A Sensor for a Date-Rape Drug, Incorporated Into a Beverage Container

A Major Qualifying Project Report
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By:

Jillian Morang _____
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Approved:

_____

Professor John C. Macdonald, Advisor

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Nomenclature

BCG- Bromocresol green

BCP- Bromocresol purple

GBL- Gamma-butyrolactone

GHB- Gamma-hydroxybutyric acid

IR- Infrared

NMR- Nuclear magnetic resonance

PDMS- Polydimethylsiloxane

SAMs- Self-assembled monolayers

TLC- Thin layer chromatography

UV-VIS- Ultraviolet- visible

I. Introduction

The objective of this project was to explore approaches for developing a beverage container that will act as a sensor indicating the presence of the date-rape drug gamma-hydroxybutyric acid (GHB) in the contained liquid. This research aimed to identify an indicator for GHB that gives a detectable color change when exposed to the typical dosage level, and to investigate methods to incorporate the indicator with a suitable material on the surface of the container. The indicators investigated were bromocresol purple and bromocresol green. The methods of incorporating the indicator that were explored were adsorption, embedding, and covalent attachment.

The use of date-rape drugs on unwilling victims is much too common. This project represents a first step toward development of surface-based sensing materials that provide continuous monitoring for the presence of date-rape drugs in liquids in contact with the sensor material. Ultimately, we hope to incorporate these materials into commercial beverage containers as a means to protect individuals against drug-induced date rape.

II. Background

2.1.1 Date Rape Drugs

A date rape drug is any drug that is given unknowingly to induce a person into a sedated or unconscious state, in order to facilitate in the sexual assault of the dosed person. [1] While there are many drugs that can be used as a date rape drug, the most commonly considered are flunitrazepam (rohypnol), ketamine, and gamma-hydroxybutyric acid (GHB), shown in Figure 1. These drugs are also included in the "club drugs" grouping along with LSD, and are often taken recreationally during rave parties. [2] [3] [4]

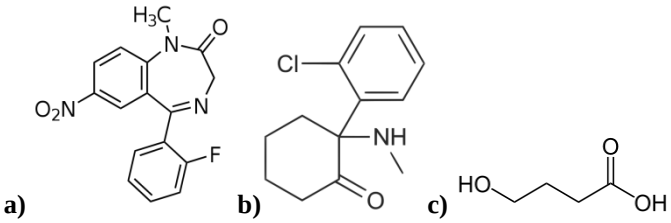

Figure 1: a) Flunitrazepam, b) Ketamine, c) Gamma-hydroxybutyric acid

2.1.2 Flunitrazepam

Flunitrazepam (Figure 1a) is known by its brand name, rohypnol, as well as many other street names such as roofies or roche. Rohypnol is a benzodiazepine sedative which has no legal uses in the United States, though it is used in some other countries to treat insomnia. This drug comes as an odorless, colorless pill and is taken in one and two milligram doses, though legally manufactured rohypnol is only available in 1mg doses and turns blue when dissolved. [2]

Flunitrazepam can cause amnesia, drowsiness, muscle relaxation, slurred speech, lowered blood pressure, impaired judgment and coordination, and nausea. These effects are intensified

when the drug is taken in combination with alcohol. The signs of having taken rohypnol begin within thirty minutes, peak after two hours, and can last for more than eight hours. [2]

2.1.3 Ketamine

Ketamine (Figure 1b) has various street names such as special K, lady K, and blind squid. Most legal use of ketamine is in veterinary anesthetic, though the drug can also be used in human anesthesia. The drug can be consumed through snorting the powder form, dissolved in a liquid and taken orally, or through intramuscular shots. The method of ingestion effects how quickly and how much the drug will effect the user. Taking ketamine intramuscularly has the quickest effect, and is the most dangerous and likely to result in overdose. [3]

The effects of low doses of ketamine include numbness, heaviness in the limbs, blurred vision, reduced hearing, and feelings of detachment and introversion. Higher doses lead to extreme changes in judgment, hallucinations, convulsions, respiratory depression, unconsciousness, and memory loss. Experiences when taking ketamine can also include an initial rush of energy, anesthesia, and muscle spasm. [3]

2.1.4 Gamma-Hydroxybutyric Acid

Gamma-hydroxybutyric acid (Figure 1c) is most often referred to as GHB, though it has many other street names including liquid ecstasy and blue nitro. GHB occurs naturally in small amounts in the human body, but too much of the drug can have adverse effects. The effects of the drug depend on the amount taken (Table 1). These effects begin within 15 to 30 minutes, peak between 2 and 4 hours, and can last 3 to 6 hours after consumption. [4]

Table 1: Effects of GHB by Dosage

Dosage	Effects
<1g	Relaxed feeling, reduced inhibitions
1-2g	Strong relaxation, slowed heart rate and respiration, and interference with blood circulation, coordination and balance
2-4g	Pronounced interference with motor and speech control, may go into a coma-like state
>4g	Respiratory depression, unconsciousness, coma, or overdose

The consumption of GHB may easily result in an over dose, especially when the drug has been home-made. This is due to the fact that the amount of GHB present in the typical spoonful dosage can vary from 0.5g to 5g, depending on the synthesis of the drug. Other side effects which can result from the intake of GHB are nausea, vomiting, delusions, hallucinations, memory-loss and seizures. [4] In this study, we focused developing a sensor for GHB because that drug is used frequently for date rape, and because the molecular structure of GHB features an organic carboxylic acid that is known to cause a colorimetric response in the presence of indicators sensitive to changes in pH.

2.2 Sensors

Drug sensors, which generally consist of a material that contains an indicator (a molecule that indicates the presence of the drug), are used to detect the presence of certain types of drugs. Many of these sensors result in a colorimetric response when in the presence of the drug for which it is indicating. This type of indication is useful because it can be detected with the human eye. The change in color of indicators most commonly is triggered chemically via a change in molecular conformation, structure, or state of protonation of the indicator upon interaction with the drug.

2.2.1 pH indicators

pH is the quantitative measure of the acidity or basicity of a solution resulting from the –log[H^+] in the solution. The pH scale typically ranges from 0 to 14: 7 representing a neutral solution, less than 7 acidic, and greater than 7 basic. [5] pH indicators are sensors that adjust color or fluorescence with a change in pH. Different indicators are effective over differing pH ranges. In this study, we chose to investigate the response of three general pH indicators—methyl red, bromocresol purple and bromocresol green (Figure 2)—because those indicators are sensitive to changes in pH in the range between 3.8 and 6.8 where the acidic carboxylic acid group on GHB was expected to give a response.

