Rapid, Visual Detection of Illicit Substances in a Variety of Environments Via Competitive, Amine-Responsive Fluorophores

Introduction

Drug facilitated sexual assault is a growing issue, especially among young adults and despite the most common date-rape drugs, such as Rophonyl and GHB, being outlawed in the US, as an over the counter alternative, many assaulters are turning to Diphenhydramine, commonly known as Benadryl. When mixed with alcoholic beverages, coined a Benadryl cocktail, the victim can experience drowsiness, blackouts, and amnesia and while date-rape detection kits exist today, none that I've found have tested for DPH and many have been pulled off the market for producing inconclusive results. The research I conducted attempts to protect victims against such acts with a simple, rapid sensor that can detect 250mg+ of DPH in a variety of alcoholic beverages. The same diagnostic system is applicable for a variety of amines including pyrrole – a compound found in marijuana for the detection of unsafe drivers under the influence of cannabis.

Synthesis of Dyes

Fluorescein Isothiocyanate (FITC) and Protoporphyrin IX (PPIX) were purchased from Sigma Aldrich and independently synthesized. Whereas FITC was used as purchased, the protoporphyrin dye (fig. 2) was synthesized with cellulose acetate then dissolved in DMSO and activated with toluene. The newly-prepared CA-PPIX now possesses a paper-friendly structure, and is ideal for placement onto a filter-paper sensor substrate (fig. 2).

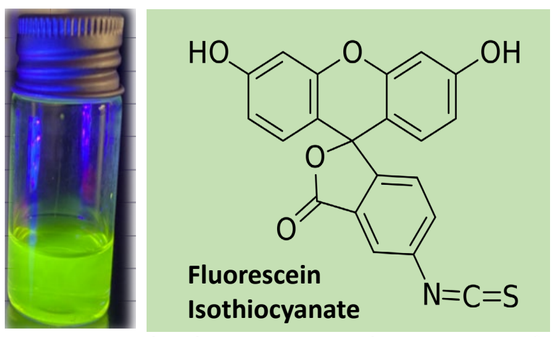


Figure 1: molecular structure and appearance of FITC in ethanol when illuminated with a 405nm excitation light

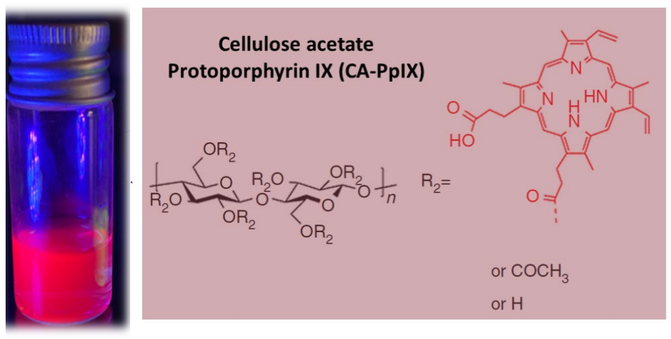


Figure 2: molecular structure and appearance of CA-PPIX in ethanol when illuminated with a 405nm excitation light

Combination of Dyes and Optimal Excitation

A 3-dimensional emission scan of a 1:1 mass ratio mixture of CA-PPIX and FITC diluted in ethanol was collected. Then, a 3D scan was taken to find overlapping excitation wavelengths between the dyes as highlighted in red (fig. 3). After this examination, various excitation wavelengths were tested to determine the optimal excitations for equivalent, balanced illumination of each dye. It was found that 410 nm provided the optimal emission of the two-dye system (fig. 4).

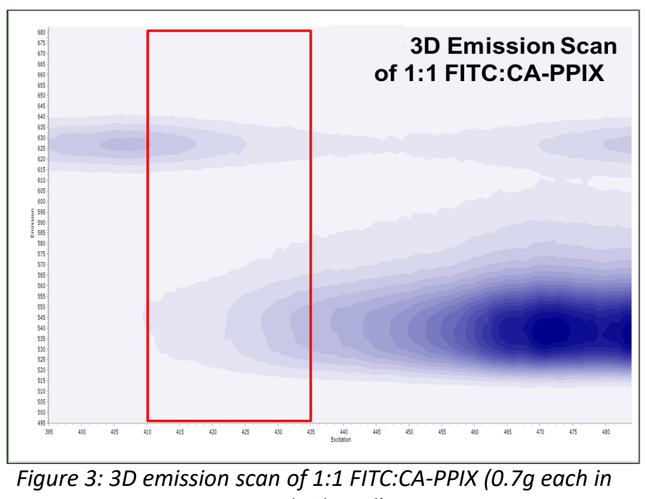


Figure 3: 3D emission scan of 1:1 FITC:CA-PPIX (0.7g each in 10ml Ethanol)

