Applications of desorption electrospray ionisation mass spectrometry and ion mobility spectrometry to petroleomic and lubricant analysis

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Applications of Desorption Electrospray Ionisation Mass Spectrometry and Ion Mobility Spectrometry to Petroleomic and Lubricant analysis

by

Caitlyn Da Costa

A Doctoral Thesis
Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

October 2015

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Thesis Abstract

The use of mass spectrometry for the analysis of petrochemical products and crude oils enables the generation of detailed molecular data essential for chemical characterisation and product development. However, the need for multistage sample preparation techniques can be time consuming and may result in the loss of information. Ambient ionisation in combination with mass spectrometry enables the direct analysis of compounds present on a surface with minimal or no sample preparation. The work presented in this thesis evaluates the application of mass spectrometry (MS) hyphenated with ambient ionisation and ion mobility for the analysis of chemical additives used in lubricant and petrochemical products and also crude oil.

A technique called desorption electrospray ionisation (DESI) pioneered the ambient ionisation field. An in-house designed and constructed DESI source has been developed to enable hyphenation of DESI with MS and ion-mobility mass spectrometry (IM-MS) for the interrogation of chemical additives used in lubricant and petrochemical oils directly from multiple surface substrates. The approach has been successfully applied to the analysis of a range of chemical additives as standards and when present in a lubricating oil matrix. Data has also shown that DESI-MS can be used to map additive deposition on a surface.

The quantitative capabilities of DESI-MS have been assessed using a lubricant antioxidant additive present in a lubricant oil matrix and deposited on a surface. The DESI-MS method showed good linearity with a limit of detection (LOD) for the antioxidant additive below that used in typical commercial formulations. The use of a suitable internal standard in the DESI-MS analysis has been shown to significantly improve the repeatability of the approach.

Hyphenation of DESI with post ionisation separation methods, such as high field asymmetric waveform ion mobility spectrometry (FAIMS), can improve mass spectral response for targeted analytes through selective transmission. The analysis of a series of corrosion inhibitor additives in a base oil matrix has been carried out using electrospray (ESI) and DESI hyphenated with FAIMS-MS. FAIMS selection of target ions improved the sensitivity of ESI and DESI through enhanced analyte transmission and a reduction in the chemical noise resulting from the oil matrix. DESI-FAIMS-MS was shown to improve target analyte response compared to DESI-MS alone using the corrosion inhibitors as model compounds, showing how the
combined technique can be used for the rapid analysis of analytes directly from surfaces with no sample preparation or pre concentration.

Direct analysis in real time (DART) is an alternative ambient ionisation approach to DESI. The use of DART-MS for the direct analysis of lubricant and oil additives has been evaluated. All selected additives were successfully detected by DART-MS as standards and in an oil matrix. The surface material, DART helium gas temperature and the presence of an oil matrix were all shown to effect the desorption and ionisation of target analytes. The quantitative capabilities of DART-MS were assessed using the antioxidant additive in a lubricant oil matrix and in the presence of an internal standard. The technique showed good linearity and repeatability. The untargeted analysis of chemical additives present in a fully formulated lubricant oil has been carried out by DESI and DART ionisation techniques. The effect of DESI electrospray solvent and DART helium temperature were both shown to impact the observed mass spectral response for the sample.

The analysis of crude oil is particularly problematic due to the high complexity of the sample. A crude oil sample has been analysed using ESI combined with high resolution MS, ESI-FAIMS-MS and DESI-MS. High resolution mass spectrometry enabled the identification of molecular ions that could be characterised using specialist software. The use of FAIMS resulted in shift in the observed chemical profile for the crude oil sample showing selective transmission of molecular species based upon the differential mobility of ions rather than factors such as polarity or solubility that are typically used for sample fractionation. Molecular species from within the crude oil sample were successfully desorbed and ionised by DESI-MS using a DESI solvent composition of 6:4 toluene:methanol.
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<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionisation</td>
</tr>
<tr>
<td>API</td>
<td>American Petroleum Institute</td>
</tr>
<tr>
<td>AP-MALDI</td>
<td>Atmospheric pressure-matrix assisted laser desorption ionisation</td>
</tr>
<tr>
<td>APPI</td>
<td>Atmospheric pressure photo ionisation</td>
</tr>
<tr>
<td>AP-TD/SI</td>
<td>Atmospheric pressure thermal desorption-secondary ionisation</td>
</tr>
<tr>
<td>ASAP</td>
<td>Atmospheric solids analysis probe</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society of the International Association for the Testing of Materials</td>
</tr>
<tr>
<td>BADCI</td>
<td>Beta electron-assisted direct chemical ionisation</td>
</tr>
<tr>
<td>CCS</td>
<td>Collisional cross section</td>
</tr>
<tr>
<td>CEM</td>
<td>Chain ejection model</td>
</tr>
<tr>
<td>CF</td>
<td>Compensation field</td>
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<tr>
<td>CI</td>
<td>Chemical ionisation</td>
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<tr>
<td>CID</td>
<td>Collision induced dissociation</td>
</tr>
<tr>
<td>CRM</td>
<td>Charge residue model</td>
</tr>
<tr>
<td>CV</td>
<td>Compensation voltage</td>
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<tr>
<td>DAPCI</td>
<td>Desorption atmospheric pressure chemical ionisation</td>
</tr>
<tr>
<td>DAPPI</td>
<td>Desorption atmospheric pressure photo ionisation</td>
</tr>
<tr>
<td>DART</td>
<td>Direct analysis in real time</td>
</tr>
<tr>
<td>DBDI</td>
<td>Dielectric barrier discharge ionisation</td>
</tr>
<tr>
<td>DBEs</td>
<td>Double bond equivalents</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>DCBI</td>
<td>Desorption corona beam ionisation</td>
</tr>
<tr>
<td>DEMI</td>
<td>Desorption electrospray/metastable induced ionisation</td>
</tr>
<tr>
<td>DESI</td>
<td>Desorption electrospray ionisation</td>
</tr>
<tr>
<td>DF</td>
<td>Dispersion field</td>
</tr>
<tr>
<td>DICE</td>
<td>Desorption ionisation by charge exchange</td>
</tr>
<tr>
<td>DMS</td>
<td>Differential mobility spectrometry</td>
</tr>
<tr>
<td>DT-IMS</td>
<td>Drift tube IMS</td>
</tr>
<tr>
<td>DV</td>
<td>Dispersion voltage</td>
</tr>
<tr>
<td>EASI</td>
<td>Easy ambient sonic spray ionisation</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersive x-ray spectroscopy</td>
</tr>
<tr>
<td>EESI</td>
<td>Extractive electrospray sonization</td>
</tr>
<tr>
<td>ELDI</td>
<td>Electrospray assisted laser desorption ionisation</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionisation</td>
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<tr>
<td>FAIMS</td>
<td>Field asymmetric waveform ion mobility</td>
</tr>
<tr>
<td>FAPA</td>
<td>Flowing atmospheric pressure afterglow</td>
</tr>
<tr>
<td>FD</td>
<td>Field desorption</td>
</tr>
<tr>
<td>FD-ESI</td>
<td>Fused droplet-electrospray ionisation</td>
</tr>
<tr>
<td>FI</td>
<td>Field ionisation</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>FTICR-MS</td>
<td>Fourier transform ion cyclotron resonance mass spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infra-red</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>HDC</td>
<td>Higher-energy collisional dissociation</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IEM</td>
<td>Ion evaporation model</td>
</tr>
<tr>
<td>IMS</td>
<td>Ion mobility spectrometry</td>
</tr>
<tr>
<td>IR-LAMICI</td>
<td>Infrared laser ablation metastable induced chemical ionisation</td>
</tr>
<tr>
<td>LAESI</td>
<td>Laser ablation electrospray ionisation</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LDESİ</td>
<td>Laser desorption electrospray ionisation</td>
</tr>
<tr>
<td>LESA</td>
<td>Liquid extraction surface analysis</td>
</tr>
<tr>
<td>LIAD-ESI</td>
<td>Laser induced acoustic desorption-electrospray ionisation</td>
</tr>
<tr>
<td>LMJ-SSP</td>
<td>Liquid micro junction-surface sampling probe</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LTP</td>
<td>Low temperature plasma probe</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>MALDESİ</td>
<td>Matrix-assisted laser desorption electrospray ionisation</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix assisted laser desorption ionisation</td>
</tr>
<tr>
<td>MOSFET</td>
<td>Metal-oxide semiconductor field-effect transistor</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>NAI</td>
<td>National Aperture Inc</td>
</tr>
<tr>
<td>ND-ESI</td>
<td>Neutral desorption extractive electrospray ionisation</td>
</tr>
<tr>
<td>n-DESI</td>
<td>Nano-desorption electrospray ionisation</td>
</tr>
<tr>
<td>NI</td>
<td>National Instruments</td>
</tr>
<tr>
<td>NI-MAX</td>
<td>National Instruments Measurement &amp; Automation Explorer</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NPL</td>
<td>National Physical Laboratory</td>
</tr>
<tr>
<td>PAOs</td>
<td>Polyalphaolefins</td>
</tr>
<tr>
<td>PESI</td>
<td>Probe electrospray ionisation</td>
</tr>
<tr>
<td>PS</td>
<td>Paper spray ionisation</td>
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<tr>
<td>Q</td>
<td>Quadrupole</td>
</tr>
<tr>
<td>RADIO</td>
<td>Radio frequency acoustic desorption and ionisation</td>
</tr>
<tr>
<td>REIMS</td>
<td>Rapid evaporative ionisation mass spectrometry</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency</td>
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<tr>
<td>S/N</td>
<td>Signal to noise</td>
</tr>
<tr>
<td>SARA</td>
<td>Saturate, aromatic, resin and asphaltene fractionation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SIMS</td>
<td>Secondary ion mass spectrometry</td>
</tr>
<tr>
<td>SIR</td>
<td>Selected ion response</td>
</tr>
<tr>
<td>TAAH</td>
<td>Tetralkylammonium halides</td>
</tr>
<tr>
<td>TAN</td>
<td>Total acid number</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TM-DART</td>
<td>Transmission mode DART</td>
</tr>
<tr>
<td>TM-DESI</td>
<td>Transmission mode DESI</td>
</tr>
<tr>
<td>ToF</td>
<td>Time-of-flight</td>
</tr>
<tr>
<td>TWIMS</td>
<td>Triwave ion mobility spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>UPLC</td>
<td>Ultra performance liquid chromatography</td>
</tr>
<tr>
<td>VAMAS</td>
<td>Versailles Projects on Advanced Materials and Standards</td>
</tr>
<tr>
<td>ZDDP</td>
<td>Zinc dialkyldithiophosphate</td>
</tr>
<tr>
<td>%RSD</td>
<td>% Relative standard deviations</td>
</tr>
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</table>
CHAPTER ONE

Introduction
1.1 Mass Spectrometry of Petroleum and Lubricants

Petroleum is derived from the processing of naturally occurring crude oil found in geological formulations beneath the earth’s surface. Drilling for crude oil has had a pivotal role in global history as a result of societies increasing dependence on the derived products to assist in the function of everyday life. Although a key use for refined petroleum is in the generation of fuels, such as petrol and diesel, petrochemicals can be found in many modern day products including lubricants, clothing, plastics and pharmaceuticals. This PhD will focus on the analysis of petrochemical additives and a key petrochemical product, oil lubricants, which are used both commercially and within the oil industry to maintain any components that could be subject to enhanced wear. In addition this introduction will discuss the general chemical composition of crude oil and possible tools to assist in molecular analysis of the raw product.