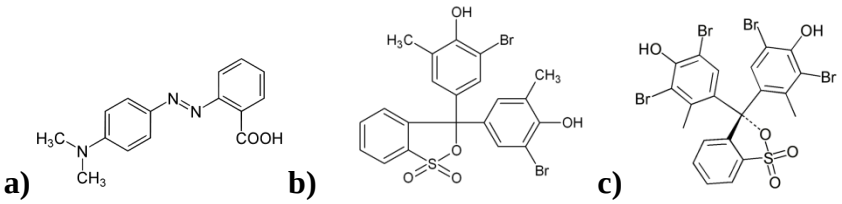

Figure 2: a) Methyl red b) Bromocresol purple c) Bromocresol green

2.2.1.1 Methyl Red

Methyl red (Figure 2a) is an indicator that changes colors when transitioning from pH 4.4 to pH 6.2. This sensor appears as red at pH lower than 4.4 and yellow at pH above 6.2, with a transition through varying levels of orange at pH values in between. This indicator is an azo-dye, having the functional group N=N.

2.2.1.2 Bromocresol Purple

Bromocresol purple, BCP, (Figure 2b) displays a color change from yellow at pH below 5.2 to purple at pH above 6.8. Cresols are compounds which contain the methylphenol functional

group. Bromocresol purple's color change is the result of the protonation/deprotonation of the hydroxyl groups on the two substituted phenols (Figure 3).

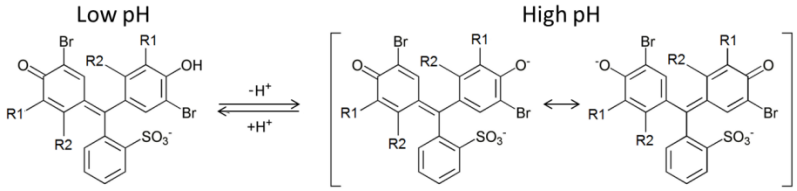

Figure 3: Bromocresol purple, bromocresol green mechanism of color change

2.2.1.3 Bromocresol Green

Bromocresol green, BCG, (Figure 2c) is a pH indicator that changes from yellow to blue when transitioning from a pH of 3.8 to a pH of 5.4. This indicator will also display various levels of green when transitioning between the two pH levels. Similar in structure to BCP, BCG's color change results from the same protonation/deprotonation mechanism (Figure 3). We anticipated that BCG would provide the greatest sensitivity as an indicator because alkyl substituted carboxylic acids such as GHB generally exhibit pKa values in the range of 4-5.

2.3 Elastomers

Elastomers are rubber-like materials, made up of long polymer chains and capable of returning to their original shape after being stretched. [6]

2.3.1 Polydimethylsiloxane

Polydimethylsiloxane, PDMS, is an elastomer consisting of repeating chains of $Si(CH_3)_2O$ making it part of the chemical group called siloxanes. PDMS is prepared by polymerizing dimethylsiloxane monomers in the presence of aqueous sodium hydroxide base, which results in a rubbery cross-linked PDMS polymer. In the presence of organic solvents such as ethanol, PDMS exhibits swelling because the elastomer is somewhat porous and therefore

somewhat permeable to organic solvents and other organic guests that can diffuse into the material. In contrast, PDMS general is stable and does not swell in the presence of water.

2.4 Adsorption

Physical adsorption (Figure 4) is the attraction of particles of gases or dissolved compounds onto the surface of a solid, without chemically bonding them to the surface. Porous solids tend to adsorb more, due to their larger surface area. Chemical adsorption results in the compound being bonded to the surface, and therefore typically takes longer to occur than physical adsorption. As described later in this report, we investigated physical adsorption of indicators onto several different substrates as a means to create a sensor for GHB.

Figure 4: Adsorption

2.5 Embedding

Embedding is the immobilization of particles within a matrix. The matrix chosen is often a polymer, where the embedded compound can be surrounded by spaghetti-like polymer chains. Another approach we investigated to create a sensor involved embedding indicators for GHB within thin films of PDMS (see above).

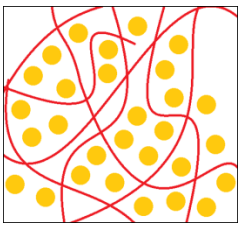

Figure 5: Embedding

2.6 Self Assembled Monolayers

Self-assembled monolayers, or SAMs, are ordered molecular assemblies formed in a single layer on the surface of a precious metals such as gold. SAMs are prepared by exposing the surface of a clean gold substrate (e.g., a gold-coated microscope slide) to a solution of a compound containing a thiol (SH) group. Spontaneous formation of covalent gold-sulfur bonds between the thiol groups and gold atoms on the surface results in uniform coverage of the surface. SAMs on gold are highly ordered and may include a large variety of functional groups in side chains and the terminal head group exposed at the surface of the SAM. [7] As such, SAMs provide a convenient means to alter the surface properties of the gold substrate and to functionalize the surface with chemical reactive groups. Later in this report, we describe an alternative approach to create a sensor for GHB that involved attaching indicators to the surface of SAMs.

2.7 Characterization Techniques

2.7.1 Infrared Spectroscopy

Most compounds containing covalent bonds will absorb various frequencies of electromagnetic radiation in the infrared, IR, region. IR spectroscopy takes advantage of the vibrational section of the IR region. When molecules absorb IR radiation, they become excited to a higher energy state. Molecules have specific energy levels, or frequencies, at which they will absorb. Due to this specificity, an IR spectrum can aid in the characterization of a compound. [8]

2.7.2 Grazing Angle IR Spectroscopy

Grazing angle IR is a form of IR spectroscopy in which the IR radiation is reflected to a surface and back to the sensor that detects the absorption frequencies of the surface. This type of

IR spectroscopy frequently is used to characterize thin molecular films on reflective surfaces such as gold, which necessarily results in weaker absorption due to the low concentration of organic material present. We used grazing-angle IR to characterize SAMs of cysteamine on gold and to verify the presence of indicators attached to those SAMs.