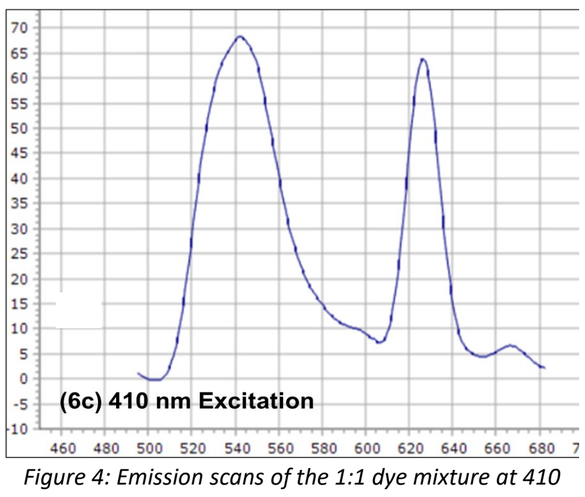


Figure 4: Emission scans of the 1:1 dye mixture at 410 nm shows optimal wavelength for balanced illumination of each dye

Selective Conjugation of DPH

The isothiocyanate group within FITC reacts with amino terminals and primary amines in proteins to form a conjugate. Given this, DPH-FITC conjugation would quench, or quiet the green fluorescence, which forms the basis for the sensor's color selectivity for the date rape drug (fig. 7).

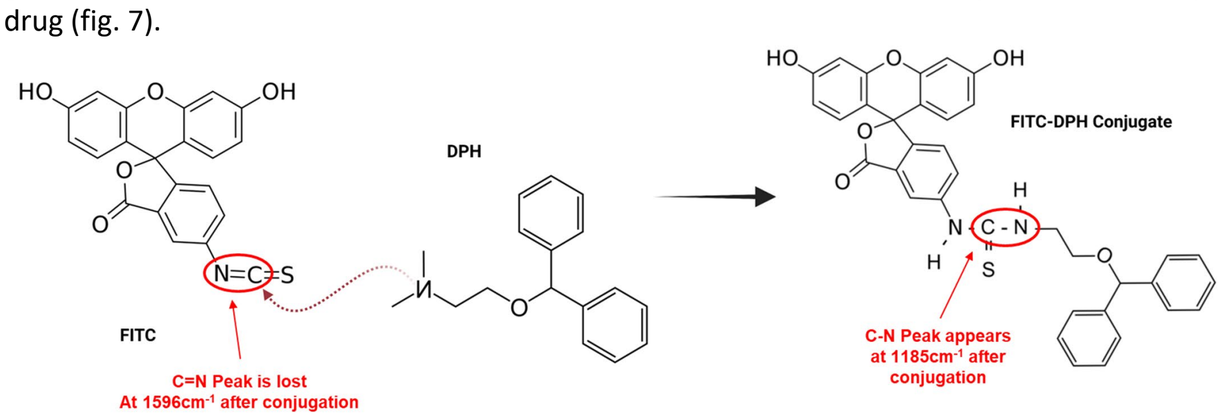


Fig. 7: Conjugation mechanism between the primary amine group of DPH, and the isothiocyanate group of FITC.

Figure 8 uses ATR-FTIR spectroscopy as analytic support for the DPH-FITC conjugate. Close examination of the dye-DPH spectrum reveals a loss of the spectral band at 1596cm^{-1}, which is attributed to the loss of the C=N bond of the isothiocyanate group of FITC. Further, a new peak arises at 1185cm^{-1}, which provides evidence for the creation of the C-N linkage between DPH and FITC.

