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## Investigation of Dibenzothiophene Removal from I Organic Phase: Enzymatic Approach vs. Ionic Liquids

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# **Investigation of Dibenzothiophene Removal from Organic Phase: Enzymatic Approach vs. Ionic Liquids**

(Spine Title: Extraction of Sulfur using Ionic Liquids)

(Thesis Format: Integrated Article)

by

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**Graduate Program in Engineering Science**

**Department of Chemical & Biochemical Engineering**

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Engineering Science

Faculty of Graduate Studies  
The University of Western Ontario  
London, Ontario, Canada

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THE UNIVERSITY OF WESTERN ONTARIO  
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entitled:

**Investigation of Dibenzothiophene Removal from Organic Phase:  
Enzymatic Approach Vs. Ionic Liquids**

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requirements for the degree of  
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## ABSTRACT

Sulfur in fuel fractions like gasoline and diesel is responsible for acid-rain. The low levels of sulfur in fuels imposed by the environmental regulations of 15 ppm by 2006, have led to the search of alternative methods to comply with these new demands. The conventional process of hydrodesulfurization is unable to achieve the desired sulfur level even with the use of high temperatures and pressures to achieve a maximum reduction of the heavy sulfur compounds.

Recently, ionic liquids have emerged as attractive “green” solvents. Enzymes have also been reported previously for oxidation of organic sulfur compounds. In this study, two approaches were adopted for sulfur removal; first an enzymatic approach was attempted for sulfur oxidation. This approach was found to be unsuccessful. Next, extractive desulfurization using an ionic liquid was investigated and successfully demonstrated using a biphasic system involving dibenzothiophene (DBT) hexane solution and 1-Butyl-3-methylimidazolium tetrafluoroborate, which forms the heavier layer in the system. The effects of different factors that may influence the extraction process with the ionic liquid were studied. The recycling of the ionic liquid used in the extraction was demonstrated by precipitating the sulfur compounds through the addition of water by 50%. After the diluted ionic liquid was concentrated by removing the water, it was used again in the extraction process. The sulfur level is reduced by 50% after each stage. The DBT Distribution coefficient in hexane/ionic liquid was determined to be around 0.73.

*(Keywords: Extractive Desulfurization, DBT, Ionic liquid)*

## **CO-AUTHORSHIP**

The following thesis is composed of two manuscripts; one is submitted and the other one is being submitted to technical journals, which is co-authored by Khaled Abdel-Gawad and Dr. Amarjeet Bassi.

All the research and experimental work presented in this thesis were performed by Khaled Abdel-Gawad. The manuscripts, which appear in Chapters 3 and 4 of this thesis were written by Khaled Abdel-Gawad and revised by Dr. Amarjeet Bassi.

*To my dad who was and will always be my inspiration.*

*To my mom who is the purpose of my achievements.*

*To my brothers and sister who gave me the strength.*

*To my best friend Mouhamed Hajar for his support.*

*To my best friends Ezat Antaky & Rima Hany back home.*

*To the one person whom I will always love @>-->--*

*To all my family for the unconditional love and support they have  
showered me with throughout this journey.*

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# List of Symbols

## Notations

ppm	Parts per million
$m$	Partition or distribution coefficient ( $Y_e / X_r$ )
$F$	Feed (mass, Quantity/tare)
$R$	Raffinate (mass, Quantity/tare)
$S$	Solvent (mass, Quantity/tare)
$E$	Extract (mass, Quantity/tare)
$X_f$	Weight fraction of solute in the Feed (ppm)
$X_r$	Weight fraction of solute in the Raffinate (ppm)
$Y_s$	Weight fraction of solute in the Solvent (ppm)
$Y_e$	Weight fraction of solute in the Extract (ppm)
$X_f/X_r$	Reduction ratio (unitless)
DBT	Dibenzothiophenes
IL	Ionic Liquid
KG1-MATR	Mechanically agitated tubular reactor

## Chapter 1: Introduction

Diesel and gasoline are considered the most usable fuels around the world. The importance of these fuel fractions drives the continuous desire to optimize their quality. Therefore, the harmful impurities' concentration in these fuels must be lowered to a value that will be acceptable to insure a high level of fuel purity.

Sulfur in petroleum products is considered one of the major contaminants that cause industrial and environmental problems. As a result, desulfurizing of petroleum derivatives was a priority since the World War II, where hydrodesulfurization first came into practice. Hydrodesulfurization (HDS) is considered the conventional process to lower the sulfur content in gasoline and diesel where hydrogen reacts with sulfur catalytically in a high temperature and pressure. Thus the sulfur will be reduced to around 300 ppm in gasoline and 500 ppm in diesel, which is not sufficient enough under the new environmental regulations.

Environmental regulations regarding the content of sulfur in diesel and gasoline have kept changing throughout the years. The main purpose was and still is to reduce the level of sulfur content in gasoline and diesel to the least practically achievable value. New catalysts have been used in the HDS to produce better results in further lowering the sulfur level. At the same time, studies have been conducted to find alternative processes to meet the legislative requirements for achieving the desired level of sulfur in diesel and gasoline.



Such approaches include oxidative and/or extractive desulfurization. Oxidative desulfurization (both chemical and bio-chemical) is considered a newer approach to achieve deep level sulfur removal from diesel and other petroleum products to satisfy the new environmental regulations.

Also, desulfurization by extraction using new solvents has been under investigation for many years in order to achieve the desired level of sulfur through multi-stage process. In this chapter, the impact of sulfur on the environment is reviewed, and then desulfurization methods are discussed.

## **1.1 Sulfur Damage**

Sulfur reduction in fossil fuels is prompted by the negative effects it has on the refinery processes and environment. Many catalysts in reformer units are sensitive to the amount of sulfur in the feed. In fact, some bimetallic reforming catalysts require the sulfur content to be limited to the vicinity of 1ppm or less. Also, the organo-sulfur content of the feed to the hydrocracker must be reduced to avoid poisoning of the hydrocracking catalyst (Gandhi & Shelef, 1991). On the other hand, the environmental impact of sulfur emission forces the air pollution control standards to be more stringent in the removal of this component up to 80% or more in various fuel oils (Braid *et al.*, 1999).

The sulfur dioxide produced from diesel and gasoline combustion in the engine is reduced at the catalytic converter to sulfate particles, which could form a coat covering

the catalyst and prevent it from converting nitrogen oxides and the volatile organic compounds to non-harmful compounds to the environment. As a result, an increase in air pollution and further reduction of the ozone layer will occur due to the harmful chemicals emitted such as sulfur nitrogen oxides. On the other hand, sulfur dioxide emitted and not reacted in the catalytic converter contributes in the formation of acid rain, which contains sulfuric acid (see equations 1.1 & 1.2). This acid is considered very harmful to the human health (Braid *et al.*, 1999):



## 1.2 Environmental Regulations

The International Joint Commission (IJC) between Canada and the U.S is a bilateral organization that focuses on providing a solution to any conflict or dispute between the United States and Canada regarding the protection of their transboundary environment through the implementation of the Great Lakes Water Quality Agreement and the improvement of transboundary air quality. With main offices located in Washington and Ottawa, the IJC has officially asked the governments of the United States and Canada to adopt uniform nationwide standards for the sulfur level in gasoline. The standards were summarized by annual average sulfur content of 30 parts per million (ppm) in gasoline and 15 ppm in diesel optimally by 2005, but certainly no later than 2006 (E.P.A, 2005). The highway diesel fuel sulfur rule must be implemented by July of 2006 in all North American refineries whereas the Ultra Low Sulfur Diesel (ULSD) implementation dates

for terminals and retail outlets are extended by 45 days. The International Air Quality Advisory Board, which is made up of U.S. and Canadian scientists and air pollution program administrators who consider trans-boundary air quality issues, gave the Commission the evidence of the necessity to propose new standards to lower the effect of sulfur on the environment (E.P.A, 2005).

Average sulfur content in gasoline currently varies from 300 ppm to 350 ppm in the United States and Canada, with some exceptions such as Ontario, which averages in the order of 550 ppm. Because of its severe air pollution, California has been the world leader in requiring cleaner fuels. Since 1996, California has legislated sulfur in gasoline be limited to either 40 ppm maximum or 30 ppm annual average with an 80 ppm maximum. Proven oil refining technology is available and currently in use to produce gasoline to this standard. The Commission is convinced that the associated production costs are more than balanced by improvements in air quality and benefits to human health (E.P.A, 2005).

In Canada, a federal-provincial Government Working Group recently released a report for public comment that suggests three options regarding the future sulfur content in gasoline: reduce to 30 ppm annual average and 80 ppm maximum in all of Canada effective January 1, 2002, with some variation possible by region (essentially the California sulfur standard) reduce to 150 ppm annual average and 200 ppm maximum in all of Canada effective January 1, 2002; or defer further action on sulfur levels in Canada and match in the future the least restrictive U.S. fuel requirement of the new national vehicle standards expected in the 2004 time frame. On the last option, it must be noted

that British Columbia has already taken regulatory action to reduce the sulfur content of gasoline sold in that province (E.P.A, 2005). Next, several methods for diesel desulfurization are discussed.

## 1.3 Desulfurization Processes

### 1.3.1 Hydrodesulfurization

Hydrodesulfurization (HDS) process has been used for many years as the conventional method in the petrochemical field to purify the liquid fuels from its sulfur contamination. This process is operated under high temperatures and pressures in order to reach the optimum level of reduction of the sulfur compounds when reacted with the hydrogen gas under the presence of a catalyst. The hydrogen sulfide product ( $H_2S$ ) is then separated from the reacted fuel and purified. The HDS process is inefficient to achieve deep desulfurization. A likely reason is the structural steric hindrance of heavy sulfur compounds with a boiling point over  $300^{\circ}C$ , which prevents them from being reduced by hydrogen (Mure *et al.*, 2001).

Dibenzothiophene (DBT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT) are two such sulfur compounds present in diesel fuels. These compounds have a very low reactivity in the sulfur reduction process. This has been shown by Ma *et al.*, (1994). Under a temperature range of  $280-4200^{\circ}C$  and a pressure of 2.9 MPa, the kinetic rate constant ( $k$ ) for DBT was found to be about  $0.1 \text{ min}^{-1}$  where as the value for 4,6-DMDBT was  $0.005 - 0.013 \text{ min}^{-1}$ .

Table 1.1 Properties of Diesel (Deshpande *et al.*, 2005)

Total Sulfur (wt %)	0.71
Boiling range	230 – 350 °C
Density (15 °C)	0.84 g/mL
Aromatics	17%
Olefins	5%
Saturates	78%
Pour point	-10 °C

### 1.3.2 Oxidative Desulfurization

An oxidative desulfurization (ODS) approach to sulfur removal serves as an alternative to the HDS process. The advantage that an ODS process has over HDS is that the sulfur compounds that are the most difficult to reduce in the HDS are the most reactive in the ODS process; i.e. this process has a reverse order of reactivity as compared to the HDS. The reason behind this is because of the reactivity of sulfur compounds for oxidation. This reactivity is related to the increased electron density on the sulfur atom. The electron donating properties of methyl groups on the aromatic rings positively influences DBT derivatives and the one with the most electron rich sulfur atom will react fastest. (Otsuki *et al.*, 2000).

The most important fact is that the oxidation of thiophenes to sulfones increases their polarity and molecular weight. The enhanced polarity makes it easier to remove them by adsorption on a solid material such as silica, alumina, clay or activated carbon. It also facilitates their separation by extraction, distillation or alkali treatment. (Yazu *et al.*, 2001). Figure 1.1 shows a schematic diagram describing the ODS pathway as reported by Yazu *et al.* (2001). The ODS process leads to the production of more polar sulfoxides.

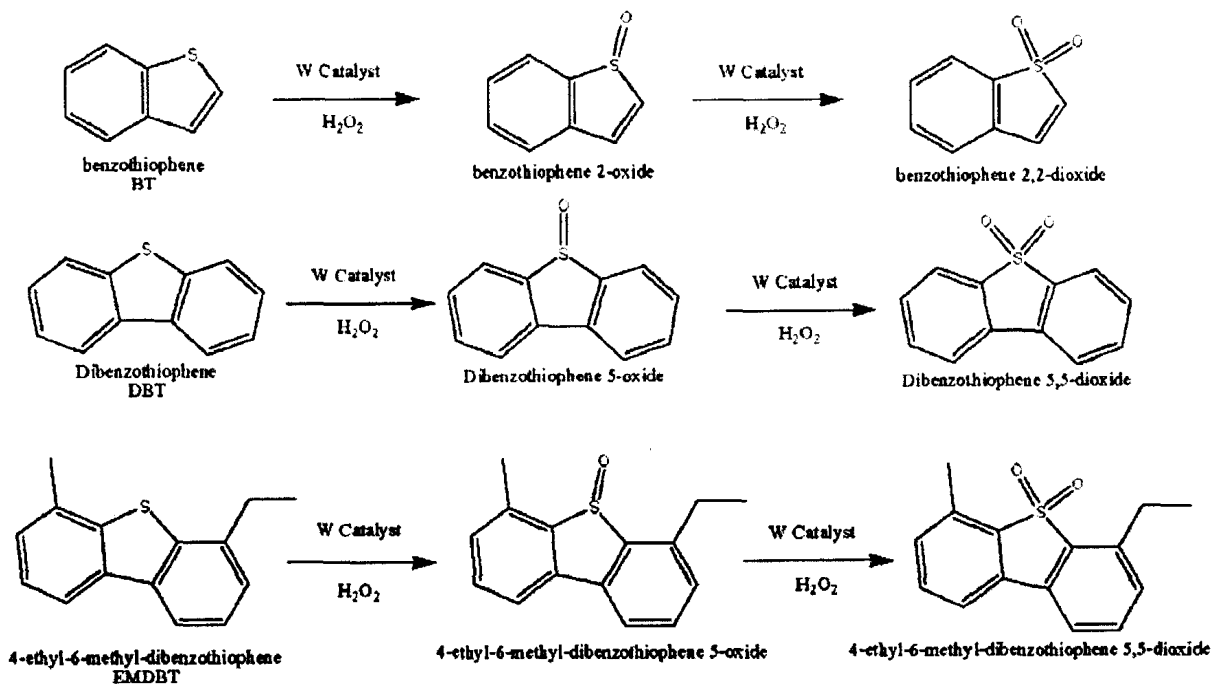


Figure 1.1 Simplified reactions of sulfur compounds following the ODS process. (Yazu *et al.*, 2001).

Oxidative desulfurization can be carried out using the following two approaches:

(1) Chemical oxidation

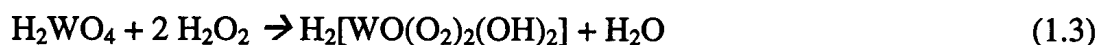
The use of pure chemical catalysts as well as an oxidizing agent is the main key in achieving the desired sulfur removal level.

(2) Bio-oxidative desulfurization

The oxidation process is executed in the presence of a biocatalyst; e.g. bacteria or enzyme.

### 1.3.2.1 Chemical-oxidative Desulfurization

Campos-Martin *et al.* (2004) studied the oxidation of different organic sulfur compounds with hydrogen peroxide in a biphasic liquid system with the existence of a chemical catalyst. The oxidation was done under atmospheric pressure and ambient temperature. These authors found that the tungsten phase transfer catalysts (PTC) are the most efficient in converting the thio-ethers into their corresponding sulfoxides and ultimately into sulfones using the hydrogen peroxide as the oxidant in the bi-phasic system. In their experiments, the tungstic acid, which is catalyst precursor  $\text{H}_2\text{WO}_4$ , is rapidly oxidized by  $\text{H}_2\text{O}_2$  with the presence of an accelerator such as phenylphosphonic acid:



The phase transfer agent ( $\text{Q}^+$ ) found in the  $\text{H}^+$ -  $\text{Q}^+$  ion exchange transfers the bisperoxotungstate ( $\text{H}_2[\text{WO}(\text{O}_2)_2(\text{OH})_2]$ ) to the organic layer in the biphasic system.

The oxidation of the thioethers to their sulfones occurs in the apolar phase by the complex catalytic compound the bis-peroxo-tungstate. The compound is regenerated again at the interface between the two layers and the sulfones produced are transferred to the polar layer due to their high solubility in the polar solutions, which results in a free-sulfur organic layer.

Different parameters were investigated in order to observe their effect on the rate of oxidation (Campos-Martin *et al.* 2004). Some of these parameters included temperature, hydrogen sulfide and the organo-sulfur compounds molar ratios, the type of solvent used,



and the substrate used in the reaction. It was found that the oxidation rate increased by increasing the temperature, and also increasing the molar ratio of H<sub>2</sub>O<sub>2</sub> and the organic sulfur molecule.

The oxidative method of desulfurization is very promising additional research was done on the biphasic system and it was found that replacing the water based solvent with acetonitrile increases the mass transfer of the sulfones since the surface tension of this solvent is very low (Campos-Martin *et al.* 2004). In addition to that, a kerosene fraction was investigated using this method and the sulfur level was lowered to 25 ppm under temperature of 353 K and H<sub>2</sub>O<sub>2</sub>: Substrate molar ratio of 2.5:1 (Campos-Martin *et al.*, 2004).

Bernal and Cedeño Caero (2005) focused their studies on the effect of solvents on the oxidative desulfurization of organic sulfur compounds. The same biphasic system used by Campos-Martin *et al.* (2004) was also implemented but with different catalyst and several solvents. The catalyst used was V<sub>2</sub>O<sub>5</sub>/Al<sub>2</sub>O<sub>3</sub> and the oxidant was hydrogen sulfide. The solvents studied were: N, N-dimethylformamide (DMF), 2-ethoxyethanol (EEOH), acetonitrile (MeCN) and gamma-butyrolactone (BuL). The results showed that the benzothiophenes reactivities decrease in the order of the following solvents: MeCN > BuL > EEOH > DMF (Deshpande *et al.*, 2005). It was also concluded that a significant percentage of the sulfur compound is transferred to the apolar layer without oxidation to their corresponding sulfones. By investigating the extractive capacity of these solvents, it was found that 70-85% of benzothiophenes by physical extraction process, using the best solvent (DMF) and only close to 30-60% with MeCN (Deshpande *et al.*, 2005). In

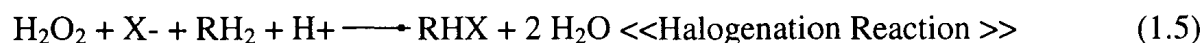
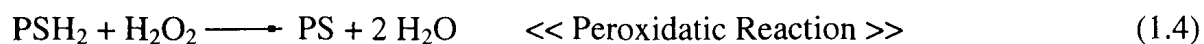
conclusion, oxidative desulfurization is a promising alternative in order to achieve a low level of sulfur in the petroleum fractions.

### **1.3.2.2 Bio-oxidative Desulfurization**

Hemo-proteins have been reported to oxidize the sulfur compounds to sulfones and sulfoxides (Ayala *et al.*, 1998) like Haemoglobin. Several enzymes such as Chloroperoxidase and Soybean peroxidase may have potential activity. However, this has not been previously explored. Some of these studies are discussed in this section.

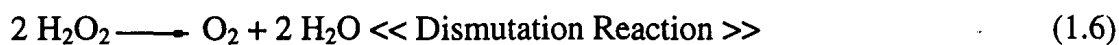
#### **1.3.2.2.1 Chloroperoxidase**

Chloroperoxidase enzyme is a 42000 Da glyco-monomeric hemo-protein from the fungus *Caldariomyces fumago*. This enzyme carries out the catalysis of the peroxidative halogenations needed by the fungus for caldariomycin biosynthesis. The enzyme also demonstrates peroxidase, catalase and Cytochrome P450- like activities (Griffin *et al.*, 1991). Chloroperoxidase (CPO) is considered a very important catalyst among the hemoproteins. CPO catalyzes reactions that use the hydrogen peroxide as a co-substrate. Therefore, this catalyst reflects characteristic of peroxidases. CPO catalyzes the iodination of some organic compounds. The peroxidatic and halogenation reactions can be represented by the following generic reactions (Libby, 2001):



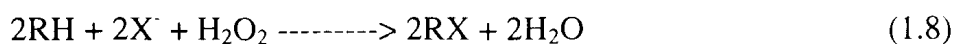
Where, PSH<sub>2</sub> is the peroxidatic substrates and RH<sub>2</sub> is the halogenation substrates.

However, unlike most other peroxidases, CPO also catalyzes the dismutation of hydrogen peroxide and some cytochrome P450 mono-oxygenase type reactions (R = oxygen acceptor substrate):



Therefore, understanding the structure and chemical mechanism of the enzyme supplies a better insight into the behavior and activity of catalases, cytochrome P 450 mono-oxygenases and peroxidases other than CPO (Libby, 2001).

With the use of chloroperoxidase, the halide ions can be oxidized except for fluoride, which is not considered a substrate for the enzyme. Since the fluoride ion competes for both the hydrogen peroxide and the halogen anion binding sites, it is considered an inhibitor of the halogenation reaction (Han *et al.*, 2002). The following equation shows the catalytic activity of the enzyme through a halogenation reaction:



It will also catalyze the peroxide-dependent oxidation when a halide ion is not present; the products of this reaction are different peroxidase substrates. Based on the substrate used, the best pH value for the enzyme to function properly is between 4-7 (Han *et al.*, 2002).

Since the CPO enzyme showed promising characteristics, genetic engineering was used to improve the CPO enzyme properties by developing more hydrophobic and thermo-tolerant clones of the enzyme to be used and a catalyst in the oxidative desulfurization of diesel in the bio-refining processes (Julia & Acero, 2002)

#### **1.3.2.2.2 Soybean Peroxidase**

Soybean Peroxidase belongs to class III of the plant Peroxidase super-family that also includes horseradish (HRP), barley (BPI) and peanut (PNP) peroxidases (oxidoreductase, EC 1.11.1.7). It is a 326-amino acid which contains glycoprotein and has a molecular mass of ~37 kDa. Soybean seed hull is considered an inexpensive source where the enzyme is present in large amounts. It has many applications especially in the physiological processes in plants (Bedard & Mabrouk, 1997).

By studying the three-dimensional crystal structure of SBP (See Figure A2), a similar overall protein arrangement was observed between SBP and HRP-C. Despite the similarities in the protein structure, SBP showed less susceptibility to permanent inactivation by hydrogen peroxide and it higher heat activity under 70 °C even after up to 12 hours (Bedard & Mabrouk, 1997). The melting temperatures of the secondary and

tertiary structures of SBP with characteristic transition midpoints,  $T_m$ , are 86 and 83.5 °C. Also, changing the pH has a great effect on the melting temperature of the secondary and tertiary structure of the enzyme (Bedard & Mabrouk, 1997). Therefore, this information must be taken into consideration during the investigation to maintain the enzyme under a temperature and pH value that will guarantee the proper behaviour of the enzyme without worrying about its biological structure. The enzyme will be tested under ambient temperatures, which will insure the safety of the protein structure.

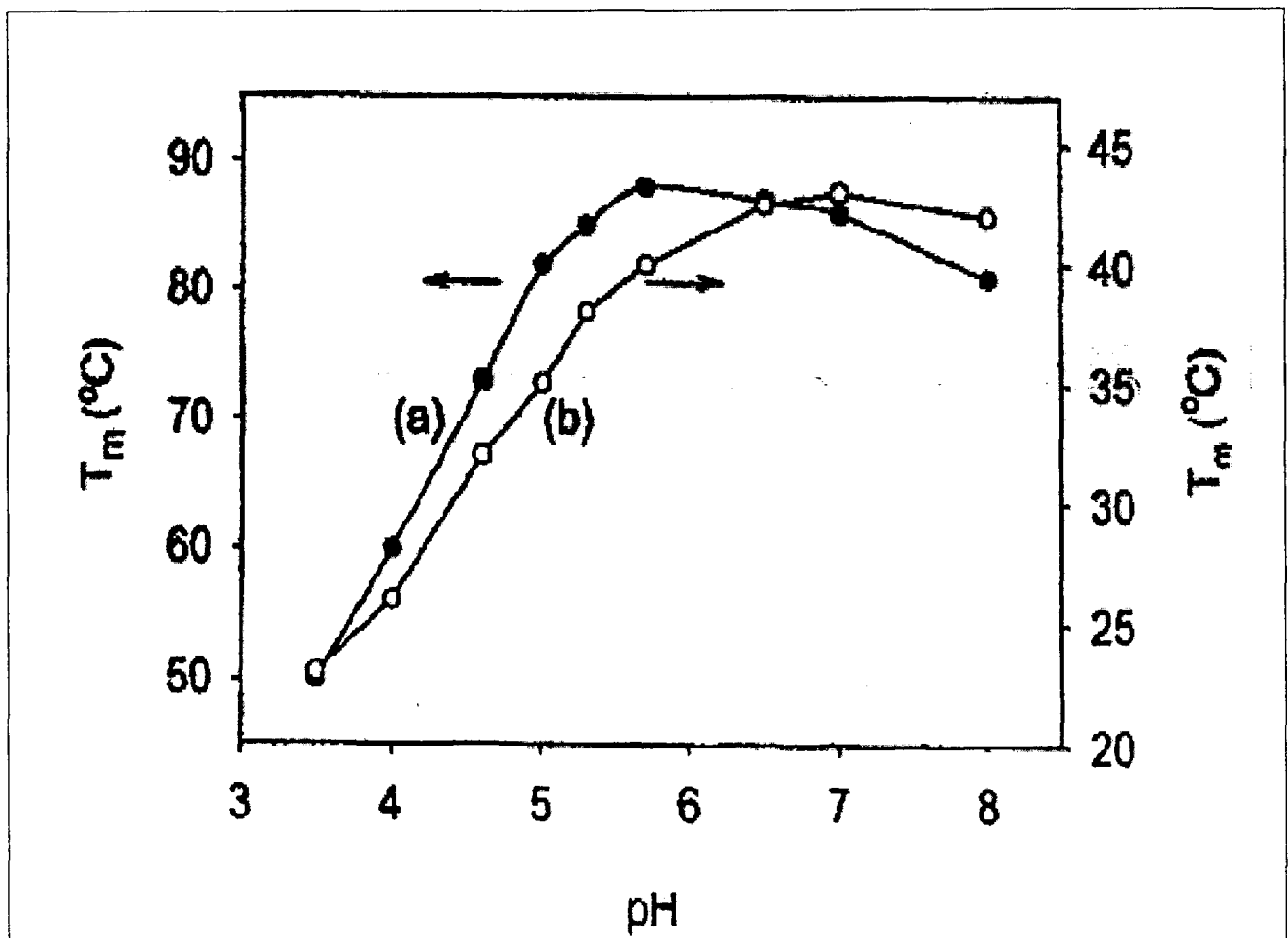


Figure 1.2 pH dependence of  $T_m$  corresponding to the melting of the secondary structure (●) and the tertiary structure (○) (Kamal *et al.*, 2002).