2.7.3 Water Contact Angle

Contact angle goniometry of a 1 microliter drop of water on a surface, or water contact angle (Figure 6), is a measure that gives a quantifiable number to the hydrophobicity, or wettability of a surface. A large value for the angle, θ, shows a hydrophobic surface that is not very wettable. A small value for θ shows a hydrophilic surface with high wettability. This can be useful in a number of ways including in the detection of a change in the composition of a surface. When comparing contact angle data it is important to ensure that the same drop size was used on tested surfaces because this can effect θ. The contact angle technique can be applied to a variety of liquids. Contact angle goniometry was used to characterize the change in surface energy associated with deposition of SAMs on gold by monitoring the change in contact angle of water droplets.

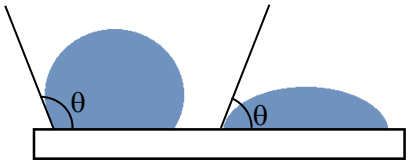

Figure 6: Water Contact Angle

2.7.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance, NMR, spectroscopy provides information about the number of magnetically distinct atoms of the type being studied. Typically hydrogen or carbon is the studied atom, though NMR is possible for many different atoms. This type of spectroscopy tells

how many different types of a certain atom are in a molecule, and provides insight into the immediate environment of those atoms. [8] NMR spectroscopy was used to characterize the structure of GHB and to verify that the sodium salt of GHB used in this study was present in solution in the ring-open form rather than in the ring-closed lactone form.

2.7.5 Ultraviolet-Visible Spectroscopy

Ultraviolet-visible, UV-VIS, spectroscopy shows the absorption of energy by a compound in the ultraviolet and visible light ranges. This type of spectroscopy is only useful in colored or fluorescent compounds. This is because other compounds are transparent in these regions and will show no absorption. [8] UV-VIS spectroscopy was used to determine the absorption maxima of the indicator BCG in bulk solutions and on the surfaces in order to determine if BCG was present on SAMs of cysteamine.

2.8 Other Products and Current Technology

There are numerous technologies currently available to test a drink for date-rape drugs. However, none of these technologies test continuously or without effort from the user. Most of the drug tests are strips, whether disguised or otherwise, which a person has to either dip into their drink or remove a small portion of their drink for testing.

III. Experimental

3.1 Synthesis of NaGHB

The procedure for the synthesis of the NaGHB salt was based on previously studied procedures. [9] NaOH (10.476g) was weighed out. H_2O (20mL) was added to the NaOH and stirred until dissolved. Gamma-butyrolactone, GBL (20mL), was added and continued to stir. The GBL sat as an oil layer on top of the NaOH solution and the flask became warm when the GBL was added. The flask was stoppered and the reaction was left at room temperature for 24 hours. The resulting solution was rotovapped and dried in a desiccator under vacuum to give the product in 86% yield.

3.2 Testing the Indicators Against NaGHB

5% and 0.04% by weight solutions of BCP and BCG in 80:20 EtOH:H_2O and methyl red in dichloromethane were prepared. Silica gel TLC plates and filter paper were submerged in each solution and dried with heat, leaving the adsorbed indicators. 1M, 0.1M, and 0.01M solutions of NaGHB in H_2O were tested against the adsorbed sensors.

3.3 Embedding BCP in PDMS

A saturated solution of BCP in ethanol was prepared. The PDMS was prepared by mixing DMS (20mL) with the saturated BCP (2mL) and the curing agent (2mL). The resulting slurry was spread in thin layers on glass slides, placed in the oven under vacuum and heated to 70°C for two hours. The heat was removed and the slides were left under vacuum for the weekend. The slides were removed and submerged in 0.02M solutions of NaGHB in ethanol and H_2O for fifteen minutes.

3.4 Attaching BCP and BCG to Cysteamine SAMs

Clean gold slides were submerged in a solution of cysteamine in ethanol (10mM, 20mL) for 24 hours. The slides were removed and washed first with ethanol followed by dH_2O. These were the cysteamine SAMs. The SAMs were submerged in solutions of BCP or BCG in ethanol (10mM, 20mL) for 24 hours. The newly coated SAMs were removed, washed first with ethanol followed by H_2O, and dried under a stream of nitrogen.

IV. Results

4.1 Synthesis of NaGHB

Analysis of the NaGHB product by IR spectroscopy (Figure 8) as well as comparison of the ^{1}H NMR spectra for GBL (Figure 9) and the NaGHB product (Figure 10), given later in the report, were used to determine the success of the synthesis of NaGHB (Figure 7) as described in the experimental section.

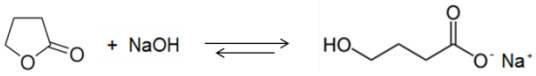

Figure 7: Mechanism for the synthesis of NaGHB

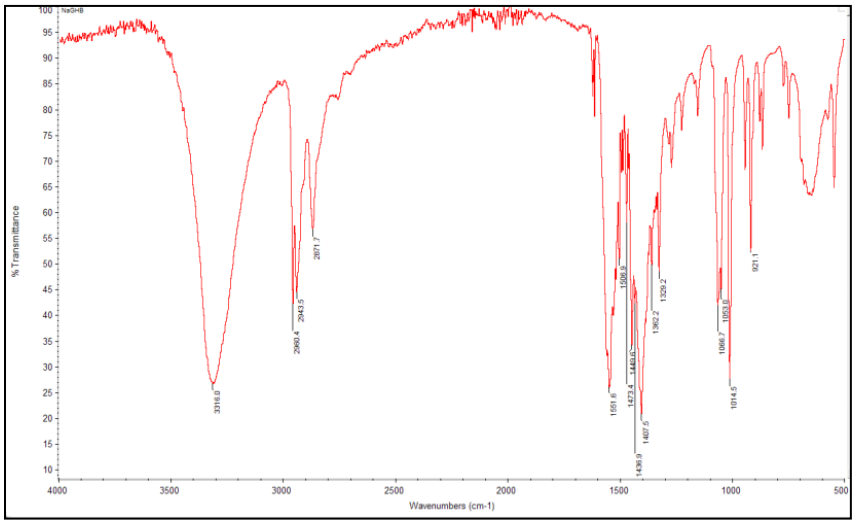

Figure 8: IR spectrum of product (NaGHB)

