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A Polymer To Detect Explosives : Towards An Effective Sensor Material

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A POLYMER TO DETECT EXPLOSIVES: TOWARDS AN EFFECTIVE SENSOR MATERIAL

By

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Abstract

A Polymer to Detect Explosives: Towards an Effective Sensor Material, Master of Applied Science, 2008, Sofija Katić. Applied Environmental Science and Management, Ryerson University.

There are upwards of 100 million landmines in over 70 countries. Nitroaromatic vapours emanate from landmines and a need exists for a sensitive and robust sensor for their detection. It is the goal of this study to develop a thin, reusable, fluorescent film composed of β-cyclodextrin polymer crosslinked with epichlorohydrin, for the detection of nitro-aromatics. The fluorescent moiety, 2-naphthol, (sensing function) was incorporated into the β-cyclodextrin-epichlorohydrin polymer (trapping function). Fluorimetry, FTIR and ^{1}H NMR were employed to characterize the polymer and examine whether 2-naphthol was covalently linked to the β-cyclodextrin polymer network. Fluorescence quenching studies were conducted using a nitroaromatic compound, nitrobenzene, to determine the quenching efficiency. The characterization studies indicate that 2-naphthol is incorporated into the β-cyclodextrin-epichlorohydrin polymer. A difference does not seem to exist in the quenching efficiency of free 2-naphthol, 2-naphthol:β-cyclodextrin complex and the polymers by nitrobenzene.

Aknowledgements

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1.0 Introduction

In spite of international efforts to control the use of landmines, they remain a major global problem. There are an estimated 50 to 100 million[1, 2] planted landmines in over 70 countries[2]. The annual rate of dead and wounded from these ordnances is between 26,000-28,000 people a year with one casualty every 20 minutes.[2] The cost associated with constructing and planting these weapons is minimal (U.S. $3-$30).[1, 2] However, the decommissioning of landmines can be upward of U.S. $1000.[2, 3]

Landmines, besides the obvious consequences to human life, also have extended detrimental effects on the environment and socio-economic development of a nation following war. The following figure, (Figure 1), illustrates the triangular relationship that exists between landmines, environmental degradation, and under-development of a society affected by these ordnances.[4]

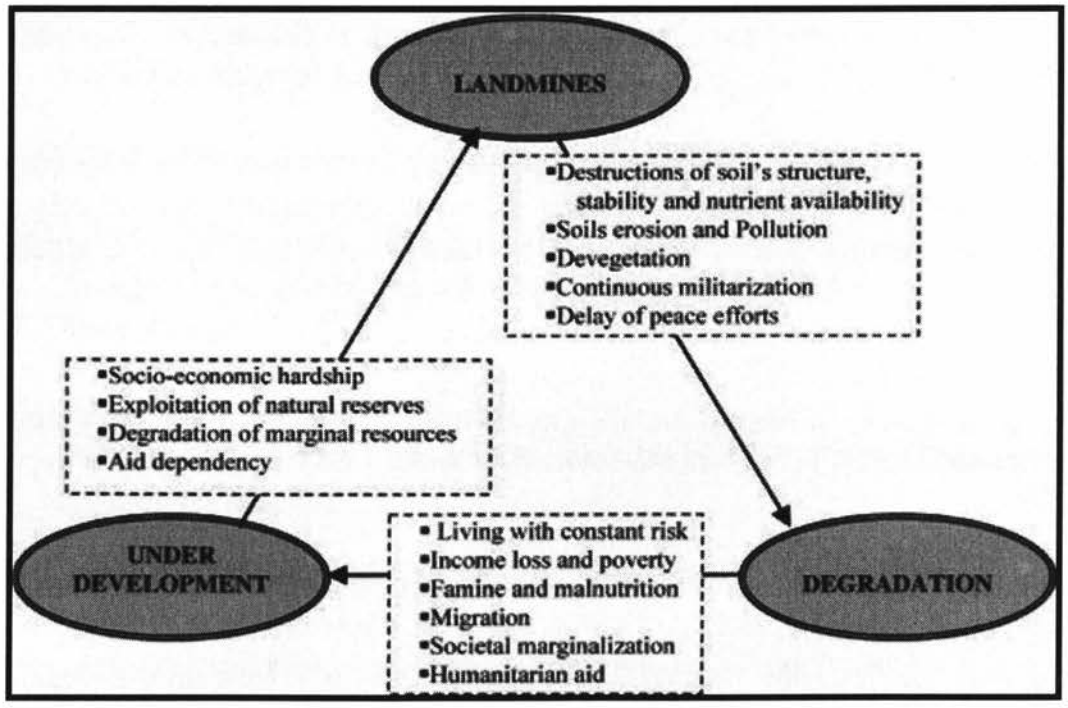


Figure 1: Triangular Relationship Between Landmines, Degradation and Under Development[4]

Some environmental consequences of landmines include soil erosion and pollution. Chemicals leaching from the landmine casing can contaminate the surrounding soil and groundwater. People living in an area that has been affected by landmines are at constant risk not only from the ordnances, but also from poverty, famine and malnutrition. Farming and access to wells is limited if the mines are planted in these areas.[4] Thus, landmines have both devastating immediate and indirect consequences to human life.

A variety of approaches have been used to detect land mines and other unexploded ordnances. The most common and widely used detection methods include: hand-held metal detectors, visual inspection, sniffer dogs and mine prodders.[3] Some of the more sophisticated analytical tools used in the field include gas chromatography with various detectors including; electron capture, ion mobility spectrometry and mass spectrometry[5] and other methods including immunoassays and immunochemical detection based systems.[5]

Recently, there has been a trend towards developing small, portable sensor devices robust enough to use in the field and yet sensitive enough to detect the low levels of explosive vapors emanating from buried mines and ordnance.[6]

This thesis describes attempts to develop a novel sensor material for explosives detection based on a polymer design that includes a sensor function (quenching of fluorescent sites within the polymer by model explosives) and a trapping function (sites within the polymer matrix which trap the desired analyte, resulting in a high local analyte concentration).

1.1 Sensors and their Applications

A sensor is a device or system that measures a response to a physical or chemical change.[7] There are two main classifications of

sensors: biosensors and chemosensors. The discriminating feature between them is the origin of the molecular component signalling the presence of matter or energy (i.e., the sensor function). The sensor function in a chemosensor is of abiotic origin while in a biosensor it is biotic in origin (e.g. antibodies and enzymes).[7, 8]

A sensor is typically made up of two main parts: an analyte sensitive/specific sensing layer and a transducer. The sensing layer is a crucial element in any sensor because it is the initial site of stimulus-sensor interaction. The initial signal is produced as a result of the contact between the target molecule and the sensing layer. The transducer is responsible for deciphering the interaction between the analyte and sensing layer. The information transduction occurring at this interface is usually carried out with electrons, photons, or sound waves that correspond to electrical, optical, and frequency measurements.[9]

Sensors have found various uses in many fields including medicine, industry and environmental monitoring. A detailed description of the multitude of sensors available is well beyond the scope of this thesis, but some examples are provided. Sensors have found uses in medicine ranging from hearing aids to immunoreaction readings.[10, 11] There are many sensors available to detect various analytes of concern in the food industry. For example, the presence of herbicides and pesticides can be detected by a biosensor (Figure 2).[7]

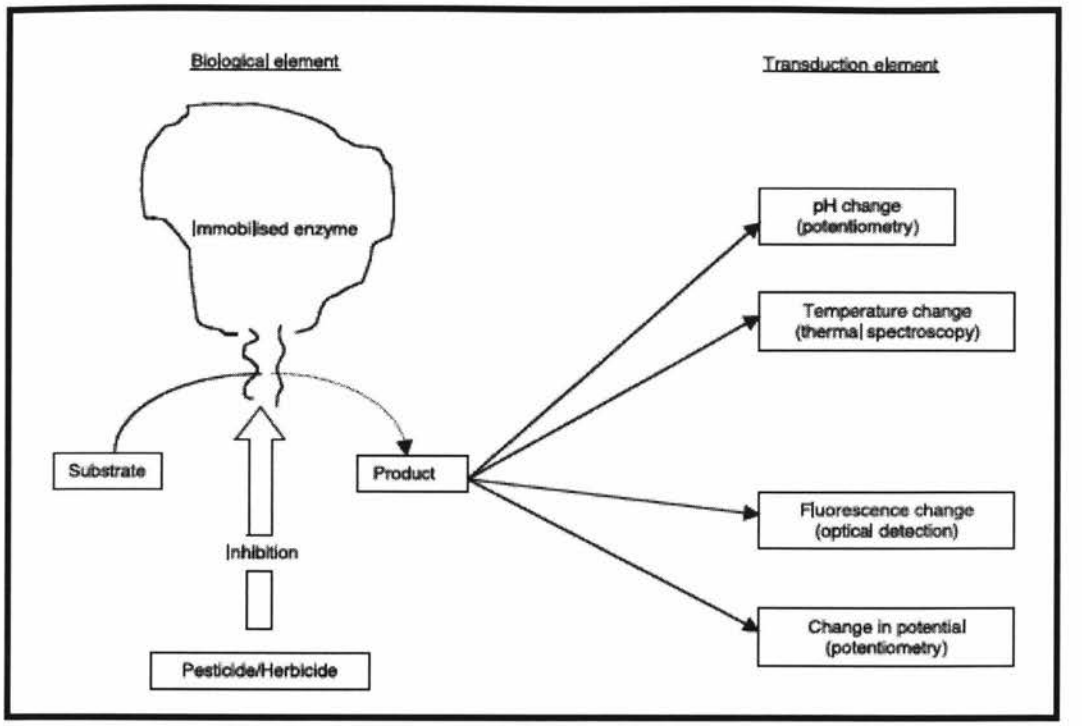


Figure 2: Schematic Illustration of a Biosensor for Pesticide/Herbicide Detection[7]

Sensors are readily used in the environmental sector for pollution monitoring and control. For example, the use of gas sensor technology has had considerable impact on pollution control. Oxygen gas sensors are used to measure exhaust emissions from vehicles.[12]

1.2 The Important Aspects of Sensors

A sensor, in order to be of practical use, should be both sensitive and selective to the target analyte and exhibit a linear response to the analyte concentration. Each of these features is discussed in the following sections, using explosive vapours from landmines as a specific example.

1.2.1 Sensor Sensitivity

Sensitivity refers to the ability of a sensor to detect the lowest possible chemical concentration. The lower the detection limit of the analyte, the greater the sensor sensitivity must be. This is very important

when the analyte in question is detectable in only minute concentrations such as parts per billion (ppb) and parts per trillion (ppt). For example, environmental contaminants are found in very small concentrations. An area of environmental research that requires extremely sensitive sensors is landmine detection. The figure below illustrates the vapour concentration of explosives in landmines.

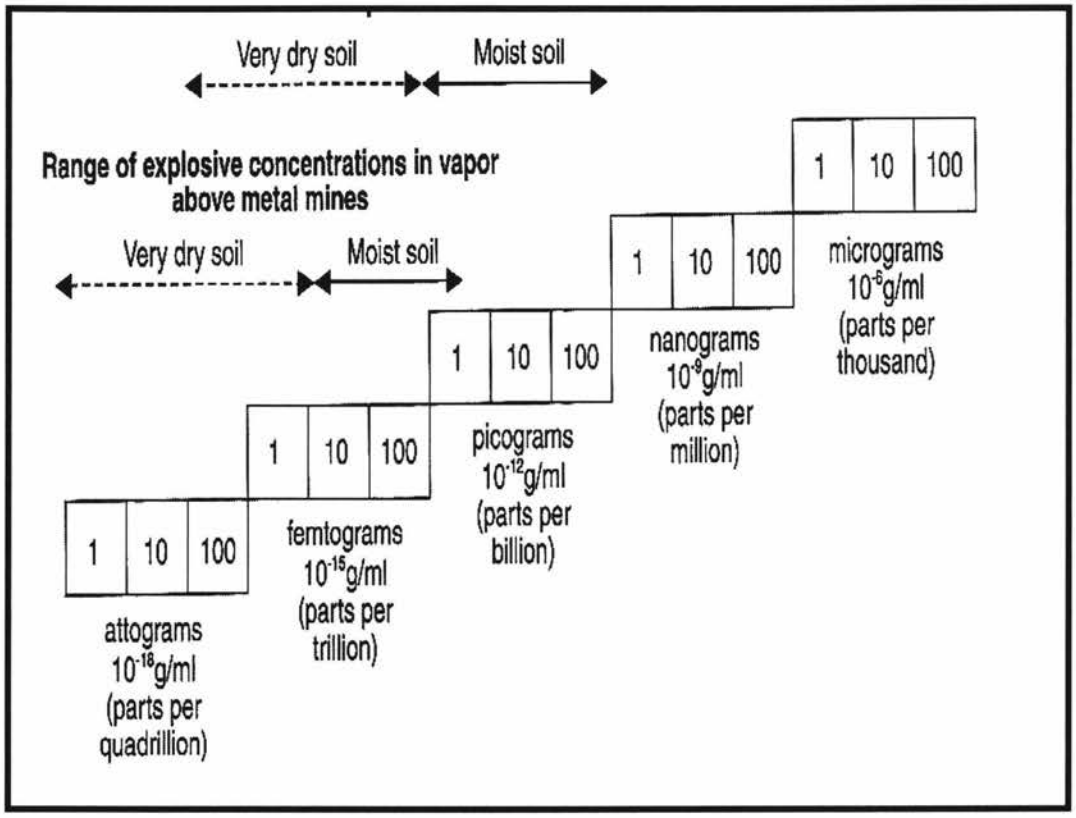


Figure 3: Required Detection Limits for Explosive Vapours[13]

Figure 3 illustrates that the detectable concentrations for explosive vapours are very low, typically ranging from ppb to parts per quadrillion (ppq).[13] The detection limits of different chemical and physical methods for landmine detection are presented in Table 1. Even the most sensitive method, fluorescence, is problematic. Interferences from naturally occurring fluorescent compounds can lead to false positives.[13, 14] Thus, the next section discusses this issue, selectivity.

Table 1: Various Sensor Categories and Detection Limits. Adopted and Modified from[13]

Sensor Category	Description	Approximate Detection Limit (g explosive per mL air
Fluorescent	Measure a change in fluorescence wavelength on the top of a polymer-coated glass fibre or on an antibody biosensor that occurs in response to the presence of explosives	10^{-15}
Electrochemical	Measure changes in electrical resistance of arrays of polymers upon contact with explosive vapours; alternatively, measure changes in electrical properties in coupled electrode pair during reduction or oxidation of explosives	10^{-12}
Piezoelectric	Measure shift in resonant frequency of various materials (thin polymer, quartz microcrystal, or other) due to mass change upon exposure to explosive vapour	10^{-11}
Spectroscopic	Compare the spectral response of a sample with that of a reference material	10^{-9}

1.2.2 Sensor Selectivity

Selectivity refers to the sensor's ability to discriminate between the target analyte and those compounds that are similar to it but not of interest. Continuing with the previous example, since interferents typically exist in higher concentration than the analyte, not only is the sensitivity of the sensor an issue but so is the selectivity. False positives will be more frequent if the sensor is not able to distinguish between the interferent and

analyte.[13] Fortunately, there are techniques available to increase both the sensitivity and selectivity of a sensor. These are discussed in detail in the following sections.

1.2.3 Linear Response to Analyte Concentration

It is important for a sensor to have a linear response to analyte concentration. The capacity of the sensor to respond over a wide range of concentrations may increase its practical use. Ideally, a linear relationship between the concentration and signal output should be observed. A deviation from a linear relationship between fluorescence intensity and concentration may be evidenced at higher analyte concentrations. Linearity is lost for various reasons but particularly due to self-quenching and the inner-filter effect.[15, 16] Self-quenching occurs because ground state analyte molecules absorb the fluorescence that is produced by the other analyte molecules.[16] The 'primary' inner-filter effect is a consequence of the incident light being absorbed before it reaches the area of the sample where luminescence is observed.[17] Thus, the linear relationship only exists at low fluorophore concentrations.[16]

1.3 Cyclodextrins

The intramolecular transglycosylation reaction resulting from the bacterial degradation of starch by cyclodextrin glucanotransferase enzyme produces a set of products that are referred to as cycloamyloses, cyclomaltoses, Schardinger dextrins, but are most commonly known as cyclodextrins (CDs).[18-20] CDs are water-soluble, non-reducing maltooligosaccharides and consist of glucose units which are linked by α-(1,4) glycosidic bonds.[18] The first generation or parent CDs are α-, β-, and γ-CD which consist of six, seven and eight α-(1,4)-linked glycosly units, respectively (Figure 4).[18, 19]

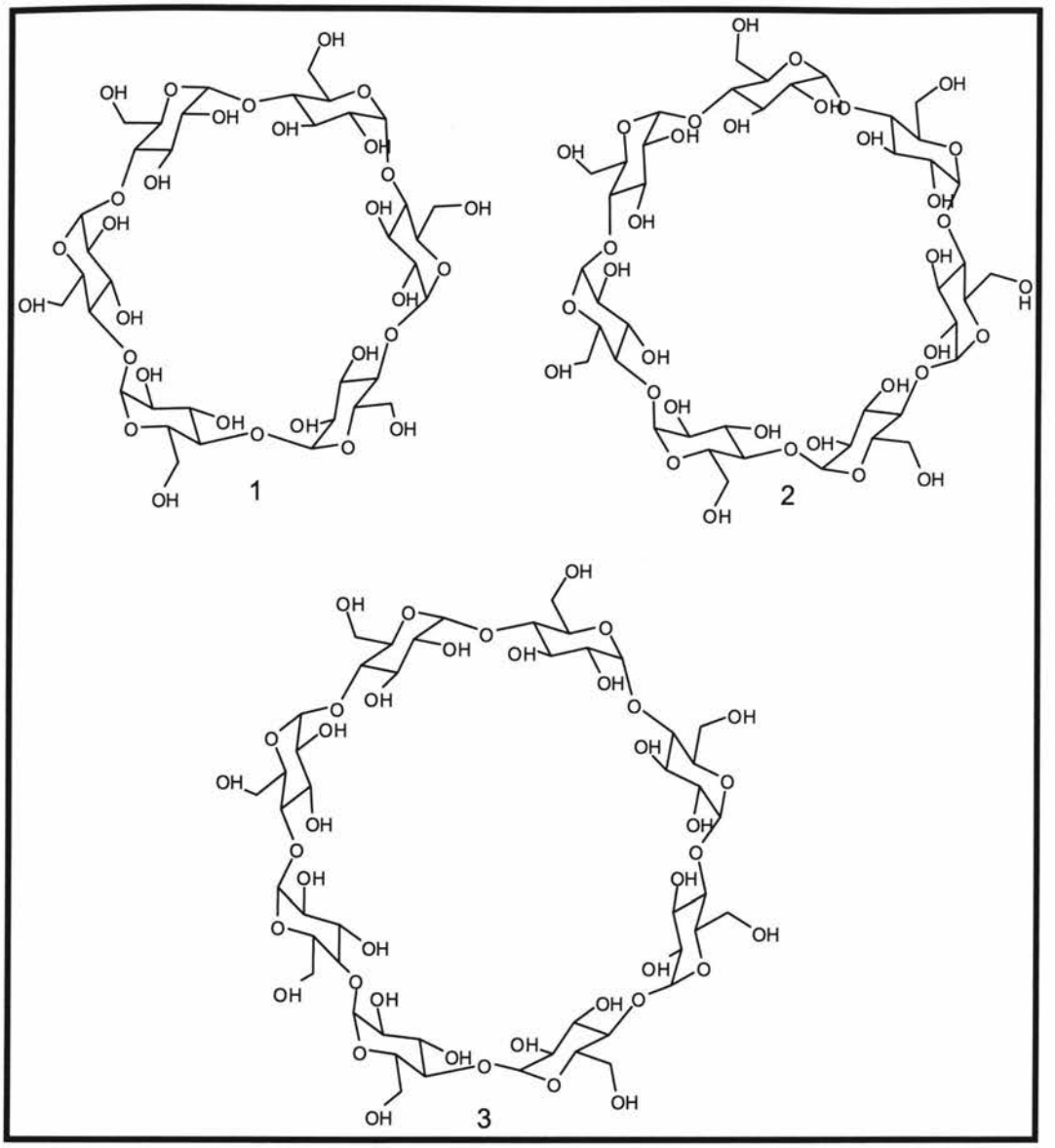


Figure 4: Structure of the Parent Cyclodextrins. 1 is α-Cyclodextrin; 2 is β-Cyclodextrin; 3 is γ-Cyclodextrin from CHEMBIODRAWULTA V.11.0