1.1.1 Crude oils: Composition and analysis

Crude oil is a naturally occurring fossil fuel generated from the extreme compression of dead organisms under the earth’s upper surface over millions of year. The result is a thick mixture of hydrocarbons and related sulphur, oxygen, nitrogen and small amounts of metallic containing species. There are four different hydrocarbon groups found in crude oil mixtures that are categorised by the hydrocarbon ratio and C-C bonds. These include paraffins, olefins, naphthalenes and aromatics. A summary is provided in Table 1.1. The chemical composition of crude oils is not uniform between different geological sites or even within the same oil reserve. The ratios of the different hydrocarbon components can fluctuate as well as the relative presence of sulphur, nitrogen, oxygen and metallic species. In general terms, typically around 90% of the crude oil formulation is made from paraffins, naphthalenes and aromatics. Table 1.2 provides an overview of typical crude oil elemental compositions.
Table 1.1: Summary of hydrocarbon classes found in crude oils$^{1,2}$

<table>
<thead>
<tr>
<th>Class</th>
<th>Chain/ring</th>
<th>Saturated/Unsaturated</th>
<th>Example</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffin</td>
<td>Chain</td>
<td>Saturated</td>
<td>Hexane</td>
<td>30</td>
</tr>
<tr>
<td>Olefin</td>
<td>Chain</td>
<td>Unsaturated</td>
<td>Hexene</td>
<td>6</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Ring</td>
<td>Saturated</td>
<td>Cyclohexane</td>
<td>49</td>
</tr>
<tr>
<td>Aromatic</td>
<td>Ring</td>
<td>Unsatuated</td>
<td>Benzene</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1.2: Elemental composition of crude oils$^2$

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>83-87</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>10-14</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.1-2</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.1-1.5</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.5-6</td>
</tr>
<tr>
<td>Metals</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

The molecular study of crude oil composition and the characterisation of petroleum products have recently been termed ‘Petroleomics’. Crude oils present highly complex matrices that contain a high concentration of non-volatile and difficult to analyse compounds. This is further complicated by the vast array of different hydrocarbon chain lengths, conformational and structural isomeric species and differing degrees of saturation that can be found within the mixture resulting in a dynamic abundance in the range of 10,000-100,000.$^3$ The detection of target analytes within this matrix and the full characterization of oils is therefore one of greatest analytical challenges posed. The desire to characterise oil samples is driven by the vast quantities of information this can provide including details regarding the age, origin, treatment and decomposition of the product. In addition it is believed that sufficient characterisation of the organic species can enable prediction of petroleum product properties improving the efficiency of oil reserve processing and “downstream” reactions.$^4$ Typically chemists have approached the analysis of petroleum oils through a range of different wet chemical methods that separate the complex mixture into chemical fractions before analysis. An example of this is the SARA fractionation method that separated crudes into 4 groups, saturates, aromatics, resins and asphaltenes, based upon differences in solubility and polarity.$^5$
Further tests centre on the investigation into an oils bulk property (viscosity index, density, conductivity, thermal stability, oxidation, pour point, vapour pressure, total acid number and water, sulphur and wax contents), as well as methods such as nuclear magnetic resonance (NMR), UV-visible and infra-red spectroscopy and chromatography. Several reviews highlight the range of different analytical techniques available for petroleum analysis.3,4,6

The application of mass spectrometry (MS) provides a unique opportunity for molecular investigation of crude oil species. This approach is not a novel concept and, typically hyphenated with separation techniques such as gas chromatography (GC) and thin layer chromatography (TLC), enabled some early progress to be made in the molecular characterisation of oils.7,8 The direct analysis of oils using mass spectrometry is incredibly problematic due to the chemical diversity of the analytes. Factors such as ionisation efficiency, mass resolution and competitive ionisation effects all require consideration.9 In the late 1990’s Fenn and colleagues coupled electrospray ionisation (ESI) with low resolution mass spectrometry for the interrogation of acidic and polar species in petroleomic samples.10 The analysis of nitrogen, oxygen and sulphur containing compounds was of particular interest because of their impact in both downstream and upstream process. The formation of NOx and SOx compounds contribute to atmospheric pollution and enhanced corrosion.4 This, along with instrumental development, led the way for a range of different ambient ionisation methods to be applied within the field including; easy ambient sonic spray ionisation (EASI),11 desorption electrospray ionisation (DESI),12 nano-DESI (nDESI),13 atmospheric pressure photoionisation (APPI),14 matrix assisted laser desorption ionisation (MALDI),15 direct analysis in real time (DART)16 and field desorption/field ionisation (FD/FI).17,18 The range of ionisation techniques reflects the complex chemical nature of crude oils, with no single method capable of ionising all species. The prevalent trend in the mass spectrometric analysis of petroleum is the use of superior mass analyses for high-resolution mass spectrometry, which has enabled the characterization and detailed analysis of such complex mixtures. High resolution mass analysers, such as Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), provide advanced mass resolving power compared to time-of-flight (TOF) or low resolution quadrupole mass spectrometers and can be coupled to a range of different ionisation sources.4,5,19–27

The use of chemometrics and data visualization tools to understand the large quantity of data generated and to “fingerprint” oils is also an expanding area of interest assisted by development of FTICR-MS.28,29
A smaller field of study, but one that requires equal consideration, is the hyphenation of ion mobility spectrometry (IMS) and high-field asymmetric waveform ion mobility (FAIMS) to the mass spectrometric analysis of oils. In IMS and FAIMS, separation occurs following the formation of gas phase ions on the basis of ion mobility, which is related to the collisional cross section (CCS) of an ion. This approach differs from the traditional wet chemical separation methods and chromatographic techniques that are primarily based upon a compounds solubility and polarity. Techniques such as IMS-MS and FAIMS-MS can provide an additional separation mechanism for oil derived ions, which can improve the peak capacity of the mass spectrometric technique and provide a secondary identification parameter for oil components. In addition to this it has the potential for interrogation into structural relationships, such as differences in gas phase conformation or aggregation, of oil components using relative differences in CCS measurements. IMS-MS has been applied to the separation of N, NO, NO₂, O and O₂ classes of compounds, isomeric separation and the analysis of diesel fuels and fuel additives. CCS measurements have been used to generate structural information for chemically related compounds and the investigation into gas phase aggregation. Additionally the use of complexing reagents to improve IM resolution has been investigated. FAIMS-MS has been applied to the separation and characterisation of NO and NO₂ species showing potential for isomer separation and the detailed analysis and structural elucidation of naphthenic acids from oil sand ore and pond water samples.

1.1.2 Petroleum and lubricant additives

Chemical additives are a necessary requirement to control many petroleomic processes and to enhance traditional performance characteristics of petroleomic products. The use of additives can be found in both midstream (extraction and transport) and downstream (refinement). In the midstream, the compounds function to minimise the negative effects of the crude oil extraction, such as acid corrosion and oxidation, and assist with the priming and maintenance of oil transport systems. There are a wide range of downstream applications, but the work presented in this PhD will primarily focus on midstream additives and those used in lubricant oils.

Lubricant oils are traditionally a petroleum product derived from the refinement process of crude oils. They principally function within tribological systems to minimise friction, heat and wear at the point of contact between two moving counterparts.
Tribology can be defined as the “mechanisms of friction, lubrication and wear of interacting surfaces that are in relative motion”. The movement of two surfaces in opposing directions will generate friction at the point of contact, which can eventually result in the break-down of the surface and wear of the structural component. A lubricant serves the purpose to reduce the friction present at the boundary of two moving counterparts within a tribological system by forming a fluid-film layer on the surface. The expected performance characteristics of advancing tribological systems have resulted in a greater demand being placed on the lubricating oil to ensure system maintenance and longevity. Untreated lubricant oils do not possess the properties to efficiently deal with current tribological demands and therefore a range of chemical additives are incorporated into the final formulation to ensure the product is fit for purpose. These chemical additives can greatly influence the physical and chemical properties of the lubricant and as a result are of an analytical interest for lubricant development.

**Lubricant base oils**

The base oil forms the bulk of the lubricant, providing a fluid layer to separate moving parts and act as a carrier for chemical additives. The performance characteristics of the base oils are modified through the use of chemical additives, but the starting chemical and physical properties of the oil also requires careful consideration. Both the low temperature characteristics, such as viscosity and pour point, and high temperature properties, such as flash point and volatility, need to be stable within the operating range of the tribological system. In addition additives must remain in solution at all times. There are two key types of base oils used in lubricant formulation; mineral and synthetic. Mineral base oil is generally formulated from crude oil during the fractionation and distillation process conducted at refineries. The chemical composition of mineral base oils is primarily hydrocarbon based containing paraffins, naphthalenes and aromatics, with trace amounts of non-hydrocarbons including organosulphur, oxygen and nitrogen containing compounds and high molecular weight resins and asphaltenes. Synthetic base oils have been developed over the years to improve the performance characteristics of lubricants that could not be achieved through simple modifications on mineral base oils. Many compounds have been tested as suitable replacements and several are now commonly used as mixtures in the synthesis of synthetic lubricants. These include polylphaolefins (PAO’s), alkylated aromatics, polybutenes (mainly isobutene), synthetic esters, polyalkylene glycols and phosphate esters.
Typical lubricant base stocks are mixtures of different oil formulations and as a result the American Petroleum Institute (API) has designated a grouping system based upon hydrocarbon content, sulphur content and viscosity index. This is summarised in Table 1.3.

