrate not only the sensitivity of chlorophyll towards NO_2 but also the destructive effect of NO_2 gas to chlorophyll pigment of plants. Further work will be directed towards improving the detection limit and applying it as NO_2 sensor for environmental monitoring.

ACKNOWLEDGMENTS

Financial support was provided by the Creative Work and Research Grant (CWRG Grant No. 2012-25) of the University of the Philippines System.

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