For steric reasons there are no known CDs containing fewer than six glucose residues. Conversely, there are reported higher order CDs containing up to twelve glucopyranose units.[21, 22]

The structure of CDs is defined by the location of the primary and secondary hydroxyl groups. The CD molecule is likened to a doughnut or

a truncated cone where all secondary hydroxyl groups are located on one of the two edges of the cone and all primary hydroxyls on the other edge. The primary hydroxyls form the smaller opening of the cone whereas the locations of the secondary hydroxyl groups yield the larger opening (Figure 5). The latter group is held quite rigidly whereas the primary hydroxyls can rotate and in part block the cavity. The internal cavity of a CD molecule is lined by hydrogen atoms bonded to the C-5 atom of the glucosyl units forming a ring and another ring or secondary belt where the C-2-OH group of one glucopyranoside unit hydrogen bonds with the C-3-OH group of a neighbouring glucopyranose unit. This intramolecular hydrogen bond formation explains the difference in water solubility between the parent CDs. The β-CD has the lowest water solubility in comparison to the α-, and γ-CD as the secondary belt in the β-CD is complete rendering a rigid structure. On the other hand, this belt is incomplete in the α-CD due to the distorted position of one of the glucopyranose units; thus, only four of the six possible hydrogen bonds are formed. The most soluble of the CDs is the γ-CD because of its noncoplanar and flexible structure. The water solubilities of α-,β-, and γ-CD in g/100mL are 14.5, 1.85 and 23.2 respectively. CDs are insoluble in tetrahydrofuran, chloroform, acetone, isopropanol, ethanol and methanol.[21, 23] There is also another ring that lines the cavity which is formed by the glycosidic oxygen atoms whereby the non-bonding electron pairs produce a high electron density (Lewis base characteristic). All of the above mentioned attributes account for the apolar cavity and hydrophilic exterior which are characteristic of CDs.[19, 21, 22]

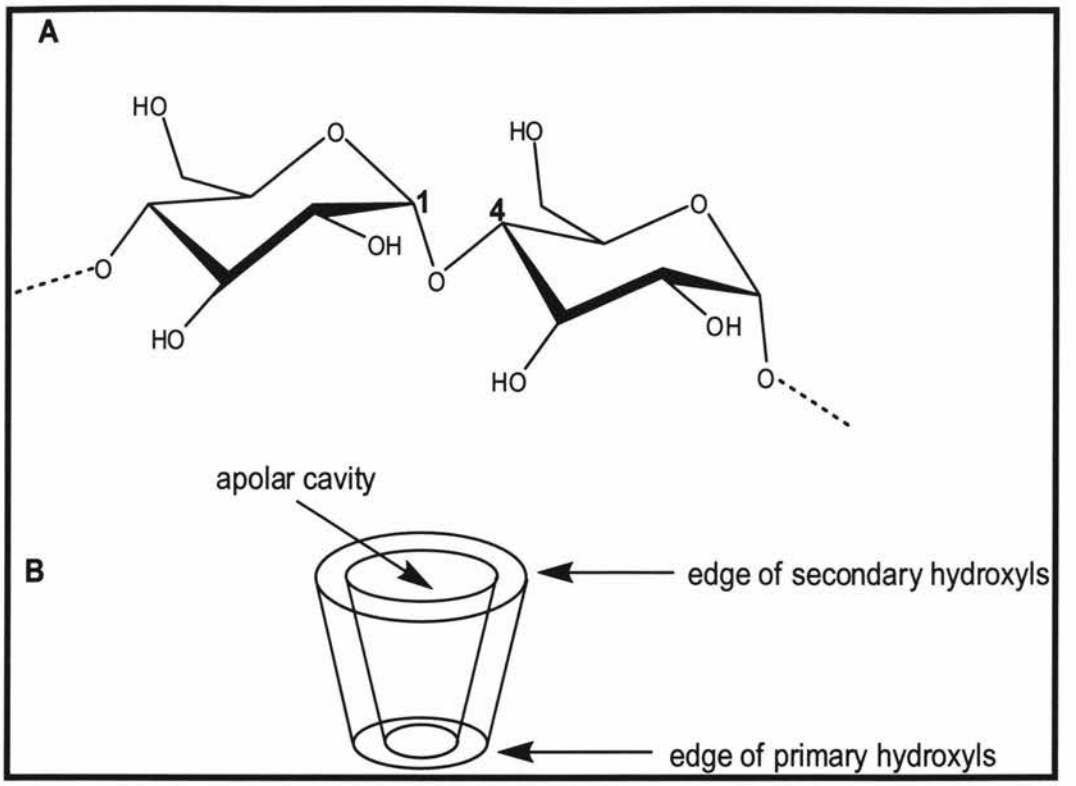


Figure 5: A) Illustration of α-1-4 Glycosidic Bond B) Location of Hydroxyl Groups [21] Adapted and Modified by CHEMBIODRAWULTRA V. 11.0

1.4 Inclusion Complexes

One of the most important properties of CDs is that they form inclusion complexes. An inclusion complex is a molecular compound formed when one compound, referred to as a host molecule, spatially encloses another, the guest molecule. The cavity of the host is often not altered, or only slightly so upon molecular complexation.[23] During the formation of an inclusion complex, (Figure 6), no covalent bonds are formed or broken and the binding is not permanent since it is controlled by non-covalent interactions. Binding is also a dynamic process.[18, 19, 22-24] In an aqueous solution the more hydrophobic guest molecules displace the water molecules that are in the CD cavity. An apolar-apolar association is

achieved along with a decrease in CD ring strain; thus, a lower energy state is achieved.[23]

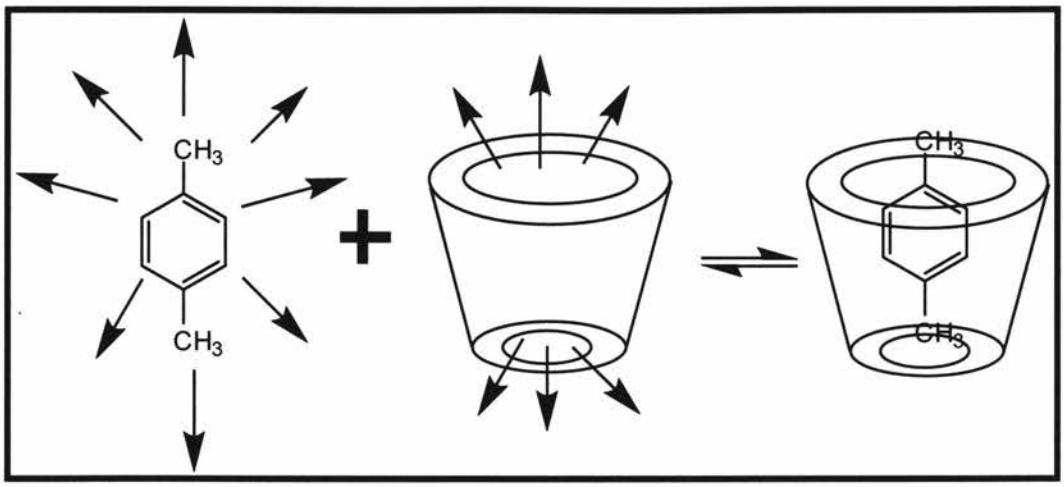


Figure 6: Schematic Illustration of an Inclusion Complex. Arrows Represent Repulsion of Water Molecules: Modified from [22] by CHEMBIODRAWULTRA V.11

Steric issues play a predominant role in determining if a CD complex will form. The size of the CD cavity relative to the size of the guest will determine if a complex between them is possible. The next important element in the formation of an inclusion complex is the thermodynamic interactions that exist between the various components of the system. A favourable net energetic driving force must exist to insert the guest into the CD cavity.[19] Interactions that may contribute to the overall thermodynamics of complex formation include: hydrophobic effects, Van der Waals interactions and hydrogen bonding.[21]

1.4.1 Ratio of Host-to-Guest Interaction In CD-Based Inclusion Complexes

The most frequently observed host: guest ratio in CD-based inclusion complexes is 1:1; however, other ratios such as 2:1, 1:2, 2:2 and other higher order equilibria are also known.[22, 25, 26] The thermodynamic equilibrium that exists between the CD and the guest, (G), in solution, is expressed in the following equation for a 1:1 complex:

$$CD + Guest \leftrightharpoons CD{:}G$$

Where CD:G refers to the cyclodextrin complex.[21-23]

The stability of the formed complex is quantitatively expressed by the following equation, (Eq.1), where the equilibrium constant is denoted by K_f:

$$K_f = \frac{[CD{:}G]}{[G][CD]}$$

The greater the value of the equilibrium constant, the more stable the complex.[21-23]

1.4.2 Changes in Guest Attributes Upon Complexation

The encapsulation of a guest molecule may change the physiochemical properties and chemical reactivity of the guest. For instance, fluorescence increases are often observed upon complexation. The increase is observed for several reasons. The fluorophore is transferred from its aqueous environment into an apolar surrounding which may impact the transition moment of an organic molecule. Furthermore, the complexed fluorophore is now protected from external quenchers, such as oxygen, and finally there is a decrease in intramolecular motion that facilitates non-radiative decay.[27] In terms of chemical reactivity, it is usually decreased upon complexation because the guest is stabilized, meaning that the inter- or intra-molecular motion that is needed for the reaction to occur may be inhibited due to the constraints of the CD cavity.[27, 28] However, in some cases the CD serves as an artificial enzyme when different reactions are accelerated and modifications in the reaction pathway may also occur.[28] Another feature that changes when the guest and CD host form a complex is a decrease in diffusion and volatility of the guest. Finally, the once hydrophobic guest becomes hydrophilic.[22]

1.4.3 Applications of Cyclodextrins

CDs have found use in various areas. For example, in chromatography they may be applied in separation methods.[29, 30] In other areas such as food processing and cosmetics they can be used to reduce odour and moisture loss.[18, 27, 31-33] Increasing the solubility of drugs is important in the pharmaceutical field and CDs have proven to be useful in this area.[33, 34] CDs also have the potential of becoming very important in the environmental field as a means of trapping pollutants.[35-38] The specific application of CDs that is evaluated in this paper is their role in sensor systems.

2.0 The Use of Cyclodextrins in Sensors

There are several attributes of CDs that lend them advantages over other sensor systems. CDs are biodegradable[22], thus from an environmental perspective they are less toxic and hazardous components than certain other substances. A certain degree of specificity for analyte detection can also be achieved because the different sizes of CD cavity available, (α, β, γ), provide the possibility of incorporating target molecules in specific size ranges into the cavity which may lead to better detection. CDs are also quite easy to functionalize. Functionalizing CDs may also potentially improve the sensitivity and selectivity of a sensor. This may be accomplished by incorporating a spectroscopically active function, such as a fluorophore, onto the CD. Now the once undetectable target molecule can be observed, for example by the changes it induces in the fluorescently labelled CD system.

2.1 Functionalizing Cyclodextrins

There are several reasons for modifying CDs, including the need to set up coordination sites to create metalloCDs and changing the external surface to make possible monolayers and micellar structures containing CDs.[39] Most important to this thesis is the introduction of photophysically

active groups for the development of chemical sensors. CDs are spectroscopically inert hosts; the addition of a chromophore, renders them spectroscopically active.[40] This modification potentially allows for the use of CDs as sensors.

Modification of the hydroxyl groups located at C-2, C-3 and C-6 does not alter the core CD cavity, (Figure 7).[21, 23] The C-6 hydroxyl groups are the most basic; C-2 hydroxyls are the most acidic; C-3 hydroxyls are the least accessible to functionalization.[41]

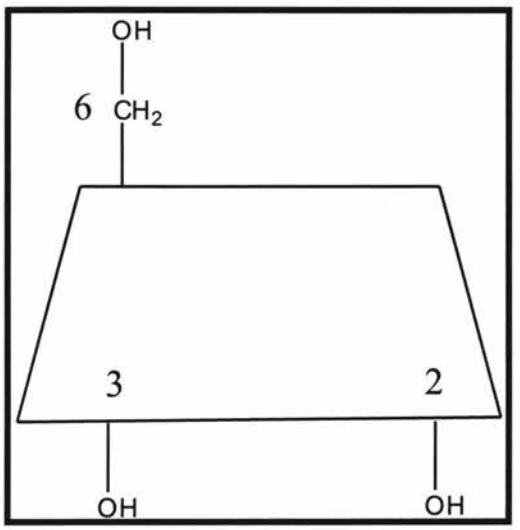


Figure 7: Schematic Illustration of Hydroxyl Groups Located on Carbon. Created using CHEMBIODRAW ULTRA V.11

The figure below (Figure 8), illustrates some of the various methods used for modifying the hydroxyl groups of CDs.

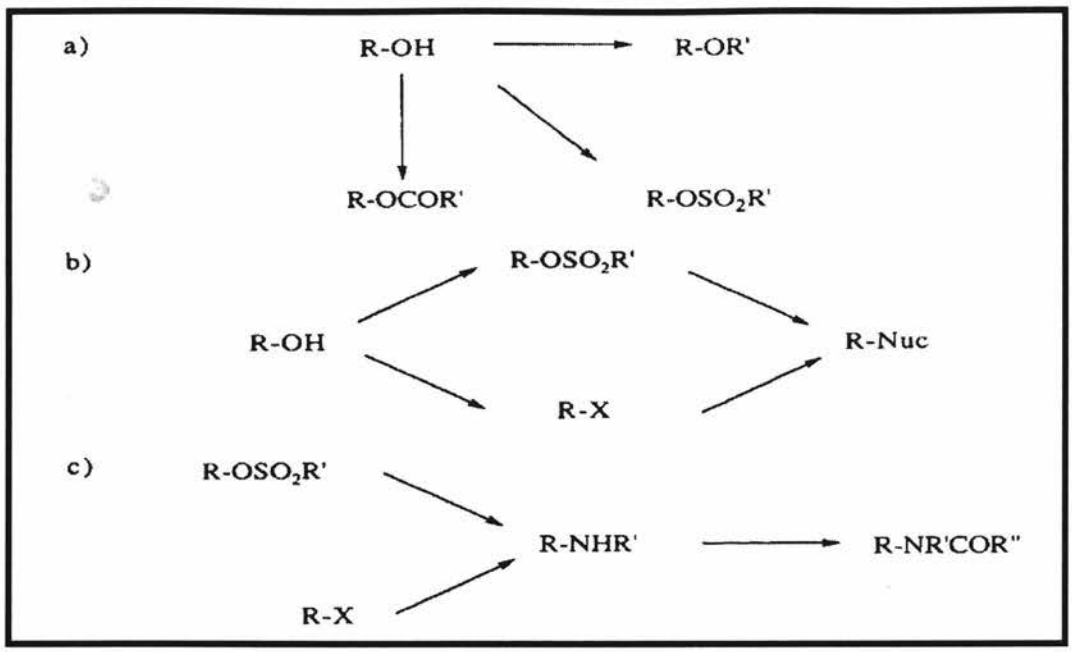


Figure 8: Various Reactions For Modifying Cyclodextrins [39]

The first reaction scheme in Figure 8 (a), is a direct method used for introducing substituents through alkylation, acylation and sulfonation. Scheme (b) illustrates the use of intermediates such as sulfonates and halides. Following this step, substituents are introduced via nucleophilic displacement. The final scheme, (c), further expands on the method outlined in (b) by incorporating amino groups which provides greater modification possibilities by providing a greater variety of nucleophilic sites.[39] These modifications alter certain characteristics of the parent CDs. For example, the solubility of CDs changes when they are derivatized which can increase their solubility in water and some organic solvents.[21, 23] Although these characteristic changes are important, the main advantage of modifying CDs is to make them spectroscopically active. These modified cyclodextrins can be used as sensors for detecting various ions and molecules

2.2 *Sensitivity and Selectivity*

Incorporating CDs into a sensor may improve the sensitivity of the system. For example, there is a fluorescence increase normally associated upon the formation of an inclusion complex.[22] For instance, a target molecule that exhibits a low fluorescence intensity is difficult to detect, but may show an increased fluorescence enhancement when it complexes with CD. Thus, a lower concentration of the target could be evaluated. Therefore, incorporating CDs into a sensor may enhance the fluorescence intensity of the target molecule making the sensor more sensitive.

When evaluating a single target molecule, the selectivity of an ideal chemical sensor is infinite, i.e. there is no noise from environmental interferents and the signal produced from the sensor is solely that of the analyte. On the other hand, an ideal chemical sensor for the detection of many analytes would contain an array of sensors. Again, each sensor would be selective to only one of the target molecules.[14] Even though achieving such an ideal sensor is very difficult, the use of CDs in a sensor system would improve the selectivity as a result of the formation of inclusion complexes which are able to selectively bind target molecules and not bind possible interferents.[42]

2.3 *Size of Cyclodextrins*

The different sized CDs also make them suitable for use in sensory systems. For example, α-CDs typically complex with compounds with long aliphatic side chains or low molecular weight molecules including benzenes. β-CDs favour complexation with aromatics varying from naphthalene to steroids and finally γ-CDs can accommodate larger molecules such as pyrene.[19] Thus, depending on the size of the analyte an appropriate CD can be used in the sensor to form an inclusion complex.

3.0 Cyclodextrin-Based Sensors

CD-based sensors can be divided into different categories. One category is the free CD, i.e. not polymerized or otherwise immobilized, using, for example, changes in fluorescence or electrochemical properties as a means of measurement. There are also immobilized CDs including coatings on surface acoustic wave devices and also polymer immobilized CDs. Each of these CD based sensors is discussed below with an emphasis placed on immobilized CDs.

