Chemical trapping of nitric oxide by aromatic nitroso sulfonates

A Thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY

By

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March, 2013

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STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University library, being made available for loan and photocopying subject to the provision of the Copyright Act 1968.

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Date: 28 March 2013

Wendy Venpin
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I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisors, attesting to my significant contribution to the joint publications.

______________________________ Date: 28 March 2013

Wendy Venpin
STATEMENT OF CONTRIBUTION OF OTHERS

We, the undersigned, attest that Research Higher Degree candidate, Wendy Koo Pao Foon Venpin, has devised the experimental program, conducted experiments, analysed data, performed computational chemistry calculations and has written all papers included in this thesis. Professors Bogdan Z. Dlugogorski, Eric M. Kennedy and John C. Mackie provided advice on the experimental program, project direction and assisted with the editing of the papers, consistent with normal supervisors-candidate relations.

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Professor Eric M. Kennedy  Date:

__________________________________________
Professor John C. Mackie  Date:
DEDICATIONS

To my late father, Charles Venpin, who provided an example of a hardworker and inspired my love for science and engineering.
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ABSTRACT

This thesis investigates the employment of spin traps as NOx scavengers to control noxious NOx formation during nitrosation reactions. Most notably, the reaction conditions studied are relevant to the sensitisation of emulsion explosives activated by the chemical gassing, where the formation of these gases can trigger serious respiratory problems to explosives users. Spin traps are widely used by biochemists to detect and measure free radicals such as nitric oxide (NO) in biological systems. The spin trapping reaction involves the addition of a radical to the spin trap, which results in the formation of a complex adduct, detectable by electron paramagnetic resonance (EPR). Intuitively, as a result of the effect of the spin trapping reaction on free radical, these spin traps can potentially play an important role in the development of a new technology to reduce NOx emission.

Four aromatic ortho substituted nitroso compounds, 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), nitrosobenzene sulfonate (NBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and 3,5-dichloro-4-nitrosobenzene sulfonate (DCNBS) were subjected to detailed experimental and theoretical investigation in this thesis. These compounds were synthesised by the oxidation of their corresponding amine compounds and characterised by infrared (IR), ultraviolet visible (UV-Vis), nuclear magnetic resonance (NMR) spectroscopy and nanostructured assisted laser desorption ionisation mass spectrometric (NALDI-MS).

The thesis initially studied the reaction of DBNBS with NO, where NO was generated in an acidic nitrite solution, conditions which are similar to the chemical gassing process of emulsion explosives, by examining gaseous and liquid products from the
reaction. Membrane inlet mass spectrometer (MIMS) analysis disclosed the presence of significant amount of nitrogen gas (N$_2$) in the gas phase, whereas ion chromatographic analysis of the reaction mixture disclosed elevated amounts of nitrate were formed during the gassing reaction.

During the reaction, DBNBS initially reacts with NO to form a short live DBNBS-NO adduct. The release of N$_2$ is a consequence of the homolytic cleavage of the C-N bond of a diazenyl radical. This assertion is based on quantum chemical calculations (Density Functional Theory) which validates the favourable formation of a diazenyl radical as well as oxygen. The elevated concentration of nitrate in solution provides additional evidence of the presence of oxygen released as a result of the decomposition of the radical intermediate (DBNBS-NO adduct). NALDI-MS analysis of liquid products in the study enabled the identification of 3,4,5-trinitrobenzene sulfonate (MW of 291.888 a.m.u) as the primary product from the reaction, and a number of other nitro compounds were also identified. Analysis of the gaseous and liquid products, in particular the NALDI-MS technique, demonstrates that the presence of nitrite leads to the formation of a competing reaction pathway whereby nitro group is introduced in the aromatic system through the coupling of nitrite with a phenyl radical.

A novel membrane NOx analyser and a stopped-flow UV-Vis spectrometer were employed to determine the rate of trapping of NO by DBNBS based on the proposed mechanism. The thermodynamic and kinetic properties of the dissociation of DBNBS dimer to its monomer, were first investigated as this step controls the trapping of NO by DBNBS. An equilibrium constant, $K_C$ of $(1.29 \pm 0.03) \times 10^{-3}$ (at 25 °C) for DBNBS dimer/monomer interchange was estimated, which indicates that, at equilibrium around 20 % of the dissolved DBNBS is present as monomer at room temperature, and
available for trapping NO under these conditions. The study of the reaction at temperatures ranging from 25 to 60 °C shows increasing monomer equilibrium concentrations as temperature rises.

Analysis of the measurements from the ex situ trapping of NO (where nitric oxide was generated via the rapid nitrosation of ascorbic acid) to a multistep reaction mechanism resulted in an estimate of the rate constant $k_{\text{Trap}}$ of 165 mol$^{-1}$ dm$^3$ s$^{-1}$. In contrast, the net rate of trapping was considerably lower with a value of 4.7 mol$^{-1}$ dm$^3$ s$^{-1}$ for the in situ reaction of DBNBS with NO, where NO is formed via the slow decomposition of nitrous acid.

The physicochemical properties of the four selected aromatic ortho substituted nitroso compounds were also examined. Since the four nitroso compounds exist in a monomer-dimer equilibrium with only the monomeric form behaving as a free radical scavenger, thermodynamic analysis of the dimer-monomer equilibrium was undertaken using UV-Vis spectrophotometer.

The reactivity of the aromatic ortho substituted nitroso compounds towards NO was investigated, to determine the effect of substituents on the aromatic ring towards trapping efficiency in an aqueous system. The production of nitrogen gas and an elevated quantities of nitrate were observed during the reactions of nitroso compounds with NO suggesting that the homolytic cleavage of aryl radicals generally occurs when nitroso compounds reacts with NO as proposed previously for DBNBS.

The capacity of the aromatic nitroso sulfonates was investigated at ammonium nitrate (AN) concentration ranging from 0 to 7.5 mol dm$^{-3}$ for trapping NO. The solubility of DCNBS in AN solutions was the most affected among the four compounds and was
reflected by a notable decrease in the efficiency of NO removal by the compound with increasing AN concentrations.

Experiments in AN explosive established that chemical trapping of NO was more efficient when the nitroso compounds were added at the time of chemical gassing, rather than being part of the discrete phase of the emulsion. All nitroso compounds demonstrated an inhibitory effect on the amount of NO released from the chemical gassing of the emulsion explosive. Owing to the reduced efficiency in NO removal in AN solutions and AN emulsion, aromatic nitroso sulfonates are good NO scavengers with removal efficiency in NO of up to 70 % that can be achieved in sensitised AN emulsion.
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<th>Description</th>
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<tbody>
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<td>AN</td>
<td>Ammonium nitrate</td>
</tr>
<tr>
<td>DBNBS</td>
<td>3,5-dibromo-4-nitrosobenzene sulfonate sodium salt</td>
</tr>
<tr>
<td>DCNBS</td>
<td>3,5-dichloro-4-nitrosobenzene sulfonate sodium salt</td>
</tr>
<tr>
<td>DETC</td>
<td>Diethyldithiocarbamate</td>
</tr>
<tr>
<td>DMNBS</td>
<td>3,5-dimethyl-4-nitrosobenzene sulfonate sodium salt</td>
</tr>
<tr>
<td>EDRF</td>
<td>Endothelium-derived relaxing factor</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>EPR</td>
<td>Electron paramagnetic resonance</td>
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<td>GC</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>Mass spectrometry</td>
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<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>NO₂</td>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>N₂O</td>
<td>Nitrous oxide</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>Nitrite</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Nitrate</td>
</tr>
<tr>
<td>NOCT</td>
<td>Nitric oxide cheletropic trap</td>
</tr>
<tr>
<td>PIBSA</td>
<td>polyisobutylene succinic anhydride</td>
</tr>
<tr>
<td>PTIO</td>
<td>2-phenyl-4,4,5,5-tetramethylimidazoline-1-yloxyl-3-oxide</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>O₂⁻</td>
<td>Superoxide</td>
</tr>
<tr>
<td>ONOO⁻</td>
<td>Peroxynitrite</td>
</tr>
<tr>
<td>SCR</td>
<td>Selective catalytic reduction</td>
</tr>
<tr>
<td>SNCR</td>
<td>Selective non-catalytic reduction</td>
</tr>
<tr>
<td>TLV-TWA</td>
<td>Threshold exposure limit time weighted average</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet visible</td>
</tr>
</tbody>
</table>
CHAPTER 1: INTRODUCTION
1.1 Background

Nitric oxide (NO) is one of the main contributors to the formation of ground level ozone, urban smog and acid rain. NO is also a noxious gas, causing detrimental health effects in humans. It acts as an asphyxiant at high concentrations and, at lower concentrations exposure, affects the central nervous system, cardiovascular, hepatic and reproductive function [1]. Additionally, NO is extremely reactive in air and undergoes rapid oxidation with atmospheric oxygen to produce the even more toxic species nitrogen dioxide; a reddish-brown coloured gas. The development of effective methods to remove or scavenge this toxic gas is essential to minimise human exposure. The focus of the research in the present study was to eliminate or reduce the evolution of NOx gases during the sensitisation of ammonium nitrate emulsion explosives.

Emulsion explosives are concentrated water-in-oil emulsions in which a discontinuous phase of inorganic oxidiser salt solution (mostly aqueous ammonium nitrate droplets) is dispersed in a continuous organic fuel phase. The continuous phase generally consists of a mixture of diesel fuel and a polyisobutylene succinic anhydride (PIBSA)-based emulsifier. The emulsifier is usually included in the emulsion for promotion and stability of the discontinuous phase. Table 1.1 shows the typical ingredients in an emulsion explosive.
This type of explosive is most commonly used worldwide as an explosive in the mining and construction industries [2] due its excellent blasting characteristics and low cost. In addition, ammonium nitrate emulsion explosives are relatively safe, since they require sensitisation on site prior to detonation [3]. The sensitisation process involves the introduction of voids or small bubbles of gas in the emulsion explosive. The gas bubbles compress adiabatically by shock wave and rapidly raise the emulsion temperature to an extent high enough to detonate the bulk emulsion explosives.

There are several methods to sensitise the emulsion explosives, including the addition of hollow particles such as glass micro balloons [4, 5], sparging the emulsion with air [6] and reacting the emulsions with one or more chemicals to form small gas bubbles [7, 8]. The latter method is known as chemical gassing, and is the most widely adopted technique for sensitising emulsion explosives, because it is relatively inexpensive and reliable, thus producing an emulsion explosive which is highly sensitive to detonation.

\begin{table}
\centering
\begin{tabular}{|l|c|}
\hline
Name & Proportion \\
\hline
Ammonium nitrate & 60-100 \% \\
Water & 10-30 \% \\
Inorganic oxidiser & 0-10 \% \\
Emulsifier & 0-10 \% \\
Oils and other oxygen negative material & 0-10 \% \\
\hline
\end{tabular}
\caption{Ingredients in an emulsion explosive}
\end{table}
One popular approach to chemical gassing involves the generation of nitrogen in the explosive via the nitrosation of ammonium ions present in the solution droplets of ammonium nitrate. Chemical gassing is commonly performed by reacting concentrated solutions of nitrite salt with the ammonium nitrate emulsion explosive to form N₂ gas, as shown by Reaction 1.1

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + \text{H}_2\text{O}
\]  

(1.1)

Concentrated acetic acid can, and often is, added during the gassing process in order to enhance the rate of gassing. However, when an emulsion explosive gases rapidly using nitrite as the gassing component, and at low pH, nitrogen oxides (NOx) particularly nitric oxide (NO) are produced via side reactions, in addition to nitrogen gas as the primary gaseous product.

\[
\text{NO}_2^- + \text{H}^+ \rightleftharpoons \text{HNO}_2
\]

(1.2)

\[
\text{HNO}_2 \rightleftharpoons \text{NO} + \text{NO}_2 + \text{H}_2\text{O}
\]

(1.3)

Gassed emulsion explosives can, in some circumstances fall or slide out of the blasthole and thereby releasing some of the sensitising gas. In particular, when stemming is applied to a blast hole with a low-density emulsion explosive in bench loading, some sensitised emulsion may collapse and gas can then be lost from the sensitised emulsion. The current OSHA standard for NO and NO₂ are 25 and 3 ppm respectively averaged over an eight-hour work shift and the production of such gas under poor ventilation conditions poses a safety hazard to the workers [9]. Thus, it is important to develop intrinsically safe explosives that are not toxic to workers prior to detonation, due to evolving NOx.
Paradoxically, the toxic nitric oxide gas plays an essential role in many biological systems and contributes to a variety of important physiological processes. It functions as an endothelium derived relaxing factor (EDRF), a vascular antioxidant, and as a messenger molecule in the cardiovascular and immune systems [10-12]. NO is also a free radical since it possesses one unpaired electron. In biological research activities related to studying NO, biochemists often employ NO spin traps, together with electron paramagnetic resonance technology, to study the multiple biological and physiological functions of this important molecule. This method entails the use of a reactive compound to trap the initial unstable free radical as a stable, paramagnetic species detectable by EPR [13]. Considering this, the different NO spin traps can be studied and subsequently applied to practical emulsion explosives systems to reduce or eliminate NO and provide a safe environment to workers. To-date, there exist very few techniques to control the release of NO during the chemical gassing of emulsion explosive. For example, the addition of concentrated acetic acid can be controlled to limit emission of NO or urea can be added as a gassing accelerator to virtually release minimum amount of NO. However none of these measures have been proved to be truly effective since they influenced the rate of chemical gassing and consequently the application of NO spin trap in emulsion explosive is innovative and well justified. The employment of NO spin traps compared to other current practices for NOx control such as absorption or adsorption will be more practical and efficient for aqueous applications and in particular to emulsion explosive systems as they can be incorporated in the aqueous emulsion to simultaneously target and inhibit the formation of NO without interfering with the chemical gassing process. Therefore, such NO trap represents a preventive measure to NOx emissions rather than a pollution control measure.
Chapter 1: Introduction

1.2 Project Objectives

The present research aims to investigate the different NO spin traps currently used by biochemists for application during the sensitisation of ammonium nitrate emulsion explosive so as to reduce NOx devolution and offer an intrinsically safe and environmental friendly gassing technology.

The specific objectives were achieved through the following steps:

- Performing a critical literature review for identification and selection of an appropriate NO trap for emulsion explosives application.
- Identifying method(s) to monitor nitric oxide and measure the traps directly and continuously.
- Assessing the trapping ability of the selected NO trap in an aqueous system, under conditions that simulate chemical gassing.
- Understanding the nitrosation reaction of the NO trap with nitric oxide and determine rates and mechanism via detailed identification and qualification of reaction products in both gaseous and aqueous phases.
- Predicting and determining if other NO traps with the same functionality of the selected NO trap can also reduce NOx formation.
- Designing and constructing an experimental facility for trapping NO formed from the chemical gassing process of ammonium nitrate emulsion explosive using the selected traps.
1.3 Thesis structure

The literature related to nitric oxide and NO spin traps is presented and assessed in the following chapter, along with discussion on the identification of aromatic nitroso 3,5-di-bromo sulfonate compound as a potential NO trap in emulsion explosive systems. Chapter 3 describes the synthesis and characterisation of nitroso compounds, the experimental setup as well as analytical and computational methodologies. Chapters 4 and 5 are concerned with the study of the nitrosation reaction of DBNBS spin trap with nitric oxide in dilute solutions, under conditions mimicking the sensitisation process. A multi-step reaction mechanism and the kinetics of these reactions are also presented in these chapters. Chapter 6 presents an experimental and theoretical study of the acid dissociation constant of nitroso compounds, knowledge of which is fundamental for understanding nitrosation reaction of nitroso compounds. Chapter 7 investigates other nitroso compounds and relates their reactivity towards the dissociation of their dimeric to monomeric forms in order to understand the effect of substituents on the dimer-monomer interchange process, while Chapter 8 explores the effect of substituents in aromatic nitroso sulfonate compounds in relation to their reactivity with NO and proposes a general reaction mechanism for the reaction of this class of compounds with NO. In Chapter 9, the trapping of NO by the nitroso compounds in ammonium nitrate solutions and emulsion explosive is investigated. This chapter evaluates the removal and the efficiency of the traps for practical applications. Finally, Chapter 10 summarises the key findings of this thesis and provides suggestions for future research.
1.4 References


Chapter 1: Introduction


CHAPTER 2: LITERATURE REVIEW
2.1 Introduction

The sensitisation of ammonium nitrate emulsion explosive via chemical gassing results in the formation nitrogen as the primary gaseous product, but under certain conditions significant quantities of nitrogen oxide can also form. Gassed emulsion explosives can drop or slide out of a blast hole, thus releasing a portion of the sensitising gas. During bench loading, sensitised emulsion may collapse and lose gas, in particular when stemming is applied to a blast-hole with a low-density emulsion explosive [1].

When nitric oxide (NO) is in contact with oxygen, the more toxic nitrogen dioxide (NO₂), with a threshold exposure limit time weighted average (TLV-TWA) limit of 3 ppm, is produced, thus creating a mixture of NO and NO₂, generally known as NOx. Under poor ventilation conditions and in confined spaces, miners can be exposed to dangerous level of NOx which can pose a serious safety hazard. The gas dissolves in the fluid in the eyes and lungs, forming nitrous and nitric acids causing irritation, and, at high concentration, can irreversibly damage the respiratory system [2]. At a concentration of 200 ppm in air, the gas is lethal [3]. Although the mining industry developed measures to minimise exposure of NOx to miners such as providing sufficient ventilation to disperse fume, accidental exposure can occur and thus the exposure to NOx still remains a hazard.

Currently, there are few techniques that have been developed to minimise the generation of NOx generated under these conditions. Urea can be employed as a gassing accelerator to virtually eliminate NOx or any other toxic gases that might be generated. Reducing the amount or concentration of acid added during the gassing will reduce the amount of NOx emitted [1]. This review will outline the key characteristics and reactions of the NO molecule. It also outlines the current NO inhibition methods.
available, and assesses new approaches being developed to tackle the problem of NOx emission and exposure.

2.2 Characteristics of nitric oxide (NO)

The first study of the nitric oxide (NO) molecule dates from 1772, when Joseph Priestly (1733-1804) (who called it "nitrous air") discovered the colourless and toxic gas [4]. NO is a diatomic molecule (Figure 2.1) which many researchers have described as a molecule possessing unique properties [5-10]. The N-O molecule contains nitrogen in the +2 oxidation state with an arrangement of 11 valence electrons. The physical and thermodynamics properties of NO are shown in Table 2.1.

The molecule is also known as a free radical species since it contains an unpaired electron which is localised on the N-O bond [11]. The N-O molecule has a bond length of 1.154 Å and a bond order of 2.5 due to the presence of an unpaired electron in an antibonding $\pi^*$ molecular orbital [12]. NO exists as a gas in its standard state, with low solubility in water; at room temperature and pressure, the maximum solubility of NO is approximately 2 mM [13]. However, NO is lipophilic and 6-8 times more soluble in polar solvent [14, 15]. For instance in methanol and acetonitrile the solubility of NO are $14.5 \times 10^{-3}$ and $14.1 \times 10^{-3}$ mol dm$^{-3}$ atm$^{-1}$ respectively [16].
The NO molecule, being a free radical, is highly reactive. It reacts rapidly with molecular oxygen in an overall third order reaction to yield powerful, oxidising nitrogen oxides such as NO₂, N₂O₃, NO₂⁻ and NO₃⁻ [19, 20]. One important radical-radical reaction of NO in biological systems is its reaction with the radical anion superoxide
(O$_2^-$), to generate the peroxynitrite anion (Equation 2.1), a reaction which occurs at a rate very close to that expected for a diffusion controlled process ($k = 6.7 \times 10^9$ mol$^{-1}$ dm$^3$ s$^{-1}$) [21]. This reaction was proposed to be responsible for the removal of excess toxic nitric oxide in the body, since once formed, ONOO$^-$ is protonated, resulting in its rapid decomposition to nitrate (NO$_3^-$) in the absence of an oxidisable substrate [22]. Therefore, O$_2^-$ will rapidly inhibit NO-mediated N-nitrosation reactions in favour of reactions catalysed by OONO$^-$ [23].

\[
\text{NO} + \text{O}_2^- \rightarrow \text{ONOO}^-
\]  

(2.1)

In aqueous solution, NO can efficiently form complexes with various transition metals such as Fe(II and III), Co(II), Ru(III), Cu(II) and Mn(II), thus giving rise to stable NO metal nitrosilic adducts [24, 25]. The reaction of a transition metal with NO has been applied most notably in catalytic converter in motor vehicles to assist in reducing nitrogen dioxide emissions [26]. Reactions of this type are also crucial in the human body to enable the function of many cytochromes and oxidases [27].

### 2.3 Toxicology of NO and environmental effects

Nitric oxide is recognised as a hazardous and corrosive gas which necessitates handling of the compound with due caution [28]. Table 2.2 summarises the Australian maximum exposure data for NOx. Exposure to NO, even at relatively low concentrations causes fatigue, shortness of breath, coughing and confusion. Acute exposure through inhalation results in collapse, rapid burning and swelling of tissues in the throat and upper respiratory tract, breathing difficulties, throat spasms, fluid build-up in the lungs and eventually death [29, 30]. In addition, NO is unstable in air and undergoes
spontaneous oxidation with atmospheric oxygen to produce unstable nitrogen dioxide, a reddish brown gas. Sokol et al. studied the oxidation of NO and employed a second order model to determine its kinetics. They demonstrated that the reaction occurs extremely rapidly, with a rate constant for NO₂ formation at 25 °C of $1.2 \times 10^{11}$ ppm⁻² s⁻¹ [31]. Nitrogen dioxide is regarded as highly toxic, with a short-term exposure limit (15 min) of 5 ppm. Even at concentrations as low as 1.5 ppm, NO₂ can be a source of acute lung injury eventually leading to pneumonic and pulmonary edema [32, 33].

### Table 2.2. Australian exposure standard data for NO and NO₂

<table>
<thead>
<tr>
<th>Material</th>
<th>TWA ppm</th>
<th>TWA mg m⁻³</th>
<th>STEL ppm</th>
<th>STEL mg m⁻³</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide</td>
<td>25</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>Australia Exposure standards</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>3</td>
<td>5.6</td>
<td>5</td>
<td>9.4</td>
<td>Australia Exposure standards</td>
</tr>
</tbody>
</table>

The release of NO and other nitrogen oxides can also have a deleterious effect on the receiving environment. In the atmosphere, nitric oxide is rapidly oxidised to nitrogen dioxide (half-life ~ 50 days), which subsequently dissolves in water to form nitric acid. Emission of oxides of nitrogen is a major contributor to acid rain [34].

Oxides of nitrogen can influence the level of ozone in the stratosphere, since their formation occurs via the photochemical reaction of nitrogen with oxygen [35, 36]. The formation of excessive levels of nitric oxide reduces the level of stratospheric ozone due
Chapter 2: Literature review

to the consumption of ozone in the conversion of NO to NO$_2$. In the lower atmosphere (troposphere), oxides of nitrogen are a major contributor to the formation of photochemical smog via a complex set of reactions that lead to the formation of a variety of nitrated organic compounds (from volatile organic matter) and excessive levels of ozone. According to plant scientists, the production of tropospheric ozone is responsible for 90% of the injury to vegetation in North America [37].

### 2.4 Preparation of nitric oxide

The preparation of solutions containing a pre-determined concentration of NO is relatively straightforward but requires careful attention to well established synthesis procedures. In particular, oxygen should be excluded from all solutions during the preparation.

\[ \text{NO}_2^- + \text{H}^+ \rightleftharpoons \text{HNO}_2 \quad (2.2) \]

Nitrous acid is a weak, monobasic acid with a pK$_a$ of 3.16 at 25 °C [39, 40]. It is unstable and decomposes (primarily) to nitric oxide, nitrogen dioxide and water (Equation 2.3). The decomposition of nitrous acid has been studied and the forward and reverse rate constants were determined to be 13.4 and 1.58 $\times$ 10$^8$ mol$^{-1}$ dm$^3$ s$^{-1}$ respectively [41]. In the laboratory, NO can be synthesised by the reduction of nitrous acid, generated in aqueous acid solution from a nitrite salt, usually sodium or potassium nitrite [38].

\[ 2\text{HNO}_2 \rightleftharpoons \text{NO} + \text{NO}_2 + \text{H}_2\text{O} \quad (2.3) \]
Nitric oxide is often prepared via the reduction of nitrous acid by ascorbic acid [42]. The reaction of ascorbic and nitrous acid rapidly produces two moles of nitric oxide per mole of ascorbic acid at pH levels below 2 as shown in Equation 2.4 [20, 43].

\[
\text{HO} + 2\text{HNO}_2 \rightarrow \text{HO} - \text{HNO}_2 + 2\text{NO} + 2\text{H}_2\text{O} \quad (2.4)
\]

2.5 NOx control measures

NOx emissions from exhaust gases, formed during the combustion of fossil fuel used in power plants, vehicles and other incineration processes are the primary anthropogenic sources of NOx emissions in the atmosphere. Approximately 50 percent of emissions are a result of fossil fuel combustion for heating and power generation [37]. Most technologies developed for NOx abatement are in relation to minimising NOx emissions from these processes. These include the adoption of process control optimisation methods and the implementation of novel technologies, such as low NOx burners and staged combustion that can directly reduce NOx level at its source [44], but also secondary measures, like selective non-catalytic reduction (SNCR) [45] and selective catalytic reduction (SCR) [46].

Other sources of NOx emissions include the manufacture of nitric acid and other nitrogenous chemicals [47], electric arc welding process [48], the use of explosives in mining [49] and farm silos [50, 51]. These are considered stationary sources of NOx and together contribute to 30 % of the NOx emissions [52]. Concerns regarding environmental and health issues have resulted in tighter regulations requiring significant
improvement to overall NOx emissions, such as a 60 percent reduction from 2003 emission levels within 10 years [53]. Thus, considerable improvement of currently employed methods, and the development of new technologies, is required to reduce NOx emissions. In the following section, attention is focussed on stationary sources of NOx, in particular chemical processes where nitric acid, nitrates or nitrites are used as reagents, with a view to learn from these technologies and thus develop a research strategy the aim of which is the net reduction of NOx generated during chemical gassing of emulsion explosives.

2.5.1 NOx abatement in chemical industry

NOx emissions from industrial sources accounts for 12 percent of global anthropogenic NOx as illustrated in Figure 2.2 where the primary sources originates from the manufacture or utilisation of nitric acid for nitrification or oxidation of organic compounds such as explosive manufacturing processes. The significance of this NOx source should not be underestimated [54], as in contrast to exhaust gases emitted from combustion processes, nitrous gases produced in this context are from a fixed, point-source location. The amount of NOx released depends on the acid concentration and generally the higher the concentration, the higher the percentage of NOx released. In addition, despite the volume being low compared to combustion processes, the concentration of NOx released is significant [55].
The conventional solution to control NOx emissions from this type of source is the deployment of technologies which enable NOx absorption in alkali solution [56], or adsorption or reduction of nitrogen oxides using hydrogen, methane or ammonia [57]. Since the release of NOx gas involves a mixture of several components, which can include N₂O, NO, NO₂, N₂O₃, N₂O₄ and N₂O₅, the absorption of NOx gas is an extremely complex process [58]. When an alkali solution is employed, the resultant products are nitrites (NO₂⁻) and nitrates (NO₃⁻). However, it is not possible to obtain flue gas in which NOx is absent, as HNO₂ is produced during absorption and converted in the presence of strong acids to nitric acid and nitric oxide, according to Equation 2.5 [59].

\[ 3\text{HNO}_2 \rightarrow \text{HNO}_3 + 2\text{NO} + \text{H}_2\text{O} \]  (2.5)
Oxidising agents such as potassium permanganate, sodium chlorite, sodium dioxide, sodium hypochlorite, hydrogen peroxide, organic hydroperoxide, peracid, ozone, oxone and ferrous chelating agent can also oxidise nitrous oxide into nitrogen compounds such as NO$_2^-$ and NO$_3^-$ [60-64]. Among these reagents, sodium chlorite (NaClO$_2$) was the most effective [65]. Yang et al. studied the absorption of dilute concentrations of NO using NaClO$_2$ [66]. They demonstrated that NO can be quantitatively oxidised to NO$_3^-$ by NaClO$_2$ in an aqueous alkali solution as shown in Equation 2.6.

\[
4\text{NO} + 3\text{ClO}_2^- + 4\text{OH}^- \rightarrow 4\text{NO}_3^- + 3\text{Cl}^- + 2\text{H}_2\text{O} \tag{2.6}
\]

This method of reducing NOx has the capacity of reducing NO emissions as well as improving the efficiency of nitric acid production since the addition of oxidants selectively generates additional quantities of nitric acid [67].

### 2.6 Analytical methods for determining the concentration of nitric oxide

As a result of the recognised importance of NO in metabolic processes, most assays developed to date are related to its measurement in biological systems. This section summarises the various techniques employed to measure NO in chemical and biological systems. These include, inter alia, commonly employed analytical methods such as chemiluminescence, mass spectrometry, electron spin resonance spectrometry and the use of species-specific electrochemical sensors. The underlying principle of the technique, the application, advantages and drawbacks of each technique are also subsequently discussed.
2.6.1 Mass spectrometry

The combination of a quadrupole mass spectrometer with a membrane inlet as a NO measurement technique is based on the diffusion of dissolved gases through a capillary, and the identification of the diffusing gases with a mass spectrometer according to differing mass to charge ratios (m/z). Figure 2.3 shows a schematic of the membrane inlet mass spectrometry. The analyte of interest passes across a membrane into the mass spectrometer three diffusion process steps [68];

1. Selective adsorption of analyte at the membrane surface (gas is adsorbed onto the surface of the membrane)
2. Diffusion through the membrane (analyte enters the membrane)
3. Desorption of the molecules into the vacuum on the other side of the membrane.

![Figure 2.3. Schematic of MIMS system. Adapted from [69]](image)

This technique, developed in the 1960’s by Hoch and Kok, established its effectiveness in the determination of CO₂, O₂ and N₂ in studies of algal and plant physiology [70].
More recently, the membrane inlet mass spectrometry (MIMS) has been widely adopted and has become popular for a wide range of clinical, pharmaceutical, environmental and analytical applications [71]. Lewis et al. were the first to report the detection of NO in aqueous solution, as well as in the gas phase, from mammalian cell cultures via the use of a semi-permeable membrane leading to the ion source of a mass spectrometer [72].

Construction and design modifications to the inlet have been reported in literature [73]. The modification schematic presented in Figure 2.4 uses a membrane inlet probe comprising of a small segment of silicon rubber tubing attached to a glass tube that leads to a mass spectrometer [73]. This configuration has the advantage that, it can be immersed repetitively in a number of different solutions.

![Diagram of the membrane inlet](image)

**Figure 2.4.** Diagram of the membrane inlet for mass spectrometric measurements. Adapted from [73].

Silicone rubber (silastic) is commonly utilised for the MIMS application because of its inertness to many chemical and biological systems. As a general rule, only relatively non-polar, volatile, low molecular-mass organic compounds such as nitric oxide are soluble in and pass through the silicone membrane matrix [72]. In addition, water is
excluded to a significant extent in the apparatus due to its polar nature and thus silastic membrane is suitable for use when detecting compounds under aqueous conditions.

A lower detection limit of 0.5 nmol dm$^{-3}$ nitric oxide was reported by Tu et al. [73] for the MIMS system shown in Figure 2.4. This limit is determined mainly by the properties of the silicon rubber tube and its thickness, as well as permeability of the surface area of the tube exposed to the solution [74, 75]. Although the sensitivity of this assay is comparable to chemiluminescence, the cost and maintenance of a mass spectrometer are limiting factors for the adaption of this technology.

### 2.6.2 Chemiluminescence analyser

The concentration of NO can be determined using a chemiluminescence reaction involving ozone. The reaction leads to the rapid formation of nitrogen dioxide in an excited state, which relaxes to the ground state emitting a photon through chemiluminescence (Equations 2.7 and 2.8) and this photon can be measured using a photo detector.

\[
\text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2 \quad (2.7)
\]

\[
\text{NO}_2^* \rightarrow \text{NO}_2 + \text{h} \nu \quad (2.8)
\]

The amount of light emitted is proportional to the concentration of NO in the sample and calibration of the detector is usually conducted with a commercially available, high purity nitric oxide source of known concentration. The method was originally developed for the determination of nitric oxide as an atmospheric pollutant but due to its high sensitivity, its application has been adopted and applied for the determination of
Chemical trapping NO production in biological systems. The method takes advantage of the low solubility of NO in aqueous solution and is thus commonly used for the analysis of NO released into the gas phase; for example from endothelial cells [76, 77], assays of denitrifying enzymes [77] and photochemical decomposition. Nitric oxide dissolved in aqueous solution can also be measured by displacement of the gas into the head space with an inert gas under vacuum conditions and its direct transfer to a chemiluminescence analyser [27]. Concentrations as low as $10^{-13}$ mol dm$^{-3}$ of NO have been measured using this method (by Zafiriou et al. [78]) with a detection threshold limit of 1-20 picomols of NO [78].

In recent years, a new procedure has been developed (Robinson et al. [79]) whereby chemiluminescence is induced by the reaction of NO to NO$_2$ with an alkaline luminol/H$_2$O$_2$ solution. The replacement of ozone with luminol was found to enhance the sensitivity of the detector by a factor of around 20. However, this new procedure lacks selectively and does not exclusively measure nitric oxide as a result of the luminol reacting with a variety of other species. This new technique is mainly used to visualise the production of NO in cells [80].

### 2.6.3 Electron paramagnetic resonance

Since NO is a paramagnetic molecule containing an unpaired electron, it can be detected and quantified using electron paramagnetic resonance (EPR) spectroscopy. The basic principle of detecting radicals by EPR spectroscopy is long established. Briefly, discrete amounts of energy and magnetic field strength; unpaired electrons are promoted to higher energy levels with subsequent relaxation from this state producing a
characteristic spectrum. NO cannot be studied directly by EPR because its fast spin-orbit relaxation renders the radical EPR undetectable at room temperature, irrespective of the presence of its unpaired electron [81]. Spin traps are thus required to interact with the free radical, producing a stable adduct that can be detected by EPR. The reaction principle of spin trapping is illustrated in Equation 2.9.

$$ R^* + ST \rightarrow R-SA^* \quad (2.9) $$

Where $R^*$ is the radical species (NO), ST is the spin trap and $R-SA^*$ is the spin trap adduct. EPR spin trapping can then provide specific information on the radical, in chemical and biological situations [82]. Different types of spin trap have been used to detect NO by EPR; these include nitroxide spin traps (nitroso and nitrone), NO cheletropic traps (NOCTs), heme protein and metal complexes.

With current EPR spectrometers, the lowest theoretical limit of detection in an aqueous solution is approximately $10^{-9}$ mol dm$^{-3}$. However, considerably higher concentrations in the range of $(0.1-10) \times 10^{-6}$ mol dm$^{-3}$ are required in practice to record and resolve detailed spectra with reasonable signal to noise ratios. The technique has drawbacks, including the need of specialised operator, high capital cost of the spectrometer and the lack of commercially available traps [10].

### 2.6.4 Electrochemical sensors

Electrochemical measurement is a direct method of NO detection which does not rely on intermediate sampling procedures and is capable of analysing relatively low concentrations of NO, in the nanomolar range. It is often described as the most
practical method for measuring NO in biological samples due to its relative simplicity and low cost and small size of electrode. There are two electrochemical methods which are widely available (i) the Clark probe, which was originally developed for the determination of oxygen in solution (detection limit of $10^{-6}$ mol dm$^{-3}$ of NO) [83] and (ii) the polymeric porphyrin electrode (detection limit of $10^{-9}$ mol dm$^{-3}$) [84]. The second method offers distinct advantages over the first as the response time is shorter, it has a very low detection limit and a wide range of linearity exists between the current and nitric oxide concentration. However for the (more typical) level of high oxide requiring measurement ($10^{-3}$–$10^{-5}$ mol dm$^{-3}$), the Clark probe is more suitable, whereas for the measurement of low NO concentration, particularly in small scale biological studies, the use of polymeric porphyrin electrode is the preferred choice.

In the Clark probe configuration, the electrochemical oxidation of nitric oxide occurs on a platinum electrode with a silver wire as the counter-electrode with the system running in amperometric mode. Shibuki was the first to report the measurement of nitric oxide in rat brain tissue using a miniature Clark-type electrode [85]. Amperometric measurements at a potential of 0.9 V showed a linear relationship between measured oxidation current and NO concentration in vitro over the range of $(1-3) \times 10^{-6}$ mol dm$^{-3}$ μM of NO. Overall, electrochemical NO sensors provide a detection limit in the range of 1 nM and a linear range up to $25 \times 10^{-6}$ mol dm$^{-3}$ depending on the design of the electrode. For this technique, fouling represents its main drawback requiring frequent cleaning of the electrode [86].
2.6.5 Fluorometric assay

This technique is based on the reaction of NO with a non-fluorescent compound forming fluorescent nitrosyl adducts. The most common probes are 2,3-diaminonaphtalene (DAN) and 4,5-diaminofluorescein (DAF-2) [87, 88]. DAN was the earliest compound used for NO detection in Fluorometric methods [89]. Although DAN provided a relatively weak fluorescent signal, when it reacts with NO to produce 2,3-naphthotriazole the fluorescence intensity increases by more than a 100-fold. The detection limit of this method is below $5 \times 10^{-8}$ mol dm$^{-3}$. The common pathway for NO quantification with 2,3-diaminonaphthalene (DAN) is illustrated in Figure 2.5.

![Fluorometric assay pathway diagram](image)

**Figure 2.5.** Common pathway for NO quantification with 2,3-diaminonaphthalene (DAN)

Fluorescent chemical transformation of DAFs relies on the reactivity of the aromatic vicinal diamines during the course of NO oxidation, leading to the formation of the highly fluorescent triazolofluorescein DAF-2T [91]. It can afford high sensitivity for
NO, with a detection limit of $5 \times 10^9$ mol dm$^{-3}$. Abe et al. [87] recently developed a fluorometric assay for detection of NO generated from a nitric oxide-releasing drug employing sesamol (3,4-methylenedioxyphenol) as a fluorometric substrate, with a detection limit of $4 \times 10^{-13}$ mol dm$^{-3}$. The sesamol is converted to a fluorescent derivative, which forms a dimer in the presence of NO.

### 2.6.6 Other methods

The methods described subsequently have been applied to the determination of nitric oxide concentration, but are less frequently encountered. The majority of these methods detect different products of the reaction with NO, thus offering an indirect measurement of NO. They are not always specific to the measurement of NO because these compounds could form as a result of other reaction pathways occurring during analysis.

- **Griess reaction** – For indirect measurement of NO by quantifying its stable decomposition products NO$_3^-$ and NO$_2^-$ using UV-Vis spectrophotometer [92, 93].
- **Fourier Transform Infrared (FTIR)** - To measure gaseous pollutants including NO in the atmosphere [94] by identifying N-O stretching vibration.
- **Gas chromatography** – Often requires the use of derivatisation reagents such as pentafluorobenzyl bromide, then enabling measurement of nitrite and nitrate using GC analysis [95].
- **Raman spectrometry** – Monitoring of nitric oxide activity either *in vivo* or *in vitro*, especially as a function of its interaction with hemoglobin or other metalloproteins.
2.7 Nitric oxide in biological systems

NO is a toxic gas but paradoxically it plays an essential role in many biological systems, and is an important component in the control of a myriad of important physiological activities. It functions as an endothelium-derived relaxing factor (EDRF), a vascular antioxidant and as a messenger molecule in the cardiovascular and immune systems [82, 92, 96]. At cellular level, NO is able to stimulate or inhibit the activity of specific enzyme systems either through its signalling properties, oxidation properties or ability to nitrosylate specific amino acids most notably those containing the thiol moiety [97]. To study this molecule, biochemists generally employed spin trapping techniques with EPR [98-100].

2.7.1 Spin trapping technique to develop NO scavengers

Since the employment of the NO spin trap stabilises free radicals and forms stable adducts detectable by EPR to allow NO measurement, NO spin traps can also be employed as NO scavengers. The concept of employing a NO spin trap as a NO scavengers is not new. In the human body, NO despite being an important regulatory molecule controlling many vital physiological functions when produced in excess can lead to some undesirable, deleterious side effects. For instance, NO can possess both injury-inducing and tissue protective properties with tissue injuries being demonstrated by models, in which excessive NO appears to be pathophysiologically significant [97].

The design of drugs which are able to scavenge and remove pathophysiological quantities of NO, while preserving some activity of NO are believed to be essential for normal biological functioning. Rather than preventing its synthesis, it represents an
efficient and alternative strategy for treating NO-mediated diseases. Many NO scavengers developed, such as haemoglobin, nitroxyl nitrooxide, dithiocarbamates and ruthenium containing compounds, were originally used as spin-trapping probes, in order to demonstrate NO production both in vitro and in vivo [97, 101]. Nitronyl nitroxide carboxy-derivative of the imidazolineoxyl N-oxide compound (PTIO), originally developed for the spin labelling technique for small molecules [102, 103] also acts as an NO scavenger in both in vitro and in vivo systems [104].

Considering this, we can exploit the ESR spin trapping technique to develop NOx scavengers as possible technology for NOx abatement to ultimately control NOx emission from industrial sources. Indeed, this is the overall objective of the present investigation.

### 2.7.2 Different types of spin traps

A variety of spin traps have been developed by biochemists to measure NO by EPR. The spin traps which have successfully been employed to detect NO are described subsequently.

#### 2.7.2.1 Metal complexes

The most reliable approached to study NO in biochemistry is to use metal complexes [82]; in particular Fe^{2+} complexes, which are extensively used to trap NO. Nitric oxide rapidly binds to Fe^{2+} complexes, to form a relatively stable iron-nitrosyl complex (Equation 2.10). Examples of such metal complex are diethylthiocarbamate (DETC) and ethylenediaminetetraacetic acid (EDTA). Aqueous Fe^{2+} (EDTA) solution have also
been used in flue gas treatment for NO\textsubscript{X} removal due to its high adsorption efficiencies for NO. In an almost instantaneous reaction, the nitrosyl complex is formed according to Reaction 2.10 with an equilibrium constant, $K_C$, of $2 \times 10^6$ mol\textsuperscript{-1} dm\textsuperscript{3} [105].

$$\left[\text{Fe}^{2+} (\text{EDTA})\right]^2^- + \text{NO} \rightleftharpoons \left[\text{Fe}^{2+}(\text{EDTA})(\text{NO})\right]^2^- \quad (2.10)$$

### 2.7.2.2 Nitronyl nitrooxide

Nitronyl nitroxides were first synthesised as molecular spin labels by Ulmann et al. [103] and were observed to convert nitrous acid and nitric oxide to a corresponding imino nitroxide and nitrogen dioxide. Nitronyl nitroxides are organic compounds having both nitrone (functional group corresponding to a spin trap) and nitroxide (functional group corresponding to a spin label) moieties [81]. Examples of nitronyl nitroxide employed as NO scavengers are 2-phenyl-4,4,5,5-tetramethylimidazolineoxyl-1-oxyl-3-oxide (PTIO) and its water soluble derivative carboxy-PTIO. These two compounds are widely used as NO scavengers and have been increasingly used in the field of NO related studies, despite the specificity of carboxy-PTIO as NO scavengers being questioned by Pfeiffer et al., 1997 [106]. PTIO was also applied to determine NO concentrations in polluted atmospheres as a solid, stable indicator when exposed to air in a canister [107].

According to Akaike et al., the reaction of PTIO with NO proceeds in a stoichiometric manner, yielding 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl (PTI) at a ratio of 1:1 in an oxygen-free solution according to Equation 2.11 [104, 108].
However Hoggs et al. employed a kinetic model to determine that, the ratio of 1:1 is only valid for rates of NO generation $< 10^{-13}$ mol dm$^{-3}$ s$^{-1}$, whereas at higher NO generation rates, 2 equivalents of NO are consumed by 1 equivalent of NNO [109].

NNO and INO are both nitroxides which can be detected and distinguished by ESR spectrometry, for which it is possible to use the reaction as a tool for detecting NO in chemical and biological systems. The advantage of these compounds is that, they have high efficiency of trapping NO, most are crystalline and water soluble and do not react with nitrite or NO$_2$.