Figure 1.2 indicates that increasing the pH of the solution where the enzyme is present increases the melting temperature till around a pH value of 6 and starts to decrease around pH value of 7. As a result, the thermal resistance of the enzyme increases by increasing the value of pH till around a value of 6 (Kamal *et al.*, 2002).

Due to its high thermal stability, SBP shows that it can be used as biocatalyst and biosensor in a wider range of temperature. It also shows a great potential in non-physiological processes such as wastewater treatment and phenolic resin synthesis (Kamal *et al.*, 2002).

The three step mechanism where SBP catalyses the oxidation of a substrate AH to form its corresponding radical product A<sup>•</sup> is (Nissum *et al.*, 2001):



Where: Compound I (Cpd I) = peroxidase-O, Compound II (Cpd II) = peroxidase-OH.

These two active species, compounds I and II, are capable of producing a free radical from obstructing an electron from the substrate used followed by coupling, disproportionation and/or reaction with molecular oxygen (Kobayashi *et al.*, 1986).

Based on the enzyme's reactive potential, it can be used to polymerize various organic pollutants and oxidize several desired compounds present in the industrial and petrochemical streams. SBP is able to retain its catalytic properties under wide ranges of pH and at elevated temperatures (Geng *et al.*, 2001). We hypothesize here that both CPO

and SBP may have a potential to oxidize DBT, however, this needs to be further investigated.

### 1.3.3 Desulfurization by Extraction

Alternative technologies are the key to provide the solution to sulfur-free clean fuels. Therefore, extractive desulfurization (EDS) is considered one of the promising methods to reach the desired levels of sulfur in fuels. The extraction process depends only on the right choice of the extracting agent. The operation is done under ambient temperatures and pressures with minimal use of any extra chemicals since no reactions are occurring during the process. The conventional units in extraction can be used in this process, which makes it easier to implement the process into a commercial stage. All these advantages makes the capital cost of this process minimal compared to the other desulfurization processes.

The extracted organo-sulfur compounds can be concentrated and recycled back into the process for further treatment if the extracting agent can be regenerated. The most important part in choosing an extracting agent is its **selectivity** property.

Room temperature ionic liquids exhibit many properties which make them potentially attractive media catalysts or solvents. They have essentially no vapour pressure, and are easy to contain. They are able to dissolve a wide range of organic, inorganic and organometallic compounds. The solubility of gases, *e.g.* H<sub>2</sub>, CO and O<sub>2</sub>, is generally good which makes them attractive solvents for catalytic hydrogenations, carbonylations,

hydroformylations, and aerobic oxidations. Polarity and hydrophilicity/lipophilicity allow them to obtain selective extractive properties. They can be readily adjusted by a suitable choice of cation/anion (see earlier). Therefore, ionic liquids have been referred to as ‘designer solvents’ (Martyn *et al.* 2000).

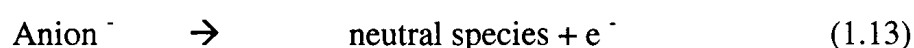
### 1.3.3.1 Properties of Ionic Liquids

Ionic liquids (IL) are actually molten organic salts with melting points under 100 °C, often even lower than room temperature (21 °C) (Candra-Broch *et al.*, 2003). These salts are manufactured from an inorganic anion and an organic cation and the delocalized charge of the salt reduced lattice energy and hence have lower melting points. The most common ILs are imidazolium and pyridinium derivatives. The lack of a measurable vapor pressure makes ILs more desirable than the conventional organic solvents (Fuller *et al.*, 1997) (Candra-Broch *et al.*, 2003).

Ionic liquids are attractive solvents as they are non-flammable, have a high thermal stability and are relatively inexpensive to manufacture. They usually exist as liquids well below room temperature up to a temperature as high as 200°C (Ki-Sub *et al.*, 2004). These solvents are used in a wide variety of chemical activities. Due to their electrochemical properties, ionic liquids are used in the deposition of metals on surfaces. In addition to that, the low volatility and thermal stability of these salts allowed them to be used in different types of chemical reactions as catalysts without worrying about the decomposition or degradation of these agents which allows more production of the desired products (Ki-Sub *et al.*, 2004).



The electrochemical stability is very important in ionic liquids since they are used as catalysts for many reactions and are deeply involved in the chemical reaction mechanism. The stability of the ionic liquids depends on the stability of the cation against electrochemical reduction-processes and the stability of the anion against oxidation-processes. Both could be represented by the following general equations (Wasserscheid *et al.*, 2002):



As a result, the electrochemistry of the ionic liquids is affected by impurities. The most common impurities that have a great effect are the residual halides since they are much easier to be oxidized which leads to a drastic decrease in the electrochemical stability of the used ionic liquid. Ultimately, ionic liquids must be purified to a high percentage before usage to insure optimum performance (Wasserscheid *et al.*, 2002).

Another important property of ionic liquids is their thermal stabilities. Even though ionic liquids have a negligible vapor pressure below their decomposition temperature, several studies showed that these systems do not possess sufficient long-term stabilities; for example, hexafluorophosphate-based ionic liquids form corrosive decomposition products such as HF and phosphoric acid when they are subjected to elevated temperatures for a long time (Forsyth *et al.*, 2004).

Wilkes *et al.* (2004) reported thermal decomposition onset temperatures of 445°C for 1-ethyl-3-methyl-imidazolium-tetrafluoroborate, 423°C for 1-butyl-3-methyl-imidazolium

tetrafluoroborate and 457°C for 1,2-dimethyl-3-propyl imidazolium bis-(trifluorosulfonyl)-imide (Forsyth *et al.*, 2004). Finally, the extractive property of the ionic liquids is one of the most important because of the promising results these extracting agents can give. Their unique chemical structure allows most of them to form a separate layer from the organic and the aqueous solutions. This leads to the possibility of forming biphasic system which results in a mass transfer to the desired compounds to the ionic liquid layer. Also, their selectivity gives an advantage to preserve the chemical structure of the organic layer processed (Paulechka *et al.*, 2003). Some of the ionic liquids are miscible with water. This can be used as a method to regenerate the spent ionic liquid where the organic compounds absorbed into the ionic liquid can be precipitated out by the addition of water (Zhang *et al.*, 2004).

#### **1.3.3.1.1 1-Butyl-3-methylimidazolium tetrafluoroborate**

The chemical formula of the 1-Butyl-3-methylimidazolium tetrafluoroborate ionic liquid is  $C_8H_{15}N_2.BF_4$ . It has a molecular weight of 226.02 g/mol and a melting point of -71 °C. It is considered more viscous than common molecular solvents. It has a viscosity value of 233 mPa.s and a density of 1.22 g/mL under 20 °C. It appears as a clear, colorless viscous liquid and is 100% miscible in water. See Table 1.2 (Ki-Sub *et al.*, 2006)

Table 1.2 Properties of 1-Butyl-3-methylimidazolium tetrafluoroborate (Ki-Sub *et al.*, 2006).

Density (20 °C)	1.22 g/mL
Viscosity (20 °C)	233 mPa.s
Surface Tension (25 °C)	46.4 mN.m <sup>-1</sup>
Melting temperature	-71 °C
Vapor pressure	~0
Solubility	<p>Insoluble in organic solutions</p> <p>Soluble in water</p>

Jochen *et al.* (2004) carried out early experiments which were focused on the selective extraction of organic sulfur compounds using different types of ionic liquids. The model fuel used was prepared by mixing *n*-dodecane with DBT-derivatives. The ratio of the sulfur concentration in the ionic liquid to the sulfur concentration in the model fuel was measured for different ionic liquids. This ratio is defined as the Nernst partition coefficient  $K_N$  (mgS/kgIL)/ (mgS/kgOil). Table 1.3 reflects the Nernst partition coefficient for each ionic liquid tested.

Nevertheless, Due to the low hydrolysis stability and some cases toxicity of the chlorometallate ILs, they are not practical to be used in a large scale process. On the other hand, the experiments on the alkyimidazolium tetrafluoroborate ([BMIM][BF<sub>4</sub>]) and hexafluorophosphate ILs ([BMIM][PF<sub>6</sub>]) showed fairly high Nernst values for the extraction of the organic sulfur compounds, which is consistent with data reported in the literature.

Table 1.3 Nernst partition coefficients  $K_N$  for extraction with ILs: Ratio of concentration of sulfur in IL over concentration of Sulfur in Oil (Jochen *et al.*, 2004)

IL	$K_N$ in mg(S) kg(IL) <sup>-1</sup> / mg(S) kg(oil) <sup>-1</sup> <sup>a</sup>
[BMIM]Cl/AlCl <sub>3</sub>	4.0
[BMIM][BF <sub>4</sub> ]	0.7
[BMIM][PF <sub>6</sub> ]	0.9 <sup>b</sup>
[BMIM][OcSO <sub>4</sub> ]	1.9
[EMIM][EtSO <sub>4</sub> ]	0.8
[MMIM][Me <sub>2</sub> PO <sub>4</sub> ]	0.7

<sup>a</sup> Model oil: 500 ppm S as DBT in *n*-dodecane: mass ratio 1 : 1. mixing time 15 min. room temperature. <sup>b</sup> 60 °C.

The most important property [BMIM] [BF<sub>4</sub>] shares with other ionic liquids is its insolubility in most common organic solvents. This is a key factor behind testing this type of solvents as extracting agents in an organic environment. In addition to that, its 100% miscibility in water shows an advantage in manipulating the functionality of the ionic liquid during the extracting process. This is discussed in more details in Chapter 4.

## 1.4 Objectives

This study focuses on two different approaches of diesel desulfurization under ambient temperature and pressure conditions. The first section of this study focuses on the enzymatic oxidation attempts of the heavy organic sulfur compounds to its corresponding sulfone. This investigation specifically deals with:

I [a] Investigation of the activity measurement of the Chloroperoxidase and the Soybean peroxidase and select the better enzyme to serve as a catalyst.

[b] Investigation of a hypothesis that oxidation of Dibenzothiophene can be carried out using CPO or SBP as a catalyst.

The second section of the study is directed to the extractive desulfurization of diesel under moderate conditions of temperature and pressure using a selected ionic liquid as an extracting solvent. This investigation specifically deals with:

II [a] Study of the extractive desulfurization of Dibenzothiophene (DBT) from a hexane solution into the 1-Butyl-3-methylimidazolium tetrafluoroborate (BMIM BF<sub>4</sub>) ionic liquid phase.

[b] Investigation of parameters influencing the extractive desulfurization of DBT.

The chapters are organized as follows:

Chapter [1] A state-of-the-Art literature research on the work done in the past regarding desulfurization processes.

Chapter [2] Materials and Methods describing the general chemicals and the equipments used in the study and the procedures followed during the investigation

Chapter [3] This describes the activity study of the enzymes CPO and SBP and the unsuccessful oxidative desulfurization investigation of DBT.

Chapter [4] This describes the study the extractive desulfurization of DBT using BMIM BF<sub>4</sub> and the effect of different parameters on the extraction capacity like solvent to fuel ratio, water effect, etc.

Chapter [5] Conclusions and Recommendations are presented.

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## Chapter 2: Materials and Methods

### 2.1 Materials

Dimethyl-dibenzothiophene (DBT) with 98% purity was purchased from Aldrich Chemical Company, Inc. Chloroperoxidase was purchased from Aldrich Chemical Company, Inc (St. Louis, MO, US) with a purity of 97 % and was always stored under 4 °C in a refrigerator. Soybean peroxidase was extracted from the soybean hull through a certain procedure explained in chapter 3. The soybean hull was purchased from ADM Agriculture Inc (Auburn University, AL, US) The solvent used for the first segment of the research was a 99.5 % pure acetonitrile (MeCN), which was purchased from MERCK, KgaA, Darmstadt, Germany. The oxidizing agent used for the oxidation attempts was a 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which was also purchased from MERCK, KgaA, Darmstadt, Germany. The second oxidizing agent tested agent tert-butyl peroxide (98 %) was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI, USA. Hexane (85 %) was purchased from EM Sciences; a branch of EM industries Inc. 1-butyl-3-methylimidazolium tetra-fluoroborate (97 %) was purchased from Fluka, Sigma-Aldrich (St. Louis, MO, US). Tween 80 (Polyoxyethylene) was purchased from Fisher Scientific Company (Steinheim, Germany). Triton X-114 (Octylphenoxy-polyethoxyethanol) was purchased from Sigma chemical Company (St. Louis, MO, US). Distilled water was used in the steps when water was needed.

## 2.2 Analysis

Gas Chromatography (GC) was applied to identify sulfur compounds in organic phases, acetonitrile and hexane. An Agilent 6890 Series GC unit with sulfur specific Flame Photometric Detector (FPD) was used to determine the DBT concentration in each sample. It also contains the HP-5 (cross linked 5% PH ME siloxane) capillary column (30m x 0.32mm x 0.25 $\mu$ m film thickness). The needle injector is used to insert the sample inside the unit where the volume of the injector is from 0-10  $\mu$ L. The analysis parameters used in the GC software are listed in Table (2.1). The analysis time for every sample was 28.4 minutes and the retention time of DBT was 8.1 - 8.4 minutes.

The Agilent 6890 Series GC system was used to analyze the samples taken from each experiment. The GC unit was turned on allowing the system to reach the desired steady state through the parameters set for the flow rates of the gases used. The oven heats up the inlet of the GC system to 95 °C then the sample is injected through the inlet that is connected to the FPD detector. The liquid sample starts to vaporize due to the high temperature. The analysis is executed allowing the oven's temperature to increase in a rate of 10 °C/minute. This results in the evaporation of the entire sample and the transfer of all the chemicals by the carrier gas to the detector through the HP-5 capillary column. The detector measures the amount of sulfur of each compound based on the time required for each chemical to pass through the column. The heavier the compound is the slower its time to reach the detector. The total run time was set to 28.4 minutes to insure the complete transfer of all the chemicals found in the sample.

Table 2.1: The Agilent 6890 Series GC system Analysis Parameters used in the construction of the calibration curve and the determination of the sulfur concentration in each sample studied

<b>Inlet Conditions</b>	<b>Detector</b>	<b>Oven</b>
Inlet Temperature 300°C	H <sub>2</sub> Flow 75 mL/min	Initial Temperature 95 °C
Pressure 25 psia	Air Flow 100 mL/min	Ramp 10 °C/min
Split Ratio 40:1	N <sub>2</sub> Flow 15 mL/min	Final Temperature 325 °C held for 5 min



## 2.3 Equipment

Figure 2.1 shows the experimental equipments used in the first section of the research, which are the oxidative desulfurization attempts. A small reactor was prepared from a 30 mL glass bottle containing the model fuel (i.e. DBT solvent in acetonitrile or hexane), the enzyme and the oxidant (hydrogen peroxide or tert-butyl peroxide). Inside the reactor, a magnetic stirrer was inserted in order to achieve a proper mixing of the mixture. Finally, a stirring plate was used to provide the magnetic field needed for the stirrer to rotate.

The equipment used in the extraction desulfurization attempt is shown in Figure 2.2. This consists of an extractor vessel, which is a small 5mL glass bottle, contained the biphasic system of DBT-Hexane solution and the BMIM-BF<sub>4</sub> ionic liquid. A vortex unit used to allow the proper mixing of the two layers by placing the glass bottle on the top of the vortex instrument. This unit was represented by either a shaft connected to a motor with several rotating blades or an ultrasound unit that provide pulses into the mixture for intense mixing. Also, a constant-temperature glass jacket was used during the testing of the temperature parameter and its effect on the extraction process. A water bath was used to provide a continuous supply of a constant temperature using a pump. The pump contained a heating element that allowed the control of the water temperature. The accuracy of the temperature control was  $\pm 1$  °C. The Ultrasound Pulse generator provided a pulse with a constant frequency of 23 kHz. The pulse cycle and amplitude ratio can be varied from 0 – 1 and 20 – 100 % respectively.

Finally, the column KG1-MATR is a mechanically agitated tubular reactor with a volume of 200 mL. It contains two columns; an inner column where the mixing occurs and an outer column functions as a jacket to control the temperature through water that is recycled from a batch. The column contains 4 inlets/outlets, which provide great flexibility in manipulating the reactor based on the goal that needs to be achieved. Two inlets at the top can be used to inject the samples into the column. Two outlets at the bottom used to extract the mixed solutions. Finally there is an outlet at the bottom of the reactor which can be used as a connector to allow a pressured air to be injected in order to increase the efficiency of the mixing or a drainage system that allows the mixed solutions to be emptied out from the bottom of the column. The rotating blades are mounted on a shaft that is connected to a motor where the speed of rotation can be controlled. The shaft was inserted into the inner column where a cylindrical plastics mounted on the shaft were used to stabilize the vibration during the rotation of the blades.

The purpose of designing the KG1-MATR column is to upscale the setup of the experiment and investigate its effect on the extraction process. The column can withstand a maximum capacity of 200 ml of a total mixture. The column was made of a Plexiglas material with two columns that have a thickness of 0.635 cm. The inner diameters of the inner and outer columns are 3.175 and 7.62 cm respectively. The total height of the column is 29.21 cm and the diameter of the inlets/outlets is 0.635 cm. The column was sealed from the top and bottom to prevent any leakage of the mixture during the operation of the unit. See Figure 2.3 for the column dimensions.

The temperature was controlled by a water bath and after every run; a sample was taken for analysis using the GC unit.

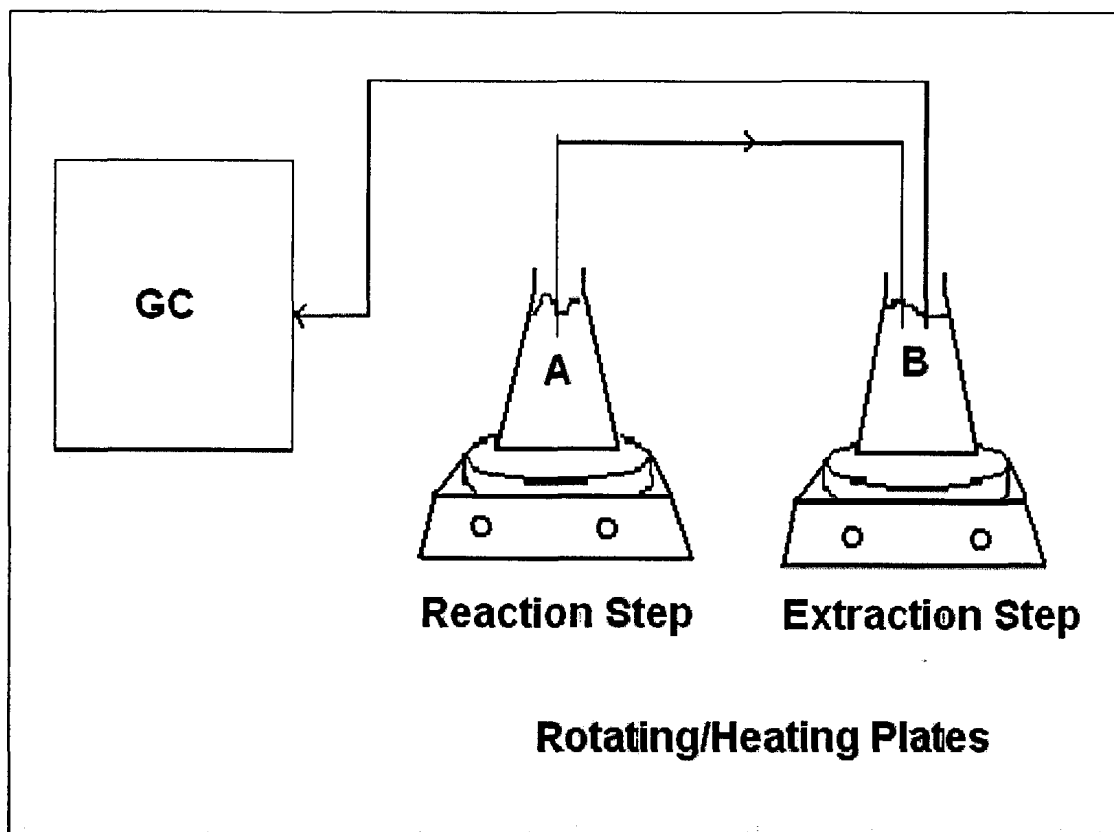


Figure 2.1 Experimental Set-up for the oxidative desulfurization (GC: Gas Chromatographer with FPD column; A: Assay that contains the fuel model (DBT-Acetonitrile), H<sub>2</sub>O<sub>2</sub> and SBP; B: Biphasic layer system used to extract the un-reacted DBT and the expected sulfone product.)