### Table 1.3: API grouping for lubricant base oil formulations

<table>
<thead>
<tr>
<th></th>
<th>Saturates (%)</th>
<th>Sulfur (%)</th>
<th>Viscosity Index</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>&lt; 90</td>
<td>&gt; 0.03</td>
<td>80-120</td>
<td>Solvent extraction, solvent or catalytic dewaxing and hydrofinishing</td>
</tr>
<tr>
<td>Group II</td>
<td>&gt; 90</td>
<td>&lt; 0.03</td>
<td>80-120</td>
<td>Hydrocracking and solvent catalytic dewaxing</td>
</tr>
<tr>
<td>Group III</td>
<td>&gt; 90</td>
<td>&lt; 0.03</td>
<td>&gt; 120</td>
<td>Special processes such as isohydromerization</td>
</tr>
<tr>
<td>Group IV</td>
<td>Polyalphaolefins</td>
<td></td>
<td></td>
<td>Synthetic</td>
</tr>
<tr>
<td>Group V</td>
<td>All other</td>
<td></td>
<td></td>
<td>Synthetic</td>
</tr>
</tbody>
</table>

**Chemical additives**

Petroleum chemical additives are an integral feature of modern day petroleomic processes and are a necessity to maintain performance characteristics of midstream and tribological systems. Additives primarily function to either inhibit or counteract negative chemical reactions, such as corrosion, oxidation and sludge formation, that would reduce the practical function of the system. There are many different types of additives that are commonly mixed into performance packages before incorporation into the base stock to generate the formulated lubricant. The range of additives and their relative abundance within the performance package will reflect the intended physical and chemical properties of the lubricant. Features for consideration include; expected lifetime, performance and temperature range of operation, additive interaction and acceptable viscosity and pH range. The function of commercial chemical additives has been summarised here:

**Detergents and Dispersants:** Compounds that function to reduce the formation of carbon and sludge deposits are classes as detergents or dispersants. Detergents commonly contain calcium, magnesium or sodium metal cation groups. They are usually alkaline and react to neutralize strong acids. They can also exhibit anti-wear
and extreme temperature properties. Dispersants are non-metallic ashless cleaning agents that suspend soot and combustion products within the body of the oil to prevent deposition of sludge. They commonly containing oxygen, nitrogen or phosphorous that, along with the hydrocarbon tail, interacts with particulates to enable a solubilizing action.

**Anti-oxidants:** The presence of oxygen and elevated temperatures cause rapid oxidation of hydrocarbon compounds, which will result in variation of the viscosity and acidity beyond acceptable ranges and the formation of sludge. Antioxidant compounds, commonly sterically hindered phenols or aromatic amines, are added to inhibit oxidation reactions and reduce the rate of oxidative breakdown of the product.

**Corrosion inhibitors:** The definition of a corrosion inhibitor is a chemical compound that reduces the rate of corrosion within a system without altering the concentration of any other corrosive agent. Corrosion inhibitors, such as sulphates, act by binding to the metal surface to create a physical barrier to prevent the access of corrosive species, such as water and oxygen.

**Viscosity index improvers:** Polymers of methacrylates, oelfins, acrylates and styrene-butadiene are added to lubricants to improve the viscosity index. The relative viscosity index of oil lubricants is used as a general measure of quality, with a high viscosity index suggesting function of the lubricant over a large temperature range.

**Pour point depressors:** The pour point of an oil describes the temperature 3°C above which the oil is no longer able to freely pour. Reducing the pour point temperature improves low temperature characteristics. Compounds such as polymethacrylates, polyacrylates and di(tetra paraffin phenol)phthalate act as pour point depressors through surface adsorption onto wax crystals resulting in a surface layer which prevents the growth of the crystals and inhibits their ability to adsorb oil.

**Anti-wear additives:** Anti-wear additives have strong attractions to the metal surfaces resulting in the formation of a film. The film of anti-wear chemicals will have a slower shear strength than the original material resulting in a preferential breakdown of the surface film rather than the metal material. Zinc dialkyldithiophosphate (ZDDP) is a common anti-wear additive.

**Anti-foam additives:** The presence of trapped air within the lubricating oil can lead to the production of foam, which can cause starvation of lubrication to an engine due to
the presence of air bubbles on contacting surfaces. Silicone polymers are common anti-foaming additives for non-aqueous foams.

**Friction Modifiers:** These additives are commonly used in boundary lubrication to reduce the coefficient of friction and improve lubricity, particularly under the effect of high temperatures where base oil lubricity is reduced. They function by binding to the surface and include fatty acids, fatty amides and other compounds with a long hydrocarbon chain.\(^{41}\)

All lubricant additives, with the possible exception of some viscosity modifiers and pour point depressors, are synthesised with a polar and non-polar region. The hydrocarbon group, of sufficient carbon length, ensures solubility in the base stock oil. The polar functionality usually contains oxygen, nitrogen, sulfur or phosphorous and influences the chemical activity of the compound. A schematic of a typical additive structure is given in Figure 1.1

![Figure 1.1: General representation of a petroleomic chemical additive molecule](image)

Functionality of the chemical additives can either be at the target surface or within the bulk of the lubricant, depending upon the compounds polar to non-polar ratio.\(^{40}\) Compounds such as detergents, dispersants and antioxidants will have a low polar:non-polar ratio and therefore carry out solution based chemistry within the oil matrix. In contrast additives such as corrosion inhibitors, antiware additives and friction modifiers will have a higher polar:non-polar ratio. This can either be a result of
increasing the polarity of the head group, or reducing the length of the hydrocarbon chain in the hydrophobic tail. The increased polarity of the molecule will enhance its affinity towards the tribological surface, where it will ideally form strong non-covalent interactions with the surface to bring about its functionality. An example of this is the antiware/multi-purpose ZDDP additive that functions to create a protective layer over the metal surface to reduce surface friction at a boundary (Figure 1.2).  

![Chemical structure of ZDDP additive](image)

**Figure 1.2: Function of surface active ZDDP lubricant additive at a boundary surface**  

1.1.3 Analysis of petroleum chemical additives

The targeted analysis of chemical additives in petrochemical products such as lubricating oils is pivotal for formulation development, and can generate information relating to application, performance characteristics, additive interactions, and product age and degradation state. The relative concentration of chemical additives in
performance packages will depend upon the intended purpose of the product and the physical and chemical environment it will be exposed to. Formulation development therefore needs to assess the additives performance and breakdown rate when exposed to extreme tribological conditions with additives as standards and in mixtures. A range of wet chemical tests provided by the American Society of the International Association for Testing and Materials (ASTM) guidelines are applied to the routine analysis of lubricant additive formulations including, total acid number, elemental analysis, ash testing, base number, density, flash point, nitrogen and sulphur content, viscosity and water content. However, the tests typically target performance characteristics of the bulk product and therefore cannot be used to specifically assess chemical additives on a molecular level. Surface analysis methods including optical microscopy, transmission electron microscopy, scanning electron microscopy and X-ray photoelectron spectroscopy have all been applied to the analysis of boundary surfaces for investigation into the deposition and activation of surface active lubricant additives.

The application of mass spectrometry to the field of petroleum additive analysis has advantages over the wet chemical tests. However, this field still remains relatively unexplored. Mass spectrometry, particularly when hyphenated with techniques such as ESI, can generate molecular data that provides information relating to the solution based chemistry of additives. This not only allows for detailed compositional information to be generated, but can be used to investigate potential additive-additive interactions and individual additive performance following exposure. Lubricant additives have been analysed by techniques such as GC-MS, ESI-MS and ESI-MS/MS.
1.2 Atmospheric Pressure and Ambient Ionisation Mass Spectrometry

The ionisation process in mass spectrometry describes the generation of gaseous phase ions, either under vacuum or at atmospheric pressure, that are extracted into mass spectrometric systems.

Atmospheric ionisation and ambient ionisation are terms that are easily confused. Atmospheric ionisation describes all ionisation methods that are carried out under atmospheric pressure, such as ESI. The field of ambient ionisation focuses on the “direct analysis of untreated samples in the open environment, whilst maintaining the native condition and spatial integrity of the sample”.

Ions generated from a section of the sample surface external to the mass spectrometer inlet act as a representation of the chemical composition of the target. This enables the rapid native state interrogation of a sample with minimal sample preparation, whilst preserving the spatial chemical information.

A recent review proposed that the term ‘ambient ionisation’ refer to techniques that have the capability of meeting several key requirements including 1) the generation of analyte ions in the open environment without spatial constraints resulting from the ionisation source, 2) direct ionisation of analytes with minimal sample pre-treatment, c) interfacable with multiple mass spectrometers and d) to generate ions with internal energies equal to or lower than ESI, atmospheric pressure-matrix assisted laser desorption ionisation (AP-MALDI), APPI and atmospheric pressure chemical ionisation (APCI).