2.7.2.3  Nitric oxide cheletropic traps (NOCT’s)

Nitric oxide cheletropic traps (NOCT’s) are an additional class of molecules that have been specifically designed to trap nitric oxide by means of formal cheletropic reactions. It involves the reaction of reactive $o$-quinodi-methane derivatives with nitric oxide to yield a stable cyclic nitroxide radical that can be readily detected by EPR spectroscopy.
The traps are insensitive to the presence of oxygen, superoxide, solvent polarity, pH and the ionic forms of nitrogen oxides but they are insoluble in water and their resultant nitroxides are thermally unstable [111-113]. Lately, in order to circumvent the problem with the unstable nitrooxide radical, a fluorescence nitric oxide cheletropic trap (FNOCT) was developed whereby a fluorophore functionality was incorporated to detect and quantify nitric oxide [111, 114]. In a typical FNOCT reaction with NO, a phenanthrene-based $\sigma$-quinodimethane system is employed to produce a fluophoric phenanthrene moiety on reaction with NO.

2.7.2.4 Aci-+nitromethane (aci NM)

In alkaline solution, nitroalkane (RCH$_2$NO$_2$) can undergo ionisation and rearrange to an aci anion RHC=NO$_2^{-}$. This base-catalysed nitro to aci transformation renders the nitroalkane useful as spin trap, according to Equation 2.12 and 2.13:

\[
RCH_2NO_2 \xrightarrow{-\text{H}^+} RCH=NO_2^- \quad (2.12)
\]

\[
RCH=NO_2^- + \text{radical} \rightarrow \text{radical} - \text{CHRNO}_2^\cdot \quad (2.13)
\]
Reszka et al. demonstrated that aci NM may be useful in the detection of nitric oxide and as well nitrogen dioxide species [115]. When NO is exposed to aci NM in a strong base such as 0.5 mol dm\(^{-3}\) NaOH (pH > 10), a distinctive EPR spectrum can be obtained, allowing the unambiguous identification and measurement of the species. In a more recent study Reszka et al. [96] showed that in alkaline solutions, nitroalkanes of the general composition RCH\(_2\)NO\(_2\) (e.g. nitroalkane, nitroethane, nitropronane) can function as NO-specific spin traps, producing persistent spin adducts which can be readily detected by EPR spectroscopy. However, aci anion is only useful when applied in strong base, since at a lower pH, the concentration of the aci anion may be too low for efficient trapping or the resulting spin adducts mono-anion radical are unstable.

2.7.2.5 Classic nitroso spin traps

C-Nitroso compounds are a class of spin trap, largely used in biological systems. These compounds have an –NO group which binds directly to carbon. Trapping of radical R’ leads to the formation of a stable nitroxide as per Equation 2.14:

\[
\text{R’} + \text{RN}≡\text{O} \rightarrow \text{N}−\text{O}
\]  

(2.14)

The addition of the spin trap molecule unambiguously proceeds via the trapping the radicals with their localised unpaired electron. Mackor et al. were the first to report the use of these compounds as radical scavengers for an alkyl radical [116]. Nevertheless, they did not suggest the use of C-nitroso compounds as free radical scavenger for more universal application.
An important disadvantage of nitroso compounds is their propensity to dimerise. Most nitroso compounds are isolated as crystalline dimers, which dissociate in solution to the coloured monomers which only then can act as free radical scavengers [117]. In some circumstances, such as for 2-methyl-2-nitroso-3-butane, UV radiation is required to dissociate the dimer to the active monomeric form [118]. Accordingly, an understanding of the dimer-monomer equilibrium is important and a prerequisite for kinetic studies of spin trapping processes.

- **Aliphatic v/s aromatic nitroso spin traps**

Both aliphatic and aromatic nitroso compounds are commonly used as spin traps. Typical examples include 2-methyl-2-nitrosopropane (MNP), nitrosobenzene and 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS). While most of aliphatic nitroso compounds are very reactive, they are toxic and are thermally and photochemically unstable with the life time of their oxygen-centred radical adducts often exceedingly short. In addition to being insoluble in water, MNP decomposes under the influence of visible radiation and on thermal heating (50 °C in dark) to decompose to give di-\(t\)-butyl nitroxide (DTBN) [119].

\[
\text{Bu}^t\text{N}=\text{O} \xrightarrow{\text{hv}} \text{NO}^- + \text{Bu}^t\xrightarrow{\text{MNP}} \text{BU}_2\text{NO}^- \quad (2.15)
\]

In order to circumvent many of the problems associated with aliphtic nitroso spin traps, such as MNP, aromatic nitroso spin traps, such as DBNBS, have been developed and are widely used. The chemical structure of DBNBS is shown in Figure 2.7. Although DBNBS readily dimerises, there is sufficient monomer, even at low concentrations of the trap, to produce a spin adduct. This nitroso spin trap is insensitive to visible light.
and displays good thermal stability [120]. As a result, it does not react to produce nitroxides on exposure to light or with minor excursions in temperature. Saturated solutions of DBNBS are pale green, and a more pronounced blue green is obtained on gentle heating. The addition of a sulfonate group to the benzene ring of a nitrosobenzene derivative improves the solubility of DBNBS in aqueous media and at the same time renders it insoluble in non-polar solvents [120, 121]. DBNBS has solubility around 100 g L\(^{-1}\) in water at 20 °C, though remaining substantially in its dimeric form [122].

![Figure 2.7. The chemical structure of the sodium salt of DBNBS](image)

- **Scavenging of NO by DBNBS**

With respect to the efficacy of trapping of NO by DBNBS, there has been considerable debate and difference of opinion among biochemists [119, 123-126]. In a study undertaken by Arroyo and Khono, where a range of nitrone and nitroso compounds were investigated, only 3,5-dibromo-4-nitrosobenzene sulfonate was demonstrated as being capable of spin trapping NO [123]. Pou and co-workers dismissed the process of NO trapped by DBNBS as being an experimental artifact, which produced misleading ESR spectra under a variety of experimental conditions [119, 126, 127]. In their study,
they employed $^{15}$NO with a nuclear spin of 0.5 in place of $^{14}$NO in order to compare the ESR spectra obtained with the $^{15}$NO/$^{14}$NO labelled DBNBS spin adducts, which should differ significantly. Since they could observe only a relative decrease in amplitude and no significant difference in the ESR pattern, they concluded that the 0.96 mT triplet observed was a result of the nitrogen of the DBNBS molecule and not from the nitrogen of the nitric oxide. In contrast, Nazhat et al. disagreed with the conclusion of Pou et al. and demonstrated that a novel, well defined radical having a characteristic ESR spectrum with hyperfine splitting of 0.96 mT is formed directly from the reaction NO with DBNBS and concluded that this was not an experimental artifact [125].

Ichimori et al. also studied the reaction of DBNBS with NO. They suggested that, an unstable adduct is produced upon reaction, and decomposes readily in aqueous solution to form a dimerised product [124]. Davies et al. also examined the reaction between DBNBS and NO under aqueous conditions. They employed high performance liquid chromatography (HPLC) and fast atom bombardment mass spectrometry (FAB-MS) and characterised the radical product as a monosodium electrostatic complex with the dianion bis (2,6-dibromo-4-sulfophenyl) nitroxyl. Their study also demonstrated that a significant amount of nitrogen gas and nitrate was formed during the reaction [128]. They suggested that, the following mechanistic scheme was occurring during reaction:

1. DBNBS reacts with NO to form an unstable DBNBS-NO adduct.
2. The formed adduct undergoes cyclisation.
3. The cyclic product decomposes to form diazonium salt and releases either oxygen or superoxide.
4. The diazonium salt decomposes, releasing nitrogen and producing a phenyl radical.
5. The phenyl radical is trapped by another DBNBS molecule to form the dianion, bis(2,6-dibromo-4-sulfophenyl) nitroxyl.

2.7.3 Selection of NO spin traps as potential NO scavenger

One of the important characteristics of the NO traps to be considered for application during the sensitisation of emulsion explosive is its ability to scavenge the generated nitric oxide, without changing the properties of the ammonium nitrate emulsion explosive or the rate of chemical gassing. In addition, the reaction of the trap with nitric oxide should not be associated with the formation of significant quantities of undesirable or noxious side products, and its presence should not alter the pH of the system which is necessary for the chemical gassing process to occur.

The deployment of metal complexes, such as iron complexes of ethylenediaminetetraacetic acid (EDTA) seems to be an attractive option for the scavenging of NO. The large equilibrium constant for the formation of nitrosyl complexes from EDTA will result in the complete scavenging of nitric oxide formed during the gassing of emulsion explosive. However, this technology offers several drawbacks that would need to be circumvented before it could be considered for application in emulsion explosive formulations, including the probable oxidation of the Fe$^{2+}$ (EDTA) complexes by oxygen and nitrite from the gasser (NaNO$_2$). In addition, the presence of a transition metal such as Fe$^{2+}$ can catalyse the decomposition of ammonium nitrate and thus greatly decrease the thermal stability of the emulsion explosive [129] and could result in the formation of N$_2$O would undermine the purpose of using the spin trap.
Likewise, nitronyl nitroxide, in particular 2-(4-carboxyphenyl)-4,4,5,5-
tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), offers the advantage of being
highly efficient in trapping NO, with a rate of approximately $10^4$ mol$^{-1}$ dm$^3$ s$^{-1}$ at room
temperature [82]. However, these compounds are rapidly reduced by ascorbate and
 glutathione and a range of other reducing agents [130]. It is also only partially effective
in acidic media, which is the condition employed for gassing of emulsion explosive,
since the $pK_a$ of C-PTIO is 3.5 [131]. In addition to representing a costly option in the
control of NO emissions for application in large scale emulsion explosive system, it is
only available in milligram quantities.

The applicability of fluorescence nitric oxide cheletropic trap (FNOCT) as a potential
NO trap in emulsion explosive is very attractive. In an early stage of the project, it was
proposed that FNOCT could be applied to emulsion systems as its mode of operation is
pH independent and the compound is water soluble [132]. However, since FNOCT
requires several complicated steps in the synthesis process as shown in Figure 2.8, its
use for this study was not continued.

![Figure 2.8. Scheme for the synthesis of fluorescence nitric oxide cheletropic trap [132]](image-url)
With respect to aci anions of nitroalkane which may function as a NO spin trap, converting nitroalkane into a useful adduct for the detection of NO as well as NO$_2$ is attractive and innovative. However, the reaction is base catalysed (pH > 10), and, under acidic conditions, the nitroalkane undergoes ionisation and rearrangement to a tautomeric aci form. This requirement is unfavourable during the gassing of the emulsion as the pH during gassing must be below 3.5.

In the present study, nitroso DBNBS has been identified to be attractive for use in emulsion explosive system. Numerous reports [124, 125, 128, 133, 134] have concluded that NO either dissolved in a buffer or generated in situ can be trapped by DBNBS. Despite reports of the compound existing in a monomer-dimer equilibrium with the monomer being the active form for trapping NO, even in dilute solutions there is sufficient monomer present to give spin adduct. The compound is reasonably soluble, approximately 100 g L$^{-1}$ in water at 20 °C, ensuring its addition in the gassing reaction and ensuring homogeneity in the emulsion explosive. In addition, with a pKa of -2.2 ± 0.5 [135], its presence will not inhibit the sensitisation of an emulsion explosive system.
2.8 Summary

There are currently many studies being undertaken to reduce global NOx emissions, most of which are related to combustion processes. However with respect to NOx formed in industrial chemical processes and more specifically during the sensitisation of emulsion explosives via chemical gassing process, existing technologies must be improved and new methods developed to handle the problem of NOx emission from these sources.

One approach to overcome the problem of NOx emissions from the chemical sensitisation of emulsion explosive is to employ NO spin traps. These traps, originally developed by biochemists for the detection of NO radical via the technique of electron paramagnetic resonance spectroscopy (EPR), were exploited as NO scavengers in drugs to reduce and control pathophysiological quantities of NO in the human body. Likewise, NO spin trap particularly nitroso 3,5 dibromo-4-nitrosobenzene sulfonate can be applied during the chemical gassing of emulsion explosive to minimise the exposure of the toxic NO gas in confined spaces.
2.9 References


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Chapter 2: Literature review


Chapter 2: Literature review


CHAPTER 3: METHODOLOGY
The primary purpose of this chapter is to present a description of the chemicals, apparatus and main experimental methods underpinning this research project. A methodology section, briefly describing the methods and apparatus utilised within the study will also be enclosed in subsequent chapters, but are elaborated on to a greater extent in the present chapter. However, the methods related to the determination of acid dissociation constant of nitrosobenzene sulfonate and its methyl ortho substituted compound and utilisation of the NALDI-MS and are presented in Chapter 6 and Appendix B respectively. In addition, the apparatus and methods employed for investigating the nitroso traps with ammonium nitrate emulsion explosive are described in detail in Chapter 9.

### 3.1 Chemicals

#### 3.1.1 Nitroso spin trap as an NO scavenger

In the literature review, several NO spin traps were identified as potential NO scavengers for use in the process of chemical gassing of ammonium nitrate emulsion explosive. Nitrosobenzene sulfonate 3,5-dibromo compound was selected for the study due to its established propensity to scavenge NO either dissolved in buffer or produced in situ. In addition, being an electrolyte and relatively unreactive to species other than NO, its presence will not interfere nor alter the pH conditions of the chemical gassing process, which proceeds in the low pH range (pH < 4). We also extended the study to determine if para substituted nitroso aromatic compounds have the capacity to reduce NO formed in aqueous solutions. Since the function of the sulfonate group on the aromatic ring in DBNBS is to increase the water solubility, we subsequently
investigated three other analogues of DBNBS compound listed in Table 3.1 with view to applications in aqueous systems.

Table 3.1. List of aromatic nitroso sulfonates used in this study

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Abbreviation</th>
<th>Structural formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-dibromo-4-nitrosobenzene sulfonate</td>
<td>DBNBS</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
<tr>
<td>nitrosobenzene sulfonate</td>
<td>NBS</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
<tr>
<td>3,5-dimethyl-4-nitrosobenzene sulfonate</td>
<td>DMNBS</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
<tr>
<td>3,5-dichloro-4-nitrosobenzene sulfonate</td>
<td>DCNBS</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
</tbody>
</table>

### 3.1.2 Synthesis of nitrosobenzene sulfonate

The initial section of the research involved the synthesis of the aromatic nitroso sulfonate compounds, and their characterisation. The synthesis process was necessary, as these compounds are not available for direct purchase; for example, DBNBS which
was previously available from Sigma Aldrich, has been discontinued due to insufficient demand. In addition, there is considerable literature which recommends the synthesis of DBNBS due to a significant quantities of commercially available DBNBS which have a surprising low content of authentic DBNBS and are thus highly contaminated with unknown species [1]. With respect to the other aromatic nitroso sulfonate compounds, none of these are available for purchase. Standard synthesis procedures, adopted from literature, are described in Appendix A.

![Chemical structure of aromatic nitrosobenzene sulfonate compounds](image)

Figure 3.1. Chemical structure of aromatic nitrosobenzene sulfonate compounds with aromatic carbon numbered.

### 3.1.2.1 3,5-dibromo-4-nitrosobenzene sulfonates sodium salt (DBNBS)

Initially, two batches of DBNBS were prepared according to the method reported by Kaur et al. [2]. Concurrently, 10 other batches of DBNBS were prepared, each with a minor modifications to the original synthesis technique, was undertaken in order to improve the process in terms of yield and reaction time. 3,5-dibromosulfanilic acid (3.53g, 10 mmol dm$^{-3}$) was added to a mixture of glacial acetic acid (30 cm$^3$), 30 % aqueous hydrogen peroxide solution (7.9 cm$^3$, 78 mmol dm$^{-3}$) and anhydrous sodium acetate (0.82 g, 10 mmol dm$^{-3}$). A volume of 0.6 cm$^3$ of catalytic concentrated sulfuric
acid was then added after gently heating the solution at 40 °C to reduce the reaction time. A solid product from the crystallisation formed readily after standing at room temperature under a fume hood for two days instead of 14 days. The solution was recrystallised to reduce the likelihood of the presence of insoluble matters and impurities and any undissolved solids were removed by hot filtration. The recrystallised solution was once more allowed to stand for two days at room temperature until solids appeared and were separated by vacuum filtration. The solids were washed sequentially 3 times with glacial acetic acid (10 cm$^3$), ethanol (10 cm$^3$) and a dry mixture of ether/dioxane (1:1) (10 cm$^3$) followed by ethanol (10 cm$^3$) and subsequently dried for 12 h under vacuum. In addition, the mother liquor that remained after filtration was kept and diluted with 3 cm$^3$ of glacial acetic acid. The mother liquor was allowed to stand at room temperature for another two days.

3.2.1.2 *Tetrabutylammonium 4-nitrosobenzenesulfonate (NBS)*

4-nitrosobenzene sulfonate was prepared according to the procedure by Priewisch and Rück-Braun [3]. Tetrabutylammonium sulfanilate was dissolved in 20 cm$^3$ DCM and the mixture oxidised using 3.51 mmol dm$^{-3}$ of oxone solution. (Procedure for the preparation of tetrabutylammonium sulfanilate is found in Appendix A). The mixture was stirred under nitrogen for 0.5 h. After separation of the layers, the aqueous layer was extracted twice with DCM. The combined organic layers were washed with 1 mol dm$^{-3}$ HCl, saturated sodium bicarbonate solution, water, brine and dried (magnesium sulfate). Removal of the solvent by vacuum evaporation yielded 720 ± 10 mg of the compound as a dark green liquid.
3.2.1.3 3,5-dimethyl-4-nitrosobenzene sulfonate sodium salt (DMNBS)

The compound was synthesised in three steps using the method by Hamilton et al. [4]. 3,5-dimethylsulfanillic acid was first prepared by heating a mixture of freshly distilled 2,6-dimethylaniline and concentrated sulphuric acid to 170 °C for 5 h. Following this, the resultant product was then converted to its sodium salt via the dropwise addition of sodium hydroxide and refluxing for 1 h. The nitroso product was finally obtained as a pale yellow solid from the oxidation with hydrogen peroxide in the presence of glacial acetic acid and sodium acetate. We prepared three batches of this compound.

3.2.1.4 3,5-dichloro-4-nitrosobenzene sulfonate sodium salt (DCNBS)

DCNBS was synthesised from 2,6-dichloroanaline, according the method described by Hamilton et al. in two steps [4]. 2,6-dichloro-sulfanilic acid was obtained by reacting 2,6-dichloroaniline with fuming sulphuric acid under nitrogen and heated at 170 °C for 5 h. The compound was then oxidised with 30 % hydrogen peroxide in the presence of glacial acetic acid and sodium acetate. The resulting solution was left to stand for 14 days at room temperature after which a portion was removed by evaporation until solid was observed. The reaction mixture was then left to stand overnight at 4 °C to finally yield after filtration and purification, 3,5-dichloro-4-nitrosobenzene sulphonate sodium salt as a cream powder. Three batches were prepared for this compound.
3.1.3 Characterisation of nitrosobenzene sulfonate compounds

Characterisation of the synthesised aromatic nitroso sulfonate compounds was performed by $^1$H and $^{13}$C-NMR, UV-Vis, infrared and NALDI MS. Spectra obtained from these techniques are annexed in Appendix A for reference.

3.1.3.1 $H$-NMR and $C$-NMR

$^1$H and $^{13}$C-NMR spectra were recorded on a Bruker Avance DPX-300 spectrometer. Samples were prepared by dissolving approximately 20 mg of the synthesised compound in 0.7 cm$^3$ D$_2$O and spectra were recorded as D$_2$O solutions and ethanol was used as the internal standard for chemical shifts ($^1$H and $^{13}$C 0.0 ppm). The chemical structure in Figure 3.1 shows the numbering of the different carbon atoms in aromatic nitroso sulfonate compounds.

3.1.3.2 UV-Vis spectroscopy

UV-Vis analysis was carried out on the different batches of the synthesised DBNBS. Samples containing 0.1 mmol dm$^{-3}$ of the nitroso compound were placed in 1 cm quartz cuvette and analysed on a Varian Cary 50Scan UV-Visible spectrophotometer. Spectra were corrected for the absorbance of solvent.
3.1.3.3 **IR spectroscopy**

IR spectra of the synthesised compound were recorded on a PerkinElmer Spectrum BX FT-IR spectrometer. The samples were grounded in a nujol mull (paraffin oil) and the IR spectrum was taken (with a background spectrum subtracted).

3.1.3.4 **Nanostructured assisted laser desorption ionisation mass spectroscopy (NALDI-MS)**

For the NALDI-MS analysis, no sample preparation was required. Aliquots of the samples (5 μL) were deposited directly on the surface of the NALDI target (Bruker Daltonics Australia) and allowed to dry at room temperature in ambient air. MS and MS/MS spectra were acquired in negative reflector mode using a Bruker Daltonics Ultraflex III MALDI time-of-flight mass spectrometer. MS data were obtained in the m/z range between 0 and 800 Da by averaging signals from 2500 laser shots using target random-walk movement. Prior to each data acquisition, the target was externally calibrated with elemental sulfur standard (Sigma Aldrich) and a blank spectrum of the NALDI target was recorded prior to analysis of samples. The FlexAnalysis 3.0 software (Bruker Daltonics) was used to analyse the data.

The detailed methodology employed for characterising aromatic nitroso sulfonate compounds by NALDI is provided in Appendix B.
3.1.3.5  Characterisation data

Table 3.3 provides the data for the characterisation of aromatic nitroso sulfonate compounds. The batches prepared using our modified procedure \((n=10)\) contained the four peaks of DBNBS compound in the \(^{13}\text{C}\)-NMR spectra and were within the same chemical shift. Similarly, the batches synthesised strictly according to Kaur et al.’s method also had the four peaks within the same chemical shift. As shown in Table 3.2, addition of catalytic concentrated sulfuric acid accelerated the reaction from 14 days to 2 days and did not affect the composition of the product. The result disagrees with the recommendation of Hamilton et al. \([1]\) which stated that manufacturers should strictly adhere to the procedure prescribed by Kaur et al. Thus, addition of concentrated sulfuric acid is strongly recommended for the synthesis of DBNBS, since it saves time in the synthesis process. Besides, with the minor modifications performed, the yield was improved to 62 % by harvesting two crops of DBNBS crystals.

<table>
<thead>
<tr>
<th>Synthesis type</th>
<th>Chemical shift of (^{13}\text{C})-NMR peak (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>110-120 ppm</td>
</tr>
<tr>
<td>A((n=2))</td>
<td>119.06 ± 0.05</td>
</tr>
<tr>
<td>B ((n=10))</td>
<td>119.02 ± 0.08</td>
</tr>
</tbody>
</table>
Chapter 3: Methodology

### Table 3.3. Characterisation data for the synthesis of aromatic nitroso sulfonate compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>DBNBS</th>
<th>NBS</th>
<th>DMNBS</th>
<th>DCNBS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yield (%)</strong></td>
<td>62</td>
<td>97</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td><strong>$^{13}$C-NMR (ppm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>119.06 (C-2 and C-6)</td>
<td>123.18 (C-2 and C-6)</td>
<td>17.96 (2 x CH3)</td>
<td>127.16 (C-2 and C-6)</td>
<td></td>
</tr>
<tr>
<td>133.98 (C-3 and C-5)</td>
<td>127.35 (C-3 and C-5)</td>
<td>126.59 (C-2 and C-6)</td>
<td>133.83 (C-3 and C-5)</td>
<td></td>
</tr>
<tr>
<td>143.93 (C-1)</td>
<td>147.92 (C-1)</td>
<td>134.38 (C-3 and C-5)</td>
<td>137.63 (C-1)</td>
<td></td>
</tr>
<tr>
<td>150.60 (C-4)</td>
<td>153.04 (C-4)</td>
<td>141.74 ppm (C-1)</td>
<td>147.91 (C-4)</td>
<td></td>
</tr>
<tr>
<td>Tetrabutylammonium salt: 58.762, 23.93, 19.65, 13.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>$^1$H-NMR (ppm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.17 (2-H and 6-H).</td>
<td>-</td>
<td>2.43 (2 x CH3)</td>
<td>8.08 (2-H and 6-H)</td>
<td></td>
</tr>
<tr>
<td>7.68 (2-H and 6-H).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IR (cm$^{-1}$)</strong></td>
<td>1554</td>
<td>-</td>
<td>1264</td>
<td>1295</td>
</tr>
<tr>
<td>(nitroso stretching band)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1283 (aromatic C-nitroso trans dimer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>UV-Vis ($\lambda_{max}$) (nm)</strong></td>
<td>308</td>
<td>281</td>
<td>230 and 303</td>
<td>295</td>
</tr>
<tr>
<td><strong>NALDI-MS (a.m.u)</strong></td>
<td>342:344:346 (1:2:1)</td>
<td>186</td>
<td>214</td>
<td>254,256,258 (9:6:1)</td>
</tr>
<tr>
<td>(negative mode)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For NBS, CDCl$_3$ was employed as solvent
3.1.4 Stability of the aromatic nitroso compounds

The aromatic nitroso compounds were generally stable at room temperature and under standard laboratory storage conditions with the exception of NBS. When stored at room temperature, NBS would transform from a dark green to yellowish green after several days, disclosing its decomposition/oxidation under standard conditions. It was also observed that, when the compound was stored below -6 °C in a freezer, the signs of decomposition occurred more slowly, notable after 2 months of storage. However, as a precaution and in order to obtain reproducible and accurate analytical results, freshly prepared NBS was always employed in the experiments.

Aromatic nitroso sulfonate compounds are thermally stable, with melting point > 300 °C with the exception of NBS due to the presence of the tetrabutyl ammonium salt with a melting point of 86 °C. Figure 3.2 shows the UV-Vis spectra of NBS as a result of heating up to 100 °C. The shift in the $\lambda_{\text{max}}$ from 281 nm to 270 nm demonstrates that, decomposition occurred when NBS was heated to 100 °C. In contrast, heating the compound up to 80 °C (below the melting point) did not cause any alteration in the UV-Vis spectra. (Spectra shown in Appendix A)
3.2 Experimental apparatus

Figure 3.3 illustrates the schematic diagram of the experimental apparatus, which consists of a 2 neck 10 cm$^3$ round bottom glass flask reactor (Ace Glassware) submerged in a temperature controlled water bath. A small volume reactor was chosen to collect well defined kinetic measurements and to save cost on chemicals. When completely filled, the 10 cm$^3$ reactor can afford a volume of 17 cm$^3$ of solution. A magnetic bar was placed inside the reactor to ensure uniform mixing of reagents during the experiments. The reactor was equipped with an inlet and outlet port made of 1/16” stainless steel tubing for the flow of inert nitrogen or argon gas that will be either used to purge the system prior to starting experiment or as a carrier gas during the experiment. A hypodermic needle was inserted into the septum port for periodic

Figure 3.2. Effect of heating of nitrosobenzene sulfonate to 100 °C
aqueous sampling for ion chromatography analysis or for the injection of reagents to trigger reaction. Depending on the analytical measurement required, the experimental setup was modified. A membrane inlet probe, connected to a quadrupole mass spectrometer, was immersed into the aqueous solution for the detection of gases in particular for detecting NO and N₂ gas (shown in Figure 3.3(a)). This sampling method is similar to those described by Lewis et al. and Tu et al. [5, 6] and offers a direct, simple and continuous measurement of nitric oxide and other gaseous components in aqueous systems simultaneously. A 3 L Tedlar gas sample bag was also attached at the outlet exhaust for collection of the mixture of gas for further analysis on a FTIR.

The measurement of nitric oxide for determining kinetics was achieved by adopting the well-established chemiluminescence method, with continuous sampling by diffusing NO through the silastic membrane. The setup was changed in order to accommodate an inlet and outlet with a 30 mm length of membrane tubing attached between them with the outflow going to a chemiluminescence NOx analyser as shown in Figure 3.3(b). This setup was employed for the measurement of the kinetics of both in situ and ex situ trapping.
Chemical trapping of NO

Figure 3.3. Schematics of experimental apparatus used to study the trapping of NO by aromatic nitroso sulfonate compounds. (a) Setup employed for membrane inlet mass spectrometry and (b) setup employed for membrane NOx analyser.
Chapter 3: Methodology

3.3 Methods

3.3.1 Generation of NO saturated solutions

Saturated NO was prepared in a reactor via reduction of nitrite using an acidified solution of ascorbic acid. This method is very well known to generate NO very rapidly and has been reported in literature [7]. Figure 3.4 shows the experimental setup for the preparation of saturated solution of NO. The apparatus employed to generate the NO solutions consisted of a 50 cm³ reaction flask, with the inlet connected to a supply of high purity nitrogen. The reactor was charged with 30 cm³ of a solution containing around 0.375 mol dm⁻³ ascorbic acid and 0.75 mol dm⁻³ HClO₄ prior to purging the system of air using N₂ at a flow rate of 1.67 cm³ s⁻¹. The N₂ purge was stopped after 30 min and a steady flow of NO initiated by adding a 25 % solution of sodium nitrite dropwise to the reactor via a syringe inserted through a rubber stopper. The NO was passed through a 2 mol dm⁻³ NaOH scrubber to remove trace quantities of NO₂ that might be formed in the first reactor. An empty vessel was also placed after the NaOH scrubber, to collect the entrained NaOH or nitrite from hydrolysis of NO₂. The NO was then bubbled in the target solution (distilled water) contained within a 10 cm³ reaction flask which was itself place in a water bath at the desired temperature. Any excess NO was then oxidised by passing in a final scrubber containing acidified potassium permanganate to prevent the excess NO escaping to the atmosphere. The NO was allowed to bubble through the solution for a period of 20 min which corresponds to 700 cm³ of NO produced at an average of 35 cm³ min⁻¹. After half of the NO had been produced, the NO solution was collected via 5 cm³ luer lock gas tight syringe for analysis.
3.3.2 Trapping reaction

In this study, the suitability of aromatic nitroso sulfonate compounds as NO traps were evaluated for both in situ and ex situ reaction with NO, despite in situ trapping being of more practical significant since it stimulates the conditions of the chemical gassing process in emulsion explosives. An in situ trapping means the trapping reaction was performed in the same reactor that generated the nitric oxide whereas ex situ trapping signifies that the reaction of the trap with NO occurred in a reactor different to where the NO was generated. However, as it will be further described, we also considered the situation when NO measurements were required for the determination of the kinetics of the ex situ trapping. In this particular situation, where NO was rapidly generated via the nitrosation of ascorbic acid and the solutions of nitroso compounds were then added after the NO level reached a maximum, the reaction was considered to be an ex situ trapping reaction although trapping was performed in the same reactor as the NO was generated.

Figure 3.4. Experimental setup for ex situ preparation of NO saturated solution.
3.3.2.1  **In situ trapping of NO / preparation of reaction mixture**

The reactor shown in Figure 3.3 was filled with nitroso compound solutions acidified with 0.1 mol dm$^{-3}$ acetic acid and flushed with nitrogen for 15 min, and then 1 cm$^3$ of 0.125 mol dm$^{-3}$ sodium nitrite solution was injected via a syringe into the reaction vessel to initiate the generation of NO. The acetic acid provided protons for the nitrosation reaction but also upon protonation with sodium nitrite formed sodium acetate which acts as a buffer in the system maintaining an almost constant pH throughout the duration of the reaction. A typical plot of the pH change during the in-situ trapping of NO is annexed in Appendix A. The final dilute nitrite concentration was 0.015 mol dm$^{-3}$, following the addition of nitrite to the reaction medium. The reaction was allowed to proceed for 1 h and when reaction mixture was required for further analysis, the pH was adjusted to 5.5 by dropwise addition of a 0.1 mol dm$^{-3}$ NaOH solution which was used to stop the decomposition of nitrous acid and quench any further reaction.

3.3.2.2  **Ex situ trapping of NO**

*Preparation of reaction product from the reaction of nitroso with NO in water*

NO was bubbled through aromatic nitroso solutions in the setup described in Section 3.3.1, where the reactor containing water was replaced by the nitroso solutions. Prior to commencing an experiment, the apparatus was degassed for 30 min by nitrogen gas at a flow of 1.67 cm$^3$ s$^{-1}$. When the nitrite was injected in the first reactor, the flow was reduced to 0.5 cm$^3$ s$^{-1}$ and the reaction was allowed to proceed for 1 h. Following the reaction, the flow of nitrogen was increased back to 1.67 cm$^3$ s$^{-1}$ for 1 h to eliminate all
NO in the reactors and subsequently to prevent formation of nitrogen dioxide if NO was in contact with atmospheric oxygen.

- **Preparation for kinetics measurements**

NO was initially generated via the rapid nitrosation of ascorbic acid. The reactor was charged such that it was completely filled with 0.3 mmol dm\(^{-3}\) of ascorbic acid acidified with 10 mmol dm\(^{-3}\) HCl.

### 3.3.3 Gaseous analysis

#### 3.3.3.1 Membrane inlet mass spectrometer

- **Design of the membrane probe**

The inlet membrane probe comprised a 10 mm length of silastic tubing of 1.5 mm ID and 2.0 mm OD (Speciality Manufacturing Inc, Saginaw, MI) which was sealed at one end with silicone sealant (Dow Corning, US) and attached to the other end to a 70 mm length of stainless steel tubing (1/16” ID). Silastic was selected as the membrane material because of its high permeability to NO and non-polar nature, it favours non-polar molecules with low molecular weight such as nitric oxide [5, 6, 8]. A helix of fine nichrome wire was inserted inside the 10 mm silastic tubing to prevent the silastic tubing from collapsing under the partial vacuum of the inlet of the mass spectrometer. The distance from the Silastic membrane to the ion source was 1000 mm. Mass spectra were obtained from quadrupole mass spectrometer (QMS) (Pfeiffer Thermostat\(^{\text{TM}}\)) at an emission current of 0.99 mA. Source pressures were approximately 2 mPa. The
resulting spectra were well determined with a return ion current relative to a baseline signal separating each mass unit. Using various mass-to-charge ratios from the MIMS (after subtracting the background signal), ratios of isotopologues were evaluated to confirm the presence, and where possible the identity, of gaseous products. Table 3.4 presents a list of the isotopes applied to calculate statistical abundances of isotopologues of species present in this Chapter 4.

Table 3.2. List of isotopes that are important for MS isotopic ratio.

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Nominal mass</th>
<th>Isotopic mass</th>
<th>Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>C</td>
<td>12</td>
<td>12.000 000</td>
<td>98.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>13.003 355</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>14.003 074</td>
<td>99.63</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>N</td>
<td>15</td>
<td>15.000 108</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>15.994 915</td>
<td>99.76</td>
</tr>
<tr>
<td>Oxygen</td>
<td>O</td>
<td>17</td>
<td>16.992 435</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>17.999 160</td>
<td>0.02</td>
</tr>
</tbody>
</table>

3.3.3.2 FTIR analysis

The Varian IR-600 FT-IR spectrometer (Varian, Australia) was employed to identify and confirm gaseous products. It was equipped with a permanently aligned gas cell with a 10 m path length (Infrared Analysis, USA). Prior to collecting the sample gas in the Tedlar air sampling bag (SKC Inc., USA) for analysis, the cell gas was evacuated.
and flushed at least 3 times with nitrogen gas and a background check was first run. Each sample was scanned 32 times over the range of 4000-50 cm$^{-1}$, at a resolution of 0.5 cm$^{-1}$. The identification of gaseous species was based on standard spectra library provided within the QASoft software package (Infrared Analysis, USA). The gaseous products identified in this study were nitric oxide, nitrogen dioxide, nitrous oxide and carbon dioxide.

### 3.3.4 Liquid product analysis

#### 3.3.4.1 Ion chromatography

A Dionex DX-100 chromatograph equipped with an Ion Pac AS14A analytical column and AG14A guard column was employed for analysis of ionic product. The eluent used contained 8.0 mmol dm$^{-3}$ Na$_2$CO$_3$ and 1 mmol dm$^{-3}$Na$_2$HCO$_3$ with a flow rate of 1.0 cm$^3$ min$^{-1}$. Data logging was achieved with a universal chromatography interface (Dionex) with Chromoleon software used to analyse the chromatograph peaks.

Samples were drawn from the reactor using gastight syringes via a septum side port and quenched with 0.1 mol dm$^{-3}$ NaOH to stop the reaction before being diluted. Concentration of nitrite, nitrate, bromide (in the case DBNBS was used) and chloride (when DCNBS was used) were determined by comparing the peak area and retention time of the sample solution to a calibration plot generated using known standard solutions. Calibration plots are annexed in Appendix A.
3.3.4.2 NALDI-MS

NALDI-MS played a major role in identifying aqueous products from the trapping reaction. Reaction mixtures of nitroso compounds, following reaction with NO, were prepared one hour before analysis on NALDI targets. The detail description of the analysis is covered in Appendix B.

3.3.5 Kinetic measurement

3.3.5.1 Stopped flow UV-Visible spectroscopy – kinetics measurement of nitroso compounds

A RX-2000 rapid mixing accessory (Applied Photophysics) coupled with a Varian Cary50 UV-visible spectrophotometer was employed to study the kinetic behaviour of the reaction of the investigated nitroso spin trap to determine both rate and equilibrium constants. UV-Vis is suitable for measuring nitroso compounds such as 3,5-dibromo-4-nitrosobenzene sulfonate as this species exhibits a characteristic monomer absorption band centered around 750-770 nm, due to n→π* transition, while the dimer displays an absorption band in the UV-Vis region at around 308 nm, as illustrated in Figure 3.5.
Chapter 3: Methodology

Chemical trapping of NO

Figure 3.5. Comparison of UV-Vis spectrum of DBNBS with reaction mixture from wavelength 800 - 200 nm. 2(b) Zoomed section from 0-0.06 Absorbance unit; arrow indicates the absorbance at wavelength of 760 nm which reached zero after the reaction.

The stopped flow apparatus facilitates the study of fast kinetics by allowing reactants to be driven at a high rate through a mixing chamber, then abruptly stopping the flow to monitor the extent of the reaction by spectrometric measurements across an observation cell. The RX 2000 possesses a dead time of 8 ms and can function with either a 10 mm or 2 mm optical path length. A circulation water bath was employed to control the temperature in the stopped flow module and the cell compartment. A thermostated cell holder regulated by a Varian Cary single cell Peltier temperature controller (Varian, Inc.) houses the observation cell.

When studying reactions requiring oxygen to be excluded, 1 g of sodium dithionite was added to the water bath and the water was degassed continuously until the completion of the experiment. The stopped flow apparatus was flushed with deoxygenated distilled deionised water and a baseline absorbance reading was recorded and subtracted from subsequent measurements. Prior to each kinetic experiment, oxygen was also removed from the reactant solutions by sparging them for at least 15 min with N₂ gas. It was
found that 15 min was sufficient to remove all the oxygen present in the solutions since longer degassing yielded identical results.

Figure 3.6. Schematic diagram of the stopped flow UV-Vis apparatus

- High performance liquid chromatography (HPLC)

HPLC was mainly employed to establish whether the kinetic reaction (i.e rate of consumption of nitroso monomer by nitric oxide) at wavelength 750-770 nm using UV-Vis was feasible. This was performed by analysing and comparing reactant and products to ensure that there would be no interference peaks arising at the studied wavelength in the reaction product. Figure 3.7 compares the chromatograms of DBNBS sample with its reaction mixture obtained from the HPLC. The results indicate that, kinetic measurements of DBNBS consumption by NO using UV-Vis spectrometer at wavelength of 760 nm are possible, since no spectra of species other than DBNBS appear at this wavelength.

In addition, HPLC was used to determine the relative purity of the nitroso compound, in particular for NBS, which decomposes at room temperature. HPLC analyses were
performed using a Varian Prostar HPLC system equipped with a UV-Vis detector. The compounds of interest were separated by reverse-phase column (Varian, Microsorb C18 5.0 µm, 4.6 × 150 mm) with a mobile phase consisting of 10-90% acetonitrile and distilled deionised water at a flow of 1.0 cm³ min⁻¹ for 10-20 min (depending on the compound). The temperature of the column was maintained in a column oven at 25 °C and injection was performed by an auto sampler in a partial loop filled mode. To detect the separated components for nitroso compounds and their reaction mixture, the UV detector was set at a wavelength of 760 nm whereas, for determining the purity of NBS detection was performed at 280 nm.

![HPLC chromatogram](image)

**Figure 3.7.** HPLC chromatogram at wavelength of 760 nm for (a) DBNBS and (b) reaction mixture

### 3.3.5.2 Membrane NOx chemiluminescence analyser - kinetic measurements of NO

The NO concentration in the solution was continuously sampled by nitrogen carrier gas flowing through the so-called membrane inlet, a 30 mm segment of silastic tubing, 1.5 mm ID and 2.0 mm OD. The membrane inlet was attached at one end to a gas inlet
Chemical trapping of NO (connected to a nitrogen stream) and, a stainless steel tube (1/16” ID) connected to the chemiluminescence analyser (Thermo Scientific Model 42i-HL). Gases from solution, including NO, that diffuse through the inlet membrane are entrained in the N₂ carrier gas, flowing at a set rate of 0.67 cm³ s⁻¹ to the analyser. The membrane inlet was immersed in the solution contained in the reactor, as shown in Figure 3.3. The solutions in the reactor were purged by continuously sparging nitrogen via a stainless steel tube inserted in the septum with outflow via a needle inserted in the rubber stopper at the top of the reactor. Upon completion of the purging process, the nitrogen commenced to flow through the membrane inlet line and solutions containing reagents were injected through a syringe into the reaction vessel. The solutions were stirred using a magnetic stirrer and stirring bead to ensure uniform mixing of the solutions. The reactor was submerged in a temperature controlled water bath to conduct experiments at the desired temperature.

- Calibration

The membrane NOx analyser was calibrated for nitric oxide over the range of concentrations that were close to the range observed in this study. This was performed by injecting solutions of known concentration of NO in the reaction vessel with continuous stirring. NO in solution was generated by reduction of nitrite using 0.012 mol dm⁻³ ascorbic acid in 0.012 mol dm⁻³ HCl. The concentration of NO detected by the NOx analyser was recorded when it reached a maximum level. A plot of NO concentration in gaseous (ppm) versus NO concentration in solution (mmol dm⁻³) was linear up to 1.9 mmol dm⁻³ as shown in Figure 3.8. Calibration of the membrane NOx analyser was repeated 3 times to ensure measurement consistency.
3.3.6 Computational techniques

Density functional theory (DFT) methods were employed to study the reaction of aromatic nitroso sulfonate compounds with NO. Calculations were performed using both Gaussian 09 [9] (Australian National University Supercomputer Facility, Canberra) and Gaussian 03 [10] (Intersect Limited in Sydney, Australia) suite of programs. Optimised geometry, vibrational frequencies and single point energy of all stationary points were determined during the study. The effect of the solvation was studied using the polarisable continuum model (PCM) [11]. Computational details are given in subsequent chapters where theoretical calculations are employed.
3.4 References


CHAPTER 4: STUDY OF TRAPPING OF NO BY 3,5-DIBROMO-4-NITROSOBENZENE SULFONATE.

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Chapter 4: Study of trapping of NO by 3,5-dibromo-4-nitrosobenzene sulfonate

4.1 Introduction

Nitric oxide (NO) is a noxious free radical, formed in several important industrial processes, and is one of the major atmospheric pollutants causing significant environmental and health effects [1, 2]. Even relatively low levels of NO can affect the central nervous system, resulting in respiratory and reproductive disfunction, while exposure to high levels of this gas can lead to asphyxiation and even death [3-9]. In biological systems, NO plays an important modulatory role through physiological and pathological processes; it regulates blood pressure, acts as a neurotransmitter and supports the immune system to fight infectious diseases and viruses [10, 11]. Since NO is paramagnetic, it is generally studied by electron paramagnetic resonance (EPR) using spin trapping techniques whereby the spin trap molecule binds to the free radical to form a stable adduct, detectable by EPR [9, 12-16]. Considering this, we can exploit the EPR spin trapping technique to develop NO scavengers to ultimately control NO emission from industrial processes and in particular during the sensitisation of emulsion explosives via chemical gassing processes. The sensitisation of emulsions by chemical gassing involves the in situ generation of very small nitrogen gas bubbles in the emulsion, produced following the injection of concentrated sodium nitrite and acetic acid solutions.

3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), a C-nitroso compound, was introduced as a spin trap for carbon-centred radicals by Kaur et al. in 1981 [17, 18]. DBNBS has been applied to successfully trap nitric oxide generated from the decomposition of sodium nitrite under acidic conditions; the resultant radical product has a characteristic electron spin spectrum consisting a triplet with $a^N = 0.96$ mT [19,
20]. Although the compound exists in a monomer-dimer equilibrium with the monomeric form being active to trap radicals, there is sufficient monomer concentration to generate spin adducts [21, 22]. In addition, the spin trap’s thermal stability, resistance to photodegradation and high solubility of 100 g L\(^{-1}\) in water make it a potential candidate as a NO scavenger in aqueous systems.