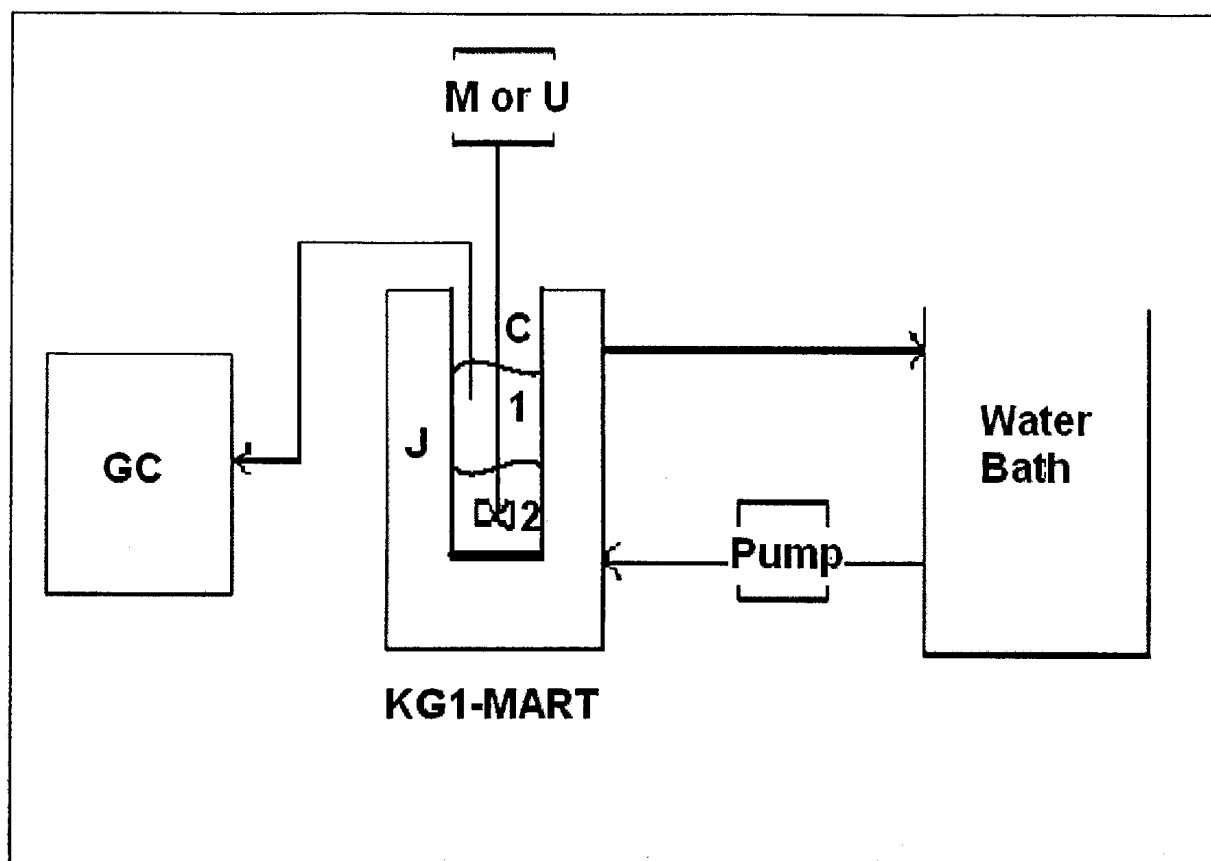


Figure 2.2 Equipments used in the extraction process, (KG1-MATR: Mechanically Agitated Tubular Reactor; GC: Gas Chromatographer with FPD Column; M: Motor allows the control of the blades' speed, J: Jacket to maintain the temperature of the column; C: The inner column that contains the biphasic layer system (1 Fuel model, 2 ionic liquid solvent); Pump: Contains a heating source to achieve the desired temperature; U: Ultrasound pulse generator (Does not include rotating blades))

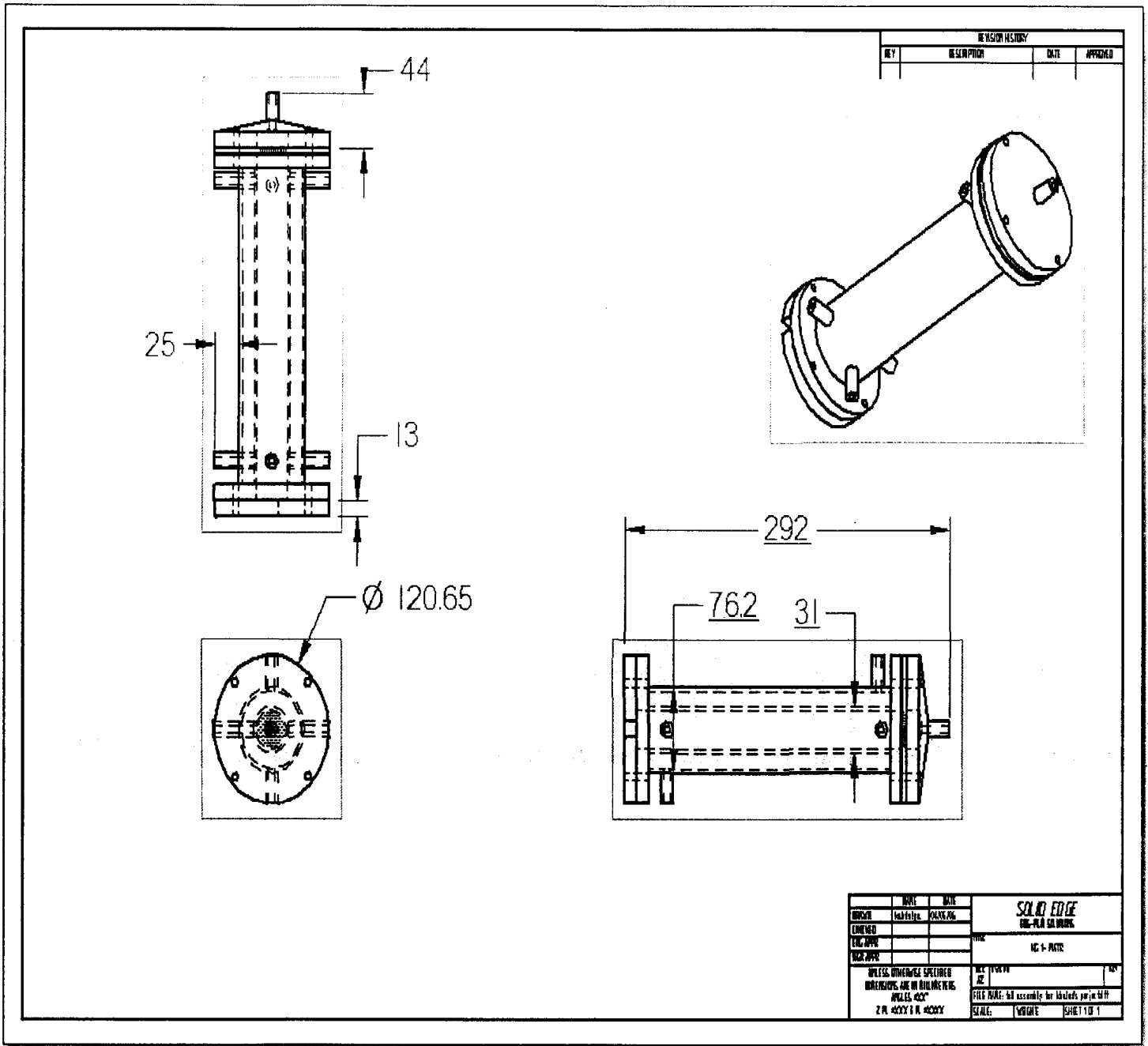


Figure 2.3 Dimensions of the mechanically agitated tubular reactor KG1-MATR.

## 2.4 Methodology of Experiments

The Bio-catalytic oxidation attempt of DBT was started by representing the diesel with different types of model fuels. DBT-MeCN and DBT-Hexane solutions were considered model fuels with a sulfur concentration of 500 ppm. The concentration was prepared by dissolving 25 mg of Dibenzothiophene in 50 ml of the desired solvent. The SBP enzyme was prepared and used as a catalyst in the oxidation process. The assay consisted of 75% of the DBT solution, 20% of distilled water, 4% of the 0.1 M tert butyl peroxide or hydrogen peroxide and 1% of the diluted SBP enzyme in the  $\text{KH}_2\text{PO}_4$  phosphate buffer (pH 6).

On the other hand, the extraction process was done by using the DBT-Hexane solution and the BMIM-BF<sub>4</sub> ionic liquid. The 500 ppm DBT solution was added to the ionic liquid with a volume ratio of 1:1 and formed a biphasic system. The preliminary studies were done under ambient temperatures and pressures and the process was done on a very small scale. A small container was used and the mixture was subjected to an intense mixing using the Vortex unit.

In addition to that, the temperature effect was investigated using the ultrasound pulse generator to insure constant mixing throughout the experiment and concentrate on the change of the extracting capacity due to the change in temperature. The biphasic layer was added in the glass bottle where the ultrasound probe was inserted into the bi-layer

solution. The mixing time was set to 15 minutes and the temperatures investigated were 20, 30, 40, 50, and 60 °C. Similar tests were conducted using the column where the mixing was done using rotating blades mounted on a shaft that is connected to a motor. Finally, several parameters like the effect of water presence and the addition of surfactants were investigated and their details can be found in Chapter 4.

# Chapter 3: Enzymatic Oxidation of Dibenzothiophene (DBT) in Acetonitrile (MeCN)/Water

## 3.1 Introduction

Many petro-chemical companies implemented new catalysts or modified their chemical processes in order to comply with the new environmental regulations forced by the government. The modification costs by the refineries were high due to the time restriction set by the environmental protection agency to lower the sulfur level in diesel to 15 ppm (E.P.A, 2005).

Since the conventional Hydrodesulfurization process could not meet the new sulfur level requirements, other processes have been tested in order to achieve the desired results of lowering the sulfur level to the acceptable concentration. Therefore, the biological approach was investigated under ambient temperature and pressure. Different types of bacteria were used to test their effectiveness in reducing the sulfur level. It was found that the Rhodococcus bacteria reduce the sulfur content by 60% (Castorena *et al.*, 2002). On the other hand, it was found that these types of bacteria must be genetically engineered in order to insure the proper reduction of the sulfur level (McFarland *et al.*, 1998). In addition to that, since bacteria are living organisms, specific nutrients and surrounding environment must be provided to achieve the optimum results. Therefore, it is not



feasible to utilize bacteria as catalysts in the large-scale process since the capital cost of providing the proper environment for the bacteria will be high.

Enzymes were previously investigated as potential catalysts in the oxidation process of the organic sulfur compounds (Han *et al.*, 2002). It was stated that enzymatic oxidation is advantageous over microbial desulfurization since the latter has mass transfer limitations. Also, enzymes show broader specificity compared to microbial cells for substrate (Libby, 2001). Chloroperoxidase is an enzyme that has the ability to catalyze reactions that use the hydrogen peroxide as a substrate, which reflects its potential to be used as a catalyst in the desulfurization process (Ayala *et al.*, 1998). It was able to chlorinate aromatic hydrocarbons assayed in the presence of hydrogen peroxide and chloride ions (Vazquez-Duhalt *et al.*, 2001).

Soybean peroxidase is also investigated in order to observe its potential as an oxidative catalyst in the desulfurization process. Its physical and chemical properties make it a great candidate to study its activity and potential catalytic capabilities (Nissum *et al.*, 2001). Since the enzyme gives a great reactivity potential, it can be utilized to polymerize and oxidize several undesirable compounds found in the industrial fields. SBP is able to retain its catalytic properties under wide ranges of pH and at elevated temperatures (Geng *et al.*, 2001).

The main hypothesis of this study is based on the catalytic oxidation of the chosen sulfur compound (DBT) to its corresponding sulfone and sulfoxide using a CPO or SBP

enzyme. The enzyme should catalyzes the addition of one or two oxygen atoms from a peroxide source to the DBT structure, which allows the formation of a hydrophilic compound that is easy to extract from the diesel phase. It is important to obtain the activity of the used enzyme since it reflects the effect of the catalysis on the oxidation system. The activity of the enzymes used in this study was measured before the oxidation studies of DBT. This step determines the feasibility of the enzyme to be used as a catalyst in this reaction. Chloroperoxidase (CPO) and Soybean peroxidase (SBP) were investigated under different procedures.

## **3.2 Results and Discussions**

### **3.2.1 Activity Measurement of Chloroperoxidase (CPO)**

Based on the literature research, Chloroperoxidase catalyzes the reaction of forming dichlorodimedon (colored) from the substrate Monochlorodimedon (MCD). The activity measurement assay consists of 2 ml of a citric acid buffer (pH = 4), 150  $\mu$ l of Monochlorodimedon (MCD) in an ethyl alcoholic solution, 120  $\mu$ l of 0.1 M KCl, 140  $\mu$ l tert-butylperoxide, and 10  $\mu$ l of concentrated Chloroperoxidase. The runs were conducted in a spectrophotometer where the wavelength used was 278 nm (VanDeurzen *et al.*, 1997).

The purpose of the experiment is to find the proper parameters to measure the optimum value of the CPO enzyme. The run is executed in a spectrophotometer unit where a specific wavelength is set to 420 nm in order to measure its absorbance in the assay. The mixture is added in a small glass container, which is placed inside the unit, and the reaction is initiated by adding the oxidant, which is in this case the peroxide solution. The increase in the absorption line indicates an observation of the enzyme activity. This activity is measured by the slope of the straight line obtained from the graph produced by the spectrophotometer unit. Each experiment lasted for 150 minutes and the run was repeated several times.

In order to see the effect of the enzymes concentration, 10  $\mu$ l of concentrated Chloroperoxidase was added to 0.5 ml of the citric acid buffer. The same procedure was executed using the diluted enzyme instead and the results were obtained from the unit.

Based on the first assay where the concentrated enzyme was used, the CPO had a measured activity of 0.0042 A/min. In addition to that, the results from the diluted-CPO assay, there was no indication of any enzymatic activity.

Figure 3.1 shows the comparison between the two assays. Even though the first assay resulted in activity presence, it was considered small and insufficient to be used as a catalyst. Also, the second mixture showed no detection of any enzymatic activity, which could be speculated that the enzyme was diluted too much and there was no sufficient concentration of the enzyme to catalyze the desired reaction.

In order to obtain a better understanding of the behavior of the enzyme, several parameters were tested like the type of oxidant used and its concentration, and the pH effect on the activity of the enzyme.

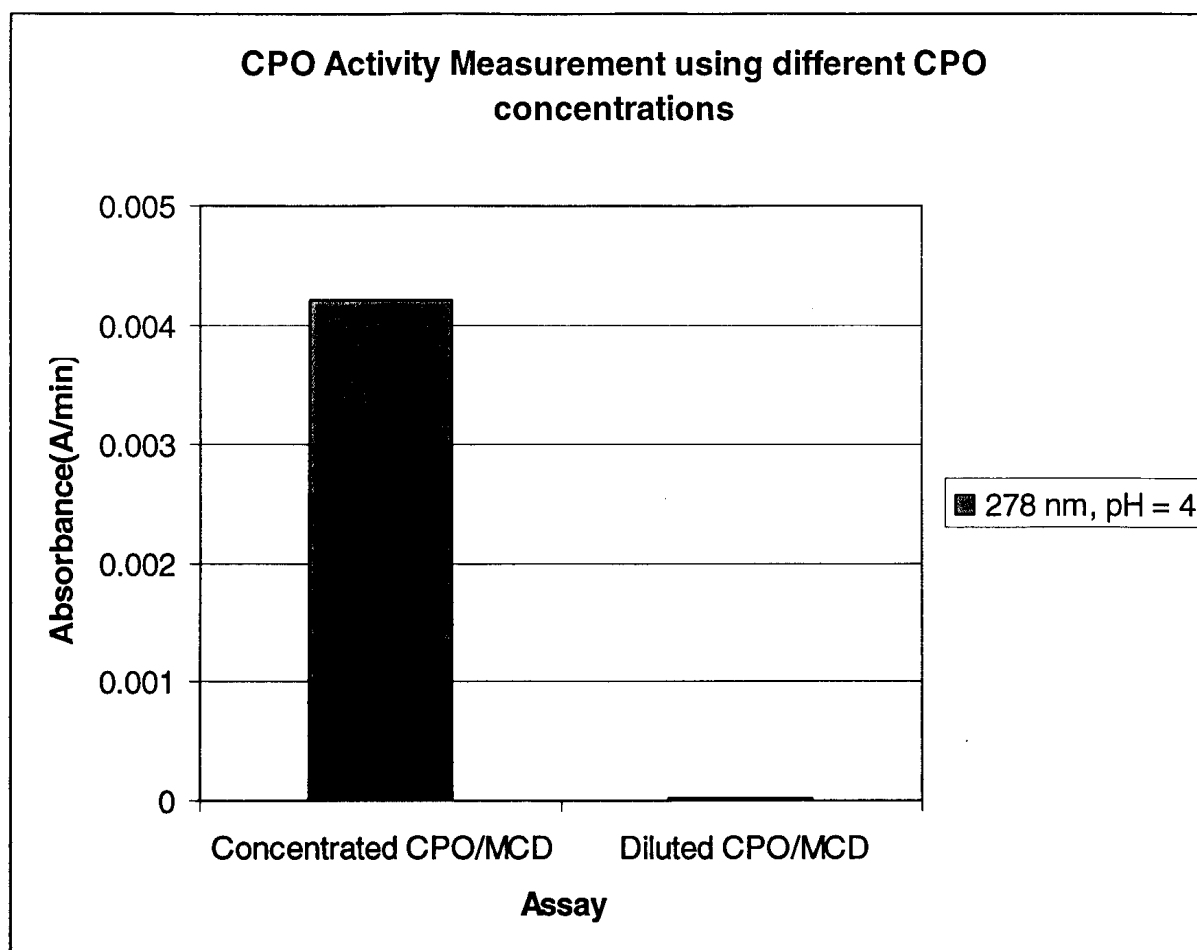


Figure 3.1 CPO Activity measurement using 2 ml of a citric acid buffer (pH = 4), 150  $\mu$ l of MCD in an ethyl alcoholic solution, 120  $\mu$ l of 0.1 M KCl, 140  $\mu$ l tert-butylperoxide, and 10  $\mu$ l of (concentrated/Diluted) Chloroperoxidase. Run time = 150 minutes, spectrophotometer wavelength = 278 nm.

### 3.2.1.1 Effect of Oxidant Type on the CPO's Activity

In order to measure the optimum activity of the enzyme, several changes had to be done to the assay. Different parameters were tested like changing the oxidant and the buffer used in the assay in order to observe the effect on the activity of the enzyme.

The second assay was maintained under the same measurements of the chemicals used. Hydrogen peroxide was used instead of the original tert-butylperoxide with different concentrations. The first run was executed with hydrogen peroxide concentration of 10 M/L and the experiment was conducted and repeated three times. The results indicated no presence of any reaction occurring in the mixture. Another sample was tested where the concentration of the hydrogen peroxide was diluted to 1M/L and several runs were investigated. The negative results obtained from these experiments indicated that the concentration of the hydrogen peroxide is too high for the enzyme to function properly.

Finally, an additional dilution of the oxidant to 0.1 M/L gave out positive results as shown in Figure 3.2 where the run was conducted for 300 minutes. This concludes that the high concentration of the hydrogen peroxide resulted in drastic hindering of the CPO enzyme activity. This concludes that the enzyme is sensitive to the concentrated hydrogen peroxide, which will result in 'killing' the enzyme. As the hydrogen peroxide concentration decreases the negative effect on the CPO enzyme activity decreases. The final dilution of  $H_2O_2$  to 0.1 M/L resulted in CPO activity measurement of 0.0045 A/min, which is almost the same as the activity measurement of 0.0042 A/min. See Figure (3.3).

As a result, the concentration of the oxidant has a major effect on the activity measurement of the Chloroperoxidase enzyme. As the concentration of the oxidant increase, the activity of the enzyme decreases.

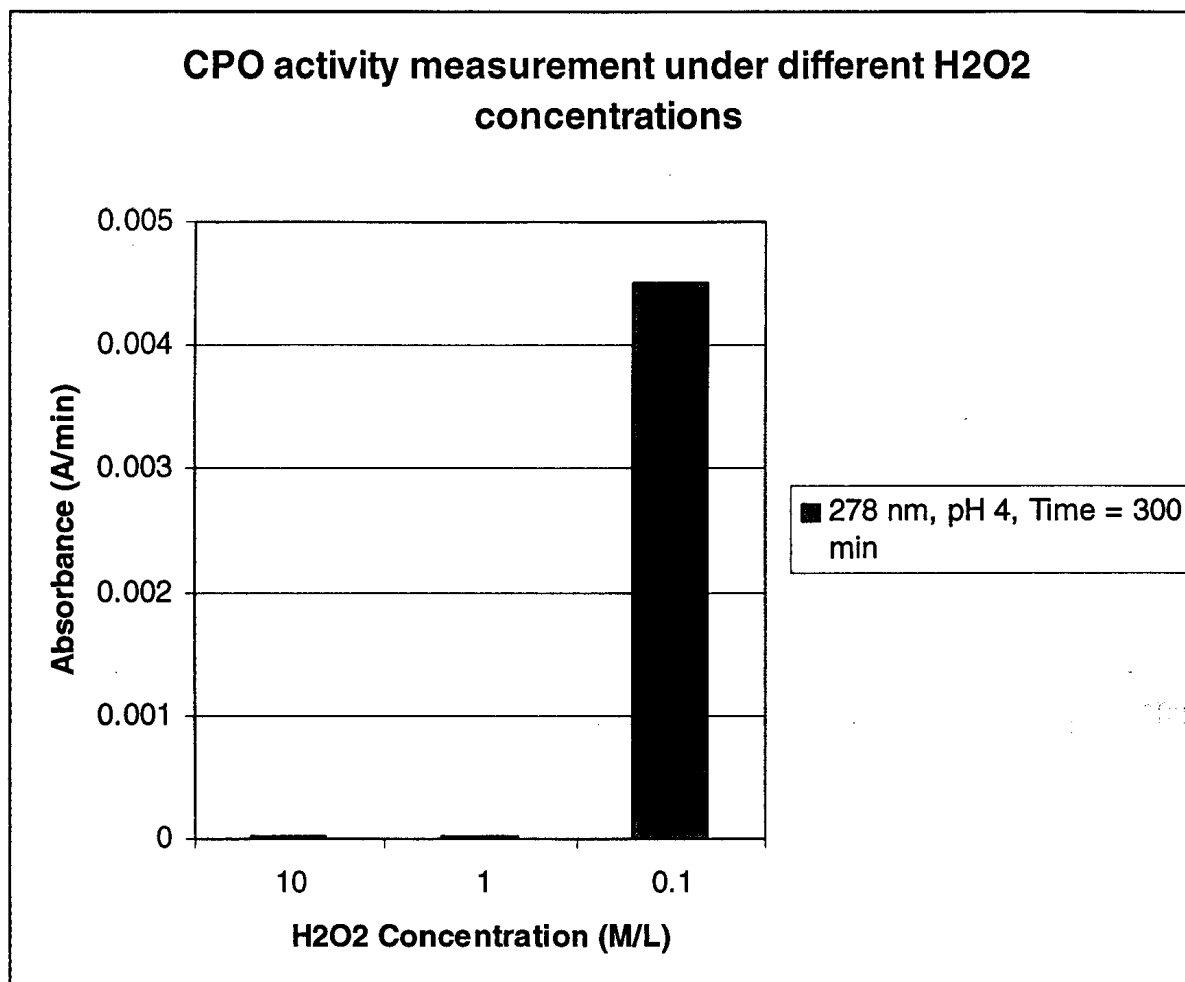


Figure 3.2 CPO activity measurement under different hydrogen peroxide concentrations (10, 1, 0.1 M/L), 2 ml of a citric acid buffer (pH = 4), 150  $\mu$ l of MCD in an ethyl alcoholic solution, 120  $\mu$ l of 0.1 M KCl, 140  $\mu$ l of 10 M/L H<sub>2</sub>O<sub>2</sub>, and 10  $\mu$ l of Concentrated Chloroperoxidase. Run time = 300 minutes, photospectrometer wavelength = 278 nm.

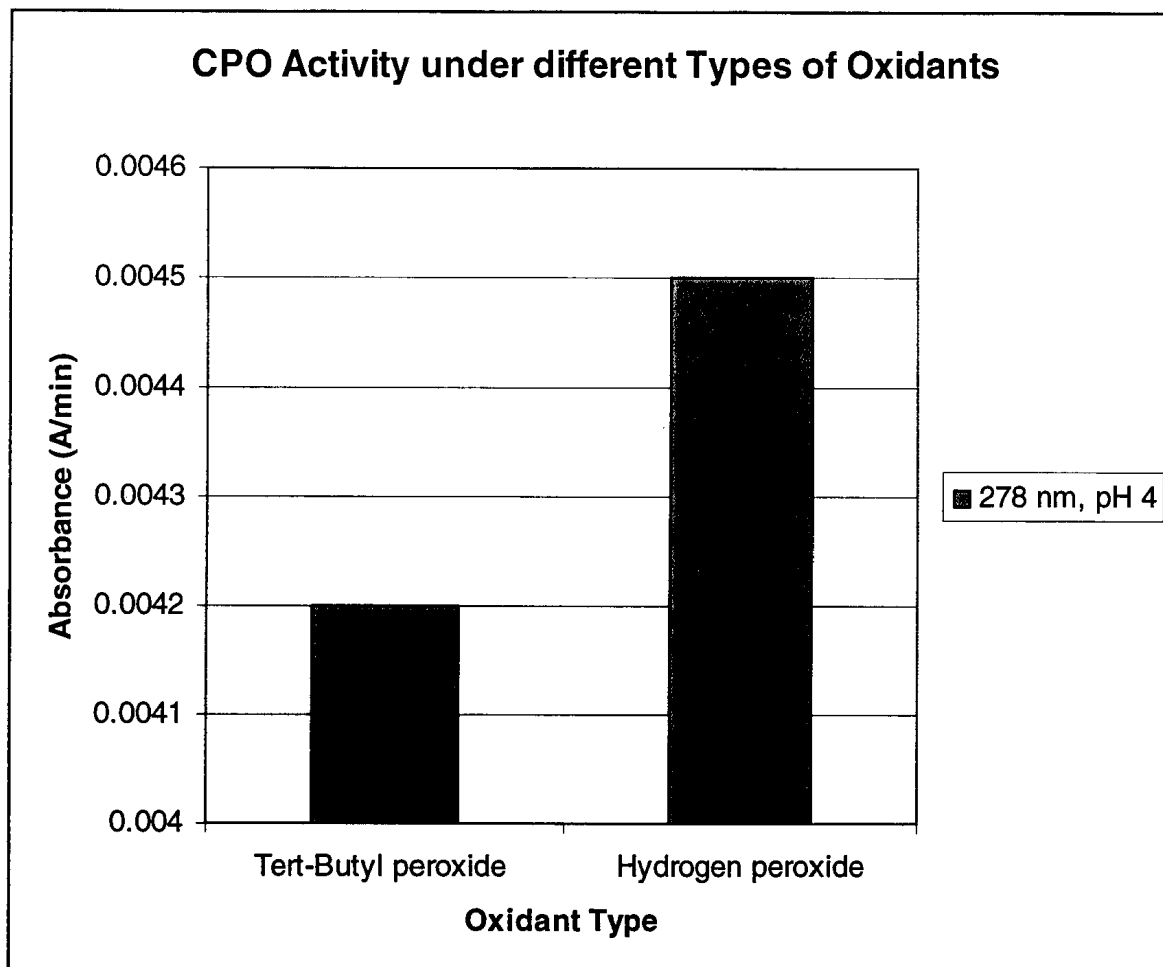


Figure 3.3 CPO activity comparison based on the oxidant used in the assay.



### 3.2.1.2 Effect of pH Value on the CPO's Activity

The citric buffer used in the assay had a pH of 4. In order to test the effect of the pH on the activity measurement of the Chloroperoxidase enzyme, another citric buffer was prepared with a pH of 2.75.

The new buffer was used to dilute the enzyme by 10 folds. The assay used included the citric buffer with a pH of 4. The run of the sample lasted for 100 minutes and was repeated several times.

The inconsistency with the pH of the buffers added resulted in a great noise and the activity value was determined to be around 0.0002 A/m that is very low compared to the previous measurements. Han *et al* (2002) found the optimum pH range for the CPO enzyme is 4-7. Therefore, it can be concluded that using the new buffer with a pH of 2.75 increased the acidity level of the assay forcing the enzyme to function out of its optimum pH range, which resulted in a very low activity value. As a result, the presence of different buffers causes the degradation of the enzymes activity.

## **3.2.2 Soybean Peroxidase (SBP) Activity Measurement**

### **3.2.2.1 Extraction & Purification Method**

The extraction and purification methods were obtained from a pre-set procedure used in the laboratory at the Chemical & Biochemical Engineering Department in the University of Western Ontario in which all the experiments were conducted.