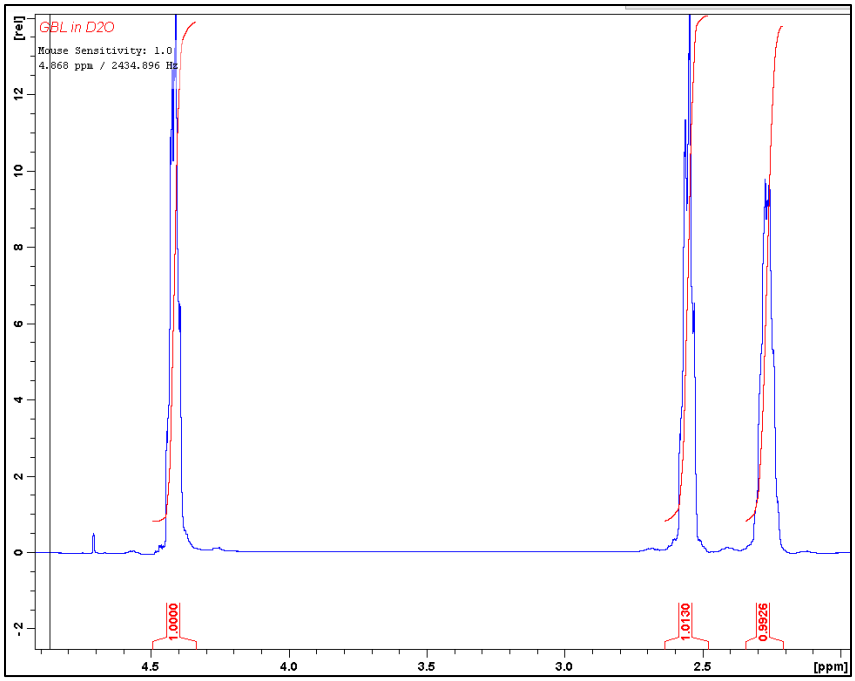

Figure 9: H-NMR spectrum of GBL

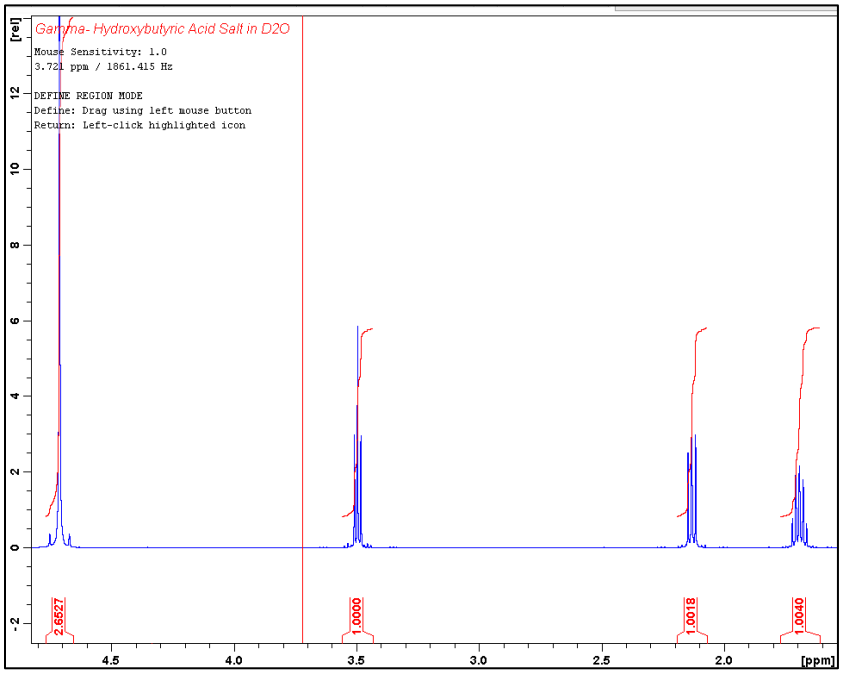

Figure 10: H-NMR spectrum of product (NaGHB)

4.2 Testing the Indicators Against NaGHB

The response of the three indicators to 1 M, 0.1 M and 0.01 M aqueous solutions of NaGHB is summarized in Table 2. This data was used to determine whether the chosen indicators gave the desired colorimetric response.

Table 2: Color change of indicators in the presence of NaGHB

NaGHB (M)	1	0.1	0.01
Indicator (% by weight) Surface Initial Color			
Methyl red (0.04) Filter paper Light red	Light red	Light red	Light red
BCP (0.04) Filter paper Light orange	Purple	Purple/brownish	Yellow
BCG (0.04) Filter Paper Light yellow	Blue	Light blue/slightly greenish	Light blue/green
Methyl Red (5) TLC Plate Dark red (almost black)	Dark red (almost black)	Dark red	Dark red
BCP (5) TLC Plate Red	Red-orange	Yellow	Yellow
BCG (5) TLC Plate Orange	Yellow/orange	Green/yellow	Green

4.3 Embedding BCP in PDMS

The PDMS/BCP coated slides were submerged solutions of NaGHB in ethanol and water, as described in the experimental section. Figure 11 and Figure 12 show the resulting color changes to the slides and solutions, to be further discussed later in this report.

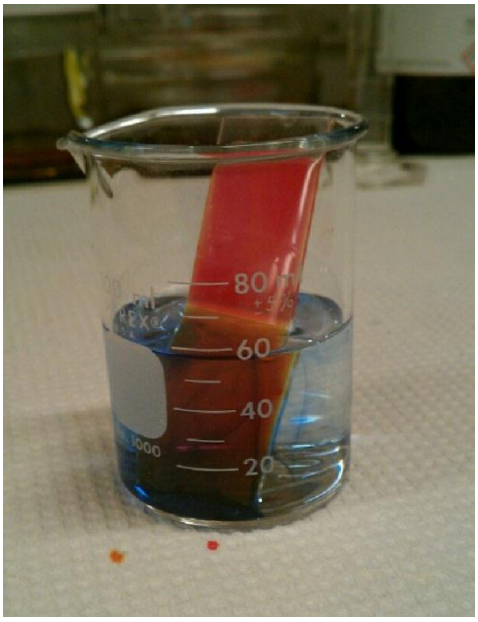

Figure 11: BCP/PDMS coated slide in ethanol

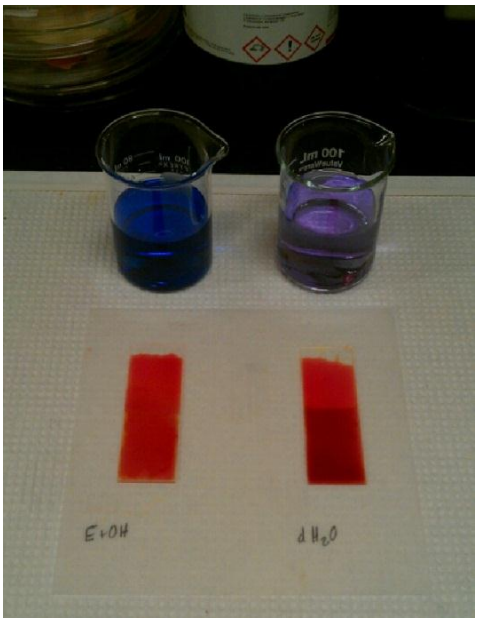

Figure 12: BCP/PDMS coated slides after 15 minutes submerged

4.4 Attaching BCP and BCG to Cysteamine SAMs

Analysis and comparisons of IR (Figure 14-Figure 20), UV-VIS (Figure 21-Figure 24), and water contact angle (Table 3) data were used to determine whether the BCP and BCG compounds were present on the surface of the SAMs, as shown in Figure 13.