In the present work, we have studied the \textit{in situ} nitrosation reaction of DBNBS spin trap with nitric oxide formed in an acidic nitrite (pH ~3.5) solution, conditions which are similar to those occurring during the sensitisation of emulsion explosives activated by chemical gassing [23]. The primary objective of this study is to demonstrate that the spin trap DBNBS can be used for other applications apart from spin trapping in EPR, especially enabling the direct reduction of free NO which may be produced during sensitisation of an emulsion explosive. Prior studies carried by Davis et al., for the \textit{ex situ} reaction of DBNBS with NO in water (in the absence of nitrite) reported the formation of a single reaction product, bis (2,6-dibromo-4-sulfophenyl) nitroxy [19]. However, the mechanism of the trap under a variety of conditions has not been studied specifically where acidic nitrite is present. Thus, we are interested to determine if similar products will be formed in the \textit{in situ} reaction. We have characterised experimentally the trapping reaction of NO with DBNBS in acidic nitrite solution by analysing both the gaseous and liquid products from the reaction using MIMS and other analytical techniques. We specifically report here the use of NALDI-MS for the matrix free desorption ionisation of low mass range liquid products produced during the reaction [24]. On the basis of these experimental data, we suggest a new pathway, occurring in the presence of nitrite, whereby nitro compounds are formed. The suggested reaction mechanism represents
valuable information for the future determination of the kinetics of trapping of NO with DBNBS.

4.2 Methodology

4.2.1 Chemicals and reagents

Reagents used were commercially available and used without further purification. DBNBS was synthesised as described in Chapter 3 and elsewhere. Silastic tubing was purchased from Speciality Manufacturing Inc (Saginaw, MI, USA) and silicone sealant was from Dow Corning (Midland, MI, USA). NALDI™ plates which are produced by Nanosys, Inc. for Bruker Daltonics Inc., were purchased from Bruker Daltonics Australia.

4.2.2 Preparation of reaction mixture of DBNBS with NO, formed via reduction of nitrite. (In situ trapping of NO)

DBNBS reaction mixture was prepared by reacting DBNBS with NO which is formed from nitrous acid decomposition. Initially a 0.015 mol dm$^{-3}$ acidified DBNBS solution with 0.1 mol dm$^{-3}$ acetic acid was flushed with argon for 15 min, then 0.125 mol dm$^{-3}$ sodium nitrite solution was injected via syringe into the reaction vessel to initiate the generation of NO. The final diluted nitrite concentration was 0.015 mol dm$^{-3}$, following the addition of nitrite to the reaction medium. After allowing the reaction to proceed for one hour, the pH of the reaction mixture was adjusted to pH 5.5 by dropwise addition of
Chapter 4: Study of trapping of NO by 3,5-dibromo-4-nitrosobenzene sulfonate

4.2.3 Preparation of reaction products from the reaction between DBNBS with NO in water. (Ex situ trapping of NO)

Nitric oxide was prepared in a reactor via reduction of nitrite, using an acidified solution of ascorbic acid. It has been shown that ascorbic acid reacts readily with nitrous acid under acidic conditions quantitatively forming NO and dehydroascorbic acid, and this procedure can be used to generate NO [25]. Before NO was bubbled into the DBNBS solution, it was passed through a 2 mol dm\(^{-3}\) NaOH scrubber to remove trace quantities of NO\(_2\) that might have been formed in the first reactor. Stainless steel tubing was employed after the NaOH scrubber to reduce measurement error due to diffusion of oxygen through the tubing walls. An empty vessel was also placed after the NaOH scrubber, to collect the entrained NaOH or nitrite formed from hydrolysis of NO\(_2\). The NO was then bubbled in the reactor containing 0.015 mol dm\(^{-3}\) DBNBS solution. Any excess NO was then bubbled through a scrubbing reactor containing acidified potassium permanganate to prevent the excess NO from escaping. Prior to starting the experiment, the apparatus was degassed for 30 min by nitrogen gas at a flow rate of 100 cm\(^3\) min\(^{-1}\). When the nitrite was injected in the first reactor, the flow of nitrogen was reduced to 30 cm\(^3\) min\(^{-1}\) and the reaction was allowed to take place for 1 h. The flow of nitrogen was then adjusted to 100 cm\(^3\) min\(^{-1}\) for 1 h to eliminate all NO in the reactors and subsequently to prevent formation of nitrogen dioxide if NO was in contact with atmospheric oxygen.
4.2.4 Analysis of gaseous products. MIMS and FTIR

4.2.4.1 Design of the membrane inlet for MIMS

The inlet membrane probe comprised a 10 mm length of silastic tubing of 1.5 mm ID and 2.0 mm OD which was sealed at one end with silicone sealant and attached to the MS capillary leading to the ion source of the mass spectrometer. The membrane was immersed in the solution, contained in a two-neck, 10 cm³ volumetric flask as shown in Figure 3.3(a) and with the details of the apparatus described elsewhere. In these experiments, the solution was purged with argon to remove oxygen and nitrogen and the purging of argon was terminated at the start of an experiment. Mass spectra were obtained from a quadrupole mass spectrometer (QMS) (Pfeiffer Thermostat™) at an emission current of 0.99 mA. Source pressures were approximately 2 mPa. The resulting spectra were well resolved, with a return of ion current relative to a baseline signal separating each mass unit. From the measured ion current for the different mass-to-charge ratios from the MIMS (after subtracting background signal), isotopic ratios were evaluated to confirm the presence, and, where possible, the identity of gaseous products.

4.2.4.2 FTIR analysis

A Varian IR-660 FT-IR spectrometer was used for the identification and confirmation of gaseous products. The gaseous products were purged from the reactor with nitrogen gas and collected in a 3 L Tedlar sampling bag. A 10 m path length cell was flushed for at least 3 times with nitrogen and a background check was run initially to ensure the absence of previous species before introducing the gaseous product collected into the
sampling bags. Gases were identified based on standard spectra using the QASoft database and software program (Infrared Analysis Inc.).

4.2.5 Analysis of liquid products

4.2.5.1 Ion chromatography and NALDI-MS

A Dionex DX-100 ion chromatograph with an Ionpac AS14A analytical column and AG14A guard column, with suppressed conductivity detection was employed for analysis of anionic products. The eluent consisted of 8.0 mmol dm$^{-3}$ Na$_2$CO$_3$ and 1.0 mmol dm$^{-3}$ NaHCO$_3$ with a column flow rate of 1.0 cm$^3$ min$^{-1}$. Data logging was achieved with the Chromeleon Chromatography data system software and was used to analyse the chromatograph peaks.

4.2.5.2 NALDI-MS

Aliquots of the samples (5 μL) were deposited directly on the surface of the target and allowed to dry at room temperature in ambient air. MS and MS/MS spectra were acquired in negative reflector mode using a Bruker Daltonics Ultraflex III MALDI time-of-flight mass spectrometer. MS data were obtained in the $m/z$ range between 0 and 800 Da by averaging signals from 2500 laser shots using target random-walk movement. Elemental sulfur standard was employed to externally calibrate the NALDI target. The FlexAnalysis 3.0 software (Bruker Daltonics) was used to analyse the data. (Further information on the utilisation of NALDI-MS is provided in Appendix B).
4.2.6 DFT study for confirmation of reaction mechanism

Calculations were performed with the Gaussian 03 software package [26]. Density functional theory calculations were performed at B3LYP/6-311+G(d,p) level [27] to optimise geometries in the gas phase and determine gas phase reaction free energies. The effect of solvation on the gas phase calculations was also investigated using the polarisable continuum model, PCM model (UAHF radii) [28] applying the optimised geometry from the gas phase calculation. In order to calculate the Gibbs energy change of an aqueous reaction, a thermodynamic cycle shown in Figure 4.1 was employed to combine the calculated solvation free energies with the gas phase free energy change of reaction.

\[
\begin{align*}
\Delta G^\circ_{\text{gas}}(A) &\rightarrow B_{\text{gas, 1 atm}} + C_{\text{gas, 1 atm}} \\
A_{\text{aq, 1 atm}} &\rightarrow B_{\text{aq, 1 atm}} + C_{\text{aq, 1 atm}} \\
\Delta G^\circ_{\text{aq, 1 atm}} &\rightarrow \Delta G^\circ_{\text{aq, 1 atm}} = \Delta G^\circ_{\text{gas}} + \Delta G^\circ_{\text{gas}}(B) + \Delta G^\circ_{\text{gas}}(C) - \Delta G^\circ_{\text{gas}}(A)
\end{align*}
\]

Figure 4.1. Thermochemical cycle for determining aqueous free energy change of reaction for typical reaction \( A \rightarrow B + C \).

Since the gas phase reaction energies have a standard state of 1 atm, whereas solution phase and solvation free energies has a standard state of 1 mol L\(^{-1}\) for the solute in both the gas phase and solution phase, a correction factor corresponding to
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\[ \Delta nRT \ln(\tilde{R}T) \] needs to be added [29]. \( \Delta n \) corresponds to the number of moles of product less reactants, \( R \) and \( \tilde{R} \) refers to the universal gas constant in units of 8.314 J mol\(^{-1}\) K\(^{-1}\) and 0.0821 L atm mol\(^{-1}\) K\(^{-1}\), respectively.

4.3 Results and Discussion

4.3.1 Gaseous analysis

Figure 4.2 shows the change in the ion signal of \( m/z \) 30 (corresponding to NO) as measured by MIMS when 0.015 mol dm\(^{-3}\) solution of DBNBS was injected following initial generation of nitric oxide. The time of injection of the 0.030 mol dm\(^{-3}\) nitrite and 0.015 mol dm\(^{-3}\) DBNBS solutions is indicated by the filled and open arrows, respectively. The addition of nitrite resulted in an immediate rise in the concentration of NO, while the subsequent addition of DBNBS, after around five minutes, caused a rapid decrease in this ion signal. Separate experiments performed with distilled deionised water revealed that the addition of DBNBS did not have a significant effect on the concentration of NO due to dilution but the apparent decrease in NO concentration was a result of the trapping of NO.
Figure 4.2. Effect of addition of 0.015 M DBNBS 5 min after starting generating NO (as measured by MIMS) at 25 °C. The repeatability of the experiment is also demonstrated in the plot by 3 runs under the same experimental conditions.

The MIMS results demonstrated that as the reaction between DBNBS and NO was proceeding, NO was being formed as suggested by the increase in the ion current at m/z = 30. However, we note that the amount of NO was considerably lower than when the trap was absent, where an 82 % reduction in NO formed was observed under these conditions, as shown in Figure 4.3. We also investigated on the effect of varying DBNBS concentration (see Figure 4.4) and, observed that, as the concentration of the trap increased the reduction in NO released became more significant.
Figure 4.3. Comparison of the effect of presence of DBNBS in the reaction medium when DBNBS was absent (a) and present (b).

Figure 4.4. Effect of varying DBNBS concentration in the reaction medium.
We confirmed the presence of NO with the experimental isotopic ratio for \( m/z = 31/30 \) of \( 4.0 \pm 0.3 \times 10^{-3} \) which is close to the expected average value of \( 4.06 \times 10^{-3} \). A significant amount of nitrogen gas was also detected and confirmed by the isotopic ratio of \( 7.04 \pm 0.21 \times 10^{-3} \) for \( m/z = 29/28 \). Unfortunately, it was not possible to accurately quantify \( \text{N}_2 \) using MIMS. Figures 4.5 show typical plots for \( m/z \) 28 and 29 applied to validate the presence of \( \text{N}_2 \) gas.

The signal at \( m/z \) 32, 33 and 34 remained relatively constant throughout the experiment, and thus the presence of oxygen in solution could not be confirmed during reaction. A small amount of \( \text{CO}_2 \) was detected during the reaction, although we suggest this is a result of \( \text{CO}_2 \) being released from a \( \text{CO}_2/\text{HCO}_3^- \) equilibrium upon acidification of the reactant solution. The presence of \( \text{CO}_2 \) could be only confirmed with ratio \( m/z \) 45/44 with a value of \( 1.2 \pm 0.5 \times 10^{-2} \) but not using the ratio \( m/z = 46/44 \). This anomalous value for the \( m/z = 46/44 \) can occur if there is contribution to the ion current from other gases present whereby the overlapping ion currents of nefarious origin are encountered when analysing mixtures of several gaseous components. In the present situation, \( \text{N}_2\text{O} \) and \( \text{NO}_2 \), which have similar daughter ions at these \( m/z \) ratios, could be also present and even small traces of these gases could change the isotopic ratio. For that reason, we could not verify the presence of \( \text{NO}_2 \) since the value of the isotopic ratio of \( 9.2 \pm 0.3 \times 10^{-2} \) for \( m/z = 47/46 \) obtained was far from the predicted average values. In addition the ratio for \( m/z = 48/46 \) could not be evaluated because the signal at \( m/z = 48 \) was constant.

Table 4.1 provides a list of product gases considered, with the corresponding isotopic ratios.
Figure 4.5. Typical plots of ion current against time for determining presence of N\textsubscript{2} during \textit{in situ} reaction of DBNBS with NO

\[
\text{m/z 29/28} = (7.0 \pm 0.2) \times 10^{-3}
\]
Table 4.1. List of product gases considered, with their corresponding isotopic ratio

<table>
<thead>
<tr>
<th>Considered gases</th>
<th>Isotopic ratio</th>
<th>Experimental isotopic ratio value</th>
<th>Expected average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>$m/z 31/30 = \frac{^{14}N^{16}O + ^{15}N^{16}O}{^{14}N^{17}O}$</td>
<td>$(4.0 \pm 0.3) \times 10^{-3}$</td>
<td>$4.06 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$m/z 45/44 = \frac{^{13}C^{16}O_2 + 2^{12}C^{17}O^3}{^{12}C^{16}O_2}$</td>
<td>$(1.2 \pm 0.5) \times 10^{-2}$</td>
<td>$1.19 \times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$m/z 46/44 = \frac{2^{12}C^{17}O^3}{^{12}C^{16}O_2}$</td>
<td>$(3.1 \pm 0.9) \times 10^{-2}$</td>
<td>$4.03 \times 10^{-3}$</td>
</tr>
<tr>
<td>N₂</td>
<td>$m/z 29/28 = \frac{2^{15}N^{14}O}{^{14}N_2}$</td>
<td>$(7.0 \pm 0.3) \times 10^{-3}$</td>
<td>$7.35 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$m/z 47/46 = \frac{^{15}N^{16}O_2 + 2^{14}N^{16}O^3}{^{14}N^{17}O}$</td>
<td>$(9.1 \pm 0.3) \times 10^{-2}$</td>
<td>$3.68 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$m/z 48/46 = \frac{2^{14}N^{17}O^3}{^{14}N_2}$</td>
<td>-</td>
<td>$4.03 \times 10^{-3}$</td>
</tr>
<tr>
<td>N₂O</td>
<td>$m/z 45/44 = \frac{2^{15}N^{14}O + ^{14}N_2^{17}O}{^{14}N_2^{16}O}$</td>
<td>$(1.2 \pm 0.5) \times 10^{-2}$</td>
<td>$7.75 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$m/z 46/44 = \frac{^{14}N_2^{15}O}{^{14}N_2^{16}O}$</td>
<td>$(3.1 \pm 0.9) \times 10^{-2}$</td>
<td>$2.03 \times 10^{-3}$</td>
</tr>
</tbody>
</table>
FTIR was employed in conjunction with MIMS to verify the presence of gaseous products, particularly when there is a mixture of different gases having similar masses. The FTIR spectrum of a typical gas sample discloses the presence of NO, NO$_2$, CO$_2$ and traces of N$_2$O as shown in Figure 4.6.

![Figure 4.6. Typical FTIR spectrum of the gas sample.](image)

### 4.3.2 Analysis of liquid products

#### 4.3.2.1 Ion chromatography

- **In situ sample**

The chromatographic analysis of anions indicated an elevated concentration of nitrate in the reaction mixture (sample representing plot (b) in Figure 4.3) compared to control A (sample representing plot (a) in Figure 4.3). The ratio of nitrite (NO$_2$) consumed to
nitrate (NO$_3^-$) formed in the reaction mixture and control A were 1:0.43 and 1:0.1 respectively. This confirms that when NO is formed from nitrous acid and is being trapped by DBNBS, nitrite is being consumed. We note that for the ion chromatographic analysis of a neat DBNBS sample, none of the targeted anions was detected.

- **Bromide formation**

From all NO scavenging experiments, bromide was detected in the reaction mixture, with the ratio of nitrite consumed to bromide formed typically around 1:0.22. The bromide in solution is suggested to be from the 2 bromine atoms present in the DBNBS compounds, which were released as bromide during the reaction. Since DBNBS was absent in control A (Figure 4.7), we did not detect bromide in these samples.

In an attempt to study further the formation of bromide during the reaction, a series of experiments were undertaken. In control B, where DBNBS was reacted with nitrite, a small amount of bromide was detected in the sample, slightly higher in concentration than in the control A experiment. When comparing control B with C, where nitrite and DBNBS reacted for 1 h (case B) or 18 h (case C), both the bromide and the nitrate were found to have increased by at least a factor of 3, while the nitrite concentration decreased. The result from these experiments implies that a trace amount of acetic acid (impurity remaining from the DBNBS synthesis process) was present and was reacting with nitrite to form nitric oxide (which was detected by MIMS). Thus the reaction of DBNBS with nitrite alone was not sufficient to induce bromide formation; rather Br$^-$ was formed in the reaction of DBNBS with nitric oxide produced from nitrous acid.
decomposition. It was also established that the presence of a significant amount of acetic acid catalyses the decomposition of nitrous acid. For instance in the reaction mixture where 0.1 mol dm$^{-3}$ acetic acid was added, and the reaction was allowed to take place for 1 hour which was sufficient time to form a significant amount of bromide.

![Ions analysis](image)

**Figure 4.7.** A graph showing the mean concentration (mmol dm$^{-3}$) of nitrite, bromide and nitrate in the reaction mixture for *in situ* trapping of NO.

- **Ex situ sample**

In the *ex situ* experiment, nitrate levels were higher, indicating that the formation of nitrate during the reaction of DBNBS with NO as shown in Figure 4.8. Unexpectedly, the concentration of nitrite measured was especially elevated, approximately 14 times...
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higher than in the water sample bubbled with NO (control D). Bromide was also detected in the \textit{ex situ} experiments.

![Graph showing ions analysis](image)

**Figure 4.8.** A graph showing the mean concentration (mmol dm^{-3}) of nitrite, bromide and nitrate in the reaction mixture for \textit{ex situ} trapping of NO

4.3.2.2 \textit{NALDI-MS}

- DBNBS sample

The NALDI spectra of a neat DBNBS sample are dominated by a peak at \textit{m/z} 343.893 which corresponds to the parent ion of the DBNBS molecule as shown in Figure 4.9. The intensity of isotopic peaks in this region was in a ratio of 1:2:1, which is characteristic of the presence of two bromine atoms in the structure. An oxidation product of DBNBS was disclosed by the presence of a peak at \textit{m/z} = 359.943 [DBNBS
+ $m/z$ 16]. Initially, this peak was attributed to the presence of 3,5 dibromonitrobenzene sulfonate, an artifact formed during the ionisation process (as is often observed in desorption ionisation mass spectrometry) [30, 31]. However, subsequent experiments carried out on the NALDI target as a function of time provided evidence that oxidation was caused by surface activity, the extent of which is strongly dependent on the storage time of the analyte on the NALDI target. Thus, a peak at $m/z = 359.943$ is always present as background signal from the NALDI target. However, as is subsequently discussed, an observed significant increase in the intensity of this peak in the sample of the reaction mixture can also be attributed to the formation of a DBNBS byproduct, which forms during NO scavanging.

![Figure 4.9](image.png)

**Figure 4.9.** A NALDI-MS spectrum of DBNBS. Peaks at $m/z$ 343.883 and 359.943 were attributed to DBNBS and its oxidation product [DBNBS + $m/z$ 16] respectively.

- Sample of the *in situ* reaction mixture
The peak at \( m/z \) 343.893 (DBNBS parent ion) was present in the sample of the reaction mixture but was lower in intensity (concentration) in comparison to the sample of the original DBNBS, signifying that DBNBS was consumed in the reaction. The ion signal associated with a molecular weight at \( m/z \) 359.943 was also present in all sample aliquots; however, the intensity varied widely from sample to sample. In the original DBNBS sample, the intensity ratio of ion signals at \( m/z \) 343.893:359.943 was 1:0.33, while that from the reaction mixture was 1:3.39 which indicates that the product was not only formed from the process of NALDI analysis but is also produced during the reaction itself. In the mass spectra obtained for the reaction mixture, ions absent in the original DBNBS sample were subsequently detected in the reaction mixture including species with \( m/z \) values of 261.903, 291.880, 324.891 and 449.449 (see Figure 4.10). The NALDI-MS spectra also showed peaks with molecular weight of 574.720, 609.286 and 655.299 in some of the samples. These peaks are as yet unidentified. Quantitative analysis from NALDI analysis was not possible in the present study, and the relative concentration of different product species is simplistically based on the relative intensities of the parent ion of each species.

The ion signal with an attributed molecular weight of 291.880 has the highest intensity in the mass spectrum, and is suggested to be the primary product formed in the reaction. Bromine is not present in this compound, as the ratio of the peaks at \( m/z \) 291.880 and \( m/z \) 293.882 is 47:1. MS/MS analysis of the \( m/z \) 291.880 ion did not elucidate a significant amount of structural information on the ion except that the loss of mass 46 was observed, indicating presence of a nitro group in the compound.

Another product which was detected in the reaction mixture was disclosed by the presence of a species at \( m/z \) 449.449. This product has two bromine atoms attached to
the benzene, with the three peaks at $m/z$ 447, 449 and 451 having an intensity ratio of approximately 1:2:1, as expected for a compound containing two bromine atoms (the natural abundances of $^{79}\text{Br}$ and $^{81}\text{Br}$ are 50.54 and 49.46%, respectively).
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Figure 4.10. Typical NALDI mass spectrum of the *in situ* reaction mixture
- **Sample of the *ex situ* reaction mixture**

The *ex situ* reaction mixture was also analysed and the liquid products were compared with *in situ* products. The mass spectrum for the *ex situ* reaction mixture was dominated by an ion with molecular weight of 680.9, as shown in Figure 4.11. The intensities of peaks at this group of ions were in a 1:4:6:4:1 ratio, suggesting the presence of four bromine atoms in the ion. We also detected other species in the *in situ* reaction mixture, associated with *m/z* values of 291.880, 343.853 and 359.918.

![Figure 4.11. Typical NALDI mass spectrum showing the peak at *m/z* 680.857 present in *ex situ* reaction mixture](image-url)
4.3.3 Nitrous acid formation and decomposition

Under acidic nitrite conditions, nitrous acid formed and slowly decomposed, primarily to nitric oxide, nitrogen dioxide, dinitrogen trioxide and water according to Reactions 4.1 – 4.4 [25, 32-34]. It is this liberated nitric oxide that we have studied and trapped \textit{in situ} using DBNBS.

\begin{align*}
\text{NO}_2^- + \text{H}^+ & \rightleftharpoons \text{HNO}_2 \quad \text{(4.1)} \\
2\text{HNO}_2 & \rightleftharpoons \text{NO} + \text{NO}_2 + \text{H}_2\text{O} \quad \text{(4.2)} \\
2\text{NO}_2 + \text{H}_2\text{O} & \rightleftharpoons \text{HNO}_2 + \text{H}^+ + \text{NO}_3^- \quad \text{(4.3)} \\
2\text{HNO}_2 & \rightleftharpoons \text{N}_2\text{O}_3 + \text{H}_2\text{O} \quad \text{(4.4)}
\end{align*}

For C-nitroso compounds, the addition of a spin trap molecule proceeds by the radical associated with a localised unpaired electron, to form an adduct. Ichimori et al. proposed that DBNBS reacts with NO in water (Reaction 4.5) to produce an unstable complex that readily decomposes in aqueous solution [35]. Despite our inability to detect a short-lived DBNBS-NO adduct, it is believed that the reaction in the present study will be similar to the generalised nitroso spin trap free radical reactions, whereby an adduct is produced when NO initially reacts with DBNBS. Reaction 5 is highly reversible with calculated aqueous free energies of reaction, \( \Delta G^\circ_{\text{rxn}} = 28.17 \text{ kJ} \) and equilibrium constant \( K = 1.15 \times 10^{-5} \text{ mol}^{-1}\text{dm}^3 \) based on B3LYP/6-311+G(d,p) results.
4.3.4 Nitrogen generation and the formation of phenyl radical

Experiments using the MIMS showed that a considerable amount of nitrogen gas was released during the reaction of DBNBS with NO. The release of nitrogen was a consequence of either the homolytic cleavage of a diazenyl radical or from the decomposition of diazonium ion, since in aqueous solution, diazonium ions are unstable (at higher temperature than 5 °C) and the N⁺≡N group tends to leave the benzene ring as N₂ [36, 37].

For the formation of a diazenyl radical to occur, oxygen (O₂) would be released during the decomposition of the radical intermediate (DBNBS-NO adduct) (Reaction 4.7), whose free electron has rearranged itself to form the radical intermediate in Reaction 4.6. On the other hand, a diazonium ion would be present if superoxide (O₂⁻) was formed when the radical intermediate decomposes (Reaction 4.8).
The high concentration of nitrite and nitrate in ex situ and in situ reactions suggested that oxygen could be formed during the reaction. Experiments with MIMS did not disclose the presence of oxygen, and in the case of oxygen formation, the MIMS data are inconclusive. In aqueous solution and in the presence of oxygen, NO oxidises to NO₂ which exist in equilibrium with its dimer N₂O₄ (Reaction 4.10 & 4.11). The association of NO₂ to N₂O₄ is fast in aqueous solutions with \( k = 4.5 \times 10^8 \) mol\(^{-1}\) dm\(^3\) s\(^{-1}\) and the solubility of N₂O₄ is around 100 times higher than NO₂. Once formed, N₂O₄ decays by a relatively fast reaction with water to produce nitrite and nitrate according to Reaction 11 (\( k = 1 \times 10^3 \) s\(^{-1}\)) \cite{38}. In addition, the small amount of NO₂ detected from the FTIR experiments add further indirect evidence that oxygen could be produced during the reaction.

\[
2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2 \quad (4.9)
\]
\[
2\text{NO}_2 \rightleftharpoons \text{N}_2\text{O}_4 \quad (4.10)
\]
\[
2\text{N}_2\text{O}_4 + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + \text{NO}_3^- + \text{H}^+ \quad (4.11)
\]
However an elevated level of nitrate in the reaction mixture can also suggest the presence of superoxide in the system. If superoxide were present in the system, under acidic conditions, it would decompose and yield hydroperoxyl radical, since the $pK_a$ of superoxide is 4.8 [39]. The hydroperoxyl radical will subsequently react with excess NO in the reactor, forming peroxynitrite/peroxynitrous acid ($pK_a =6.8$) (Reaction 12) [40-42], which decays in a first order process producing nitrate with $k = 1.2 \, s^{-1}$ at 25 °C (Reaction 4.13) [43, 44]. This process could contribute to the high level of nitrate in both the in situ and ex situ reaction mixtures.

$$\text{O}_2^- + \text{H}^+ \rightarrow \text{HO}_2$$  \hspace{1cm} (4.12)

$$\text{HO}_2 + \text{NO} \rightarrow \text{HOONO}$$  \hspace{1cm} (4.13)

$$\text{HOONO} \rightarrow \text{NO}_3^- + \text{H}^+$$  \hspace{1cm} (4.14)

Given the difficulties in differentiating experimentally if superoxide or oxygen (or indeed both molecules) were formed during reaction, a DFT study was performed to determine the $\Delta G^\circ_{\text{rxn}}$ at 298 K of the reactions leading to the formation of oxygen and superoxide respectively. The DFT study predicts a favourable reaction for the formation of diazenyl radical and oxygen both in the gas phase as well in the aqueous phase with a $\Delta G^\circ_{\text{gas}}$ of 1.77 kJ and $\Delta G^\circ_{\text{Soln}}$ of -8.72 kJ. On the other hand, the reaction yielding the diazonium ion and superoxide was highly unfavorable, due to the endoergic nature of the reaction with a highly positive $\Delta G^\circ_{\text{gas}}$ and $\Delta G^\circ_{\text{Soln}}$ of 303.16 and 78.15 kJ respectively. The mechanism describing the release of nitrogen and concurrent formation of a phenyl radical is presented in Figure 4.12.
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Figure 4.12. Pathways leading to the generation of nitrogen gas and formation of phenyl radical from the reaction of DBNBS with NO

4.3.5 Ex situ reaction

Davies et al. proposed the formation of a mono-sodium bis (2,6-bromo-4-sulphonyl) nitroxyl with molecular weight of 680.9 when DBNBS reacts with NO in water (Figure 4.13) [19]. They showed the presence of the product by FAB-MS, following separation of their reaction mixture using HPLC. The present results agree with Davies et al. as this species was also identified by NALDI-MS in our experiments from the reaction of DBNBS with NO in distilled deionised water.

Figure 4.13. Pathways for the formation of mono-sodium bis (2,6-bromo-4-sulphonyl) nitroxyl from the ex situ reaction of DBNBS with NO
4.3.6 *In situ* reaction

During experiments undertaken in the presence of added nucleophiles such as nitrite (*in situ* reactions), it is proposed that a primary product from the reaction of DBNBS with NO (in the presence of nitrite/nitrous acid), has a molecular weight of 291.9, which is characterised by a nitro group attached in the benzene ring and where bromine is absent as a result of bromide being released during the reaction. In the following discussion, we explore data obtained from NALDI analysis to elicit the composition of the major reaction products produced during spin trapping reaction. It should be noted that while the molecular weight and therefore composition of reaction products were accurately determined, distinguishing an isomeric structure for each compound was not possible, and discussion related to the formation of specific isomers is speculative.

The diazenyl radical formed by decomposition of the intermediate adduct, undergoes homolytic cleavage of the C-N bond to yield gaseous N₂ and electrophilic π-aryl radical, which reverts to a stable σ-aryl radical [45]. The radical may then couple with a nitrite anion to produce a π* radical anion via a σ* radical anion, which subsequently reacts with electrophile N₂O₃ to yield nitrobenzene sulfonate as shown in Figure 4.14. The phenomenon of phenyl radical being trapped in aqueous solution by anions of nitromethane, ethane and propane as well as nitrite ion-yielding radical anions was observed by Russell et al [46]. The major driving force for the capture of the phenyl radical by nitrite ion, instead of reacting with another radical, is the stability of the nitrobenzene radical anion.
Eventually, when an electron withdrawing nitro group is bonded to an aromatic system, it produces centres of low electron density located at certain ring carbon atoms which are subsequently preferentially attacked by nucleophilic reagents [47, 48]. This phenomenon occurs in particular when there are nitro functional groups positioned ortho to the halide leaving group [49, 50]. As a result of the activating influence of the nitro group in halogen displacement, the presence of nitro group in the benzene ring will enhance further substitution by nitrite, replacing the bromine atoms. This would explain the absence of bromine in M+2 peak of m/z 291.880 since the ratio was 47:1 and the bromide ion detected when the reaction mixture was analysed by ion chromatography. The other peaks detected at 324.891, 359.918 confirm this hypothesis and represent intermediate species, present as precursors to the trinitrobenzene sulfonate final product.

It is suggested that the presence of the product of trinitrobenzene sulfonate, also detected in the ex situ reaction mixture, results from the appearance of a small concentration of nitrite produced as a consequence of the formation of oxygen from the reaction. A very
small amount of nitrite is enough to enable a new competing pathway to occur, where a nitro group is introduced in the system. However, since DBNBS is present in relatively high concentrations in the ex situ experiment, the pathway whereby a second DBNBS molecule reacts with NO will prevail.

4.3.7 Formation of side products

In general, nucleophiles react with electron deficient arene such as nitrobenzene derivatives (due to the presence of the powerful electron withdrawing nitro groups) by direct attack and addition to ortho or para position occupied by halogen resulting in the formation of $\sigma^X$ adducts [50]. This causes spontaneous departure of the halide anion $X^-$ from the $\sigma^X$ adduct affording products of nucleophilic aromatic substitution of halogen $S_{N Ar}$. However, the addition of nucleophile is also possible in positions occupied by hydrogen to form $\sigma^H$ adducts. This type of reaction was reviewed by Mąkosza and Winiarski who noted that these reactions proceed faster than in equally activated positions occupied by halogens [51, 52]. However, the produced $\sigma^H$ adducts may then undergo conversion into products of substitution of halogen and formation of $\sigma^X$ via ring opening elimination of $X^-$ anion from the anionic acyclic intermediate followed by ring closure; reaction termed addition of nucleophile ring opening ring closure, ANRORC [53]. This therefore results in the same product as in the conventional $S_{N Ar}$ process. In the present case, addition of nitrite nucleophile may have also occurred in positions occupied by hydrogen at the meta position, although in literature nucleophilic substitution at the meta position is very rare and only few examples have been found [54, 55]. Figure 4.15 illustrates the reaction pathway for the production of a side product with molecular
weight of 449.510 as a result of nucleophilic substitution at position occupied by hydrogen.

\[
\begin{array}{c}
\begin{array}{c}
\text{3,5-dibromo-2,4,6-} \\
\text{trinitrobenzene sulfonate}
\end{array}
\end{array}
\]

\[m/z\ 449.510\]

**Figure 4.15.** Pathway leading to the formation of by product 3,5-dibromo-2,4,6-trinitrobenzene sulfonate

Another important reaction product (a result of the ambidentate behaviour of the nitrite ion [56, 57]) is phenol (hydroxyl-dinitrobenzene sulfonate), detected at \(m/z\ 261.903\), formed through the attack of oxygen atom in the reaction mixture, as outlined in Figure 4.16. Dokunikhin et al. found that sodium nitrite reacts with 2,3,6,7-tetrachloroanthraquinone in N,N-dimethylformamide to yield isomeric \(\beta\)-dinitro-\(\beta\)-dihydroxyanthraquinone, showing that aryl halide reaction can be influenced by the ambidentate nature of nitrite ion [58]. However, it appears that in the present reaction, the attack was mainly from the nitrogen atom of the nitrite ion, since the peak of the main product was more intense.
Figure 4.16. Formation of phenol (hydroxyl-dinitrobenzene) sulfonate as a result of the ambidentate nature of the nitrite ion.
4.4 Conclusion

In this study, we have confirmed the formation of 3,4,5 trinitrobenzene sulfonate in the reaction of DBNBS with NO in the presence of acidic nitrite, and developed a new competing reaction pathway for its formation. However, at low nitrite concentrations and high DBNBS concentrations as observed in *ex situ* reactions where a small amount of nitrite (due to the reaction NO with oxygen) is present, a DBNBS molecule reacts with the phenyl radical to form the radical product dianion bis (2,6-dibromo-4-sulfophenyl) nitroxyl, as reported by Davis et al. Although caution should be applied to employ DBNBS as a spin trap for the detection and quantification of NO in biological systems as a result of the formation of other products from competing reaction, it can potentially be used for the scavenging of NO to control NO emissions from chemical industrial processes.
4.5 References


Chapter 4: Study of trapping of NO by 3,5-dibromo-4-nitrosobenzene sulfonate


Chapter 4: Study of trapping of NO by 3,5-dibromo-4-nitrosobenzene sulfonate


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CHAPTER 5: THERMODYNAMICS AND KINETICS OF THE REACTION OF NO WITH 3,5-DIBROMO-4-NITROSOBENZENE SULFONATE
5.1 Introduction

A mechanistic study, based on Reactions 1-3, was described in Chapter 4, the aim of which was to understand the trapping reaction of NO by DBNBS in acidic nitrite solution. It was established that N₂ gas was released as a result of the homolytic cleavage of the C-N bond of a diazenyl radical which was itself formed by the decomposition of the DBNBS-NO adduct.

\[
\text{DBNBS} + \text{NO} \rightleftharpoons \text{DBNBS-NO adduct} \quad \text{(R5.1)}
\]

\[
\text{DBNBS-NO adduct} \rightarrow \text{O}_2 + \text{Diazenyl radical} \quad \text{(R5.2)}
\]

\[
\text{Diazenyl radical} \rightarrow \text{N}_2 + \text{Aryl radical} \quad \text{(R5.3)}
\]

This chapter investigates the thermodynamic and kinetic properties of the dissociation of DBNBS dimer, as this step controls the trapping of NO by DBNBS. We experimentally determined the rate at which DBNBS traps NO, where NO was generated \textit{(in situ)} from the decomposition of nitrous acid. This measurement required the development of a new experimental technique which involved sampling of NO by diffusion through a polymeric membrane and the quantification of the sampled gases by a chemiluminescence analyser. The membrane probe was immersed in the reactor at all times.

In \textit{ex situ} experiments, NO was generated from the rapid nitrosation of L-ascorbic acid. Once the reaction reached completion and a known quantity of NO released, DBNBS was then added to initiate the trapping reaction. Concurrently, the rate of DBNBS consumption by the saturated solution of nitric oxide was measured using ultraviolet-visible (UV-Vis) spectrometry and compared with chemiluminescence.
measurements of NO sampled in the *in situ* experiments. Knowledge of the kinetics of the reaction of DBNBS with NO will be critical to develop practical traps for NO generated during chemical gassing of ammonium nitrate emulsion explosives.

### 5.2 Methodology

#### 5.2.1 Chemicals and reagents

DBNBS was synthesised from 3,5-dibromosulfanilic acid, sodium salt (Sigma Aldrich, Australia), as described in Chapter 3 and elsewhere. L-ascorbic acid, glacial acetic acid and sodium nitrite were of AR grade, purchased from Sigma Aldrich (Castle Hill, Australia). Hydrochloric acid solution was made by dilution of 37 % HCl (Sigma Aldrich). Silastic tubing was from Dow Corning (Midland, MI, USA). Saturated solutions of NO were prepared by bubbling nitric oxide generated via the reduction of nitrite by ascorbic acid into the target solutions. Prior to bubbling NO gas through the aqueous solutions, the system was flushed with nitrogen for at least 30 min in order to remove any oxygen present in the system and therefore eliminate the formation of NO$_2$ via oxidation of NO. The nitric oxide saturated solutions were collected using 10 cm$^3$ luer lock gas tight syringes for further study.
5.2.2 Thermodynamic and kinetic study of the equilibrium reaction of DBNBS with its trans dimer

5.2.2.1 Determination of DBNBS dimer-monomer equilibration, $K_C$

Samples for UV-visible spectrum analysis were prepared by dissolving the as-synthesised DBNBS (final concentration 0.05-8 mmol dm$^{-3}$ monomer added) in distilled, deionised water. UV visible spectra (400-900) nm were measured at 25 °C on a Varian Cary 50 Scan UV-Visible spectrophotometer coupled with a single cell Peltier thermostated cell holder (Varian, Inc) using a sealed quartz cell (optical length, 1 cm).

5.2.2.2 Measurement of the rate of formation of DBNBS monomer

The dilution of a known concentration of DBNBS with distilled deionised water was studied using a stopped flow apparatus (RX-2000, Applied Photophysics) coupled to a UV-Vis spectrometer (Varian Cary 50). Rapid dilution of DBNBS in water facilitates the dissociation of the DBNBS dimer and shifts the equilibrium towards formation of DBNBS monomer. We examined a range of DBNBS solutions, with initial concentrations between 3.75 and 10 mmol dm$^{-3}$. The absorbance at 760 nm was used to determine the concentration of DBNBS monomer using an extinction coefficient of DBNBS of 34 mol$^{-1}$ dm$^3$ cm$^{-1}$ [1].
5.2.3 Measurement of rate of consumption of DBNBS monomer by saturated solution of nitric oxide (ex situ trapping of NO)

A stopped flow UV-Vis spectrometer was also employed to study the consumption of DBNBS by NO at DBNBS concentrations ranging from 0.45 to 3.6 mmol dm\(^{-3}\). The absorbance at 760 nm served to determine the concentration of DBNBS monomer (\(\varepsilon_{760} = 34 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}\)). A circulating bath delivered water at constant temperature to the surrounding manifold maintaining the drive syringe and its contents at 25 ± 0.5 °C. The cell holder was also thermostated by a single cell Peltier temperature controller device to ensure a constant temperature in the cell. All experiments were performed under anaerobic conditions in an effort to minimise the concentration of oxygen and thus reduce the conversion of NO to HNO\(_2\). In order to ensure anaerobic conditions in the stopped flow cell, 1 g of sodium dithionite was added to the water bath and the water was degassed continuously throughout the duration of the experiment. In addition, DBNBS solutions were degassed with nitrogen at least 15 min prior to each experiment and the stopped flow cell flushed with deoxygenated distilled, deionised water. A baseline was then acquired and subtracted from subsequent measurements.

5.2.4 Measurement of NO by membrane NOx analyser

Measurement of NO was performed using a membrane inlet NOx analyser (Thermo Scientific Model 42i-HL). The inlet membrane probe comprised a 30 mm length of silastic tubing of 1.5 mm ID and 2.0 mm OD (Dow Corning, US) attached at one end to a stainless steel tube (1/16” ID) flowing pure N\(_2\) into the probe, and, at the other to separate segment of stainless steel tube (1/16” ID) that led to the NOx analyser (see
Figure 3.3(b)). Gas in solution diffuses through the inlet membrane and is then entrained in a stream of nitrogen gas set at a flow of 0.67 cm$^3$ s$^{-1}$ into the analyser.

The membrane inlet was immersed in a solution contained in a two-neck, 10 cm$^3$ volumetric flask which was completely filled with solution, as described in Chapter 3 and elsewhere. A second neck was fitted with a septum which served as an injection port. Solutions in the reactor were purged prior to reaction by continuously bubbling nitrogen, via stainless steel tubing pierced through a septum with outflow via a needle inserted through the rubber stopper at the top of the reactor. Upon completion of the purging process, the nitrogen flow was directed to the membrane inlet line and solution-containing reagents were injected through a syringe into the reaction vessel. The reactor contained a small magnetic stirrer to ensure uniform mixing of the solution and was itself immersed in a water bath for temperature control.

Calibration of the membrane inlet and NOx analyser was performed by injecting a solution of known concentration of NO into buffered solutions contained in the reaction vessel. (Refer to Chapter 3 for detailed description of calibration).

5.2.4.1 Ex situ trapping of NO

In ex situ trapping experiments, NO was generated via the rapid nitrosation of ascorbic acid prior to reaction with DBNBS. This was performed by charging the reactor with 18 cm$^3$ of 0.3 mmol dm$^{-3}$ ascorbic acid, acidified with 10 mmol dm$^{-3}$ HCl and purging the solution for 15 min with nitrogen gas. When the purging process was complete, 1 cm$^3$ of nitrite solution was injected (the total nitrite concentration following addition was 0.6 mmol dm$^{-3}$) to initiate the rapid generation of NO. The reaction proceeded until
all the nitrite was consumed and the NO concentration was found to reach a maximum, after which DBNBS solution was immediately injected to trigger the trapping reaction.

5.2.4.2 In situ trapping of NO

During in situ trapping experiments, the reactor was completely filled with acidified solution of DBNBS previously mixed with 0.1 mol dm$^{-3}$ acetic acid and flushed with nitrogen for 15 min. Subsequently, 1 cm$^3$ of 0.125 mol dm$^{-3}$ sodium nitrite solution was injected (via syringe) into the reaction vessel to initiate the generation of NO. The final, diluted nitrite concentration was 0.015 mol dm$^{-3}$, following the addition of nitrite to the reaction medium. The reaction was allowed to proceed for a period of 1 h.