#### **Extraction Method**

The SBP enzyme was extracted from the soybean seed hulls using the following procedure; 62 g of soybean seed hulls was soaked in 500 mL of potassium phosphate buffer (pH 6.3). The mixture was kept in cold room with a temperature of 4 °C for 24-hour retention time. These parameters are considered the optimum values which allow maximum extraction to the Soybean peroxidase enzyme. The supernatant was extracted using a two-step centrifugation; 10000 rpm for 30 minutes and 10000 rpm for 10 minutes.

The activity of the crude enzyme concentrated in the supernatant was measured before the purification step.

#### **Purification Method**

A saturated Ammonium sulfate was used to purify the crude enzyme. The 100% saturation of ammonium sulfate was prepared by adding 76 g of ammonium sulfate to 100 mL of potassium phosphate buffer (pH 6.3). To achieve 30 % saturation of ammonium sulfate in the crude enzyme, 10.7 mL of saturated ammonium sulfate was

added to every 25 mL of SBP crude enzyme. The mixture was kept in a cold fridge with temperature of 4 °C and duration of 24 hours. Using a centrifuge with 10000 rpm for 10 minutes, the supernatant and the pellets were separated and the activity of each was tested.

### **3.2.2.2 Measurement of the SBP activity**

Geng *et al* found that the activity of SBP could be measured under pH range between 2.2 and 8. The activity was measured based on the formation of tetra-guaiacol from its original substrate guaiacol in the presence of the enzyme and a certain type of buffer that gave a value of pH between 2 to 10. Based on the highest rate, the relative activity of the enzyme is a function of the acidity of the buffer solution used (Geng *et al.*, 2001).

The activity of SBP increased with the increase of the pH till it reached an optimum value which equals to pH = 6. Nicell and Wright also obtained similar results with small deviation in pH for SBP activity in aqueous phenol. These results indicate that SBP has the ability to react with various substrates over wide range of pH (Geng *et al.*, 2001).

#### ***3.2.2.2.1 Activity Measurement of the Crude Enzyme***

The guaiacol substrate was diluted by adding 100 µl of guaiacol to 10 ml of distilled water to get 1% guaiacol solution. Also, H<sub>2</sub>O<sub>2</sub> was diluted from 30% to 0.3% hydrogen peroxide solution by adding 100 µl of H<sub>2</sub>O<sub>2</sub> to 10 ml of distilled water. A buffer was

prepared with a pH of 6.3 (KH<sub>2</sub>PO<sub>4</sub> Buffer) to be used in the enzyme activity measurement assay.

The assay used consisted of 2ml of KH<sub>2</sub>PO<sub>4</sub> buffer pH 6.3, 500 µl of 1% guaiacol, 10 µl of the crude enzyme and 500 µl H<sub>2</sub>O<sub>2</sub>. The assay except the hydrogen peroxide is prepared and displayed into the photo-spectrometer where the wavelength was set at 420 nm. The hydrogen peroxide was added one second before starting the run. The run took an average time of three minutes and three runs were done. Figure 3.4 shows the result of the average absorption detected. The average activity value of the crude soybean peroxidase enzyme was 3.71 A/min. The un-purified enzyme showed a great potential in its performance since its activity is greater than the CPO enzyme by 3.0x 10<sup>2</sup> folds.

The purification step should increase the activity of the enzyme since less protein extracted from the hull will compete for the substrate found in the assay.

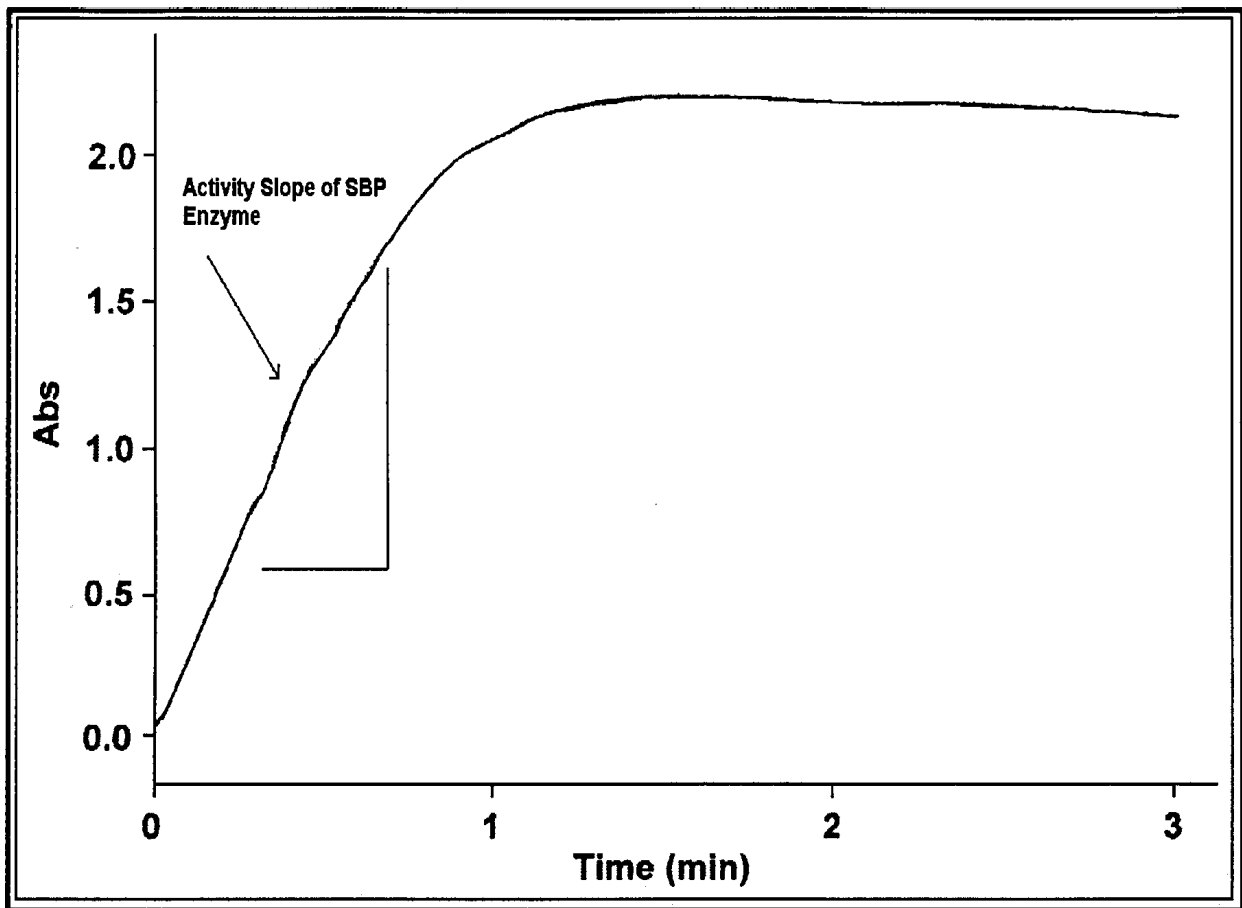


Figure 3.4 Activity measurement of the crude enzyme. Assay consists of 2ml of  $\text{KH}_2\text{PO}_4$  buffer pH 6.3, 500  $\mu\text{l}$  of 1% guaiacol, 10  $\mu\text{l}$  of the crude SBP, 500  $\mu\text{l}$   $\text{H}_2\text{O}_2$ .

Spectrophotometer Wavelength: 420nm. Time: 3 minutes.

### ***3.2.2.2.2 Activity Measurement of the Purified supernatant and the pellets***

The purified supernatant after the first purification step was tested through the preparation of the following assay. 2mL of  $\text{KH}_2\text{PO}_4$  buffer pH 6.3 was used as the assay base and 500  $\mu\text{L}$  of 1% of guaiacol was added to the buffer. The supernatant (10  $\mu\text{L}$ ) was added before the addition of the 500  $\mu\text{L}$  of hydrogen peroxide, which will result in the initiation of the activity measurement reaction. The average activity measurement of the enzyme tested in the supernatant was around 0.0008 A/min. This very low value of the enzymes activity is a result of the low existence of the enzyme in the supernatant.

Therefore, it can be concluded that the enzyme is concentrated in the pellet extracted after the purification step. Thus, the activity of the pellet must be tested to determine the existence of the SBP enzyme.

The pellet extracted from the purification step was diluted in 1 mL of the phosphate buffer (pH 6.3). A sample of the diluted pellet was used in the assay to test the activity of the enzyme. 2mL of  $\text{KH}_2\text{PO}_4$  buffer pH 6.3 was used as the assay base and 500  $\mu\text{L}$  of 1% of guaiacol was added to the buffer. 10  $\mu\text{L}$  of the diluted pellet was added before the addition of the 500  $\mu\text{L}$  of hydrogen peroxide, which will result in the initiation of the activity measurement reaction. The run was repeated three times to insure the validity of the results.

The activity of the SBP enzyme was averaged out to be ~ 40 A/min. Figure 3.5 shows a comparison between the SBP solutions which can conclude that purifying the solution increased the enzyme's activity by 10 folds. This increase can be attributed to the fact that using ammonium sulphate in the purification step eliminated any other proteins competing for the same substrate allowing the SBP enzyme to function with a high capacity.

Finally, it can be concluded that the SBP is better than the CPO to be used as a potential catalyst in the oxidation of DBT.

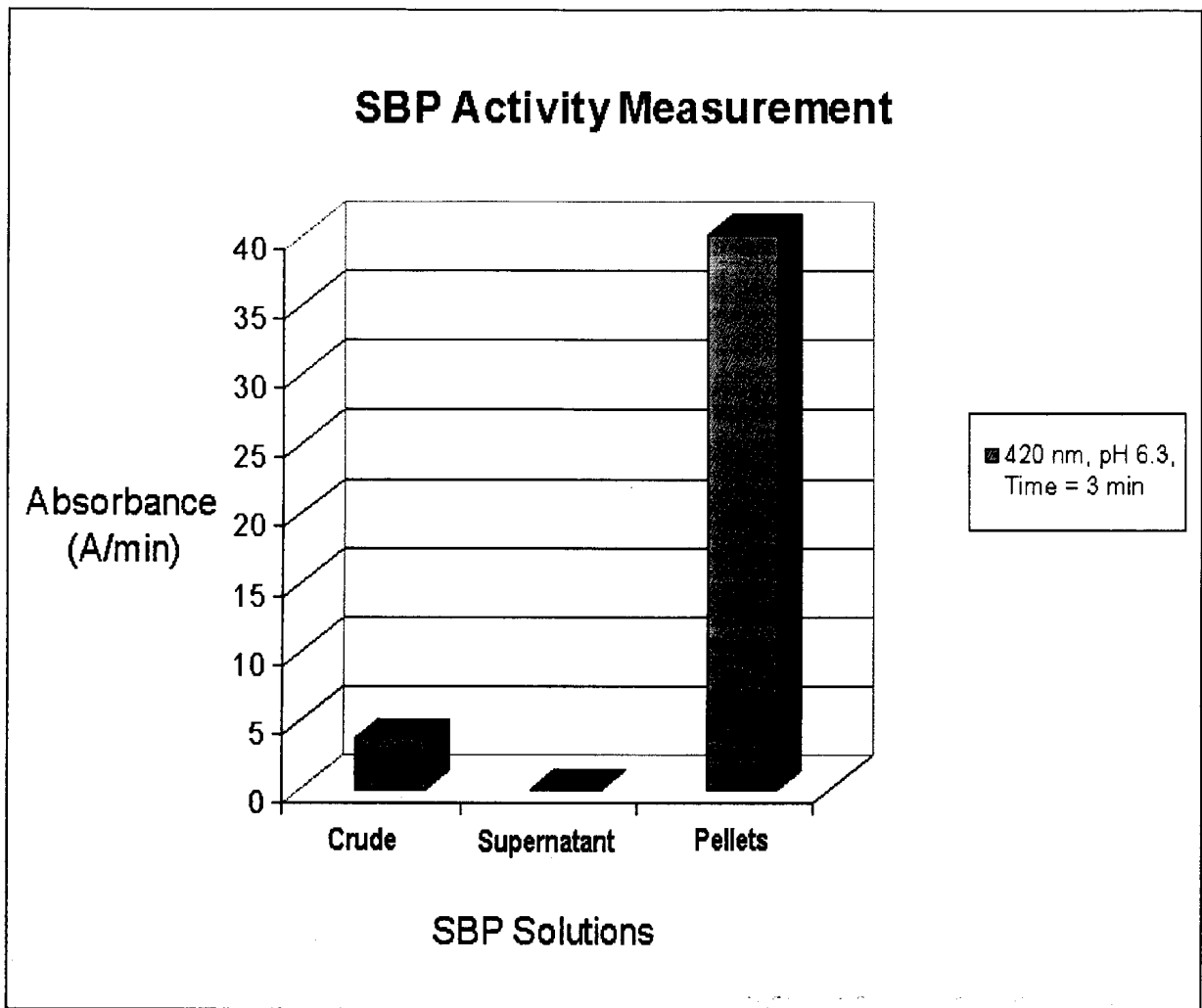


Figure 3.5 SBP Activity measurement in different Soybean solutions (Crude= 3.7 A/min, Supernatant = 0.0008 A/min, Pellets = 40 A/min). Assay consists of 2ml of  $\text{KH}_2\text{PO}_4$  buffer (pH 6.3), 500  $\mu\text{l}$  of 1% guaiacol, and 10  $\mu\text{l}$  of the SBP solution, 500  $\mu\text{l}$   $\text{H}_2\text{O}_2$ . Spectrophotometer Wavelength: 420nm. Time: 3 minutes.



### 3.2.3 Enzymatic Oxidation of DBT

The oxidation of DBT was attempted using the soybean peroxidase as the driving catalyst of this reaction. The first assay tested consisted of 75% of the DBT solution (500 ppm DBT in Acetonitrile), 20% of distilled water to provide an aqueous environment for the enzyme, 4% of the 0.1 M hydrogen peroxide and 1% of the diluted SBP enzyme in the phosphate buffer (pH 6).

All the components of the assay were added together except the hydrogen peroxide since it was the initiator of the expected reaction. After the addition of the hydrogen peroxide, the assay went under an extensive mixing for 30 minutes to allow the completion of the reaction. A white precipitation was observed and a sample was taken and dissolved in a pure acetonitrile. After testing the sample using the GC unit, the results were negative. No presence of the expected DBT sulfones was found which allowed the conclusion that the reaction did not occur. It was speculated that the white precipitation found in the mixture was the precipitation of the enzyme itself.

Different parameters were investigated like the water effect, the enzyme concentration, and the oxidant concentration in order to allow the reaction to occur. The distilled water was eliminated to allow a direct sampling of the mixture at the end of each run since the GC is sensitive to water. A new sample was investigated and the same white precipitation occurred. After measuring the concentration of the DBT in the mixture, it was found that

the value of the concentration was relatively the same, which indicated that no reaction occurred in this new assay.

More concentrated and purified enzyme was used in order to determine whether the level of purity or concentration of the enzyme has any effect on the negative occurrence of the reaction. The same runs were done with and without the addition of the distilled water to the mixture and no presence of DBT sulfone were found.

The final parameter that needed to be tested was the concentration of the hydrogen peroxide and its measurement in the mixture. A new assay was prepared where only the DBT-Acetonitrile solution and a concentrated hydrogen peroxide were added to the mixture without the presence of the Soybean peroxidase. After testing a sample using the GC unit, a presence of the DBT sulfone was detected and indicated the occurrence of the reaction. The Soybean peroxidase was added to the mixture to see if it will increase the concentration of the product. A white precipitation was observed and the results showed that the addition of the enzyme did not have any effect on lowering the activation energy of the reaction and allowing a higher reaction rate. This confirmed that the white precipitation was the enzyme itself and not the product of the expected reaction.

In conclusion, even though the soybean peroxidase showed a great potential activity, the functionality of the enzyme died out in its presence in an organic harsh environment. This makes it not feasible from the perspective of achieving a very low sulfur level fuel under ambient parameters, which is friendlier to the environment.

In addition to that, the difficulty faced in extracting the “un-reacted” DBT using another organic solvent in order to test it in the GC, triggered the importance of extraction in the field of this study.

Therefore, the interest of this study was shifted to focus on the pure extraction of DBT using new extracting agents called “Ionic Liquids”.

### **3.3 Conclusion**

From the tests done on both enzymes, the maximum activity obtained for the CPO enzyme was around 0.004 A/min whereas; the optimum activity value for the SBP enzyme was around 40 A/min. By comparison, it is obvious that the Soybean peroxidase is better than the CPO enzyme by around 1000 folds.

After measuring the activity of the Chloroperoxidase and the Soybean peroxidase, it was concluded that the SBP has a greater activity, which indicates its feasibility in implementing this enzyme as a catalyst in the oxidation attempt of the Dibenzothiophene. The biotechnical approach to solve the sulfur level problem in diesel and gasoline shows enormous potential to satisfy the new regulations. Unfortunately, under the parameters used in the oxidation attempt of DBT, there was no indication of any reaction occurrence. Even though Soybean peroxidase is considered one of the enzymes that show great chemical and thermal characteristics that are suitable for catalytic performance, it failed to achieve the desired goal of oxidizing the DBT in the experimental system. This reflects the difficulty in manipulating the enzyme that functions very well in an aqueous solution to be implemented to work properly in harsh organic surroundings.

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# **Chapter 4: Extractive Desulfurization of Dibenzothiophene using BMIM BF<sub>4</sub> Ionic Liquid as an Extracting Agent: Effect of Parameters on Extraction Process**

## **4.1 Introduction**

The reduction of sulfur level in diesel and gasoline is becoming so critical since the deadline to meet the environmental regulations passed by the U.S. Environmental Protection Agency (EPA) is approaching and the concentration of sulfur in these fuels must be lowered to 15 ppm by the year 2006 (E.P.A, 2003). The conventional hydrodesulfurization process was not able to achieve the concentrations set for sulfur even with the increase of the reactor's parameters like temperature and pressure (Te *et al.*, 2001). Even though a lot of modifications were done on the conventional process, high levels of economical costs were reached without achieving the desired goal of lowering the sulfur level to the set value (Zhang *et al.*, 2004).

Even though the comparability of the capital cost between the existing commercial process of diesel desulfurization and the extraction process using ionic liquids cannot be achieved practically. The new extractive process shows economical potential if it reaches to the commercial stages for several reasons. Even though the price of the ionic liquid is considered fairly high, its ability to be recycled will offset its high value and result in

achieving profitable outcome in the long run. Also, its negligible volatility due to its low vapour pressure even under high temperatures drastically causes a reduction in the cost of replacing any lost quantity in the process which in turn reduces the emission of any chemicals that can be harmful to the environment and human health. Finally, its ability to be thermally stable under high temperature values, its use under high temperatures will reduce the cost needed to lower the treated fuel to a low value before it is subjected to extraction by the utilized ionic liquid.

These are general interpretations of the positive potential of the new extractive desulfurization process using ionic liquid but a detailed economical study must be done to verify these conclusions.

Researchers investigated different alternatives to achieve the goal of producing the ultra low sulfur fuels. One of the processes that are under investigation is the extractive desulfurization method (EDS). Since the extraction process depends on the mass transfer of the desired chemical from one phase to another, it is critical to insure the success of the process by selecting the right extracting solvent that possesses the right selectivity of extracting the unwanted compounds (Jochen *et al.*, 2004).

In order for this method to become feasible, the process must be executed under ambient temperature and pressure and it must be implemented in conventional extracting units when it reaches the commercial stage. The promising process shows a great economical savings, which can be directed to choose the proper extracting agent to achieve the

desired goal (Candra-Broch *et al.*, 2003). The most important part in choosing an extracting agent is its selectivity property.

Ionic liquids are considered new solvents that can be used in several areas of the chemical field. Their unique properties allowed them to introduce the concept of “Green Chemistry” into the industrial fields (Ki-Sub *et al.*, 2004). They possess the capability of forming virtually no vapour pressure under elevated temperatures, which is considered an advantage over the regular organic solvents that are currently used (Fuller *et al.*, 1997). Most of the ionic liquids are immiscible with the aqueous and organic solutions, which allow expanding its implementation to both phases in the extraction field (Forsyth *et al.*, 2004). On the other hand, some ionic liquids are miscible in water but they form a separate phase when mixed with an organic solution (Zhang *et al.*, 2004). These types of ionic liquids can be used in order to achieve the desired level of extraction and also recycle the exhausted ionic liquid (Jochen *et al.*, 2004). Using the ionic liquids in the extraction process to eliminate the heavy sulfur compounds from diesel can be used as a replacement of the conventional hydrodesulfurization process to deeply desulfurize the fuel fractions to values below 15 ppm S or even lower. Therefore, specific ionic liquid (1 Butyl-3-methylimidazolium tetrafluoroborate) was chosen as an extracting agent since it had many desirable properties that can be used in the extraction process (Wasserscheid *et al.*, 2002).



### 1-Butyl-3-methylimidazolium tetrafluoroborate $C_8H_{15}N_2.BF_4$

The 1-Butyl-3-methylimidazolium tetrafluoroborate ionic liquid has a melting point of -71 °C and viscosity of 233 mPa.s. This ionic liquid is considered more viscous than common solvents. It has a molecular weight of 226.02 and a density of 1.22 g/mL under 20 °C. It appears as a clear, colorless viscous liquid and is 100% miscible in water. (Kisub *et al.*, 2004)

Early experiments using a model fuel of dissolving DBT-derivatives in n-dodecane were implemented to observe the difference in the extraction selectivity of several ionic liquids (Jochen *et al.*, 2004). The Nernst partition coefficient  $K_N$  (mgS/kgIL)/ (mgS/kgOil) was the measuring parameter in order to determine the feasibility of the ionic liquid. The experiments on the alkyimidazolium tetrafluoroborate ([BMIM][BF<sub>4</sub>]) and hexafluorophosphate ([BMIM][PF<sub>6</sub>]) ILs showed fairly high Nernst values of 0.7 and 0.8 respectively for the extraction of the organic sulfur compounds (Jochen *et al.*, 2004).

The most important property [BMIM] [BF<sub>4</sub>] shares with other ionic liquids is its insolubility in most common organic solvents. This is a key factor behind testing this type of solvent as extracting agent in an organic environment. In addition to that, its 100% miscibility in water shows an advantage in manipulating the functionality of the ionic liquid during the extracting process. This leads to the possibility of forming biphasic system which results in a mass transfer of the desired compounds to the ionic

liquid layer. Also, their selectivity gives an advantage to preserve the chemical structure of the organic layer processed (Paulechka *et al.*, 2003).

The objective of this research is to study the ability of the ionic liquid BMIM BF<sub>4</sub> to extract DBT from a hexane solution under different parameters, which were not investigated in a previous research like temperature, water, and surfactant effect. This ionic liquid was chosen because of its selective extractive property and its ability to completely mix with water which could be easily manipulated to recycled compared to the ionic liquids that are immiscible with water or the conventional extracting agents that need more complicated purification and recycling steps which in the long run result in an increase in the capital cost.

## 4.2 Materials and Methods

The DBT solution was prepared by dissolving 25 mg of DBT in 50 mL of Hexane, which resulted in the formation of a 500 ppm model fuel. The 1-butyl-3-methylimidazolium tetra-fluoroborate (97 %) was purchased from Fluka, Sigma-Aldrich (St. Louis, MO, US). Different parameters like run-time, temperature and water effect were investigated to determine their effect on the ionic liquid capability in extracting DBT in the biphasic solution.

It is important to optimize the duration of every experiment since it represents the minimum time required for a maximum extraction capacity and after several experiments it was determined to be around 15 minutes. In addition to that, the diluting effect on the ionic liquid is also investigated to determine whether it has positive or negative results on the extraction process. Also, the temperature is considered a critical factor in deciding upon one of the optimum conditions that allow maximum extraction.

Additional variables were studied like surfactant addition and ratio effect. The surfactant addition to the biphasic layer should reduce the surface tension between the two layers allowing more mass transfer to occur in the system. Finally, the ratio between the ionic liquid and the model fuel is studied to determine the minimum amount required of the ionic liquid for a maximum extraction results. Therefore, several ratios were investigated in order to choose the optimum ratio for the biphasic system, which was decided to be 1:1 ratio.

The experiments involved a 1:1 ratio between the DBT solution (500ppm) and the concentrated ionic liquid [BMIM BF<sub>4</sub>] in order to obtain the maximum extraction capacity of the ionic liquid. During the mixing of the assay a sample was taken every 5 minutes from the hexane top layer in order to test the concentration change of DBT with time. All experiments were repeated at least three times. The error bars are the standard deviations from the average taken of the three runs for every experiment

### 4.3 Results and Discussion

The extractive desulfurization involves the mass transfer of dibenzothiophene (DBT) from the model fuel to the ionic liquid layer. Therefore, the organic solution that represents the diesel fuel must be prepared from a material like hexane that will form a separate layer from the ionic liquid during the extraction process.

Figure 4.1 shows that the DBT concentration undergoes a rapid decrease in the hexane layer during the first 15 minutes of mixing followed by a gradual decrease till it reaches 20 minutes where the concentration levels off. The dip showing in this graph is due to the over saturation state, which occurs because of the intense mixing of the bi-phasic mixture. This test gives the time required for the mass transfer to reach completion and the ionic liquid to reach a maximum DBT saturation.

The initial time determined for every run was 20 minutes. Figure 4.2 shows a graph that represents two-stage extraction of 1:1 ratio of the biphasic system between the ionic liquid and the DBT solution. The first stage lasted for 20 minutes and a sample was taken from the DBT solution layer every 5 minutes. After the first 20 minutes, the intense mixing is stopped and the hexane solution layer that contains that non-extracted DBT is separated from the used ionic liquid layer. The second stage extraction starts with the addition of the separated hexane solution containing residual DBT to a new ionic liquid under the same volume (1:1 ratio). Then, the further mixing lasts for 20 more minutes with a sample obtained every 5 minutes. As a final result, the DBT concentration is reduced from 500 ppm to  $165 \pm 3$  ppm after the second stage.