The report by Cooks and co-workers in 2004 detailing a novel ionisation approach called DESI for the direct analysis of compounds on a surface is considered to be the first publication of ambient ionisation methods. In the ensuing years over 30 ambient ionisation methods have been reported. A summary is given in Tables 1.4-1.7. The majority of the techniques have been developed upon the foundations of established ESI and chemical ionisation (CI) methods for the generation of molecular ion species. As a result they are generally considered to be a “soft” ionisation approach, generating molecular ion data with minimal fragmentation. While many of the techniques have comparable mechanistic features for analyte ion formation, the array of different geometric configurations and sample introduction approaches has widened the field of application. The use of ambient ionisation methods has been reported within the fields of drug and pharmaceutical research, food and environmental analysis, chemical warfare and safety, ‘omic’ based studies and chemical profiling experiments.
Table 1.4: Spray based ambient ionisation methods\textsuperscript{46,48}

<table>
<thead>
<tr>
<th>Ionisation method</th>
<th>Acronym</th>
<th>Year invented</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid micro junction-surface sampling probe</td>
<td>LMJ-SSP</td>
<td>2001</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Fused droplet-electrospray ionisation</td>
<td>FD-ESI</td>
<td>2002</td>
<td>Biological\textsuperscript{51}</td>
</tr>
<tr>
<td>Desorption electrospray ionisation</td>
<td>DESI</td>
<td>2004</td>
<td>Food and environmental, security, biological imaging, pharmaceuticals</td>
</tr>
<tr>
<td>Easy ambient sonic-spray ionisation</td>
<td>EASI</td>
<td>2006</td>
<td>Food analysis, ink analysis, polymer analysis</td>
</tr>
<tr>
<td>Extractive electrospray ionization</td>
<td>EESI</td>
<td>2006</td>
<td></td>
</tr>
<tr>
<td>Probe electrospray ionisation</td>
<td>PESI</td>
<td>2007</td>
<td>Food and environmental, biological imaging</td>
</tr>
<tr>
<td>Neutral desorption electrospray ionisation</td>
<td>ND-EESI</td>
<td>2007</td>
<td>Food analysis, forensics</td>
</tr>
<tr>
<td>Atmospheric pressure thermal desorption-secondary ionisation</td>
<td>AP-TD/SI</td>
<td>2010</td>
<td>Security</td>
</tr>
<tr>
<td>Liquid extraction surface analysis</td>
<td>LESA</td>
<td>2010</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Paper spray ionisation</td>
<td>PS</td>
<td>2010\textsuperscript{52}</td>
<td>Biological\textsuperscript{52}, Security</td>
</tr>
<tr>
<td>nano-DESI</td>
<td>n-DESI</td>
<td>2010\textsuperscript{54}</td>
<td>Biological imaging\textsuperscript{54}</td>
</tr>
</tbody>
</table>

Table 1.5: Chemical based ambient ionisation methods\textsuperscript{46,50}

<table>
<thead>
<tr>
<th>Ionisation method</th>
<th>Acronym</th>
<th>Year invented</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct analysis in real time</td>
<td>DART</td>
<td>2005</td>
<td>Food and environmental, forensic and security, pharmaceutical, polymer analysis, biological analysis</td>
</tr>
<tr>
<td>Desorption atmospheric pressure chemical ionisation</td>
<td>DAPCI</td>
<td>2005</td>
<td>Food analysis, pharmaceutical analysis</td>
</tr>
<tr>
<td>Atmospheric solids analysis probe</td>
<td>ASAP</td>
<td>2005</td>
<td>Polymer analysis</td>
</tr>
<tr>
<td>Dielectric barrier discharge ionisation</td>
<td>DBDI</td>
<td>2007</td>
<td>Analysis of organic compounds</td>
</tr>
<tr>
<td>Flowing atmospheric pressure afterglow</td>
<td>FAPA</td>
<td>2008</td>
<td>Environmental, polymer fingerprinting</td>
</tr>
<tr>
<td>Low temperature plasma probe</td>
<td>LTP</td>
<td>2008</td>
<td>Food and environmental, security, imaging</td>
</tr>
<tr>
<td>Beta electron-assisted direct chemical ionisation</td>
<td>BADCI</td>
<td>2009</td>
<td>Pharmaceutical analysis</td>
</tr>
<tr>
<td>Desorption corona beam ionisation</td>
<td>DCBI</td>
<td>2010</td>
<td>Environmental analysis</td>
</tr>
</tbody>
</table>
### Table 1.6: Laser based ambient ionisation methods\textsuperscript{46,48}

<table>
<thead>
<tr>
<th>Ionisation method</th>
<th>Acronym</th>
<th>Year invented</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrospray assisted laser desorption</td>
<td>ELDI</td>
<td>2005</td>
<td>Food analysis, metabolomics</td>
</tr>
<tr>
<td>Matrix-assisted laser desorption electrospray ionisation</td>
<td>MALDESI</td>
<td>2006</td>
<td>Pharmaceutical analysis, proteomics</td>
</tr>
<tr>
<td>Laser ablation electrospray ionisation</td>
<td>LAESI</td>
<td>2007</td>
<td>Forensic, imaging, metabolomics</td>
</tr>
<tr>
<td>Laser desorption electrospray ionisation</td>
<td>LDESI</td>
<td>2009</td>
<td>Proteomics</td>
</tr>
<tr>
<td>Infrared laser ablation metastable induced chemical ionisation</td>
<td>IR-LAMICI</td>
<td>2010</td>
<td>Pharmaceutical imaging</td>
</tr>
</tbody>
</table>

### Table 1.7: Other ambient ionisation methods\textsuperscript{46,48}

<table>
<thead>
<tr>
<th>Ionisation method</th>
<th>Acronym</th>
<th>Year invented</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desorption atmospheric pressure photo ionisation</td>
<td>DAPPI</td>
<td>2007</td>
<td>Environmental analysis, security, imaging</td>
</tr>
<tr>
<td>Desorption electrospray/metastable induced ionisation</td>
<td>DEMI</td>
<td>2009</td>
<td>Pharmaceutical</td>
</tr>
<tr>
<td>Laser induced acoustic desorption-electrospray ionisation</td>
<td>LIAD-ESI</td>
<td>2009</td>
<td>Proteomics</td>
</tr>
<tr>
<td>Radio frequency acoustic desorption and ionisation</td>
<td>RADIO</td>
<td>2009</td>
<td>Proteomics</td>
</tr>
<tr>
<td>Rapid evaporative ionisation mass spectrometry</td>
<td>REIMS</td>
<td>2009</td>
<td>Biological studies</td>
</tr>
<tr>
<td>Desorption ionisation by charge exchange</td>
<td>DICE</td>
<td>2010</td>
<td></td>
</tr>
</tbody>
</table>
1.2.1 Electrospray ionisation (ESI)

The successful formation of gaseous phase ions is pivotal for the mass spectrometric analysis of compounds. While techniques such as APCI and APPI are suited to small volatile analytes, the analysis of larger and less volatile species requires an alternative approach. ESI provides a means of analysing molecules directly from the liquid phase to generate molecular ion species. The technique was first reported in work carried out by Dole and co-workers in 1968,\(^{55}\) and further developed by Fenn and colleagues in the late 1990’s.\(^ {56}\) The attraction of ESI was the ability to ionise larger organic molecules, such as proteins and macromolecules, without the extensive fragmentation observed with chemical based ionisation approaches, which often limited the chemical data obtained. The potential applications of ESI were further advanced by the simple hyphenation to liquid phase chromatography for the analysis of complex liquid mixtures such as biological samples.

In ESI target analytes, present in a solution, are introduced into the ionisation source using a mobile phase, commonly consisting of an organic-aqueous mixture. Ionisation of analyte species occurs in solution and can be promoted through the use of additives such as formic acid or ammonium hydroxide. The solution is passed through capillary tubing using positive pressure from either a syringe or a high/ultra performance liquid chromatography (HPLC/UPLC) pump towards the ESI source. The ESI source is comprised of a metal capillary tube, commonly 0.02 mm outer diameter and 0.01 mm inner diameter, that has an applied voltage of 1-4 kV and is positioned 1-3 cm from a counter electrode (Figure 1.3).\(^ {57}\)

![Figure 1.3: Schematic diagram of an ESI source\(^ {57}\)](image_url)
The applied voltage, either positive or negative depending upon the target analytes, generates a strong electric field of $10^6 \text{ V m}^{-1}$ at the tip of the capillary, which induces polarization of the mobile phase and will cause the accumulation of ions at the capillary tip. Ions of an opposing charge to the applied voltage will migrate towards the capillary walls and be neutralised, resulting in the formation of a droplet with an overall net charge. The downfield forces present as a result of the polarization will cause distortion of the droplet and the formation of a Taylor cone (Figure 1.4 and 1.5). When the applied field is sufficiently high the Taylor cone will become unstable. The resulting breakdown generates an electrospray plume consisting of small droplets of solvent and analyte ions with a net charge. Solvent evaporation, assisted by the flow of a desolvation gas and heightened temperatures, reduces the size of the droplets while the net charge is maintained, increasing the repulsion between ions within the droplet as they travel towards the counter electrode. This process continues until the point at which Rayleigh’s Limit is exceeded.

Figure 1.4: Generation of an electrospray plume of charged solvent droplets. Polarisation of the mobile phase causes the accumulation of ions at the capillary tip leading to the formation of a Taylor cone. The breakdown of the Taylor cone occurs when the applied voltage is sufficiently high.\textsuperscript{61}
Figure 1.5: Photograph of the breakdown of the Taylor cone and resulting plume of charged droplets in an ESI source.\(^{61}\)

The solvent droplet is exposed to two key forces; the surface tension and the Coulombic repulsive forces of the ions. The Rayleigh Limit is the point at which the charge of the droplet is in equilibrium with the surface tension, Equation 1.1.\(^{62}\)

\[ q_R = 8\pi(\varepsilon_0\gamma a^3)^{1/2} \]  

Equation 1.1

Where \( q_R \) is the charge of the droplet, \( \varepsilon_0 \) is the electrical permittivity, \( \gamma \) is the surface tension of the solvent and \( a \) is the radius of the droplet.