3.1 The Use of Fluorescence for Cyclodextrins in Solution

Fluorimetry is a very useful analytical technique due to its low detection limits.[15] Modified CDs that are free in solution can serve as chemosensors. In many instances, a fluorophore is attached to the CD. The fluorescence is monitored and changes in the fluorescence intensity indicate the complexation between the analyte guest and the host (modified CD). There have been several reports describing the use of modified CDs for the detection of molecules using fluorescence to detect their presence.[38, 40, 43-45] Whether a decrease or increase in fluorescence intensity is observed depends on the modification of the CD. Wang and Ueno[38] used naphthol–modified β-CD as a fluorescent sensor for detecting contaminants in drinking water (Figure 9).[38] The contaminants they investigated include geosmin and 2-methylisoborneol, which are odorants, and several alkyl halides that included carbon tetrachloride and chloroform. The CDs were modified with two different naphthol moieties that resulted in different fluorescence responses.

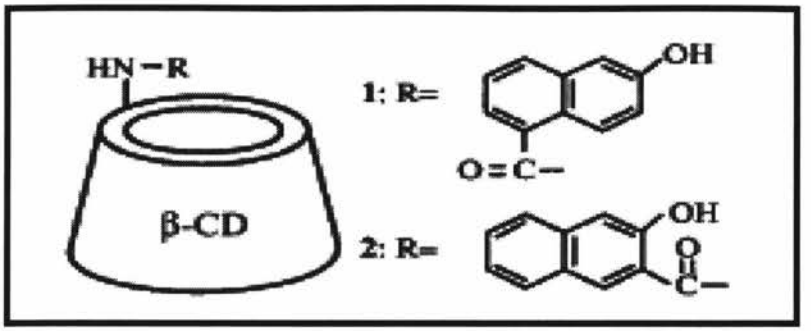


Figure 9: Substituted CD with Naphthol Moieties[38]

When the concentration of an introduced guest species was increased, the fluorescence intensity of β-CD modified with moiety 1 increased, while that modified with moiety 2 decreased. The change in fluorescence may be a result of the change in location of the naphthol moiety from inside the CD cavity to the outside of the cavity due to a guest complexing with the CD. The authors also evaluated the sensitivity of the system, estimated by the parameter $\Delta I/I_o$, where ΔI is the change in fluorescence intensity observed upon binding of the guest and I_o is the original fluorescence intensity. They also evaluated the binding constants for each of the guest analytes. The sensitivities and binding constants of the guests for each moiety is illustrated in Table 2.

Table 2: Sensitivity and Binding Constants for Moiety 1 and 2[38].

Guest	$\Delta I/I_0 \times 10^2$		K in $mol^{-1} \cdot dm^3$	
	1	2	1	2
CH_2Cl_2	1	>–1	10	12
$Cl(CH_2)_2Cl$	4	–1	60	40
$CHCl_3$	14	–3	200	155
CCl_4	191	–35	2150	2120
C_6H_6	20	–3	300	140
Geosmin[b)]	259	–93	6500	7200
2-MIB[b)]	438	–113	9800	8900

Both moieties exhibited much larger sensitivities for the geosmin and 2-methylisoborneol odorants compared to the alkyl halides. Also larger binding constants where observed with the geosmin and 2-methylisoborneol odorants indicating that a stronger complex is formed.[38] Thus, the results demonstrate that the use of modified, free CDs has potential value for the design of a sensor system that is both sensitive and selective.

3.2 Electrochemical Cyclodextrin Systems

CDs can also be used as electrochemical sensors. Carbon paste electrodes modified with CDs have been used for the determination of various molecules ranging from antidepressants to environmental pollutants including polycyclic aromatic hydrocarbons (PAH).[46-48]

Results from an experiment by Ferancová *et. al.*,[45] comparing a β-CD modified carbon paste electrode and one that was not modified are illustrative of the electrochemical approach. The analyte tested was thioridazine, a drug used in the treatment of schizophrenia, whereby a significant increase in the signal was observed for the modified electrode because of accumulation of the analyte at the electrode surface due to the presence of CD binding sites (Figure 10).[45]

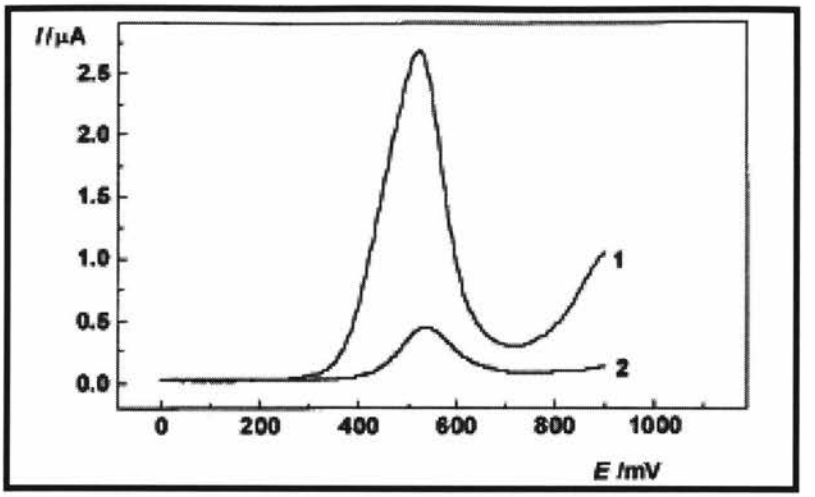


Figure 10: Differential Pulse Voltammograms of (1) β-CD Modified Carbon Paste Electrode (2) Unmodified Electrode[46]

The modified electrode showed a linear response in the range of 10^{-8} to 10^{-7} mol dm^{-3}.[46] This system represents the preparation of an inexpensive, sensitive and disposable sensor.

3.3 Immobilized Cyclodextrins

With modification of the hydroxyl groups of a CD molecule, immobilization of the CD onto a substrate can be accomplished. A commonly used supporting material is a cellulose membrane because of its inexpensive cost and availability, which is important when constructing a disposable sensor.[49, 50] The immobilization of fluorescent CDs has led to the possibility of CD-based sensory tools. As mentioned earlier, CDs are spectroscopically inert; thus, the CD is modified by the addition of a fluorophore. Tanabe *et. al.*, chose dansyl moieties as fluorophores in their research because they are well studied and characterized.[49, 50] The two dansyl-modified CDs studied are dansly glutamate-(DnsGlu-β-CD) and dansylglycine-modified CDs (DnsC4- β-CD). Figure 11 illustrates the dansyl moiety and the DnsGlu-β-CD-membrane.

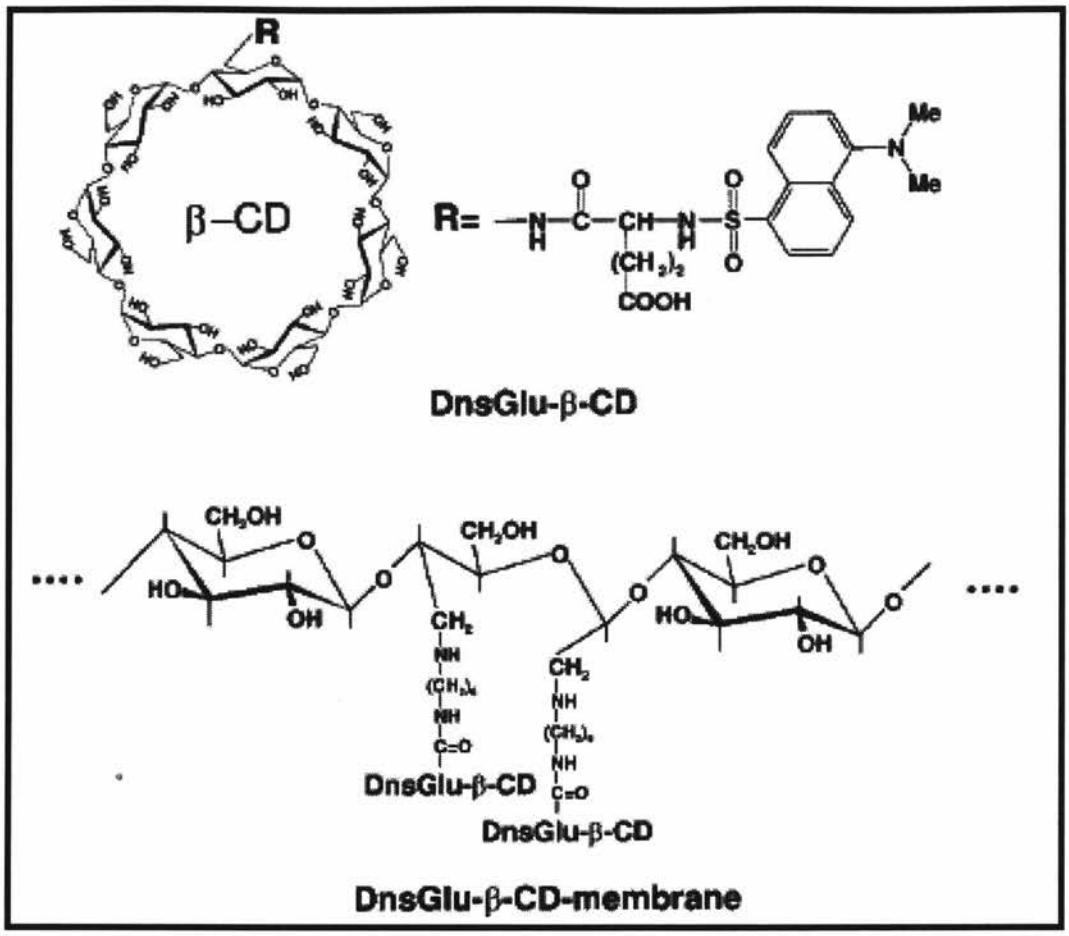


Figure 11: Structure of DnsGlu-β-CD and the Membrane[49]

Both the DnsGlu-β-CD and the DnsC4-β-CD systems show similar results in solution and when immobilized on the cellulose membrane. Fluorescence is observed in both states and a decrease in fluorescence is observed upon addition of a guest molecule. The guest molecules used in the DnsGlu-β-CD experiment included 2-adamantanol, (+/-)-borneol, deoxycholic acid, (DCA), ursodeoxycholic acid, (UDCA), hyodeoxycholic acid, (HDCA), and chenodeoxycholic acid, (CDCA),.[49] The analytes tested in the DnsC4-β-CD experiment were the same as those just mentioned but also included cyclohexanol and cholic acid and instead of 2-adamantanol, used 1-adamantanol.[50] These guests were chosen because they are all known to complex with CD.[49, 50] The reduction in

fluorescence is attributed to the dansyl-moiety being excluded from the CD cavity by the guest to the bulk water solution.[40, 49, 50] Binding constants were calculated for DnsGlu-β-CD in both solution and the membrane, but only for the membrane system in the case of DnsC4-β-CD. The binding constants for the DnsGlu-β-CD membrane were higher for the acid analytes than those determined in solution. However, a decrease in binding was observed for the (-)-borneol and an increase in binding for (+)-borneol when comparing the membrane to the solution. The possible explanation is that a free carboxyl group exists in the DnsGlu-β-CD which may account for the differences in guest binding.[49] The results of the binding experiments for the DnsC4-β-CD showed that the greatest binding strength was for the UDCA which also demonstrated the highest sensitivity.[50]

Recovery of fluorescence was evidenced when the membrane was washed and conversely no fluorescence was observed in the washed solution; thus a reversible system had been synthesized. This also indicates the dansyl-modified CD is immobilized on the cellulose membrane.[49, 50] From the point of constructing a CD-based sensor, immobilized CDs may have more practical applications than free CDs.

3.4 Cyclodextrin Polymers

The use of CD polymers in sensor systems has several advantages. The making of a polymer is a relatively simple and low cost process that does not normally require the use of special clean rooms or processes that require high temperatures. They can be deposited on several different substrates using various methods such as spin-coating, spraying, dip-coating, vacuum deposition processes and the Langmuir-Blodgett technique.[51]

Cyclodextrin polymers are often prepared by a polycondensation reaction using di- or polyfunctional compounds such as aldehydes,

ketones, allyl halides, and ethyleneglycol to name a few.[23, 36, 52] The most popular and well studied bifunctional cross-linking agent is epichlorohydrin, (Epi), despite it being a potential carcinogen and a hazardous environmental pollutant.[21, 23] The polymerization occurs under basic conditions, (NaOH), and a proposed mechanism is illustrated in Figure 12.

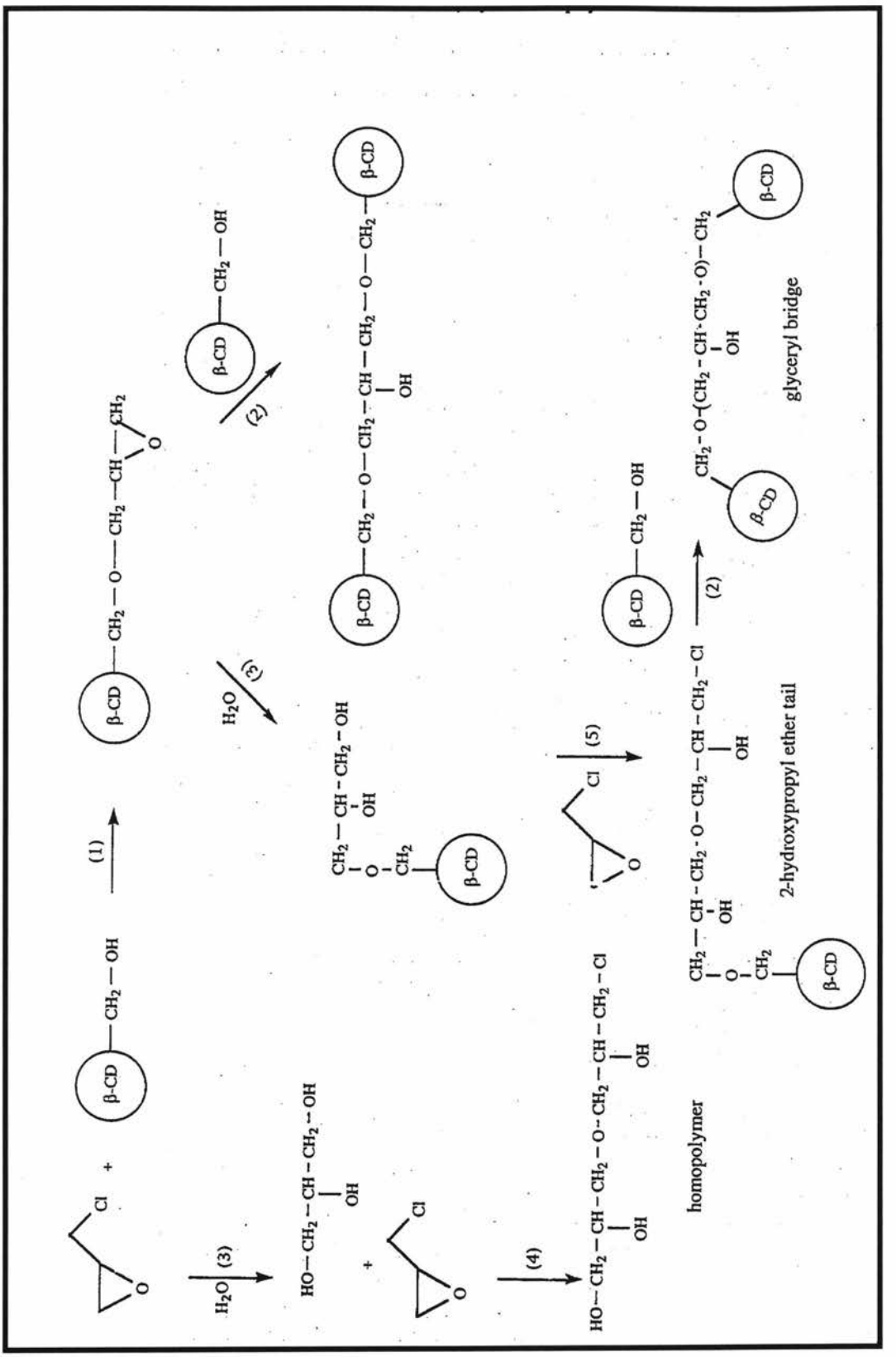


Figure 12: Condensation Reaction of β-CD with Epichlorohydrin[53]

Since Epi contains two reactive functional groups, there exist two possible structures: A network of crosslinked cyclodextrins and/or formation of a polymerized Epi chain.[21, 35] The amount of Epi added to the system will determine the water solubility of the polymer with larger amounts leading to an insoluble polymer due to extensive cross-linking.[21, 36, 53] However, the ratio of Epi to cyclodextrin is not the only variable that determines the solubility of the polymer. Other factors such as sodium hydroxide (NaOH) concentration and temperature play a role.

It has been determined that if the NaOH concentration is greater than 50% the precipitation of CD occurs instantly. On the other hand, at lower NaOH concentrations the polycondensation reaction is supported. An intermediate concentration of 33% NaOH has proven to provide the most favorable environment for the reaction. The highest ratio of Epi: CD content in the polymer and the highest percentage of CD content occurs with a NaOH concentration less than 16%. However, the reaction time under these conditions is extremely long (60 hours). At a NaOH concentration of 33% the reaction time is significantly decreased to approximately four hours yielding a product with a Epi:CD ratio of 14.3 and CD content of 52%. The dramatic difference in reaction rates with varying NaOH concentrations is a result of the relative reactivities of the hydroxyl groups located at C-2, C-3 and C-6.[53]

The most commonly employed and sensitive method for determining the substitution site on the CD is ^{13}C-NMR. Results of ^{13}C-NMR studies have demonstrated that at NaOH concentrations less than 33%, substitutions occur at all three-mentioned hydroxyl groups. However, when the concentration is increased to 33%, the resonances of C-2 and C-3 are not affected, but the resonance of the C-6 is shifted indicating that substitution is occurring predominately on the primary alcohols. These results explain why at lower NaOH concentrations there

is a higher CD content and lower Epi:CD in the polymer and extended reaction time.[53]

^{1}H NMR is also used to determine, approximately, the amount of β-cyclodextrin that is in the polymer.[53-55] The anomeric proton attached to the C-1 of the glucose unit is not available for any substitution.[53-55] This proton appears at 5.04 ppm whether the β-cyclodextrin is in its native form or polymerized.[53-55] Since this peak is well resolved from the hydroxypropyl ether linker resonances, it is commonly used to estimate the amount of β-cyclodextrin incorporated into the synthesized polymer. For example, Sainz-Rozas *et al.*,[55] found that the β-cyclodextrin water soluble polymer they synthesized contained 64% β-CD for a low molecular weight polymer and 42% for a higher weight polymer.[55]

Temperature does not seem to have as significant an effect on the reaction as the NaOH concentration. However, at higher temperatures, (90°C), the reaction does occur faster.[53]

β-CD-Epi cross-linked polymers can be used to trap organic pollutants.[36, 37, 56] The detection of environmental contaminants is of concern and the use of these polymers in sensor systems is an emerging technique that shows great promise. For example, an insoluble β-CD-Epi cross-linked polymer was synthesized for the determination of Bisphenol A (BPA), an environmental pollutant, which demonstrated great potential for being adapted from the laboratory to the field.[56] The sensing membrane solution was prepared by dissolving the β-CD-Epi polymer in poly(vinyl chloride), (PVC), and di(2-ethylhexyl) sebacate (DOS) and tetrahydrofuran (THF)-chloroform mixed solvent. The results illustrated that without the β-CD-Epi polymer in the PVC membrane the fluorescence of BPA is weak. On the other hand, the incorporation of the polymer significantly increased the fluorescence emission, which signals the incorporation of BPA in the CD cavity.[56] Other features including high selectivity and reversibility make this system very plausible for field use.