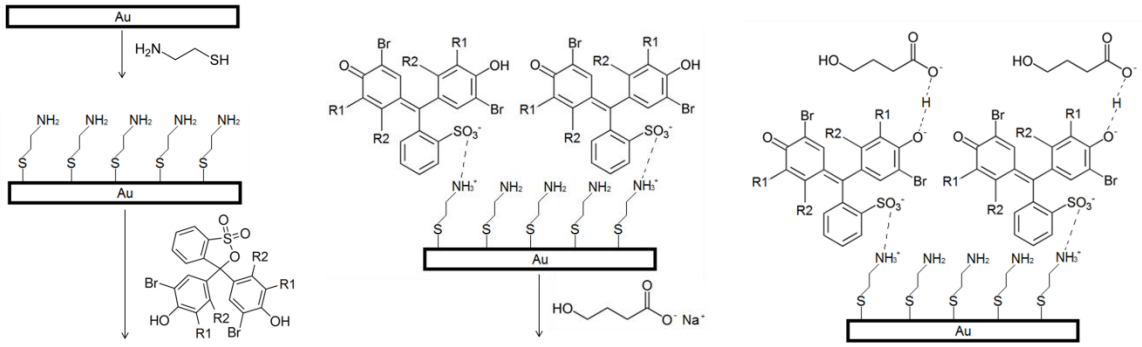

Figure 13: Mechanism for the attachment of BCP/BCG to a gold surface

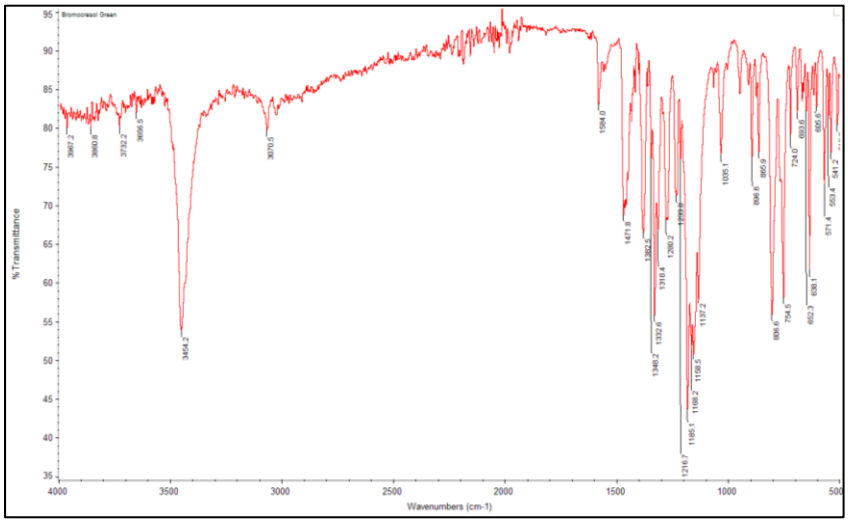

Figure 14: IR spectrum of BCG

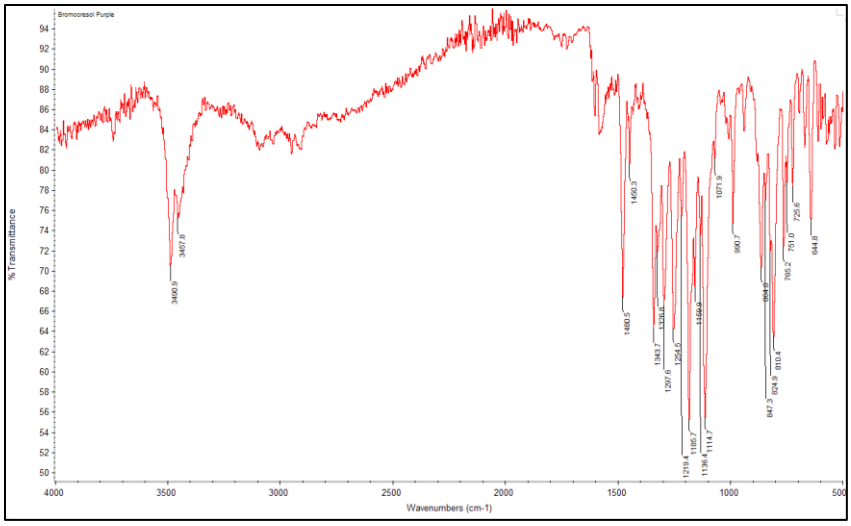

Figure 15: IR spectrum of BCP

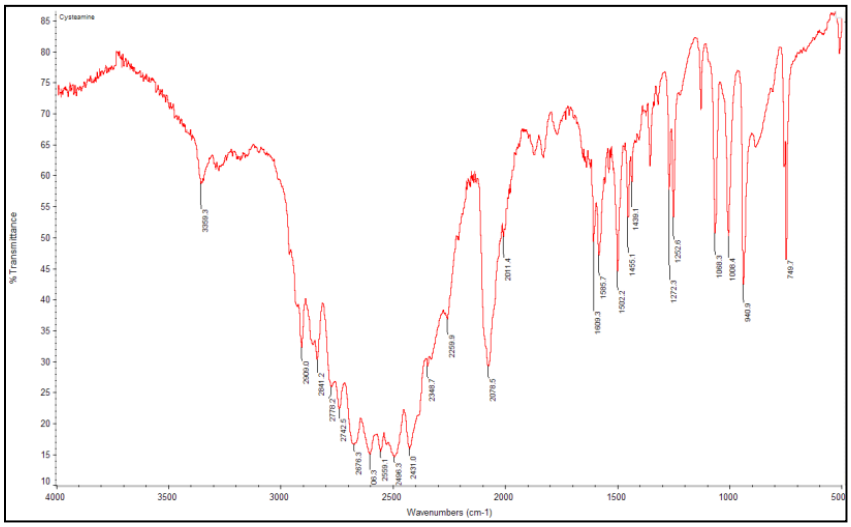

Figure 16: IR spectrum of cysteamine

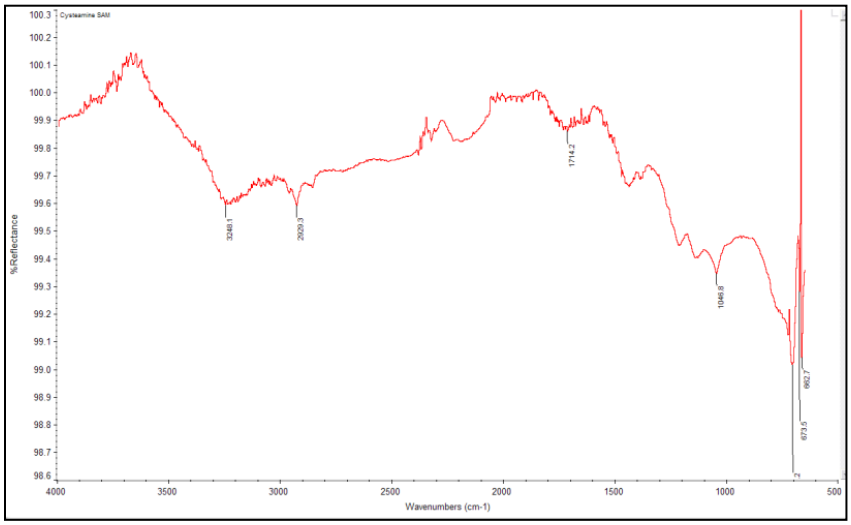