After observing the behavior of the graphs in Figure 4.1 and 4.2 it was determined that the run time must be reduced to 15 minutes, which is the time around which the extraction reached its maximum capacity. Therefore, the same procedure was repeated again in Figure 4.3 where the run time was 15 minutes and the final result after the second stage was the reduction of the DBT concentration to  $165 \pm 4$  ppm. The experiment was repeated three times and the average result was obtained.

The KG1-MATR column was used to run the extraction process on a larger scale. This has several advantages like increasing the volume of the biphasic system, which gives more realistic results. Also, it allows better control over the mixing and agitation of the mixture, and a practical control of the temperature under which the column is operating.

A one-stage extraction step was executed using the column and the run was repeated three times where 50 ml of ionic liquid was added to 50 ml of the DBT-Hexane solution. Each run lasted 20 minutes and a sample was taken every 5 minutes for analysis. Figure 4.4 shows that the concentration of DBT was reduced to around  $230 \pm 10$  ppm when the mixing time reached the 15 minutes mark. Therefore, the scaling up the process by using the column has the same result as using the small flasks where the DBT concentration in the model fuel was reduced by almost a factor of 2 in every stage.

Therefore, the time required for a complete saturation of the ionic liquid layer in a 1:1 ratio system was determined to be 15 minutes. This is an important parameter since it reflects the minimum time required for the system to reach equilibrium.

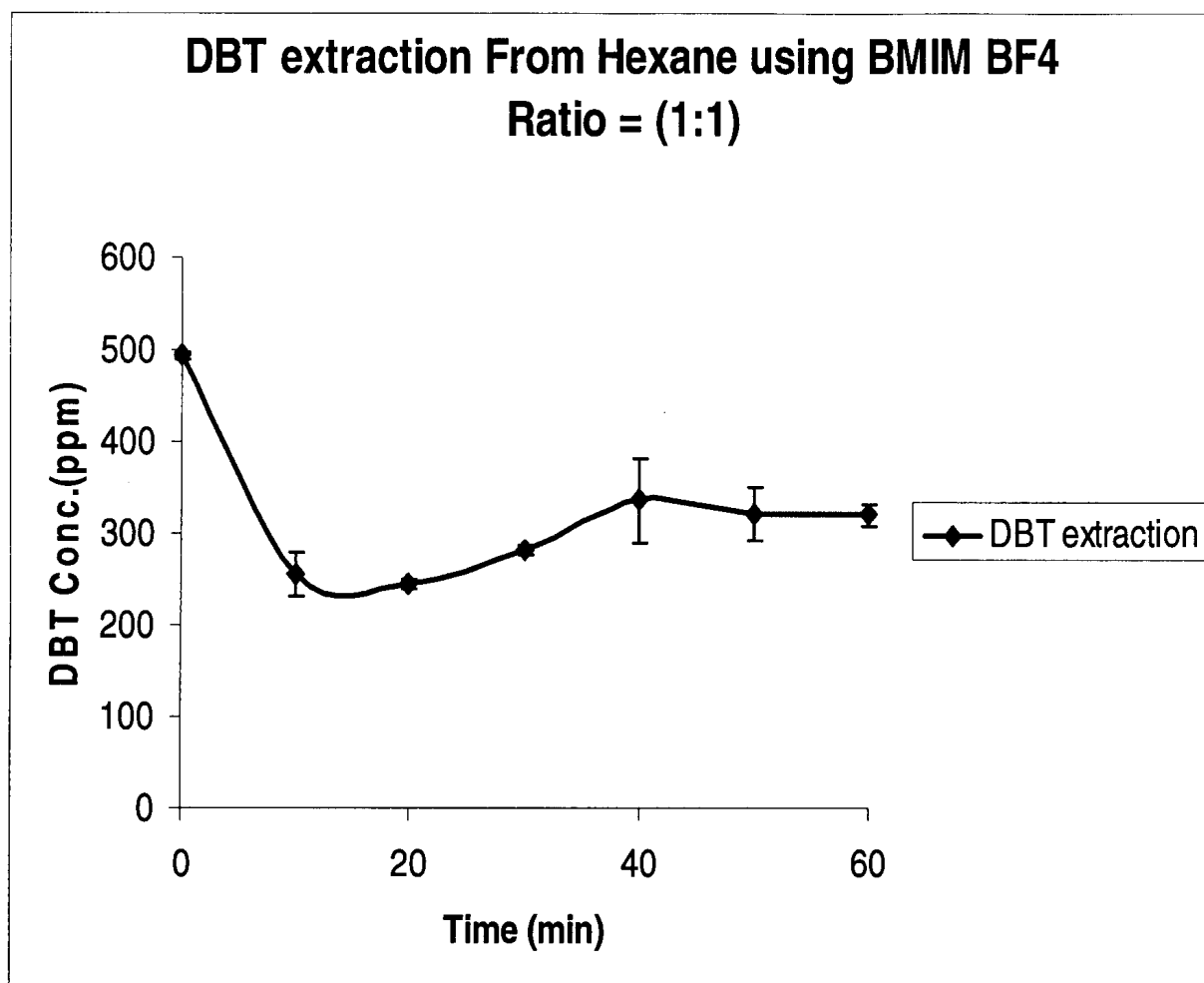


Figure 4.1 Initial experiment of 500ppm DBT solution extraction using BMIM BF<sub>4</sub> ionic liquid, Duration: 60 minutes, Ratio DBT: IL = 1:1. Experiment repetition = 3 times. Minimum DBT concentration is  $270 \pm 5$  ppm at run time = 15 minutes

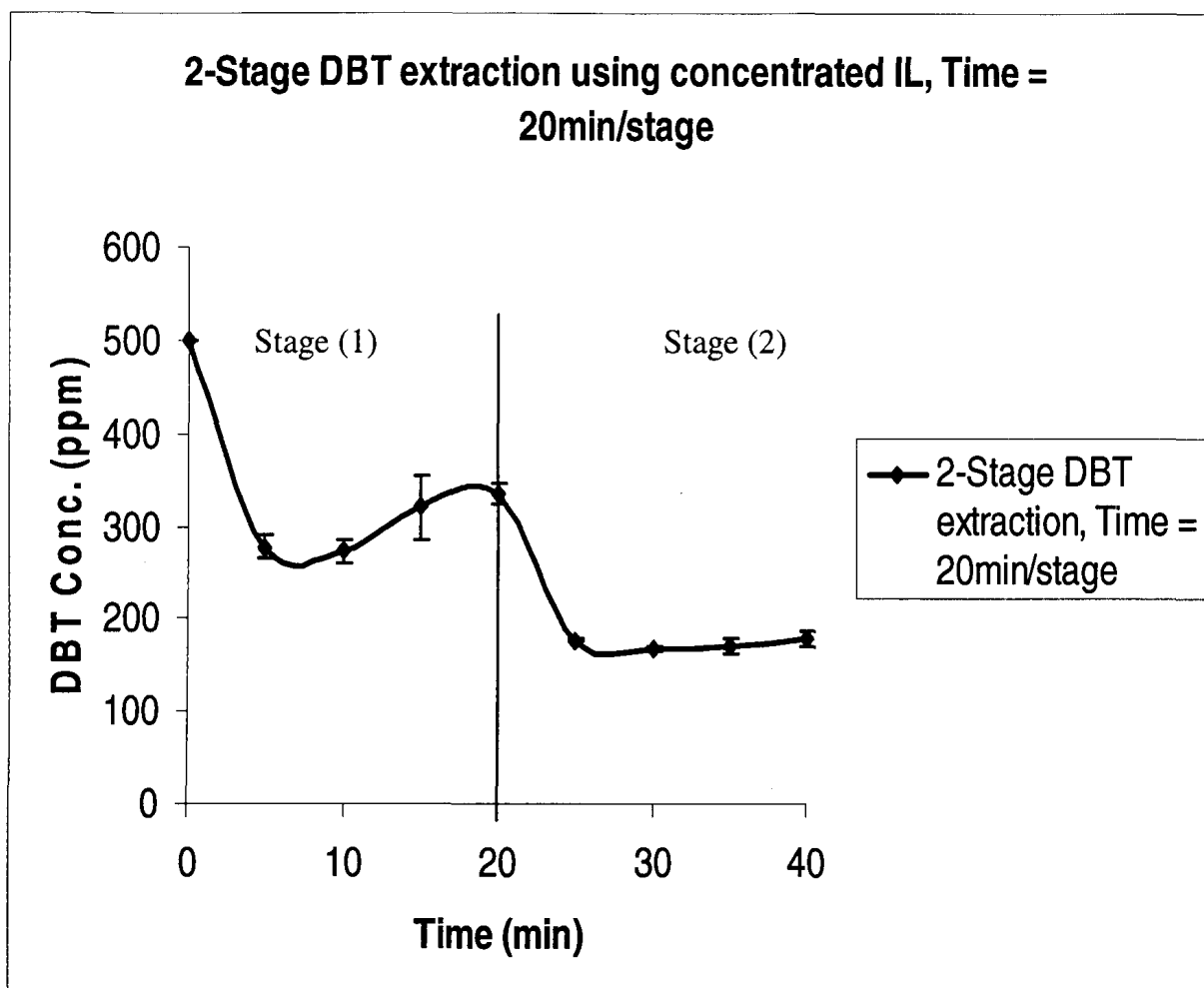


Figure 4.2 Initial experiment of 2-Stage 500 ppm DBT solution extraction using BMIM BF<sub>4</sub> ionic liquid, Duration: 20 minutes/stage, Ratio DBT: IL = 1:1.  
Final DBT concentration = 176 ± 8 ppm.

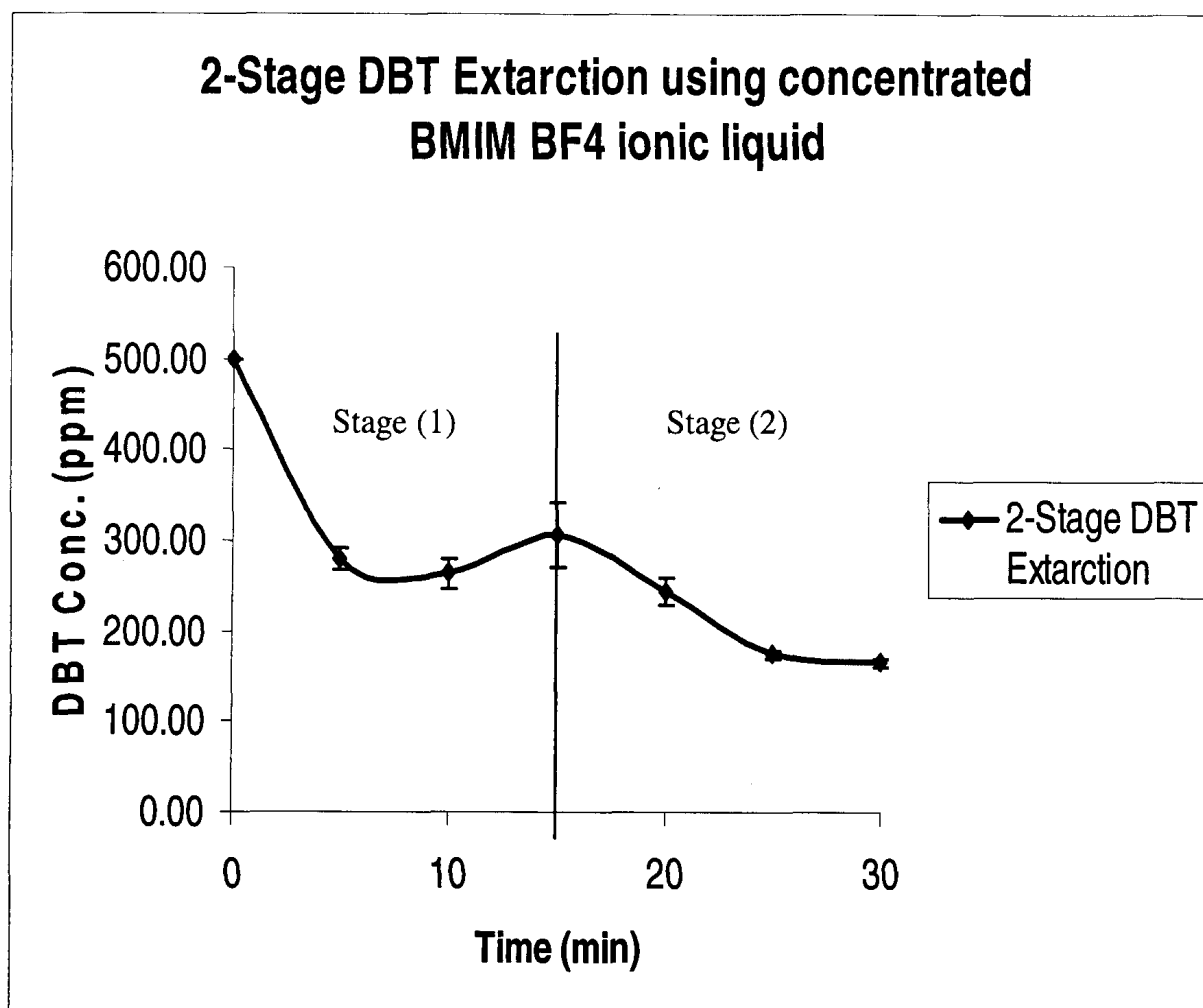


Figure 4.3 Initial experiment of 2-Stage 500 ppm DBT solution extraction using BMIM BF4 ionic liquid, Duration: 15 minutes/stage, Ratio DBT: IL = 1:1.

Final DBT concentration =  $165 \pm 4$  ppm.



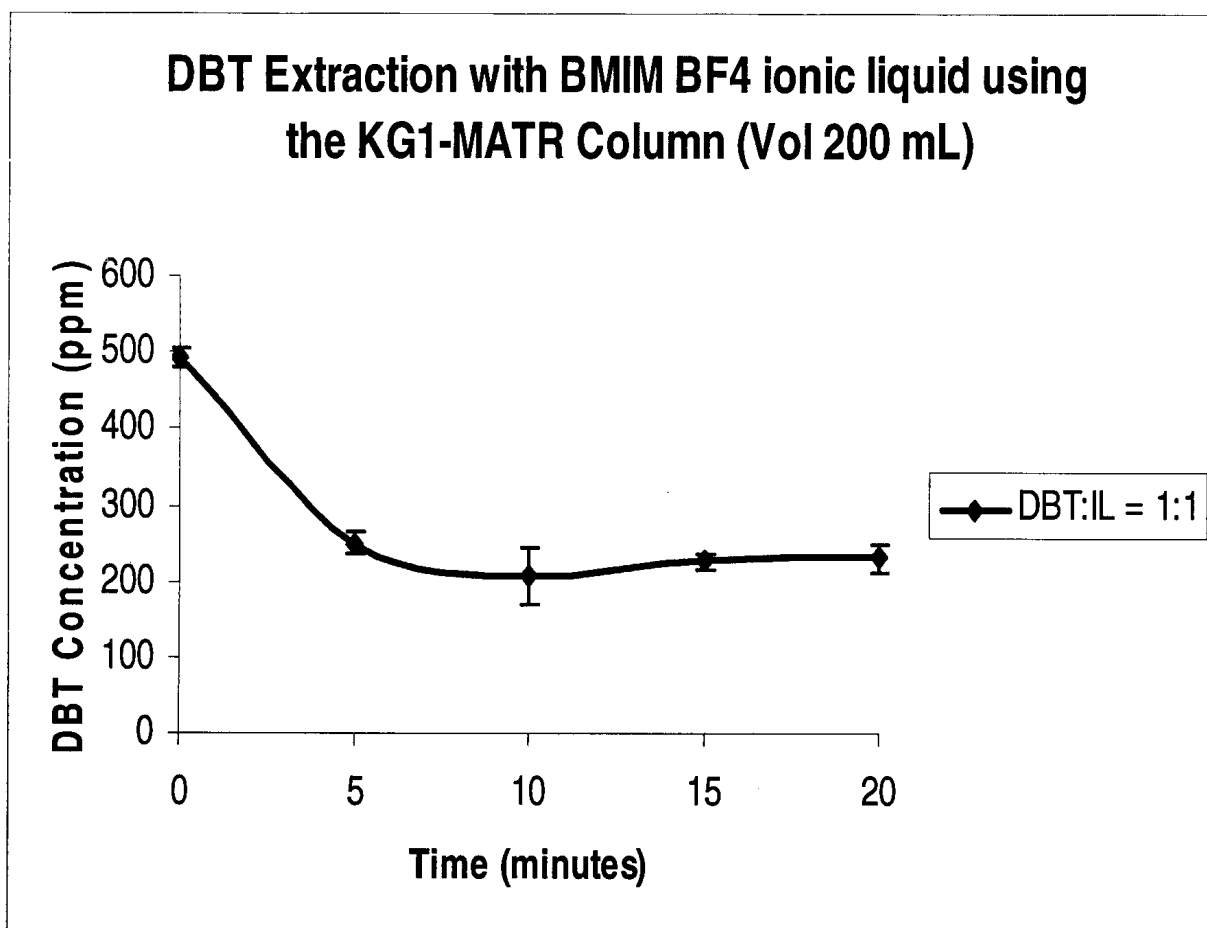


Figure 4.4 1-Stage extraction process using the KG1-MATR column. Extraction of DBT from the model fuel using BMIM BF<sub>4</sub> ionic liquid, Duration: 20 minutes/stage, Ratio DBT: IL = 1:1. Final

DBT Concentration =  $230 \pm 10$  ppm

### 4.3.1 Investigation of Run Time and Ionic Liquid Extraction

#### Capacity

After determining the time required for extraction in one stage, the experiment was repeated with a mixing duration of 15 minutes and ratio of 1:1. Two separate stages were done in order to check the consistency of the extracting agent capacity in decreasing the DBT concentration in the DBT-Hexane layer to the desirable level. After the end of every stage, The DBT solution was separated from the spent ionic liquid and added to a new layer of a fresh ionic liquid for a second stage of extraction.

The DBT concentration showed a decrease in the hexane layer to almost half the initial concentration value. It went down from 500 ppm to around 260 ppm in the first stage of extraction. Similar results were obtained in the second stage of extraction where the value of the DBT concentration was decreased from 300 ppm to 160 ppm.

By comparison, Jochen *et al.* (2004) confirmed that time required for every stage of extraction should be at 15 minutes since all the experiments done in their research was based on a mixing time of 15 minutes and a model fuel to ionic liquid ratio of 1:1. This shows a consistency in the extraction behavior of the ionic liquid, which results in lowering the sulfur level of the fuel model by half in each stage. See Figure 4.5.

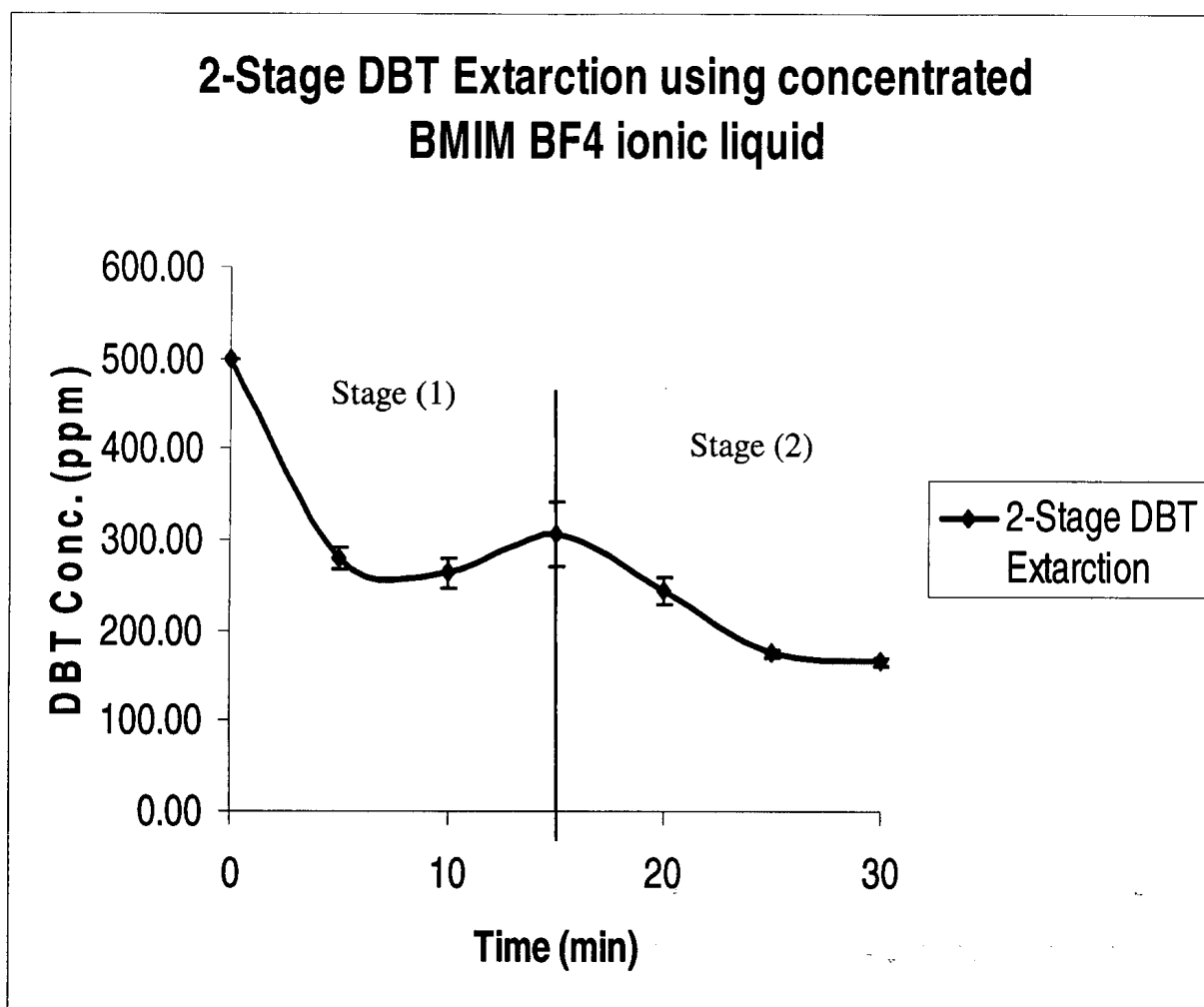


Figure 4.5 2-Stage DBT extraction using BMIM BF<sub>4</sub> ionic liquid, Duration: 15 minutes/stage,

Ratio DBT: IL = 1:1. Final DBT concentration =  $165 \pm 4$  ppm.

### 4.3.2 Investigation of Dilution Effect on BMIM BF<sub>4</sub>

Most of ionic liquids have a unique property that allows them to form a separate layer from an organic or aqueous solution. Even though 1-butyl-3-methylimidazolium tetrafluoroborate forms a separate layer from the organic solution, it is totally miscible with water. Therefore, it was very important to investigate the effect of water presence on the extractive capacity of the ionic liquid. The ionic liquid was diluted with distilled water and it was used to extract the DBT from the hexane solution under two-stage extraction process. Four runs were made where the dilution increased gradually. The first run was executed with the concentrated ionic liquid and the last run was done with a dilution ratio of IL: H<sub>2</sub>O = 7: 3. The results showed as the ratio of the BMIM BF<sub>4</sub> ionic liquid dilution increases, its ability to extract more DBT from the hexane solution decreases. Figure 4.6 shows that the DBT concentration was lowered to around 165 ppm after the second stage using the concentrated ionic liquid. Whereas, the maximum diluted ionic liquid decreased the concentration of DBT to only around 390 ppm, which reflects the great effect of water presence on the functionality of the ionic liquid.

The reason behind the decrease in the extractive capacity of the ionic liquid is the DBT hydrophobic characteristic. DBT is considered a heavy organic sulfur compound that is dissolvable in organic solutions only and does not dissolve in water-based solutions. Therefore, diluting the ionic liquid decreases the concentration of the BMIM BF<sub>4</sub> molecules in the total solution which decreases the overall space allowed for the DBT solutions to enter the ionic liquid layer and it would be more preferable to maintain its

presence in an organic environment. As a result, the presence of water has a negative effect on the extraction property of the BMIM BF<sub>4</sub> ionic liquid.