Exceeding Rayleigh’s Limit occurs when \( q_R > \gamma \) resulting from the reduction in \( a \). This induces fission of the droplet into smaller progeny droplets, a process called Coulomb fission.\(^{60}\) This process continues until formation of very small droplets, approximately 10 nm in diameter, has been achieved.\(^{63}\)

The mechanism for the generation of single gaseous phase ions is widely debated. There are currently three methods proposed which include the charge residue model (CRM), the ion evaporation model (IEM) and the chain ejection model (CEM). It is believed that the process involves multiple mechanisms and could be a combination of all of the different models. A summary diagram of the models is given in Figure 1.6.
The CRM and IEM are well established and the different mechanistic features have been studied. The CRM, first proposed by Dole et al.,\textsuperscript{55} suggests that the formation of singular charged gaseous ions from the small charged droplets of analyte is as a result of continued Coulomb fissions that eventually lead to the formation of droplets containing a singular analyte ion. Continued solvent evaporation leads to the formation of free gaseous ions. The IEM is an alternative mechanism proposed by Iribarne and Thompson.\textsuperscript{64} They found direct emission of ions from within the droplet occurs once the radius of the droplet falls below 10 nm, ejecting a gaseous analyte ion from the droplet. The formation of a Taylor cone at the surface of misshapen droplets occurs as surface ions repel each other. When the Coulomb repulsion from neighbouring ions exceeds the surface tension of the solvent droplet the charge will be emitted as a singular gaseous ion. The ejection of surface ions will continue in a ‘machine gun’ like manner until the surface tension of the droplet inhibits the process. In 2012 Konnerman and colleagues reported a “mathematically possible method” for the formation of ions from polymer chains that are disordered, partially hydrophobic and capable of binding excess charge carriers, called the CEM. They suggested protein unfolding, triggered by solution-based factors such as pH, leads to the formation of a disordered protein conformer with exposed hydrophobic residues that would immediately migrate to the droplet surface. In the event of one chain terminus being ejected from the droplet then this would be followed by a sequential ejection of

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**Figure 1.6: Summary diagram of the proposed models for the formation of gaseous phase ions in ESI.**

The CRM and IEM are well established and the different mechanistic features have been studied. The CRM, first proposed by Dole \textit{et al.},\textsuperscript{55} suggests that the formation of singular charged gaseous ions from the small charged droplets of analyte is as a result of continued Coulomb fissions that eventually lead to the formation of droplets containing a singular analyte ion. Continued solvent evaporation leads to the formation of free gaseous ions. The IEM is an alternative mechanism proposed by Iribarne and Thompson.\textsuperscript{64} They found direct emission of ions from within the droplet occurs once the radius of the droplet falls below 10 nm, ejecting a gaseous analyte ion from the droplet. The formation of a Taylor cone at the surface of misshapen droplets occurs as surface ions repel each other. When the Coulomb repulsion from neighbouring ions exceeds the surface tension of the solvent droplet the charge will be emitted as a singular gaseous ion. The ejection of surface ions will continue in a ‘machine gun’ like manner until the surface tension of the droplet inhibits the process. In 2012 Konnerman and colleagues reported a “mathematically possible method” for the formation of ions from polymer chains that are disordered, partially hydrophobic and capable of binding excess charge carriers, called the CEM. They suggested protein unfolding, triggered by solution-based factors such as pH, leads to the formation of a disordered protein conformer with exposed hydrophobic residues that would immediately migrate to the droplet surface. In the event of one chain terminus being ejected from the droplet then this would be followed by a sequential ejection of
the protein ion. There is currently little experimental evidence to support the CEM. However, general consensus believes ESI occurs as a combination of multiple processes. The CRM is proposed to be the preferred method to describe the formation of gaseous ions for larger molecules such as proteins and the IEM is believed to be more applicable for smaller ions.

Ion formation in ESI is as a result of the electrochemical and desolvation processes that dictate the accumulation of charge within the electrospray droplets, therefore mono-charged ions, or multiply charged ions for larger molecules such as proteins, are often presented in the mass spectrum. For example, ions typically observed in positive ion mode include \([M+H]^+\), \([M+nH]^n+\), \([M+Cat]^+\), \([M+L+H]^+\) where L is a ligand (primarily for non-covalent complexes). The approach is a ‘soft’ ionisation technique that induces little fragmentation, therefore information regarding solution phase chemistry can be retained. However, target molecules are required to form charged ions primarily through the proton transfer or adduct formation. As a result ESI is more suited to the analysis of polar species. The impact of analyte characteristics on ESI response needs to be considered when interpreting the spectra generated. The ionisation efficiency of target compounds will be determined by the ability to carry a charge, competitive ionisation effects such as gas phase proton affinity and surface activity of the target analyte in the solvent droplet. Differences in these can result in equimolar analytes having different ESI-MS responses when analysed under the same instrumental parameters. The effect of competitive ionisation can be minimized for the analysis of complex mixtures through the use of HPLC separations prior to ESI-MS.

The development of ESI has revolutionised the way in which mass spectrometry is used. As a result, ESI is one of the most commonly used ionisation methods and is applied to a vast array of different analytical challenges for both qualitative and quantitative measurements.

1.2.2 Desorption electrospray ionisation (DESI)

DESI is a spray based ambient ionisation technique that enables direct surface analysis using mass spectrometry. Developed by Cooks and co-workers in 2004 to overcome the inherent problems of many traditional ionisation techniques, such as the necessity for vacuum regions leading to an inaccessible sampling space, DESI
has since become one of the most popular and versatile ambient ionisation methods. DESI describes a unique ionisation process that utilizes the formation of an electrospray to directly desorb and ionise analytes from a surface in the open air resulting in ESI-like mass spectra.

The mechanism by which analyte ions are formed during DESI is something that has been well studied but never fully elucidated. In DESI a sample surface is positioned under an aqueous organic spray, commonly methanol-water, generated using an electrospray. The gaseous ions formed are sampled directly using a mass spectrometer equipped with an atmospheric pressure inlet. A schematic diagram of a DESI source is provided in Figure 1.7.

![Figure 1.7: Schematic diagram of a traditional DESI source](image)

Early theory on the DESI mechanism centred around two contributing techniques; a single-stage droplet pick-up mechanism combined with chemical sputtering. The generation of analyte ions was thought to occur through transfer of a heterogeneous electron, proton or other ions from low kinetic energy solvent clusters impacting the sample surface. Provided enough momentum was present from the electrospray phase it was proposed that the charged analytes could be released from the surface and sampled by the mass spectrometer.

In more recent years, however, subsequent experiments have been carried out to provide a more detailed insight into the mechanism of DESI, and it has since been proposed that the formation of analytic ions involves multistage momentum transfer...
and “droplet splashing” events, discounting the earlier suggestions of a single-stage droplet pick-up mechanism.\textsuperscript{46,47}

Initially “surface wetting” of the sample, in which droplets of electrospray phase approximately 10 μm in diameter and accelerating at > 100 m/s\textsuperscript{69} form a localised thin liquid layer of mobile phase on the sample surface, promoting a solid/liquid extraction process of surface analytes into the solvent film. The extraction process presents itself as a ‘solvation delay’ where a reduced signal is observed for the initial 0.1 min of the DESI analysis of a sample.\textsuperscript{70} Subsequent droplet collisions from the electrospray phase onto the wetted surface lead to the formation analyte-containing secondary droplets in a “splashing” mechanism. The interaction of two liquid phase droplets can be described using 4 different mechanisms; bounce, coalescence, disruption and fragmentation.\textsuperscript{71} A summary diagram is shown in Figure 1.8.\textsuperscript{71} The processes of droplet bounce, where the coalescence of the two droplets is inhibited by a thin layer of air, and stable coalescent, which occurs when the two droplets combine to form a single larger droplet, would not lead to the generation of analyte-containing secondary droplets for mass spectrometric analysis. Therefore, they will not contribute to the DESI mechanism. The temporary coalescence of two droplets followed by the formation of post collision droplets can occur through disruption events, where there is a disproportionate transfer of material between the two droplets, and fragmentation, where the collision event causes the formation of secondary droplets. The contribution of both mechanisms to the DESI process is viable and fit with data generated by Costa and Cooks, who used numerical multiphase fluid dynamic simulations to image the “splashing” process (Figure 1.9)\textsuperscript{72} and detail the formation of secondary droplets from a wetted surface, confirming the process as a momentum transfer event.\textsuperscript{72}
Figure 1.8: Mechanisms for liquid droplet interactions

Figure 1.9: Image of the formation of secondary analyte containing droplets via a splashing mechanism in DESI. The image has been generated using multiphase fluid dynamic simulations to elucidate the DESI mechanism
The ionisation process in DESI occurs primarily through solution based charge transfer events from solvent and/or additive ions present in the electrospray solution to the neutral analyte species. The process of liquid phase ESI-type ionisation has been confirmed for larger molecules, such as multiply charged proteins. This is followed by ESI-like processes involving Coulomb fission and the CRM and/or IEM methods for the formation of gas phase ions, detailed in Section 1.2.1 of this thesis. This is supported by similarities in the internal energies of the analyte ion in both DESI and ESI when measured with the thermometer ion method.\textsuperscript{72,73}

A vapour phase ionisation process has also been proposed by Takats \textit{et. al.} for more volatile species. It was postulated that the difference in spray geometry results in slight mechanistic changes to the ionisation mechanism, leading to the preferential ionisation of certain compound types. Evidence was reported to suggest that the ionisation process was dictated by experimental parameters and the physical and chemical properties of the target analytes resulting in two distinct groupings: ESI type compounds and APCI type compounds (Figure 1.10).\textsuperscript{68} For analytes typically observed using APCI, such as cholesterol and TNT, ionisation in DESI occurs when the potential difference between the sprayer and the surface exceeds 2 kV, leading to gas-phase ion-molecule reactions.\textsuperscript{68}

Figure 1.10: Optimal combination of spray impact angle and spray position for different compounds (glass surface, 10 ng of each compound, 1 µL/min methanol/water; optimization was performed to obtain the best S/N).\textsuperscript{68}
The successful desorption and ionisation of target analytes is a highly complex process, influenced by a wide range of variable factors that are both sample and surface specific. The experimental parameters used in a DESI-MS method can cause the selective ionisation of specific analytes, and impact the sensitivity of the technique. Such factors include: the geometric configuration of the DESI source, spray effects, electrospray solvent composition, substrate effects and analyte chemistry, which will be discussed in further detail below.