5.2.5 Data analysis

Both the NO and DBNBS measurements collected during the ex situ trapping experiments were separately fitted to a nine-step reaction mechanism using the DynaFit program [2] to obtain rate of trapping, $k_{\text{trap}}$. The reaction mechanism was written into a DynaFit script file, and rate constants were included for all known reactions. The DynaFit program automatically integrates a system of ordinary differential equations describing the reaction mechanism, and adjusts the rate constants in these equations to minimise the error between modelled values and experimental data using a Levenberg-Marquardt algorithm. Several initial estimates of the fitted rate constant were trialed to ensure convergence of the program to a global minimum.
Chapter 5: Thermodynamics and kinetics of the reaction of NO with 3,5-dibromo-4-nitrosobenzene sulfonate

5.3 Results and discussion

5.3.1 Thermodynamic and kinetic analysis of the equilibrium DBNBS and its trans dimer

The dimer-monomer equilibrium constant, $K_C$, (Reaction 5.4) for our synthesised DBNBS was evaluated using the specific concentration obtained from the visible absorption band of the monomer and applied to Equation 5.1 [1]. The derivation of Equation 5.1 is provided in Appendix C.

\[
\text{DBNBS dimer } \rightleftharpoons 2 \text{ DBNBS monomer} \quad k_4, k_{-4} \quad \text{(R5.4)}
\]

\[
\frac{D}{C_i L} = \left( \frac{D^2}{C_i L^2} \right) \left( \frac{2}{K_C \varepsilon} \right) + \varepsilon \quad \text{(E5.1)}
\]

where $D$ is the optical density, $C_i$ is concentration of initial concentration of equivalent monomer (mol dm$^{-3}$), $\varepsilon$ is the molar extinction coefficient per centimetre of cell length and $K_C$ is the equilibrium constant. The equilibrium constant $K_C$ is taken as a dimensionless number with all concentration values referenced to a standard state. From the plot of $D/C_i L$ against $D^2/C_i L^2$ (Figure 5.1), we obtained a value for the extinction coefficient of DBNBS at 760 nm of 34.44 mol$^{-1}$ dm$^{3}$ cm$^{-1}$. Applying this value of the extinction coefficient, we determined the dimer-monomer equilibrium constant to be $(1.29 \pm 0.03) \times 10^{-3}$ at 25 °C. These values are in good agreement with the reported values of $34$ M$^{-1}$ for the extinction coefficient and an equilibrium constant of $1.3 \times 10^{-3}$ [1]. The $K_C$ value obtained confirms that, at equilibrium, approximately 20
% of the dissolved DBNBS (10 mmol dm\(^{-3}\)) is present as monomer at room temperature, and available for trapping NO under these conditions.

![Plot of D/C\(_t\) against D\(^2\)/C\(_t\)\(^2\)](image)

**Figure 5.1.** Plot of D/C\(_t\) against D\(^2\)/C\(_t\)\(^2\)

Reaction 5.4 was fitted to the experimental data for the dissolution of dimer to monomer using DynaFit for determining rates \(k_4\) and \(k_{-4}\) for DBNBS dimer/monomer equilibrium. Values of 0.03 s\(^{-1}\) and 21.6 mol\(^{-1}\) dm\(^3\) s\(^{-1}\) for \(k_4\) and \(k_{-4}\) respectively, describe well the dissociation of the DBNBS dimer to monomer. Figure 5.2 illustrates the experimental measurements and model fits for the formation of monomer from the dissociation of dimer to monomer in aqueous solutions. From these rate constants, a value for \(K_C\) of 1.31 \(\times\) 10\(^{-3}\) was evaluated, consistent with the value of \(K_C\) reported by Ide et al. The observed data highlight the relatively slow rate of dissociation to the monomer since it took roughly 34 s for the monomer concentration to its reach equilibrium concentration.
Figure 5.2. Comparison of experimental measurements (points) with the model (solid lines) of the formation of DBNBS monomer for initial concentration of (Δ) 3.75 mmol dm\(^{-3}\), (○) 5 mmol dm\(^{-3}\) and (×) 10 mmol dm\(^{-3}\) DBNBS. For clarity, only every 10\(^{th}\) data point is shown.

5.3.1.1 Temperature dependence and thermodynamic analysis of the formation of DBNBS monomer

We examined the rates of the forward and reverse reactions, as well the equilibrium constant \(K_C\) for the DBNBS monomer=dimer reaction over a temperature range of (25–60) °C. The results obtained at five temperatures are tabulated in Table 5.1. The plot of \(\ln(k)\) versus \((1/T)\) yielded an activation energy of 79.7 ± 0.3 and 27.9 ± 0.4 kJ mol\(^{-1}\) for the forward and reverse reaction respectively as illustrated in Figure 5.3. \(K_C\) increased with increasing temperature, confirming the favorability of monomer formation as temperature rises.
Table 5.1. Rate constants and Equilibrium data for the dissociation of dimer to monomer for temperature range of 25-60 °C

<table>
<thead>
<tr>
<th>( T / ^\circ C )</th>
<th>( K_c )</th>
<th>( k_1 / s^{-1} )</th>
<th>( k_{-1} / \text{mol}^{-1} \text{ dm}^3 \text{ s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>( 1.29 \times 10^{-3} )</td>
<td>( (2.9 \pm 0.1) \times 10^{-2} )</td>
<td>( 21.6 \pm 0.4 )</td>
</tr>
<tr>
<td>30</td>
<td>( (1.97 \pm 0.1) \times 10^{-3} )</td>
<td>( (4.9 \pm 0.1) \times 10^{-2} )</td>
<td>( 25.4 \pm 0.5 )</td>
</tr>
<tr>
<td>40</td>
<td>( (4.02 \pm 0.2) \times 10^{-3} )</td>
<td>( 1.48 \times 10^{-1} )</td>
<td>( 37.9 \pm 0.9 )</td>
</tr>
<tr>
<td>50</td>
<td>( (5.59 \pm 0.4) \times 10^{-3} )</td>
<td>( (3.22 \pm 0.1) \times 10^{-1} )</td>
<td>( 61.1 \pm 1.5 )</td>
</tr>
<tr>
<td>60</td>
<td>( 14.10 \times 10^{-3} )</td>
<td>( 8.9 \times 10^{-1} )</td>
<td>( 63.8 \pm 1.8 )</td>
</tr>
</tbody>
</table>

Figure 5.3. Arrhenius plot for dissociation of DBNBS dimer to monomer. Solid and no fill symbols represents data for the forward and backward reaction respectively.
5.3.2 Generation of NO via nitrosation of ascorbic acid

Nitric oxide in the *ex situ* experiments was generated via the reduction of nitrous acid by ascorbic acid, as shown in Reaction 5.5 [3-5]. Ascorbic acid rapidly and quantitatively generates NO (from NO$_2^-$) at pH levels below 2 [6, 7] and has been used in previous studies as a calibration method when care is taken to eliminate all oxygen present in the aqueous ascorbic acid solution. In the trapping experiments involving the reaction of DBNBS with NO, the NO was generated and DBNBS was injected into the solution once the level of NO reached a maximum level (signifying that all nitrous acid present has reacted with ascorbate.)

\[ \text{Reaction 5.5} \]

\[
\text{C}_6\text{H}_8\text{O}_7\text{H}^+ + 2\text{HNO}_2 \rightarrow \text{C}_6\text{H}_8\text{O}_7\text{H}^+ + 2\text{NO} + 2\text{H}_2\text{O}
\]

5.3.2.1 Effect of varying ascorbic acid concentration on the trapping of NO

Varying the concentration of ascorbic acid, while maintaining concentration of DBNBS constant showed that, if the concentration of ascorbic acid is much greater than the concentration of DBNBS, complete trapping of NO was not observed (Figure 5.4). The results indicate that oxygen was produced during the reaction since nitrous acid formed, most likely via reaction with NO and oxygen. This observation is consistent with the results reported by Beake et al. who proposed the following mechanism to quantitatively describe their experimental results [7]:

\[ \text{O}_2^+ + \text{NO} \rightarrow \text{NO}_2 + \text{O}_2 \]
Here H₂A is the dissociated ascorbic acid molecule, A the dehydroascorbic acid product and HA⁻ the radical generated from the O-nitrosated species, which itself is not kinetically significant in this scheme. The mechanism predicts that each mole of oxygen regenerates 4 moles of nitrous acid through steps R5.7 and R5.8.

As a result of these observations, it is clearly important to maintain the stoichiometry of ascorbic to nitrite in a ratio of 1:2 (or higher) in order to minimise the undesirable side reaction producing nitrous acid. In conditions where significant nitrous acid is formed, it will completely consume the ascorbic acid and consequently generate nitric oxide.
Figure 5.4. NO concentration at various initial concentrations of ascorbic acid (AA) ranging from 0.9 to 9.9 mmol dm$^{-3}$

5.3.3 Kinetic analysis of the trapping of NO _ex situ_

5.3.3.1 _Model development_

The proposed reaction mechanism is illustrated in Table 5.2. Reaction 5.4 describes the monomer-dimer interchange of DBNBS with the rate constants $k_4$ and $k_{-4}$ for DBNBS monomer formation which were determined separately as described above. Reaction 5.11 (see Table 5.2) describes the overall trapping reaction of NO by DBNBS, with a rate constant denoted by $k_{\text{Trap}}$. It is the result of the combination of Reaction 5.1-5.3 yielding oxygen, nitrogen and aryl radical. We group this overall step for the trapping reaction instead of determining individual rate constants as we are unable to
Chapter 5: Thermodynamics and kinetics of the reaction of NO with 3,5-dibromo-4-nitrosobenzene sulfonate

individually measure the species involved in Reactions 5.1-5.3. Reactions 5.12-5.14 are the various pathways of nitrosation, as a result of the formation of oxygen from the trapping Reaction 5.11. The values of rate constants, \( k_{12}, k_{13}, k_{-13}, k_{14}, k_{-14}, k_{15} \) and \( k_{-15} \) were all available from the existing literature.

The formation of a mono-sodium bis (2,6-bromo-4-sulfonyl) nitroxyl species as observed by Davis et al. [8] dominates when the concentration of DBNBS is relatively high and in excess. However, under the concentration range of DBNBS of 0.3 -1.2 mM used in the present study, the mono-sodium bis (2,6-bromo-4-sulfonyl) nitroxyl compound was not observed. Thus under the conditions studied, the only pathway we considered for the aryl radical is reaction with a nitrite ion to yield radical anion (R 5.16) which itself subsequently reacts to form nitro compounds by coupling with \( \text{N}_2\text{O}_3 \) present in the system (R5.17). Reactions 5.16 and 5.17 were assumed to be fast and irreversible, occurring at the diffusion controlled limit with \( k_{16} \) and \( k_{17} = 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1} \).

\[ \text{5.3.3.2 Mass transfer} \]

For NO measurement by the membrane inlet NOx analyser, an additional step to account for the mass transfer of NO from the liquid to gaseous phase (R5.18) is included in the reaction mechanism. Separate experiments, whereby the rate of removal of NO from the aqueous to gaseous phase by rapid injections of known concentrations of NO-saturated solutions into the reactor, established a mass transfer constant \( k_{\text{MT}} \) of \( 0.75 \times 10^{-3} \text{ s}^{-1} \). (Refer to Appendix C for further details)
5.3.3.3 Effect of pH on the trapping reaction

The reaction of DBNBS with NO in buffered solutions of pH 3.5 and 7 and also in unbuffered solution at a pH 4.5 established that the reaction was not dependent on pH. As such, we did not include the reaction involving the protonation of the DBNBS in the proposed model. Details of these experiments and results are provided in Appendix C.

Table 5.2. Proposed reaction mechanism for the ex situ trapping reaction

<table>
<thead>
<tr>
<th>Equ</th>
<th>Reaction</th>
<th>Rate constant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5.4</td>
<td>DBNBS dimer ⇌ 2DBNBS</td>
<td>$k_1 = 0.03 \text{ s}^{-1}$; $k_{-1} = 21.55 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$</td>
<td>Measured in this study</td>
</tr>
<tr>
<td>R5.11</td>
<td>DBNBS + NO → O₂ + N₂ + Aryl radical</td>
<td>$k_{\text{Trap}} = 164.8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$</td>
<td>Fitted parameter in the current study</td>
</tr>
<tr>
<td>R5.12</td>
<td>2NO + O₂ → 2NO₂</td>
<td>$k_{12} = 2.1 \times 10^6 \text{ mol}^2 \text{ dm}^6 \text{ s}^{-1}$</td>
<td>Ref [9]</td>
</tr>
<tr>
<td>R5.13</td>
<td>2NO₂ + H₂O ⇌ HNO₂ + NO₃⁻ + H⁺</td>
<td>$k_{13} = 7.0 \times 10^6 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$; $k_{-13} = 0.0089 \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1}$</td>
<td>Ref [10]</td>
</tr>
<tr>
<td>R5.14</td>
<td>HNO₂ ⇌ NO₂⁻ + H⁺</td>
<td>$k_{14} = 6.93 \times 10^6 \text{ s}^{-1}$; $k_{-14} = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$</td>
<td>Ref [11]</td>
</tr>
<tr>
<td>R5.15</td>
<td>2HNO₂ + NO₂⁻ + H⁺ ⇌ N₂O₅ + H₂O</td>
<td>$k_{15} = 32000 \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1}$; $k_{-15} = 6400 \text{ s}^{-1}$</td>
<td>Ref [11]</td>
</tr>
<tr>
<td>R5.16</td>
<td>Aryl radical + NO₂ → Radical anion</td>
<td>$k_{16} = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$</td>
<td>Assumed value</td>
</tr>
<tr>
<td>R5.17</td>
<td>Radical anion + N₂O₅ → nitro + NO₂ + NO</td>
<td>$k_{17} = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$</td>
<td>Assumed value</td>
</tr>
<tr>
<td>R5.18</td>
<td>NO (aq) → NO(gas)</td>
<td>$k_{\text{MT}} = 0.75 \times 10^{-3} \text{ s}^{-1}$</td>
<td>Estimated in this study</td>
</tr>
</tbody>
</table>
Chapter 5: Thermodynamics and kinetics of the reaction of NO with 3,5-dibromo-4-nitrosobenzene sulfonate

Figure 5.5 demonstrates the model fits as compared to measurements obtained for the DBNBS monomer concentration from the UV-vis spectrophotometer at 25 °C with an initial NO concentration of 0.95 mmol dm\(^{-3}\) and DBNBS concentrations ranging from 0.3 to 1.32 mmol dm\(^{-3}\). The proposed model describes the trapping reaction of NO by DBNBS by assuming a value 164.8 mol\(^{-1}\) dm\(^{3}\) s\(^{-1}\) for \(k_{\text{Trap}}\) at 25 °C. We subsequently assessed \(k_{\text{Trap}}\) with a set of independent data obtained from aqueous NO measurements obtained from the NOx analyser. The plot in Figure 5.6 demonstrates that the proposed model successfully predicts the change in NO concentration with time during the reaction of DBNBS with NO at 25 °C.

Figure 5.5. Model fit as compared to the measurements for the time change of initial (○) 0.3 mmol dm\(^{-3}\), (×) 0.5 mmol dm\(^{-3}\), (+) 0.86 mmol dm\(^{-3}\) and (Δ) 1.32 mmol dm\(^{-3}\) DBNBS at 25 °C for. Solid lines represent model predictions. For clarity every 10\(^{th}\) point is shown.
Based on these experimental results, the rate of DBNBS monomer formation was determined to be the rate determining step in the overall trapping reaction. This was highlighted by measurements of DBNBS consumption when the initial DBNBS concentration was 1.32 mmol dm$^{-3}$, and, where upon reaction with NO, the concentration decreased for the first 5 seconds, followed by an increase in monomer concentration, to finally reach equilibrium. The result also suggests that the rate of trapping is strongly dependent on the concentration of DBNBS which is controlled by the DBNBS dimer-monomer equilibrium.

![Figure 5.6. NO concentration versus time for the ex situ trapping of 0.6 mM NO using 0.3 – 1.2 mM DBNBS. (Δ) 0.1 mol dm$^{-3}$, (□) 0.15 mmol dm$^{-3}$, (○) 0.3 mmol dm$^{-3}$, (⋆) 0.6 mmol dm$^{-3}$ and (⊙) 1.2 mmol dm$^{-3}$. Solid lines represent model fit.](image-url)
5.3.4 In situ trapping of NO.

The trapping of nitric oxide formed in situ from nitrous acid decomposition was studied at concentrations of DBNBS ranging from 0.005 to 0.365 mol dm$^{-3}$ using the membrane inlet NOx analyser. The decomposition of nitrous acid in aqueous solution has been extensively studied [7, 12-16] due to the widespread implications of NOx pollution in the atmosphere. The decomposition pathway for nitrous acid principally involves two equilibria shown in Reactions R5.19 and R5.20.

\[
\text{NO}_2^- + H^+ \rightleftharpoons HNO_2 \quad \text{(R5.19)}
\]

\[
2\text{HNO}_2 \rightleftharpoons \text{NO} + \text{NO}_2 + \text{H}_2\text{O} \quad \text{(R5.20)}
\]

\[
2\text{NO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HNO}_2 + H^+ + \text{NO}_3^- \quad \text{(R5.21)}
\]

However, it is often represented by the overall Reaction 5.22 as it has been demonstrated to have an overall stoichiometry of 3 moles of nitrous acid decomposing to 2 moles of nitric oxide and one mole of nitric acid [6].

\[
3\text{HNO}_2 \rightarrow 2\text{NO} + \text{NO}_3^- + H^+ + \text{H}_2\text{O} \quad \text{(R5.22)}
\]

\[
\frac{d[\text{NO}]}{dt} = \frac{2k_{\text{fwd}}[\text{HNO}_2]^4}{[\text{NO}]^2} - k_{\text{MT}}[\text{NO}] \quad \text{(E5.4)}
\]

We employed the simplified rate law based on Reaction 5.22, considering mass transfer of the aqueous to gaseous to determine the rate constant for the decomposition of nitrous acid to produce NO. The rate law is based on elementary equations taking the assumption that NO$_2$ hydrolysis is the overall rate limiting step. The ODE in Equation
5.4 provided an excellent fit to the experimental data, with the value of \( k_{\text{fwd}} \) of \( 1.32 \times 10^{-6} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1} \) which is consistent with the recent value of \( 1.34 \times 10^{-6} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1} \) reported by Rayson et al. [17]. (Refer to Appendix C for derivation of ODE). Rayson et al. studied the reaction using UV-Vis spectrometry whereas we obtained the rate constant using a NOx analyser thus showing that the membrane NOx analyser provided accurate measurements for NO data. The experimental data for nitrous acid decomposition in the absence and presence of DBNBS at 25 °C are compared in Figure 5.7.

We derived an expression (Equation 5.5) for determining NO concentration formed \textit{in situ} during the trapping reaction based on the proposed reaction mechanism for the \textit{ex situ} reaction. The model involved the following terms: (i) the generation of nitrous acid, (ii) mass transfer between aqueous and gaseous phases and (iii) trapping of NO.

Applying the model to the experimental data for different initial equivalent concentration of DBNBS monomer ranging from 0.005 to 0.365 \( \text{mol dm}^{-3} \), an effective net rate of \( 4.7 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1} \) was obtained. The set of ODE’s describing the in-situ reaction mechanism is provided in Appendix C.

\[
\frac{d[\text{NO}]}{dt} = \frac{2k_{\text{fwd}}[\text{HNO}_2]^4}{[\text{NO}]^2} - k_{\text{Trap}}[\text{DBNBS}][\text{NO}] - 2k_{12}[\text{O}_2][\text{NO}]^2 + k_{17}[\text{N}_2\text{O}_3][\text{Aryl radical}] - k_{M1}[\text{NO}]
\]

(E5.5)

Figure 5.8 shows experimental measurements of NO generation from the trapping of nitric oxide by DBNBS for initial equivalent DBNBS concentrations between 0.005 and 0.36 \( \text{mol dm}^{-3} \). According to the measurements, the kinetics are similar to the decomposition of nitrous acid; most notable is the rapid initial decomposition.
Chapter 5: Thermodynamics and kinetics of the reaction of NO with 3,5-dibromo-4-nitrosobenzene sulfonate

whereby NO is formed. However, the amount of NO generated by this initial rapid decomposition was approximately half the amount compared to conditions where DBNBS was absent. Following this rapid generation of NO, a rapid decrease in the concentration of NO was observed, suggesting that the rate of NO trapping dominates over the rate of nitrous acid decomposition. We also note that when the concentration of DBNBS monomer was significantly increased and equal to the concentration of nitrite, the initial rapid rate of decomposition was 3-fold lower compared to conditions where DBNBS was absent. Complete trapping of NO was never observed under the conditions employed but a very large reduction of 97.8% was observed.

From the kinetics analysis, the value of 164.8 mol\(^{-1}\) dm\(^3\) s\(^{-1}\) obtained from the model of the ex situ reaction represents the apparent rate constant for the trapping of NO, \(k_{\text{Trap}}\). We found that that the experimental data from the in situ reactions did not fit the model using the apparent rate constant and rather the effective rate during the in situ reaction was 35 fold slower compared to the rate predicted from the ex situ experiments. This suggest additional chemical reaction(s) are occurring when the concentration of nitrite is relatively high. Such difficulties has been reported in a number of studies where nitrite was involved and rate involving these reactions were unable to be quantified experimentally [18, 19]. The possibility of a slower rate of trapping should also be considered as the rate of reverse reaction for Reaction 5.1 could become significant when there is simultaneously elevated concentration of nitrous acid the formation of nitric oxide is relatively slow. This can therefore reduce the overall rate constant \(k_{\text{Trap}}\).
Figure 5.7. Experimental nitrous acid decomposition in the absence of DBNBS (□) and 0.015 mol dm$^{-3}$ (○) DBNBS at 25 °C.

Figure 5.8. Change in NO concentration versus time for the in situ trapping for different quantities of DBNBS and initial 0.015 mol dm$^{-3}$ sodium nitrite at 25 °C. (○) 0.005 mol dm$^{-3}$, (×) 0.015 mol dm$^{-3}$, (□) 0.03 mol dm$^{-3}$ and (Δ) 0.365 mol dm$^{-3}$.
An additional set of experiments was undertaken to determine whether NO could be completely removed following the initial decomposition of nitrous acid. In these experiments DBNBS was introduced into the solution well beyond the point of rapid increase in NO as illustrated in Figure 5.9. Even under these conditions, complete reduction of NO was not achieved. This is most likely due to the continuous and relatively slow decomposition of nitrous acid. Nevertheless under these conditions a 98% reduction in NO was observed after 1 hour of reaction with the trap.

Figure 5.9. Effect of addition of different concentrations of DBNBS after the initial rapid generation of NO by nitrous acid decomposition. Open arrow indicates the time of injection of DBNBS. (○) 0 mol dm^{-3}, (+) 0.005 mol dm^{-3}, (Δ) 0.010 mol dm^{-3} and (×) 0.015 mol dm^{-3}.

Chemical trapping of NO
5.4 Conclusion

A kinetic study of the trapping of NO under *ex situ* conditions was undertaken and the resulting measurements were also fitted by a proposed mechanistic model. Agreement between experimental results and model for the current mechanistic understanding of the trapping reaction suggests the key reaction steps are included in the model, although the kinetic parameters of one reaction step $k_{\text{Trap}}$, were estimated by fitting of experimental data. The study also demonstrated that the model obtained under *ex situ* conditions cannot be directly applied to NO trapping under *in situ* conditions where further additional, and as yet unknown, steps due to the presence of added nitrite are needed to be included in the *in situ* reaction model. *In situ* experiments revealed that complete trapping of NO could not be achieved, most likely because of the slow generation of NO from the decomposition of nitrous acid in this reaction. However, an overall reduction of 97.8 % NO was achieved under *in situ* conditions, highlighting the potential of DBNBS as a NO scavenger for environmental control of NOx emissions.
5.5 References


CHAPTER 6: DETERMINATION OF THE ACID DISSOCIATION CONSTANT OF NITROSOBENZENE SULFONATE AND 3,5-DIMETHYL-4-NITROSOBENZENE SULFONATE
Chapter 6: Determination of the acid dissociation constant of nitrosobenzene sulfonate and 3,5-dimethyl-4-nitrosobenzene sulfonate

6.1 Introduction

Nitroso compounds represent a major class of important chemicals in various biological metabolic processes [1]. For example, biochemists employ S-nitrosothiols (RSNOs) as non-toxic nitric oxide (NO) donors that pharmacologically release NO in vivo [2]. Some C-nitroso compounds arising as reactive intermediate in biological system either by oxidation of amines or by reduction of nitro compounds act as toxins [3]. Some nitroso compounds also serve as trapping agents in the detection of short-lived free radicals such as nitric oxide by forming a stable adduct with characteristic hyperfine splitting detectable by electron paramagnetic resonance spectrometry (EPR) [4, 5]. Two such compounds, nitrosobenzene sulfonate (NBS) and 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS), initially developed as spin traps, act as potent scavengers of nitric oxide (NO).

Our interest has been focused on the application of these compounds to trap NO formed during the sensitisation of ammonium nitrate emulsion explosives. However, unlike 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) NBS and DMNBS constitute of relatively weak acids existing in solution in both protonated and deprotonated form [6]. DBNBS is another potent scavenger of NO which is a relatively strong acid with $pK_a$ of around -2.2. The sensitisation of ammonium nitrate explosives via chemical gassing occurs in the pH range of 3.5 to 4.5, and, as such, it is possible that under these pH conditions NBS and DMNBS exist in both protonated and unprotonated states (see Equation 6.1). The trapping reaction will not proceed efficiently if nitroso compounds remain protonated at the N=O site (the active site for trapping NO). Consequently, knowledge of the acid dissociation constant of these two nitroso compounds is essential.
to perform kinetic analysis of their rate of reaction with nitric oxide during the trapping reaction.

**Chemical trapping of NO**

\[
\begin{align*}
\text{X}= & \text{CH}_3 \text{ or H} \\
\end{align*}
\]

Among the techniques currently available for determining an acid dissociation constant, which include potentiometric titration, UV-Vis spectroscopy, NMR spectroscopy, ion conductivity, calorimetry, capillary zone electrophoresis, potentiometry constitutes the most common approach due to its accuracy and reproducibility [7-9]. During potentiometric titration, a sample is titrated with acid or base using a pH electrode to monitor the course of the pH during titration. The value of \( pK_a \) is estimated from a change in slope of titration curve compared with that of a blank titration where the sample is absent [10].

Meanwhile with the recent progress in computation chemistry and the introduction of dielectric continuum models [11, 12], a substantial amount of work has been performed to investigate the solvent effect on the thermodynamics of chemical reactions. Proton transfer reactions, which enable the determination of the acid dissociation constant, remain by far the most studied due to their importance in chemical and biological...
The prediction of the acid dissociation constant via quantum chemical calculations represents a technically challenging task, as an error of 1.36 kcal/mol in the change of free energy of reaction results in an error of 1 \( pK_a \) unit [14]. Nevertheless, it remains worthwhile to develop a method to determine the aqueous \( pK_a \) for nitroso compounds which can be employed to predict \( pK_a \) values of other nitroso compounds. Moreover, \( pK_a \) values can be estimated by computational methods on the basis of molecular structure, and together with experimental data, can assist in understanding factors which influence the acidic character of substituted nitrosobenzene sulfonates.

The present Chapter aims to establish the \( pK_a \) of NBS and DMNBS experimentally via potentiometric titration. The technique, standards and analysis method were validated using dichloroacetic acid as a model compound, whose \( pK_a \) is well established. A computational method, which utilises the polarisable continuum model with the 6-31+G basis set together with BB1K and B3LYP density functional methods, was followed for the calculation of aqueous \( pK_a \) values and comparison of the experimentally determined \( pK_a \) values. In addition, commercially available software assisted in the estimation of \( pK_a \) values [15]. The software calculates the partial charge distribution on each of the atoms, which is very sensitive to protonation of acid/base active sites and can be used to determine the \( pK_a \) of a molecule. Results obtained from the theoretical model and software methods were compared with the experimentally obtained \( pK_a \) data.
6.2 Methodology

6.2.1 Potentiometric pKₐ determination

Titrations in aqueous solution were performed using a Metrohm 665 Dosimat burette for the addition of NaOH, controlled by a PC using Matlab software. In all experiments, the temperature was maintained at 25 ± 0.5 °C using a thermostated with water circulating through the jacket. All experiments involved solutions adjusted to a constant ionic strength of 0.1 mol dm⁻³ NaNO₃. Titrations were performed in a nitrogen gas atmosphere. Nitrogen was first bubbled for 10 min prior to performing titrations through the solution contained in an air-tight jacketed vessel and then passed over the continuously stirred solution throughout the titration. A Metrohm pH electrode (3 mol dm⁻³ KCl filled) was used to monitor the change in pH, with an output reading in mV. A solution of 0.1 mol dm⁻³ NaOH (1–3 cm³) was added stepwise (0.002–0.005 cm³ increments) from the Dosimat to a solution (10 cm³) of 0.01 mol dm⁻³ nitrosocompounds and 0.01 mol dm⁻³ HCl in order to adjust the initial pH to ~12. Triplicate titrations of each system were performed. The log β (which is equivalent to pKₐ, as the compound is assumed to be monoprotic from pH 0 to 14) value and species distribution curves for the different titrations were estimated using commercially available software. The software estimates the acid dissociation constant using a non-linear refinement that requires initial values of logβ to be entered as input data [16].
6.2.2 Calibration of electrode

In the procedure for calibrating the electrode, a strong acid was titrated with a strong base. A volume of 10 cm$^3$ of 5 mmol dm$^{-3}$ HCl was added in the titration vessel and was titrated with 10 mmol dm$^{-3}$ NaOH. The HCl was standardized by titrating against a solution of NaOH of known concentration. A computer program was then employed to assist with the calibration of the glass electrode in terms of hydrogen ion [17]. The program provides the pseudo-Nernstian standard potential and slope of the electrode and, in particular, an estimate of the carbonate contamination of the base allowing the experimental calibration curve to be fit within experimental error over the pH ranges 2.5 to 4.5 and 10.7 to 11.5.

6.2.3 Theoretical model

A variety of theoretical techniques and methods have been developed to estimate acid dissociation constants. Most make use of thermodynamic cycles in order to improve the accuracy of solution-phase free energies. In the present study, we employed the thermodynamic cycle A depicted in Figure 6.1, based on Reaction 6.1 to determine $\Delta G_{\text{aq}}$.

$$
\begin{align*}
\text{HA}_{(aq)} & \xrightarrow{\Delta G_{\text{aq}}} \text{A}^-_{(aq)} + \text{H}^+_{(aq)} \\
\Delta G_{\text{solv}}(\text{HA}) & \quad \Delta G_{\text{solv}}(\text{A}^-) \quad \Delta G_{\text{solv}}(\text{H}^+) \\
\text{HA}_{(g)} & \xrightarrow{\Delta G_{\text{gas}}} \text{A}^-_{(g)} + \text{H}^+_{(g)}
\end{align*}
$$

Figure 6.1. Proton-based thermodynamic cycle A
Chapter 6: Determination of the acid dissociation constant of nitrosobenzene sulfonate and 3,5-dimethyl-4-nitrosobenzene sulfonate

In Figure 6.1, $\Delta G_{aq}$ represents the overall change in free energy of the reaction in solution, $\Delta G_{gas}$ is the change in the gas-phase free energy and $\Delta G_{solv}$ is the change in free energy of solvation. Based on the diagram, $pK_a$ is calculated using the following equations:

\[ pK_a = \Delta G_{aq}RT \ln(10) \]  \hspace{1cm} (6.2)

\[ \Delta G_{aq} = \Delta G_{gas} + \Delta \Delta G_{solv} \]  \hspace{1cm} (6.3)

Where,

\[ \Delta G_{gas} = G_{gas}(H^+) + G_{gas}(A^-) - G_{gas}(HA) \]  \hspace{1cm} (6.4)

and

\[ \Delta \Delta G_{solv} = \Delta G_{solv}(H^+) + \Delta G_{solv}(A^-) - \Delta G_{solv}(HA) \]  \hspace{1cm} (6.5)

Thus $pK_a$ can be obtained using theoretical values of $\Delta G_{gas}$ and $\Delta G_{solv}$ of the protonated and deprotonated species of nitroso compound, with an experimental value for the solvation free energy of proton, $\Delta G_{solv}(H^+)$. We used an experimental value of $-259.5$ kcal/mol for $\Delta G_{solv}(H^+)$ [18]. Since the gas phase reaction energies have a standard state of 1 atm, whereas solution phase and solvation free energies have a standard state of 1 mol L$^{-1}$ for the solute in both the gas phase and solution phase, a correction factor corresponding to $\Delta nRT\ln(\bar{R}T)$ was added to Equation 6.3. $\Delta n$ corresponds to the difference in the number of moles of product and reactants, and $R$ and $\bar{R}$ refer to the universal gas constant in units of 8.314 J mol$^{-1}$ K$^{-1}$ and 0.0821 L atm mol$^{-1}$ K$^{-1}$, respectively.
6.2.4 Computational details

All calculations were performed with the Gaussian 09 software package [19]. The geometries of the neutral and the protonated species were fully optimised at the BB95/6-31G and B3LYP/6-31G levels of theory [20]. Frequency calculations were carried out at the same level of theory to characterise the stationary points obtained. For each of the compounds investigated, the gas-phase Gibbs free energy change was first calculated. The solvation free energies were then calculated by applying the polarisable continuum model method using the same DFT level and basis set which were used for geometry determination in the gas phase. We assumed that reaction occurs in an implicit water model, described by the conductor-like polarisable continuum model (PCM) [21]. We used the united atom topological model applied on atomic radii of the universal force field (UFF) for the cavity of molecules, which is the default of Gaussian 09.

6.2.5 Empirical method for predicting $pK_a$

ChemAxon Marvin 5.3.8 software was run using the default options on a Windows XP computer [15]. Molecules were analysed using the protonation option of MarvinSketch, to estimate $pK_a$. 
6.3 Results and discussion

6.3.1 Analysis methods for potentiometry

The analysis method to derive $pK_a$ from titration curves is based on a least squares non-linear regression, and was developed from the acid-base equilibrium. For a monoprotic weak acid, the volume base added to the titration ($V_B$) at any pH can be determined using Equation 6.6 [22, 23].

$$V_B = V_A \frac{C_A \alpha_1 - [H^+] + [OH^-]}{C_B + [H^+] - [OH^-]} \quad (6.6)$$

The fraction of conjugate base, $\alpha_1$ is expressed by

$$\alpha_1 = \frac{K_a}{[H^+] + K_a} \quad (6.7)$$

where, $V_A$ is the total volume of a monoprotic acid (titrate)

$V_B$ is the calculated volume of base (titrant) added to the titration

$K_a$ is the acid dissociation constant

$C_A$ and $C_B$ are the concentrations of acid and base, respectively.

The theoretical volume of base added to reach a certain pH can be calculated from Equation 6.7. Coincidently, the experimentally determined volume of base added, $V_{EB}$,
to reach the same pH can be directly measured during titration. The dissociation constant, $K_a$, can therefore be computed by minimising the sum of $(V_{EB} - V_B)^2$.

### 6.3.2 Ionic strength correction

The acid dissociation constant of a weak monoprotic (HA) in an aqueous solution with a controlled ionic strength is defined as

$$K_a = \frac{[H^+][A^-]}{[HA]} \cdot \frac{\gamma_{HA}^{H^+} \gamma_{A^-}}{\gamma_{HA}}$$  \hspace{1cm} (6.8)$$

Where $\gamma_{H^+}$, $\gamma_{HA}$ and $\gamma_{A^-}$ are the activity coefficient of proton, acid and its conjugate base respectively. Given that activity coefficient of HA is assumed as unity [3], and the pH of the solution as $-\log (\gamma_{H^+} [H^+])$, Equation 6.8 can be related to the $pK_a$

$$pK_a = pH - \log \frac{[A^-]}{n[HA]} \cdot \log \gamma_{A^-}.$$  \hspace{1cm} (6.9)$$

The activity coefficient can be calculated by Güntelberg approximation

$$\log \gamma = -0.51z^2 \frac{\sqrt{I}}{(1+\sqrt{I})}$$  \hspace{1cm} (6.10)$$

Where $I$ is the solution ionic strength (M) and $z$ is the ion charge.

By combining Equations 6.9 and 6.10 we obtained Equation 6.11 which was employed to correct the effect of ionic strength on $pK_a$ determination.

$$pK_a = pH - \log \frac{[A^-]}{n[HA]} + 0.51(z_{HA}^2 - z_{A^-}^2) \frac{\sqrt{I}}{(1+\sqrt{I})}$$  \hspace{1cm} (6.11)$$
6.3.3 Method validation

The applicability of the analysis was established by the potentiometric titration of dichloroacetic acid. Based on the experimental data obtained, and applying the software analysis followed by the correction for the effect of ionic strength, a $pK_a$ was determined to be $1.50 \pm 0.03$. This value is in good agreement with literature value of 1.48 [24]. The result also indicates that the use of least squares regression is an appropriate method for determining the $pK_a$ of a weak acid. The titration curve for dichloroacetic acid is shown in Figure 6.2. From the plot, Sigma (σ) is a measure of overall goodness of fit.

![Figure 6.2](image)

**Figure 6.2.** Titration curve for determining the $pK_a$ of dichloroacetic acid. The symbol (◊) indicates the experimental value and the dotted line indicates the theoretical titration curve. Red and blue line denote unprotonated and protonated species respectively.
6.3.4 Experimental measurement of $pK_a$ values of nitroso compounds

In order to obtain an estimate for the acid dissociation constant, the experimentally obtained potentiometric data were fitted using Hyperquad software. The model provides a reasonably good fit to the experimental values for both compounds. The measurements for the titrations of NBS and DMNBS in diluted HCl with NaOH solution are shown in Figures 6.3 and 6.4, respectively.

![Titration curve for nitrosobenzene sulfonate](image)

**Figure 6.3.** Titration curve for nitrosobenzene sulfonate. The symbol (◊) indicates the experimental value and the dotted line indicates the theoretical titration curve. Red and blue lines denote unprotonated and protonated species respectively.
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Figure 6.4. Titration curve for 3,5-dimethyl-4-nitrosobenzene sulfonate. The symbol (○) indicates the experimental value and the dotted line indicates the theoretical titration curve. Red and blue lines denote unprotonated and protonated species respectively.

pKₐ values for NBS and DMNBS were estimated to be 1.26±0.38 and 2.28±0.04 respectively based on analysis of the experimental data. These data suggest that, these two compounds are relatively weak acids in contrast to DBNBS and DCNBS. The presence of bromine (in DBNBS) and chlorine (in DCNBS) in the ortho position of the nitroso compounds (which are more electronegative than either hydrogen or methyl) enhances the electron density at the halogen moiety. The consequently reduced electron density of the nitroso group in these compounds therefore results in net increase in acid strength. We also noted that the pKₐ value of DMNBS is slightly higher than that of NBS. The CH₃ group present in DMNBS increases the basic character of the compound, due to its electron donating ability in comparison with hydrogen.
6.3.5 Theoretical calculation of $pK_a$

Initially, $pK_a$ values were calculated for NBS and DMNBS using various basis sets with the B3LYP simulation method (Table 6.1). The use of diffuse functions, is to provide a more accurate description of anion or neutral molecules with unshared electron pairs. In addition, processes that involve a change in the number of unshared pairs, such as protonation, are better modelled if diffuse functions are included. Comparison of the computational results with the experimental data shows that best agreement for nitroso compounds was obtained using the 6-31+G(2d,p) basis set while the addition of a second diffuse function did not improve the results. Larger basis sets such as 6-311+G(d,p) or 6-311++G(2d,p) did not systematically improve the accuracy of the results, in fact it was found that disagreement was larger in these examples.

Since the error in the computation of $pK_a$ by the B3LYP method was quite significant, in particular for DMNBS, different DFT methods such as CBS, MP2 and BB1K were also attempted. Calculations with CBS-QB3 and MP2 were not possible since a high level of energy calculation is difficult to extend to larger molecules such as DMNBS. On the other hand, BB1K, developed by the Truhlar group and usually employed for predicting kinetics data [25], somewhat surprisingly gave estimations closer to the experimentally determined $pK_a$ values for both compounds when compared to B3LYP results, as shown in Table 6.2.
### Table 6.1

Calculated p$K_a$ values of nitroso compounds using different basis sets with B3LYP

<table>
<thead>
<tr>
<th>Basis set</th>
<th>NBS p$K_a$</th>
<th>Error in p$K_a$</th>
<th>DMNBS p$K_a$</th>
<th>Error in p$K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-31+G(d)</td>
<td>-3.56</td>
<td>4.82</td>
<td>-4.44</td>
<td>6.72</td>
</tr>
<tr>
<td>6-31+G(d,p)</td>
<td>-1.20</td>
<td>2.46</td>
<td>-1.88</td>
<td>4.16</td>
</tr>
<tr>
<td>6-31+G(2d,p)</td>
<td>-0.22</td>
<td>1.48</td>
<td>-0.50</td>
<td>2.78</td>
</tr>
<tr>
<td>6-31+G(2d,2p)</td>
<td>-3.65</td>
<td>4.91</td>
<td>-0.75</td>
<td>3.03</td>
</tr>
<tr>
<td>6-31++G(d)</td>
<td>-7.46</td>
<td>8.72</td>
<td>-4.42</td>
<td>6.70</td>
</tr>
<tr>
<td>6-31++G(2d)</td>
<td>-6.33</td>
<td>7.59</td>
<td>-3.34</td>
<td>5.62</td>
</tr>
<tr>
<td>6-311+G(d,p)</td>
<td>-5.79</td>
<td>7.05</td>
<td>-3.63</td>
<td>5.91</td>
</tr>
<tr>
<td>6-311++G(2d,p)</td>
<td>-7.85</td>
<td>9.11</td>
<td>-1.17</td>
<td>3.45</td>
</tr>
</tbody>
</table>

*Calculated as the difference between the experimental and the theoretical values.
Table 6.2. Gas phase Gibbs free energies, solvation energies and calculated p$K_a$ values of nitroso compounds using different level with B3LYP/6-31G and BB1K/6-31G

<table>
<thead>
<tr>
<th>Compound</th>
<th>B3LYP/6-31+g(2d,p)</th>
<th>BB1K/6-31+G(2d,p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G_0$ (a.u.)</td>
<td>$\Delta G_{sol}$ kcal mol$^{-1}$</td>
</tr>
<tr>
<td>NBS</td>
<td>-984.85</td>
<td>-58.48</td>
</tr>
<tr>
<td>NBS-H</td>
<td>-985.29</td>
<td>-35.02</td>
</tr>
<tr>
<td>DMNBS</td>
<td>-1063.44</td>
<td>-60.13</td>
</tr>
<tr>
<td>DMNBS-H</td>
<td>-1063.87</td>
<td>-37.64</td>
</tr>
</tbody>
</table>

$^\dagger$Calculated as the difference between the experimental and the theoretical values.

6.3.6 Comparison of p$K_a$ determination methods

The method employed for predicting p$K_a$ of organic molecules relies on empirically estimated physico-chemical parameters that are obtained from ionisation site-specific regression equations [26]. The calculated p$K_a$ values of the monoprotic molecules are assumed to be the sum of the next three variables as outlined in Equation 6.12 [15].

$$pK_a = a^*Q + b^*P + c^*S + d^*a \quad (6.12)$$

where, $Q$ is the partial charge increment, $P$ the polarisability increment, $S$ sum of the structure specific increments, and $a$, $b$, $c$, and $d$ are regression coefficients specific to the ionisation site.
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Szegezdi et al. examined 1670 molecules to assess the performance of the pK_a estimation model, by comparing the calculated pK_a values with experimentally determined values [26]. Their study showed a good correlation (r=0.95, s=0.72, n=1670) was obtained for a number of organic compounds and pharmaceutical molecules.

From Table 6.3, the pK_a values of NBS and DMNBS obtained by using the software is in good agreement with the experimental values obtained by potentiometric titration. Thus accordingly, the empirical method for the pK_a prediction employed provides reasonably accurate results for nitroso compounds with 0.12-0.32 pK_a unit error. This approach for determining pK_a is quick and undemanding in terms of CPU time and memory as opposed to quantum chemical methods (DFT).

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK_a determination method</th>
<th>a Determined by potentiometric titration</th>
<th>Estimated using commercial software</th>
<th>b Based on quantum chemical simulation, with basis set BB1K/6-31+G(d,p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="" /></td>
<td>1.26</td>
<td>1.14</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td><img src="image2" alt="" /></td>
<td>2.28</td>
<td>1.96</td>
<td>2.08</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated by fitting the potentiometric data to the program Hyperquad 2008
* The solvation calculations were carried out using the BB1K/6-31+G(d,p) and the PCM model.
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6.4 Conclusion

We investigated the acid dissociation constant of NBS and DMNBS using experimental and computational techniques. Potentiometric titration was used to obtain a titration curve and $pK_a$ values of 1.26 and 2.28 at 25 °C were determined for NBS and DMNBS respectively. These values suggest that both compounds are weakly acidic, with DMNBS being more basic due to the presence of the electron donating methyl group.