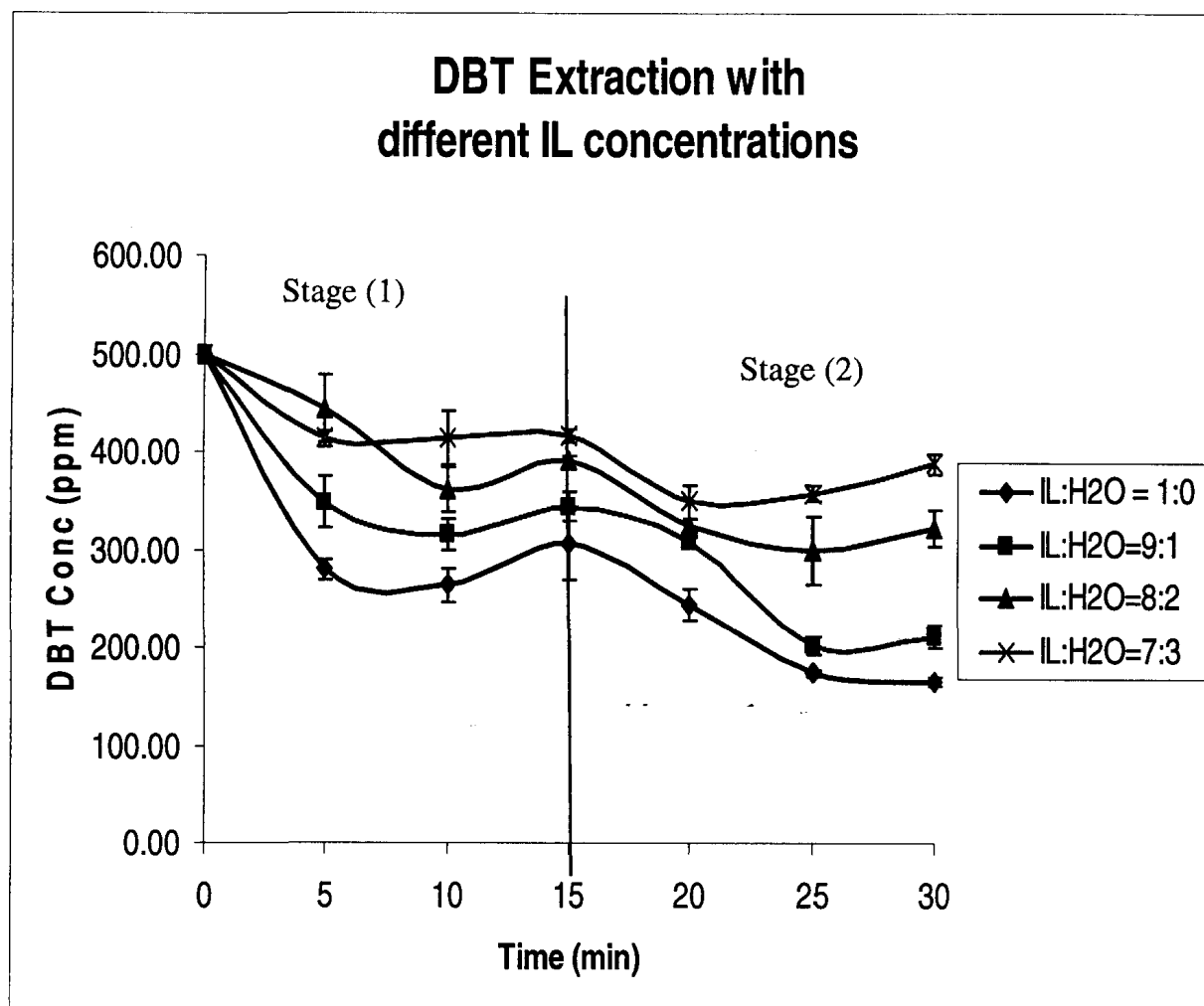


Figure 4.6 Effect of water presence on the extractive capacity of the BMIM BF<sub>4</sub> ionic liquid in the 2-stage extraction process. Final DBT Concentration =  $165 \pm 4$  ppm (IL: H<sub>2</sub>O = 1:0),  $211 \pm 12$  ppm (IL: H<sub>2</sub>O = 9:1),  $322 \pm 15$  ppm (IL: H<sub>2</sub>O = 8:2),  $388 \pm 9$  ppm (IL: H<sub>2</sub>O = 7:3),

### 4.3.3 Surfactant Effect on the Extraction Capacity

Since the ionic liquid formed a separate layer than the hexane solution, a surface tension between the two layers was created to separate the two. An attempt was done to enhance the mass transfer of the DBT through the separating surface by the addition of different surfactants. Since surfactants are known for decreasing the surface tension between two different layers, it gave a promising step to increase the extraction ability of the ionic liquid. Two different surfactants were studied (Tween 80 and Triton X-114) and compared to the pure extraction by concentrated ionic liquid. The concentrated BMIM BF<sub>4</sub> was added to the DBT-Hexane solution with 1:1 ratio and 10  $\mu$ L of the selected surfactant was added to 1 mL of the total mixture. The extraction process was executed with 15 minutes of mixing for every stage. Figure 4.7 shows the results obtained from this experiment.

The addition of the Tween 80 surfactant lowered the DBT concentration to around 140 ppm after the second extraction stage. Whereas the Triton X-114 surfactant lowered the sulfur level even more to around 110 ppm. Therefore it can be seen that the Triton X-114 has a better effect in lowering the surface tension and enhancing the extracting capacity of the ionic liquid since the pure ionic liquid decreased the sulfur level to only 165 ppm after the second stage of extraction.

As a result, adding Triton X-114 enhances the extraction capacity of the ionic liquid and further decrease the DBT concentration from 165 ppm to 110 ppm after two stages of extraction.

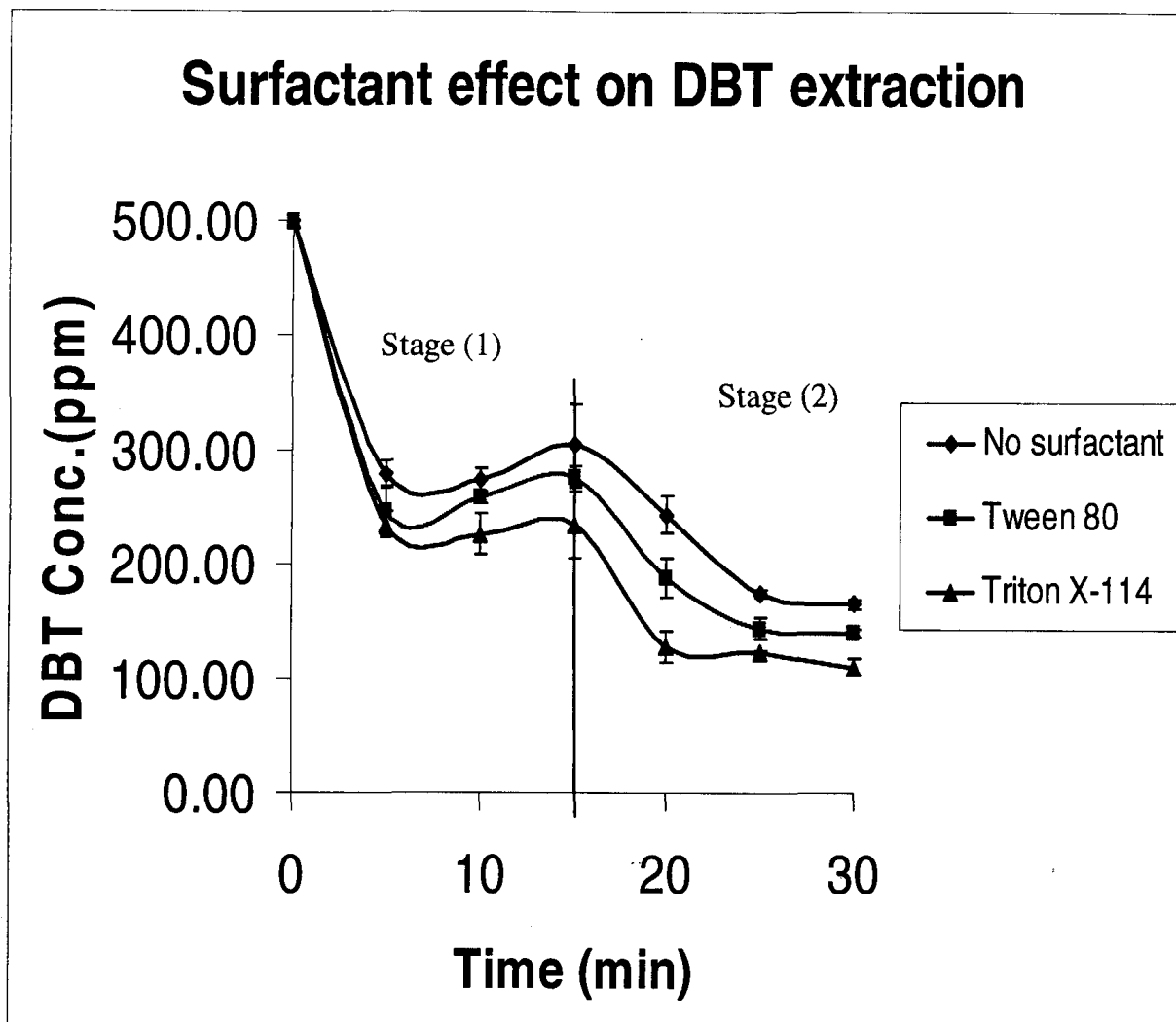


Figure 4.7 Effect of surfactants (Tween 80, Triton X-114) on the extractive capacity of the BMIM BF<sub>4</sub> ionic liquid in the 2-stage extraction process (10  $\mu$ L for every 1mL of total mixture). Final DBT Concentration =  $165 \pm 4$  ppm (No Surfactant),  $140 \pm 3$  ppm (Tween 80),  $109 \pm 8$  ppm (Triton X-114),

### 4.3.4 Investigation of Temperature Change

Since the BMIM BF<sub>4</sub> is considered a viscous solvent, it was very critical to investigate the temperature effect on the extraction of DBT. An ultrasound unit was used for mixing where a probe was immersed into the biphasic system and impulses of 1 cycle per second and amplitude of 100% were used to provide an intense mixing of the system. The system was prepared in a glass flask, which was sealed from the top, and only the probe was allowed to penetrate through. Also, the flask was immersed in a water bath, where a heater pump controlled its temperature.

The extraction process was investigated under 20, 30, 40, 50 and 60 °C where a one-stage extraction was executed and a sample of the DBT solution was taken after 15 minutes of intense mixing. Three runs were done under each temperature and the average was taken to represent the value of the DBT concentration. Figure 4.8 shows the gradual decrease in the DBT concentration with the increase of temperature till the value reaches 40 °C, and then the DBT value increases in the processed model fuel when the temperature exceeds the value of 40 °C. The value of the DBT concentration after one stage extraction under this temperature was around 230 ppm.

Increasing the temperature allowed the ionic liquid to be less viscous which reduced the surface tension of the solvent allowing the DBT to be extracted more efficiently. On the other hand, allowing the temperature to exceed the optimum value caused a decrease in the efficiency of the extraction. This could be related to the excessive reduction of the



surface tension of the ionic liquid allowing the back extraction effect to take place since the organic sulfur compounds are more stable in an organic environment.

The extraction column was used to validate the results obtained and a one stage extraction step was repeated using the column. One sample was taken every 5 minutes and the temperature was maintained at the selected optimum temperature which was 40 °C. Three runs were executed and the average was represented by Figure 4.9. This figure shows that the DBT concentration was reduced to  $230 \pm 15$  ppm at the end of the extraction stage. By comparing these results with the value obtained from the previous graph, it can be reconfirmed that the optimum temperature for the extraction operation is 40 °C.

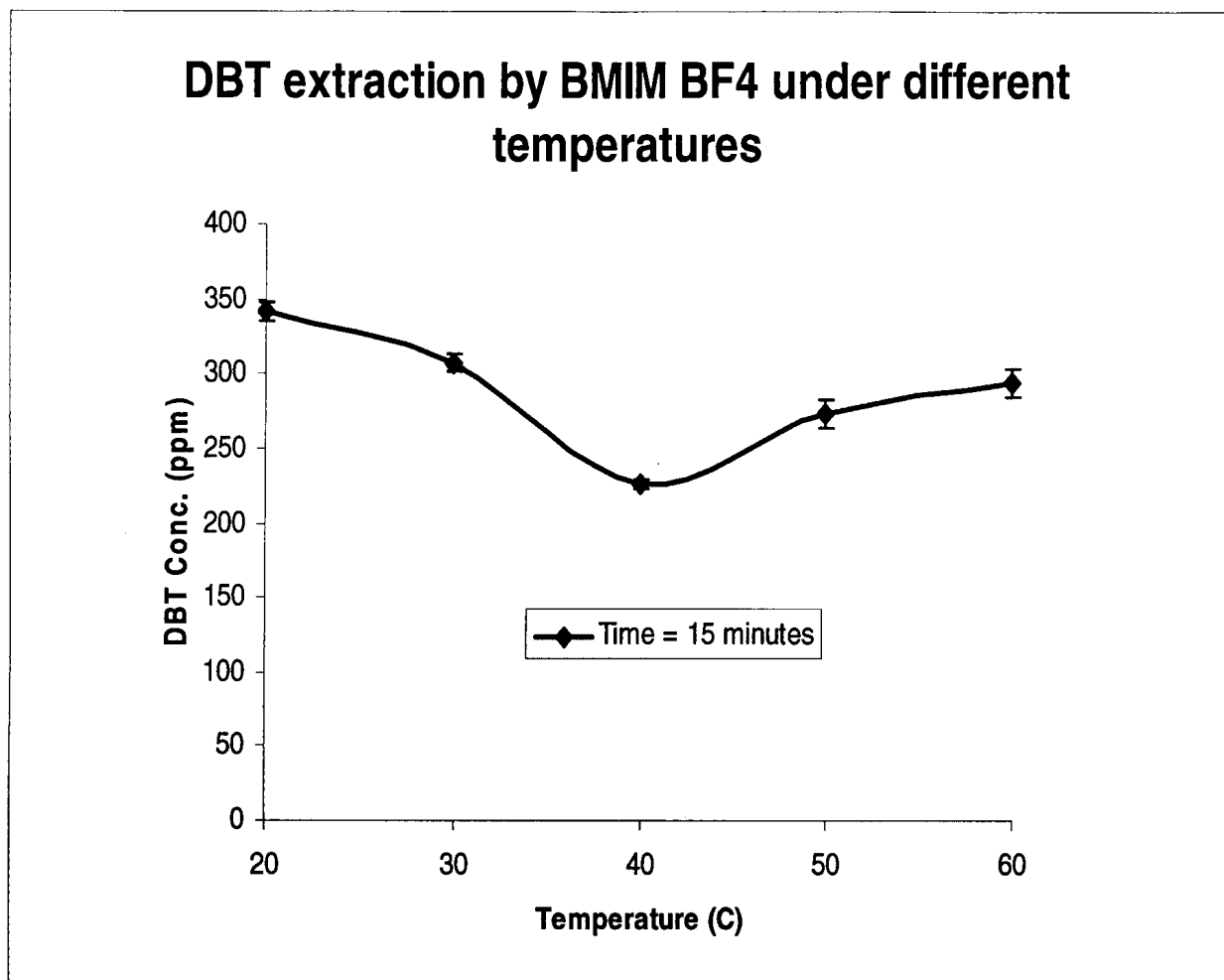


Figure 4.8 Effect of temperature on the extractive capacity of the BMIM BF<sub>4</sub> ionic liquid in the 1-stage extraction process (time = 15 minutes. Ultrasound mixing: cycle = 1, Amplitude = 100%).

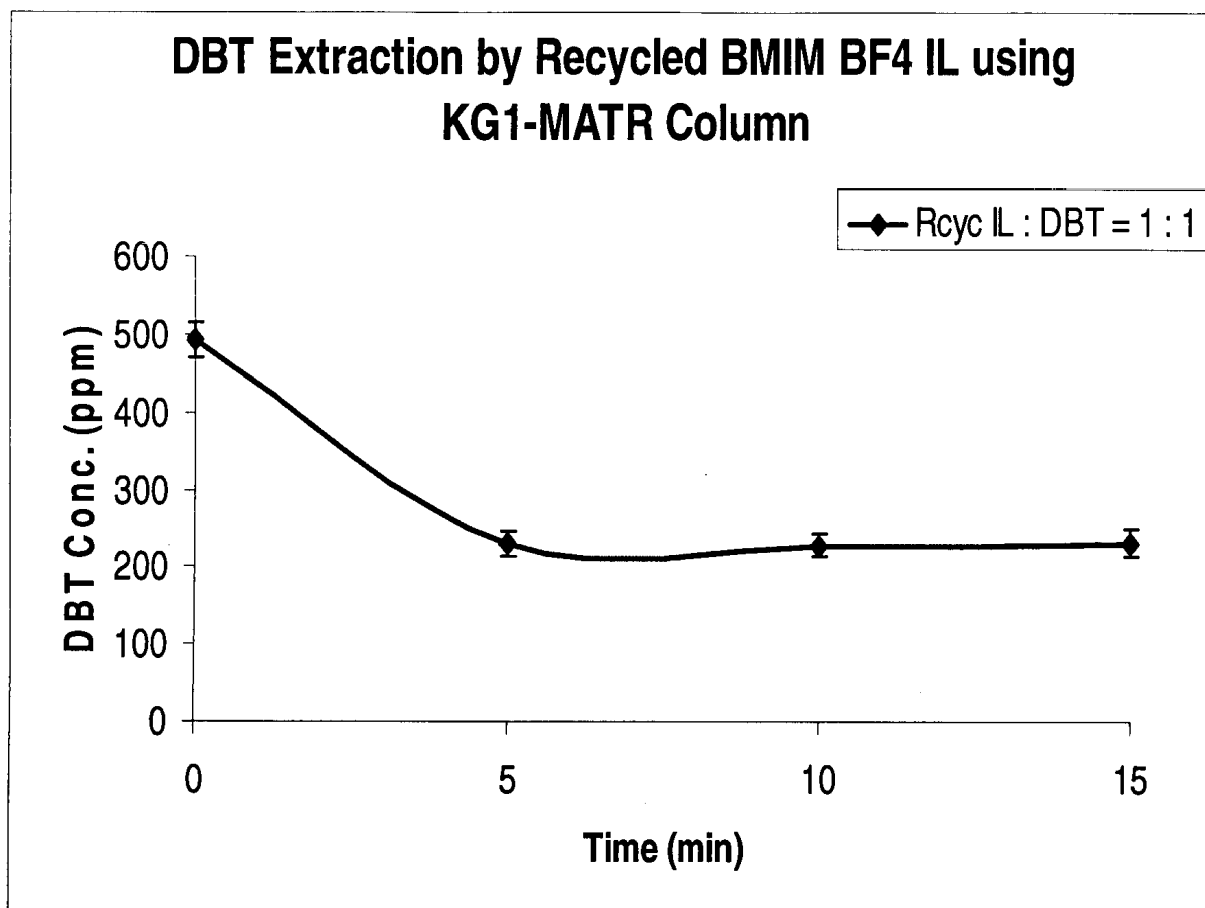


Figure 4.9 DBT extraction from the hexane solution using a recycled BMIM BF<sub>4</sub> ionic liquid in the KG1-MATR Extraction column (DBT: IL = 1:1, Mixing Time = 15 minutes, Temperature = 40 °C). Final DBT concentration = 230 ±15 ppm.

### 4.3.5 Ionic Liquid to DBT Solution Ratio

The ratio between the DBT solution and the ionic liquid is very important in determining the suitable ratio to be economically and practically feasible.

In order to obtain the optimum ratio between the model fuel and the ionic liquid, several ratios were investigated and compared. The ratios studied were DBT: IL = 4:1, 2:1, 1:1, 1:2, and 1:4.

All the ratios were tested under one-stage extraction and mixing time of 15 minutes. Figure 4.10 shows the effect of the quantity of the ionic liquid on the extraction capacity of the solvent. As predicted, the ionic liquid extractive capacity increases by the increase of its quantity. The final DBT concentration after one-stage extraction can be observed in Table 4.1

Table 4.1 Results of DBT concentration after one-stage extraction under different DBT solution to BMIM BF<sub>4</sub> ionic liquid ratios

DBT: Ionic Liquid Ratio	DBT Concentration (ppm)
4:1	440
2:1	403
1:1	265
1:2	215
1:4	146

This shows a similar pattern observed in literature. Jochen *et al.* (2004) obtained the effect of ratio on the extraction process using different ratios of a selected ionic liquid. Figure 4.11 reflects the effect of mass ratio for crosscurrent extraction of model oil, where the model oil used was 500 ppm DBT in n-dodecane and the ionic liquid used was 1-*n*-butyl-3-methylimidazolium octylsulfate ([BMIM] [OcSO<sub>4</sub>]). Since the 1:1 ratio lowers the sulfur level to around half the original concentration of DBT and a reasonable amount of the ionic liquid is used to achieve this acceptable level of sulfur, it was chosen to be the optimum ratio.

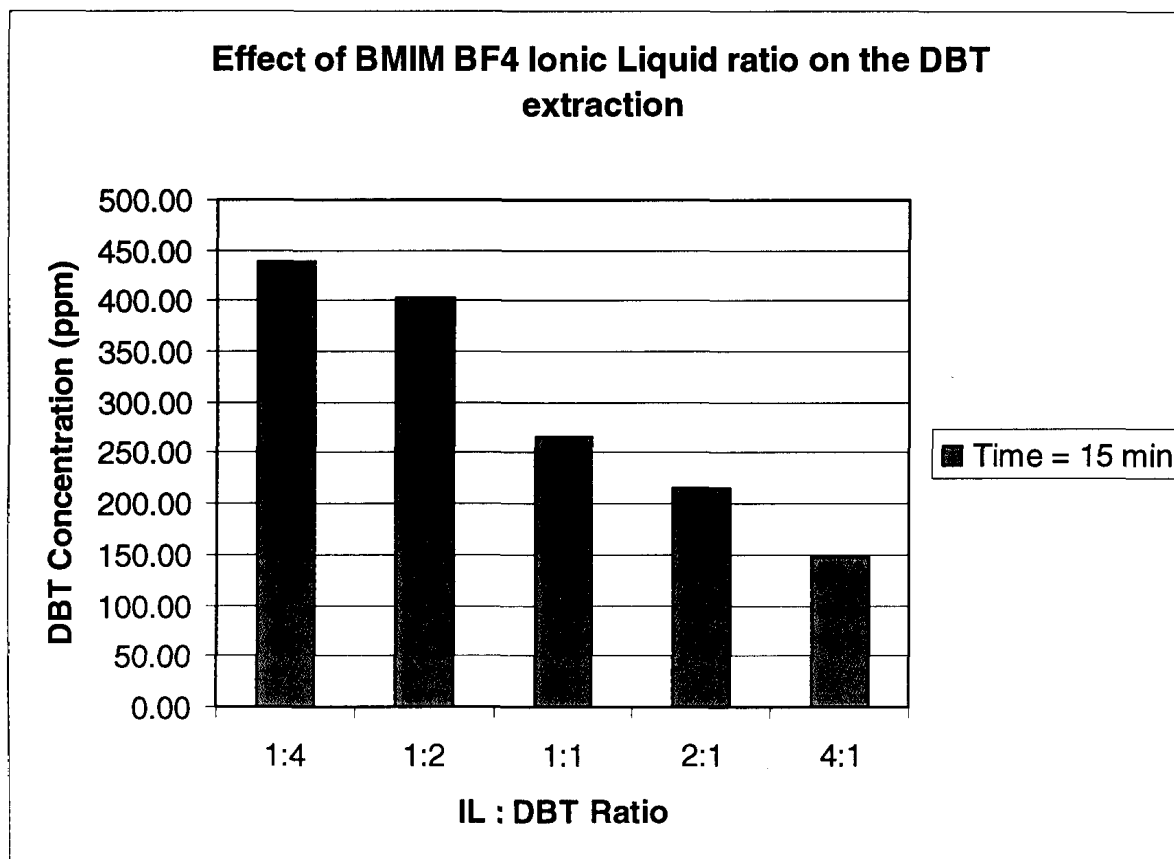


Figure 4.10 Effect of ionic liquid BMIM BF<sub>4</sub> ratio with DBT solution on the extractive desulfurization of the model fuel in the 1-stage extraction process (Mixing time = 15 minutes.)

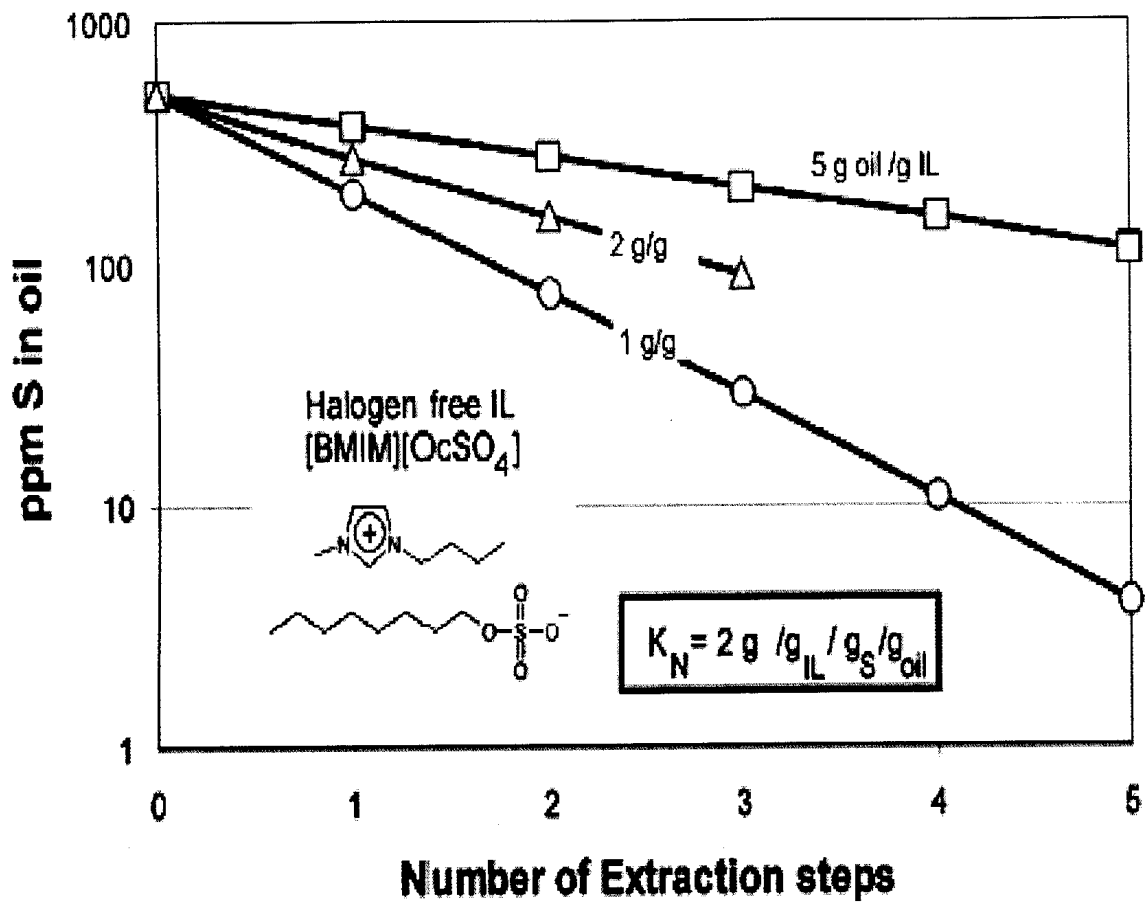


Figure 4.11 Influence of the oil to IL mass ratio for cross-current extraction of a model oil (500 ppm S as DBT in *n*-dodecane; IL: [BMIM] [OcSO<sub>4</sub>], room temperature) (Jochen *et al.*, 2004).