Source Geometry

A key feature of ambient ionisation is the ability to analyse a range of surface topographies in the ambient environment. The geometry of the DESI source must therefore be accessible to a wide range of sample shapes whilst enabling optimum ionisation/desorption for target analytes. However, source geometry is known to be one of the main contributing factors to variability in DESI-MS studies, and impacts both the ionisation efficiency and sensitivity of the technique. A schematic of a “traditional” DESI source configuration is shown in Figure 1.11, where the spray impact angle ($\alpha$) is at approximately $55 \, ^\circ$, the collection angle ($\beta$) is $10 \, ^\circ$ and the distances are typically within 2-10 mm.$^{72}$ In the work carried out by Cooks et al. into DESI source optimisation and mechanistic features, it was shown that the spray impact angle will determine the ionisation efficiency and approach$^{68}$ of the technique, while the collection angle will affect the mass spectrometer sampling of the secondary droplets, and therefore the sensitivity.$^{68,74}$

![Figure 1.11: Schematic of a DESI source highlighting parameters that require optimization.](image-url)
A retrofit commercial DESI source has been developed (Prosolia, Indianapolis, USA) that follows the traditional DESI configuration. The source can be interfaced to various mass spectrometers, enabling full sample specific optimisation to be carried out. The secure platform and automated user interface are beneficial for rapid screening analyses following standard protocols. However, dependence upon such designs leaves little room to further push the capabilities of the technique. Many DESI studies are still carried out using sources built in-house that can be optimised to suit the specific nature of the investigation. Several papers have reported modifying the traditional DESI source design to enable different applications. In 2007 Venter et al. designed a “Geometry Independent DESI Source” (Figure 1.12a) featuring a 90 ° incident angle and a 90 ° collection angle with the desorbed area of the sample surface enclosed. The source is described as safer, robust and easier to use, as less optimization experiments are required. Alternatively liquid or solid samples can be deposited onto a mesh positioned in line with both the electrospray and mass spectrometer inlet in a technique called transmission mode DESI (TM-DESI) (Figure 1.12b). In this configuration the spray incident angle is reduced to 0° so that analytes are sampled as the spray passes through the mesh rather than as a result of the splashing mechanism as the electrospray plume is deflected off a solid surface. TM-DESI again reduces the requirement for source geometry optimisation and can enhance sample throughput.

Figure 1.12: a) Image of a “Geometry independent DESI Source” and b) Schematic of an TM-DESI source, both designed to reduce the number of geometric parameters that require optimisation
An area of special interest in the adaption of DESI sources is to enable the non-proximate analysis of surfaces away from the mass spectrometer inlet. This reduces the spatial confinements of the DESI source and can therefore enable the analysis of a greater range of surface areas. In the initial paper on DESI, Cooks reported the use of a flexible ion transfer line for the passage of ions desorbed from the surface into the mass spectrometer,\(^4\) therefore extension of the ion transfer line is a logical progression to expand the applications of DESI. This idea was further promoted with the development of a “DESI wand” in 2005, described as a two part system comprising a miniaturized DESI source and a long ion transfer tube that can enable “free access to a sample surface”\(^6\). In 2006, a paper was published reporting the non-proximate DESI detection of explosives and chemical warfare agents in which a long stainless steel ion transfer tube was used to detect trace quantities of analytes up to 3 meters from the mass spectrometer inlet.\(^7\) The transfer of ions, assisted by the vacuum of the mass spectrometer, was successful for the target analyte and, although a drop in molecular ion signal was noted with the increase in ion transfer length, a corresponding drop in noise ensured good signal to noise ratios (S/N) were maintained. The effects of material temperature and the addition of a reagent gas to ion transfer tubes have also been investigated for a range of target analytes.\(^8\) Comparison of solid stainless steel tubing and flexible conductive silicone tubing showed little variation in sensitivity with silicone tubing having the advantage of easy manipulation for the analysis of different surfaces. Successful ion transfer was achieved at ambient temperature. However, heating above 100 °C promoted fragmentation of some compounds reducing molecular ion intensity.

**Spray Effects**

The general term "spray effects" covers the parameters relating to formation of an electrospray, including capillary voltage, nebuliser gas flows and solvent flow rate. The generation of an electrospray plume of charged solvent droplets using a charged capillary is required for surface wetting, progeny droplet generation and charge transfer. The voltage applied to the electrospray capillary in DESI will determine the charge present on the spray droplets and the potential present between the capillary tip and the counter electrode, having a direct influence upon molecular ion intensity in the mass spectra. An applied voltage in the range of 2-5 kV to the electrospray capillary is typically used in DESI-MS studies, which is similar to ESI. It has been observed that increasing the capillary voltage, to 5 kV, increases ion yield, as more charge is available for the generation of analyte ions (Figure 1.13b).\(^6\) However, above 5 kV little increase in molecular ion intensity is observed, which could be a
result of charge build-up on insulating surfaces, causing instability in molecular ion formation. The flow and pressure of the nebuliser gas will influence both the size and speed of the impacting electrospray droplets. Increasing the nebuliser flow rate will reduce the initial size of the impact droplet, increase impact velocity and improve desolvation, aiding the formation of secondary droplets and therefore improving the efficiency of the ionisation process (Figure 1.13c). If the nebuliser gas flow becomes too high, however, this will result in evaporation of the spray droplets before collision with the sample surface, inhibiting the ionisation process. High nebuliser gas flow rates can also cause damage to some sample surfaces through erosion. The solvent flow rate will impact the size distribution and average charge of droplets which will bring about several effects. At very low solvent flow rates the velocity of the droplets may not be enough to prevent evaporation of the droplets between the spray tip to the sample surface. Increasing the solvent flow rate to an optimum point will increase the wetted area of the sample surface and therefore increase both the quantity of analyte available for desorption and also the efficiency of the desorption/ionisation process. However, if the solvent flow rate exceeds the optimum this may result in excessive accumulation of solvent on the surface, washing of the sample from the surface, surface erosion and a reduction in ionisation, as shown in Figure 1.13d. The nebuliser gas and solvent flow rates will be influenced by the volatility of the electrospray phase solvent composition and target substrate.

![Figure 1.13: Optimisation of DESI parameters using melittin as a model compound investigating the effect of a) incident angle of electrospray (α), b) electrospray voltage, c) nebulizing gas flow and d) solvent flow rate on ion intensity.](image_url)
Electrospray solvent composition

The electrospray solvent composition is a key parameter for the selective DESI-MS analysis of target compounds deposited on the surface. The solvent needs to generate an electrospray plume when passed through the charged capillary, enable extraction of analytes from the surface through solid/liquid extraction processes, and promote the formation of analyte ions through charge transfer events. Common solvents include methanol, water, acetonitrile and mixtures similar to those used in ESI, although the effect of other solvent systems has been investigated. The solubility of target analytes in the electrospray solvent composition will dictate the chemical profile observed in the mass spectra. Insoluble species will not be extracted into the wetted area on the surface, and therefore not be sampled by the DESI process. This can be used to impart a level of selectivity in the DESI method for the targeted analysis of compounds present in complex mixtures. As DESI is an ambient ionisation method for the direct analysis of surfaces there is little scope for sample preparation or pre-separation methods for the analysis of complex mixtures, which can lead to high levels of chemical background in the resulting mass spectra and problems with competitive ionisation effects. Careful selection of the electrospray solvent composition, or the use of additive to target specific analytes, can overcome the inherent problems associated with ambient ionisation methods.

The term “reactive DESI” was first used in 2005 for the direct DESI-MS analysis of explosives from a range of surface materials. Additives such as trifluoroacetic acid, HCl and NaCl were doped into the electrospray phase to form adducts with RDX and HMX explosives to enhance target analyte response and improve analyte desorption. In a similar manner reactants can be added to the electrospray to undergo in-line reactions with the target analyte when present in the secondary droplets generated during the DESI mechanism. These chemical reactions occur at a faster rate in DESI/ESI than when carried out on the bench top due to the heightened physical and chemical environment within the solvent droplet as desolvation occurs. The technique has been widely applied to a range of analytical challenges including the use of oxidising electrosprays for the detection of a specialised polymer, dicationic ion-pairing agents for imaging of biological fatty acids and lipids, modified phenylboronic acids for saccharide analysis, hydroxylamine for the rapid screening of anabolic steroids in urine and the in line monitoring of reactions and their intermediates.
Substrate Effects

The optimization of experimental parameters, such as source geometry and spray effects, is dependent upon the target analyte and substrate. Studies into the effect of surface properties on DESI-MS response have shown that composition, topography and conductivity are the primary factors of interest. The composition of a surface will influence its chemical and physical properties, having an effect on both desorption and ionisation of target analytes. Typical surface materials include PTFE, glass, and filter paper. However, the in situ nature of DESI has resulted in the direct analysis of samples from surfaces including TLC plates, biological tissues and pharmaceutical tablets. Non-conductive materials with little affinity towards target analytes are favourable as they have good “wettability” and promote desorption processes. The “wettability” of a surface, i.e. the energy of the surface, will impact the behaviour of droplets. Surfaces with low surface energy in the open air, such as PTFE, will reduce the non-directional movement of polar solvent droplets focusing the movement towards the counter electrode. The reduced affinity towards the target analyte will encourage dissolution of compounds into the localised solvent droplet. The wetted area of the surface will also be influenced by the surface tension between the electrospray solvent and substrate and the spray parameters. Surface topography, or the roughness of a surface, has been found to affect the splashing process in the DESI mechanism. Rough or etched surfaces can improve DESI signal and reduce carry over resulting from washing of the sample into the mass spectrometer inlet. Finally, the conductivity of the surface material will affect charge transfer and the desorption/ionisation process of the DESI mechanism. The formation of ions relies upon the spray solvent wetting the surface and charged droplets being released, which requires a charge on the surface. Insulating surfaces build up charge quickly following initiation of the spray, which is maintained over a long period of time. The charge distribution on a PTFE surface used in a DESI experiment was measured using a captive probe coupled to a metal-oxide semiconductor field-effect transistor (MOSFET) static charge detection circuit. The experiment found two localised areas of high charge density, one in the desorption region and one in front of the mass spectrometer inlet that could result from the DESI spray and subsequent splashing effects. In contrast, conducting surfaces such as metals are often neutralized and it has been proposed that conducting surfaces may require the application of a voltage for successful desorption of target analytes.
As with all ionisation techniques the chemical and physical properties of target analytes needs to be considered when developing suitable methods for analysis. DESI-MS is routinely applied to the analysis of small to large polar molecules present as standards and mixtures, either deposited onto a sample surface or present in situ. Many of the factors impacting the successful desorption and ionisation processes in DESI have already been discussed. However, it is also necessary to consider the properties of the target compounds. The surface activity of compounds, either to the target substrate or at the liquid/air interface, the solubility and dissolution rate of compounds from the surface into the wetted area, and the presence of matrix ions and competitive ionisation events will impact the desorption and ionisation efficiency of analytes. Compounds with a high dissolution rate and preferentially present at the liquid/air interface of the solvent droplet are more likely to be present in progeny droplets following splashing events compared to those with high surface activity situated at the solid/liquid interface. This will subsequently influence the relative abundance of observed ions in the resulting mass spectra.