The $pK_a$ values of these compounds were also compared with results of quantum chemical calculations based on functional and basis sets and with empirical methods. With respect to the quantum chemical calculations, the aqueous phase $pK_a$ of nitroso compounds were estimated via the proton exchange thermodynamic cycle whereby the estimation of the gas phase acidities and solvation free energies were used. As $pK_a$ values are directly proportional to $\Delta G_{solv}$, the free energy change for the transition from protonated to deprotonated state, a relatively minor error in estimating $\Delta G_{solv}$ can lead to a significant error for $pK_a$, such is the case when the B3LYP method was applied. In contrast, the application of empirical methods for predicting $pK_a$ compared reasonably well with experimental results for aromatic nitroso sulfonate compounds, with the advantage that the computational requirements for empirical simulations are very favourable when compared to the use of DFT methods.
6.5 References


Chapter 6: Determination of the acid dissociation constant of nitrosobenzene sulfonate and 3,5-dimethyl-4-nitrosobenzene sulfonate


CHAPTER 7: COMPARATIVE STUDY OF PHYSICOCHEMICAL PROPERTIES OF ORTHO SUBSTITUTED AROMATIC NITROSO COMPOUNDS

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7.1 Introduction

The release of nitric oxide formed during the sensitisation of ammonium nitrate emulsion explosives can affect the health of underground miners, unless they are trained to avoid excessive acidification of the sensitised emulsion or are required to evacuate during the gassing process [1]. Thus, there is a practical need to develop new technologies that minimise the risk of exposure of workers to NO. In Chapter 4, we described the application of 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) under conditions simulating the chemical gassing process of emulsion explosives to effectively reduce the net amount of nitric oxide released from the reaction mixture. The N=O moiety in the nitroso compound is generally believed to be responsible for the trapping of the nitric oxide radical, and thus potentially any nitroso trap, which is soluble in aqueous conditions and does not affect the pH of the chemical gassing reaction, could act as a nitric oxide scavenger. As established in Chapter 5, the rate of DBNBS monomer formation greatly influences the overall rate of reaction of DBNBS with NO. The pace of monomer formation is the rate-determining step in the overall reaction. Consequently, if the nitroso compound exists primarily in a monomeric form, then the rate of the reaction is at or close to its maximum rate.

When determining the equilibrium constant, $K_C$, for the DBNBS dimer-monomer equilibrium, it was established that the majority of dissolved DBNBS was in a dimeric form and only a relatively small proportion of DBNBS existed as monomers, suitable for participating in the trapping reaction. Fortunately in aromatic nitroso compounds, conjugation between the electron donating substituent in the para position and the nitroso group enhances the stability of the monomeric form of the nitrosobenzene with respect to its dimer [2]. Conversely, the stabilisation of the dimer form relative to
monomeric derivative is caused by the ortho-substitution and is most pronounced when both ortho-positions are occupied by substituent groups other than hydrogen [5]. In the case of DBNBS, the dimer configuration is favoured due to the presence of bromo moieties at both ortho positions. With the aim of developing efficient nitroso traps, which by necessity exist primarily in monomeric form, we note that nitrosobenzene should have less electron withdrawing substituent at the para position and less bulky groups at the ortho position.

Based on these considerations, we investigated a range of nitroso compounds, namely nitrosobenzene sulfonate (NBS), 3,5 dichloro-4-nitrosobenzene sulfonate (DCNBS) and 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS). These compounds are congeners of DBNBS, and all have the common feature of the presence of a sulfonate group attached to benzene ring in the para position with respect to the nitroso group which enhances their water solubility. They are distinct, however, as they all have different substituents at the ortho position of the aromatic ring; bromine (DBNBS), chlorine (DCNBS), methyl (DMNBS) and hydrogen (NBS).

Thermodynamic properties were determined by means of UV-Vis spectrometry at temperatures ranging from 25 to 60 °C. Absorption UV-Vis spectrophotometry is an ideal analytical tool for quantitative analysis of nitroso compounds as a function of concentration. This is because nitroso compounds in solutions have a characteristic monomer absorption band centred in the range of 630 to 790 nm due to n-π* transition of non bonded electrons localised on nitrogen [4].

An understanding of the equilibrium constant, \( K_C \), is essential in order to calculate the concentration of the monomer capable of trapping NO, and is important during the analysis of kinetic data obtained from the trapping reaction with nitroso spin traps in
chemical systems. We also study the kinetics of the dissociation of aromatic nitroso sulfonate dimer to monomer at different temperatures to calculate the activation parameters: enthalpy of activation (\(\Delta H^\dagger\)), entropy of activation (\(\Delta S^\dagger\)) and Gibbs free energy of activation (\(\Delta G^\dagger\)).

### 7.2 Methodology

#### 7.2.1 Material

Nitroso compounds (3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), 3,5-dichloro-4-nitrobenzene sulfonate (DCNBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and nitrosobenzene sulfonate (NBS)) were synthesized following published methods and procedures [5-7]. Refer to Chapter 3 for detailed methods.

#### 7.2.2 Calculated chemical structure of aromatic nitroso sulfonate

All calculations were carried out with Gaussian 09 software package [8]. The geometries of aromatic nitroso sulfonate compounds were optimised at the B3LYP/6-311+G(d,p) level of theory. Enthalpies of solvation were determined at the same level of theory, with the polarisable continuum model (PCM) for water as solvent, as implemented in Gaussian 09 program [9].

\[
\begin{align*}
\text{O}_3\text{S} & \quad \text{X} \quad \text{N} \quad \text{O} \\
\text{O}_3\text{S} & \quad \text{X} \quad \text{N} + \text{O} \\
\text{where X}= & \text{H, CH}_3, \text{Cl, Br}
\end{align*}
\]
The C-N bond dissociation energy (BDE) in aromatic nitroso sulfonate compounds was calculated using Equation 7.1 [6].

\[
\text{BDE}(\text{R-NO}) = \Delta H_f^\circ (\text{R}) + \Delta H_f^\circ (\text{NO}) - \Delta H_f^\circ (\text{R-NO})
\]  

(7.1)

where the standard state is a hypothetical 1 molar solution at 298.15 K.

7.2.3 Thermodynamics of aromatic nitroso sulfonate dimer/monomer interchange

7.2.3.1 Experimental procedure

Samples for UV-visible spectrum analysis were prepared by dissolving synthesised DBNBS, NBS, DCNBS and DMNBS (final concentration \((0.05 - 8) \times 10^{-3} \text{ mol dm}^{-3}\) monomer added) in distilled, deionised water. For each solution, the visible spectra 400-900 nm were recorded at 25, 30, 40, 50 and 60 °C on a Varian Cary 50 Scan UV-Visible spectrophotometer coupled with a single cell Peltier thermostated cell holder (Varian, Inc) using a sealed quartz cell (optical length, 1 cm). A single-cell Peltier-thermostated cell holder (Varian, Inc.) served to control the temperature of the quartz cell (± 0.5 °C).
7.2.3.2 Monomer-dimer equilibrium constant

The dimer-monomer equilibrium is shown in Reaction R.1 and the corresponding equilibrium constant is given in Equation 7.2

\[
\text{nitrnoo dimer} \rightleftharpoons 2 \text{nitrnoo monomer} \quad k_1, k_1 \quad (R7.1)
\]

\[
K_C = \frac{[\text{Monomer}]^2}{[\text{Dimer}]} \quad (7.2)
\]

Under the assumption that the light absorption at or near the wavelength of maximum absorption is only due to the monomer, Holmes et al. [3] used Equation 7.2 and the Beer law to obtain Equation 7.3. Derivation of Equation 7.3 is presented in Chapter 5.

\[
\frac{D}{C_i L} = \frac{D^2}{C_i L^2} \left( \frac{2}{K_C} \right) + \quad (7.3)
\]

Where, \(D\) is the optical density, \(C_i\) is concentration of initial equivalent monomer of nitroso compound (M), \(\varepsilon\) is the molar extinction coefficient per centimetre of cell length, \(L\) is the optical path length (cm) and \(K_C\) is the equilibrium constant for the overall reaction. The equilibrium constant \(K_C\) is taken as a dimensionless number with all concentration values referenced to a standard state. From Equation 7.3, a plot of \(D/C_i L\) against \(D^2/C_i L^2\) should be linear, and the intercept allows an estimation of the extinction coefficient and the slope yields \(K_C\).
7.2.3.3 Thermodynamics parameters

The standard enthalpy change, $\Delta H^\circ_R$, and the standard entropy change $\Delta S^\circ_R$ for Reaction 7.1 were determined by applying the van’t Hoff equation (Equation 7.5), using the $K_C$ values obtained at different temperatures

$$\ln K = \frac{\Delta H^\circ_R}{RT} + \frac{\Delta S^\circ_R}{R}$$  \hspace{1cm} (7.5)

Where, $K$ is the equilibrium constant, $R$ is the gas constant $= 8.314472 \text{ J K}^{-1} \text{ mol}^{-1}$.

7.2.4 Kinetics of aromatic nitroso sulfonate dimer/monomer interchange

7.2.4.1 Experimental procedure

The rapid dilution of a known initial concentration of aromatic nitroso sulfonate with distilled deionised water was studied by stopped flow UV-Vis spectrophotometer (RX-2000 rapid mixing accessory from Applied Photophysics coupled to a Varian Cary 50 UV-Visible spectrophotometer). Rapid dilution allows the dissociation of nitroso dimer favouring formation of the monomer and shifts the equilibrium to favour monomer formation. Different (initial) concentrations of aromatic nitroso sulfonates $(3.75 - 10) \times 10^{-3} \text{ mol dm}^{-3}$ were examined. Absorbance at the wavelength of maximum absorption was measured in order to determine the concentration of monomer using extinction coefficients for each aromatic nitroso sulfonate species. The effect of temperature $(25$ to $60 \degree \text{C})$ on the monomer/dimer equilibrium distribution was also performed to determine the enthalpy and entropy of activation for the reaction of each aromatic...
nitroso compound. A circulation water bath was employed to control the temperature in the stopped flow module and the cell compartment.

Reaction 7.1 was incorporated into software package (DYNAFIT) [12] and the rate of formation of monomer (k₁) and dimer (k₃) were obtained from global non-linear least square fitting of monomer concentration profile. The effect of temperature on the rate of the reaction of dimer-monomer interchange was studied using the Arrhenius equation. The Arrhenius equation shown in Equation 7.6 relates the rate constant of a reaction to the temperature and the activation energy, $E_a$.

$$k_A(T) = A e^{-E_a/RT}$$ (7.6)

Where $A = \text{pre-exponential factor}$

$E_a = \text{activation energy, kJ mol}^{-1}$

$R = \text{gas constant} = 8.314 \text{ J mol}^{-1}$

$T = \text{absolute temperature, K}$

### 7.2.4.2 Activation parameters $\Delta H^\ddagger$, $\Delta S^\ddagger$ and $\Delta G^\ddagger$

An alternative to Arrhenius theory is the transition-state theory which was introduced by Eyring et al. in 1935 [11, 12] (See Equation 7.7). In this approach, the transition state is considered to be a distinct chemical species whose potential energy is the highest in the reaction pathway and in equilibrium with the reactants whilst the rate of reaction corresponds the product of the concentration of the activated complex and the frequency with which this species passes to the product state. Subsequently, an Eyring
plot can be employed to determine the activation parameters $\Delta S^\ddagger$ and $\Delta H^\ddagger$ from the temperature dependence of the rate constant.

$$\ln \frac{k}{T} = - \frac{\Delta H^\ddagger}{RT} + \ln \frac{k_B}{h} + \frac{\Delta S^\ddagger}{R}$$  \hspace{1cm} (7.7)

Where,

- $k$ is reaction rate constant
- $T$ is absolute temperature
- $\Delta H^\ddagger$ is enthalpy of activation
- $h$ is the Planck's constant
- $k_B$ is the Boltzmann's constant
- $R$ is the gas constant
- $\Delta S^\ddagger$ is entropy of activation.

Thus a plot of $\ln k/T$ versus $1/T$ results in a straight line with a slope of $-\Delta H^\ddagger/R$ from which the enthalpy of activation can be derived, and with an intercept of $\ln(k_B/h) + \Delta S^\ddagger/R$ from which the entropy of activation is obtained. The free energy of activation $\Delta G^\ddagger$ was evaluated using Equation 7.8.

$$\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$$  \hspace{1cm} (7.8)
7.3 Results and discussion

7.3.1 Structure and behaviour of aromatic nitroso sulfonate

The structural data estimated for aromatic nitroso sulfonate compounds are summarised in Table 7.1. All four molecules were found to be planar, in good agreement with the results of an experimental study on nitrosobenzene carried out by Hanyu et al. [13]. The presence of an electron withdrawing group substituent at the ortho position in the aromatic ring leads to a weakening of the C-N bond relative to the unsubstituted parent compound (NBS). However, we note that, for all aromatic nitroso sulfonate compounds, regardless of substitution, the BDE of C-N are estimated to be in excess of 191 kJ mol\(^{-1}\) and thus these compounds are considered to be thermally stable. The C-N-O angle increases with increasing molecular weight of the nitroso compounds. This discloses the presence of conjugation between the nitroso group and the phenyl ring since greater delocalization tends to result in an enhanced C-N-O bond angle. The latter may be demonstrated by the dimension found for DCNBS and DBNBS compared to the one obtained for NBS.
Table 7.1. Structural parameters of the aromatic nitroso sulfonate compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>BDE (C-N)/kJ mol(^{-1})</th>
<th>C-N bond length/Å</th>
<th>N-O bond length /Å</th>
<th>CNO/degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS</td>
<td>230</td>
<td>1.42</td>
<td>1.22</td>
<td>116</td>
</tr>
<tr>
<td>DMNBS</td>
<td>215</td>
<td>1.41</td>
<td>1.23</td>
<td>117</td>
</tr>
<tr>
<td>DCNBS</td>
<td>200</td>
<td>1.42</td>
<td>1.22</td>
<td>118</td>
</tr>
<tr>
<td>DBNBS</td>
<td>191</td>
<td>1.32</td>
<td>1.19</td>
<td>133</td>
</tr>
</tbody>
</table>

7.3.2 Monomer-dimer equilibration

Based on the data presented in Figure 7.1, the value for the extinction coefficient of 34.4 dm\(^3\) mol\(^{-1}\) cm\(^{-1}\) for DBNBS was determined, in good agreement with value of 34 dm\(^3\) mol\(^{-1}\) cm\(^{-1}\) reported by Ide et al. [14]. Table 7.2 summarises the equilibrium data for nitrosobenzene compounds at 25 °C. We note that the extinction coefficients of the nitroso compounds investigated were within the range of 30-35 dm\(^3\) mol\(^{-1}\) cm\(^{-1}\) except for DMNBS, whose value was around 20.2 dm\(^3\) mol\(^{-1}\) cm\(^{-1}\). Theoretically, the relatively low value for the extinction coefficient in the visible regions in nitroso compounds indicates a strong tendency for dimerisation in aromatic compounds in which the resonance interaction between the benzene ring and the NO-group is weak [15]. A lack of resonance between the NO-group and the benzene ring is normally a result of charge polarization, i.e. the influence of strong electron-attracting substituents or steric crowding induced by ortho substituents. In contrast, the methyl group at the ortho position in DMNBS is neither a strong electron attracting substituent nor sterically hindering as compared to bromine and chlorine atoms. The monomer/dimer equilibria
Chapter 7: Comparative study of physicochemical properties of ortho substituted aromatic nitroso compounds

of a series of 2,6-dichloro-4-substituted-nitrosobenzenes have been studied in benzene solvent and electron releasing substituents have been found to favour dissociation of the dimer to monomer [16]. Similarly, as methyl is an electron releasing group, this would qualitatively account for the large value of $K_C$ for DMNBS, despite the latter having a relatively low extinction coefficient.

Accordingly, the value of $K_C$ of the nitroso compounds were determined to be in the following order: NBS>DMNBS>DCNBS>DBNBS. The result is in good qualitative agreement with the supposition that ortho groups other than hydrogen favour the dimerisation of aromatic nitroso compounds [17]. For instance, it is well known that 2,6-dichlorobenzene is strongly dimerised even in dilute solution while dimerisation is much less favoured in p- and m-chloronitrosobenzene and 3,5-dichloronitrosobenzene [5]. We also observed an effect attributable to steric inhibition in DBNBS, as a result of the relatively large kinetic diameter of bromine as compared to the other compounds. Thus, our data are consistent with the premise that dimerisation is caused by steric inhibition due to size of ortho substituent in nitrosobenzene compounds.

In Table 7.2, we also included $K_C$ values which were evaluated by Holmes et al. for 2,6-dichloro-4-bromonitrosobenzene and 2,4,6-trichloronitrosobenzene [16] to compare with the value for DCNBS. Despite the fact that halogen groups are generally known to have electron withdrawing character by induction, their presence at the para position in these two compounds, favouring dissociation to monomer, suggests that halogen may also act as electron releasing substituents as reported by Holmes et al. [16]. The presence of a sulfonate group, which brings strong electron withdrawing character in DCNBS, favours dimer formation; the relevant $K_C$ values vary between 2.5 to 3 times lower that those obtained for 2,6-dichloro-4-bromonitrosobenzene and 2,4,6-
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trichloronitrosobenzene, respectively. We can nevertheless note that the data obtained by Holmes et al. and those reported in the present contribution, despite the compounds being slightly different in chemical structure, are within the same order of magnitude.

**Figure 7.1.** Plot of $\frac{D}{C_L}$ against $\frac{D^2}{C_L^2}$ at 25 °C for nitrosobenzene sulfonate compounds. (□ NBS, △ DMNBS, × DCNBS, ○ DBNBS)
Table 7.2. Equilibrium constant ($K_C$) of nitroso monomer-dimer in aqueous solution at 25 °C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$</th>
<th>$K_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBNBS</td>
<td>34.4 ± 0.5</td>
<td>$1.29 \times 10^3$</td>
</tr>
<tr>
<td>NBS</td>
<td>35.0 ± 1.0</td>
<td>$(5.11 \pm 0.4) \times 10^3$</td>
</tr>
<tr>
<td>DMNBS</td>
<td>20.2 ± 0.7</td>
<td>$(3.46 \pm 0.5) \times 10^3$</td>
</tr>
<tr>
<td>DCNBS</td>
<td>31.3 ± 1.1</td>
<td>$(1.97 \pm 0.2) \times 10^3$</td>
</tr>
<tr>
<td>2,6-dichloro-4-bromo nitrosobenzene(a)</td>
<td>-</td>
<td>$5.7 \times 10^3$</td>
</tr>
<tr>
<td>2,4,6-trichloronitrosobenzene(a)</td>
<td>-</td>
<td>$4.9 \times 10^3$</td>
</tr>
</tbody>
</table>

(a) In benzene solution at 25 °C [16]

7.3.3 Temperature dependence of $K_C$

The effect of temperature on the $K_C$ on the four nitroso compounds was also investigated. The equilibrium data and the extinction coefficient of aromatic nitroso compounds over a temperature range of 25 to 60 °C are provided in the Appendix D. The extinction coefficient for the nitroso compounds remained essentially the same over the temperature range studied. While an increase in the equilibrium constant with increasing temperature was observed for the four nitroso compounds under investigation. The increase in $K_C$ as a function of temperature provides evidence that nitroso monomer is favoured upon heating.
7.3.4 Thermodynamics of aromatic nitroso sulfonate dimer/monomer interchange

The standard enthalpy change of the reaction of dimer forming two monomers, $\Delta H_R^{\circ}$ and the standard entropy change $\Delta S_R^{\circ}$ were evaluated on the basis of the van’t Hoff Equation, using the $K_C$ values obtained in the temperature range from 25 to 60 °C. The results are listed in Table 7.3. Relatively little variance in $\Delta H_R^{\circ}$ is observed across the series, ranging from 47.6 to 53.1 kJ mol$^{-1}$, with the highest enthalpy change for the reactions of DBNBS monomers formed by the decomposition of the dimer. Likewise, the entropy as well the free energy changes of the reactions were essentially equivalent for all aromatic nitroso sulfonates investigated in the present study. Nevertheless, a small stepwise increase can be observed in these thermodynamic parameters as the size of the ortho substituent increases, suggesting bulkier substituent groups favour the dimer reactants.

Table 7.3. Standard enthalpy, entropy and free energy changes for reactions forming two monomers from a dimer (reaction R1), at 298.15 K

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda$/nm</th>
<th>$\Delta H_R^{\circ}$/kJ mol$^{-1}$</th>
<th>$\Delta S_R^{\circ}$/J K$^{-1}$ mol$^{-1}$</th>
<th>$\Delta G_R^{\circ}$/kJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS</td>
<td>755</td>
<td>47.6 ± 0.3</td>
<td>116 ± 0.9</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>DMNBS</td>
<td>770</td>
<td>49.0 ± 0.4</td>
<td>117 ± 1.3</td>
<td>14.1 ± 1.4</td>
</tr>
<tr>
<td>DCNBS</td>
<td>765</td>
<td>50.1 ± 0.5</td>
<td>119 ± 1.2</td>
<td>15.2 ± 1.3</td>
</tr>
<tr>
<td>DBNBS</td>
<td>760</td>
<td>53.1 ± 0.5</td>
<td>123.0 ± 1.7</td>
<td>15.3 ± 2.0</td>
</tr>
</tbody>
</table>
7.3.5 **Kinetics of aromatic nitroso sulfonate dimer/monomer interchange**

The rate of dissociation of aromatic nitroso sulfonate dimer to monomer was investigated by UV-Vis spectrophotometer. The results obtained for $k_1$ and $k_{-1}$ from fitting experimental concentration profiles of the four aromatic nitroso sulfonate compounds for the dissociation of the dimer are tabulated in Appendix D. Figure 7.2 shows the measurement of NBS monomer with time employed for the determination for determining rate of dissociation of NBS dimer to monomer at 25°C. (Plots for DMNBS and DCNBS are provided in Appendix D.) The model was found to fit well the experimental data with the values of 0.121 s$^{-1}$ and 23.72 mol$^{-1}$ dm$^3$ s$^{-1}$ for $k_1$ and $k_{-1}$ respectively from the dissociation of NBS dimer.

![Kinetics of formation of NBS monomer](image)

**Figure 7.2.** Measurement of NBS monomer concentration for determining rate of dissociation of dimer to monomer at 25°C. + 5 mmol dm$^{-3}$, □ 7.5 m mol dm$^{-3}$ and Δ 10 mmol dm$^{-3}$. Solid lines represents model predictions.
7.3.6 Activation parameters

We summarise in Table 7.4 the activation parameters for the dissociation of dimer to monomer which were obtained from the Arrhenius and Eyring plots. Arrhenius plots for the forward and backward reaction of the dimer-monomer interchange of nitroso compounds, as well associated Eyring plots, are provided in the supporting information. Aromatic nitroso sulfonate dimers undergo dissociation to the monomer in a first order process with activation energies ranging from 59 to 79 kJ mol\(^{-1}\). Activation barriers for dimerisation are from 11.5 to 27.9 kJ mol\(^{-1}\). The activation energies for the dissociation of dimer obtained for the aromatic nitroso sulfonate is lower compared to the reported values for aliphatic nitroso compounds which ranges from 83.7 to 150.7 kJ mol\(^{-1}\) \[18-21\]. Likewise, we noted that Δ\(H^\ddagger\) was lower than the reported Δ\(H^\ddagger\) for aliphatic nitroso compounds \[22\]. This variation is doubtlessly a distinctive characteristic between aromatic and aliphatic nitroso compounds as noted by Azoulay et al., when they compared o,o'-azodioxytoluene and azodioxylcyclohexane in acetonitrile \[23\]. The possible existence of a conjugation between the N=N bond and the aromatic ring could have lowered the barrier to rotation about the N=N bond. Thus the rate of dissociation of aromatic nitroso dimers is considerably faster as compared to aliphatic nitroso dimers.
Chapter 7: Comparative study of physicochemical properties of ortho substituted aromatic nitroso compounds

Table 7.4. Activation parameters for the dissociation of aromatic nitroso sulfonate dimer

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$E_a$/kJ mol$^{-1}$</th>
<th>$\Delta H^\ddagger$/kJ mol$^{-1}$</th>
<th>$\Delta S^\ddagger$/kJ mol$^{-1}$ K$^{-1}$</th>
<th>$\Delta G^\ddagger$/kJ mol$^{-1}$ (b)</th>
<th>Arrhenius factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS</td>
<td>59.2 ± 0.44</td>
<td>56.6 ± 0.43</td>
<td>-73.2 ± 1.10</td>
<td>78.4 ± 1.18</td>
<td>2.7 × 10$^9$</td>
</tr>
<tr>
<td>DMNBS</td>
<td>62.9 ± 0.33</td>
<td>60.3 ± 0.33</td>
<td>-63.7 ± 1.13</td>
<td>79.2 ± 1.17</td>
<td>8.4 × 10$^9$</td>
</tr>
<tr>
<td>DCNBS</td>
<td>71.4 ± 0.61</td>
<td>68.9 ± 0.62</td>
<td>-45.6 ± 1.97</td>
<td>82.6 ± 2.06</td>
<td>6.9 × 10$^{10}$</td>
</tr>
<tr>
<td>DBNBS</td>
<td>79.7 ± 0.28</td>
<td>77.0 ± 0.25</td>
<td>-15.8 ± 0.81</td>
<td>81.8 ± 0.85</td>
<td>2.7 × 10$^{12}$</td>
</tr>
<tr>
<td>DMNB$^{(a)}$</td>
<td>-</td>
<td>84.5 ± 2.0</td>
<td>-6 ± 3</td>
<td>86.3 ± 3.6</td>
<td>-</td>
</tr>
</tbody>
</table>

$^{(a)}$ Measurement performed in acetonitrile [24], $^{(b)}$ calculated at 298.15 K

While it is desirable to compare the activation parameters obtained in the current study with reported literature values for other nitroso compounds, most aromatic compounds are insoluble in water and were therefore investigated in solvents such as acetonitrile and dichloromethane. However compounds dissociated in polar solvents such as methanol or acetone have entropies of activation lower in comparison to circumstances where the compound was dissolved in a non-polar solvent such as benzene or toluene. This indicates that the solvent effectively associates with the bond which is about to break in the dissociation [25]. For instance Gowenlock and Batt [21, 26] studied the dissociation of 3-methyl-3-nitrosobutanone in various solvents and reported a
decreasing entropy of activation with increasing dielectric constant. They reported that lower values of entropy change are evident with polar solvents such as ethanol or acetonitrile. Taking this into consideration, and using water as solvent with a dielectric constant, $\varepsilon$ of 80, it is anticipated that the activation of entropy for aromatic nitroso sulfonate will have a lower value when dissolved in water compared to acetonitrile ($\varepsilon = 36.6$). For this reason, the entropy of activation for DMNBS in water was lower in comparison to DMNB in acetonitrile.

In general, the variance in the enthalpy of activation for the nitroso compounds studied increased slightly with increasing ortho substituent size. In comparison, the entropy of activation highlighted significant differences in reaction rates. The value of $\Delta S^\ddagger$ for a reaction provides an estimate of the change in the order of the system proceeding from the reactants to a transition state. The relatively large and negative activation entropy in NBS suggests that the transition structure is more ordered than the dimer. While $\Delta H^\ddagger$ and $\Delta S^\ddagger$ provide important information with respect to the reaction mechanism, $\Delta G^\ddagger$ determines the rate of a reaction and is particularly useful in understanding and comparing group of compounds. Based on the results of the present study, we observe a relatively small difference in activation free energy, and accordingly the rate of dissociation of the dimer to monomer for NBS was the highest among the four compounds studied. Overall, the results disclose that, electronic and steric characteristics as a result of minor changes in the structural variations may change significantly the relative amount of monomer present in the system.
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7.4 Conclusion

The physicochemical properties of four substituted aromatic nitroso sulfonate compounds were studied, in order to improve the understanding of the reactivity of these compounds to scavenge NO. Based on the BDE of C-N bond of the aromatic nitroso sulfonate, we established that these compounds are thermally stable. Since it is necessary for the aromatic nitroso compounds to favour the monomeric form for effective NO trapping reaction, an investigation on the kinetic and thermodynamic parameters of the dimer-monomer interchange was undertaken. Based on these data, it is evident that aromatic ring substituents strongly influence reactivity. Particularly, substituents at the ortho position can greatly influence the rate of scavenging of nitric oxide. Increasing the size of ortho substituent favours the stability of the dimer form of these compounds, making such ortho substituted species less reactive for scavenging NO.
Chapter 7: Comparative study of physicochemical properties of ortho substituted aromatic nitroso compounds

7.5 References


Chapter 7: Comparative study of physicochemical properties of ortho substituted aromatic nitroso compounds


Chapter 7: Comparative study of physicochemical properties of ortho substituted aromatic nitroso compounds


CHAPTER 8: COMPARATIVE STUDY OF THE TRAPPING OF NITRIC OXIDE BY ORTHO SUBSTITUTED NITROSO SULFONATES
8.1 Introduction

In Chapters 4 and 5, we demonstrated that, 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), an aromatic nitroso spin trap, can effectively scavenge the nitric oxide formed from the decomposition of nitrous acid under conditions that mimic the sensitisation of AN emulsion explosive via chemical gassing. In an extension to this research, we synthesised a series of ortho substituted aromatic nitroso sulfonates, namely 3,5-dimethylnitrosobenzene sulfonate (DMNBS), 3,5-dichloro-4-nitrosobenzene sulfonate (DCNBS) and nitrosobenzene sulfonate (NBS). The presence of sulfonate group at the para position in DBNBS results in enhanced water solubility to the compound and thus in the new series of compounds, all containing the sulfonate group, were considered for investigation.

In the present chapter, we compare the reactivity of ortho substituted aromatic nitroso sulfonates towards nitric oxide, in order to gain an insight into the effect of substituents on the aromatic ring on the trapping efficiency. The ultimate purpose of the study was to assess whether aromatic nitroso compounds (in general) can scavenge nitric oxide formed from nitrous acid decomposition. Furthermore, we present a mechanism for the reaction of each of the aromatic nitroso sulfonate compounds with nitric oxide. In our previous study, the reaction of NO with DBNBS was distinguished by the formation of nitrogen gas and the relatively high concentration of nitrate found in the reaction mixture. It is therefore of great interest to establish if similar products are produced with the other aromatic nitroso sulfonate compounds.
Membrane inlet mass spectroscopy (MIMS) and FTIR have been applied to measure the gaseous products from the reaction of these aromatic nitroso sulfonate compounds with nitric oxide. Together, these techniques enable the measurement of a variety of gases including nitrogen and nitrogen oxides. The liquid fraction sampled from the reaction mixture were analysed by ion chromatography (IC) and nanostructure assisted laser desorption and ionisation mass spectroscopy (NALDI-MS). We also fitted the kinetics measurements for the concentration of nitric oxide and aromatic nitroso sulfonate compounds using membrane inlet NOx analyser and UV-Vis stopped flow apparatus in the development of a generalised mechanism which was then used to evaluate and compare the rate constants for the trapping reaction.

8.2 Methodology

8.2.1 Material

Nitroso compounds (3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), 3,5-dichloro-4-nitrobenzene sulfonate (DCNBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and nitrosobenzene sulfonate (NBS)) were synthesised following published methods and procedures [1-3]. Refer to Chapter 3 for detailed methods. L-ascorbic acid, sodium nitrite and 37% hydrochloric acid were AR reagents and purchased from Sigma Aldrich (Castle Hill, Australia). Glacial acetic acid was purchased from Ajax-fine Chem. Silastic tubing was purchased from Speciality Manufacturing Inc (Saginaw, MI, USA) and silicone sealant was from Dow Corning (Midland, MI, USA). Nanostructured assisted laser desorption ionisation (NALDI\textsuperscript{TM}) plates, which are manufactured by
Nanosys, Inc. for Bruker Daltonics Inc., were purchased from Bruker Daltonics Australia.

8.2.2 Experimental apparatus

The apparatus employed to study the in situ trapping of nitric oxide formed from nitrous acid decomposition was a 2 neck 10 cm³ round bottom flask submerged in water bath for temperature control and is described in detail in Chapter 3. Briefly, the reactor contained 0.015 M aromatic nitroso sulfonate compound solution acidified with 0.1 M acetic acid which was flushed with argon for 15 min at a flow rate of 1.33 cm³ s⁻¹. The flow of argon was then reduced to 0.67 cm³ s⁻¹ and 1 cm³ of 0.125 M sodium nitrite solution was injected, which trigger the reaction generating NO. The membrane probe, which was completely immersed in the reactor solution, enabled the direct measurement of the composition of the dissolved gases, while the exhaust gas stream was collected and sampled in a 3L tedlar sampling bag (SKC Inc.) for later analysis by FT-IR. After allowing the reaction to proceed for 1 hour, the reaction was quenched through the dropwise addition of 0.1 mol dm⁻³ NaOH. Samples of the liquid reaction mixture were collected for subsequent analysis.

8.2.3 Gaseous analysis

8.2.3.1 Membrane inlet mass spectroscopic analysis

An inlet membrane probe comprising a 10 mm long semi-permeable silastic tubing sealed at the end with silicone sealant (Dow Corning, US) was constructed and attached

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to the vacuum inlet leading to the ion source of a quadrupole mass spectrometer via a 1/16” stainless steel tubing. The probe was immersed in the reaction solutions, allowing detection of species present in aqueous solution across the membrane leading to a quadrupole mass spectrometer (QMS). In these experiments, the purging of argon was terminated at the start of an experiment.

8.2.3.2 Fourier transform infrared analysis

The product gases evolving from the 2 neck round bottom 10 cm$^3$ volumetric flask were purged from the reactor with high-purity nitrogen gas, dried by passage over a column of drierite and collected in gas sampling bag for analysis on a Shimadzu, IRaffinity-1 Fourier transform spectrometer, equipped with a 10 m long-path gas cell (Infrared Analysis, USA) with a spectral resolution of 0.5 cm$^{-1}$. QASoft and Gram software packages aided in the detection of product gases from the measured spectra.

8.2.4 Liquid analysis

8.2.4.1 Ion Chromatograph (IC)

Analysis of the anions present in the reaction mixture was performed using a Dionex-DX-100 ion chromatograph with an eluent containing 8 mmol dm$^{-3}$ Na$_2$CO$_3$ and 1 mmol dm$^{-3}$ mM NaHCO$_3$, equipped with an Ionpac AS14A analytical column and AG14A guard column, with suppressed conductivity detection. Samples were withdrawn from the reactor using a gas tight syringe via a septum side port and were diluted 50 fold for analysis. All samples were analyzed in triplicate with a mobile phase
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flow rate of 1.0 cm$^3$ min$^{-1}$. Nitrite, nitrate, bromide and chloride concentration were obtained by comparing peak area with the area obtained with standard solutions. Instrument control, data acquisition, processing, and report generation were accomplished through the Chromeloon® Chromatography Data System.

8.2.4.2 NALDI-MS

Sample volumes of 5 µL of reaction mixtures were spotted directly on a NALDI target. For comparative purposes, samples of corresponding starting aromatic nitroso sulfonate were also spotted. NALDI-TOFMS spectra were acquired using a Bruker Daltonics Ultraflex III MALDI time-of-flight mass spectrometer, operated in negative ion, reflectron mode. Prior to each analysis, the target was calibrated using an elemental sulfur standard (Sigma Aldrich, Castle Hill, Australia). MS data were acquired in the $m/z$ range between 0 and 800 Da by averaging signals from 2500 laser shots using target random-walk movement.

8.2.5 Measurement of NO by membrane NOx analyser

To assist in the development of the mechanism involving the spin trapping of NO, a novel membrane NOx analyser (Thermo Scientific Model 42i-HL) apparatus was employed, as described in Chapters 3 and 5. The membrane probe of 30 mm length of silastic tubing was immersed completely in the reactant solution. Solutions in the reactor were purged with nitrogen flowing at a rate of 1.33 cm$^3$ s$^{-1}$ via 1/16” stainless steel tubing placed through a septum with outflow via a second syringe. Once the solution was purged for 30 min, the needle was plugged by inserting a 5 cm$^3$ syringe,
and the stainless steel tubing removed. Nitrogen flow was then connected to the membrane inlet line, and adjusted to 0.67 cm$^3$ s$^{-1}$. Sodium nitrite solution was then injected via syringe into the reaction vessel via an injection port to trigger the NO generation reaction.

8.2.5.1 Ex situ trapping

In these experiments, NO was first generated via the rapid decomposition of ascorbate and, when the NO concentration attained a maximum level signifying that all the nitrous acid present has reacted with ascorbate, the aromatic nitroso sulfonate compound was then injected in the solution to initiate the trapping reaction. (Refer to Chapter 3 for detailed procedure).

8.2.5.2 In situ trapping

The reactor was completely filled with the desired concentration of aromatic nitroso sulfonate solutions which was acidified with 0.1 mol dm$^{-3}$ acetic acid. Nitrite was then injected to initiate the in situ trapping reaction.

8.2.6 Measurement of aromatic nitroso sulfonate by UV-Vis stopped flow apparatus

Stopped flow device coupled to a UV-Vis spectrometer was used to study the consumption of aromatic nitroso sulfonate by NO. UV-Vis spectrometry was appropriate for analysis as the aromatic nitroso sulfonate compounds have characteristic
monomer absorption band centred in the range of 700-790 nm due to n-\(\pi^*\) transition. Also preliminary study performed by HPLC showed that, products of reaction mixture showed no absorption in the visible region.

### 8.3 Results and discussion

#### 8.3.1 Gaseous analysis

**8.3.1.1 MIMS and FTIR**

MIMS analyses disclosed the formation of nitrogen (N\(_2\)) gas during the in situ experiments for each of the aromatic nitroso sulfonate compounds with NO. Determination of the isotopic ratio of the \(m/z\) 29/28 ion currents was used to confirm the presence of N\(_2\). The experimental values of the isotopic ratio of \(m/z\) 29/28 obtained from the reaction of the nitroso compounds with NO were all within the range of the expected value of 7.35 \(\times\) 10\(^{-3}\), assuming these ion currents are due to the measurement of \(^{14}\text{N}^{14}\text{N}^+\) and \(^{14}\text{N}^{15}\text{N}^+\). Plots obtained for \(m/z\) 28 and 29, when DCNBS was reacted with NO in situ, are illustrated in Figure 8.1.

The volume of N\(_2\) gas generated from the reaction of DCNBS with NO was significantly higher in comparison to the other nitroso compounds. For example, there was approximately 50 \% more N\(_2\) produced from DCNBS in comparison to the reaction gas mixture from NBS. Figure 8.2 plots the ion current (against time) for \(m/z\) 28 (corresponding to N\(_2\)) for the compounds; the plot for DBNBS was also included for comparative purposes.
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Figure 8.1. Plot of ion current against time for m/z 28 and 29 for DCNBS.

Figure 8.2. Detection of N₂ gas by MIMS from the NO trapping reaction by the aromatic nitroso sulfonate compounds. ○ NBS, △ DMNBS, ◊ DCNBS and * DBNBS
Other gases, in addition to N\textsubscript{2} detected were NO, CO\textsubscript{2}, and N\textsubscript{2}O. The small amount of CO\textsubscript{2} detected is believed to originate from the release of dissolved CO\textsubscript{2}/HCO\textsubscript{3}\textsuperscript{-} upon the acidification of the reactants.

The amount of CO\textsubscript{2} is estimated to be approximately 1.5 ppm by FTIR analysis, which represents roughly 1.5 \% of all the gases detected. Likewise, a small amount of N\textsubscript{2}O was identified corresponding to approximately 5 \% of the gases present in the mixture, which is believed to be a side product as a result of the decomposition of nitrous acid. N\textsubscript{2}O is not likely to arise from the trapping reaction as in blank experiments conducted in the absence of the aromatic nitroso sulfonate traps, a similar amount of N\textsubscript{2}O was again detected. Figure 8.3 illustrates a typical FTIR spectrum obtained from the trapping reaction (product gases from the reaction with DCNBS is illustrated as an example), while Figure 8.4 compares the FTIR spectra of product gases released from nitrous acid decomposition with and without aromatic nitroso sulfonate trap (product gases from reaction with NBS is used as an example in this case). There was no detectable difference in terms of the gases identified although the intensity of the absorbance reflects a lower concentration of these gases in the sample where the traps were present.
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**Figure 8.3.** FTIR spectrum of sample gas from the trapping reaction with the library spectra for CO₂, N₂O, NO₂ and NO. Sample gas was from the *in situ* reaction of DCNBS with NO.

**Figure 8.4.** Comparison of spectra of product gases evolved from nitrous acid decomposition; with and without spin trap. Spectrum illustrated is for the nitroso sulfonate trap reaction of NBS with NO.
In general, gas analysis using MIMS and FTIR demonstrated that most of the gaseous products were initiated from the decomposition of nitrous acid with the exception of N₂, which was a product from the trapping reaction. As previously reported, MIMS is not a standalone analytical technique for the identification of gases, in particular when the gases have a number of common daughter fragment ions, and thus a complementary technique such as FTIR is required for the confirmation of the identity of gaseous species with MIMS.

8.3.2 Liquid analysis

8.3.2.1 Ion chromatography

The mean concentration of nitrite and nitrate from reaction mixtures obtained with and without aromatic nitroso sulfonate compounds are illustrated in Figure 8.5. The results reveal a considerably higher concentration of nitrate and a lower concentration of nitrite in the samples where the aromatic nitroso sulfonate compounds were present. This implies there is an additional reaction taking place during the trapping reaction, which is responsible for the consumption of nitrite. When DCNBS was present in the reaction mixture, the steady state concentration of nitrite decreased to 9 mmol dm⁻³, from an initial nitrite concentration of 15 mmol dm⁻³. This trend was most notable for the chlorinated substituted nitroso sulfonate (DCNBS), which contained the highest concentration of nitrate but also the lowest amount of nitrite among the four compounds. In contrast, in the reaction mixture of DMNBS, the nitrate concentration was approximately three times lower and the nitrite concentration was twice as high in the sample of DCNBS reaction mixture. The relative amount of nitrate and nitrite
present in the samples are a preliminary indication of the efficiency of DCNBS compared to DMNBS.

In addition to nitrite and nitrate, chloride ions were detected in the reaction mixture of DCNBS, which suggests that the chlorine atoms in the compounds were replaced with nitrite by nucleophilic substitution. A typical ion chromatogram for the reaction mixture of DCNBS is shown in Figure 8.6. The concentration of chloride ions in the DCNBS solution reached 5.8 mmol dm$^{-3}$, or roughly 38.6% of the original DCNBS in the system. In the samples of reaction mixtures for NBS and DMNBS, only nitrite and nitrate ions were detected.

![Figure 8.5](image)

**Figure 8.5.** Comparison of the mean concentration of nitrate and nitrite in the reaction mixture with and without aromatic nitroso sulfonate compounds for the *in situ* trapping reaction.
Samples of the reaction mixtures from the \textit{ex situ} experiments, where NO gas was bubbled in solutions of nitroso compounds, was also analysed by ion chromatography. The concentration of nitrate was considerably higher compared to the NO saturated solution, which represents the reaction mixture without nitroso traps. In contrast to the \textit{in situ} experiments, in the \textit{ex situ} experiments nitrite is initially absent in the NO saturated solution, and as a result the concentration of nitrite was subsequently higher in the sample with the nitroso traps. Figure 8.7 illustrates the mean concentration of nitrite and nitrate in the reaction mixtures for the \textit{ex situ} trapping. In the sample of DCNBS and DBNBS, chloride and bromide were detected, respectively.

\textbf{Figure 8.6.} Ion chromatograph for the reaction mixture of DCBNBS

\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{ion_chromatograph.png}
  \caption{Ion chromatograph for the reaction mixture of DCBNBS}
  \end{figure}
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### 8.3.2.2 NALDI-MS

- **Analysis of in situ solution mixture**

Figure 8.8 compares the NALDI mass spectra of the *in situ* reaction solution mixtures of the four nitroso compounds after reaction time of 1 h. Unexpectedly, an ion at *m/z* of 291.9, which was also present in the reaction mixture of DBNBS, was common to all reaction mixtures, with the exception of the reaction mixture of DMNBS. The ion at *m/z* = 291.9 represents the molecule of 3,4,5-tri-nitrobenzene sulfonate. In the sample of the reaction mixture for DCNBS, the only product identified was at a *m/z* value of

---

**Figure 8.7.** Comparison of the mean concentration of nitrate and nitrite in the reaction mixture with and without aromatic nitroso sulfonate compounds for the *ex situ* trapping reaction.
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291.9, whereas in the reaction mixture of DBNBS, a series of ions with $m/z$ values including 291.9, 261.9, 324.9, and 449.5 were detected.