3.5 Surface Acoustic Wave

The sensing mechanism used in acoustic wave sensors depends on changes in the properties of a mechanical or acoustic wave transmitting on or through a material. The velocity and/or amplitude of the acoustic wave is affected when any modifications occur in its propagation path as it transmits on or through the surface of the material. In order to monitor the changes in velocity, the phase or frequency characteristics of the sensor are measured. The substances that are most commonly used in sensors to produce the acoustic wave are piezoelectric materials. The most frequently utilized piezoelectric substrate materials are quartz and lithium tantalite.[57] A mechanical stress is created when an electrical field is applied to a piezoelectric material and vice versa, an electric field is created when a mechanical stress is applied.[57]

Surface acoustic wave sensors, (SAW), are one of the most commonly utilized surface wave devices. These devices are made up of comb like electrode patterns which are referred to as inter-digitized transducers (IDT). These IDT are placed onto a piezoelectric substrate in a particular orientation. The electrodes serve to send a surface acoustic wave across the device surface. A selective absorbent film for a specific molecule can be deposited on the surface of the device; thus, increasing the sensitivity (Figure 13).[57-59] The selective layer is a crucial feature since it is responsible for producing the primary signal when the analyte contacts the sensing layer.[60]

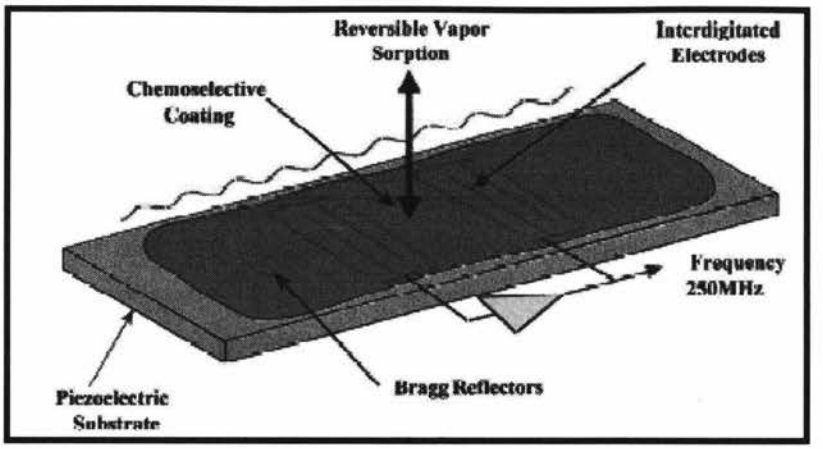


Figure 13: Basic Components of a SAW Sensor [59]

The sensitivity of the sensor depends on two principal features. Firstly, it involves the affinity of the sensing layer toward the analyte and secondly, on the ultimate sensitivity of the SAW device itself.[9]

CDs as films and monolayers have been applied to SAW devices.[9, 61] Self-assembled monolayer films (SAM), which are simply ordered monolayers of organic compounds, such as CDs, on a surface, have also been applied to SAW devices. The following figure illustrates a CD monolayer on a SAW microsensor.[9]

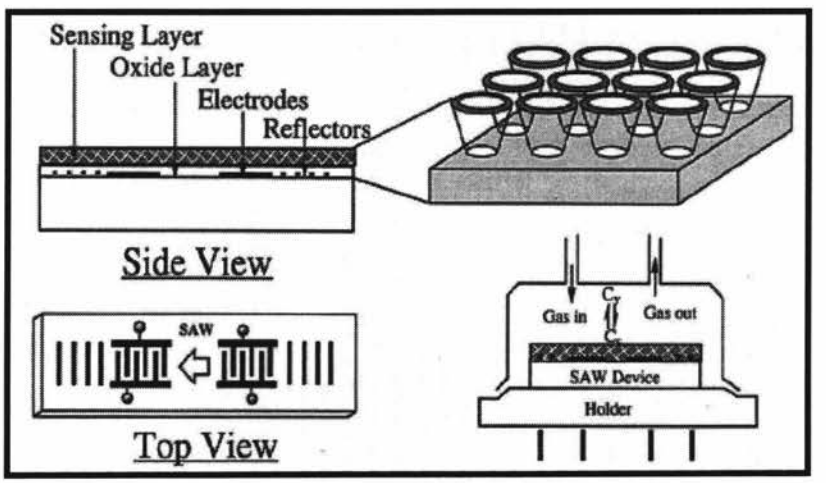
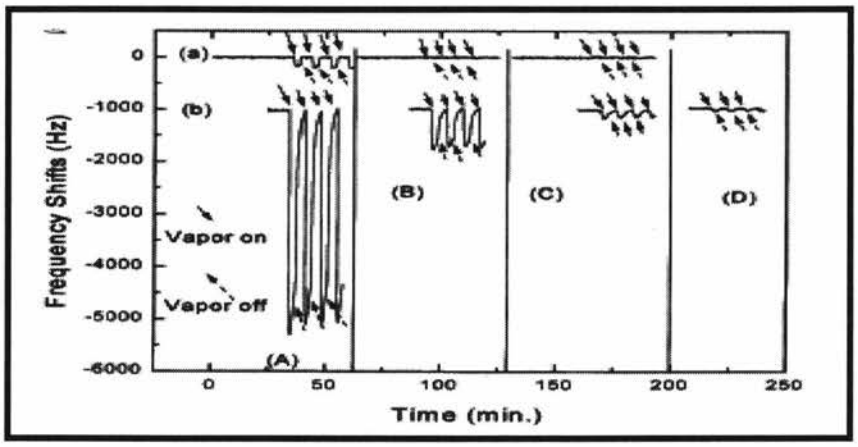


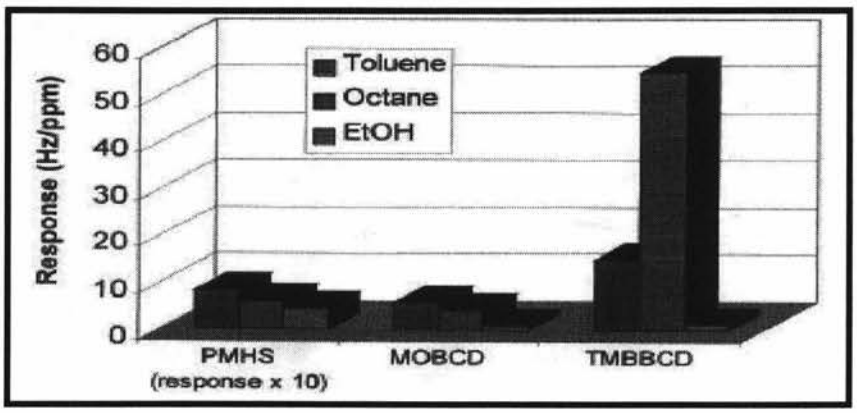
Figure 14: Self-Assembled Monolayer on a SAW Microsensor [9]

Results from a study conducted by Li and Ma who employed the use of monolayers on a SAW device observed a detection limit in the range of 50-5 ppm when analyzing organic molecules.[9]

In a study conducted by Yang *et. al.*,[61] two films were prepared and each was applied to the transducer surface. One of the films was a polymethylhydrosiloxane, (PMHS), and the other film incorporated permethylated β-CD, (MOBCD) or 2,4,6-trimethylbenzyl substituted β-CD, (TMBBCD). The films that incorporated the CDs showed both greater sensitivity and selectivity to the analyte, which was *o*-nitrotoluene, than the SAW coated device with only PMHS. The following two figures validate the incorporation of CD.



A



B

Figure 15: (A) Frequency Shifts to Polymers Exposed to Analyte (B) Film Selectivity[61]

In Figure 15 (A), the capital letters A, B, C and D refer to *o*-nitrotoluene concentrations of 6ppm, 600ppb, 60ppb and 6ppb respectively. The lower case, "a", refers to the device coated only with the PMHS polymer and the "b" refers to the MOBCD film. As is evident from Figure 15 (A), the device coated with only the PMHS polymer shows no response, as evidenced by the frequency shift, to the analyte at a concentration of 600 ppb. However, the film including the MOBCD illustrates high sensitivity even at the lowest concentration.

The difference in the selective nature of the films is illustrated in Figure 15 (B). The first bar represents toluene, the second is octane and ethanol is the third. The TMBBCD sensor coating offers the greatest selectivity and sensitivity of all three films. This film is able to discriminate between polar vapours and hydrocarbons and is less sensitive to aromatics than to alkanes. Thus, molecular recognition is important in improving the sensitivity and selectivity of films for the use of chemical sensors.[61]

3.6 Fluorescence Quenching

A key tool in this study will be the measurement of fluorescence and its quenching by model explosive analytes as a sensor mode (see below, section 3.6.1). This sub-section briefly outlines the concepts of fluorescence and fluorescence quenching.

3.6.1 Principles of Fluorescence

The primary method of quantifying analyte interaction with the sensor material in this study was fluorescence spectroscopy. Fluorescence is a well established analytical technique for organic molecules that have a fluorophore.[62] In fluorescence measurements, a solution containing fluorescent molecules is excited by a beam of photons to a higher electronic energy state. The relaxation of the molecule back to its ground state results in a release of energy in the form of a photon.

When there is no change in the electron spin, the process is referred to as fluorescence.[62]

In more technical terms, the molecule has a total spin angular momentum of S. If all the electrons in a molecule have their spins paired, then the compound is said to have a multiplicity of 1 and an S value equal to zero. A molecule with this type of configuration is said to be in the singlet state. The lowest energy singlet state is usually also the ground state of an organic molecule and is denoted as S_0. When an electron absorbs energy from a photon, it is excited to a higher energy level. Provided that the excitation does not change the electron spin, the total spin angular momentum is still zero and this first excited state is also a singlet state, called S_1. The excitation of the electron places it in an excited vibrational energy level of S_1. A process of vibrational relaxation occurs via collisions with solvent molecules to bring the electron down to the lowest vibrational energy level of S_1. At this stage, fluorescence occurs if the electron undergoes a radiative transition across the energy gap between the lowest energy vibrational level of S_1 (excited state) and a vibrational energy level of S_0. A fluorescent transition results in the release of the majority of the previously absorbed energy. After fluorescence, the electron is not in the vibrational ground state of S_0; rather, it is at a higher vibrational energy level, and must relax through collisions with solvent molecules.[62] A simplified Jablonski diagram serves to summarize and clarify this process:

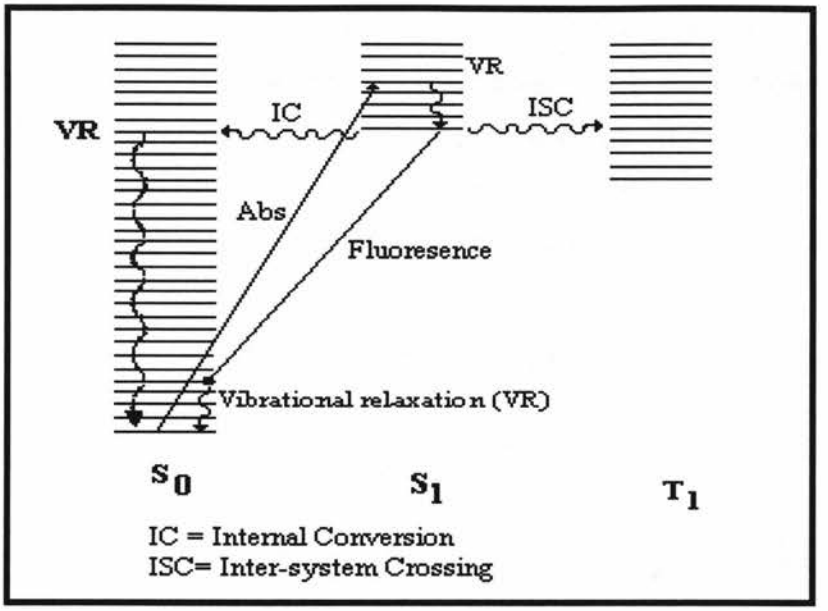


Figure 16: Simplified Jablonski Diagram[62]

For any fluorescent molecule, the fluorescence emission occurs with a specific intensity (I). The numerical value for I is dependent on several factors, one of which is concentration. However, fluorescence is not the only energetic pathway for relaxation. A molecule can relax via inter-system crossing, internal conversion (Figure 16), or quenching by dissolved oxygen. Energy lost through any of these pathways will result in lower observed fluorescence intensity.

3.6.2 Fluorescence Quenching

Quenching is a process whereby initial fluorescence of a substance is decreased via interaction with another molecule (the "quencher").[15] A quenching process can occur via various routes, which include: excited state reactions, energy transfer, charge transfer, electron transfer.[15] In addition to these quenching mechanisms, there are two broad modes of quenching behaviour; dynamic quenching and static quenching. These two processes will be described in detail because they are believed to be most applicable to the present work.

Dynamic quenching is also known as collisional quenching. This process is dependent upon the lifetime of the fluorophore in the excited state. If the quencher diffuses to the fluorescing substance during this specific time period and makes contact, a photon will not be emitted; consequently, there will be no observable fluorescence as a result of this diffusional encounter.[15] This process can be described mathematically by the Stern-Volmer, (S-V), equation (Eq.2):

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q]$$

Where F_0 is the fluorescence intensity in absence of quencher
F is the fluorescence intensity in presence of quencher
k_q is the bimolecular quenching constant
τ_0 is the lifetime of the fluorophore in absence of quencher
$K_{SV} = k_q \tau_0$ = Stern-Volmer quenching constant
[Q] is the concentration of the quencher [15].

K_{SV} is the slope of the plot of F_0/F versus the quencher concentration. If there is no deviation from linearity in the Stern-Volmer plot, most probably one fluorophore class exists in the sample and all fluorescent sites are equally available to the quencher.[15] The bimolecular quenching constant, k_q, can also be obtained if the lifetime of the unquenched fluorescent state is known; thus providing useful information regarding the quenching efficiency or fluorophore accessibility.[63]

A linear Stern-Volmer plot often reflects dynamic quenching but is not in fact diagnostic for it. A linear plot may also be observed if static quenching is at play.

Static quenching follows the same principle described by Equation 2 with the exception that the quenching constant becomes identified with the association constant of a complex between the ground state fluorophore and the quencher.[15, 63] The mechanism of this process does

not involve the excited state directly as with dynamic quenching but rather a non-fluorescent complex is formed. The fluorescence that is observed in samples subject to static quenching is due to the fraction of fluorophores that are not complexed.[15, 63] Note that there is a second form of static quenching in which any fluorophore which finds itself having a quencher molecule within a certain volume known as the "sphere of action" will be subject to static quenching.[15] This mechanism usually only becomes important at high quencher concentrations as the sphere of action volume is quite small.

The following figure, (Figure 17), schematically represents the differences between the two quenching processes.

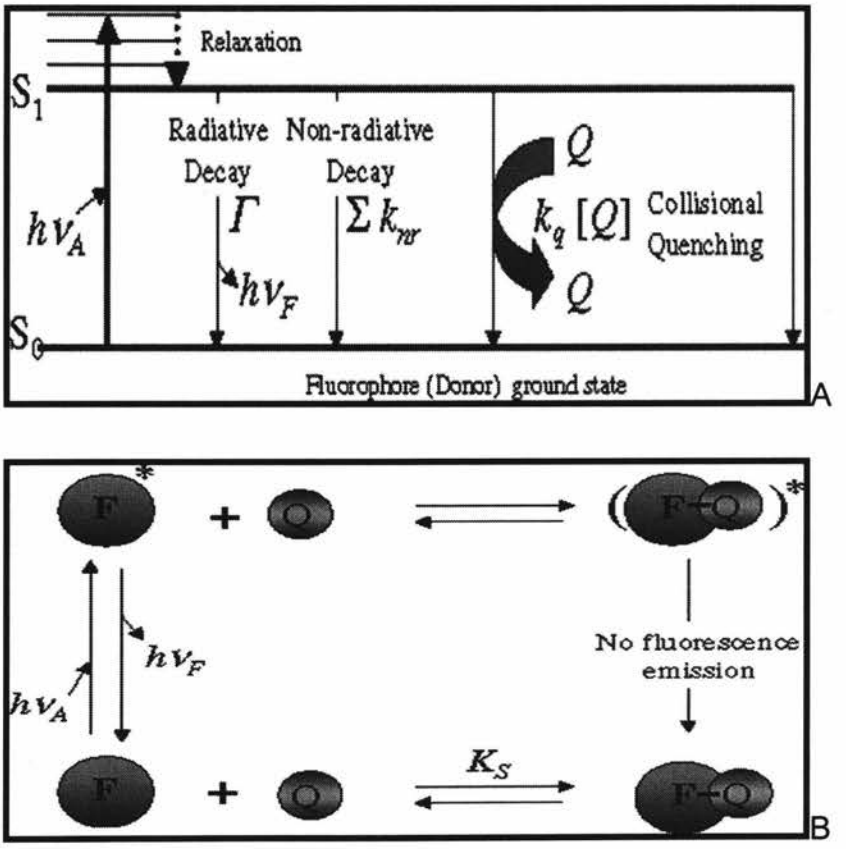


Figure 17: Schematic Illustration of the Differences Between Dynamic and Static Quenching. A) Dynamic Quenching B) Static Quenching[63]

3.6.3 Differentiating between Dynamic and Static Quenching

3.6.3.1 Temperature Experiment

Steady-state fluorescence quenching data alone are not sufficient to determine which quenching process is at work. Additional experimental work is required and one of the simplest methods is to perform quenching studies at an elevated temperature.[15] If static quenching is the process involved the quenching constant will decrease since the non-fluorescent ground state complex is destabilized. Conversely, if the quenching is predominantly dynamic, the increase in the diffusion rate with increasing temperature will result in enhanced quenching efficiency.[15, 63]

3.6.3.2 Lifetime measurements

Fluorescence lifetime measurements are also used to differentiate between dynamic and static quenching. For dynamic quenching, the ratio of the fluorophore lifetime in the absence and presence of the quencher is related to fluorescence measurements by the following equation:

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} \quad \text{Eq. } 3^{15}$$

This is not the case if static quenching is present. The lifetime measurements for static quenching would yield:

$$\frac{\tau_0}{\tau} = 1 \quad \text{Eq. } 4^{15}$$

This difference relates back to the definition of each process. Dynamic quenching involves the diffusion of the quencher to the fluorophore in the excited state to depopulate it. On the other hand, static quenching is independent of fluorescence lifetime since a non-fluorescent ground state complex is formed, leaving the uncomplexed fraction unperturbed.[15]

4.0 Objectives of the Thesis

There are up to 100 million landmines worldwide.[3] There are various techniques used for landmine detection including biological sniffers, simple visual inspection, hand-held metal detectors and chemical detection.[64] Each technique has certain advantages but also several limitations. The majority of landmines still contain nitro-aromatic explosives such as TNT.[3] The ultimate goal of this project is to develop a thin, reusable, fluorescent β-cyclodextrin polymer film for the detection of nitro-aromatics. The film should be highly selective and sensitive to nitro-aromatics. The purpose of developing such a film is for the potential use as a chemosensor for the detection of landmines. While the importance of clearing landmines has obvious security implications, the contamination of soil and ground water by nitro-aromatic explosive residues from landmines, unexploded ordinances and weapons storage sites is also a concern

The scientific concept selected to achieve this goal is to design a polymer material that contains both a *trapping function* (selective capture of contaminant molecules) and a *sensing function* (respond to contaminants). Selective trapping will give the polymeric sensor a higher sensitivity (lower detection limit) compared to non-trapping sensors. Our specific sensor designs are built upon fluorescent polymers incorporating cyclodextrin (CD) cavities. The CD-polymer will be coated on glass (i.e., fibre optics) and the fluorophore performs the sensing function (the observable is a reduction in fluorescence intensity due to quenching by an analyte) while host-guest interaction of CD cavities with contaminants provides the trapping function (Figure 18).