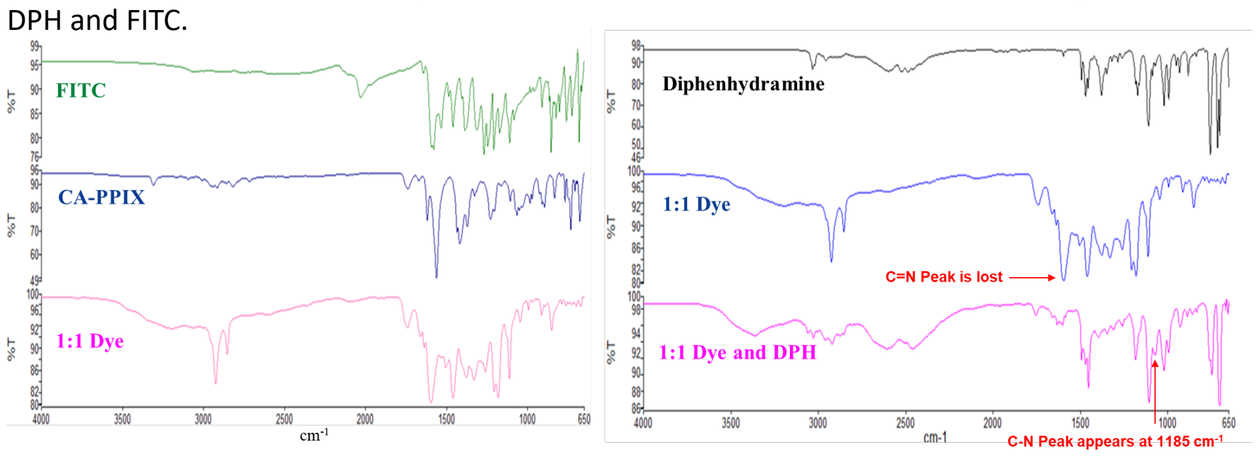


Fig. 8: Inspection of the ATR-FTIR spectra of DPH, the Dye mix, and the Dye-DPH conjugate provides direct evidence for the formation of the FITC-DPH conjugate via linkage at the isothiocyanate group.

All images were created by the student researcher

Quenching of FITC Fluorescence via Formation of the DPH-FITC Conjugate

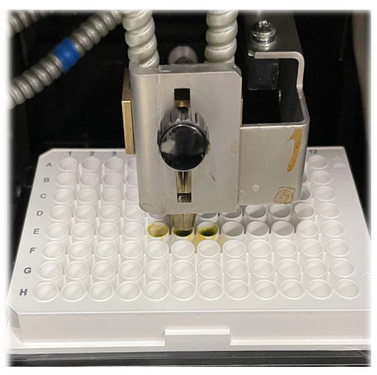
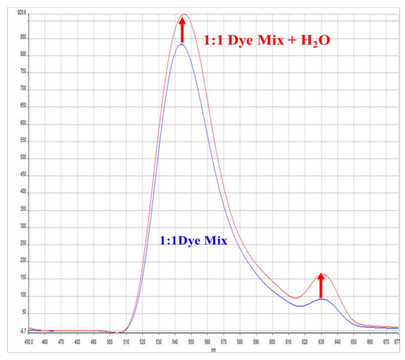


Figure 9: LSB50B well plate reader



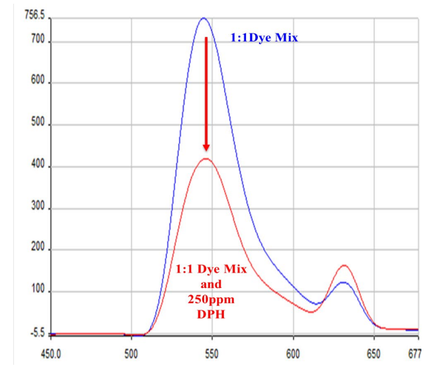


Figure 10: Emission spectrum FITC:CA-PPIX solution with 250ppm DPH in water added. The 540nm green fluorescence is notably quenched

The emission spectrum of the dye mixture was measured via an LSB50B well plate reader accessory (Fig. 9). When 250ppm DPH (in water) was added to 200µl of the dilute 1:1 solution, there is a measurable quenching of the green FITC fluorescence attributed to the DPH-FITC conjugate (fig. 10).