In relation to NBS, the ion at $m/z$ 201.9 (representing the nitrobenzene sulfonate species) was higher in intensity compared to the ion at $m/z$ 291 and considered to be the primary product from the trapping reaction. Therefore, unlike in the reaction mixtures of DBNBS and DCNBS, the ion at $m/z$ 291.9 is believed to be a secondary product.

The mass spectra of neat DMNBS and its reaction mixture are similar in term of the ions detected, although the ion at the $m/z$ 229.9, which corresponds to the parent ion of DMNBS + [m/z 16], was more intense in the sample of the reaction mixture in comparison to the neat DMNBS sample ($m/z$ 213.9). The ratio of $m/z$ 213.9:229.9 is 1:0.3 and 1:2 in the DMNBS and the sample of the reaction mixture respectively. This indicates that the ion at $m/z$ 229.9 is a product formed as a result of the reaction of the compound with NO. The presence of the latter ion in the neat DMNBS sample suggests the formation of oxidation product on the surface of the NALDI target during the analysis process. However, since both samples were spotted and analysed at approximately same time, it seems likely that, the product represented by the ion at $m/z$ 229.9 is not only due to the oxidation of nitroso compound from NALDI-MS analysis, but also a product as a result of the nitration reaction (vide infra)
Figure 8.8. Comparison of NALDI-MS spectra of the reaction mixtures of aromatic nitroso sulfonate compounds
• Analysis of the ex situ reaction mixture

Ex situ reaction mixture of aromatic nitroso products was analysed and compared with the results of the in situ experiments. The ex situ reaction mixture did not differ significantly from the in situ in terms of product distribution, although the ion intensities were lower in the ex situ mixtures. In the concentration range that the experiments were performed, dimerisation products such as those reported by Davies et al. and Ichimori et al. [4, 5] were not detected. Comparative spectra of in situ and ex situ for each aromatic nitroso compounds are presented in Appendix D the supporting document.

8.3.3 Reaction mechanism.

8.3.3.1 Release of nitrogen gas and the formation of aryl radical

Based on the release of N$_2$ gas from the reaction mixture from all four substituted aromatic nitroso sulfonate compounds, we present a generalised mechanism for the formation of nitrogen from reaction of aromatic nitroso sulfonate with NO, as illustrated in Figure 8.9. This generalised mechanism is based on the reaction of DBNBS with NO (Refer to Chapter 4). As outlined in the mechanism, it was established that N$_2$ gas was released as a result of the homolytic cleavage of the C-N bond of a diazenyl radical which was itself formed by the decomposition of the nitroso-NO adduct. In addition, we predicted the formation of oxygen as a consequence of the decomposition of the short-lived nitroso-NO adduct. These reaction steps arise, as a result of elevated amounts of nitrate and nitrite present in solution, which was observed in the present
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experiments, where significant quantities of nitrite and nitrate ions were detected in *ex situ* and *in situ* reactions.

\[
\begin{align*}
\text{Aromatic nitroso sulfonate} & \quad \text{Nitro-NO adduct} \\
\text{Nitro-NO adduct} & \quad \text{Aromatic nitro product}
\end{align*}
\]

**Figure 8.9.** Generalised reaction pathway for the release of N₂ gas and formation of aryl radical from the trapping reaction of NO with aromatic nitroso sulfonate compounds

\[
\begin{align*}
\text{Aryl diazenyl radical} & \quad \text{Aryl radical}
\end{align*}
\]

\[
X = \text{H, CH₃, Cl and Br}
\]

**8.3.3.2 Formation of aromatic nitro products**

The introduction of nitro group, which occurred via the coupling of the aryl radical with nitrite to produce a π* radical anion (which in turn reacted with electrophile N₂O₃ to
yield nitrobenzene sulfonate) (Refer to Chapter 4 for further detail) is considered to occur in all the four nitroso compounds as nitrite was formed in these systems.

On the basis of the NALDI-mass spectra obtained for the reaction mixture for NBS, it is evident that the ion at $m/z$ 291.9 formed from the reaction is a secondary rather than a primary product, as is the case when NO reacted with DBNBS and DCNBS respectively. The product is expected to originate from the nucleophilic substitution of hydrogen at the ortho position to the nitro group. This type of reaction commonly known as nucleophilic aromatic substitution for hydrogen (NASH) and has become a widely used technique in organic synthesis [6, 7]. The reaction proceeds via the initial addition of a nucleophile to the aromatic ring, with the formation of a $\sigma$-complex type intermediate. This reaction typically takes place with a nitroarene at ortho and para position [8]. Figure 8.10 demonstrates the nucleophilic aromatic substitution for hydrogen in NBS system.

![Figure 8.10. NASH taking place in the nitrobenzene sulfonate by nitrite](image)

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Only one product at \( m/z \ 229.976 \) (corresponding to 3,5-dimethyl nitrobenzene sulfonate) was detected during the NALDI-MS analysis of the sample of the reaction mixture of DMNBS, and the absence of a peak at \( m/z \ 291.9 \) suggests that further reaction did not occur following the introduction of the nitro group into the benzene.

The presence of methyl groups, which are generally known to be poor leaving group compared to halides, inhibits nucleophilic substitution by nitrite, consistent with the observation of the occurrence of 3,5-dimethyl-4-nitrobenzene sulfonate as the sole reaction product. In contrast, the presence of chloride and bromide ions in the sample of reaction mixture of DCNBS and DBNBS respectively facilitate aromatic nucleophilic substitution as shown in Figure 8.11.

\[
\begin{align*}
\text{3,4,5-trinitrobenzene sulfonate} & \quad m/z \ 291.951 \\
X = \text{Cl or Br}
\end{align*}
\]

**Figure 8.11.** Nucleophilic substitution of halogen, \( S_NAr \), in 3,5-dichloro and 3,5 dibromo nitrobenzene sulfonate
8.3.4 Kinetic analysis of the reaction of NO with aromatic nitroso sulfonate compounds

A kinetic analysis of the overall reaction R1 was studied by monitoring the decrease in NO concentration using the membrane NOx analyser in the presence of ortho substituted aromatic nitroso sulfonate compounds.

\[
\text{D}_X\text{NBS} + \text{NO} \rightarrow \text{N}_2 + \text{O}_2 + \text{Aryl radical} \quad k_{\text{Trap}} \quad (R8.1)
\]

where X represents the ortho substituent, which was H, CH$_3$, Cl or Br.

The initial concentration of NO was 0.6 mM, generated by the rapid nitrosation of ascorbic acid and allowed to reach a maximum concentration, following which an equimolar quantity of the nitroso compound was added to the reactor. In these experiments, it is assumed that the nitroso compounds react exclusively with NO. Under the conditions examined, the overall rate of the reaction of these nitroso sulfonate compounds with NO was found to be in the following order: DCBNBS > DBNBS > NBS > DMNBS with the reaction of DCNBS with NO having the highest rate among the four compounds studied. Figure 8.12 plots the change in NO concentration in the presence of the aromatic nitroso compounds at 25 °C. It was expected that the rate of reaction of NBS and DMNBS would be faster than the rate with DCNBS or DBNBS, considering their equilibrium constant favours monomer formation. Surprisingly, however the results with DMNBS reacting with NO were much slower than the rate of reaction for the other three nitroso spin traps studied.
To investigate the relatively slow rate of reaction of DMNBS with NO, the reaction was studied at varying concentration of DMNBS, keeping pH and temperature constant at 1.5 and 25 °C, respectively. The results reveal that the rate of trapping of NO by DMNBS is strongly influenced by the initial concentration of DMNBS, as a five-fold increase in concentration of DMNBS led to a corresponding five-fold decrease in the time required for removal of NO (See Figure 8.13). It is estimated that the concentration of DMNBS would need to be increased by a factor of 30 under the conditions employed to ensure complete (greater than 99%) removal of NO in comparison to DCNBS.

**Figure 8.12.** Change of NO concentration during the trapping reaction by different aromatic nitroso sulfonate compounds
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Figure 8.13. Effect of varying DMNBS concentrations (0.6 – 3.0 mmol dm$^{-3}$) with 0.6 mmol dm$^{-3}$ NO at pH 1.5 and 25 °C

When the pH was varied between 1.5 and 3.5 during the reaction of DMNBS with NO the rate of NO trapping increased with increasing pH (See Figure 8.14). In related experiments, the effect of pH on the removal of NO was studied for the other aromatic nitroso sulfonate compounds. Figure 8.15 shows the effect of varying pH for the reaction of NBS with NO at 25 °C. The scavenging of NO with NBS was also influenced by a change in pH, although it is noted that above pH 2 the rate of trapping did not increase as notably as was the case during reaction with DMNBS.

Because the reactivity of both NBS and DMNBS is pH dependant, determining the $pK_a$ values for both species, which are currently unknown, is essential. We note that
in contrast to NBS and DMNBS, varying the pH for DCNBS and DBNBS does not have a significant influence on the rate of NO scavenging for these species as illustrated in Figures 8.16 and 8.17 respectively.

The value of $pK_a$ for a substance is an indication of the acidity of the substance, based on this value it enables the determination of the relative amounts of the compound existing in deprotonated or protonated [9] form. The $pK_a$ of NBS and DMNBS in a previous study were determined to be 1.26 and 2.28 respectively, as measured by potentiometric titration [10]. The relatively low values of $pK_a$ for NBS and DMNBS highlight that under the pH conditions employed in the current study (1.5) the N=O moiety in either compound is not fully deprotonated, and as such smaller proportion of these compounds are available to react with NO. Therefore, for NBS and DMNBS reactions below a pH 2 and 4 respectively, the rate limiting step in the reaction is most likely the protonation of the N=O moiety, which would explain why the rate of reaction of NO with NBS or DMNBS is substantially slower, relative to DCNBS and DBNBS. In addition, the low value of $pK_a$ for NBS is consistent with the observation that the rate of reaction of NO with NBS at pH conditions greater than 2 did not significantly change as under these conditions as the N=O moiety is almost completely deprotonated, as shown in Figure 8.15.
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Figure 8.14. Effect of varying pH from 1.5 -3.5 for the reaction of 1.8 mmol dm$^{-3}$ DMNBS with 1.8 mmol dm$^{-3}$ NO at 25 °C

Figure 8.15. Effect of varying pH from 1.5-3.5 for the reaction of NBS with NO at 25 °C
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Figure 8.16. The reaction of DCNBS with NO at 25 °C at pH 3.5 and 1.5. (♦) pH 1.5 and (○) pH 3.5

Figure 8.17. The reaction of DBNBS with NO at 25 °C at pH 3.5 and 1.5. (♢) pH 1.5 and (●) pH 3.5
8.3.5 Model development

Table 1 summarises the chemical reactions constituting the kinetic model for the *ex situ* reaction of aromatic nitroso sulfonates with NO. Reaction 8.1 involves the important trapping reaction step, of the nitroso compounds with NO and is described as an overall reaction yielding oxygen, nitrogen and aryl radical, with the rate of trapping denoted as $k_{\text{Trap}}$. As shown in Figure 8.9, the reaction $k_{\text{Trap}}$ is suggested to occur in three steps: (1) the coupling of the NO radical with the nitroso sulfonate compound to form a short-lived adduct (2) the decomposition of the short live adduct to produce oxygen and a diazenyl radical and (3) the homolysis cleavage of the diazenyl radical to yield nitrogen and aryl radical. Reaction 8.2 describes the monomer-dimer interchange existing in these aromatic nitroso sulfonate compound. The rate constants for the dimer/monomer equilibrium were determined in Chapter 6. Reactions 8.3-8.7 are the various reaction pathways for the nitrosation in the presence of oxygen and water. Rate constants employed for the Reactions 8.3-8.7 are based on those available in the open literature. Reaction 8.7 describes the reaction of the aryl radical with a nitrite ion, forming radical anion which in turns reacts with the electrophile $\text{N}_2\text{O}_3$ to yield the nitrobenzene sulfonate product.
### Table 8.4. Chemical reactions and rate constant employed in the model for the ex situ trapping of NO by aromatic nitroso sulfonate compounds

<table>
<thead>
<tr>
<th>Eqn</th>
<th>Reaction</th>
<th>Rate constant</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>R8.1&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>DXNBS+NO → N₂+O₂+Aranyl radical</td>
<td>(k_{\text{Trap}} = ?)</td>
<td>Determined in this study</td>
</tr>
<tr>
<td>R8.2&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>DXNBS dimer ⇌ 2DXNBS</td>
<td>(k_2/k_2)</td>
<td>Estimated in Chapter 7</td>
</tr>
<tr>
<td>R8.3</td>
<td>2NO + O₂ → 2NO₂</td>
<td>(k_3 = 2.1 \times 10^6 \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1})</td>
<td>Ref [11]</td>
</tr>
</tbody>
</table>
| R8.4 | 2NO₂ + H₂O ⇌ HNO₂ + NO₃⁻ + H⁺                                           | \(k_4 = 7.0 \times 10^6 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}\)  \\
|      |                                                                         | \(k_{4} = 0.0089 \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1}\)         | Ref [12]             |
| R8.5 | HNO₂⇌NO₂⁻+H⁺                                                            | \(k_5 = 6.93 \times 10^{6} \text{ s}^{-1}\)  \\
|      |                                                                         | \(k_5 = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}\) | Ref [13]             |
| R8.6 | 2HNO₂ + NO₃⁻ + H⁺ ⇌ N₂O₃ + H₂O                                           | \(k_6 = 32000 \text{ mol}^{-2} \text{ dm}^6 \text{ s}^{-1}\);  \\
|      |                                                                         | \(k_6 = 6400 \text{ s}^{-1}\)         | Ref [13]             |
| R8.7 | Aranyl radical + NO₂⁻→Radical anion                                      | \(k_7 = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}\) | Assumed value        |
| R8.8 | Radical anion+N₂O₃→ nitro + NO₃⁻+NO                                        | \(k_8 = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}\) | Assumed value        |
| R8.9 | NO<sub>(aq)</sub> → NO<sub>(g)</sub>                                       | \(k_{\text{MT}} = 0.75 \times 10^{3} \text{ s}^{-1}\)                 | Estimated in this study |
| R8.10<sup>(b)</sup> | DXNBS ⇌ DXNBS + H⁺                                                       | \(k_{10} = 1 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}\) | Estimated in Chapter 6 |

(a) \(X= H, \text{CH}_3, \text{Cl} \text{ and Br}\)  \\
(b) \(X = H \text{ and CH}_3 \text{ only}\)

---

Chapter 8: Comparative Study of the Trapping of Nitric Oxide by Ortho Substituted Nitroso Sulfonates
Experimental measurements obtained from the NOx analyser and UV-Vis separately were applied to estimate kinetic parameters of the proposed reaction mechanism using commercial software [14]. For the measurement of NO using the NOx analyser, Reaction 8.9 was included to account for mass transfer of NO from the aqueous to gaseous phase. Independent experiments were conducted with NO formed from rapid nitrosation of ascorbic acid to determine the rate of mass transfer based on the reduction in NO with time. A rate constant of $0.75 \times 10^{-3}$ s$^{-1}$ was found to fit the data reasonably well as previously determined in Chapter 5.

Reaction 8.10 has been included to describe the deprotonation of the compounds in the mechanism and is used in the model exclusively for DMNBS and NBS, as neither compound are fully deprotonated (at the N=O site) under the pH conditions studied. The rate constant for the reaction between the hydrogen ion and the base was assumed to occur at the diffusion controlled limit ($10^{10}$ mol$^{-1}$ dm$^{-3}$ s$^{-1}$ at 298 K) [13, 15] with the dissociation reaction rate constant determined from the equilibrium constant.

The proposed reaction mechanism was first fitted to the experimental measurement of aromatic nitroso from UV-Vis spectrophotometer and the rate constant for known reactions was included where these are available. The software program then generated a system of ordinary differential equations describing the reaction mechanism and provides a “best fit” to reduce the difference between the model and experimental data using a global fit analysis routine on the basis of a Levenberg-Marquart algorithm. For each mechanism, several initial values were trialled to ensure convergence to a global minimum. The second order rate constants reported were derived from at least four independent experiments employing different concentration of the nitroso compound and specified as less 2 % standard error. The obtained values of $k_{\text{Trap}}$ were then tested.
against an independent set of data from the aqueous NO measurement obtained from the NOX analyser. Figure 8.18 and 8.19 show the measured data for the consumption of DCNBS by NO and the NO measurements respectively for determining rate of trapping. The plots for NBS and DMNBS used to determine rate constant $k_{\text{Trap}}$ are provided in Appendix E.

![Graph showing the consumption of DCNBS by NO](image)

**Figure 8.18.** Model prediction as compared to measured data for the time change of initial ($\Delta$) 0.45 mmol dm$^{-3}$, (+) 0.9 mmol dm$^{-3}$, (○) 1.2 mmol dm$^{-3}$ and (×) 1.8 mmol dm$^{-3}$ DCNBS at 25°C. Solid line represents model predictions. For clarity every 10th point is shown.
Figure 8.19. NO concentration versus time for the ex situ trapping of 0.6 mM NO using 0.1 -1.2 mM DCNBS. (▽) 0.1 mmol dm$^{-3}$, (+) 0.15 mmol dm$^{-3}$, (○) 0.3 mmol dm$^{-3}$, (□) 0.6 mmol dm$^{-3}$, (△) 1.2 mmol dm$^{-3}$. Solid lines represent model fit.

The results of the kinetic study under ex situ and in situ conditions are summarised in Table 8.2. In comparison with the previously determined second order rate constant $k_{Trap}$ for the ex situ trapping of NO by DBNBS, the value of $k_{Trap}$ determined under identical conditions was 1.8 fold higher for DCNBS. This highlights the activity of DCNBS as an effective NO scavenger. However, the rate of NO scavenging was significantly slower for DMNBS with the rate being 16 fold lower compared to DCNBS.
Table 8.5. Summary of rate parameters for the aromatic nitroso sulfonate compounds under

_in situ_ and _ex situ_ conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>$pK_a^{(a)}$</th>
<th>$k_2 / \text{s}^{-1}^{(b)}$</th>
<th>$k_3 / \text{s}^{-1}$</th>
<th>$K_c = k_3/k_1$</th>
<th><em>Ex situ</em> $k_{Trap}$</th>
<th><em>Net effective rate $k</em>{Trap}$ in situ / mol$^{-1}$ dm$^3$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS</td>
<td>1.38</td>
<td>$1.2 \times 10^1$</td>
<td>23.7</td>
<td>$5.11 \times 10^3$</td>
<td>75.6</td>
<td>0.47</td>
</tr>
<tr>
<td>DMNBS</td>
<td>2.4</td>
<td>$8.4 \times 10^2$</td>
<td>24.4</td>
<td>$3.46 \times 10^3$</td>
<td>10.1</td>
<td>0.43</td>
</tr>
<tr>
<td>DCNBS</td>
<td>-2.2</td>
<td>$1.8 \times 10^2$</td>
<td>9.3</td>
<td>$1.97 \times 10^3$</td>
<td>293</td>
<td>4.7</td>
</tr>
<tr>
<td>DBNBS</td>
<td>-2.2</td>
<td>$2.9 \times 10^2$</td>
<td>21.6</td>
<td>$1.29 \times 10^3$</td>
<td>165</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*(a)* Determined in Chapter 6, Ref [16]

*(b)* Refer to Chapter 7

Figure 8.20 compares the concentration of NO as measured from the NOx analyser, from the four aromatic nitroso compounds at initial 0.015 mol dm$^{-3}$ sodium nitrite and 0.1 mol dm$^{-3}$ acetic acid. The measurements from the _in situ_ reaction were characterised by a rapid rise followed by a steady state decrease in the concentration of NO. The time when the NO concentration starts to drop in these reactions was the point when the rate of NO trapping has become greater than the rate of decomposition of nitrous acid. This is in contrast to the case where the nitroso compounds were absent. The kinetics of the latter reaction clearly demonstrates an initial rapid rise in NO after which accumulation of NO would induce a continued slow increase in the rate of formation of NO. The overall net rate constants in the _in situ_ reactions are significantly lower compared to the _ex situ_ rate constants, highlighting the net effect of the continual...
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generation of NO in the in situ reaction. The overall net rate constant for trapping of NO by DCNBS was estimated to be approximately the same value as determined for DBNBS. However, because of the relatively slow rate of recombination of the monomer to dimer of DBNBS compared to the other nitroso compounds, the concentration of active monomer available for the trapping reaction was elevated, which subsequently led to an increase in its overall efficiency as a NO scavenger.

Figure 8.20. In situ trapping of NO by aromatic nitroso sulfonate compounds at initial 0.015 mol dm$^{-3}$ NaNO$_2$ and 0.5 mol dm$^{-3}$ acetic acid at 25 ºC. (♦) without nitroso compounds, (○) 0.015 mol dm$^{-3}$ NBS, (♦) 0.015 mol dm$^{-3}$ DMNBS, (♦) 0.015 mol dm$^{-3}$ DCNBS and (△) 0.015 mol dm$^{-3}$ DBNBS.
Complete reduction of NO by the aromatic nitroso compounds under the conditions examined was not achieved. The addition of a nitroso compounds after the initial rapid generation of NO showed a dramatic effect in reducing the concentration of NO for all the four nitroso compounds (Figure 8.21). However, identical to the situation of DBNBS, complete removal of NO was not attained, even under these conditions.

**Figure 8.21.** Effect of addition of aromatic nitroso sulfonate compounds after the initial rapid generation of NO by nitrous acid decomposition; initial nitrite concentration of 0.015 mol dm$^{-3}$. Open arrow indicates the time of addition of nitroso compounds. (◊) without nitroso compounds, (○) 0.015 mol dm$^{-3}$ NBS, (⁎) 0.015 mol dm$^{-3}$ DMNBS, (+) 0.015 mol dm$^{-3}$ DCNBS and (Δ) 0.015 mol dm$^{-3}$ DBNBS.
8.3.6 Comparison of nitroso spin traps

The efficacy of aromatic nitroso compounds for scavenging of nitric oxide was compared with other types of NO spin traps (nitronyl nitroxide and metal complexes) (see Table 8.3). The rate constants for four nitroso compounds studied in the present are significantly lower than those reported for nitronyl nitroxide and iron complexes. Iron complexes (which have the highest efficiency of NO trapping) are four orders of magnitude higher than that of nitroso compounds. The nitronyl nitroxides have rate constants ranging from \((5 - 10) \times 10^3\) mol\(^{-1}\) dm\(^3\) s\(^{-1}\) roughly an order of magnitude higher than the rate constants for aromatic nitroso compounds examined in the present investigation.

Also compared are the rate constants for the trapping reaction of DBNBS of NO using inorganic radicals that have been generated in situ. Bors et al., determined that rate constant of spin trap DBNBS with various radical by pulse radiolysis [17]. Their results, together with the data obtained in the present study for the in-situ trapping of NO formed via the decomposition of nitrous acid are summarised in Table 8.4. The trapping of radicals formed by radiolysis (as a specific source of radical) was associated with a very high rate constant which shows clearly that DBNBS is a highly effective spin trap reacting with the radicals at or near diffusion controlled limits. We noted that the formation of radicals via pulse photolysis are very fast processes. For instance, the formation of tert butoxyl radical by using laser flash photolysis has a rate constant of \(6.5 \times 10^8\) mol\(^{-1}\) dm\(^3\) s\(^{-1}\) [18]. In contrast, the generation of nitric oxide via nitrous acid decomposition occurs at a much slower rate associated with a rate constant of \(10^6\) mol\(^{-1}\) dm\(^3\) s\(^{-1}\). Consequently, the rate of trapping of NO in the in situ experiments was relatively slow, demonstrating the rate of generation of NO is also important and has a
major influence on the net rate at which the radical will be trapped. According to Rosen et al., the ability for spin trapping to identify any of the radicals depends on several factors including the rate of radical production [19]. In addition, the stoichiometry of the reaction of NO with carboxy-PTIO, a nitronyl nitroxide spin trap was reported to vary with the rate of NO formation [20]. This underscores the important influence that the rate of NO formation has on the rate of trapping, as observed in the present examination.
<table>
<thead>
<tr>
<th>Spin trap</th>
<th>Rate constant / ( \text{mol}^{-1} \text{dm}^{3} \text{s}^{-1} )</th>
<th>Experimental condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTIO (nitronyl nitroxide)</td>
<td>( 5.15 \times 10^3 )</td>
<td>Aqueous phosphate buffered solutions of NO at pH 7.4</td>
<td>Ref [21]</td>
</tr>
<tr>
<td>N(Me)(_3)-PTIO</td>
<td>( 6.0 \times 10^3 )</td>
<td>Aqueous phosphate buffered solutions of NO at pH 7.4</td>
<td>Ref [22]</td>
</tr>
<tr>
<td>Fe(MGD)(_2)</td>
<td>( 1.2 \times 10^6 )</td>
<td>Aqueous phosphate buffered solutions of NO at pH 7.4</td>
<td>Ref [23]</td>
</tr>
<tr>
<td>Fe(PDTC)(_2)</td>
<td>( 1.1 \times 10^8 )</td>
<td>Aqueous phosphate buffered solutions of NO at pH 7.4</td>
<td>Ref [24]</td>
</tr>
<tr>
<td>DBNBS</td>
<td>( 1.68 \times 10^2 )</td>
<td>NO saturated solution in water</td>
<td>Current study</td>
</tr>
<tr>
<td>DCNBS</td>
<td>( 2.93 \times 10^2 )</td>
<td>NO saturated solution in water</td>
<td>Current study</td>
</tr>
<tr>
<td>NBS</td>
<td>( 0.75 \times 10^2 )</td>
<td>NO saturated solution in water</td>
<td>Current study</td>
</tr>
<tr>
<td>DMNBS</td>
<td>( 0.10 \times 10^2 )</td>
<td>NO saturated solution in water</td>
<td>Current study</td>
</tr>
</tbody>
</table>
Table 8.7. Rate constant of trapping of radicals by DBNBS

<table>
<thead>
<tr>
<th>Radical</th>
<th>Rate constant / $\text{mol}^{-1} \text{dm}^3 \text{s}^{-1}$</th>
<th>$In situ$ generating method</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH$^-$</td>
<td>$3.95 \times 10^9$</td>
<td>Pulse radiolysis</td>
</tr>
<tr>
<td>CO$_2^-$</td>
<td>$15 \times 10^8$</td>
<td>Pulse radiolysis</td>
</tr>
<tr>
<td>O$_2^-$</td>
<td>$4.3 \times 10^7$</td>
<td>Pulse radiolysis</td>
</tr>
<tr>
<td>$\cdot$N$_3$</td>
<td>$2.4 \times 10^8$</td>
<td>Pulse radiolysis</td>
</tr>
<tr>
<td>NO</td>
<td>4.7</td>
<td>Nitrous acid decomposition</td>
</tr>
</tbody>
</table>
8.4 Conclusion

A generalised reaction mechanism from the reaction of aromatic nitroso sulfonate compounds with NO is presented. The trapping reactions are distinguished by the formation of nitrogen gas and an elevated amount of nitrate in the reaction mixture suggesting the homolysis cleavage of an aryl radical is a reaction step common to all the traps examined. Evidence of presence of nitro compounds in the liquid mixture by NALDI-MS also supports the proposed mechanism. A kinetic study, where a single parameter $k_{\text{Trap}}$ was fitted, showed that the ex situ trapping proceeds through the rate limiting dimer dissociation to monomer, while the in situ reaction involves a process where the constant generation of NO via nitrous acid decomposition is rate limiting. Despite the larger equilibrium constant of dimer-monomer of NBS and DMNBS, DCNBS was demonstrated to be the most effective NO scavenger among the four compounds in the ex situ reaction, with a $k_{\text{Trap}}$ of 293 mol$^{-1}$ dm$^3$ s$^{-1}$. However, under in situ conditions, the net reaction rate of NO trapping decreased considerably for all nitroso compounds studied. In addition, NO was completely reduced in the ex situ reactions while in the in situ reactions, NO could only be partially removed. The addition of spin traps following the initial rapid generation of NO did not reduce the NO concentration further, indicating that the net rate of NO scavenging is strongly influenced by the rate of generation of NO. A significant reduction in NO concentration can be achieved using the aromatic nitroso sulfonate compounds in particular with DBNBS.
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8.5 References


Chapter 8: Comparative Study of the Trapping of Nitric Oxide by Ortho Substituted Nitroso Sulfonates


Chapter 8: Comparative Study of the Trapping of Nitric Oxide by Ortho Substituted Nitroso Sulfonates


CHAPTER 9: NO TRAPPING IN AMMONIUM NITRATE SOLUTIONS AND AN EMULSION EXPLOSIVES BY AROMATIC NITROSO SULFONATE COMPOUNDS

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9.1 Introduction

Ammonium nitrate emulsion explosives are widely used throughout the world in the mining and construction industries [1]. They consist of concentrated water in oil emulsions, in which a (discontinuous) phase of inorganic oxidiser salt solution, primarily ammonium nitrate solutions droplets, is dispersed in a (continuous) organic fuel phase. The continuous phase is generally a mixture of diesel fuel and a polyisobutylene succinic anhydride (PIBSA) based emulsifier [2]. The emulsifier is usually included in the emulsion to enhance the stability of the discontinuous phase.

Ammonium nitrate emulsion explosives are considered to be relatively safe, as they require sensitisation (which is usually performed on site) prior to detonation [3]. The sensitisation process results in the introduction of void spaces in the emulsion matrix, which undergo adiabatic compression during detonation, leading to a rapid rise in the temperature of the explosive, sufficient to initiate an explosion [4]. The most widely adopted procedure for sensitisation is via a chemical gassing process, which generates nitrogen gas bubbles \textit{in situ} via nitrosation reactions [5, 6]. To create the nitrogen bubbles, a concentrated nitrite solution is added to the emulsion. This reaction proceeds relatively slowly and glacial acetic acid is often added during gassing to accelerate the gas-generation process [5]. However the rapid chemical gassing reaction can lead to the production of hazardous side products such as nitric oxide (NO). The formation of NO poses occupational safety and health issues for miners, especially when they are working in confined spaces such as in underground mines. Nitric oxide rapidly reacts with oxygen to produce another toxic compound, nitrogen dioxide [7]. According to international exposure standards for atmospheric contaminants in the occupational environment, the permissible exposure limit of nitrogen dioxide over an average period
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of 8 h is 3 ppm [8, 9]. Given the risk of exposure to NO or NO₂, there is a considerable interest in developing methods which minimise and control the release of these gases.

In the previous Chapters, aromatic nitroso sulfonate compounds were identified as potential scavengers of nitric oxide (NO) under conditions which were similar to those encountered during the chemical gassing process. In Chapter 4, we studied the reaction mechanism of 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) with nitric oxide mainly by determining the gaseous and liquid products of the reaction whilst Chapter 8 comparatively investigates on the mechanistic and kinetics of the reaction of three other aromatic nitroso sulfonate namely nitrosobenzene sulfonate (NBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and 3,5-dichloro-4-nitrosobenzene sulfonate. In the present Chapter, we examine the capacity of a series of aromatic nitroso sulfonate compounds, at concentrations of ammonium nitrate solutions up to 7.5 mol dm⁻³ with sodium nitrite for trapping of NO. The results of these experiments afford a comparison of the efficiency of the nitroso traps in concentrated AN solutions and in AN emulsions.

The investigation then examines the effect of aromatic nitroso sulfonate during the gassing of ammonium nitrate emulsion explosive, and compares the efficacy of four aromatic nitroso compounds. For the purpose of the emulsion trapping studies, we have constructed a perspex, cylindrical reactor, mounted with a variable speed stirrer. The stirrer enables the uniform and thorough mixing of the viscous emulsion. A continuous gas purge line sweeps NOₓ product gases from the reactor into a dedicated NOₓ analyser.
9.2 Methodology

9.2.1 Material

Nitroso compounds; 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), 3,5-dichloro-4-nitrobenzene sulfonate (DCNBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and nitrosobenzene sulfonate (NBS) were synthesised following published descriptions (Refer to Chapter 3 for detailed methods). Ammonium nitrate prills were generously supplied from Dyno Nobel Asia-Pacific Australia. Acetic acid, sodium nitrite and calcium nitrate were purchased from Sigma Aldrich (Castle Hill, Australia).

9.2.2 Preparation of ammonium nitrate emulsions (standard emulsion)

The oxidiser (discrete) phase was prepared by weighing all the oxidiser phase components into a stainless steel jug. The mixture was then heated to 75 °C (with continuous stirring) to enable the complete dissolution of the ammonium nitrate prill. Once the ammonium nitrate was dissolved, the pH of the oxidiser was measured and adjusted to between 4.8-5.4 via the dropwise addition of concentrated sodium hydroxide solution. In a separate vessel, the oil phase was weighed and heated to 60 °C. The emulsion was prepared at a ratio of 92 % aqueous phase to 8 % oil phase, formed by slowly pouring the oxidiser phase to the fuel phase with continual stirring at 600 rpm for 60 s. The speed of the mixer was then increased from 600 rpm to 1500 rpm over a period of 120 s until the viscosity of the emulsion had attained an apparent viscosity of between 22 and 28 Pa s. The apparent viscosity was estimated using a Brookfield RVDVII+ viscometer with no. 7 spindle at 20 rpm.
9.2.3 Preparation of emulsion explosive blended with NBS and DBNBS

The aromatic nitroso trap, which constituted 1% of total emulsion, was added to the oxidiser phase and the mixture then heated to 75 °C. The remainder of the procedure was identical to that used for the preparation of a standard emulsion.

9.2.4 Determination of the rate of chemical gassing

The chemical gassing of an emulsion explosive in the laboratory requires the addition of concentrated acetic acid (45 % w/w solution in water) and sodium nitrite solutions (25 % w/w solution in water) to the emulsion with continuous mixing for 30 s. Upon completion of mixing, a stainless steel cup, of known volume and mass, was slightly overfilled with the gassed emulsion and levelled off to the rim using a spatula, with the initial mass recorded. As the gassing reaction proceeded, the emulsion expanded and at regular intervals (of 2-5 min), excess emulsion was scraped off from the rim of the cup and the weight of the cup re-measured. The density change of the emulsion, and thus the rate of chemical gassing, can be determined, based on the rate of change of the mass of the emulsion remaining in the cup, and the known volume of the stainless steel cup.

9.2.6 NO measurement in ammonium nitrate solutions

The concentration of nitric oxide was measured using a membrane NOx analyser system, as described and illustrated in Figure 3.3(b) of Chapter 3. The 10 cm$^3$ round bottom flask reactor was completely filled with 17 cm$^3$ of freshly prepared ammonium nitrate solutions in 0.5 mol dm$^{-3}$ acetic acid buffered. For trapping experiments, the
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Nitroso solutions were prepared in acidified AN solutions. The reactor was flushed with nitrogen gas at a flow rate of 1.33 cm$^3$ s$^{-1}$ prior to commencing the experiment. The nitrogen gas used for flushing the reactor was introduced through a side port and exited the vessel through a needle inserted in a rubber stopper at the top of the reactor. Once the flushing process was completed, the needle was plugged with a 5 cm$^3$ syringe (used subsequently to maintain a relatively constant pressure in the vessel during gassing) and all stainless steel tubing was removed. The nitrogen flow was then connected to the membrane manifold and adjusted to 0.67 cm$^3$ s$^{-1}$. Reaction was initiated by injecting 1 cm$^3$ of a concentrated sodium nitrite solution through a needle inserted through the septum side port of the reactor. The nitric oxide diffusing through the membrane was collected over a 1 h period by the flowing stream of N$_2$ for analysis using a Thermo 42i-HL NOx analyser. The pressure inside the reactor was maintained at atmospheric by allowing expansion of the syringe plunger to accommodate for the formation of N$_2$ during the reaction. Calibration of the NOx analyser was achieved with a 1000 ppm calibration gas (Coregas Pty Australia). The membrane NOx inlet was calibrated at ammonium nitrate concentrations up to 7.5 mol dm$^{-3}$ using the same procedure as outlined in Chapter 3. Calibration plots are illustrated in Figure 9.1. The trapping reaction of NO by aromatic nitroso sulfonates was studied in ammonium nitrate solutions concentration up to 7.5 mol dm$^{-3}$. 
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9.2.7 NO measurement during chemical gassing of emulsion explosive

9.2.7.1 Glove box

Preliminary experiments for measuring the concentration of NO were first carried out within a sealed glove box of 50 L similar to the apparatus described by Rayson (2012) [10]. The outlet of the plastic glove box was connected to the Thermo 42i-HL NOx analyser while the inlet was attached to a cylinder of nitrogen and a flow meter to enabled the glove box to be purged with nitrogen gas prior to each experiment. A recipient containing a known mass emulsion, syringes holding the chemical gassing
reagents and a plastic spatula were placed in the glove box before closing it. Once the box was sealed to the atmosphere, it was purged with nitrogen for 15 min at a flow of 10 L min\(^{-1}\). The flow was then reduced to 0.41 cm\(^3\) s\(^{-1}\) and the chemical gassing reagents were added consecutively and mixed with the aid of the plastic spatula to initiate the gassing process. After a period of 1 h, the gas was stirred out of the emulsion using the plastic spatula to release any NOx contained with the gas bubbles. Throughout the duration of the experiment, the NO level was constantly being monitored at an averaging time of 10 s by the NOx analyser. Figure 9.2 shows the schematic diagram of the apparatus.

![Figure 9.2. Diagram of glove box for NOx measurement during emulsion gassing](image)

*Figure 9.2. Diagram of glove box for NOx measurement during emulsion gassing*
9.2.7.2 Perspex cylindrical 750 cm³ reactor

A perspex cylindrical reactor with a volume of 750 cm³ was constructed. The reactor was equipped with an in-built stirrer, controlled by a variable motor. A schematic diagram of the apparatus is shown in Figure 9.3. For each experiment, the reactor was charged with 75 g of emulsion. The stirrer was a two blade impeller, which enabled rapid and thorough mixing of the emulsion with the gassing reagent. The speed of the mixing was controlled by an overhead variable driven motor. The reactor was sealed to the atmosphere with four screws and a rubber gasket on the top cover. The vessel was equipped with an inlet line, which was used for purging with nitrogen gas prior to the commencement of each experiment and an outlet line connected to the Thermo 42i-HL NOx analyser for continuous monitoring of the concentration of NO. To initiate a gassing experiment, the gassing reagents were injected, via the injection port located on the top cover of the rig, and the impeller mixing the emulsion was set to 50 rpm. The reaction was allowed to proceed for a period of 1 h. During this period, the NO released from the emulsion was swept by the purge gas and the concentration of NO exiting the reactor was measured using the NOx analyser. NO formed in the decomposition of HNO₂, with the acid produced by protonation of NO₂⁻ [11]. As opposed to the previous setting where the chemical gassing occurred within a sealed glove box, the present apparatus enables constant and uniform mixing of the emulsion and thus provides more consistent and repeatable results. In addition, the 750 cm³ reactor requires less time to be flushed by nitrogen gas.
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9.3 Results and discussion

9.3.1 Ammonium nitrate solution experiments

9.3.1.1 Effect of presence of ammonium nitrate solutions

The effect of AN concentration on nitrous acid decomposition and NO solubility is shown in Figure 9.4. The concentration of nitric oxide in solution decreased substantially with increasing ammonium nitrate concentration, signifying a reduced solubility of nitric oxide with increasing ammonium nitrate concentration. This observation is in agreement with Schumpe who generalised that a dissolved electrolyte leads to a decrease in the solubility of a gas compared to its solubility in pure water [12]. The effect of increasing salt concentration, which is more broadly known as
salting out, causes an increase in activity coefficient of the electrolyte. Rayson investigated the solubility of NO in ammonium nitrate solutions at 25 °C and found that the solubility of nitric oxide decreased significantly with increasing concentration of ammonium nitrate and, based on the relationship developed by Setschenow, estimated the salting out parameter $K_{\text{NH}_4\text{NO}_3}$ to be 0.052 mol$^{-1}$ dm$^3$ [10].

![Figure 9.4](image_url)  
**Figure 9.4.** Effect of varying the concentration of ammonium nitrate up to 7.5 mol dm$^{-3}$ on the concentration of NO (aqueous) produced during nitrous acid decomposition.

As a result of a decreasing solubility of NO with increasing concentration of AN, the apparent net efficiency of NO removal was also reduced, as the concentration of NO in the gas phase increased. Nonetheless, the NO concentration was observed to decrease in the presence of DBNBS, and a similar trend was also observed for the other aromatic
nitroso sulfonate compounds. Figure 9.5 illustrates the removal of NO from the reaction with the aromatic nitroso sulfonate compounds at different concentration of ammonium nitrate solution.

The removal was evaluated using the Equation 9.1 where $C_{fb}$ and $C_{fn}$ are the final concentrations recorded after one hour of reaction from the decomposition of nitrous acid in ammonium nitrate in the absence and presence of aromatic nitroso sulfonate compounds respectively. It is noted that at concentrations up to 2 mol dm$^{-3}$, there was little variation in the NO removal efficiency of NBS, DMNBS and DBNBS, whereas in contrast, DCNBS effected a significant influence on NO removal, even at relatively low concentrations of AN.

$$\text{Percentage removal} = 100 \times \frac{C_{fb} - C_{fn}}{C_{fb}}$$

(9.1)
Figure 9.5. The removal of NO by aromatic nitroso compounds at concentrations of AN up to 7.5 mol dm$^{-3}$. (□) DBNBS, (×) DMNBS, (△) NBS, (◇) DCNBS

Generally, the rates of NO removal by NBS and DMNBS were similar, while the rate for DBNBS was slightly higher. Based on the results from Chapter 8, it is expected that DCNBS would be the most efficient NO scavenger as the results obtained in our previous aqueous system concluded that the percentage NO removal was the highest with DCNBS, and it had the fastest rate of NO removal. However, the larger slope obtained for DCNBS is inconsistent with this previous study in particular when compared to the activity of DMNBS which had the slowest rate of trapping reaction in our previous study. Indeed, the slope of NO removal by DCNBS dropped drastically with increasing ammonium nitrate concentration. In addition, it was observed (as opposed to the other aromatic nitroso sulfonate compounds) that the solubility of...
DCNBS diminished drastically as the concentration of AN was increased. Based on these observations, it can be deduced that the decreasing solubility of the compound contributed to the notable decrease in the NO removal with increasing AN concentrations.

### 9.3.1.2 Effect of ammonium nitrate concentration on the solubility of aromatic nitroso compounds

Salting out is believed to have a strong influence on the solubility of the aromatic nitroso compounds when they are dissolved in ammonium nitrate solutions of increasing ionic strength. The magnitude of the salting out effect depends on both the Setschenow constant and the concentration of the inorganic salt, according to the Setschenow Equation (9.2).

\[
\log \gamma = \log \frac{S_{\text{salt}}}{S_{\text{water}}} = -K_{\text{salt}}C_{\text{salt}} \quad (9.2)
\]

Where \(S_{\text{salt}}\) is the solubility of the organic compound in aqueous salt solution

\(S_{\text{water}}\) is the solubility of the organic compound in pure water

\(C_{\text{salt}}\) is the molar concentration of an electrolyte

\(K_{\text{salt}}\) is the empirical Setschenow constant.