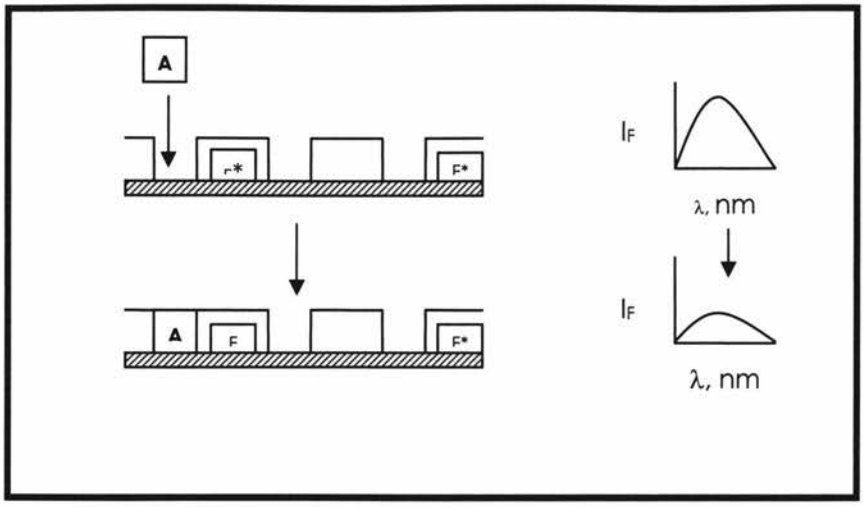


Figure 18: Schematic Illustration of Polymer Sensing by Quenching of CD-Polymer Fluorescence. "A" Refers to the Analyte of Interest

The first step towards achieving the goal of a robust and sensitive CD-based polymeric sensor is to develop a synthetic method for producing the material. The initial objective of the thesis is, thus, to optimize the preparation and clean-up of the polymer. In this case, the material is an epichlorohydrin crosslinked β-cyclodextrin polymer in which fluorescent moieties of β-naphthol are included. Furthermore, upon completion of the initial objective, the material must be examined to ensure it remains sufficiently fluorescent to be useful in a sensor design. If, after cleaning the polymer, the fluorescence remains adequate, quenching studies will be conducted in order to ensure the material's fluorescence can be quenched by a model nitro-aromatic, such as nitrobenzene, and if this quenching is enhanced by the prepared polymer system.

5.0 Materials and Methods

All chemicals were used as received without further purification. The β-cyclodextrin hydrate, epichlorohydrin, (99%), 2-naphthol, (99%), D_2O, (99.9%), and nitrobenzene (99 %), were purchased from Sigma-Aldrich. The sodium hydroxide and hydrochloric acid were purchased from BDH and J.T. Baker respectively. Both acetone and ethanol were DGA – grade purchased from Fisher Scientific. The KBR was purchased from Aldrich and used as received. The water used was purified through a Millipore –Milli-Q- Academic system equipped with two cartridges. At the point-of-use the water was filtered through a 0.2μm hydrophilic PVDF filter.

5.1 Preparation of β-cyclodextrin-2-Naphthol-epichlorohydrin polymer

The procedure for preparing a water-soluble polymer was adapted from Renard *et al.,* and modified.[53] The first step consists of solubilisation of β-cyclodextrin, (see Table 3), in 15mL of 33% sodium hydroxide solution in a 125 mL Erlenmeyer flask. The solution is maintained in a water-bath at 65°C while stirring at 500rpm using an IKA RCT basic hotplate/stirrer coupled to an IKA ETS-D4 fuzzy electronic temperature control. Secondly, the 2-Naphthol, (see Table 3), is added to this solution. Once the solution of cyclodextrin and naphthol is solubilised, the cross-linker, epichlorohydrin, (see Table 3), is added all at once through a dropping funnel attached to a condenser. After the addition of epichlorohydrin, the water bath is isothermally maintained at 65°C for 5 hours and then reduced to 30°C for 19 hours. The polymer is precipitated with acetone and the supernatant is discarded. Minimal amounts of water are added to the precipitate which is then neutralized with HCl. Acetone is used again to precipitate the polymer. The precipitate is then dried in a Fisher vacuum oven at 65°C for 24 hrs after which it is ground using a mortar and pestle.

5.1.2 Preparation of: 2-Naphthol-Epichlorohydrin Polymer

The 2-naphthol-epichlorohydrin-polymer synthesis is procedurally identical to that of a β-cyclodextrin-2-naphthol-epichlorohydrin-water-soluble polymer excluding the addition of β-cyclodextrin.

5.1.3 Preparation of β-cyclodextrin-epichlorohydrin water-soluble polymer

The control, β-cyclodextrin-epichlorohydrin water-soluble polymer, is prepared under the same conditions as those described above, excluding the addition of 2-naphthol. The amounts of each substance used for the above described polymers are shown in Table 3.

Table 3: Recipes for the Different Polymers Synthesized. The Columns are Molar Ratios

Polymer	B-CD:NOH	Epi:CD	Epi:NOH
P1	1:1	15:1	15:1
P2	2:1	15:1	29:1
P3	3:1	15:1	44:1
P4	4:1	15:1	58:1
P5	5:1	15:1	73:1
P6	6:1	15:1	87:1
P7	7:1	15:1	102:1
P8	N/A	N/A	15:1
P9	N/A	N/A	29:1
P10	N/A	N/A	44:1
P11	N/A	N/A	58:1
P12	N/A	N/A	73:1
P13	N/A	N/A	87:1
P14	0.3:1	48:1	16:1
P15	3:1	20:1	61:1
P16	5:1	20:1	102:1
P17	N/A	9:1	N/A

5.2 Polymer Cleaning

The polymers were cleaned to remove any unreacted materials via a Soxhlet extraction on a GCA/Precision Scientific apparatus. The sample

was wrapped in a Whatman #52 filter paper and then placed in a Whatman cellulose double thickness extraction thimble. The Soxhlet was run for a total of 7 days. Every 24 hours the round bottom flask was removed and solvent evaporated using a Brinkmann rotovapor R110. The fraction of material remaining in the flask was removed and dried in a vacuum oven for 24 hrs at 65°C. Fresh ethanol was added to the round bottom flask and extraction continued. The material remaining in the thimble was removed and dried after day 3 and day 7.

5.3 Instrumentation

A PerkinElmer LS50B luminescence spectrometer was used to study the polymer fluorescence in solution using a standard 1cm x 1cm quartz cuvette with a path length of 10mm purchased from VWR. All initial polymer stock solutions were prepared at a concentration of 0.1% (w/v) and diluted to a final concentration of 0.01% (w/v) for fluorescence measurements. The spectrometer parameters used are listed in Table 4.

Table 4: Parameters For Fluorescence Study

Parameter	Setting
Excitation wavelength	280 nm
Excitation slit width	5.0 nm
Emission slit width	variable
Scan speed	100 nm/sec
Scan start-Scan end	320 nm – 520 nm
Emission wavelength monitored	354 nm free 2-naphthol (protonated) 350 nm polymer 420 nm free 2-naphthol (deprotonated)

A Perkin Elmer Spectrum One Fourier Transform Infra-Red (FTIR) spectrometer was used to compare the prepared polymers to those in the literature. A KBr pellet of the polymers was prepared and scanned from 4000 cm^{-1} to 450 cm^{-1} at a scan speed of 0.20 cm^{-1}/sec.

^1H NMR samples were prepared in D_2O. A Bruker 400Hz instrument was used for ^1H NMR analysis. The amount of sample used for this analysis was strictly dependent on two factors: sample amount available after the fraction utilized in the fluorescence studies and solubility in D_2O.

5.4 Quenching Studies

A stock solution of 5mM nitrobenzene in water was prepared. The "in cuvette" concentration used in the quenching studies ranged from 0.005mM-0.05mM. The initial fluorescence of each polymer solution was measured and then sequential additions of the quencher were added via injections with a Hamilton microlitre syringe, while the solution was stirring. One minute after the quencher was added the fluorescence was monitored.

6.0 Results

The β-cyclodextrin-β-naphthol-epichlorohydrin polymers were prepared by first solubilising β-cyclodextrin and β-naphthol in basic (NaOH) solution. After this step was completed the cross-linker, epichlorohydrin, was added. The β-cyclodextrin-epichlorohydrin polymer and the β-naphthol-epichlorohydrin polymers were prepared in the same fashion, excluding the β-naphthol and β-cyclodextrin monomers, respectively. After the addition of the cross-linker, the temperature of the solution was held constant at 60°C for 5 hrs and then 35°C for 19 hrs. The polymer was precipitated with acetone and neutralized with HCl. The precipitate was dried in a vacuum oven at 65°C for 24 hrs. All the polymers were cleaned via a Soxhlet apparatus using ethanol for a period of 7 days.

Different starting ratios of cross-linker to β-cyclodextrin and cross-linker to β-naphthol were attempted. However, many of these led to water-insoluble polymer systems. There were two main problems with the water-insoluble polymers. Firstly, establishing whether the β-naphthol was permanently bound to the polymer network or just trapped in the network itself could not be decisively determined by the analytical methods employed. Secondly, the coatings onto glass-slides using a simple dipping technique resulted in unevenly coated slides. Thus, in order to determine the location of the β-naphthol in the β-cyclodextrin-epichlorohydrin polymer system, water-soluble polymers were produced. Also, it is believed that a more evenly distributed slide would result with water-soluble polymers.

The compound used to fluorescently label the epichlorohydrin-CD polymer in this study was β-naphthol. β-naphthol, also commonly referred to as 2-naphthol, (2-NOH), is a fluorescent pH sensitive, ambident nucleophile.[65] 2-naphthol is somewhat water-soluble In water.[69] 2-NOH

exhibits two emission maxima which are dependent upon the pH of the aqueous solution. The p*Ka* of 2-NOH in the ground state is 9.2, but decreases to 2.0 in the excited state.[15] If the solution is acidic, emission at 354nm for the protonated form is observed; under basic conditions the fluorescence of the naphtholate anion at 420nm is observed.[66,67] In a solution with an intermediate pH, emission from both species is observed.[68,69] By contrast, alkyl substituted analogues of 2-NOH, such as 2-methoxynaphthalene, exhibit only a single fluorescence maximum at a wavelength close to that of the protonated 2-NOH emission. This pH dependent characteristic of the 2-NOH emission behaviour can be used to provide evidence for covalent association of 2-NOH with the epichlorohydrin-crosslinked β-cyclodextrin polymer.

In order to determine if the prepared β-cyclodextrin-2-naphthol-epichlorohydrin polymer exhibited properties of pH dependence, its fluorescence was monitored before and after cleaning. The fluorescence spectrum of the polymer was compared to that of 2-naphthol and 2-methoxynaphthalene to help determine if the polymer was undergoing O or C alkylation as a mode of attachment for the 2-NOH moieties.

Figures 19 and 20 show typical fluorescence spectra obtained in water for polymer P1 before and after exhaustive clean-up by Soxhlet. The results of changing pH on the observed fluorescence of P1 are illustrated in Table 5, along with the influence of pH on the observed fluorescence of 2-NOH and its alkyl substituted analogue, 2-methoxynaphthalene.

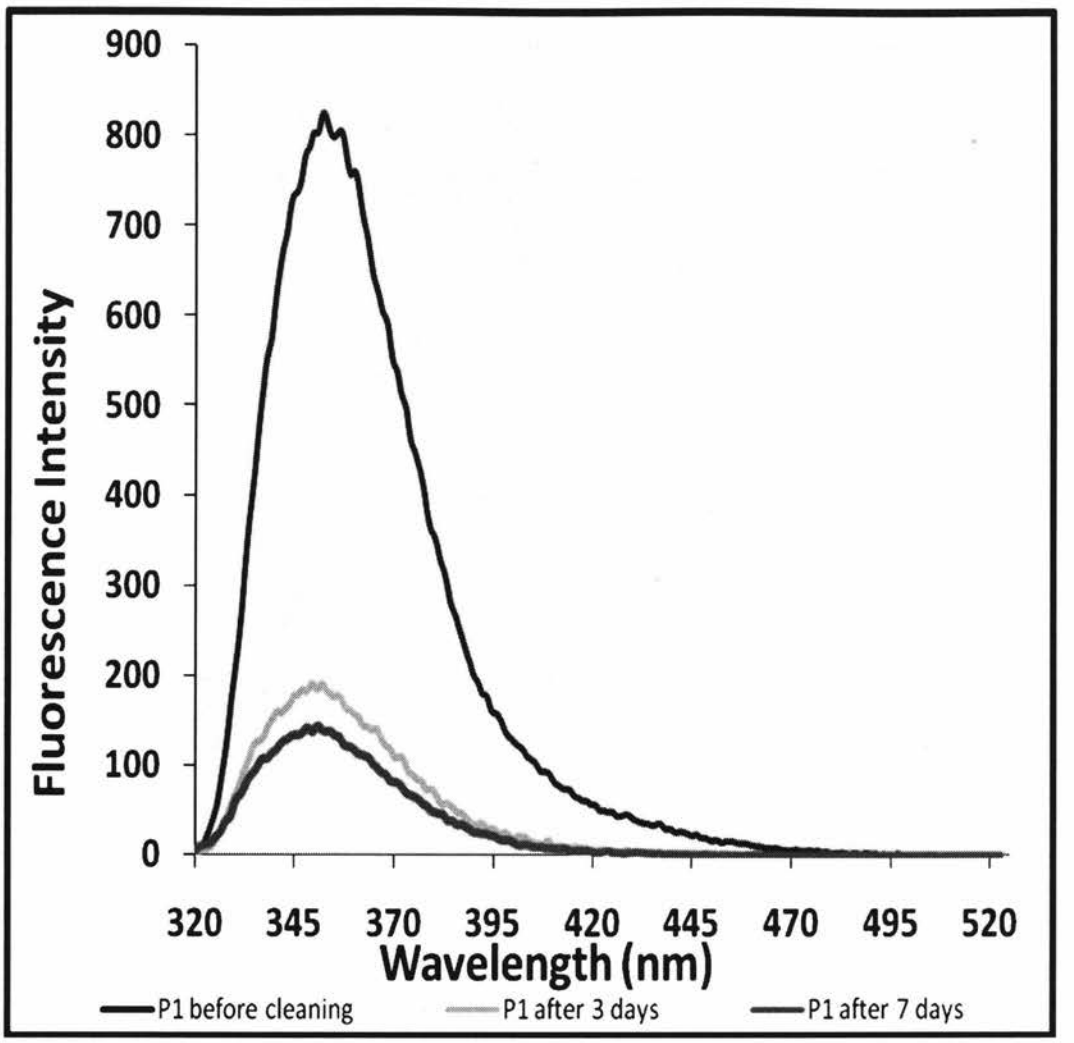


Figure 19: Fluorescence Spectra of Polymer 1, (P1), at Different Stages of Cleaning (monitored at 354nm, pH 2.0)

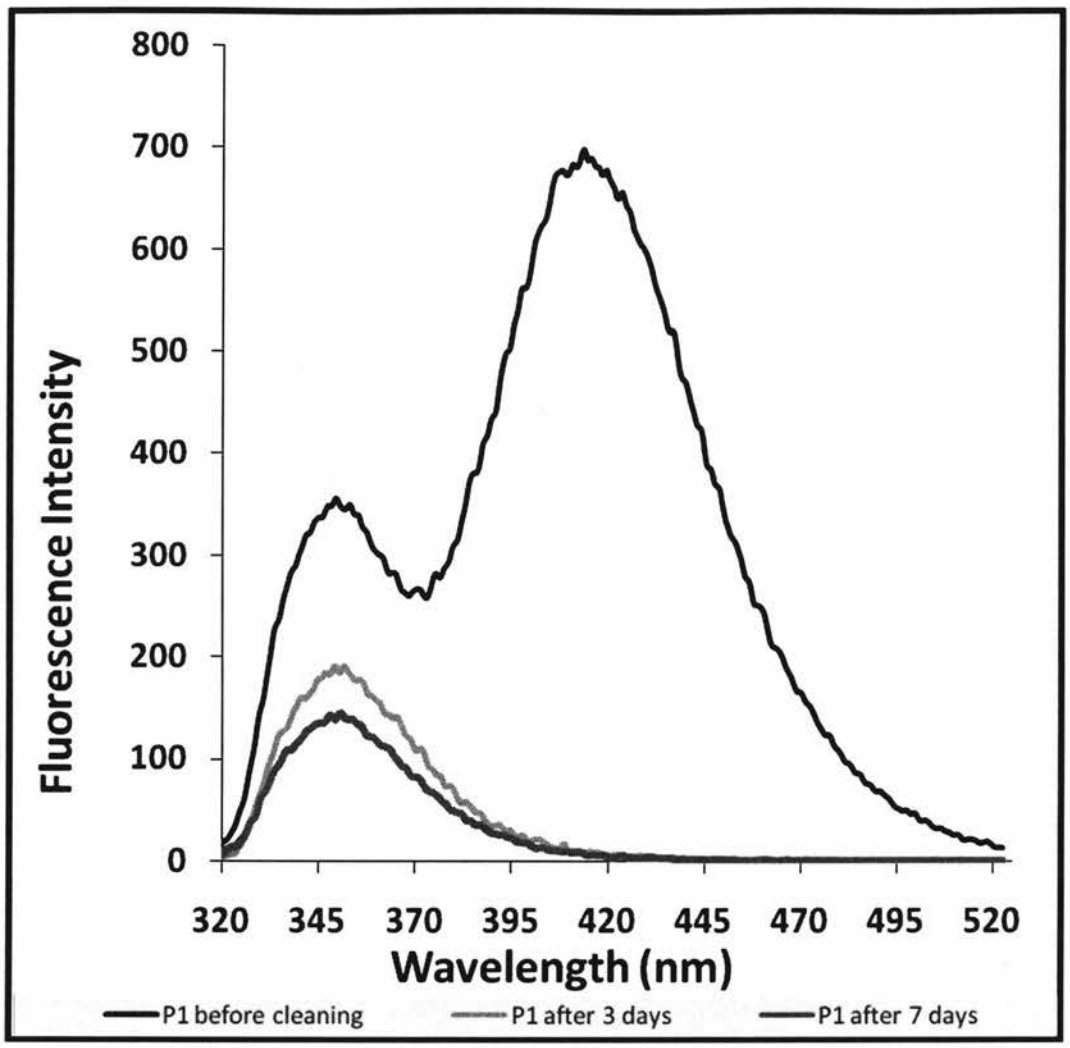


Figure 20: Fluorescence Spectra of Polymer 1, (P1), at Different Stages of Cleaning (monitored at 354nm, pH 13.0)

Table 5: Fluorescence Intensity as a Function of pH for Free Fluorophores and Polymers at 354nm and 420nm

	pH	Fluorescence at 354nm	Fluorescence at 420nm
2-naphthol	2.0	762	46
2-naphthol	13.0	0.2	838
2-methoxynaphthalene	2.0	419	71
2-methoxynaphthalene	13.0	430	79
P1 before cleaning	2.0	855	57
P1 before cleaning	13.0	367	667
P1 after cleaning	2.0	164	3
P1 after cleaning	13.0	166	3

The results at 354nm, the maximum of the protonated 2-NOH fluorescence, illustrate the expected behaviour for 2-NOH, (pH dependent fluorescence), and 2-MNOH, (pH independent fluorescence), respectively. The polymer shows pH dependence prior to cleaning but not afterwards. Table 5 also includes results at 420 nm, a wavelength where the emission of 2-NOH is almost exclusively from the deprotonated excited state. As expected, this emission intensity increases at higher pH for 2-NOH and for the uncleaned polymer. For 2-MNOH, which has no ionisable protons, the pH response is essentially flat. For the cleaned polymer the intensity decreases. If there are no ionisable groups, i.e. free 2-NOH, after cleaning then this is the expected behaviour, indicating the free fluorophore has been successfully removed by extraction.