Figure 17: Grazing angle IR spectrum of cysteamine SAM

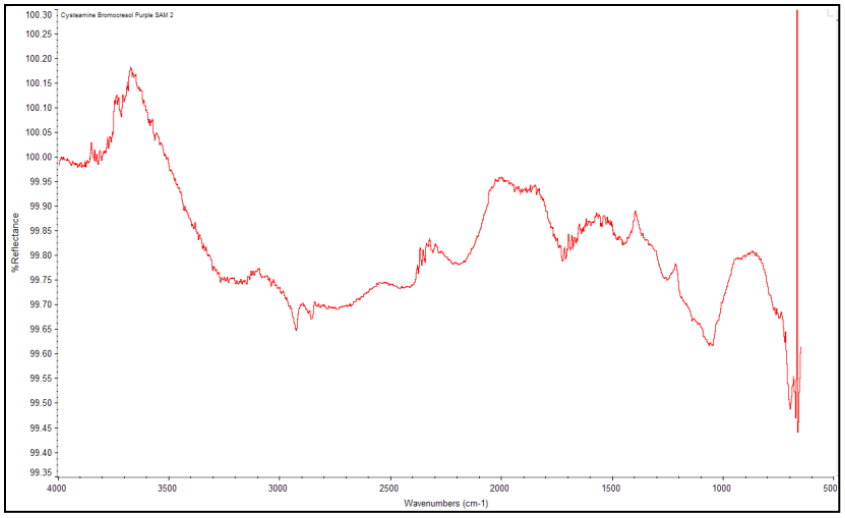

Figure 18: Grazing angle IR spectrum of BCP on cysteamine SAM

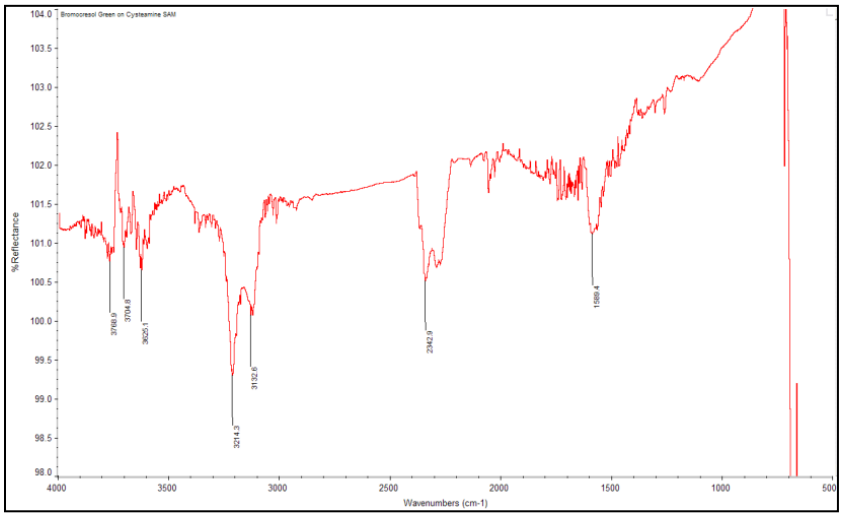

Figure 19: Grazing angle IR spectrum of BCG on cysteamine SAM

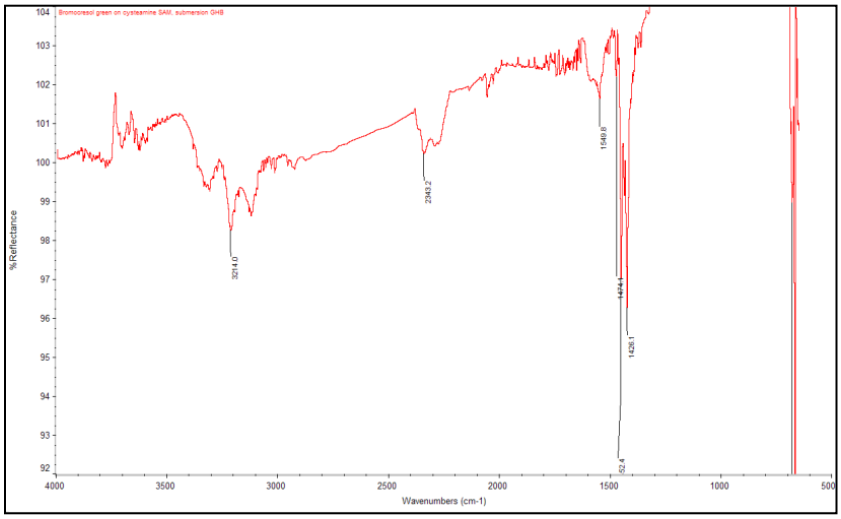

Figure 20: Grazing angle IR spectrum of BCG on cysteamine SAM, after NaGHB exposure