A summary schematic diagram of the solvent, substrate and analyte chemistry effects on DESI-MS response is shown in Figure 1.14:

![Figure 1.14: Summary schematic diagram of solvent substrate and analyte chemistry effects on DESI-MS response](image-url)
DESI-MS is a rapid and sensitive method for the analysis of compounds present on a surface that has the capability of generating, *in situ* molecular data. The approach is described as a “soft” ionisation technique generating ESI-like mass spectra with little fragmentation. The technique has been applied to a wide range of analytical areas that has been summarised in Table 1.4. The application of DESI as a rapid screening tool for the detection of explosives and the analysis pharmaceuticals has been advanced through the development of commercial sources and techniques such as TM-DESI, enabling the ambient ionisation method to become a more common analytical tool. As our understanding of DESI mechanism has increased, so has the potential applications of the technique and the diversity of molecular compounds successfully desorbed and ionised. Molecular species up to 66 kDa have been analysed by DESI-MS showing potential for proteomic studies. Although still primarily considered a qualitative technique, many groups have explored the quantitative capabilities of the method for the analysis of additives, foodstuffs, aerosols, drug screening and biological samples. The results are often described as semi-quantitative in nature due to the larger variability observed with DESI compared to ESI, resulting from uneven sample deposition, influence of the ambient environment and sample movement under the electrospray. However, the reports depict limit of detection (LOD’s) in the pg range, % relative standard deviations (% RSD’s) > 20 % and R² values < 0.99 which are consistent with ESI generated data. For example, Talaty and co-workers analysed alkaloids by ESI and DESI to compare the precision on the methods and found the % RSD values to be 9.8 % and 5.2 % respectively. Similarly the analysis of pesticides from food stuffs by DESI and ESI showed comparable LOD’s for several compounds. To improve the quantitative abilities of DESI investigations into the use of internal standards has been carried out. The presence of an internal standard can greatly influence the performance capabilities of quantitative DESI, demonstrated by Cooks and co-workers in the quantitative analysis of small drug molecules. A suitable internal standard must be soluble in both the spotting solution and the spray solution and have similar proton affinities to the target analyte. Two different internal standards, atenolol and propranolol-d₇, were used for the analysis of propranolol and the linearity and reproducibility were assessed to demonstrate this. The results showed the atenolol internal standard produced R² values of 0.82 for the calibration and there was poor reproducibility (RSD ≤ 35 %). In contrast R² > 0.99 and RSD ≤ 15 % values were achieved with the use of the deuterated internal standard.
One area of continuous growth and development is the application of DESI to the in situ imaging studies of compounds on surfaces. Traditional imaging techniques include microscopy, histology, MALDI and secondary ion mass spectrometry (SIMS). Observation methods, such as optical microscopy, are still used for clinical diagnoses but can be time consuming and rely upon human interpretation. Mass spectrometric imaging techniques have either used a matrix (MALDI) for sample ionisation or have been carried out in a vacuum (SIMS), both of which will cause damage to biological or other samples and can generate complex mass spectra for data interpretation. DESI imaging uses a pixelated method of sampling a surface area, in which a sample spot is analysed before movement of the surface under the electrospray probe to build up the chemical profile. The advantage of DESI is the ambient nature of the ionisation method, enabling the analysis of samples within the atmospheric environment with no sample preparation or modification. The disadvantage of the method is the analytical spot size, which relates to the resolution of the DESI image. A typical analytical spot is for imaging studies in DESI is 100-250 μm in diameter, which is larger than MALDI (>25 μm) and SIMS (100 nm). The technique has, however, been applied successfully to the analysis of many biological samples such as human prostate cancer tissue, adrenal glands, spinal cord lipids following injury, fingerprint analysis.

The key advantage of DESI, the direct and rapid analysis of samples on a surface, can also be one its biggest limitations when applied to the analysis of complex mixtures and matrices. The absence of sample preparation methods, such as the pre-concentration of analytes or chromatographic separations, can cause problems with ion suppression and ion identification. Many different approaches to overcome this have been investigated over the years and have shown differing levels of success. Early reports show the hyphenation of DESI-MS with TLC, where separation of mixtures was carried out using TLC before the plate was directly analysed to generate molecular data. This has been followed by the hyphenation of DESI-MS with LC, however both approaches introduce a level of sample preparation that can be time consuming and can destroy any data on localisation of the sample on the native surface substrate. Alternative approaches include the use of high resolution mass spectrometry, such as the Orbitrap instrument and FT-ICR-MS, or the application separation methods such as IMS and FAIMS, to separate ions in the gaseous phase. High-resolution mass analysers can generate highly detailed mass accurate information of complex samples without the need for extensive sample preparation, which can increase confidence in molecular ion identification.
This approach has been shown for the analysis of peptides, proteins, drugs and polymers.$^{108,109,110}$ Post ionisation separation techniques are easily hyphenated with ambient ionisation methods such as DESI and can rapidly enhance target analyte response by filtering out chemical background. The use of DESI-IM-MS has been applied to the analysis of a range of compound including chemical warfare agents,$^{111}$ pharmaceutics and$^{86,87}$ peptides$^{112}$ and DESI-FAIMS-MS to the analysis of counterfeit pharmaceuticals and the imaging of biological tissues.$^{113,114}$ The combined technique can be used to improve both selectivity and sensitivity for targeted analyses and provide an additional identifiable parameter in drift time, collisional cross section (CCS) or compensation/dispersion voltages.

1.2.3 Direct analysis in real time (DART)

In contrast to DESI, which is a solvent/spray based ionisation method, DART can carry out the instantaneous ionisation of solids, liquids and gases through chemical based ionisation processes under ambient conditions. Introduced in 2005 by Cody et al.$^{115}$ and commercialised by JEOL (Tokyo, Japan) and IonSense (Massachusetts US), DART uses a heated gas flow of metastable atoms, predominantly He, generated using a plasma discharge to desorb and ionise target analytes directly from a surface.

The DART source is fully enclosed and is comprised of an ionisation compartment containing a needle electrode held at a few kilovolts relative to a grounded perforated disk electrode, a second chamber containing another perforated electrode, followed by an exit grid electrode and insulator cap. A schematic diagram of the ionisation source is shown in Figure 1.15.$^{116}$ In the source a helium or nitrogen gas flow is directed through the ionisation compartment, where it is introduced to a plasma of electrons generated as a result of the high-voltage potential between the needle electrode and grounded perforated disk electrode.$^{58}$ The corona discharge generates a cold plasma of ions, electrons and excited atoms (metastable species). Continuation of the plasma through the secondary lens electrodes acts to heat the gas stream and remove the charged ions and electrons using a repulsive force, to leave a stream of metastable species that can be directed towards a target surface to desorb and ionise analytes. The mass spectrometer inlet is commonly fitted with an additional pumping stage, a Vapur interface, that counteracts the flow of He towards the inlet and maintains the internal vacuum of the mass spectrometer.$^{116}$
In DART, analytes undergo gas phase ionisation, similar to APCI and APPI. Target analytes present in the liquid or solid phases are therefore required to be converted into the gas phase prior to ionisation events. Desorption of target analytes from the surface is facilitated through both thermal desorption, as a result of the heated gas flow, and by energy transfer from the metastable atoms and molecules to the surface. Several different ionisation mechanisms have been proposed in DART that are dependent upon the type of carrier gas used, analyte concentration, polarity and ionisation efficiency. The dominant mechanism is believed to occur through a combination of Penning ionisation and secondary ionisation events from atmospheric ions generated by the flow of the metastable gaseous species. If we first examine the positive ion mode, the simplest explanation involves the transfer of energy from the excited metastable atoms ($G^*$) to the target analyte ($M$), leading to radical cation formation:

$$M + G^* \rightarrow M^{+} + G + e^- $$  \hspace{1cm} \text{Equation 1.2}

The ability to transfer energy from the metastable species to the target analyte will depend upon the ionisation potential of both compounds. Nobel gases are predominantly used in DART due to efficiency at which they can enter an electronically excited state when exposed to a corona discharge. The energy stored in Nobel gases decreases from He*>Ne*>Ar*>Kr*, therefore He is the most widely used DART gas. The ionisation potential of an excited state He atom is 19.8 eV, due to the $2^3S$ excited electron configuration, which is above the ionisation energy of any potentially relevant molecule. However, typically upon leaving the DART source excited state He atoms will interact with nitrogen, oxygen and water present in the source.
surrounding environment causing a process of initial ionisation that results in the formation of analyte ions through secondary ionisation events. This is summarised below, where A denotes atmospheric O, N or H₂O molecules:

\[
\text{He}^* + A \rightarrow \text{He} + A'' + e^-
\]  

Equation 1.3

This reduces the probability of direct Penning ionisation of molecules, but causes ionisation through a process of charge transfer events in which the ionisation and excitation of N₂ leads to the formation of protonated water clusters that can act as reagent ions for analyte ion generation through chemical ionisation. An example of this pathway is given below:\(^\text{116}\)

\[
\begin{align*}
\text{He}^* + \text{N}_2 & \rightarrow \text{He} + \text{N}_2'' + e^- \\
\text{N}_4'' + \text{N}_2 & \rightarrow \text{N}_4'' \\
\text{N}_4'' + \text{H}_2\text{O} & \rightarrow 2\text{N}_2 + \text{H}_2\text{O}'' \\
\text{H}_2\text{O}'' + \text{H}_2\text{O} & \rightarrow \text{H}_3\text{O}'' + \text{OH}^- \\
\text{H}_3\text{O}'' + n\text{H}_2\text{O} & \rightarrow [(\text{H}_2\text{O})_n + \text{H}]^+ \\
\text{M} + [(\text{H}_2\text{O})_n + \text{H}]^+ & \rightarrow [\text{M} + \text{H}]^+ + n\text{H}_2\text{O}
\end{align*}
\]