Determining the Setschenow constant is important, as it enables the prediction of the solubility of an organic compound in a salt solution. Many researchers have developed
theoretical models to predict $K_{\text{salt}}$, in particular for sodium chloride since it is the most widely studied [13-16]. Xie et al. demonstrated that $K_{\text{salt}}$ can be estimated by $K_{\text{salt}} = 0.0018 V_{\text{LeBas}}$, where $V_{\text{LeBas}}$ is the molar volume, as determined by the method of LeBas, whereas, Gould et al. have employed the intrinsic solubility of the solute, $S_{o}$, to determine $K_{\text{salt}}$ [17]. More recently, Ni et al. developed an empirical relationship (Equation 9.3) based on a linear correlation between $\log K_{\text{ow}}$ to $K_{\text{salt}}$ to predict the effect of NaCl on the solubility of nonelectrolytes [18]. The salting out effect is known to be influenced by the polarity of the non-electrolyte, and accordingly, an empirical estimation based on $K_{\text{ow}}$ has provided a convenient correlation to estimate $K_{\text{salt}}$ since the octanol-water partition coefficient is a descriptor of the overall polarity of the solution [12, 19].

$$K_{\text{salt}} = 0.040 \log K_{\text{ow}} + 0.114 \quad (9.3)$$

Table 9.1 summarises the estimates of $K_{\text{salt}}$ in sodium chloride solution for the aromatic nitroso compounds studied in the present investigation. All $\log K_{\text{ow}}$ were obtained by using ClogP® 4.0 software (Bio Byte Corp., Claremont, CA). The values of $K_{\text{salt}}$ based on these values of $K_{\text{ow}}$ was estimated and are the following order NBS>DMNBS>DCNBS>DBNBS with NBS having the highest solubility of all the compounds in sodium chloride solution. Based on the Setschenow equation, a high value for $K_{\text{salt}}$ signifies a reduced solubility in the electrolyte solution. Although $K_{\text{ow}}$ was calculated for the trapping agents using the solution of NaCl, it gives a trend in $K_{\text{salt}}$ that is consistent with the present results for NH$_4$NO$_3$; compare the slopes for NBS,
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DMNBS and DBNBS in Figure 9.5. The exception is DCNBS, for which the solubility was predicted to be slightly higher than that of DBNBS.

Table 9.1. Estimated Setschenow constants for aromatic nitroso sulfonates

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $K_{ow}$</th>
<th>$K_{salt}$ (Setschenow constant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS</td>
<td>-0.76</td>
<td>0.0836</td>
</tr>
<tr>
<td>DMNBS</td>
<td>0.24</td>
<td>0.1236</td>
</tr>
<tr>
<td>DCNBS</td>
<td>0.97</td>
<td>0.1528</td>
</tr>
<tr>
<td>DBNBS</td>
<td>1.27</td>
<td>0.1648</td>
</tr>
</tbody>
</table>

Extrapolating the experimental data of a solution of 7.5 mol dm$^{-3}$ to a solution of 13 mol dm$^{-3}$ ammonium nitrate (which represents a typical concentration of AN in an emulsion explosive) affords an estimation of the removal efficiency of the aromatic nitroso sulfonate compounds in a sensitised emulsion explosive system (See Figure 9.5). Accordingly, DBNBS is predicted to be the most effective NO scavenger in emulsion explosive system while DCNBS would be predicted to be the least effective with only 35 % reduction in NO trapping capacity.
9.3.2 Ammonium nitrate emulsion experiments

9.3.2.1 Effect of aromatic nitroso compounds on the density of the emulsion.

Determination of the gassing rate was undertaken by estimating the density change of the emulsion in a stainless steel cup at 25 °C. The density of gassing emulsion explosive was estimated by dividing the mass of the emulsion remaining in the cup by the constant cup volume of 125 cm$^3$. Figure 9.6 depicts the density profile obtained during the gassing of standard emulsion. The figure also compares the density profile of the standard emulsion with emulsion where gassing was performed in the presence of the aromatic nitroso sulfonates. The density profile shows three distinct steps occurring in chemical gassing process. Initially, there is a short lag period which can be attributed to the time required for the aqueous phase to be saturated with dissolved nitrogen gas. da Silva et al. [20] proposed that this short lag time is a result of molecular diffusion of the gassing reagents through the emulsion. Following this initial stage, a steady decrease in the density of the emulsion is encountered followed by a plateau in the density, as the rate of the gassing reaction starts to decrease. The rate of gassing of a standard emulsion was found to be independent of the presence of the nitrosobenzene sulfonate traps, incorporated in the emulsion.
Figure 9.6. Density profiles of emulsion with aromatic nitroso compounds. (⊙) Standard emulsion, (×) DCNBS, (+) DMNBS, (△) NBS, (□) DBNBS

9.3.2.2 Gassing of emulsion explosive in presence of nitroso compounds

Figure 9.7 compares plots for the measurement of NO obtained from the chemical gassing of a standard emulsion blended with NBS and DBNBS. (These measurements were obtained from the preliminary experiments using the sealed glove box.) For the first hour of the reaction, the NO released is due to the diffusion of NO from the emulsion to the gaseous phase, which is then swept by the purge gas to the NOx analyser. A similar trend was observed for the emulsion blended with all nitroso compounds. It was noted however that when the emulsion was destroyed, and the gas released from the emulsion, the amount of NO released was significantly reduced in
comparison with the standard emulsion. The NO removal from the collapsed emulsion in DBNBS and NBS were estimated to be 60 % and 14 % of the total amount of NO released from the standard emulsion. With respect to NBS, the amount of NO scavenged was inconsistent with the data obtained in the aqueous system. It is suspected that the reason for this lower than expected efficiency of NBS was a consequence of the decreased concentration of NBS due to its decomposition during the preparation of the emulsion.

The addition of the nitroso trap during the gassing process had a significant influence on the net amount of NO removed. When DBNBS and NBS were premixed with the gassing reagents, the net removal of NO was 72 and 60 % respectively. Figure 9.8 compares NO removal from an emulsion blended with DBNBS with one where DBNBS addition was performed at time of gassing. The addition of the nitroso compounds at the time of gassing results in a greater proportion of NO being trapped, especially in the case of NBS.
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Figure 9.7. Comparison of experimental results for the NO formation from standard emulsion with emulsion blended with DBNBS and NBS.

Figure 9.8. Comparison of NO formation from gassing a standard emulsion blended with DBNBS and gassing a standard emulsion with DBNBS mixed in gassing reagents.
9.3.2.3 Chemical gassing experiments in the 750 cm$^3$ Perspex reactor

Figure 9.9 shows the plot of concentration profile of NO from the chemical gassing a 75 g of standard emulsion with initial nitrite and acetic acid of 0.015 mol dm$^{-3}$ and 0.03 mol dm$^{-3}$ respectively using the 750 cm$^3$ Perspex reactor. The repeatability of the experiment is also shown in the plot by 3 runs under the same experimental conditions. We can observe reasonable reproducibility of the data in particular the maximum NO recorded from these run were 307.8 ± 4.1 ppm and an area under the curve of these plots of $(4.64 ± 0.25) \times 10^5$.

![Figure 9.9. Plots of concentration profile of NO from the chemical gassing a 75 g of standard emulsion with initial nitrite and acetic acid of 0.015 mol dm$^{-3}$ and 0.03 mol dm$^{-3}$ respectively.](image-url)
We compared the results obtained from trapping NO by aromatic nitroso compounds in the 10 cm$^3$ (2 neck round bottom flask) and the 750 cm$^3$ Perspex cylindrical reactors (See Figure 9.10). The removal in NO by nitroso compounds when the 750 cm$^3$ reactor was employed, followed the same trend as the results obtained from the 10 cm$^3$ flask with an error varying from 0.85 - 3.50 % depending on the compounds.

**Figure 9.10.** Comparison of results obtained from the trapping in 7.5 mol dm$^{-3}$ AN solution using a 10 cm$^3$ and 750 cm$^3$ reactor. (□) 10 cm$^3$ and (○) 750 cm$^3$ reactor.
9.3.2.4  Effect of varying stirrer speed on NO formation

A series of experiments was undertaken at 25 °C with 75 g of emulsion to investigate the effect of the stirring speed. The experiments employed an initial nitrite and acetic acid of 0.015 and 0.03 mol dm$^{-3}$ respectively. Figure 9.11 illustrates the effect of the speed of mixing on the NO concentration during the chemical gassing of a standard emulsion. The amount of NO liberated when the emulsion was not continuously mixed was minimal in comparison to experiments where mixing was performed throughout the duration of the experiment. In the case where stirring was not conducted, the maximum concentration of NO was 25 ppm, which represents around 3.5 % of the initial nitrite being converted into NO. This scenario would be similar to the conditions where chemical gassing was undertaken on site, and illustrates that the release of NO is minimal compared to conditions where mixing is applied. When mixing speeds were set to 50 and 150 rpm, the maximum concentration of NO in the gas phase was 306 and 316 ppm respectively, where the rise in NO observed upon stirring would correspond to between 47 and 48 % of the initial nitrite being converted into NO. Also noted is that the effect of an increase of the speed of the stirrer from 50 rpm to 150 rpm did not significantly enhance the total amount of NO formed during the chemical gassing process.
Figure 9.11. The effect of varying speed during the gassing of a standard explosive with an initial nitrite concentration of 0.015 mol dm$^{-3}$ at 25 °C

9.3.2.5 Effect of presence of aromatic nitroso sulfonate compounds

We examine the effect of mixing at 150 rpm on the emission of NO for emulsions where nitroso compounds were added in the gasser. The result of these experiments, shown in Figure 9.12, which clearly suggests the inhibitory effect of the traps on the amount of NO released. The rapid rise in NO concentration, followed by an exponential decay, for the experiment with DCNBS, occurred due to an advanced commencement of this experiment by 8 min, in comparison to other experiments. The presence of chloride ion, formed from the nucleophilic substitution reaction taking place in the emulsion, are believed to strongly influence the course of the reaction, catalysing the initial formation of NO from the decomposition of nitrous acid. Amado et al. have examined the nitrosation of thione-thiol and demonstrated from their kinetics analysis
that halide ions catalysed the course of the reaction [21], while da Silva et al. investigated the formation of nitrosating agents from the equilibrium reaction of nitrous acid with nucleophilic catalyst such as chloride, bromide thiocyanate and iodide [22]. According to these studies, nitroso group reactivity towards aniline-type compounds increases with increasing nitrosating agent electrophilicity (i.e. decreasing nucleophilicity). On this basis, bromide ions (in the case of DBNBS) would be less effective than chloride in catalysing the formation of nitric oxide. If an iodo compound was employed, the iodide ion would be the least effective halide ion. Indeed based on the data in Figure 9.13, the rate of initial decomposition of nitrous acid in the following order DCNBS > DBNBS > NBS > DMNBS.

![Figure 9.12](image_url)

**Figure 9.12.** Effect of presence of aromatic nitroso sulfonate compounds on the gassing of emulsion explosive with initial nitrite and acetic acid of 0.015 and 0.03 mol dm$^{-3}$, respectively.
Figure 9.13. Zoomed plot, highlighting the effect of presence of aromatic nitroso sulfonates during the period from 0-1000 s

The area under the NO concentration time curve was used to estimate the efficiency of the different aromatic nitroso compounds in emulsion (The results are shown in Figure 9.14). As expected from the preliminary results with AN solutions, DBNBS is found to be the most efficient trap, with approximately 70% of the NO removed, followed by NBS. The NO removal efficiency using DCNBS was, as predicted, to be the lowest among the four aromatic nitroso sulfonate compounds. The efficiency of DCNBS as an NO scavenger was reduced due its relatively low solubility in the saturated ammonium nitrate solution when compared to the other nitroso compounds. Indeed, as a result of the decrease in NO removal with increasing ammonium nitrate concentration observed.
with all the four aromatic nitroso compounds, the solubility of all the traps studied was affected in highly ionic solvent but not to the same extent as for DCNBS.

**Figure 9.14.** Effect of nitroso compounds on the removal of NO during the chemical gassing of 7.5 mol dm$^{-3}$ ammonium nitrate solution and emulsion (13 mol dm$^{-3}$ AN). ($\times$) DCNBS, (○) DMNBS, (□) NBS, (△) DBNBS
9.4 Conclusion

In this Chapter, the effect of the presence of aromatic nitroso sulfonate compounds during the chemical gassing of ammonium nitrate solutions and emulsion was examined. Based on a series of preliminary experiments in aqueous solution, the incorporation of the aromatic nitroso compounds at the time of gassing was observed to achieve the highest level of reduction in NO released. Based on a comparison of the density profile from the gassing of emulsion in the presence of aromatic nitroso compounds in the emulsion, it seems that the presence of the aromatic nitroso compounds does not influence the gassing process significantly. Adopting the Setschenow equation and the decreasing solubility of nitroso compounds, the efficacy of the aromatic nitroso compounds are predicted to decrease with increasing ammonium nitrate concentrations. Notwithstanding these limitations, up to 70 % removal efficiency of NO can be obtained in sensitised AN emulsion when an aromatic nitroso compounds is added during gassing.
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9.5 References


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CHAPTER 10: CONCLUSION & RECOMMENDATIONS
Chapter 10: Conclusion & Recommendation

10.1 Conclusion

The formation of nitric oxide gas (NO) from the sensitisation of ammonium nitrate emulsion explosive can have deleterious effects on the health of those exposed to its fumes. Exposure to nitric oxide gas in low concentrations produces respiratory and reproductive dysfunctions in the human body while acute exposure to the gas causes asphyxiation. There is a practical need to develop new technologies to minimise the risk associated with the exposure of workers and the environment to NO. From these perspectives, this thesis has explored potential methods to scavenge or reduce NOx formation associated with the sensitisation process of emulsion explosive. In the literature review, we identified spin traps as potential NO scavengers. Spin traps are commonly employed by biochemists to stabilise free radicals and forms stable adducts that can be detected by EPR. This then enables the measurement of NO in biological systems, and these traps could likewise serve to trap NO and eventually reduce the amount of the gas released when added to a chemical process in situ.

Among the spin traps we have reviewed, nitroso spin traps, in particular 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), were recognised to be the most appropriate for use in aqueous systems such as ammonium nitrate emulsion explosives. This thesis consequently focused on examining the trapping capacity of nitroso compounds towards NO. Our initial study was on DBNBS, followed by a series of other nitroso compounds; nitrosobenzene sulfonate (NBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and 3,5-dichloro-4-nitrosobenzene sulfonate (DCNBS). Our objective was to determine whether they could be practically applied during the sensitisation of ammonium nitrate explosives.
Chapter 4 examined the *in situ* reaction of 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) with nitric oxide (NO), generated via the reduction of nitrite under acidic conditions at room temperature. Significant quantities of N₂ were detected during reaction using the membrane inlet mass spectrometer (MIMS), which suggested the homolytic cleavage of the C-N bond of a diazenyl radical, formed by decomposition of a DBNBS-NO adduct. Measurements by nanostructured assisted laser desorption ionisation mass spectrometer (NALDI-MS) established that the primary product from the reaction was 3,4,5-trinitrobenzene sulfonate, with a molecular weight of 291.880 amu. The technique also disclosed the formation of other nitrobenzene products. The results obtained provide evidence of a new, competing reaction pathway occurring in the presence of added nitrite, whereby a nitro group is introduced in the aromatic system by coupling with a phenyl radical to yield radical anion, which reacts readily with the electrophile N₂O₃, also present in the system. However, a different pathway was observed in *ex situ* reactions where a small amount of nitrite (due to the reaction NO with oxygen) is present, a DBNBS molecule reacts with the phenyl radical to form the radical product dianion bis (2,6-dibromo-4-sulfophenyl).

Chapter 5 assessed the reaction kinetics of the trapping of nitric oxide (NO) by 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) by application of a novel membrane inlet NOx chemiluminescence analyser. An important aspect of the mechanism development was the estimation of the value of equilibrium constant $K_C$ of $(1.29 \pm 0.03) \times 10^{-3}$ (at 25 °C) and forward and backward rate constants of 0.03 s⁻¹ and 21.6 mol⁻¹ dm³ s⁻¹ respectively for the dissociation reaction of the dimeric form of DBNBS to its monomeric state. These parameters were also evaluated over a
temperature range of 25 to 60 °C, providing the following thermodynamic parameters: standard enthalpy change, $\Delta H^0 = 53.1 \pm 0.5$ kJ mol$^{-1}$; standard entropy change, $\Delta S^0 = 123 \pm 1$ J mol$^{-1}$ K$^{-1}$; enthalpy of activation, $\Delta H^\dagger = 77.0 \pm 0.3$ kJ mol$^{-1}$; entropy of activation, $\Delta S^\dagger = -15.8 \pm 0.8$ J K$^{-1}$ mol$^{-1}$.

Under conditions where nitric oxide was generated via the rapid nitrosation of ascorbic acid (ex situ experiments), a rate constant for trapping of nitric oxide with DBNBS was estimated to be $k_{\text{Trap}} = 165$ mol$^{-1}$ dm$^3$ s$^{-1}$. However, when nitric oxide was generated under conditions which are similar to those encountered in industrial processes, specifically by the decomposition of nitrous acid via the acidification of aqueous solution of sodium nitrite (in situ experiments), the net rate of trapping was significantly reduced, to 4.7 mol$^{-1}$ dm$^3$ s$^{-1}$ if the same model for the ex situ experiments was employed. This is likely to be the result of as yet undetermined side reaction(s) occurring in the presence of added nitrite and (or) limitations imposed on the net efficiency of trapping of nitric oxide due to its continuous generation from the decomposition of nitrous acid. The results confirm that complete reduction in nitric oxide is not likely under in situ reaction conditions, as the nitric oxide is being continuously produced. However, a reduction in NO of almost 98 % after a reaction duration of sixty minutes highlights the efficiency of DBNBS as a nitric oxide scavenger, for application in a practical chemical system to reduce NOx formation.

In Chapter 6, we determined the acid dissociation constant, $pK_a$ of nitrosobenzene sulfonate (NBS) and 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) using experimental and theoretical methods. $pK_a$ values of $1.26 \pm 0.38$ and $2.28 \pm 0.04$ were
determined by potentiometric titration for NBS and DMNBS respectively. The results confirmed that both compounds are weakly acidic with DMNBS being slightly more basic compared to NBS due to the presence of electron donating methyl group in the compound. Quantum chemistry calculations (density functional theory, DFT) and empirical methods were also employed in the study to predict the $pK_a$ of these compounds. Aqueous $pK_a$ of nitroso compounds were calculated using quantum chemistry calculation via a proton transfer thermodynamic cycle that combined accurate gas phase acidities with solvation free energies obtained from polarisable continuum model (PCM). The introduction of relatively minor error in the estimation of the free energy change for the transition from protonated to deprotonated state, $\Delta G_{\text{solv}}$, in particular when applying B3LYP method, could lead to significant error in the $pK_a$ in view of its direct relationship with $\Delta G_{\text{solv}}$. Conversely, the utilisation of commercially available softwares that apply empirical methods for predicting $pK_a$ is highly recommended since it compared reasonably well with experimental results for aromatic nitroso sulfonate compounds.

Chapter 7 presented the physicochemical properties of the four selected aromatic ortho substituted nitroso compounds. All four molecules are planar according to their optimised geometries obtained from quantum chemical calculations with their C-N bonds being weakened as a result of the presence of an electron withdrawing group substituent at the ortho position in the aromatic ring. However, regardless of substitution, all four aromatic nitroso compounds are considered to be thermally stable with the C-N enthalpies estimated to be in excess of 191 kJ mol$^{-1}$. These compounds, like most C-nitroso spin traps, exist in a monomer-dimer equilibrium with only the monomeric form behaving as a free radical spin scavenger. The investigation on the
influence of the substituent on the dissociation of dimer to monomer using UV-Visible spectrophotometry determined that an increase in the size of ortho substituent would favour the stability of the dimer form of these compounds, making such ortho substituted species less reactive for scavenging NO.

Chapter 8 evaluated a range of aromatic nitroso sulfonate compounds with respect to their efficacy to scavenge nitric oxide (NO). The reaction mechanism of three aromatic nitroso sulfonate compounds; NBS, DCNBS and DMNBS, with nitric oxide radical formed via the decomposition of nitrous acid were investigated. The study employed a similar approach to that undertaken in Chapter 4 for the detection and quantification of liquid and gaseous products, which included analytical tools such as membrane inlet mass spectrometry (MIMS), ion chromatography (IC) and nanostructured assisted laser ionisation mass spectrometry (NALDI). Based on the distinguished formation of nitrogen gas in the head space and an elevated amount of nitrate in the reaction mixture during reactions of all the compounds with NO, we presented in Chapter 8 a generalised reaction mechanism to demonstrate the homolysis cleavage of aryl radicals. In order to undertake a quantitative evaluation of the scavenging capacities of these nitroso compounds, rate constants of the reaction of the aromatic nitroso sulfonate compounds with NO were determined under ex situ conditions. The overall rate of the reaction of these nitroso sulfonate compounds with NO was found to be in the following order: DCBNBS> DBNBS>NBS> DMNBS with the reaction of DCNBS with NO ($k_{\text{Trap,DCNBS}} = 293 \text{ mol}^{-1} \text{ dm}^{3} \text{ s}^{-1}$) having the highest rate among the four compounds studied. Measurements show slower reaction with NO for NBS and DMNBS despite their equilibrium constant flavoured formation of monomer. Rather their higher pK$_a$ values, compared to DBNBS and DCNBS, have resulted in slower rate of reacton since the N-O
moiety was not fully deprotonated under the conditions employed. The overall net rate
constants in the \textit{in situ} reactions were significantly lower compared to the \textit{ex situ} rate
constants, highlighting the net effect of the continual generation of NO in the \textit{in situ}
reactions. In all cases, complete reduction of NO by the aromatic nitroso compounds
under the conditions examined was not achievable. Nevertheless, the addition of a
nitroso compounds after the initial rapid generation of NO showed a dramatic effect in
reducing the concentration of NO for all the four nitroso compounds and satisfactory
reduction can be achieved with these traps for \textit{in situ} conditions.

Finally, we examined the chemical trapping of nitric oxide (formed during the chemical
gassing of water-in-oil emulsion) using the four aromatic nitroso compounds in Chapter
9. Experiments involved a semi batch 750 cm$^3$ stirred reactor, with an outlet purge gas
stream interfaced to a NO$_x$ analyser to continuously monitor the concentration of NO.
We investigated the trapping of NO generated both in concentrated solutions of
ammonium nitrate (up to 7.5 mol dm$^{-3}$) and during gassing of ammonium nitrate
eмуlсіоn$. Experiments conducted in concentrated ammonium nitrate solutions
demonstrated a decreasing solubility of nitroso compounds, which signifies the efficacy
of the aromatic nitroso compounds is predicted to decrease with increasing ammonium
nitrate concentrations. In contrast to our previous study, where DCNBS had the fastest
trapping rate in aqueous system, in concentrated ammonium nitrate solution, the
efficacy of DCNBS to scavenge NO decreased due to the drastic drop in the solubility
of the compound with increasing AN concentrations. Chemical trapping of NO was
found to be more efficient when the nitroso compounds were added at the time of
chemical gassing, rather than being part of the discrete phase of the emulsion. Among
the four compounds studied, DBNBS was the most efficient trap with the capacity to
trap up to 70% of the NO formed in the emulsion.

Overall, the application of spin traps aromatic nitroso compounds was found to be
successful in reducing the formation of NO during the chemical gassing of emulsion
explosive. This technology represents an innovative and efficient manner to prevent the
release of NO without interfering with the gassing process which could be potentially
extended to other industrial applications where control of NO is required.

10.2 Recommendations

In this study, we have demonstrated that NALDI-MS represents a valuable analytical
method for the characterisation of aromatic nitroso sulfonate compounds as well for the
detection of their reaction mixtures with NO. Despite its ease of use for qualitative
purposes, quantitation of aromatic nitroso compounds remains an outstanding research
problem. The addition of surrogate and internal standards could be a possible way
forward to perform quantitative analysis. We suggest synthesising $^{13}\text{C}$ and $^2\text{H}$ labelled
3,5-dibromobenzene sulfonates (DBBS) that could either be added to the reacting
mixture prior to experiments (as a surrogate standard) or just after the reaction is
completed (as an internal standard). Labelled DBBS should be more inert than
DBNBS, as they would not undergo nitrosation reactions. Should an attempt to deploy
$^{13}\text{C}$ and $^2\text{H}$ labelled DBBS be unsuccessful, future work ought to include careful
selection and development of other surrogate and internal standards based on their
ability to mimic the chemical and physical properties of the analyte with a view that
aromatic nitroso sulfonate compounds are water soluble and most of nitroso compounds have poor water solubility.

From the comparison study of aromatic nitroso sulfonate in Chapter 7 and 8, it should be possible to extend the series of chemical species to iodo and fluoro ortho substituted nitrosobenzene sulfonates. The objective would be to examine the effect of these halides substituents in nitroso compounds towards reactivity with NO, including determination of the equilibrium between their monomers and dimers. The compounds would be prepared according to the procedure employed for the species studied in the present thesis; i.e., from aniline precursors, then converted to sulfanilic acid followed by oxidation by hydrogen peroxide in the presence of glacial acetic acid.

In Chapter 9, in order to gain an understanding into the decrease solubility of nitroso compounds as the ionic strength of the solutions is increased, predicted Setchenow constants for aromatic compounds in sodium chloride were applied to explain the results of germane systems. The work may be expounded by determining the solubilities of aromatic nitroso sulfonate compounds at different temperature in ammonium nitrate solutions at varying concentration using the method developed by May et al. [1]. The method, which has been reported by many researchers, consists of a combination of generator column method and spectrophotometric technique [2]. Generating saturated solutions of non electrolytes could comprise passing the solution acting as a solvent through a column packed with glass beads covered with non-electrolyte. This would thus increase the surface area while decreasing significantly the time required for reaching the saturation. The concentration of the aromatic nitroso compounds would then be determined by a UV-Vis spectrophotometer.
As a final recommendation, one could explore potential NO traps based on reactive oxygen species (ROS). Like nitroso compounds, ROS represent another potential method to decrease NO formation effectively in system of emulsion explosives. In biological systems, reactive oxygen species (ROS) formed in cells modulate various fundamental physiological functions and play an essential part of aerobic life. However, during time of environmental stress, ROS level can increase resulting in oxidative stress and significant damage to the cell structure. Recent studies have demonstrated that, nitric oxide acts as a potent antioxidant by scavenging ROS [3]. As a free radical, nitric oxide reacts with reactive free radical species with diffusion control rate of \( (1-3) \times 10^9 \) and \( 10 \times 10^{10} \) mol\(^{-1}\) dm\(^3\) s\(^{-1}\), for peroxyl and hydroxyl radicals, respectively [4]. In the atmosphere, peroxyl radical normally reacts with nitric oxide, to generate the more reactive radicals, nitrogen dioxide and an alkoxyl radical as shown in Reaction 10.1 [5].

\[
\text{RO}_2^\cdot + \text{NO} \rightarrow \text{RO}^\cdot + \text{NO}_2^\cdot \quad (10.1)
\]

Peroxyl radical also reacts with nitric oxide at higher pressures, to generate peroxynitrite

\[
\text{RO}_2^\cdot + \text{NO} \rightarrow \text{ROONO} \quad (10.2)
\]

Alkoxyl radicals, a known class of strong oxidants with a redox potential of 1.6 V can react rapidly with NO forming inert nitrogen products [6].

\[
\text{RO}^\cdot + \text{NO} \rightarrow \text{RONO} \quad (10.3)
\]

\[
\text{RO}^\cdot + \text{NO} \rightarrow \text{R}^\cdot \text{CHO} + \text{HNO} \quad (10.4)
\]
Since NO reacts very rapidly with ROS, one could investigate whether ROS can act as a potential scavenger for nitric oxide during the sensitisation of ammonium nitrate emulsion explosive. Such investigation would also need to focus on the effect of ROS on stability in emulsion explosive system. Peroxides such as dicyclohexyl phthalate and tert-butyl peroxy benzoate could be utilised to generate ROS \textit{in situ}, since both compounds can initiate safe and fast formation of radicals at the desired range temperature of (20-60) °C.

10.3 References


APPENDIX A

SUPPORTING DOCUMENT FOR CHAPTER 3
A.1 Synthesis of nitroso compounds

A.1.1 Synthesis of DBNBS (based on the method by Kaur et al., 1981)

A solution of 3,5-dibromosulfanilic acid (10 mmol dm$^{-3}$) in a mixture of glacial acetic acid (30 cm$^3$), 30% aqueous hydrogen peroxide solution (70 mmol dm$^{-3}$) and anhydrous sodium acetate (10 mmol dm$^{-3}$) was warmed gently to bring the solids into solution. The solution was allowed to stand at room temperature for 14 days, when straw-coloured blade-shaped crystals which had formed were separated and washed with acetic acid (5 cm$^3$) and three times with dried ether (50 cm$^3$). Residual acetic acid was removed by crystallisation from ethanol to give an analytically pure sodium 3,5-dibromo-4-nitrosobenzene sulfonate as a pale yellow powder. The yield was 34%.

A.1.2 Synthesis of tetrabutylammonium sulfanilate

Tetrabutylammonium hydroxide 30-hydrate (1.39 g, 1.73 mmol dm$^{-3}$) was dissolved in 12.5 cm$^3$ of H$_2$O. To this solution sulfanilic acid (300 mg, 1.73 mmol dm$^{-3}$) was added and the mixture was sonicated until the solid completely dissolved. Evaporation of the solvent in rotary evaporator yielded 737 mg as pale yellow.

$^1$H NMR (CDCl$_3$, 200 MHz, $\delta$): 7.66 (d, J = 8.80, 2H), 6.57 (d, J = 8.80, 2H), 3.89 (s, br., 2H), 3.20 – 3.00 (m, 8H), 1.70 – 1.20 (m, 16H), 0.95 (t, J = 7.32, 12H);

$^{13}$C NMR (CDCl$_3$): 147.4, 137.3, 127.3, 113.4, 58.2,
A.1.3 3,5-dimethyl-4-nitroso benzene sulfonate (DMNBS)

A.1.3.1 3,5-Dimethylsulphanilic acid

Freshly distilled 2,6-dimethylaniline (25 cm$^3$, 0.20 mol) was added cautiously to concentrated sulfuric acid (37.5 cm$^3$) with cooling and stirring. When addition was complete, the reaction mixture was heated at 170 °C for 5 h, and then allowed to cool to 70 °C before being poured into cold water (4 °C.). The precipitate was filtered after standing for 15 min and was then dissolved in 2M sodium hydroxide (600 cm$^3$) and heated with decolourising charcoal for 15 min. The mixture was filtered and allowed to cool. The solution was then acidified to pH 3 (with caution) using 2M hydrochloric acid. Upon cooling to 4 °C, the product crystallised as a white solid. This was filtered and dried over silica gel under vacuum. Yield 18.4 g (45%), IR: 1153 cm$^{-1}$ (Indicative of SO$_3^-$), NALDI MS: Peak observed at 200 a.m.u.

A.1.3.2 3,5-Dimethylsulphanilic Acid, Sodium Salt

Aqueous sodium hydroxide (2 mol dm$^{-3}$) was added dropwise to a suspension of 3,5-dimethylsulphanilic acid (8.3 g; mmol dm$^{-3}$) in water (50 cm$^3$) until all the acid had dissolved and the solution was just basic (pH 11). The solution was refluxed for 1 h; the solvent was removed under reduced pressure to give a white solid which was dried over P$_2$O$_5$ under vacuum. Yield 8.0 g (87%), I.R. 1167 cm$^{-1}$ (Indicative of SO$_3^-$)
A.1.3.3 3,5-Dimethyl-4-nitrosobenzenesulphonate, Sodium Salt (DMNBS)

Anhydrous sodium acetate (3.09 g, 37.7 mmol dm$^{-3}$) was dissolved in glacial acetic acid (84.6 cm$^3$) with stirring. To this solution was added 3,5-dimethylsulphanilic acid, sodium salt (8.39 g, 37.7 mmol dm$^{-3}$) and hydrogen peroxide (30% w/v, 30.2 cm$^3$, 0.294 mol). The reaction mixture was heated at 60 °C. for 1 hr and then stirred at room temperature for 2 h. The reaction mixture was left to stand at room temperature overnight to give a crystalline product. The product was filtered and washed with glacial acetic acid (40 cm$^3$), ethanol (40 cm$^3$), dioxane/diethyl ether (1:1) (40 cm$^3$) and ethanol (40 cm$^3$) to give a pale yellow solid.

A.1.4 Synthesis of 3,5-dichloro-4-nitrosobenzene sulfonate

A.1.4.1 3,5-Dichlorosulphanilic Acid

2,6-Dichloroaniline (22.0 g; 0.136 mol dm$^{-3}$) was added cautiously to concentrated sulphuric acid (50 cm$^3$) under nitrogen, with cooling and stirring. When addition was complete the reaction mixture was heated under nitrogen at 170 °C. for 5 h, and then allowed to cool to 50 °C. before pouring into cold water (4 °C.). The precipitate was filtered and then heated with decolourising charcoal (2 g) in boiling water (500 cm$^3$) for 15 min. After filtration the solvent was removed and the crude product was recrystallised from water to give a crystalline solid, which was dried over silica gel overnight, to give 3,5-dichlorosulphanilic acid as a white powder. I.R. 1153 cm$^{-1}$ (SO$_3$)
A.1.4.2 3,5-Dichloro-4-nitrosobenzenesulphonate, Sodium Salt (DCNBS)

3,5-Dichlorosulfanilic acid (2.0 g; 8.26 mmol dm$^{-3}$) and 30% hydrogen peroxide (5.9 cm$^3$, 0.058 mol) were added to a solution of sodium acetate (0.68 g; 8.26 mmol dm$^{-3}$) in glacial acetic acid (14 cm$^3$) and stirred until the solid was completely dissolved. The resulting solution was left to stand at room temperature for 14 days, after which time a portion of the solvent was removed on the rotary evaporator (water bath temperature 40-50 $^\circ$C) until a solid product was just observed. The reaction mixture was then left to stand overnight at 4 $^\circ$C. The product was filtered and washed with glacial acetic acid (5 cm$^3$), absolute ethanol (10 cm$^3$), dioxane/diethyl ether (1:1) (10 cm$^3$) and absolute ethanol (10 cm$^3$). The product was dried over silica gel overnight, to give 3,5-dichloro-4-nitrosobenzenesulphonate, sodium salt as a cream powder. Yield 0.92 g (40%).
A.2 C-NMR, H-NMR, IR, NALDI-MS and UV-Vis spectra

**Figure A.1.** Typical C-NMR spectrum of the synthesised DBNBS with minor modifications

**Figure A.2.** H-NMR spectrum of synthesised DBNBS
Figure A.3. C-NMR spectrum of Tetrabutylammonium 4-nitrosobenzenesulfonate

Figure A.4. H-NMR spectrum of Tetrabutylammonium 4-nitrosobenzenesulfonate
Appendix A

Chemical trapping of NO

Figure A.5. C-NMR spectrum of 3,5-dichlorosulfanilic acid sodium salt

Figure A.6. C-NMR spectrum of 3,5-dichloro-4-nitrosobenzene sulfonate sodium salt
Figure A.7. C-NMR spectrum of 3,5-methylsulfanilic acid sodium salt

Figure A.8. C-NMR spectrum of 3,5-dimethyl-4-nitrosobenzene sulfonate sodium salt
Appendix A

Chemical trapping of NO

Figure A.9. IR spectrum of 3,5-dichlorosulfanilic acid

Figure A.10. IR spectrum of 3,5-dichloro-4-nitrosobenzene sulfonate sodium salt
Figure A.11. IR spectrum of 3,5-dimethylsulfanilic acid

Figure A.12. IR spectrum of 3,5-dimethyl-4-nitrosobenzene sulfonate sodium salt
Figure A.13. NALDI-MS spectrum of DBNBS in negative ionisation mode
Figure A.14. NALDI-MS spectrum of NBS in negative ionisation mode
Figure A.15. NALDI-MS spectrum of DMNBS in negative ionisation mode
Figure A.16. NALDI-MS spectrum of DCNBS in negative ionisation mode
Figure A.137. UV-Vis spectrum of aromatic nitroso sulfonate. (a) DBNBS, (b) NBS, (c) DMNBS and (d) DCNBS
A.3 Effect of heating NBS to 80 °C

Figure A.148. Effect of heating nitrosobenzene sulfonate to 80 °C
A.4 Calibration plot for ion chromatograph

![NO2 calibration plot](image1)

\[ y = 49.95x \]
\[ R^2 = 0.9932 \]

![NO3 calibration plot](image2)

\[ y = 58.829x \]
\[ R^2 = 0.9970 \]

![Cl calibration plot](image3)

\[ y = 58.068x \]
\[ R^2 = 0.9919 \]

![Br calibration plot](image4)

\[ y = 81.161x \]
\[ R^2 = 0.9973 \]

**Figure A.159.** Calibration plots for ion chromatogram. (a) Nitrite, (b) nitrate, (c) chloride and (d) bromide calibration plots.
A.5 pH change during nitrous acid decomposition

![Graph showing pH change during nitrous acid decomposition](image)

**Figure A.20.** pH change during the nitrosation
APPENDIX B

CHARACTERISATION OF 4 ORTHO-SUBSTITUTED AROMATIC NITROSO SULFONATES BY LASER DESORPTION TIME-OF-FLIGHT MASS SPECTROMETRY

This appendix documents the methodology developed for the utilisation of nanostructured assisted laser desorption ionisation mass spectrometry (NALDI-MS)
## B.1 Introduction

Among the various methods used to analyse low molecular weight compounds, such as nuclear magnetic resonance (NMR), X-ray crystallography or infrared spectroscopy, mass spectrometry (MS) is the technique which provides the highest degree of accuracy and component detection sensitivity. Not surprising then, GC-MS (where gas chromatography (GC) is used for component separation) is the most popular tool for separating mixtures and providing definitive identification of individual compounds. However, a fundamental drawback of GC-MS is the requirement of a volatile, thermally stable analyte. Although derivatisation can circumvent this problem \[1\], the process requires additional, complicated and multiple preparation steps \[2-4\] which adds considerably to analysis cost and can introduce contamination via these multiple handling procedures. In the present study, attempts to derivatise DBNBS and its NO reaction products with a silylation reagent with and without methoxyamine hydrochloride (MOX) were unsuccessful as GC-MS analysis of derivitised DBNBS did not produce compounds which eluted and subsequently detected by GC-MS.

With the introduction and rapid development of electrospray ionisation mass spectrometry (ESI-MS), this technique has become the preferred analytical tool analysis of complex liquid mixtures since it provides a sensitive, robust and reliable tool for studying, at femto-mole quantities in micro-litre sample volumes, non volatile molecules \[5, 6\]. ESI-MS is generally associated with a liquid chromatographic step to enable separation of compounds in the eluent \[7\]. It is generally recognised that when analysing ionisable compounds, it is important to control the pH of the mobile phase using a pH buffer \[8\]. In particular, the separation of nitroso compounds reaction mixtures in acidic solution requires a phosphate buffer to improve peak shape and
facilitate reproducibility of analyses. Conversely, the presence of a non-labile phosphate buffer in the ESI-MS can result in ion suppression and adducts formation [9, 10], severely deteriorating sample analysis.

The exploration of ESI-MS has lead to the development of a wide range of related mass spectroscopic analysis techniques, most notably matrix-assisted laser desorption ionisation (MALDI) mass spectrometry, which is an attractive alternative technique to ESI-MS as it has a high tolerance for mixtures containing a multitude of contaminants and allows relatively straightforward spectrum interpretation as singly charged molecules are yielded [11]. However, the matrix itself can give rise to daughter ions in the low mass range, less than 800 amu or daltons, which can obscure species of interest, which are often in this mass range [12]. More recently, nanostructured assisted laser desorption ionisation (NALDI) mass spectrometry has been developed as an innovative analysis technique, which eliminates the need of a matrix and thus offers significantly reduced background signal in the low mass range and thus enabling the reliable analysis of small molecules [13]. The NALDI surfaces are applied directly onto standard stainless steel target plates. The target is designed to fit in a sample adaptor and this provides a stable surface that can be used and indeed is a standard equipment accessory in MALDI-MS instrument configuration.

The ionisation process in NALDI-MS is similar to MALDI-MS, often termed a “soft” ionisation technique whereby intense, short wave length laser pulses (under high vacuum) are employed to ionise sample molecules and release into the gas phase [14, 15]. An electrode is then used to accelerate the positive or negatively charged ions (depending on the set up polarity) towards the mass analyser [8]. Soft ionisation techniques tend to produce a mass spectra with little or no fragment ion content, thus
facilitating the analysis of mixtures [16]. In addition, and unlike other mass spectrometric analysis techniques, NALDI does not require elaborate sample preparation procedures as analytes are directly deposited onto the surface of the NALDI target which are then transferred to the NALDI ready for analysis [17].

[Figure B.1. Pictorial representation of the NALDI target and adaptor (Photocourtesy: Bruker Daltonics, [18])]

We will describe how a commercially available NALDI target was used as a support for the desorption, ionisation and analysis of 4 ortho substituted aromatic nitroso sulfonate compounds. Our recent study showed that 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), an aromatic nitroso spin trap commonly used for the detection of NO in the biological systems [19, 20], is an efficient nitric oxide scavenger, and has the potential to be employed for the control of NOX emissions from industrial processes [21].

Following this, we will demonstrate that the aromatic nitroso sulfonates nitrosobenzene sulfonate (NBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and 3,5-dichloro-4-nitrosobenzene sulfonate (DCNBS) can also be applied as nitric oxide scavengers. As these compounds are not commercially available they were synthesised, and we applied
the technique of NALDI-MS as part of characterisation process for the synthesis of the starting material as well as the subsequent nitroso compounds.

Mass calibration of the NALDI target, which is an important component of the main part of the study, was performed using elemental sulfur. Elemental sulfur is an excellent material for the low mass range calibration of TOF mass spectrometers and has been recently been used by Krugel et al. [22]. To our knowledge this is the first report of the use of NALDI-TOF MS to characterise ortho substituted aromatic nitroso sulfonate compounds.

**B.2 Methodology**

**B.2.1 Material**

Nanostructued assisted laser desorption ionisation (NALDI\textsuperscript{TM}) plates, which are produced by Nanosys, Inc. for Bruker Daltonics Inc., were purchased from Bruker Daltonics, Australia. Sulfur (purchased from Sigma Aldrich) was used without further purification for calibration of the NALDI targets. Nitroso compounds; 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), 3,5-dichloro-4-nitrobenzene sulfonate (DCNBS), 3,5-dimethyl-4-nitrosobenzene sulfonate (DMNBS) and nitrosobenzene sulfonate (NBS) were synthesised following published descriptions (Refer to Chapter 3 for details). Reaction mixtures of nitroso compounds, following reaction with NO, were prepared one hour before analysis on NALDI targets by reacting the nitroso compounds with NO which was generated from nitrous acid decomposition [23] under argon. This NO generation technique was performed by initially acidifying the nitroso compounds
with acetic acid and degassing with argon gas for 15 min in a 10 cm$^3$ round bottom flask. Bubbling was then terminated and a 0.015 mol dm$^{-3}$ sodium nitrite solution (1 cm$^3$) was injected into the solution using a syringe to trigger the commencement of an experiment. In the pH controlled experiments, the pH of the reaction mixture was adjusted to 5.5 using 0.1 mol dm$^{-3}$ NaOH solution to quench the decomposition of nitrous acid which in turn terminated the generation of NO.

**B.2.2 Calibration of NALDI-MS**

Sulfur (10 mg) samples were dissolved in 1 cm$^3$ each of the following solvents; acetone, water, toluene, acetonitrile and ethanol. Aliquots of these samples (5 µL) were deposited directly onto the surface of the target and allowed to dry at room temperature and in ambient air. The samples were also heated in a heating block to enhance solubility of the sulfur and were spotted on NALDI plate for comparison with non-heated solvent. MS data was acquired in the $m/z$ range between 0 and 800 by averaging signals from 2500 laser shots using the target random-walk movement. The energy of the laser photon beam was adjusted such that the signal intensity of the ions attained the desired level. This was performed by adjusting the laser beam attenuator which varies the intensity of the laser impacting on the sample; 40 % laser attenuation was employed for the calibration process.
B.2.3 Sample preparation for NALDI-MS

Aliquots of the samples (5 µL) were directly deposited on the surface of the target and allowed to dry at room temperature in ambient air.

B.2.4 Mass spectrometry analysis

MS and MS/MS spectra were acquired in negative reflector mode using a Bruker Daltonics Ultraflex III MALDI time-of-flight mass spectrometer equipped with nitrogen laser operating at 337 nm, a 2 GHz sampling rate digitiser, pulsed ion extraction source and reflectron. The laser pulse width was 3 ns and the maximum power of the laser pulse was 200 mW. The primary ion source voltage was fixed at 25 kV, and mass spectra were recorded at a reflector voltage of 26.5 kV. FlexAnalysis 3.0 program (Bruker Daltonics) was used to analyse the data.

B.2.5 Study of oxidation dependence on time

Samples were deposited in triplicate on the NALDI target, on three different rows. The first row was analysed immediately and the mass spectrum of the intact analytes was obtained. The second and third rows were measured after dwell times of approximately 24 and 48 hours respectively.
B.3 Results and discussion

B.3.1 Blank

Each time a sample was analysed, a blank spectrum was also recorded to determine which peaks in the spectra were from background. Figure B.2 shows a typical background mass spectrum of the NALDI target.