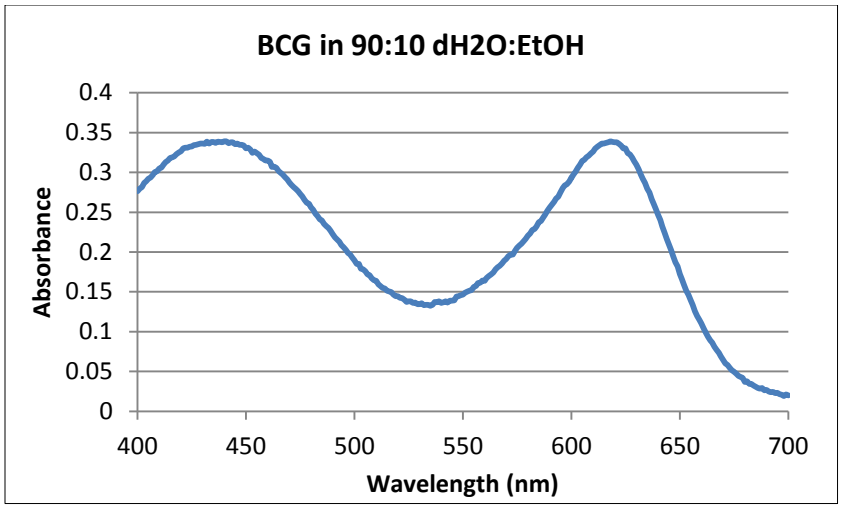

Figure 21: UV-VIS spectrum of BCG

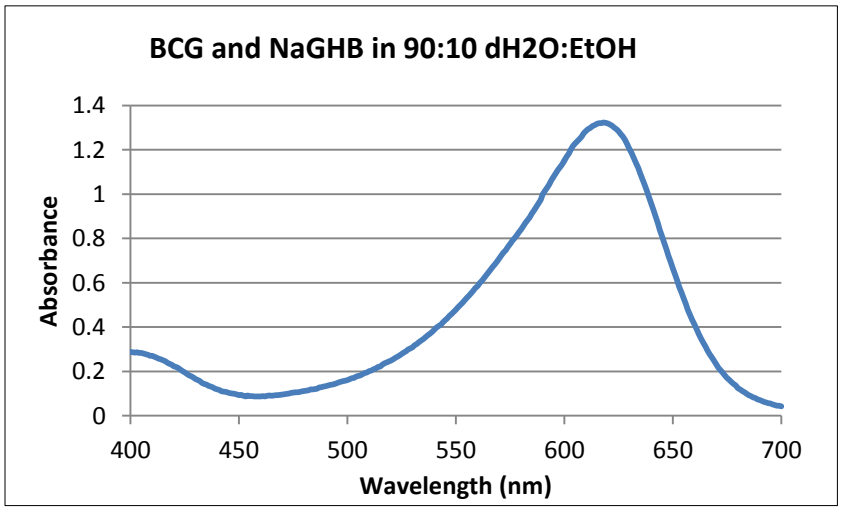

Figure 22: UV-VIS spectrum of BCG in presence of NaGHB

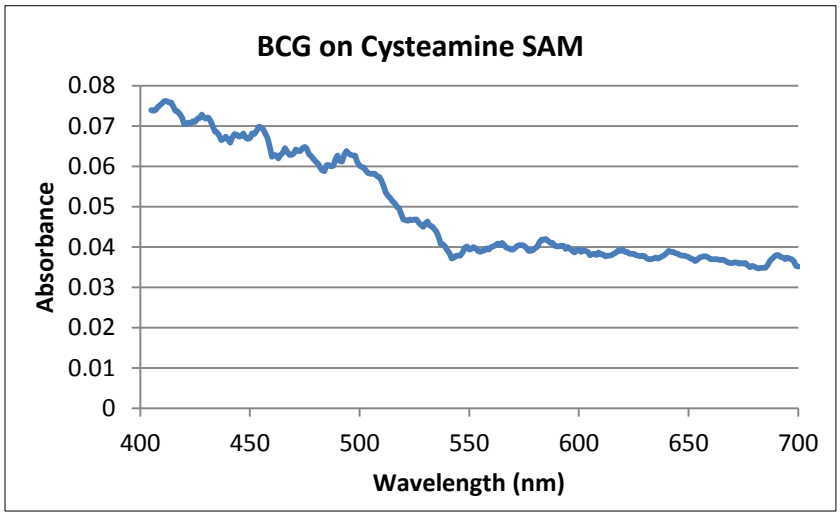

Figure 23: UV-VIS spectrum of BCG on cysteamine SAM

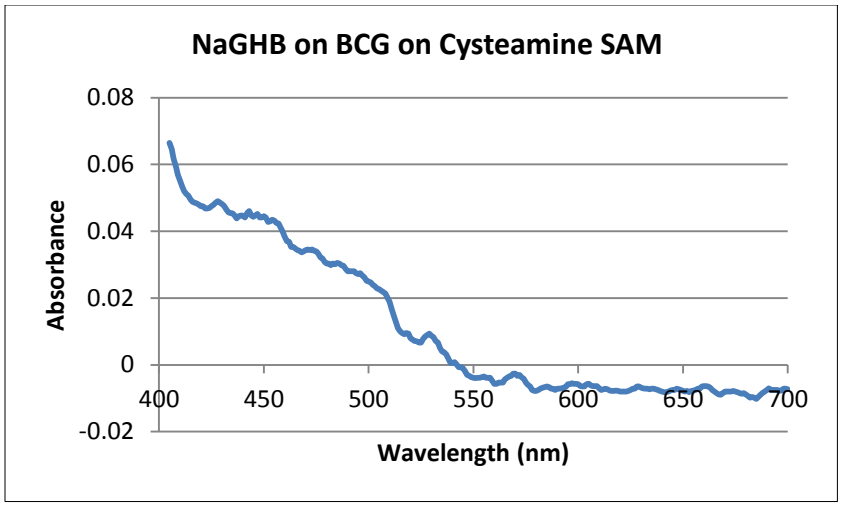

Figure 24: UV-VIS spectrum of BCG on cysteamine SAM, after exposure to NaGHB

Table 3: Water contact angle

2µL Drops	
Surface	**Angle (degrees)**
Gold	63.7
Cysteamine SAM on Gold	80.6
BCP on Cysteamine SAM	88.0
BCG on Cysteamine SAM	113.7
NaGHB on BCG on Cysteamine SAM	101.5