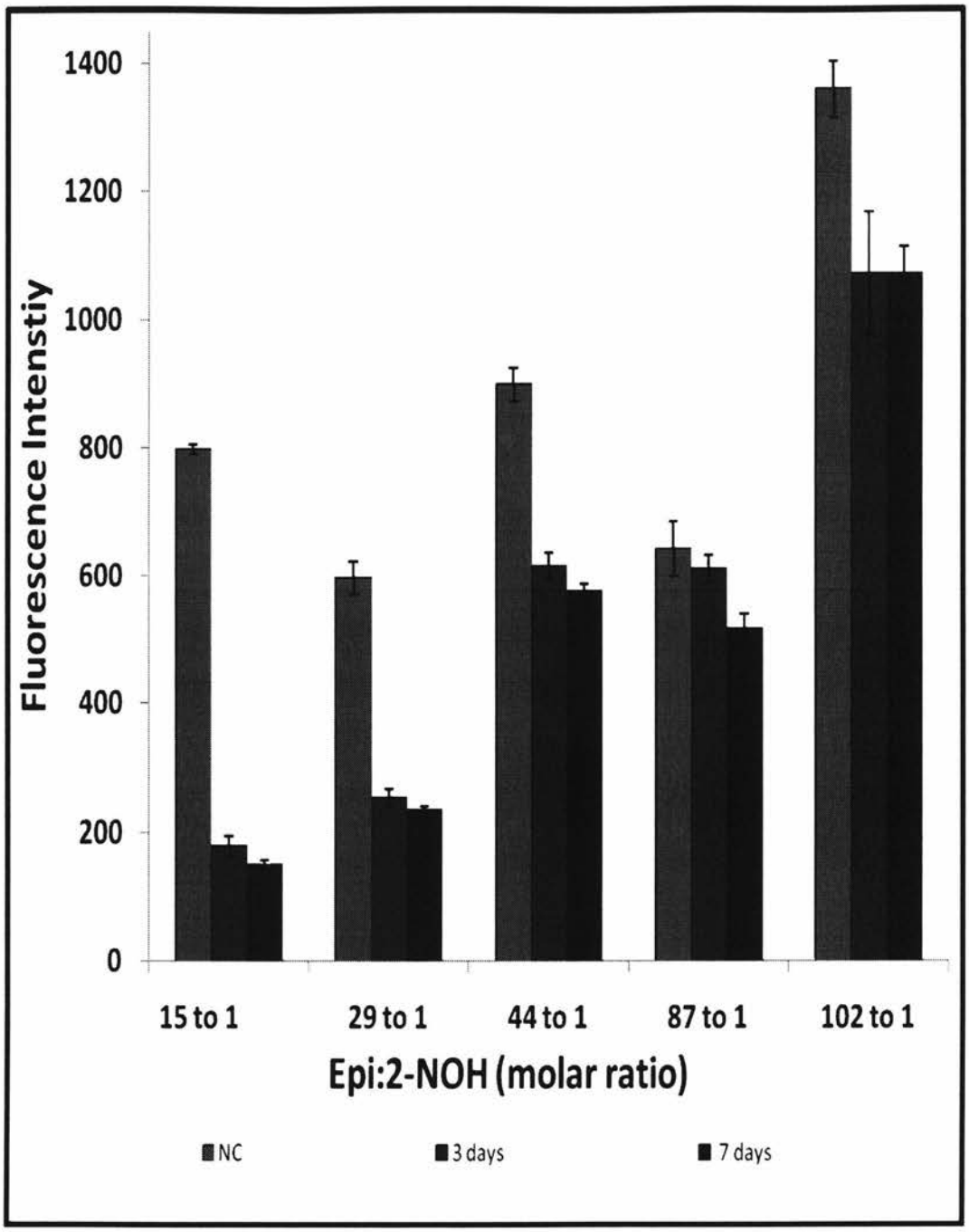


Figure 21: Observed Fluorescence (Monitored at 354 nm) of Polymer Samples from Soxhlet Thimble at pH 2.0 after Various Times of Soxhlet Extraction, NC=not cleaned

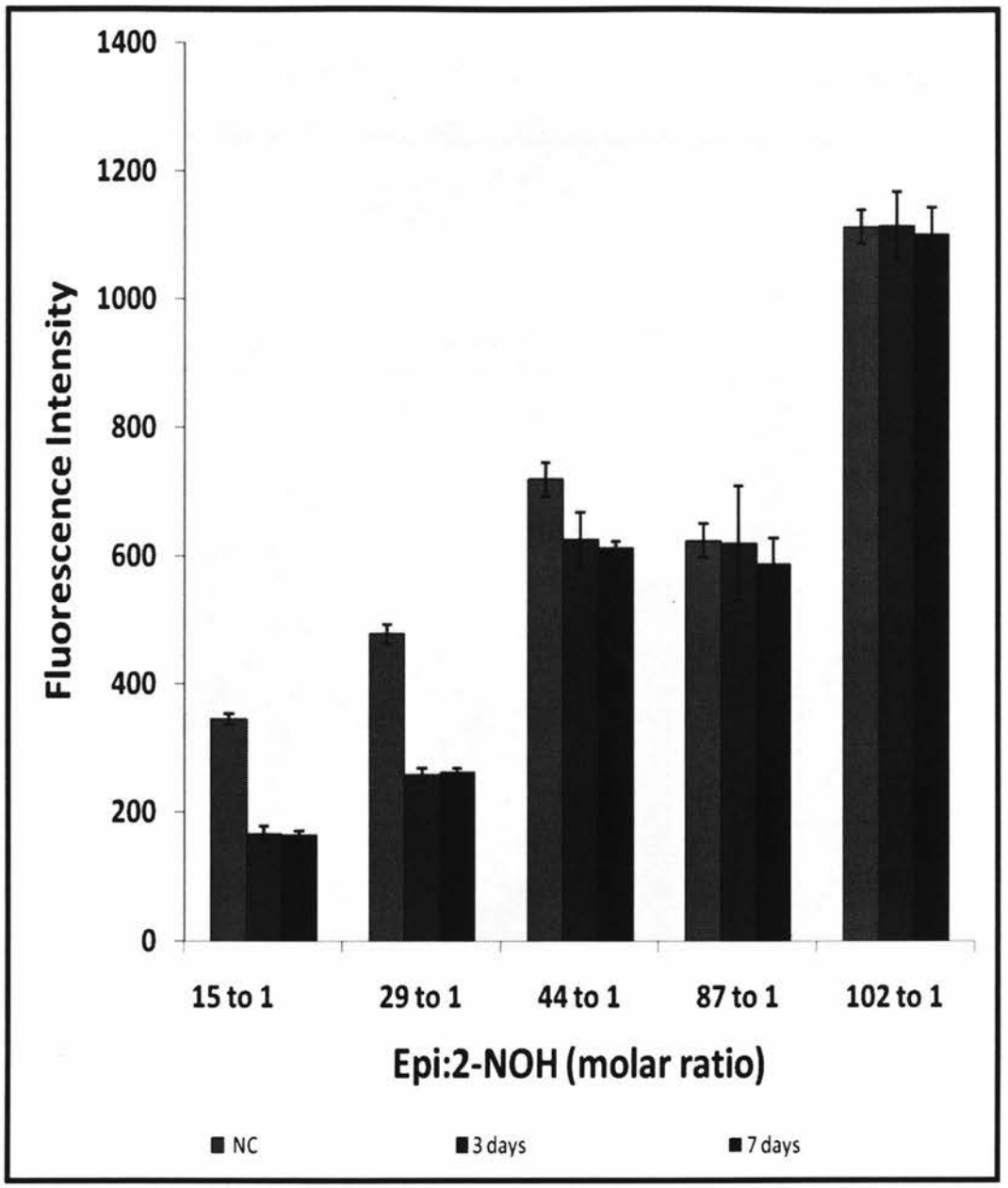


Figure 22: Observed Fluorescence (Monitored at 354 nm) of Polymer Samples from Soxhlet Thimble at pH 13.0 after Various Times of Soxhlet Extraction, NC=not cleaned

Figure 21 illustrates the fluorescence intensity of pH 2.0 adjusted polymers with varying Epi:2-NOH molar ratios after Soxhlet washing intervals of 0, 3 and 7 days. Figure 22 are the same polymers, except the pH was adjusted to 13.0 for the washing. The Epi:βCD ratio in all the above polymers was held constant at a molar ratio of 15:1. The figures

demonstrate that without extensive cleaning of the polymers, the observed fluorescence is likely a mixture of both bound and free 2-naphthol.

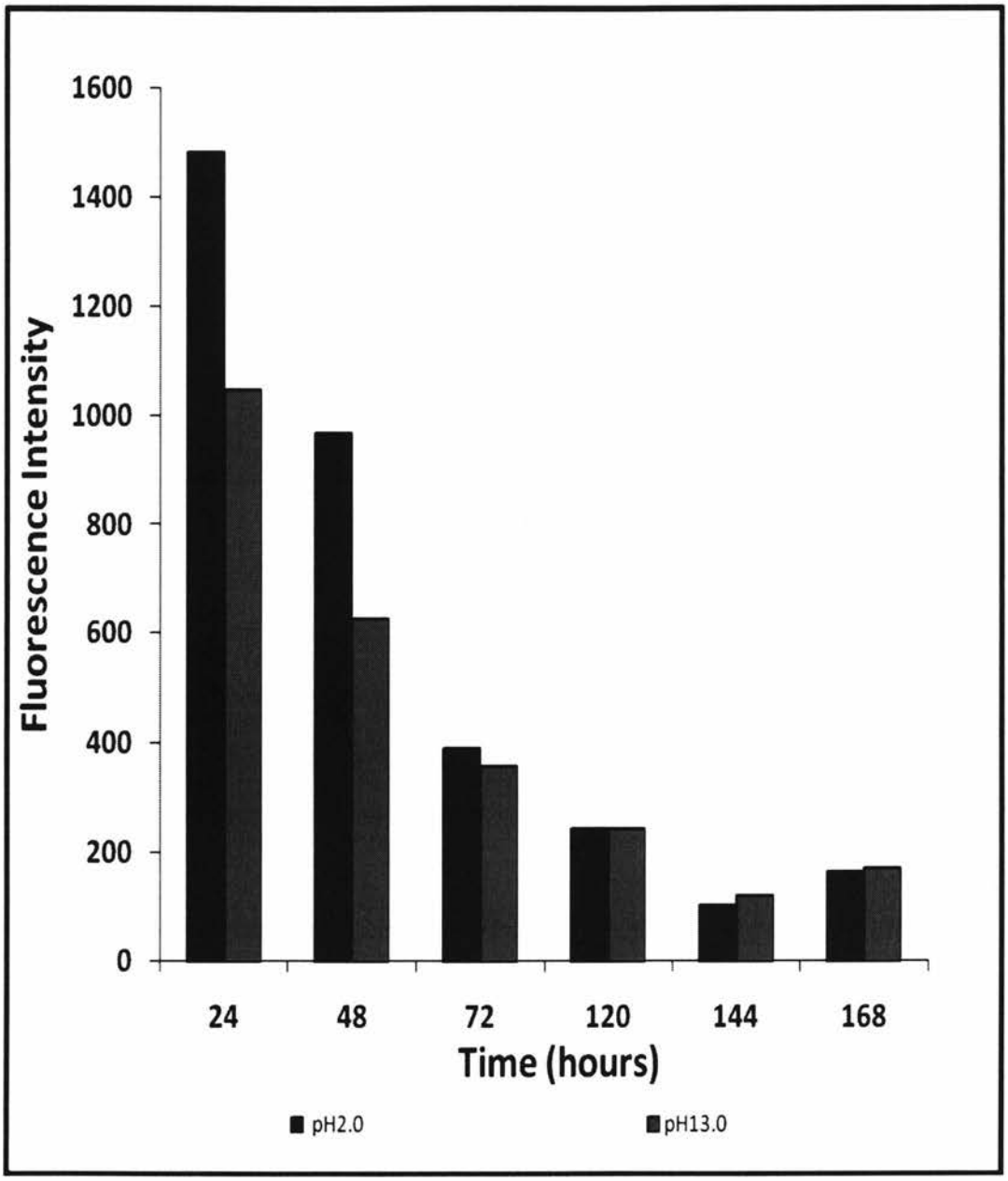


Figure 23: Monitoring of Soxhlet Washings Collected in the Round Bottom Flask For Polymer 2, (P2)

The polymer behaviour illustrated in Figure 23 (for polymer P2) is representative of all the polymers tested. The amount extracted

decreases with time for both pH 2.0 and 13.0. The difference in fluorescence intensity between time points during the early stages of extraction is more pronounced. As the extraction continues, this difference decreases suggesting that all free fluorophore has been successfully removed.

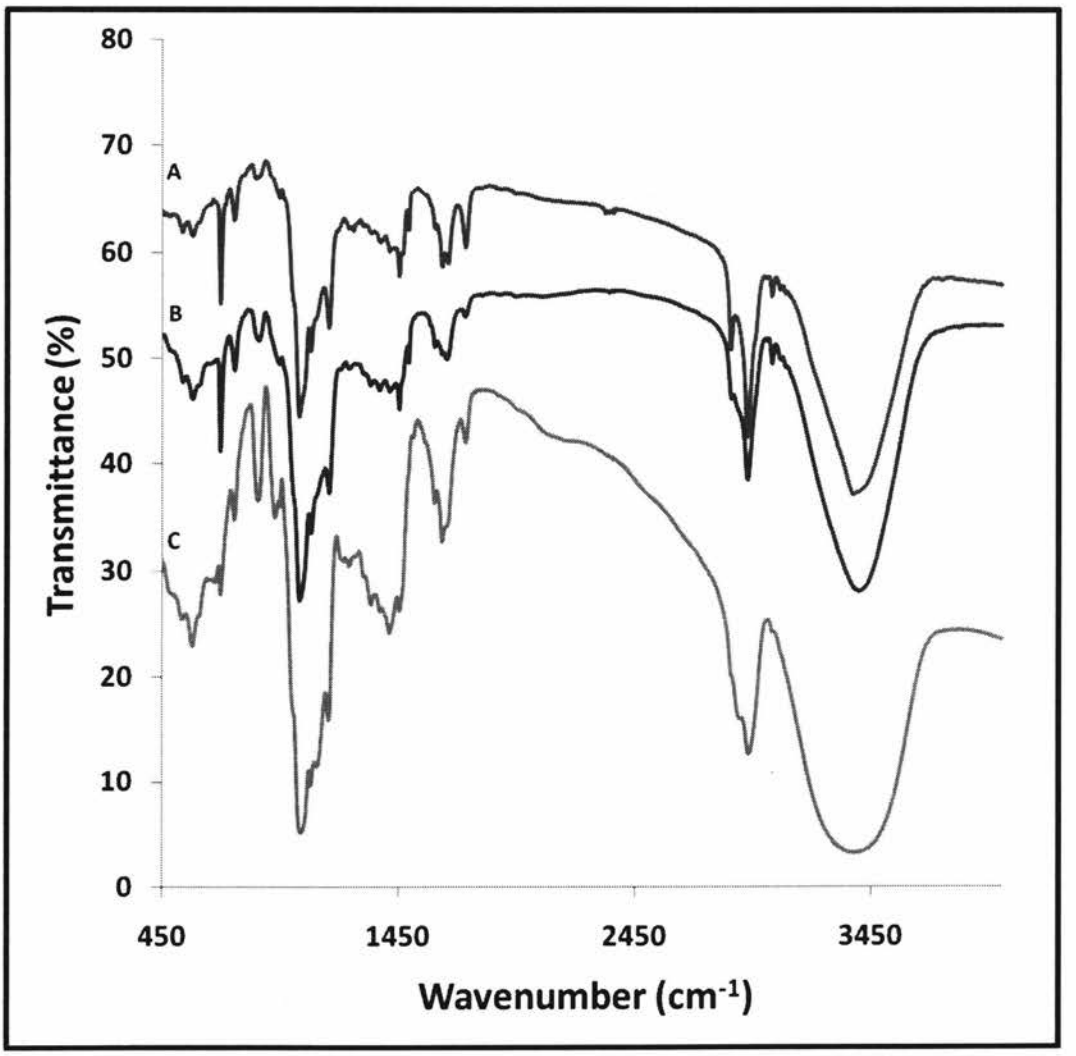


Figure 24: FTIR spectra of Synthesized Polymers (P2) in KBr

Figure 24 presents FTIR spectra for several samples relevant to the polymer preparation and clean up. Spectrum A represents a cleaned, (7

days), sample of β-cyclodextrin-2-naphthol-epichlorohydrin polymer (P2), B is the control sample, β-cyclodextrin-epichlorohydrin polymer; C is the uncleaned polymer of P2. Both spectra A and C show the following characteristics: O-H stretch at 3400 cm^{-1} , 3057 cm^{-1} for CH stretch (aromatic), 1634-1493 cm^{-1} for aromatic C=C, 1110-1044 cm^{-1} C-O stretch. There is no evidence of an epoxide ring at 850-790 cm^{-1} or the epichlorohydrin peak at 1265 cm^{-1}. Polymer B was used as the control sample in order to compare the differences between a polymer that is synthesized in the presence of 2-napthol and in its absence.