## 4.4 Recycling of the BMIM BF<sub>4</sub> Ionic Liquid

In order for this process to be feasible, it is important to find a way to recycle the exhausted ionic liquid used in the extraction process. The unique property of the BMIM BF<sub>4</sub> ionic liquid of being able to be miscible with water allowed the simple method of recycling which will offset the fairly high price of the ionic liquid. The water miscibility property that this ionic liquid obtains allows the precipitation of the extracted DBT from the used ionic liquid. Therefore, it was found that adding the same ratio of distilled water to the used ionic liquid forces the extracted DBT to settle down as solids after a few minutes of mixing the diluted used ionic liquid.

Since the amount of the DBT is very small and some of it may stay floating in the vicinity of the solution, a centrifuge was used to force the precipitated DBT to be collected at the bottom of the container. After the separation of the purified diluted ionic liquid from the precipitated DBT, the mixture is subjected to a temperature of 100 °C and a vacuum environment to allow the fast evaporation of the water and concentrating the ionic liquid to be prepared for the extraction process.

The recycled ionic liquid was tested to measure how much DBT was precipitated during the recycling process. A one-stage extraction process was done where a ratio of 1:1 between the DBT solution and the recycled ionic liquid was used.

Figure 4.12 shows that the ionic liquid lowered the DBT concentration of the hexane solution to almost half of its original concentration (From around 500 ppm to  $258 \pm 2$

ppm). Also, Figure 4.13 confirms the validity of the recycling effect by reducing the sulfur level to around  $230 \pm 15$  ppm using the extracting column, which also reflects the effect of temperature on extraction. See Figures 4.12a and 4.13a for a comparison between the fresh and the recycled ionic liquid used to extract DBT from a hexane solution in the small reactor and KG1-MATR column respectively, which reflects the feasibility and success of the recycling step

As a result, the recycling method used to purify the exhausted ionic liquid is considered feasible and the recycled extracting agent has virtually zero concentration of the extracted DBT from a previous extraction step. The block diagram in Figure 4.14 represents the whole extraction and recycling process.

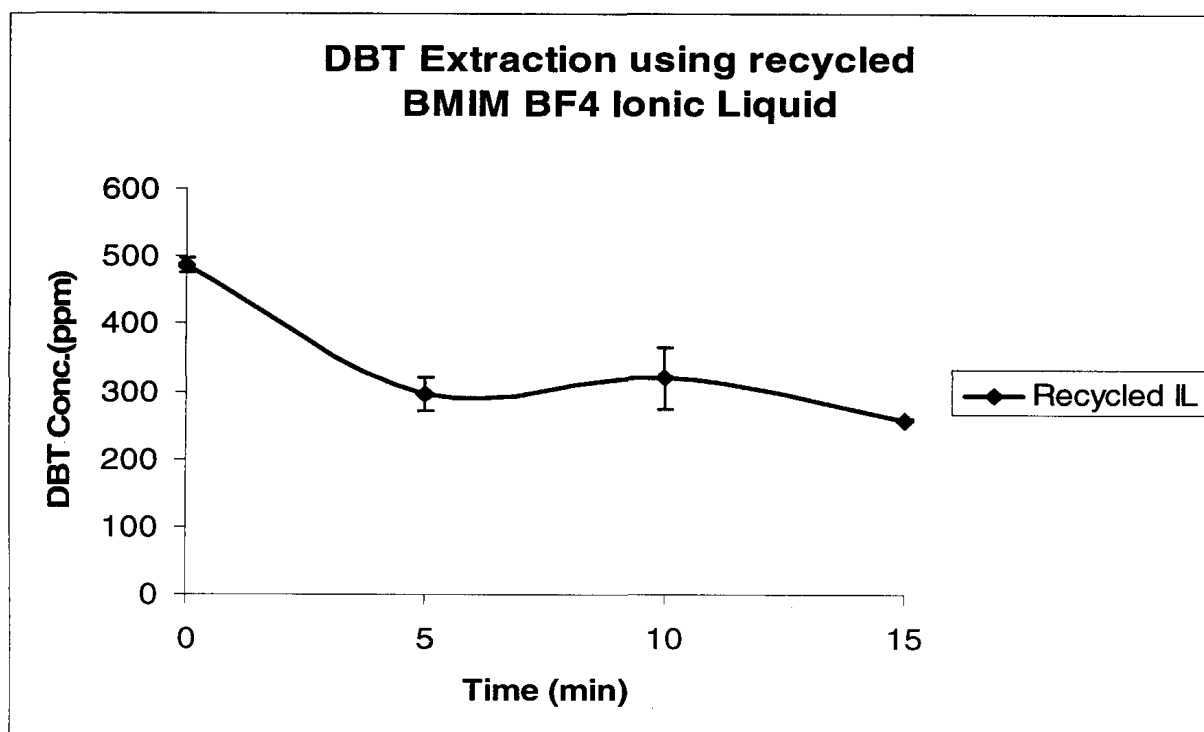


Figure 4.12 DBT extraction from the hexane solution using a Recycled BMIM BF<sub>4</sub> ionic liquid (DBT: IL = 1:1, Mixing Time = 15 minutes). Final DBT Concentration =  $258 \pm 2$  ppm.



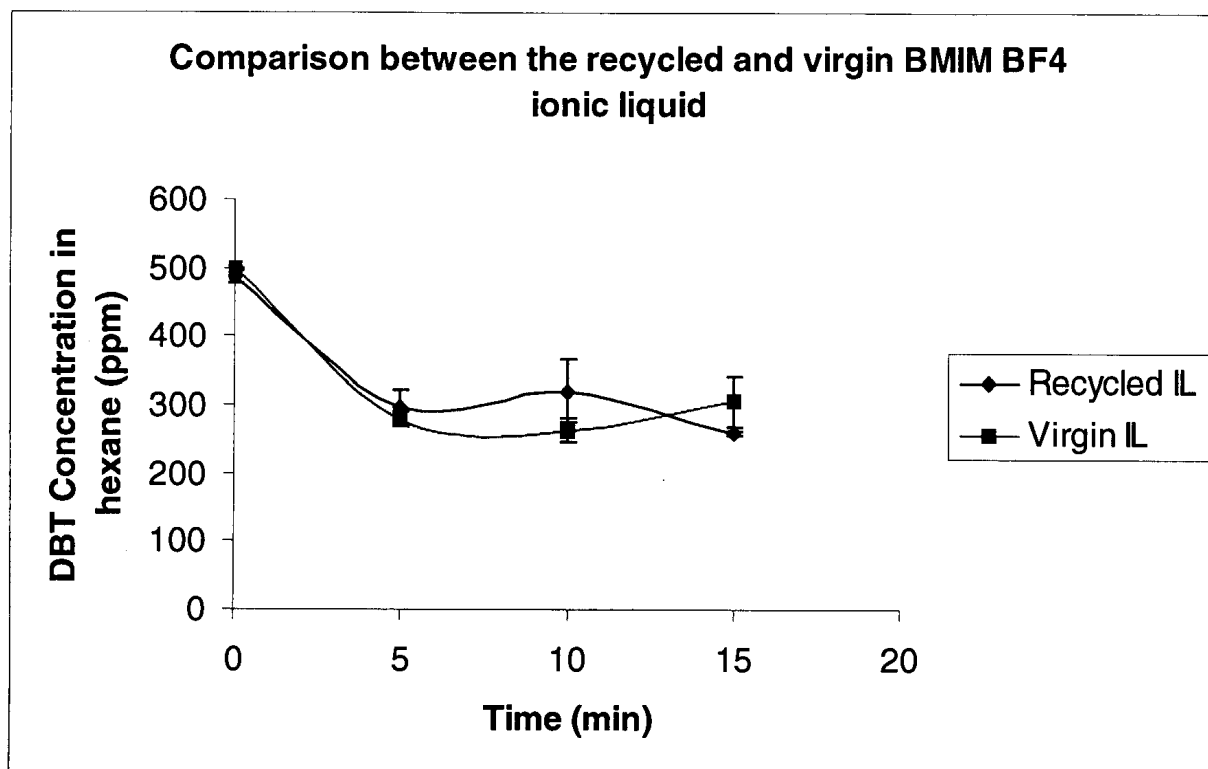


Figure 4.12a Comparison of extraction capacity between the fresh and recycled BMIM BF<sub>4</sub> ionic liquid

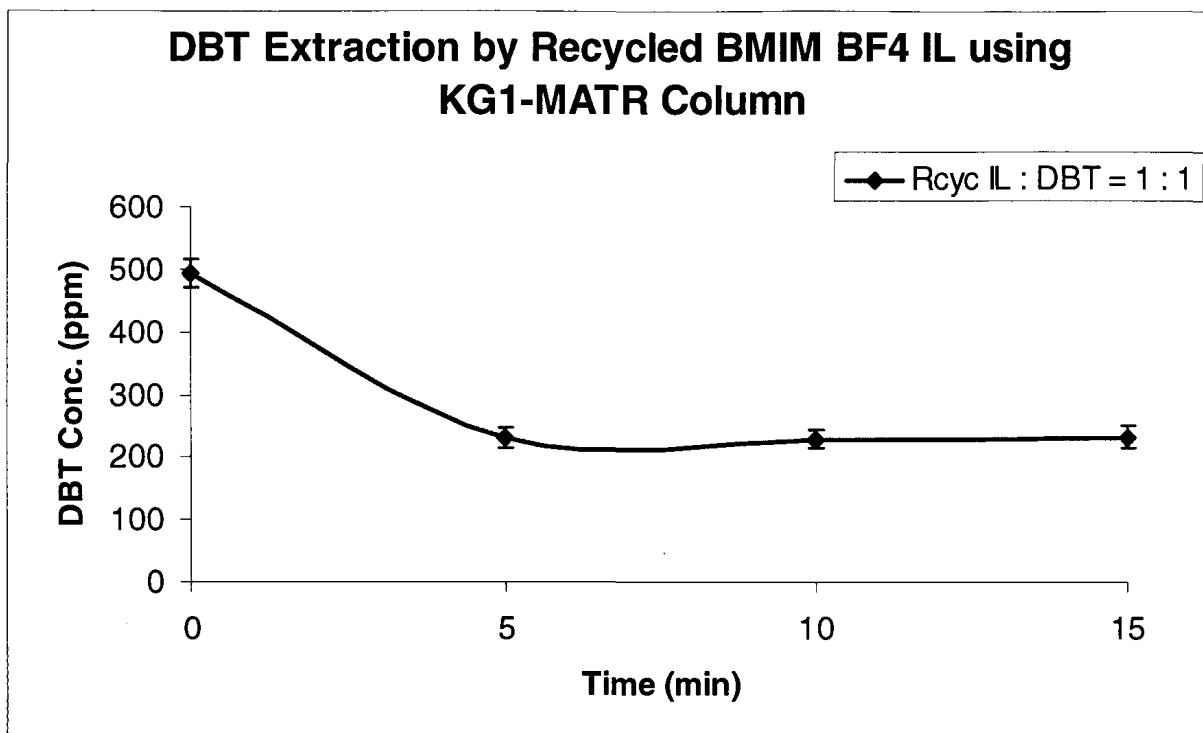


Figure 4.13 DBT extraction from the hexane solution using a recycled BMIM BF<sub>4</sub> ionic liquid in the KG1-MATR Extraction column (DBT: IL = 1:1, Mixing Time = 15 minutes, Temperature = 40 °C). Final DBT Concentration = 230 ± 15 ppm.

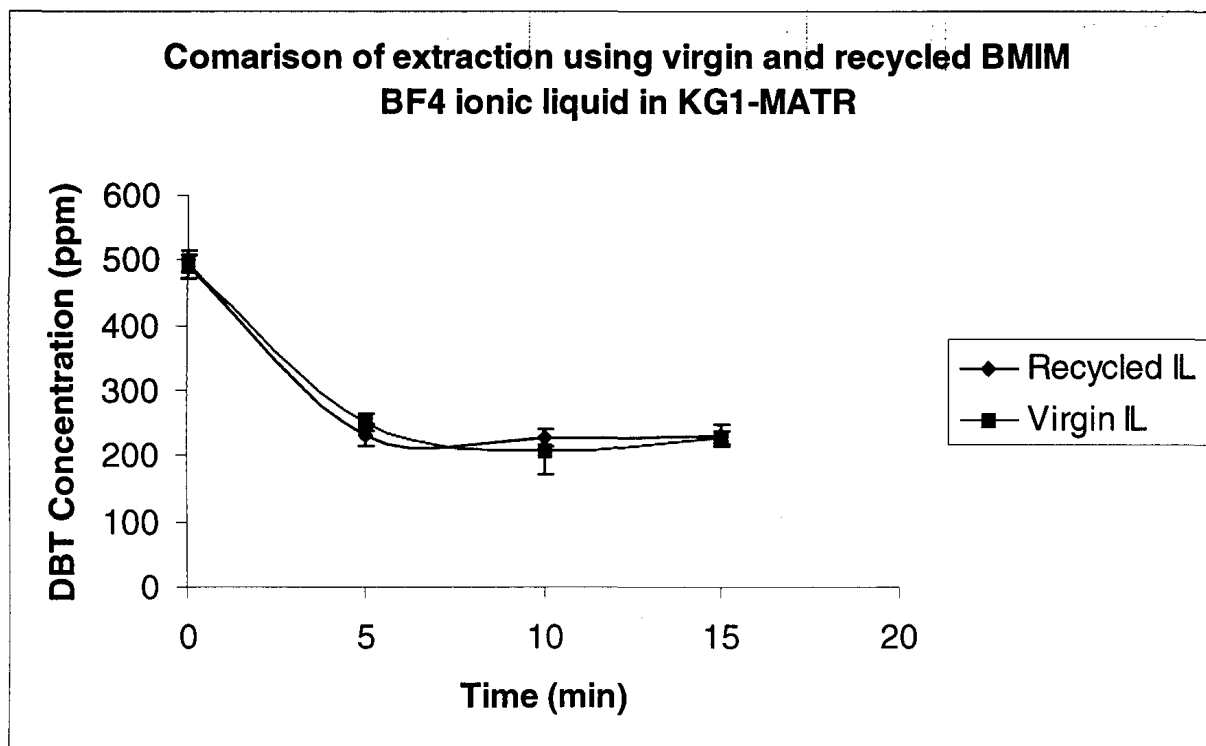


Figure 4.13a Comparison of extraction capacity between the fresh and recycled BMIM BF<sub>4</sub> ionic liquid using the KG1-MATR column

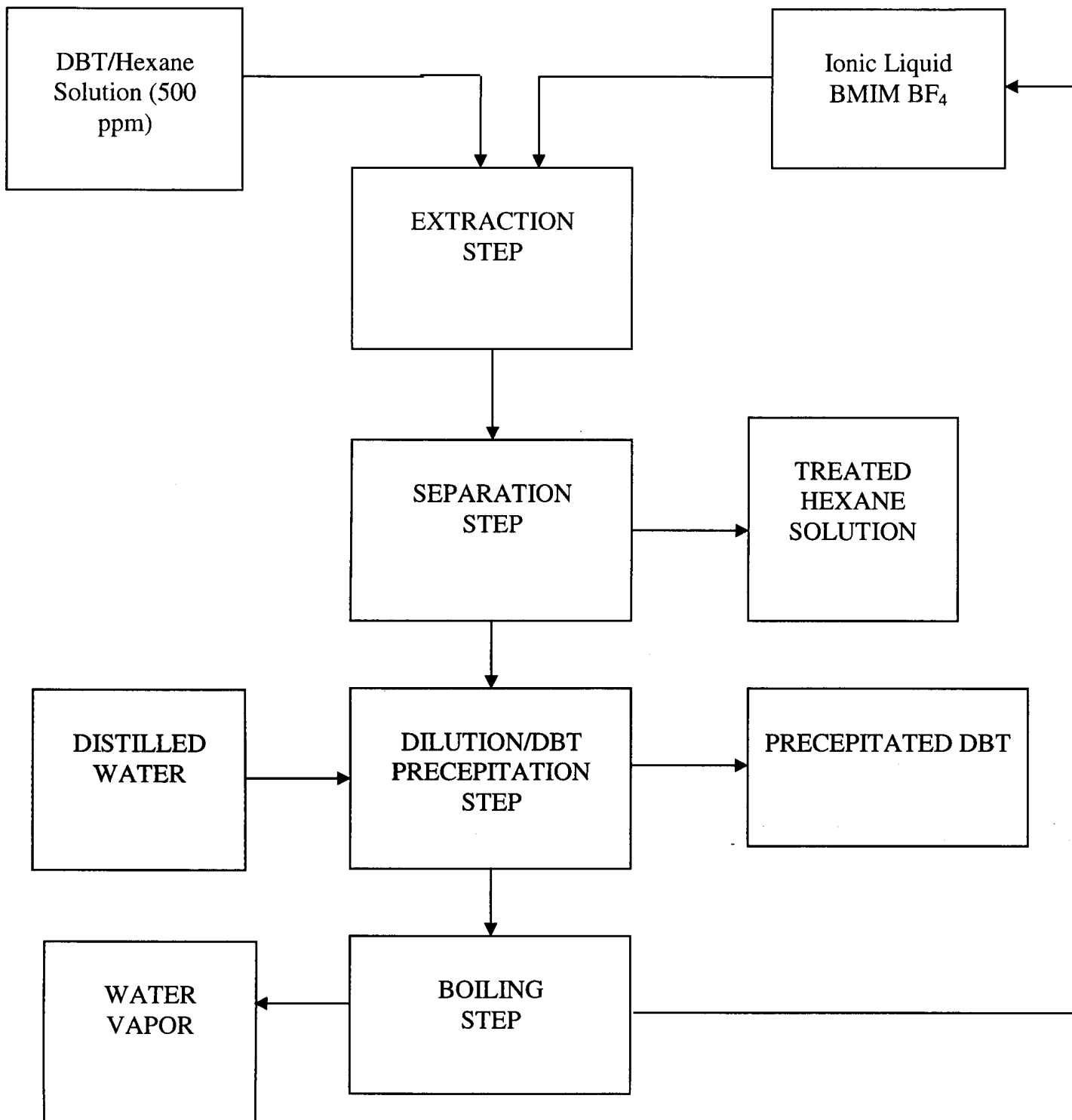


Figure 4.14 Block diagram of the DBT Extraction and the BMIM BF<sub>4</sub> Ionic Liquid Recycling

## 4.5 Modelling

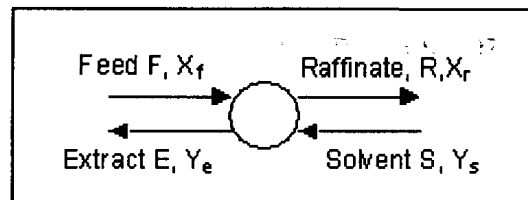
Liquid-Liquid extraction is the process under which a desired chemical is extracted from one liquid to another when the biphasic system undergoes an intensive mixing. Based on the nature of the process, several parameters could have an effect on the yield and selectivity of the overall output. Some of these variables are temperature, pH and residence time. The pressure can be neglected since it has almost no effect on the extraction performance, unless the vapour pressure of one or both liquids have a significant effect on the functionality of the process. The pH effect should be taken into consideration if the process includes a biological or reactive effect, which is governed by the pH value of the system. Therefore, the investigation of pH change was not taken into consideration in the experiment since it is governed by pure chemical extraction without the existence of any chemical reactions. On the other hand, temperature is very important in the selective process since it has a direct effect on keeping the viscosity low and ultimately reduces the mass transfer resistance.

Due to the intense mixing of the bi-phasic system, the mass transfer coefficient could be assumed negligible and there is no need to derive the value of the mass transfer coefficient of DBT between the ionic liquid than the hexane solution. Different type of mathematical model must be used to analyze the results obtained from the previous experiments.

Since the two-phase system undergoes an intense mixing during the extraction process, the only efficient method to measure the capacity of the mass transfer between the two phases is through the Distribution Coefficient, which is the same as the Nernst Partition Coefficient.

Since all the experiments done in this study were under a batch mode, it can be easily compared to a cross current operation system. Therefore, the variables used in the cross current model will be implemented on the feeds and products obtained from the batch mode system used in this thesis.

The cross current operation is used under a batch mode. This type of operation was the basis of all the runs done in this experiment. A one-stage extraction can be represented by the following diagram (Chopey, 2005):



Where:

$F$  = Feed quantity / rate, mass.       $R$  = Raffinate quantity / rate, mass

$S$  = Solvent quantity / rate, mass.       $E$  = Extract quantity / rate, mass

$X_f$ ,  $X_r$ ,  $Y_s$ , and  $Y_e$  are the weight fractions of solute in the feed, raffinate, solvent and extract, respectively. Partition coefficient 'm' is defined as the ratio of  $Y_e$  to  $X_r$  at equilibrium conditions.

$$m = Y_e / X_r \quad (4.1)$$

Assuming that the feed and the solvent are immiscible, we can anticipate that  $F = R$ , and  $S = E$ . Also, from the results obtained in the experiment of one-stage extraction of DBT, The average weight fractions are:  $X_f = 500$  ppm,  $Y_s = 0$  ppm and  $X_r = 260$  ppm at equilibrium. By a simple subtraction we can find the experimental value of  $Y_e$  to be 240 ppm.

Therefore, the partition coefficient of this stage is:  $m = Y_e / X_r = 240 / 260 = 0.9231$ .

By doing a mass balance across the extraction stage, the following can be derived:

$$F X_f + S Y_s = R X_r + E Y_e \quad (4.2)$$

Several conditions were assumed or given:

- The feed and solvent are immiscible
- $F = R$  and  $S = E$ .
- The weight fraction of the solute in the solvent is  $Y_s = 0$
- From the partition coefficient definition:  $Y_e = m X_r$

By using all the information given above, the mass balance equation can be simplified to:

$$S = F/m (X_f/X_r - 1) \text{ or } X_f/X_r = 1 + m S/F \quad (4.3)$$

Since the solvent to feed ratio was determined at the beginning of the experiments to be 1:1, we can determine the reduction ratio  $X_f/X_r$ .

$$X_f/X_r = 1 + m S/F = 1 + (0.9231)*(1) = 1.9231, \text{ or}$$

$$X_r/X_f = 1 / 1.9231 = 0.5199 \sim 0.52 \quad (4.4)$$

From the second-stage extraction, the concentration of DBT was decreased from 300 ppm to 160 ppm. This gives the following weight fractions:  $X_f = 300$  ppm,  $Y_s = 0$  ppm,  $X_r = 160$  ppm and  $Y_e = 140$  ppm at equilibrium.

Therefore, the partition coefficient of this stage is  $m = Y_e / X_r = 140 / 160 = 0.875$ , and the inverse of the reduction ratio  $X_r/X_f = 160 / 300 = 0.5333 \sim 0.53$ .

Finally, from the third-stage extraction, the concentration of DBT was decreased from 160 ppm to 90 ppm. This gives the following weight fractions:  $X_f = 160$  ppm,  $Y_s = 0$  ppm,  $X_r = 90$  ppm and  $Y_e = 70$  ppm at equilibrium.

Therefore, the partition coefficient of this stage is  $m = Y_e / X_r = 70 / 90 = 0.778$ , and the inverse of the reduction ratio  $X_r/X_f = 90 / 160 = 0.5625 \sim 0.56$ .

As we can see, the distribution coefficient is not constant from experimental point of view and in order to understand the difference between the practical and theoretical results, a comparison will be made to see how many stages needed to reduce the sulfur level to the lowest value possible.

Table (4.2): Comparison of the theoretical and experimental distribution coefficient effect on DBT concentration.

Number of Stages	Distribution Coefficient (Theoretical)	DBT Concentration (Theoretical)	Distribution Coefficient (Experimental)	DBT Concentration (Experimental)
1	0.73	289.02	0.92	260.42
2	0.73	167.06	0.875	162.70
3	0.73	96.57	0.778	91.51
4	0.73	55.82	0.6595	55.15
5	0.73	32.26	0.6562	33.30
6	0.73	18.65	0.6558	20.11

Therefore, the experimental data shows that it needs 6 stages in order to reduce the sulfur level to almost 20 ppm which almost replicates the theoretical results where the theoretical distribution coefficient was determined from the average of the experimental ones. The difference in results is acceptable since the experimental results are affected by several factors that play a role in altering the results from the calculated ones, like the human error factor.