Fabrication and Application of DPH Sensor

1. Design and Fabrication of the DPH-Sensor

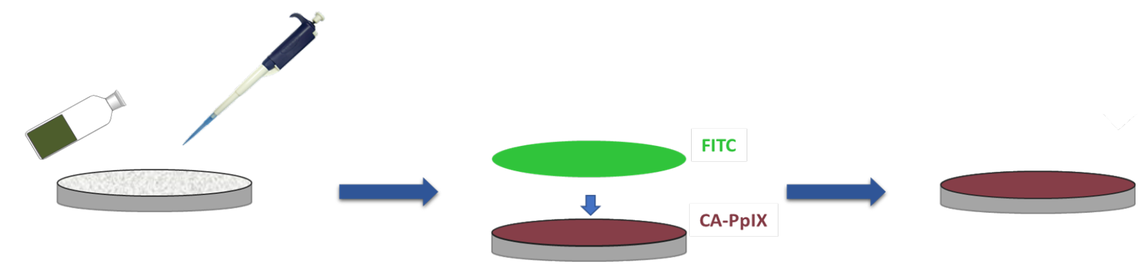


Fig. 11 (above): Construction of the DPH-Sensor

2. Application – Use of the DPH-Sensor

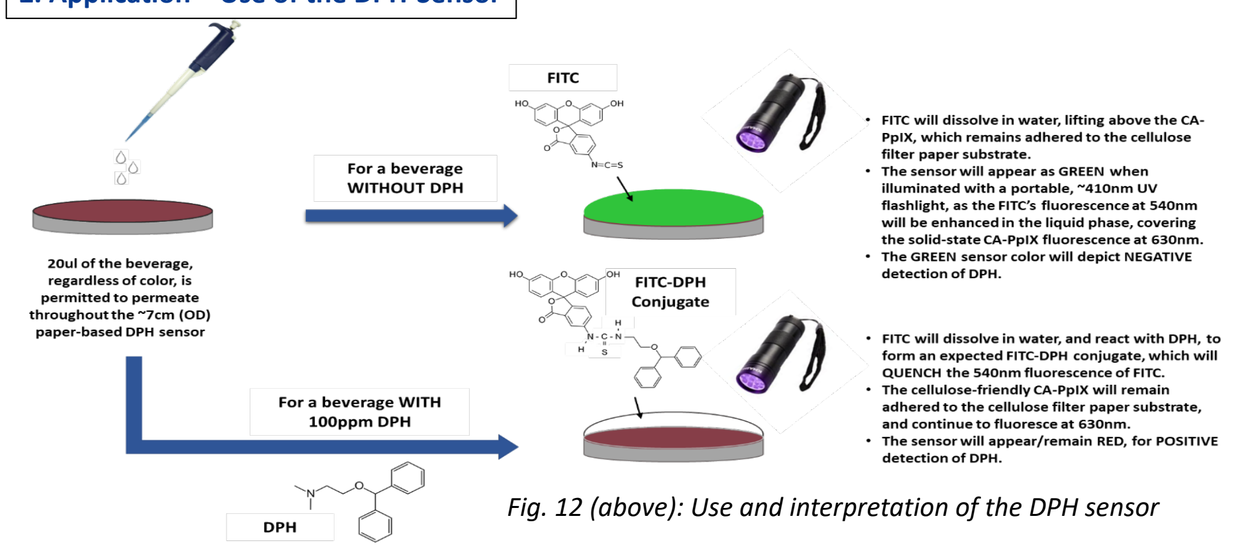
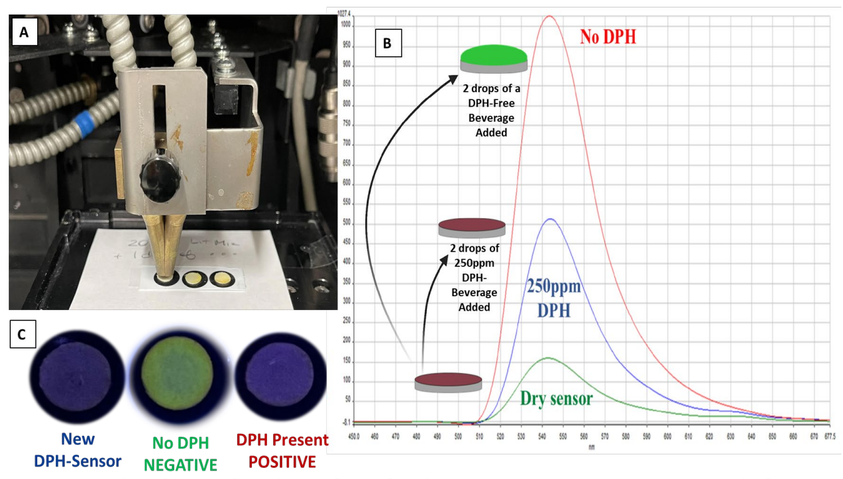


Fig. 12 (above): Use and interpretation of the DPH sensor

The visual, color-change of the DPH-Sensor was established via front surface fluorescence measurement. As indicated in Fig. 13b. Visually, the sensor appears green, as the soluble FITC 540nm fluorescence dominates, on the **GREEN-NEGATIVE** result sensor. Conversely, the addition of 2-drops of 250ppm DPH causes the anticipated quenching of FITC, and the DPH-Sensor remains **RED**, for a **POSITIVE** result (fig 13c).