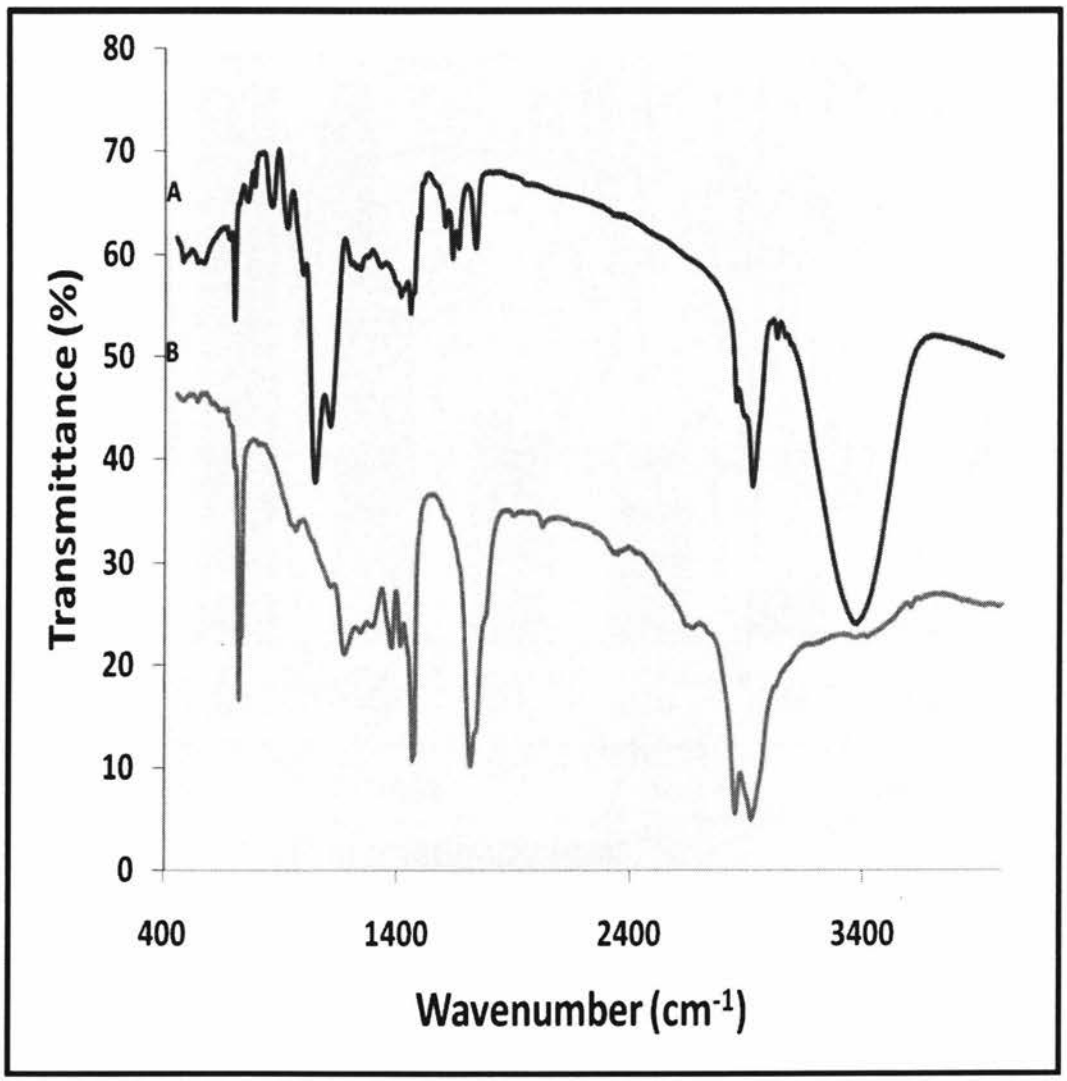


Figure 25: FTIR Spectra of 2-Naphthol-Epichlorohydrin Polymer in KBr. Spectrum A is Uncleaned P9; B is the Same Polymer Extracted for Three Days

The spectrum in Figure 25 A represents an uncleaned 2-naphthol-epichlorohydrin polymer, (P9), spectrum B represents the cleaned polymer after 3 days of extraction. The characteristic OH band at 3400 cm^{-1} from unreacted 2-NOH is absent in the cleaned polymer. Another distinguishing feature between the two spectra is the peaks between 1634-1493 cm^{-1} (aromatic C=C), evident in polymer A, but not B. These data indicate that much of the naphthol is removed by extraction as was found for the β-cyclodextrin-2-naphthol-epichlorohydrin polymer (see above).

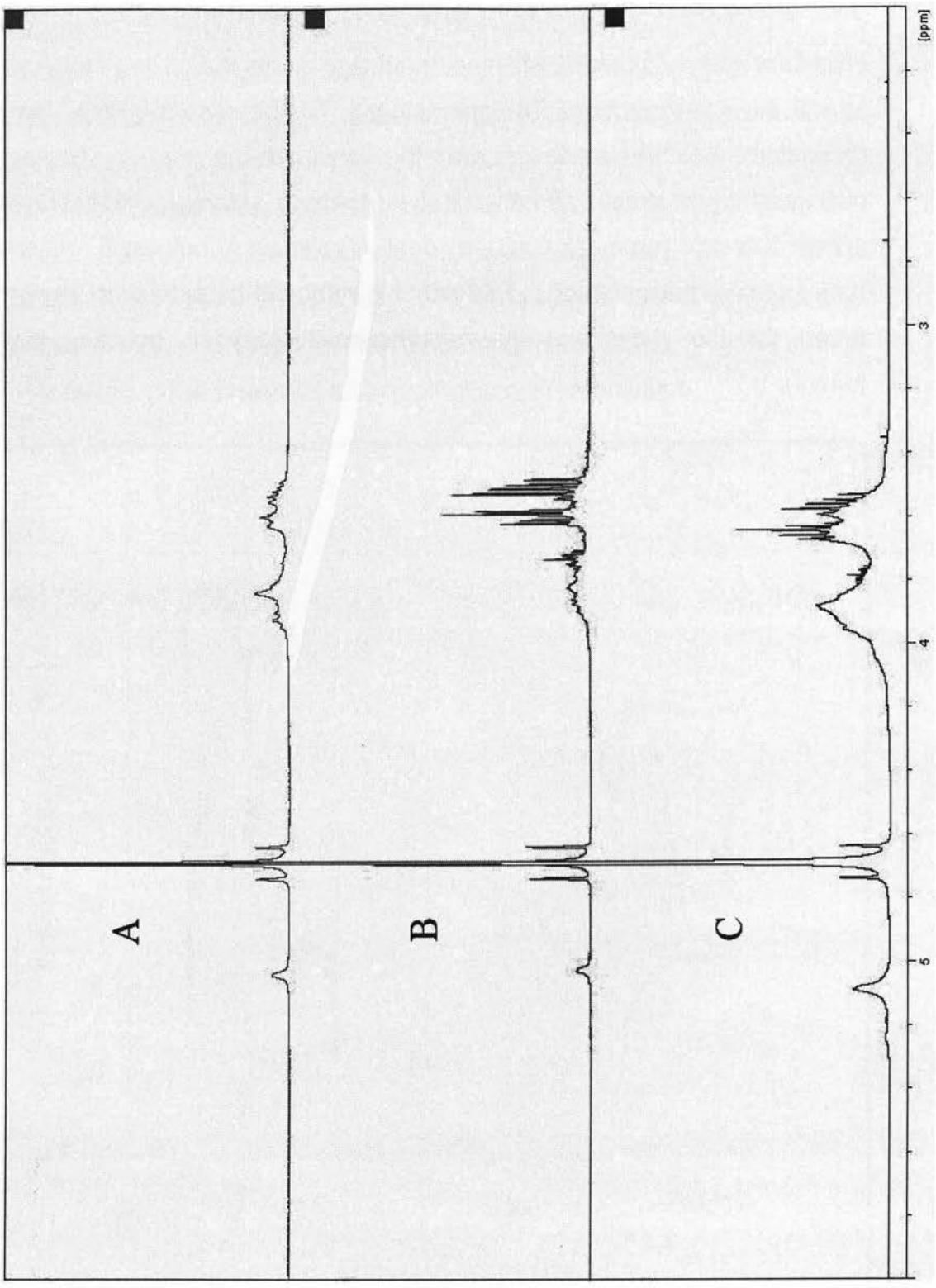


Figure 26: NMR Spectra of Polymers at Different Stages of Cleaning (A) Polymer 2, (P2) after 3 Days of Soxhlet Cleaning (B) P2 Not Cleaned (C) Control Polymer

Figure 26 shows ^{1}H NMR spectra of polymer P2 after 3 days of Soxhlet extraction. The spectra demonstrate that after cleaning the characteristic peak of the anomeric C at 5.04 ppm is still present. The spectra match that found in the literature of a water-soluble β-cyclodextrin-epichlorohydrin polymer.[54,55]

Table 6: Results of Fluorescene Quenching Studies with Nitrobenzene at 22°C

	K_{SV} (M^{-1})	k_q (10^9) (M^{-1} sec^{-1})
2-NOH	7.6	1.47
CD+NOH	7.3	1.41
P1	9.3	1.79
P7	8.2	1.59
P14	7.2	1.38
P15	6.7	1.28
P16	7.7	1.47

Table 7: Results of Fluorescence Quenching Studies with Nitrobenzene at 65°C

	K_{SV} (M^{-1})	k_q (10^9) (M^{-1} sec^{-1})
2-NOH	7.2	1.39
CD+NOH	6.3	1.21
P1	7.7	1.48

All of the quenching experiments were conducted in an aqueous solution of pH 2.0. The free 2-naphthol, β-cyclodextrin:2-naphthol and all the polymers were monitored at 354 nm.

Table 6 presents results obtained from quenching studies by nitrobenzene conducted at ambient temperature. Table 7 presents the results of quenching studies at 65°C. In all cases, the Stern-volmer plots were linear, (see Figure 27). The fact that increasing temperature has little impact on the quenching behaviour suggests that static quenching is not important in these systems. The k_q values were obtained by using the reported lifetime of 2-naphthol in water, 5.2 nsec[69], and making the

assumption that the lifetime of the naphthol moiety in the polymers is the same as that of free 2-NOH .

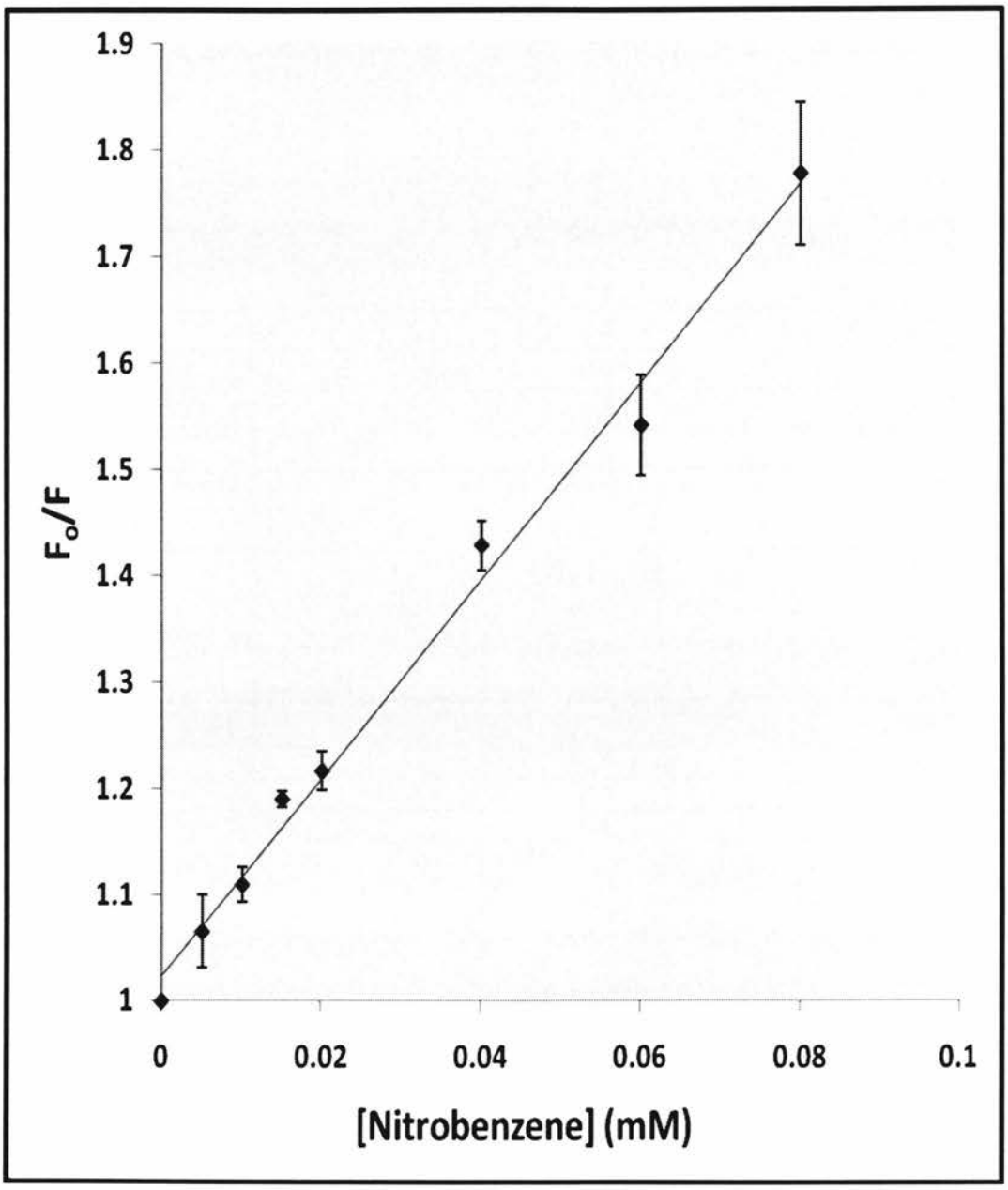


Figure 27 Stern-Volmer Plot for Polymer 1 at 22°C

7.0 Discussion

7.1. Polymer Characterization

Three different analytical methods, fluorimetry, FTIR and NMR, were employed to assist in the verification and characterization of the prepared polymers. Separately, each technique provided useful information in this regard but collectively all three methods afforded a clearer picture of the organization of the monomers, β-cyclodextrin and 2-naphthol, involved in the formation of the polymer.

One possible interfering reaction in the synthesis of the β-cyclodextrin-2-naphthol-epichlorohydrin polymer is the formation of a crosslinked system involving epichlorohydrin and 2-naphthol. The use of the bifunctional cross-linking agent, epichlorohydrin, to polymerize/oligomerize naphthol has been reported. Jovanović and colleagues[70,71], in attempts to synthesize 1-(1-naphthyloxy)-2,3-epoxypropane, better known as the prescription β-blocker propranolol, via the alkylation of α-NOH with epichlorohydrin, produced several side products, (Figure 28). The objective of their work was to eliminate the side products and obtain a pure propranolol product for pharmaceutical use. Jovanović *et al.,*[70] proposed that the most probable mechanism in the alkylation of α-NOH with epichlorohydrin is a S_N2 type aliphatic nucleophilic substitution where the nucleophilic agent is the naphthoxide anion. From a theoretical point of view, both O and C alkylation of the ambident nucleophiles, 1 or 2-NOH is possible. In a solution where the pH is greater than the p*K*a of the naphthol, (the experimental conditions in their work), the conjugate base will predominate, leading to O-alkylation. However, the conjugate base of 1-NOH is stabilized by resonance which may, conversely lead to C-alkylation and to product E (Figure 28). The authors argued that the negative charge on the oxygen was stabilized by clusters of water molecules thereby eliminating or, at least, significantly

decreasing C-alkylation. The experimental evidence illustrates that all products form, but the conditions in which the synthesis occurs determines the purity and quantity of the product.

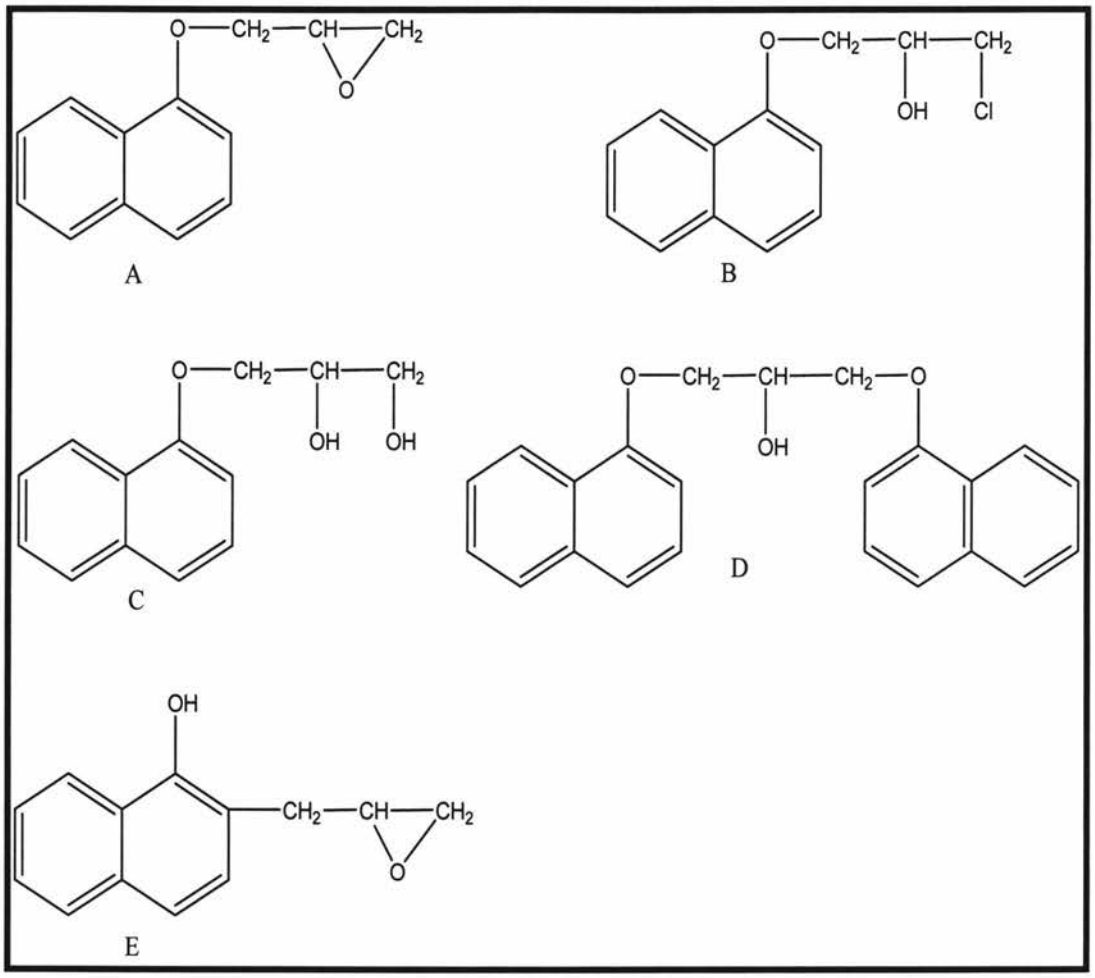


Figure 28 Products from the Reaction of α-Naphthol with Epichlorohydrin Adapted and Modified From[70] using ChemBioDrawUltra V.11.0

The characterization studies carried out here sought, in part, to evaluate to what extent the formation of naphthol-epichlorohydrin polymers/oligomers might interfere with the preparation of the key product, the β-cyclodextrin-2-naphthol-epichlorohydrin polymer.