V. Discussion

5.1 Synthesis of NaGHB

Through the execution of the previously described synthesis for NaGHB, I was able to produce the desired product with an 86% yield. The identity of this product as NaGHB was determined through the use of IR and NMR spectroscopy. The IR spectrum of the product (Figure 8) shows a broad peak at 3316cm^{-1} indicative of the hydrogen bonded O-H stretching of NaGHB. [8] This peak would not be present in GBL, since it does not have a hydroxyl group in its structure. This spectrum also shows evidence of alkane C-H stretching at 2960cm^{-1} and C=O stretching at 1551cm^{-1}. [10] If the lactone form, GBL, was present a peak would be expected at about 1750cm^{-1}. [10]

The H-NMR spectra of GBL and the resulting NaGHB were also compared. The spectrum of GBL shows three groups of hydrogen of equivalent intensity. This correlates to the three hydrogen groups on GBL, each containing two hydrogen atoms. The presence of the small peak, at about 4.7ppm is attributed to the solvent, D_2O. The spectrum of the NaGHB product shows three peaks of equivalent intensity, correlating to the three hydrogen groups, each with two hydrogen atoms, on the carbon chain of the molecule. In addition to these peaks, a strong peak at about 4.7ppm with a slightly broadened base is representative of the hydroxyl hydrogen, overlapping the solvent peak. [8] This additional peak indicates that the sample is NaGHB.

5.2 Testing the Indicators Against NaGHB

The color changes which occurred when testing the indicators, adsorbed onto filter paper as well as silica gel TLC plates showed that the BCP and BCG both react with an obvious color change to varying concentrations of NaGHB in solution, while the methyl red showed little if

any color change when in the presence of the drug. Due to the lack of reaction by methyl red, it was not used in any further testing for the presence of NaGHB.

5.3 Embedding BCP in PDMS

The BCP was successfully embedded into the PDMS and coated onto glass slides. The presence of the indicator did not affect the curing process of the elastomer. When the PDMS/BCP coated slides were submerged in ethanol and dH_2O the indicator leeched out of the PDMS and into the solvents (**Error! Reference source not found.**Figure 11). The slides themselves also showed very little color change and would not indicate the presence of the drug. The indicator came out of the PDMS at a faster rate when submerged in ethanol than when in water, as can be seen by the darker color present in the ethanol after submersion for the same length of time (Figure 12**Error! Reference source not found.**).

Due to the leeching out of the indicator into the solvents as well as the lack of response by the embedded BCP, embedding would not be an effective method for attaching the indicator on a drinking glass. The indicator would contaminate the user's beverage, which can be potentially harmful to their health. Also, the still embedded BCP would not noticeably indicate the presence of the GHB in the beverage.

5.4 Attaching BCP and BCG to Cysteamine SAMs

IR spectra of the bulk samples of BCP, BCG, and cysteamine were taken for comparison to the spectra of the gold slides after attachment was attempted. The first step in the synthesis was to create a cysteamine SAM on gold slides. The grazing angle IR spectrum of the SAM showed peaks at $3248cm^{-1}$, $2929cm^{-1}$, and $1714cm^{-1}$, which match peaks on the IR spectrum of the bulk sample of cysteamine occurring at $3359cm^{-1}$, $2778cm^{-1}$, and $1609cm^{-1}$. These correlating

peaks indicate the presence of the cysteamine on the surface of the gold slide. Following this step, the BCP and BCG indicators were to be attached to the cysteamine SAM. The grazing angle IR spectrum of the BCP on cysteamine SAM very closely resembled that of the cysteamine SAM. There were no additional peaks present, indicating that the BCP did not attach to the terminal amine group, and the slide only held the cysteamine SAM. The grazing angle IR spectrum of the BCG on cysteamine SAM was compared to that of the bulk IR of the BCG. In addition to the cysteamine peaks, this spectrum showed strong peaks at $3525cm^{-1}$, $3132cm^{-1}$, and $1589cm^{-1}$, which correspond with peaks in the IR spectrum of the bulk sample of BCG occurring at $3454cm^{-1}$, $3070cm^{-1}$, and $1255cm^{-1}$. This correlation between the additional peaks on the spectra of the product, with that of the bulk sample, indicates the presence of the BCG on the cysteamine SAM. The presence or lack thereof of each compound, at the various steps of this attachment method, was also confirmed by the changing of the water contact angle on the surfaces.

The BCP did not attach to the amine group, and therefore would not be useful as an indicator when using this type of attachment method. The BCG, however, did attach to the amine group of the cysteamine SAM, creating a surface that is coated with the indicator. Once it was determined that the BCG was successfully attached to the surface, its reactivity to the presence of NaGHB in solution was tested. Attached to the cysteamine SAM the concentration of the BCG was too low to see a color change. This being the case, after exposure to the NaGHB in solution the IR spectra was taken and compared with that of the bulk IR of the NaGHB. This IR spectra showed sharp peaks at $1426cm^{-1}$ and $1452cm^{-1}$, which correlate with those on the spectra of the bulk sample occurring at $1407cm^{-1}$ and $1436cm^{-1}$. These peaks show the presence of the NaGHB on the surface of the BCG. The UV-VIS spectra of BCG bulk samples in solution with and

without NaGHB present were taken and compared with the UV-VIS spectra of the BCG on cysteamine SAM before and after the submersion in a solution containing the NaGHB. The UV-VIS spectrum of the slide after exposure to NaGHB did not show the same color shift as the solutions, but this may be because the NaGHB must be in solution to react with the BCG.

5.5 Future Experiments

In continuation of this project, the next step would be to attempt the attachment of the BCG indicator to a polymer containing many terminal amine branches, so that the concentration of the indicator would be high enough for an observable color change. The polymer chosen should be one that can be applied as a coating on a glass or plastic surface, or can be used as the material which a beverage container is made from. If the attachment is successful, then the material should be tested against appropriate solutions of NaGHB to see if the indicator will show the color change. Other factors that should be considered in the future include finding indicators which can detect the presence of other date-rape drugs, and can be similarly incorporated into a beverage container as the BCG.

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