The average partition coefficient is 0.73, which is very close to the value found by Jochen *et al.* (2004) of 0.7 (the partition coefficient was referred to as Nernst partition coefficient  $K_N$ ).



The extraction process can be represented by a cross solubility triangle. Figure 4.15 shows a general representation of the solubility triangle between the solute (DBT) and the two liquid (model fuel and the ionic liquid). It is technically irrelevant to derive the solubility diagram since the concentration of the DBT representing the sulfur content in the oil phase is very small. It is important to concentrate only on the lower portion of the triangle where the extraction occurs. Therefore, Figure 4.16 shows the results of a one-stage extraction where the initial concentration is at 500 ppm. The plot of the enlarged section of the solubility triangle is a representation of the extraction step between the ionic liquid and the model fuel under 1:1 ratio.

Even though it was assumed that there was no cross solubility between the ionic liquid and the model fuel during the experimental runs, it is important to determine the practical value of the cross solubility between the tested ionic liquid and the actual fuel in order to incorporate it in the calculations of the process cost. Since the used ionic liquid must be regenerated, increasing the value of the cross solubility will increase the capital cost to eliminate the sulfur compounds and the oil extracted into the ionic liquid layer.

The model fuel used in these experiments showed a negligible cross solubility with the BMIM ionic liquid which was determined to be below 2% and it was independent from the ratio between the ionic liquid and the model fuel. Therefore, it is important to choose a suitable extracting agent, which will minimize this problem.

## 4.6 Conclusions

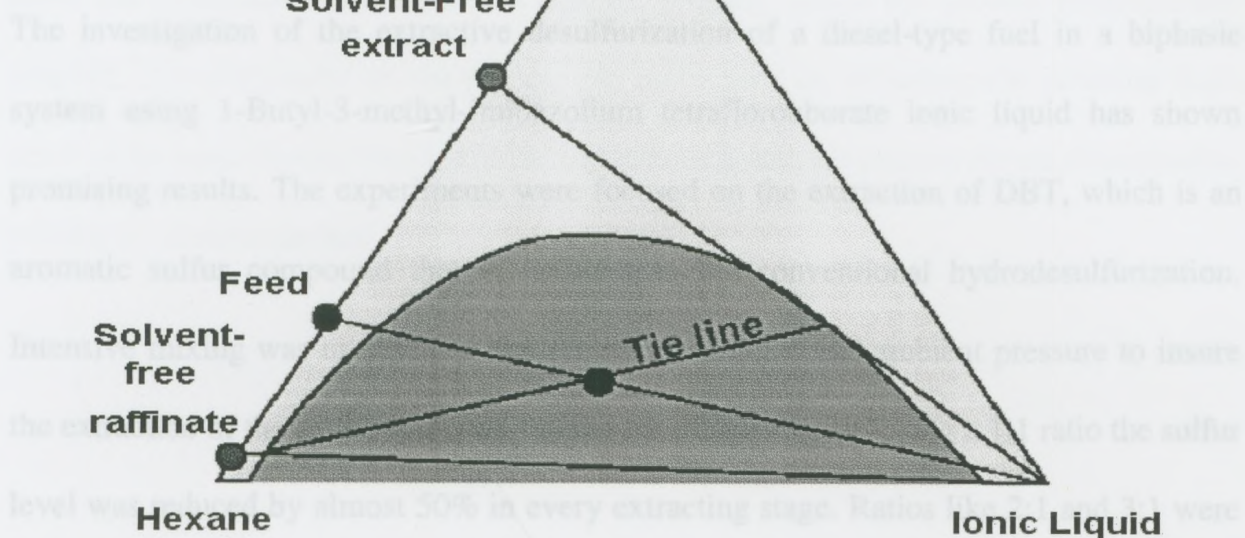


Figure 4.15 General cross solubility between the fuel, ionic liquid and the solute DBT.

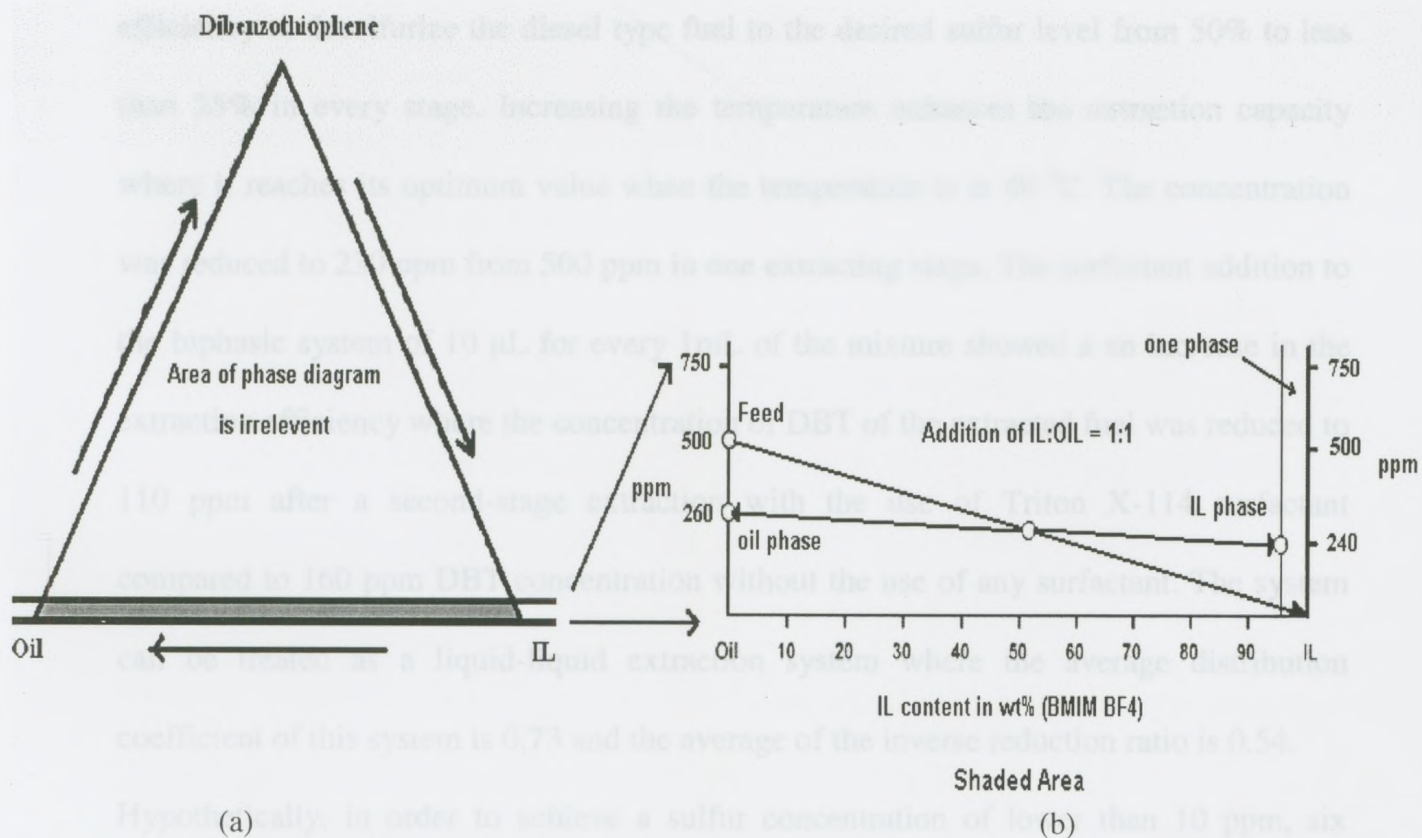


Figure 4.16 One-stage extraction representation on the bottom cut out of the solubility triangle between BMIM ionic liquid-model fuel-DBT.

## 4.6 Conclusions

The investigation of the extractive desulfurization of a diesel-type fuel in a biphasic system using 1-Butyl-3-methyl-imidazolium tetrafluoroborate ionic liquid has shown promising results. The experiments were focused on the extraction of DBT, which is an aromatic sulfur compound that is resistant to the conventional hydrodesulfurization. Intensive mixing was used under moderate temperatures and ambient pressure to insure the extraction of the sulfur compound to the ionic liquid layer. Using a 1:1 ratio the sulfur level was reduced by almost 50% in every extracting stage. Ratios like 2:1 and 3:1 were found to give a much better results but the 1:1 ratio was decided upon due to the moderately high price of the chosen ionic liquid. Diluting the ionic liquid decreased its efficiency to desulfurize the diesel type fuel to the desired sulfur level from 50% to less than 25% in every stage. Increasing the temperature enhances the extraction capacity where it reaches its optimum value when the temperature is at 40 °C. The concentration was reduced to 230 ppm from 500 ppm in one extracting stage. The surfactant addition to the biphasic system of 10 µL for every 1mL of the mixture showed a an increase in the extraction efficiency where the concentration of DBT of the extracted fuel was reduced to 110 ppm after a second-stage extraction with the use of Triton X-114 surfactant compared to 160 ppm DBT concentration without the use of any surfactant. The system can be treated as a liquid-liquid extraction system where the average distribution coefficient of this system is 0.73 and the average of the inverse reduction ratio is 0.54. Hypothetically, in order to achieve a sulfur concentration of lower than 10 ppm, six stages of extraction must be used.

This process can be easily implemented with the existing hydrodesulfurization process to improve the quality of the diesel fuel. Using the already existing units for stage extraction under the moderate temperature and ambient pressure makes the process economically feasible. The investment on the process can be focused on off-siding the moderate high price of the ionic liquid, which will be recycled back into the process by the precipitation process or another method.

## 4.7 References

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## Chapter 5: Conclusions and Recommendations

### 5.1 Conclusions

Since the conventional process of hydrodesulfurization could not reach the desired level of sulfur that meets the new environmental regulations, new methods were investigated in order to overcome the obstacles faced in the original process.

Even though the activity of the Soybean peroxidase showed promising results in the oxidative desulfurization of DBT, the results obtained showed that the enzyme could not withstand the organic surroundings of the assay and resulted in the precipitation of the enzyme. On the other hand, the extractive method with the 1-Butyl-3-methylimidazolium tetrafluoroborate used showed great results. The solvent's ability to extract around 50% of the original sulfur concentration in every stage under moderate temperatures and ambient pressure shows the feasibility of the process. The optimum temperature was at 40 °C, which resulted in decreasing the sulfur concentration to below 50% in the first stage. Also, increasing the solvent to fuel ratio increased the extraction capacity in every stage. The reduction ratio was considered high and ranged from 1.5 to almost 2 and the average distribution coefficient was 0.73. The extractive selectivity of the ionic liquid favours this green solvent over the conventional solvents used in the industry.

This method is promising since the extraction units and technology are already in use and the extractive desulfurization by ionic liquid can be merged in with the existing hydrodesulfurization process to meet the USLD environmental regulations.

## 5.2 Recommendations

This study was dedicated to find a method to reduce the sulfur level of diesel throughout oxidative desulfurization or by pure extractive desulfurization.

In the enzymatic oxidative desulfurization, it is important to choose an enzyme that has a good activity but at the same time it must be able to endure the harsh environment of the organic fuels.

In the extractive desulfurization method, a detailed testing of the temperature and the model fuel can be considered in order to validate the optimum temperature needed and reduce the differences between the real diesel and imitated fuel. Therefore, the hexane solution could be replaced by a solvent that has a lower vapour pressure and closer carbon number to the average carbon number of diesel to make the model fuel. Also, different types of ionic liquids can be tested to have a wider range of data, which allows a better decision-making in the future regarding the optimum ionic liquid that can be used in the extraction process.

In order to understand the process in more details, an intensive study must be done to cover all the aspects of this new method. More than one type of organic sulfur compound must be tested and an integration of several types of sulfur compounds must be used to mimic the real presence of sulfur in diesel in order to have a better observation in solving the sulfur problem in fuel fractions. The preliminary model of the liquid- liquid extraction was implemented and shown promising results. Also, cross solubility triangle method could be used to validate these results and build on it the next step. In addition to that, integration between an oxidative desulfurization and extractive desulfurization can be studied where the hydrogen peroxide is the oxidizing agent and the ionic liquid is the



extracting agent in order to enhance the reduction of the sulfur concentration in diesel. Finally, successful inorganic catalysts that are used in the oxidation of the organic sulfur compound in diesel can be combined with the ionic liquid extraction stage in order to maximize the reduction level of the sulfur concentration to the acceptable value. Therefore, an investigation must be conducted to study the ability of the chosen ionic liquids to extract the products that would result from the oxidation step like the sulfones and sulfoxides of the dibenzothiophenes.

## Appendix

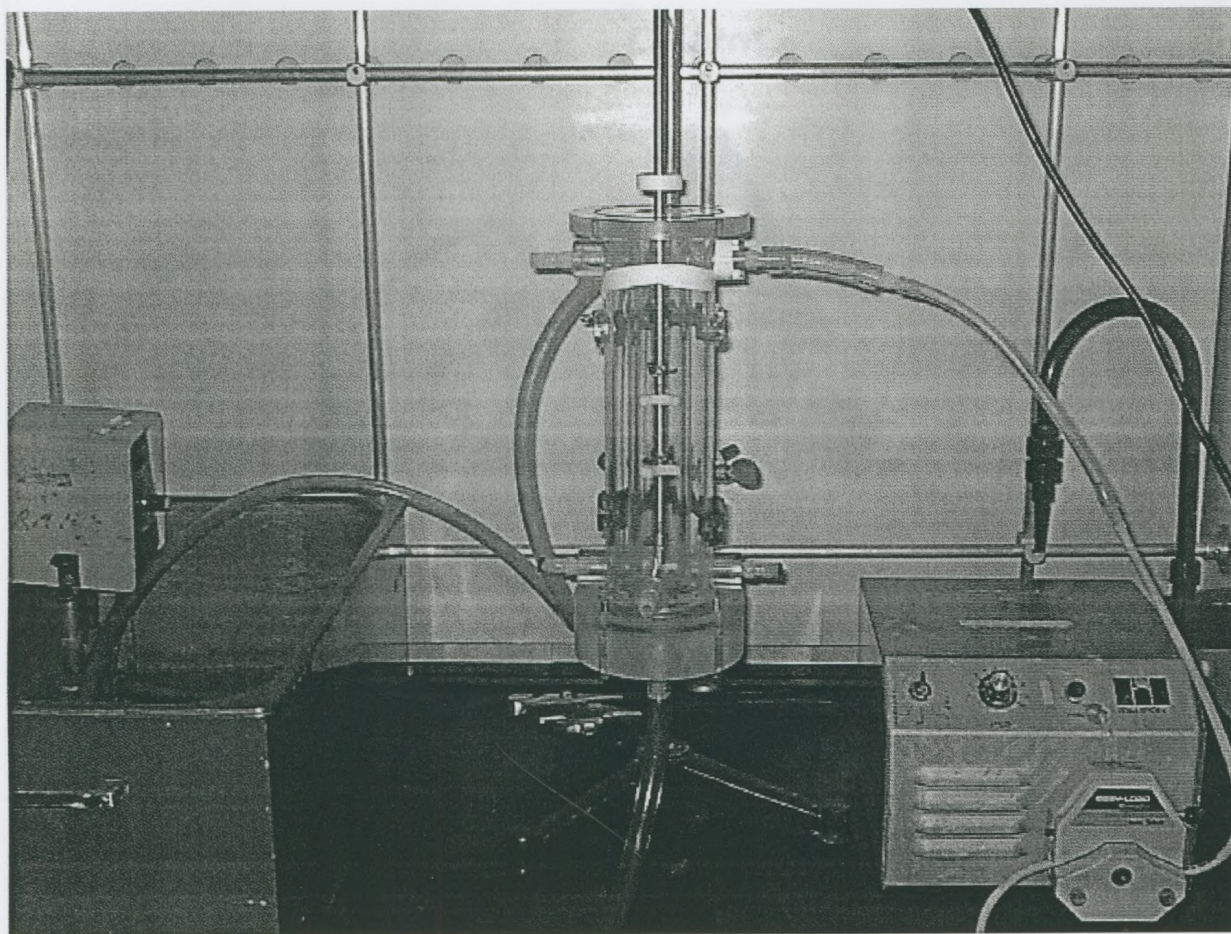


Figure A1 Picture of the experimental setup of the DBT extraction by ionic liquid.

Figure A2 Three-dimensional Crystal Structure of DBT (Kang, I.K., et al., 2002).

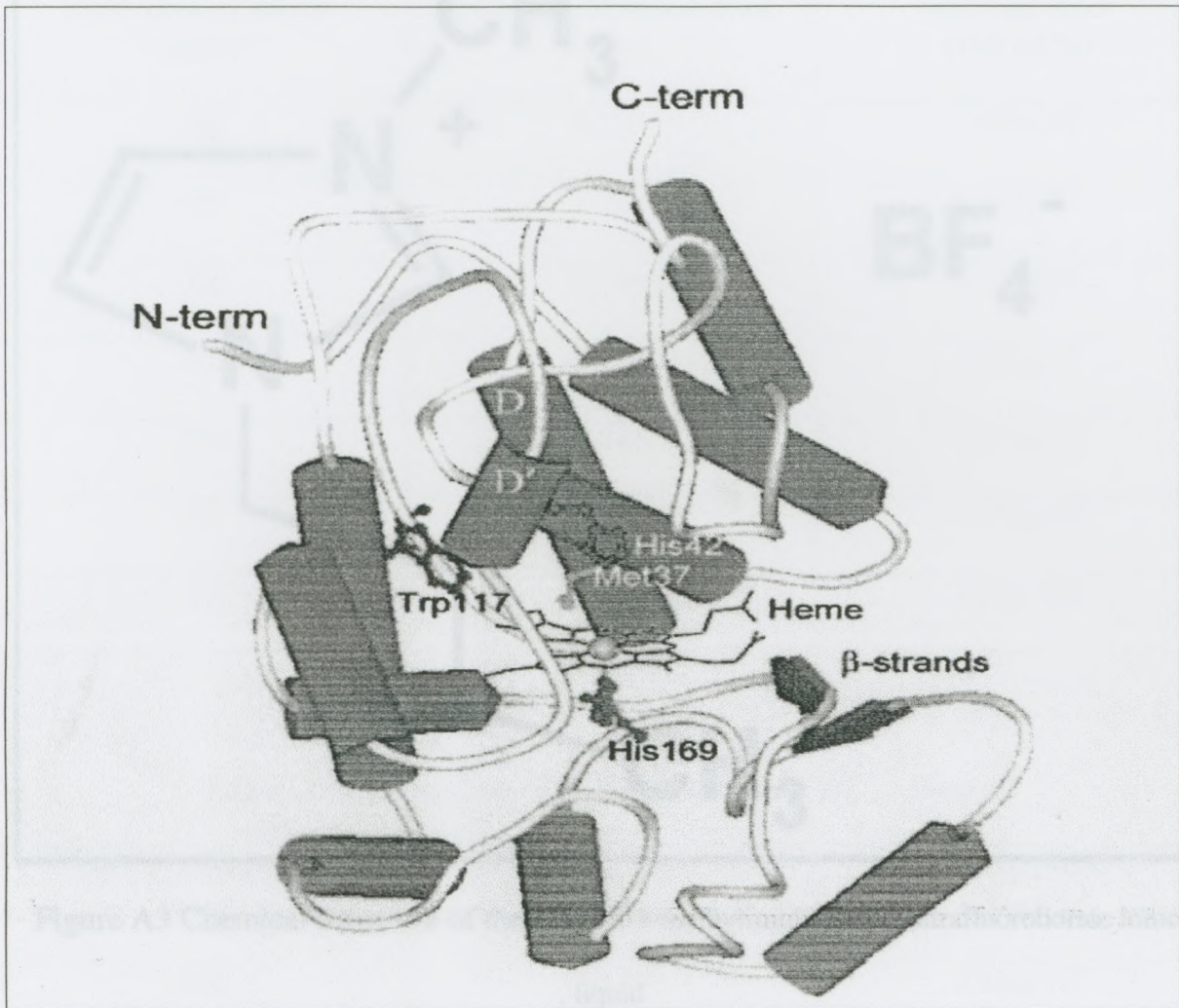


Figure A2 Three-dimensional Crystal Structure of SBP (Kamal J.K. *et al.*, 2002).



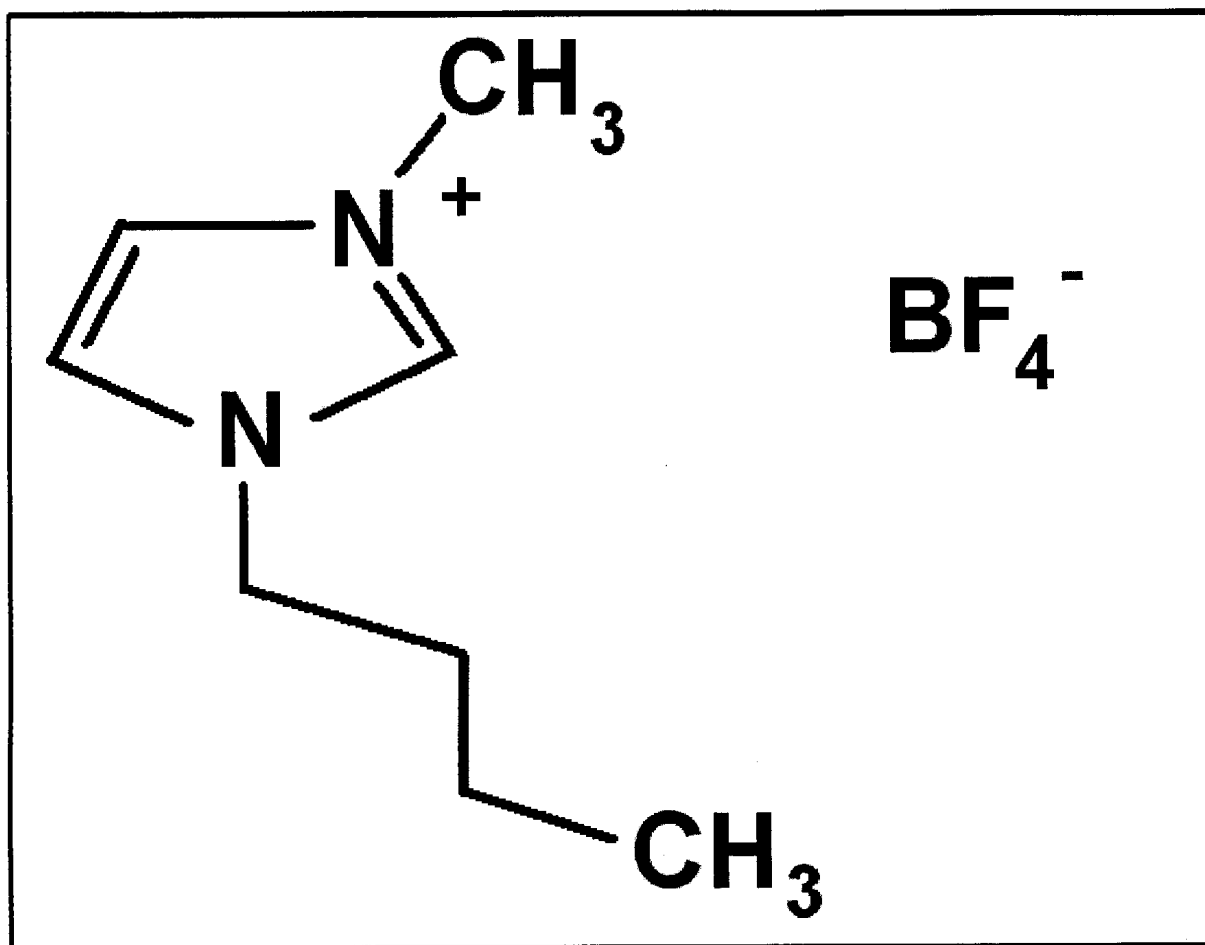


Figure A3 Chemical Structure of the 1-Butyl-3-methylimidazolium tetrafluoroborate ionic liquid

**Table A1 Calibration of GC: Calibrating DBT in Acetonitrile**

DBT Concentration (ppm)	Average Residence Time (min)	Average Area (150 pA*s)
500	8.201	$5.956 \times 10^5$
400	8.199	$5.325 \times 10^5$
300	8.196	$4.569 \times 10^5$
200	8.200	$3.502 \times 10^5$
100	8.189	$2.213 \times 10^5$
50	8.187	$7.152 \times 10^4$
25	8.195	$3.034 \times 10^4$

**Table A2 Calibration of GC: Calibrating DBT in Hexane**

DBT Concentration (ppm)	Average Residence Time (min)	Average Area (150 pA*s)
500	8.099	$2.618 \times 10^5$
400	8.100	$1.950 \times 10^5$
300	8.102	$8.087 \times 10^4$
200	8.0925	$6.119 \times 10^4$
100	8.101	$5.244 \times 10^3$
50	8.095	$4.497 \times 10^3$
25	8.100	$1.626 \times 10^3$

## **Calibration**

In order to calibrate the GC unit, different concentrations of the Dibenzothiophenes were taken from 500 ppm to 100 ppm with an interval of 100 ppm and also the concentrations 50 and 25 ppm were calibrated. The corresponding areas were obtained using the FPD detector and more than one run was done for every concentration and the average was taken to construct the calibration curve that was used to determine the concentration of the sulfur level during the experimental runs.