7.1.1 Fluorescence Investigation

As discussed in Section 6.0, the emission of 2-naphthol is pH dependent because of its ability to deprotonate. The emission spectra of 2-methoxynaphthalene is independent of pH owing to its lack of a hydroxyl group and resulting inability to deprotonate. The purpose of 2-methoxynaphthalene in the present work is to illustrate the differences between O and C-alkylation. The availability of the hydroxyl group allows 2-naphthol to potentially undergo either O or C-alkylation.[70, 72-75] If 2-naphthol is linked to the polymer system via its hydroxyl group, then its emission spectra would resemble that of 2-methoxynaphthalene since the hydroxyl group of 2-naphthol would no longer be available for deprotonation. Conversely, if 2-naphthol is linked to the polymer via C-alkylation, the hydroxyl group would be free to deprotonate and would show similar behaviour to that of free 2-naphthol.

As is evident from Table 5, the unwashed polymer shows pH dependent fluorescence after the incorporation of 2-naphthol. The emission spectra of unbound 2-naphthol in an aqueous solution at pH13 shows a single maximum at 420nm because the hydroxyl group is deprotonated. In an aqueous solution at pH 2.0 the single fluorescent maximum is at 320nm because the fluorophore is now exclusively in its protonated state. However, at pH 5.0, (not presented), two maxima appear: a stronger one at 320 nm and a weaker one at 420 nm. This is a result of 2-naphthol undergoing deprotonation in its excited state. Table 5, illustrates that two emission peaks exist for the unwashed polymer in a pH13.0 environment; one at 354 nm and one at 420nm. This indicates that the fluorophore may exist in both the bound and unbound state within the polymer. However these results also suggest that the fluorophore may bind via either O or C-alkylation. The presence of the fluorophore within the polymer as free 2-naphthol, naphthol bound via O-alkylation and/or bound via C-alkylation are not mutually exclusive. Thus, it is probable that

all three forms are present. This leads to a polymer system that is a heterogeneous mixture that may contain:

1. β-cyclodextrin-epichlorohydrin polymer
2. 2-naphthol-epichlorohydrin polymer linked via O and/or C alkylation
3. β-cyclodextrin-2-naphthol-epichlorohydrin polymer linked via O and/or C alkylation

In order to investigate whether 2-naphthol was present as a group covalently linked to the β-cyclodextrin-epichlorohydrin polymer network or as a naphthol-epichlorohydrin polymer itself, further examination into the system was needed.

All the polymers were extensively cleaned via Soxhlet using ethanol. Ethanol was chosen because both 2-NOH and epichlorohydrin are extremely soluble in this solvent while β-cyclodextrin is sparingly soluble and the β-cyclodextrin-epichlorohydrin polymer is not soluble at all. Therefore, if free 2-NOH existed or if unreacted cross-linker was present, both would be removed from the polymer matrix during exhaustive extraction. In addition, utilizing the analogy that the β-cyclodextrin-epichlorohydrin has increased solubility in water compared to the unbound cyclodextrin at the relatively low cross-linker levels used here,[76] it was reasoned that if a 2-naphthol-epichlorohydrin polymer was also synthesized, it would share equal or greater solubility in ethanol than free 2-naphthol. This would suggest that if a 2-naphthol-epichlorohydrin polymer is formed concurrently with the β-cyclodextrin-epichlorohydrin polymer and is not covalently linked to the main network, it would also be removed from the matrix along with free epichlorohydrin and 2-naphthol. In order to verify this, samples from both the extraction solvent and original sample in the thimble were tested for fluorescence.

7.1.2 Polymer containing both β-cyclodextrin and 2-Naphthol

Ethanol extraction via Soxhlet was utilized to remove the reactants that did not take part in polymerization. Therefore, the extracted material and the sample were monitored for pH dependence to determine the presence and form of 2-naphthol. The results illustrated in Figures 21 and 22 show that after 7 days of washing the produced polymer (thimble) had pH independent fluorescence. Figure 23 illustrates that the amount of material extracted decreases with time and also that the pH of this material becomes independent. These results suggest that naphthol is covalently linked to the polymeric network via O-alkylation. It is important to note however, that this does not preclude the possibility that a simultaneous reaction forming two distinct polymers is occurring, namely 2-naphthol-epichlorohydrin and β-cyclodextrin-epichlorohydrin polymers.

7.1.3 Polymer containing only 2-Naphthol

An attempt was made to synthesize a cross-linked 2-NOH polymer in order to verify that if a concurrent synthesis was occurring that it could be removed from the polymer matrix. These polymers were cleaned in the same manner as those initially synthesized with β-CD. The fluorescence of this set of polymers were very different from those that containing β-cyclodextrin. After 3 days of washing there was no observable fluorescence in the sample. This result would indicate that if the polymer was successfully synthesized it is, like its monomer, very soluble in ethanol and was extracted. The fluorescence spectra of the samples that were extracted and collected from the round bottom flasks illustrated a major fluorescence red shift of 25nm after 48 hrs of washing. This shift may be attributed to the increased conjugation in the polymer.[77] This shift is not seen in the β-cyclodextrin-2-naphthol epichlorohydrin polymer because the fluorophore is attached to a β-cyclodextrin polymer system that does not affect the conjugation of the fluorophore. To investigate this issue further FTIR and ^{1}H NMR were used.

7.2 FTIR

FTIR was used to establish whether a polymer was in fact synthesized and to determine if, after cleaning, the characteristic OH band of a hydroxyl group is present. The spectrum of CD-naphthol-epi polymer P2, after and prior to cleaning is illustrated in Figures 24 A and C respectively. P2 was chosen from the P1-P7 set because the amount of material extracted from the Soxhlet thimble was limited. Unfortunately, in order to conduct fluorescence, FTIR and ^{1}H NMR studies on this set of polymers no single sample yielded enough material to conduct all the experiments. However, it is believed that this sample is representative of the set of polymers synthesized with both cyclodextrin and naphthol, (P1-P7). The characteristic C=C band for an aromatic ring appears between 1600 and 1450 cm^{-1},[78] which is seen both before and after cleaning the polymer. In the reference spectra of β-cyclodextrin-epichlorohydrin polymer this band is not present, since there is no aromaticity in this polymer. The reference sample, C, matches that found in the literature.[79]

The difference between spectra A and B in Figure 25 is far more pronounced than those in Figure 24. The band representing an OH stretch, (3400 cm^{-1}), is completely absent from the washed 2-naphthol-epichlorohydrin polymer (P9). This indicates that if a 2-naphthol-epichlorohydrin polymer is simultaneously synthesized alongside a β-cyclodextrin-epichlorohydrin or a β-cyclodextrin-2-naphthol-epichlorohydrin polymer it will be removed from the matrix. The spectrum of P9, (Figure 25 B), is characteristic of an epichlorohydrin polymer.[79] As described in Section 3.4, epichlorohydrin can self-polymerize; and is most likely also occurring in the present system.

7.3 NMR

The purpose of using the ^{1}H NMR was to verify that the β-cyclodextrin-epichlorohydrin polymer was synthesized in the presence of

2-naphthol. Figure 26 illustrates the characteristic anomeric proton of a crosslinked β-cyclodextrin polymer appearing at 5.04 ppm for the cleaned polymers synthesized with β-cyclodextrin and 2-naphthol. This result confirms that the polymer was indeed synthesized even in the presence of 2-NOH. Also, the spectra obtained exactly match those found in the literature[79] for β-cyclodextrin polymers synthesized with epichlorohydrin. This result is significant because it confirms that the addition of the monomer, 2-naphthol, does not destroy the crosslinking ability of epichlorohydrin to. β-cyclodextrin.

7.4 Quenching Studies

After synthesizing and cleaning the polymers, quenching studies were conducted to evaluate if a fluorescently labelled β-cyclodextrin polymer would be effective at binding nitroaromatics and therefore useful as a sensitive sensor for such compounds. As mentioned in the introduction, nitroaromatics are a common feature of explosives in landmines and unexploded ordnances,[3] therefore nitrobenzene was chosen as a model target analyte. A highly sensitive detector is important because the concentration of landmine-derived nitroaromatics in soils is exceptionally low.[13]

The detection of nitroaromatics occurs by the quenching of fluorophores, such as 2-naphthol. Since nitrobenzene contains an electron-withdrawing group it will strongly interact with molecules that are electron donating, like 2-naphthol, via a charge transfer mechanism. The quenching of 2-naphthol by nitrobenzene is very rapid; thus, having the potential to produce a highly sensitive and rapid detection system.

7.4.1 Effect of Cross-linker to Fluorophore and Cyclodextrin to Fluorophore Ratios on Quenching Behaviour

Quenching occurs through the interaction of nitrobenzene and 2-naphthol. For this encounter to occur, the quencher must first reach the

naphthol moiety in the polymer network. This process may be influenced by certain properties of the polymer network such as the various crosslinking ratios which can impact the mobility of a small molecule like nitrobenzene in the polymer. To examine this effect the ratios of cross-linker to fluorophore and β-cyclodextrin to 2-naphthol were studied with the results illustrated in Table 6. The K_{SV} values determined are the Stern-Volmer quenching constants and, k_q is the bimolecular rate constant for quenching. The results in Table 6, illustrate that very little difference exists in both the obtained K_{SV} and k_q values between free 2-NOH, 2-NOH:β-CD, and the polymers. With respect to cross-linker ratios, P1 and P7 have the same initial molar ratio of Epi:CD but differ with respect to Epi:NOH and βCD:NOH starting molar ratios. These two polymers were chosen from the group with Epi:βCD ratio held constant at 15:1 because the difference in fluorescence after cleaning was significant (Figure 21) and the difference in ratio of cross-linker to fluorophore, 15:1 and 102:1, for P1 and P7, respectively, was the greatest. The difference in K_{SV} and k_q between these two polymers was minimal. Neither the starting ratios of βCD:NOH or Epi:NOH, although very different, seem to affect the efficiency of quenching, therefore, polymers with increased Epi:CD ratios were synthesized. It was reasoned that if the starting molar ratio between the cyclodextrin and fluorophore and the cross-linker to fluorophore displayed minimal difference then perhaps the ratio of cross-linker to cyclodextrin would be significant. This is reasonable as the polymers network is primarily a crosslinked network of CD cavities[53] and greater degrees of crosslinking of the CD should create a more rigid internal environment.

7.4.2 Effect of increased cross-linker to β-cyclodextrin ratio

In order to evaluate if the cross-linker ratio of epichlorohydrin to β-cyclodextrin would impact quenching behaviour, polymers with increased starting molar ratios of epichlorohydrin to β-cyclodextrin were synthesized.

P15 and P16 had the following initial molar ratios; βCD:NOH 3:1 and 5:1; Epi:NOH 61:1 and 102:1 respectively with the Epi:CD ratio constant at 20:1. Again, the difference in the K_{SV} and k_q not only between these polymers but also in comparison to P1 and P7 is minimal. Perhaps the difference between 15:1 and 20:1 of cross-linker to cyclodextrin is not significant enough to demonstrate a difference. Thus, one final attempt was made to investigate if this ratio played a role in the quenching of 2-naphthol via nitrobenzene. It was decided to substantially increase the starting ratio of Epi:CD by more than 3 times to 48:1, (P14). As is evident from the results in Table 6, P14 showed no observable difference compared to the other polymers tested. These results indicate with certainty that the ratio of cross-linker to cyclodextrin does not play a significant role in the quenching of nitrobenzene.

It is somewhat surprising that the quenching efficiency is the same for free 2-NOH as it is for CD-complexed 2-NOH and for the naphthol-like fluorophore in the polymer systems. It is quite common that encapsulation of a fluorophore by a CD cavity protects it from quenching.[69,80] In the present case, 2-NOH is known to bind to free, (i.e., unpolymerized) β-CD with a binding constant of K_f = 590 M^{-1} in water.[69] Nitrobenzene also binds to β-CD with a value of K_f of 154 M^{-1} at pH 7.0 and 32 M^{-1} at pH 13.[81] Thus a significant amount of the added nitrobenzene will associate with free β-CD, even in the presence of 2-NOH which may account for the lack of difference in quenching efficiency observed in the absence and presence of CD for the unpolymerized systems.

In the polymeric systems, the fact that quenching efficiency seems to be independent of preparation parameters, especially the degree of crosslinking, is also somewhat unexpected. However, all the polymers tested are water soluble which means that the solvent may have good access to all domains of the polymer. This in turn would allow more-or-

less unhindered access of the quencher to the various fluorescent sites on the polymers.

One of the goals of this study was to determine if this polymer design could be used as a sensor for nitroaromatics. Our quenching data show that it is possible to detect a nitroaromatic compound with the polymers. However, the polymers show no enhancement in quenching efficiency compared to quenching of free 2-NOH or CD-complexed 2-NOH. That is, the presence of the CD cavities in the polymer systems does not seem to enhance the sensitivity of the quenching process although the model analyte (i.e., the quencher nitrobenzene) is known to associate moderately strongly with β-CD in water.

7.4.3 Static or Dynamic Quenching

In order to explore whether static or dynamic quenching was involved in the quenching process, the temperature of the study was increased from ambient to 65°C. Recalling section 3.6.3.1, an increase in K_{SV} at elevated temperatures can be associated with dynamic quenching while a decrease is indicative of static quenching.[15]. Only free 2-naphthol, 2-naphthol and cyclodextrin, and P1 were tested at 65°C. The K_{SV} results, (Table 7) for the above mentioned systems exhibit only small variations between measurements at room temperature and at 65°C. The change for 2-NOH is approximately 4% while that for P1 is about 20%, with a decrease in both cases. Even though the difference in the P1 was greater, a firm decision regarding the type of quenching could not be drawn.

8.0 Conclusion and Future Recommendations

8.1 Conclusions

From the results obtained utilizing fluorescence studies, FTIR and ^{1}H NMR it is concluded that 2-naphthol was successfully incorporated into the crosslinked polymer network. After extensive cleaning there was no pH dependence of the polymer indicating that the unincorporated fluorophore was successfully removed and that remaining fluorophore was bound to the system via O-alkylation. The FTIR results indicated that even if a concurrent synthesis of a naphthol polymer was occurring with that of a β-cyclodextrin-2-naphthol-epichlorohydrin polymer, its solubility in ethanol removed if from the system, leaving only the intended product: β-cyclodextrin-2-naphtol-epichlorohydrin water-soluble polymer.

The quenching studies indicate that the water soluble crosslinked polymers are responsive to nitroaromatics and may have some value as sensor materials. However, there was no difference in quenching efficiency between free 2-naphthol, β-cyclodextrin-complexed 2-naphthol and the different polymers. Irrespective of the starting molar ratios of each system, there was no detectable difference in the quenching by nitrobenzene. This indicates that including the CD cavities affords no enhanced sensitivity to the putative sensor at least when the polymer is water soluble. It would be of value to try quenching experiments with the polymer coated onto a solid substrate, (e.g. glass), as well as to test water-insoluble preparations. Also, the temperature experiments used as a method for determining the mode of quenching did not lead to any conclusive result regarding the mode of quenching: dynamic, static or mixed.

The ultimate objective of this thesis was to coat the prepared polymer onto a glass slide, functioning as the fibre optic portion of a chemosensor, was not achieved. However, important advancements

were made in understanding the prepared polymer system. In order to obtain a purified polymer, it must be cleaned after synthesis to remove any unreacted materials and side-products. The use of Soxhlet as a means for extracting these materials has proven to be beneficial.

8.2 Future Recommendations

As mentioned earlier, the lifetime of free 2-napthol is 5.2 nsec.[69] This value was used to calculate all the bimolecular quenching constants, k_q. The results indicated that there was no difference between the systems. However, the lifetime of 2-naphthol may be different when covalently bound to a polymer network. Thus, in order to obtain more accurate bimolecular quenching constants, lifetime measurements of 2-naphthol in the polymer system are required. Since lifetime measurements in the presence and absence of a quencher are also used as a way to determine the mode of quenching, a lifetime study would give a definite answer to the question of the role of static quenching in these systems.

^{1}H NMR is a powerful analytical tool. It was, by no means, utilized to its full potential. The additional information that could be obtained from the ^{1}H NMR includes the amount of β-cyclodextrin in the polymer system and 2-naphthol. This could be done, as explained in Section 3.4, by integrating the anomeric proton of β-cyclodextrin appearing at 5.04 ppm to the hydroxypropyl ether segments. The amount of 2-naphthol in the system could possibly be obtained in a similar fashion

From the literature review conducted, the highest ratio of epichlorohydrin to β-cyclodextrin yielding a water-soluble polymer is 16:1.[55] In the present study a starting molar ratio of cross-linker to cyclodextrin of 20:1, (P15 and P16), and even higher at 48:1, (P14), resulted in a water soluble system. Since 2-naphthol is only sparingly soluble in water, the possibility of the fluorophore increasing the solubility

of the polymer is not likely. The results of the FTIR and ^{1}H NMR are conclusive in the sense that a cross-linked system does exist. However, it may be that instead of a large molecular weight polymer, the end product is oligomers of β-cyclodextrin crosslinked by epichlorohydrin with 2-naphthol covalently bound. The use of gel permeation chromatography, (GPC), would give the molecular weight of the product which would indicate definitively if a polymer or chains of oligomers was present.

In the future a different approach in the experimental procedure is suggested. In the present study, both monomers were added before the introduction of the cross-linker. Perhaps synthesizing and cleaning a prepared water-soluble β-cyclodextrin-epichlorohydrin polymer before the addition of the fluorophore would yield a high molecular weight polymer. The incorporation of the fluorophore could possibly be achieved by redissolving the prepared water soluble β-cyclodextrin-epichlorohydrin polymer, then after the addition and solubilisation of the fluorophore in the polymer solution, the cross-linker could be added. However, it is also recommended that a very low starting ratio of β-cyclodextrin to epichlorohydrin is initially used in case the extra addition of epichlorohydrin, further crosslinks the already produced polymer.

One final suggestion would be to use a different fluorophore. One suggestion would be to try 1-hydroxypryene. Since it contains a hydroxyl group it could also be covalently linked to the polymer system. Also, since pyrene has more conjugation than 2-naphthol, the quenching by nitroaromatics may be more efficient.

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