*Figs. 13a-c: The addition of ~2 drops of DPH-free beverage causes a **GREEN Sensor result**, for SAFE, whereas addition of 250ppm DPH-loaded beverage causes the **DPH Sensor to remain RED**, indicating an **UNSAFE** drink.*

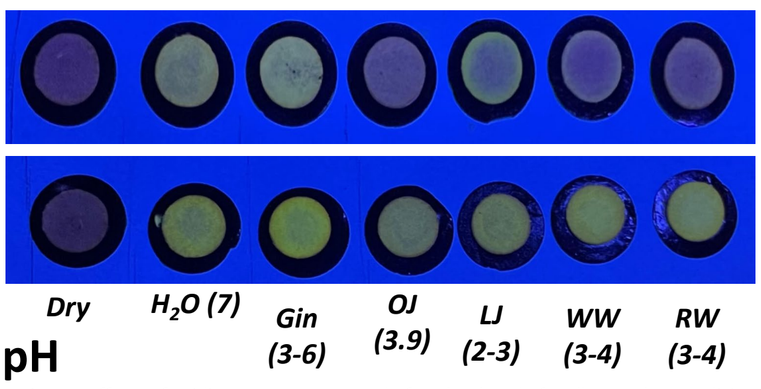


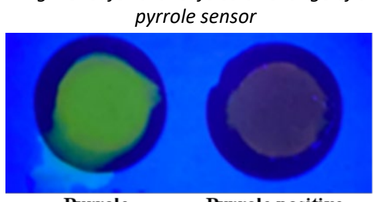
Fig. 14: effects of acidic pH on DPH sensor before (top) and after (bottom) neutralizing demonstrating successful remedy of protonation of diagnostic system

In practical usage, the sensor must consider the many contents and colors that common beverages might posses. Notably, issues arose within acidic beverages producing false negatives (fig. 14 top). To remedy this, the beverage in question is neutralized using the 6.2 methyl red pH indicator and a .02M NaOH concentration ensuring accurate results regardless of color or acidity (fig. 14 bottom).

Extension of Use – Marijuana Breath Detection via Pyrrole

Recent legalizations and increased recreational use of marijuana has led to the increased operation of automobiles while under the influence creating a significant risk for others. Research by Graves, et al. highlight the various components of marijuana and cigarette smoke including the secondary-amine pyrrole found in Marijuana but absent from cigarettes. The relative content of marijuana pyrrole to tobacco-cigarette nicotine was found to be 8:33. This allows for the translation of pyrrole content within marijuana to nicotine exposure from tobacco usage.

Fig. 15: safe vs. unsafe color-change of a pyrrole sensor



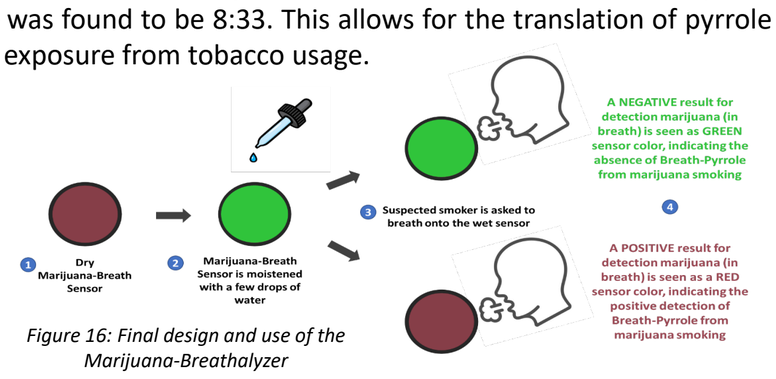


Figure 16: Final design and use of the Marijuana-Breathalyzer

In use, the DPH sensor is first wet with 2 drops of deionized water, followed by simulated exhale of 0.021 µg pyrrole (the calculated relative concentration of marijuana found in one's breath) in 1.1L of gas. The DPH-Sensor turned RED for pyrrole exposure, indicating recent use of marijuana, while remaining GREEN for simulated exhalation of normal room air, indicating marijuana-free breath (Fig. 15).