![Figure B.2. Typical NALDI background mass spectrum.](image)

B.3.2 Calibration of the NALDI target using elemental sulfur

Calibration of the NALDI target in the low mass region is not straightforward due to the presence of many interfering matrix ions and their fragments. In previous studies, where NALDI TOF MS was applied, researchers developed a calibration procedure using peptides to calibrate the NALDI-MS [24, 25]. The peptide calibration covers the mass range of 700-3200 Da and thus is not appropriate for the low mass (below 700 Da) region.
One interesting characteristic of the main group of non-metallic elements is the wide variety of allotropic forms which have been identified. Recently, Hearley et al. showed that laser desorption of elemental sulfur, selenium, tellurium and phosphorus generates a range of polynuclear ions [26], and this feature is very attractive for use in mass spectrometer calibration. Indeed, the chemistry of elemental sulfur is distinguished by a typically complex molecular behavior since the element exists in more than 20 allotropes including metastable forms [27]. The combination of mass spectrometry with the Knudsen effusion method is usually applied to the study of vapour phase species in equilibrium of the stable form of elemental sulfur and this method was employed in the study by Hearley et al. to identify $\text{S}_n^+$ ions with $n = 2 - 8$ (Hearley 2002).

Balogh et al., 1999 reported the use of spark source mass spectrometry to confirm the formation of these $\text{S}_n^+$ cations, while laser ablation/He jet methods with $\text{S}_8$ vapour have shown the existence of $\text{S}_n^+$ clusters with $n = 8, 16, 24, 32, 40$ formed from a parent $\text{S}_8$ solid [28]. As a result of the generation of series of singly charged radical cations and anions by laser ionisation, elemental sulfur is an excellent material for low mass region calibration [22]. The aim in the present study is to use sulfur for the calibration of the NALDI-MS in the range of 30-600, which signifies the generation of a series of singly charged radical anions ($\text{S}_n^-$) and cations ($\text{S}_n^+$).

Among the different solvents used, sulfur was completely soluble in toluene at room temperature. For most of the other solvents studied, the sulfur remained in suspension (particularly in the case of water). However heating of the solvent to 55 °C significantly increased the solubility of sulfur, and resulted in the generation of a wider range of charged radical anions. The only exception was acetonitrile, in which sulfur was not soluble, even after heating. Figure B.3 compares the mass spectra of sulfur in
the 5 solvents studied, where the solvent was not heated. It is clear that toluene and ethanol generated the highest number of singly charged ion peaks and as shown in Figure B.4, the heating of toluene resulted in a series of singly charged anions up to \( n = 19 \), ideal for the calibration process.

We also noted that although sulfur deposits produced a series of intense peaks in both negative and positive ionisation mode, ionisation in the negative ion mode enabled the entire \( \text{S}_1^- \) to \( \text{S}_{19}^- \) series to be observed. In contrast, when the positive ionisation mode was employed, relatively few mass spectral peaks were observed, as shown in Figure B.5.

In comparison to toluene, sulfur dissolved in ethanol did not generate peaks greater than \( n = 13 \), even following heating of the solvent (results not shown). Low mass ions such as \( m/z \) 32 were obscured, in which case lower power attenuation (higher power) was required. However, the use of the high power tends to deteriorate spectral resolution, shape and intensity of the peak of the most abundant ions. This is a well known limitation of the laser ionisation TOF instrument which is mainly due to the initial increased kinetic energy distributions of ions caused by the laser ablation process. [13]. Nevertheless, this is not a severe limitation in the present study as the mass range of interest tends to be greater than 32 Da.
**Figure B.3.** Comparison of the negative ion mass spectra of sulfur in 5 different solvents.
Figure B.4. Negative ion NALDI MS peak profiles obtained from elemental sulfur in toluene following heating to 55°C.
Figure B.5. Positive ion NALDI MS peak profiles obtained from elemental sulfur.
During the calibration process, it was also possible to identify sulfur-related peaks in the mass spectrum, since this element exists in nature as a mixture of four stable isotopes: $^{32}$S (94.93%), $^{33}$S (0.76%), $^{34}$S (4.29%) and $^{36}$S (0.02%) [29]. The difference of 2 m/z between the two major peaks in each series and their relative abundances allow the identification of sulfur peaks. For instance as shown in Figure B.6, the ratio of 100:45 for the peak at 319.721:321.717 observed under negative ionisation allows us to identify the peaks as an isotope series attributed to $(^{32}\text{S}_{10})^{-}$ and $(^{32}\text{S}_{9} + ^{34}\text{S}_{1})^{-}$. A list of calculated masses for anions of sulfur clusters used for fine tuning during calibration of the NALDI-MS is shown in Table B.1. The presence of well defined isotopes in the calibration standard is an important asset that can assist in mass spectral tuning thus enabling the instrument to automatically achieve a target resolution in each acquired mass spectrum. In addition to using the molecular ion, the system can also employ the isotopic peaks to improve mass calibration.

After applying the accurate mass assignments obtained through calibration, the relative mass error (the difference between the actual and theoretical values) was around 50 ppm, which is within the level of error for the low region for the mass spectrometer used in this study [30]. The result highlights the advantages of using sulfur for low mass calibration of NALDI-MS. Using the technique described singly charged $S_n^-$ anions up to $n = 19$ by laser desorption ionisation have been achieved as compared to the reported literature value of $n=15$ [22]. In addition to being relatively low cost, the method does not require complicated preparation procedures, whereby a simple dissolution of sulfur in toluene is all that is required.
Figure B.6. Isotope pattern observed for $^{32}\text{S}_{10}$.
Table B. 1. Calculated masses of radical anions of sulfur clusters.

<table>
<thead>
<tr>
<th>n</th>
<th>$^{32}\text{S}_n\cdot$</th>
<th>Abundances</th>
<th>$^{32}\text{S}_{n-1} + ^{34}\text{S}_1\cdot$</th>
<th>Abundances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.972619</td>
<td>100</td>
<td>33.968415</td>
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<tr>
<td>2</td>
<td>63.944689</td>
<td>100</td>
<td>65.940485</td>
<td>9.04</td>
</tr>
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<td>3</td>
<td>95.916759</td>
<td>100</td>
<td>97.912555</td>
<td>13.56</td>
</tr>
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<td>127.888829</td>
<td>100</td>
<td>129.884625</td>
<td>18.08</td>
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<td>5</td>
<td>159.860899</td>
<td>100</td>
<td>161.856695</td>
<td>22.6</td>
</tr>
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<td>6</td>
<td>191.832969</td>
<td>100</td>
<td>193.828765</td>
<td>27.12</td>
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<td>100</td>
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<td>100</td>
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<td>54.24</td>
</tr>
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<td>415.637459</td>
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<td>58.76</td>
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<td>447.609529</td>
<td>100</td>
<td>449.605325</td>
<td>63.28</td>
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<td>100</td>
<td>481.577395</td>
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<td>100</td>
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<td>72.32</td>
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<td>543.525739</td>
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<td>100</td>
<td>577.493605</td>
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<td>19</td>
<td>607.469879</td>
<td>100</td>
<td>609.465675</td>
<td>85.88</td>
</tr>
</tbody>
</table>
B.3.3 Characterisation of nitroso compounds

During the synthesis of the four nitroso compounds, NALDI-MS was an essential tool which enabled the definitive analysis of these compounds, as part of the characterisation and confirmation of the identity of the compound. Table B.2 lists the theoretical mass values and experimental mass values of the 4 nitroso compounds, as well as their (inferred) structural information. Note that in positive ion mode, the molecular ion of DBNBS was not detected.
Table B.2. Structure of nitrosobenzene sulfonate compounds and their associated molecular ion mass.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Abbreviated name</th>
<th>Chemical Structure</th>
<th>Formula weight. Cal</th>
<th>Formula weight. Exp (NALDI-MS)</th>
<th>Delta error</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-dibromo-4-nitrosobenzene sulfonate</td>
<td>DBNBS</td>
<td><img src="image" alt="DBNBS structure" /></td>
<td>343.957</td>
<td>343.893</td>
<td>-0.064</td>
</tr>
<tr>
<td>nitrosobenzene sulfonate</td>
<td>NBS</td>
<td><img src="image" alt="NBS structure" /></td>
<td>185.986</td>
<td>185.961</td>
<td>-0.025</td>
</tr>
<tr>
<td>3,5-dimethyl-4-nitrosobenzene sulfonate</td>
<td>DMNBS</td>
<td><img src="image" alt="DMNBS structure" /></td>
<td>214.017</td>
<td>214.004</td>
<td>-0.013</td>
</tr>
<tr>
<td>3,5-dichloro-4-nitrosobenzene sulfonate</td>
<td>DCNBS</td>
<td><img src="image" alt="DCNBS structure" /></td>
<td>253.908</td>
<td>253.924</td>
<td>+0.016</td>
</tr>
</tbody>
</table>
B.3.3.1 Determining the elemental composition based on isotope peak intensities

Based on the isotope abundance of bromine (DBNBS) and chlorine (DCNBS), it was possible to compare the pattern of experimental isotopic peaks with that of the theoretical isotopic distribution, thus establishing which peaks in the mass spectra could be attributed to fragments produced from the target compounds. The data in Table B.3 was used to determine the intensities of the peaks depending on the composition of the ion.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Mass</th>
<th>% Abundance</th>
<th>Relative peak height</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{35}$Cl</td>
<td>34.9689</td>
<td>75.77</td>
<td>100</td>
</tr>
<tr>
<td>$^{37}$Cl</td>
<td>36.9659</td>
<td>24.23</td>
<td>32.0</td>
</tr>
<tr>
<td>$^{79}$Br</td>
<td>78.9183</td>
<td>50.69</td>
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<tr>
<td>$^{81}$Br</td>
<td>80.9163</td>
<td>49.31</td>
<td>97.3</td>
</tr>
</tbody>
</table>

*Contribution to the intensity of the X+1 or X+2 peak for each atom present.

The mass spectrum produced by the NALDI for DBNBS in Figure B.7 was close to the theoretical value (within 50 ppm). Also, the intensity of the isotopic pattern agreed well with the theoretical pattern, with the intensities of the mass peaks around the $m/z$ 343.9 being in a ratio of 1:2:1, characteristic of two bromine atoms in the parent molecule. Similarly, for the chloro substituted compounds, the presence of two chlorine atoms was
confirmed by the isotope intensity pattern around the \textit{m/z} 253.9 which were in the ratio of 9:6:1 (Figure B.8).

The measurements reported in Table B.2 are in satisfactory agreement with the theoretical mass values, where the error in most cases is less than 0.07 Da (70 ppm) although the error for DMNBS was 0.013 Da (130 ppm). A possible reason for these discrepancies may be a result of some observed instabilities inherent in the external calibration procedure used. However when combined with the isotope pattern evidence, the mass measurements are sufficient to confirm the identities of the synthesised compounds.

In addition to being a tool to characterise the synthesised nitroso compounds, the NALDI-MS served to determine if synthesis was successful by comparing the spectra of the starting material with the synthesised product. For example, in the case of 3,5 dimethyl-4-nitrosobenzene, the starting material (3,5-dimethylsulfanilic acid) required synthesis. By the application of this novel MS method, we were able to rapidly determine whether all the starting material had oxidised to the corresponding nitroso compound during the synthesis process. As shown in Figure B.9, the peak at 200.001 Da, which corresponds to the ion of 3,5-dimethylsulfanilic acid, was absent in the spectra of methyl substituted nitroso sulfonate and a new peak at 214.004 Da was now present. This is consistant with the complete consumption of the starting material, which has been oxidised.
Figure B.7. Zoomed section of mass spectrum of DBNBS. Isotope pattern observed for peak m/z 343.883
Figure B.8. Zoomed section of mass spectrum of DCNBS; Isotope pattern observed for peak 253.924
Figure B.9. NALDI MS for comparison of starting material (b) 3,5-dimethyl-sulfanilic acid with product (b) 3,5-dimethyl-4nitrosobenzene sulfonate. Mass spectrum (a) is the blank.
B.3.4 Increased oxidation product formation as a function of time

An oxidation product of DBNBS was observed and made evident by the presence and increased intensity of a peak at \( m/z = 360 \) [DBNBS + \( m/z \) 16] Da. This mass was initially attributed to 3,5 dibromonitrobenzene sulfonate which is formed during the ionisation process. This is a commonly observed phenomenon which is known to occur during desorption ionisation mass spectrometry [32, 33]. However further experiments undertaken on the NALDI target as a function of time confirmed that oxidation was caused by surface activity and was strongly dependent on the storage time of the analyte on the NALDI target as is subsequently discussed below. We also observed the same phenomenon in the other three nitroso compounds studied, which is presumably due to the presence of the N=O bond in these compounds which also oxidises to the corresponding nitro compounds.

In order to investigate possible source(s) of oxidation products observed during the NALDI-MS process, we studied the time dependency of the oxidation products on the NALDI target. We observed only oxidation products for DBNBS itself; in contrast no oxidation products were detected in the reaction products. One possible explanation for the relatively facile oxidation of DBNBS is the presence of the N=O moiety (nitroso) in the molecule which is oxidised to the corresponding nitro compound. Following analysis of samples after a dwell time of 24 hours, the ion signals attributed to the oxidation products of DBNBS increased in intensity with time for all samples. After a dwell time of 48 hours, the intensity of the peak + \( m/z \) 16 was essentially equivalent to the spectrum taken after a dwell time of 24 hours. Table B.4 denotes the ratio of DBNBS ion signal to its oxidation product (DBNBS + \( m/z \) 16) for all the samples analysed. The data in Table B.3 underscores the changes in the abundance of the
oxidation products, and suggests that the formation of the oxidation products is strongly dependent on the storage time. This result confirms that oxidation occurs on the NALDI surface and does not occur during the laser desorption ionisation process. Parvásková et al., also detected oxidation product when studying their analyte DOPC on the NALDI target [24]. They suggested that oxidation could be a result of the catalytic activity of the NALDI surface due to the high surface site density on the target that could allow the presence of oxygen molecules on the surface as a result of the absorption and conversion of the atmospheric oxygen molecule. Our results tend to support the observation of Parvásková et al., as we observe time dependence oxidation product for DBNBS, as well for the other ortho substituted aromatic nitroso sulfonate compounds. These studies underscore the importance of analysing sample on NALDI target immediately after being spotted in order to prevent formation of oxidation products.

Table B.4. Ratio of m/z 343.9: 359.9 for DBNBS and its reaction mixtures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DBNBS</th>
<th>pH controlled rxn mix</th>
<th>Non-pH controlled rxn mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st spot (20 min)</td>
<td>1:0.33</td>
<td>1:3.39</td>
<td>1:1.81</td>
</tr>
<tr>
<td>2nd spot (24 h)</td>
<td>1:3.05</td>
<td>1:3.48</td>
<td>1:3.05</td>
</tr>
<tr>
<td>3rd spot (48 h)</td>
<td>1:3.08</td>
<td>1:3.50</td>
<td>1:3.05</td>
</tr>
</tbody>
</table>
B.3.5 Identification of liquid products in reaction mixtures

We were also able to analyse reaction mixtures from the reaction of nitroso compounds with NO which occurred under various reaction conditions. Analysis of the reaction mixture was possible because the NALDI-MS employs a “soft” ionisation technique, and subsequently minimal fragmentation of the analyte occurs during the ionisation process. With the NALDI-MS analysis, when reaction mixture was analysed, it was possible to determine if the reactants have been consumed over time. The spectra of a neat DBNBS sample with its reaction mixtures are shown in Figure B.10. The peak at $m/z$ 343.893 was also present in reaction mixture samples but was lower in intensity in comparison to the original DBNBS sample, signifying that DBNBS was consumed in the reaction. The compound with a molecular weight at $m/z$ 359.8 was present in all the samples; however the intensity of this peak varied between samples.

In the neat DBNBS sample, the ratio of the intensity peaks of $m/z$ 343.893:359.943 was 1:0.33, while the ratio of these peak intensities of the reaction mixture obtained under pH controlled and non-pH controlled were 1: 3.39 and 1:1.81 indicating that the product is not only produced during the NALDI analysis but also a product formed from the reaction of DBNBS with NO. Figure B.11 compares the mass spectra of DMNBS and its reaction mixture, and in these spectra we observe that the $m/z$ 229.9 is also present in the DMNBS sample, however the ratio of the $m/z$ 213.9:([213.9 +$m/z$ 16]) is 1:0.3 and 1:2 in the DMNBS and reaction mixture sample respectively. Thus similar to the bromo substituted aromatic nitroso sulfonate, oxidation of DMNBS occurred during the NALDI analysis as well as from the compound’s reaction with NO since the ratio of its molecular weight to its oxidation product was higher in the latter case.
Figure B.10. NALDI-MS comparing (b) DBNBS sample with (c) non-pH controlled and (d) pH controlled reaction mixtures.
Figure B.11. NALDI-MS of reaction mixture and 3,5-dimethyl-4-nitrosobenzene sample.
In the mass spectra obtained for the DBNBS reaction mixture, ions (which were absent in the neat DBNBS sample) were detected at molecular weight of 261.903, 291.880, 324.891 and 449.449. The ion with a molecular weight of 291.880 is believed to be the primary product since this ion has the highest intensity in the mass spectrum. The ion does not contain bromine, since the ratio of the peaks at \( m/z \ 291.880 \) and \( m/z \ 293.882 \) is 47:1. Figure B.12 depicts the zoomed spectra showing ions at \( m/z \ 291.880 \) for DBNBS scavenging product. The nitrogen rule for molecular ion suggests an odd number of nitrogen atoms present in the compound, assuming that the nominal mass of the peak is 292 (by rounding up 291.880). MS/MS analysis on \( m/z \ 291.9 \) did not elucidate structural information of the ion although the loss of mass 46 and 64 indicates the presence of a nitro group and sulfonate in the compound, as shown in Figure B.13.

Coincidently, the primary product from the reaction of the di-chloro substituted nitroso compound has a molecular weight of 291.944 (Figure B.14). This suggests that an analogous reaction mechanism is occurring when these nitroso compounds are reacted with nitric oxide.
Appendix B

Figure B.117. Zoomed section showing ions at m/z 291.880

Figure B.13. MS/MS analysis on m/z 291.9

Chemical trapping of NO
Figure B.14. NALDI mass spectra of 3,5 dichloronitrosobenzene sulfonate and its reaction mixture with NO
B.4 Conclusion

NALDI analysis is a rapid and reproducible method for analysis and characterisation of aromatic nitroso sulfonate compounds. In contrast to other analytical methods such as NMR and IR spectroscopy, NALDI-MS has a number of advantages including the relatively small quantity of sample required (5 µL) and the low detection limits. In the present study, elemental sulfur was used to enable calibration of the laser desorption ionisation time of flight instrument for low masses. In addition to its very low cost, sample preparation is straightforward and its use provided calibration with minimal error (roughly 50 ppm). The NALDI target surface has been shown to induce oxidation of the analyte, in particular for nitroso compounds where the presence of a N=O moiety, which can rapidly oxidise on the NALDI target. NALDI MS assists in the characterisation process of nitroso compounds and also the identification of reaction products and construction of reaction mechanism from the reaction of these compounds with NO. This technique should also be possible for a wide range of nitroso, nitro and structurally similar compounds.
B.5 References


Appendix B


APPENDIX C

SUPPORTING DOCUMENT FOR CHAPTER 5
C.1 Derivation of Equation 5.1 for determining equilibrium constant of DBNBS

Equation 5.1 was derived by Holmes et al., using Equation C.1 and the Beer Law. It was assumed that the light absorption at wavelength 760 nm is only due to the monomer.

\[ K_C = \frac{C_{\text{Monomer}}^2}{C_{\text{Dimer}}} \]  
\[ (C.1) \]

\[ C_t = C_{\text{Monomer}} + 2C_{\text{Dimer}} \]  
\[ (C.2) \]

Replacing Equation C.2 in C.1

\[ K_C = \frac{2C_{\text{Monomer}}^2}{C_t - C_{\text{Monomer}}} \]

\[ C_t - C_{\text{Monomer}} = \frac{2C_{\text{Monomer}}^2}{K_C} \]

Where, \( D \) is the optical density, \( C_t \) is concentration of initial concentration of equivalent monomer (mol dm\(^{-3}\)), \( \varepsilon \) is the molar extinction coefficient per centimetre of cell length and \( K_C \) is the equilibrium constant for reaction. The equilibrium constant \( K_C \) is taken as a dimensionless number with all concentration values referenced to a standard state.

\[ D = \varepsilon C_{\text{Monomer}} L \]

\[ C_t - \frac{D}{\varepsilon L} = \frac{2D^2}{\varepsilon^2 L^2 K_C} \]
\[
\frac{C_c \delta L - D}{\delta L} = \frac{2D^2}{\epsilon^2 L^2 K_c}
\]

\[
C_c \delta L - D = \frac{2D^2}{\epsilon L K_c}
\]

\[
D = C_c \delta L - \frac{2D^2}{\epsilon L K_c}
\]

\[
\frac{D}{C_c L} = \epsilon - \frac{2D^2}{\epsilon L^2 K_c C_t}
\]

\[
\frac{D}{C_c L} = -\left(\frac{D^2}{C_t L^2}\right)\left(\frac{2}{K_c \epsilon}\right) + \epsilon
\]

(E1)

**C.2 The effect of pH on the rate of trapping**

NO saturated solutions were also prepared in buffered solution to study the effect of pH on the rate of *ex situ* trapping of NO by DBNBS at 25 °C using stopped flow UV-Vis spectrometer. Buffer solutions at 0.01 mol dm\(^{-3}\) ionic strength and pH 3.5 and 7 were prepared from the acid and sodium salt of acetate and phosphate.

Figure C.1 illustrates the results obtained at 25 °C with initial concentration of both NO and DBNBS monomer concentration of 0.95 mmol dm\(^{-3}\) at pH 3.5 and 7. Plot of the reaction using unbuffered NO saturated solutions was also included for comparison showing the reaction is independent of the pH.
Figure C.1. Change in DBNBS concentration with time during the reaction of NO with DBNBS at 25 °C at initial NO and DBNBS concentrations of 0.95 mmol dm$^3$. (Δ) NO saturated solution buffered at pH 3.5, (○) NO saturated solution buffered at pH 7.0 and (×) NO solution in distilled and deionised water pH 5.8.

C.3 Mass transfer coefficient

Since there was transport of NO from aqueous solution to gas phase through the membrane, an expression accounting for the mass transfer should be included in the mass balance for NO. The liquid mass transfer coefficient at the membrane is denoted $k_{MT}$ with the surface area $A_M$. 
In the absence of reactions, a simple mass balance equation which describes the depletion of NO from the liquid is shown in Equation given that there is no NO in the gas phase

\[
\frac{d[NO]}{dt} = -\left(\frac{k_{MT}A_M}{V}\right)[NO]
\]  (1)

Where \( V \) is the aqueous volume = 18 cm\(^3\)

\( A_M \) is the surface area of membrane in contact with the liquid = 1.885 cm\(^2\)

\( k_{MT} \) can be referred as lumped quantity since surface area and volume will remain constant in the experiments.

The mass transfer coefficient was determined in separate experiments by rapidly generating NO in the reactor via the reduction of nitrous acid by ascorbate.

Figure C.2. Plot of NO measurement against time for mass transfer. (◊) Experimental NO measurement and dotted line is the model fit.
Appendix C

C.4 Output script file from Dynafit software for fitting measured data DBNBS monomer for the ex situ trapping of NO.

Program DynaFit version 3.28.070 [04-21-2010]
Execution started Wed July 04 20:44:39 2012

-----------------------------------------------
SCRIPT FILE
reaction mechanismDBNBS consumption.txt
-----------------------------------------------

TASK
Fit of progress curves

DATA
file .\DBNBScomp1.32mM.txt
file .\DBNBScomp0.86mM.txt
file .\DBNBScomp0.5mM.txt
file .\DBNBScomp0.3mM.txt

REACTION MECHANISM
DBNBS2 <====> DBNBS + DBNBS : k1  k-1
DBNBS + NO ---> O2 + N2 + D : ktrap
NO + NO + O2 ---> NO2 + NO2 : k3
NO2 + H2O <====> HNO2 + NO3- : k4  k-4
HNO2 <====> NO2- : k5  k-5
HNO2 <====> H2O + N2O3 : k6  k-6
D + NO2- ---> E : k7
N2O3 + E ---> NO + NO2- + F : k8

DIFFERENTIAL EQUATIONS

\[ \frac{d[DBNBS2]}{dt} = -k1[DBNBS2]+k-1[DBNBS][DBNBS] \]
\[ \frac{d[DBNBS]}{dt} = +k1[DBNBS2]+k1[DBNBS][DBNBS]-k-1[DBNBS][DBNBS]-ktrap[DBNBS][NO] \]
\[ \frac{d[NO]}{dt} = -ktrap[DBNBS][NO]-k3[NO][NO][O2]-k3[NO][NO][O2]+k8[N2O3][E] \]
\[ \frac{d[O2]}{dt} = +ktrap[DBNBS][NO]-k3[NO][NO][O2] \]
\[ \frac{d[N2]}{dt} = +ktrap[DBNBS][NO] \]
\[ \frac{d[D]}{dt} = +ktrap[DBNBS][NO]-k7[D][NO2-] \]
\[ \frac{d[NO2]}{dt} = +k3[NO][NO][O2]+k3[NO][NO][O2]-k4[NO2][H2O]+k-4[HNO2][NO3-] \]
\[ \frac{d[H2O]}{dt} = -k4[NO2][H2O]+k-4[HNO2][NO3-]+k6[HNO2]-k-6[H2O][N2O3] \]
\[ \frac{d[HNO2]}{dt} = +k4[NO2][H2O]-k-4[HNO2][NO3-]-k5[HNO2]+k-5[NO2-] \]
\[ \frac{d[N2O3]}{dt} = +k4[NO2][H2O]-k-4[HNO2][NO3-] \]
\[ \frac{d[E]}{dt} = +k5[HNO2]-k-5[NO2-]+k7[D][NO2-]+k8[N2O3][E] \]
\[ \frac{d[N_2O_3]}{dt} = +k_6[HNO_2] - k_6[H_2O][N_2O_3] - k_8[N_2O_3][E] \]
\[ \frac{d[E]}{dt} = +k_7[D][NO_2] - k_8[N_2O_3][E] \]
\[ \frac{d[F]}{dt} = +k_8[N_2O_3][E] \]

**OUTPUT**

### LEAST-SQUARES FIT

- **mean square**: 3.88396e-010
- **standard deviation**: 1.97078e-005
- **log(determinant)**: -1
- **Marquardt parameter**: 0.00781
- **execution time (sec)**: 0.090
- **datapoints**: 1885
- **parameters**: 1
- **iterations**: 10
- **subiterations**: 4
- **function evaluations**: 17
- **error status**: 0

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<tr>
<td>ktrap</td>
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</tbody>
</table>

### PLOTS

- Best-fit plot: DBNBSconc\tab\fit_0101.tab
C.5 Derivation of rate law for nitrous acid decomposition

\[ 2\text{HNO}_2 \rightleftharpoons \text{NO} + \text{NO}_2 + \text{H}_2\text{O} \quad k_{11}, k_{-11} \quad (R5.20) \]

\[ \text{NO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HNO}_2 + \text{H}^+ + \text{NO}_3^- \quad k_{12}, k_{-12} \quad (R5.21) \]

Overall equation:

\[ 3\text{HNO}_2 \rightarrow 2\text{NO} + \text{NO}_3^- + \text{H}^+ + \text{H}_2\text{O} \quad (R5.22) \]

The total concentration of nitrous acid can be defined by Equation S.3

\[ [\text{HNO}_2]_T = [\text{HNO}_2] + [\text{NO}_2^-] \quad (C.3) \]

Taking the assumption that Reaction 5.20 is at equilibrium and Reaction 5.21 is the rate limiting step:

\[ \frac{d[\text{HNO}_2]_T}{dt} = 3k_{12}[\text{NO}_2]^2 \quad (C.4) \]

\[ \frac{d[\text{NO}]}{dt} = 2k_{12}[\text{NO}_2]^2 \quad (C.5) \]

\[ K_{11} = \frac{[\text{NO}][\text{NO}_2]}{[\text{HNO}_2]^2} \quad (C.6) \]

\[ [\text{NO}_2] = \frac{K_{11}[\text{HNO}_2]^2}{[\text{NO}]} \quad (C.7) \]

Substituting Equation C.7 into C.4
Appendix C

Chemical trapping of NO

\[
\frac{d[HNO_2]_T}{dt} = \frac{3K_{11}^2k_{12}[HNO_2]^4}{[NO]^2} \quad (C.8)
\]

\[
\frac{d[NO]}{dt} = \frac{2K_{11}^2k_{12}[HNO_2]^4}{[NO]^2} \quad (C.9)
\]

\[
k_{\text{fwd}} = K_{11}^2k_{12} \quad (C.10)
\]

Thus,

\[
\frac{d[NO]}{dt} = 2k_{\text{fwd}}[HNO_2]^4 \quad (C.11)
\]

Derivation of integrated form of Equation used for kinetic fitting

\[\text{NO}_2^- + H^+ \rightleftharpoons HNO_2 \quad (R5.19)\]

From Reaction R19

\[
K_a = \frac{[NO_2^-][H^+]}{[HNO_2]} \quad (C.12)
\]

\[
[HNO_2] = \frac{[HNO_2]_{\text{Total}}[H^+]}{K_a + [H^+]} \quad (C.13)
\]

Because excess H+ is employed in the experiments, the ratio of \([H^+] / (K_a + [H^+])\) was assumed to be constant variable C

\[
C = \frac{[H^+]}{K_a + [H^+]} \]

Thus,

\[
\frac{d[NO]}{dt} = \frac{2K_{11}^2k_{12}C^4[HNO_2]^4}{[NO]^2} \quad (C.14)
\]
Or

\[
\frac{d[NO]}{dt} = \frac{2k_{fwd}[HNO_2]^4_{Total}}{[NO]^2} \tag{C.15}
\]

C.6 Ordinary differential equations for the *in situ* trapping reaction of DBNBS with NO

\[
\frac{d[NO]}{dt} = \frac{2k_{fwd}[HNO_2]^4_{Total}}{[NO]^2} - k_{Trap}[DBNBS][NO] - 2k_3[O_2][NO]^2 + k_6[N_2O_3][Aryl \ radical] - k_{MT}[NO]
\]

\[
\frac{d[HNO_2]}{dt} = \frac{-3k_{fwd}[HNO_2]^4_{Total}}{[NO]^2} + k_4[NO_2][H_2O] - k_4[HNO_2] - k_6[NO_2][HNO_2] + k_6[H_2O][N_2O_3]
\]

\[
\frac{d[DBNBS]}{dt} = 2k_1[DBNBS \ dimer] - 2k_1[DBNBS]^2 - k_{Trap}[DBNBS][NO]
\]

\[
\frac{d[DBNBS \ dimer]}{dt} = -k_1[DBNBS \ dimer] + k_1[DBNBS]^2
\]

\[
\frac{d[O_2]}{dt} = k_{Trap}[DBNBS][NO] - k_3[NO]^2[O_2]
\]

\[
\frac{d[N_2]}{dt} = k_{Trap}[DBNBS][NO]
\]

\[
\frac{d[NO_2]}{dt} = 2k_3[O_2][NO]^2 - k_4[NO_2][H_2O] + k_4[HNO_2][NO_2]
\]

\[
\frac{d[Aryl \ radical]}{dt} = k_{Trap}[DBNBS][NO] - k_7[Aryl \ radical][NO_2]
\]

\[
\frac{d[N_2O_3]}{dt} = k_4[NO_2][H_2O] - k_4[HNO_2][NO_3]
\]
\[
\frac{d[H_2O]}{dt} = -k_4(NO_2)[H_2O]+k_4[HNO_2][NO_3]\frac{d[NO_2]}{dt} - k_6[NO_2][HNO_2] \\
+ k_6[H_2O][N_2O_3]-k_7[Aryl radical][NO_2]+k_8[N_2O_3][Radical anion]
\]

\[
\frac{d[N_2O_3]}{dt} = k_6[NO_2][HNO_2] - k_6[H_2O][N_2O_3]+k_8[N_2O_3][Radical anion]
\]

\[
\frac{d[Radical anion]}{dt} = k_7[Aryl radical][NO_2]-k_8[N_2O_3][Radical anion]
\]

\[
\frac{d[Nitro compound]}{dt} = k_8[N_2O_3][Radical anion]
\]
APPENDIX D

SUPPORTING DOCUMENT FOR CHAPTER 7
### Table D.1. Temperature dependence of the monomer-dimer equilibrium constant for aromatic nitrososulfonate compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>DBNBS</th>
<th>NBS</th>
<th>DMNBS</th>
<th>DCNBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/°C</td>
<td>ε/ dm³ mol⁻¹ cm⁻¹</td>
<td>K&lt;sub&gt;c&lt;/sub&gt;</td>
<td>ε/ dm³ mol⁻¹ cm⁻¹</td>
<td>K&lt;sub&gt;c&lt;/sub&gt;</td>
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<td>25</td>
<td>34.4 ± 0.5</td>
<td>1.29 × 10⁻³</td>
<td>35.0 ± 1.0</td>
<td>(5.11 ± 0.4) × 10⁻³</td>
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<tr>
<td>30</td>
<td>34.4 ± 0.7</td>
<td>(1.97 ± 0.1) × 10⁻³</td>
<td>34.6 ± 1.0</td>
<td>(7.16 ± 0.6) × 10⁻³</td>
</tr>
<tr>
<td>40</td>
<td>35.0 ± 0.7</td>
<td>(4.02 ± 0.2) × 10⁻³</td>
<td>34.2 ± 0.6</td>
<td>(1.25 ± 0.1) × 10⁻²</td>
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<tr>
<td>50</td>
<td>35.6 ± 0.6</td>
<td>(5.59 ± 0.4) × 10⁻³</td>
<td>35.3 ± 0.6</td>
<td>(2.01 ± 0.2) × 10⁻²</td>
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<tr>
<td>60</td>
<td>35.3 ± 0.3</td>
<td>1.41 × 10⁻²</td>
<td>35.6 ± 0.5</td>
<td>(4.13 ± 0.4) × 10⁻²</td>
</tr>
</tbody>
</table>
Figure D.1. Van’t Hoff plot for the determination of standard change in enthalpy and entropy for the dissociation of nitroso dimer to monomer. ◊ DBNBS, ○ DCNBS, Δ DMNBS, □ NBS⁻
Figure D.2. Measurement of DCNBS monomer concentration for determining rate of
dissociation of dimer to monomer at 25°C. + 5 mmol dm$^{-3}$, □ 7.5 mmol dm$^{-3}$ and ▄ 10 mol dm$^{-3}$

$^3$ Solid lines represents model
Figure D.3. Measurement of DMNBS monomer concentration for determining rate of dissociation of dimer to monomer at 25°C. + 5 mmol dm$^{-3}$, □ 7.5 mmol dm$^{-3}$ and △ 10 mmol dm$^{-3}$. Solid lines represents model
### Chemical trapping of NO

**Appendix D**

**Table D.2.** Kinetic data for dimer/monomer interchange of aromatic nitroso sulfonate

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DBNBS</th>
<th>NBS</th>
<th>DMNBS</th>
<th>DCNBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_1$ / s$^{-1}$</td>
<td>$k_{1_2}$ / dm$^3$ mol$^{-1}$ s$^{-1}$</td>
<td>$k_1$ / s$^{-1}$</td>
<td>$k_{1_2}$ / dm$^3$ mol$^{-1}$ s$^{-1}$</td>
</tr>
<tr>
<td>T/ °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>$(2.9 \pm 0.1) \times 10^{-2}$</td>
<td>$21.6 \pm 0.4$</td>
<td>$1.2 \times 10^{-1}$</td>
<td>$23.7 \pm 0.5$</td>
</tr>
<tr>
<td>30</td>
<td>$(4.9 \pm 0.1) \times 10^{-2}$</td>
<td>$25.4 \pm 0.5$</td>
<td>$1.7 \times 10^{-1}$</td>
<td>$23.7 \pm 0.6$</td>
</tr>
<tr>
<td>40</td>
<td>$1.48 \times 10^{-1}$</td>
<td>$37.9 \pm 0.9$</td>
<td>$(3.7 \pm 0.1) \times 10^{-1}$</td>
<td>$29.6 \pm 0.1$</td>
</tr>
<tr>
<td>50</td>
<td>$(3.22 \pm 0.1) \times 10^{-1}$</td>
<td>$61.1 \pm 1.5$</td>
<td>$(6.1 \pm 0.1) \times 10^{-1}$</td>
<td>$30.6 \pm 0.3$</td>
</tr>
<tr>
<td>60</td>
<td>$8.9 \times 10^{-1}$</td>
<td>$63.8 \pm 1.8$</td>
<td>$1.6$</td>
<td>$39.0 \pm 0.7$</td>
</tr>
</tbody>
</table>
Figure D.4. Arrhenius plot for nitroso dimer-monomer interchange. Solid and no fill symbols represent data for the forward and backward reaction respectively. ◊ NBS, ○ DMNBS, △ DCNBS and □ DBNBS.
Figure D.5. Eyring plot for nitroso dimer dissociation to monomer. ♦ NBS, ○ DMNBS, △ DCNBS and □ DBNBS.
Dynafit Script for DCNBS dimer-monomer interchange

[task]

\[
\text{task} = \text{fit} \\
\text{data} = \text{progress}
\]

[mechanism]

\[
\text{DCNBS}_2 \leftrightarrow \text{DCNBS} + \text{DCNBS} : k_1 \quad k^{-1}
\]

[constants]

\[
k_1 = 10? \\
k^{-1} = 10?
\]

[responses]

\[
\text{DCNBS} = 1
\]

[progress]

directory .

extension txt

file DCNBS5 |conc DCNBS= 1.34e-3, DCNBS2= 1.83e-3
file DCNBS7.5 |conc DCNBS= 1.69e-3, DCNBS2= 2.90e-3
file DCNBS10 |conc DCNBS= 1.99e-3, DCNBS2= 4.01e-3

[output]

directory ./equilibriumconstantDCNBS

[end]
Result from fitting the script

Program DynaFit version 3.28.070 [04-21-2010]

Execution started Thu Mar 1 07:34:17 2012

Copyright 2010

SCRIPT FILE
\script for monomer-dimer DCNBS.txt

TASK
Fit of progress curves

DATA
file DCNBS5.txt
file DCNBS7.5.txt
file DCNBS10.txt

REACTION MECHANISM
DCNBS2 <===> DCNBS + DCNBS : k1 k-1

DIFERENTIAL EQUATIONS
\[ \frac{d[DCNBS]}{dt} = +k1[DCNBS2]+k1[DCNBS2]-k-1[DCNBS][DCNBS]-k-1[DCNBS][DCNBS] \]
\[ \frac{d[DCNBS2]}{dt} = -k1[DCNBS2]+k-1[DCNBS][DCNBS] \]

OUTPUT

LEAST-SQUARES FIT
mean square 1.3131e-009
standard deviation 3.62368e-005
log(determinant) -0.229
log(condition number) -2.48
Marquardt parameter  0.000122
execution time (sec)  0.060
datapoints            1606
parameters            2
iterations            16
subiterations         3
function evaluations  22
error status          0

FINAL GRADIENT

<table>
<thead>
<tr>
<th>Set</th>
<th>Parameter</th>
<th>Gradient G</th>
<th>G x Fitted</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>k1</td>
<td>-1.59232e-008 -2.95561e-010 -0.000140153</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k-1</td>
<td>2.93066e-011 2.69923e-010 0.000127996</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PARAMETERS & STANDARD ERRORS

<table>
<thead>
<tr>
<th>Set</th>
<th>Parameter</th>
<th>Initial</th>
<th>Fitted</th>
<th>Error</th>
<th>%Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>k1</td>
<td>10</td>
<td>0.01856</td>
<td>0.00017</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>k-1</td>
<td>10</td>
<td>9.21</td>
<td>0.094</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

COVARIANCE MATRIX

<table>
<thead>
<tr>
<th>Set</th>
<th>Parameter</th>
<th>Covariances</th>
</tr>
</thead>
<tbody>
<tr>
<td>k1</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>k-1</td>
<td>b</td>
<td>99</td>
</tr>
</tbody>
</table>

EIGENVECTORS AND EIGENVALUES

Eigenvectors
Eigenvalues 0.01 1.99

log(C) 2.48 0.00

Set Parameter 1 2

k1 1 -70 -70

k-1 2 -70 70

CONDITION INDICES

Index 2 0

Set Parameter

k1 49 49

k-1 49 49

PLOTS

Best-fit plot: equilibriumconstantDCNBS\tab\fit_0101.tab

Execution terminated Thu Mar 1 07:34:28 2012

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APPENDIX E

SUPPORTING DOCUMENT FOR CHAPTER 8
E.1 N$_2$ formation

![Figure E.6. N$_2$ formation from the reaction of DMNBS with NO](image)

![Figure E.7. N$_2$ formation from the reaction of DMNBS with NO](image)
Figure E.8. Comparison of NALDI mass spectra of neat DCNBS sample and the sample of the \textit{in situ} reaction mixture
Figure E.9. Comparison of NALDI mass spectra of neat NBS sample and the sample of the reaction mixture.
Figure E.10. Comparison of *ex situ* and *in situ* reaction mixture of NBS
Figure E.11. Comparison of *ex situ* and *in situ* reaction mixture of DMNBS
Appendix E

Chemical trapping of NO

Figure E.12. Zoom spectra for the comparison *ex situ* and *in situ* of reaction mixture of DMNBS
Figure E.13. Comparison of *ex situ* and *in situ* reaction mixture of DCNBS
E.2 Plots for kinetics measurements of reaction of nitroso with NO (ex situ and in situ)

**Figure E.9.** NO concentration versus time for the *ex situ* trapping of 0.6 mmol dm$^{-3}$ NO using 0.1 – 1.2 mmol dm$^{-3}$ NBS. (○) 0.1 mmol dm$^{-3}$, (⊙) 0.15 mmol dm$^{-3}$, (□) 0.3 mmol dm$^{-3}$, (△) 0.6 mmol dm$^{-3}$ and (×) 1.2 mmol dm$^{-3}$ initial equivalent NBS monomer. Solid line represents model predictions.
Figure E.140. Model prediction as compared to measured data for the time change of initial
(○) 0.9 mmol dm$^{-3}$, (⊙) 1.8 mmol dm$^{-3}$ and (×) 3.6 mmol dm$^{-3}$ initial equivalent concentration of
NBS monomer at 25 °C for NBS consumption by NO under ex situ conditions. Solid line
represents model predictions. For clarity every 10$^{th}$ point is shown.
Figure E.11. NO concentration versus time for the *ex situ* trapping of 0.6 mM NO using 0.1 – 1.2 mM DMNBS. (◊) 0.1 mmol dm$^{-3}$, (○) 0.15 mmol dm$^{-3}$, (□) 0.3 mmol dm$^{-3}$, (▲) 0.6 mmol dm$^{-3}$ and (×) 1.2 mmol dm$^{-3}$ initial equivalent DMNBS monomer. Solid line represents model predictions.
Figure E.152. Model prediction as compared to measured data for the time change of initial

(○) 0.45 mmol dm$^{-3}$, (∆) 0.9 mmol dm$^{-3}$ and (∗) 1.8 mmol dm$^{-3}$ initial equivalent DMNBS

monomer at 25 °C for DMNBS consumption by NO. Solid line represents model predictions.

For clarity every 10$^{th}$ point is shown.
Figure E.163. Change in NO concentration during the *in situ* trapping of NO by NBS at 25 °C. (◊) 0.103 mol dm$^{-3}$, (◯) 0.03 mol dm$^{-3}$, (Δ) 0.015 mol dm$^{-3}$ and (□) 0.005 mol dm$^{-3}$

Figure E.14. Change in NO concentration during the *in situ* trapping of NO by DMNBS at 25 °C. (◊) 0.175 mol dm$^{-3}$, (Δ) 0.03 mol dm$^{-3}$, (×) 0.015 mol dm$^{-3}$ and (○) 0.005 mol dm$^{-3}$
Figure E.15. Change in NO concentration during the in situ trapping of NO by DCNBS at 25 °C. (○) 0.175 mol dm$^{-3}$, (Δ) 0.03 mol dm$^{-3}$, (×) 0.015 mol dm$^{-3}$ and (⊙) 0.005 mol dm$^{-3}$ initial equivalent DCNBS monomer.