Equation 1.4

Alternatively direct charge transfer from N₄'', O₂'', and NO⁺ species to analyte molecules can occur:

\[
\begin{align*}
\text{N}_4'' + \text{M} & \rightarrow 2\text{N}_2 + \text{M}'' \\
\text{O}_2'' + \text{M} & \rightarrow \text{O}_2 + \text{M}'' \\
\text{NO}^+ + \text{M} & \rightarrow \text{NO} + \text{M}''
\end{align*}
\]

Equation 1.5

Equation 1.6

Equation 1.7

In the negative ion mode, the formation of analyte ions occurs in several stages. Initially the formation of high energy electrons will be generated through Penning ionisation reactions of the metastable gas (G*) with the surface material. The electrons are released into the surrounding environment where they collide with atmospheric pressure gas to form thermal electrons (e^-\text{\text{slow}}). The thermal electrons undergo electron capture by atmospheric oxygen to form O₂-. The O₂- will react with target analytes to produce anions:\(^\text{115}\)
The chemical properties of the analyte can also enable a range of different reaction for the formation of analyte ions, including:

\[
\begin{align*}
G^- + \text{surface} & \rightarrow G + e^- \\
e_{\text{fast}}^- + G & \rightarrow e^-_{\text{slow}} \\
e^-_{\text{slow}} + O_2 & \rightarrow O_2^- \\
O_2^- + M & \rightarrow [M + O_2]^- \\
[M + O_2]^- & \rightarrow M^- + O_2
\end{align*}
\]

Equation 1.8

The successful ionisation of target compounds is dependent upon their ionisation efficiency, for example in the positive ion mode target analytes need to have a greater proton affinity than the metastable gas species or the protonated water clusters, and their volatility. The desorption of analytes is primarily a thermal process therefore suitable compounds are required to have a degree of volatility as well as thermal stability.\(^{91}\) DART is typically limited to an upper mass range of 800-1000 Da and does not generate multiply charged species but is capable of the ionisation of both polar and non-polar target analytes.\(^{48}\) The presence of background chemicals in the surrounding environment, such as ammonium or chloride, can lead to the formation of adduct ions which can be used for selective ionisation of target analytes and enhancement in labile analyte response through solvent doping.\(^{117,118}\) Fragmentation events are common in DART primarily resulting from thermal degradation of analytes in the desorption process, in-source collision events and labile ion dissociation due to high internal energies.\(^{119,120}\) The DART-MS spectra have similarities to APCI, due to the Penning ionisation process. A summary of the different ions typically observed in DART-MS is given in Table 1.8.\(^{118}\)
Table 1.8: Analyte ions frequently observed in DART-MS

<table>
<thead>
<tr>
<th>Analyte polarity</th>
<th>Positive ions</th>
<th>Negative ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium polar to polar</td>
<td>[M+H]⁺, [M+O+H]⁺, [M+NH₄]⁺ (other adducts possible when counter ions are present)</td>
<td>[M-H]⁻, [M-OH]⁻, [M+CN]⁻, [M+Cl]⁻ (other adducts possible when counter ions are present)</td>
</tr>
</tbody>
</table>

Optimisation experiments in DART primarily involve adjustments to the gas type, temperature, flow rate, and source geometry. The gas type, typically He but N₂ is becoming more common, will determine the ionisation efficiency of the technique as described above. The temperature of the gas will impact both the desorption efficiency of the method and the extent of analyte ions fragmentation observed. A typical range for DART gas temperature is from room temperature to 550 °C. However, it is important to note that the set temperature of the metastable gas stream is not necessarily the temperature of the metastable species interacting with the sample surface. Heat dissipation and sample orientation/proximity have been shown to affect energy transfer rates to exposed analytes. Increasing the flow of the heated metastable gas can reduce the dissipation effects, however this can result in an increased pressure within the source region that can result in fragmentation of analyte ions. In commercial devices the gas flow rate is dependent upon the set temperature and cannot be controlled as an independent parameter.

A DART source can be operated in two geometric configurations; surface desorption mode or transmission mode. In surface desorption mode the DART source is positioned at an angle (typically 45°) so the flow of metastable gas is directed towards the sample located just below the mass spectrometer inlet orifice. In this mode the system can be used to analyse a wide range of solid, liquid and gaseous samples. While the positioning of the sample is important for the sensitivity of the technique, the sample surface material and topography do not have a large effect due to the thermal desorption of analyte ions prior to ionisation. Alternatively, transmission mode DART (TM-DART) is becoming more popular because it removes variability in sample positioning within the source enabling a more rapid and reproducible analysis. In TM-DART the source is positioned on-axis to the mass spectrometer inlet, the sample is deposited onto a fine mesh and placed between the
path of metastable atoms and the mass spectrometer (Figure 1.16). The desorbed sample directly interacts with the metastable gas flow and passes into the mass spectrometer. Perez and co-workers first reported the technique in 2010 for the direct analysis of insecticide treated malaria nets. Progression of TM-DART has seen the development of commercial sample cards and automated sample movement for rapid screening studies. Although this technique cannot be used for the direct analysis of solid samples, such as pharmaceutical pills or bank notes, it provides an easy alternative for the rapid analysis of liquid samples, such as blood serum.

**Figure 1.16: Schematic diagram of a transmission mode DART configuration**

The ability to analyse a wide range of sample states expands the potential applications of DART-MS. Typical applications of DART-MS include the trace detection of illicit drugs on a range of surface materials including fabrics and banknotes, screening for explosives and the direct analysis of biological samples. The technique has also been applied to the direct analysis of gas phase samples such as aerosols, human breath and monitoring gas-surface reactions. The capability for the direct analysis of liquid and gaseous samples has enabled the hyphenation of DART-MS with separation methods such as TLC, GC and LC to reduce matrix effects and improve sensitivity. In the case of coupling DART with LC-MS an additional advantage is the ability to use non-MS compatible eluents, such as phosphate buffers, for target analyte separation without ion suppression or source contamination. More recently DART has been coupled with IMS for the analysis of acetaminophen tablet and the analysis of α-tocopherol directly from almond surface to improve spectral quality and increase S/N. The quantitative capability of the technique has been investigated for forensic, food and...
environmental samples.\textsuperscript{135–138} The quantitative DART-MS analysis of cholesterol in egg pasta showed a linear dynamic range of 5-1500 mg/L with $R^2>0.99$, repeatability measurements between 1-8 \% RSD (n=3), an LOD of 0.03 mg/g and an LOQ of 0.05 mg/g.\textsuperscript{136}

DART and DESI have widely been considered complementary ambient ionisation techniques for the analysis of polar (DESI) and less polar species (DART). The alternative desorption and ionisation mechanisms enable the two methods to be used for the analysis of a wide range of target analytes. Studies using both DART and DESI have been carried out for the detection of dithiocarbamate fungicides in fruit,\textsuperscript{139} analysis of insecticide treated nets for malaria control\textsuperscript{123} and hyphenation experiments with a fieldable mass spectrometer\textsuperscript{140} targeting drugs, foods and explosives. The results show that when used in conjunction the techniques have the ability to expand the analytical capabilities of direct ambient ionisation methods.
1.3 Ion mobility spectrometry (IMS)

Separation methods, such as gas and liquid chromatography, have been established as the gold standard for the mass spectrometric analysis of complex mixtures. Separation of compounds based upon their polarity and affinity to a stationary phase can improve mass spectrometric response through reduction in chemical background, minimising ion suppression effects, enhancing resolution and providing an additional identification parameter.\(^5\) However, chromatographic methods can often be time consuming, as separations can take anything from a few minutes (LC) to hours (GC), and in the case of LC, it can be difficult to ensure compatibility of the mobile phase with mass spectrometry while maintaining a good degree of separation. Furthermore, it is not always possible to hyphenate these chromatographic techniques with more novel ambient ionisation approaches. The alternative is the separation of gaseous phase ions after the ionisation event using IMS.

1.3.1 Introduction to IMS

The term IMS, originally referred to as plasma chromatography, refers to the principals and mechanistic approach of characterising compounds based upon the velocity of the gas phase ions derived from a substance when present in buffer gas and exposed to an electric field.\(^4\) The underlining principals describing the movement of gas phase ions under an electric field has been known since the 1950’s through work carried out by Mason and Schamp.\(^5\) This is the foundation to our current understanding of the technique. Commercialisation of IMS has led to the development of both stand-alone devices and those hyphenated with mass spectrometry. The application of IMS is now routinely used in homeland security for the rapid detection of explosives, narcotics and chemical warfare agents. Hyphenation of IMS with ionisation sources such as ESI has also expanded the separation technique to the analysis of less volatile samples that can be present in the solid or liquid phase.\(^6\) Progression of IMS has resulted in two distinct fields; low-field mobility techniques such as drift tube IMS (DT-IMS) and triwave ion mobility spectrometry (TWIMS), and high-field methods such as differential mobility spectrometry (DMS) or high-field asymmetric waveform ion mobility spectrometry (FAIMS), where modifications to the electric field strength have given arise to alternative methods of ion separation. The mechanistic features and potential applications for each method will be discussed.
1.3.2 Low-field ion mobility spectrometry

Low-field ion mobility spectrometry has advanced directly from the work carried out by Mason and others, including Schamp, Viehland and McDaniel. Their contributions to the study of gaseous ion mobility and collision theories when ions are exposed to electric fields under a controlled atmospheric environment, both theoretically and experimentally, resulted in the development of the first IMS systems. The use of IMS has been growing in analytical laboratories since the late 1990’s. Advances in engineering and instrumental development has led to the production of smaller IMS systems that are either bench top or portable in size and the incorporation of IMS into mass spectrometers has enabled simultaneous mobility and mass measurements of molecules to be carried out.

Drift-tube IMS

The use of a drift tube configuration for the IMS separation of ions gave rise to the mechanistic understanding of IMS theory and is still applied to many analytical problems. In general, the system acts to measure the time taken for an ion to pass through a cell of known length when exposed to an electric field gradient in the presence of a buffer gas. The rate at which an ion passes through the drift region will be determined by its CCS. A schematic diagram of a drift-tube IMS cell is provided in Figure 1.17.

![Schematic diagram of a drift-tube IMS cell](image)

Figure 1.17: Schematic diagram of an linear IMS drift tube
The IMS cell is comprised of three regions, the ionisation zone, the drift tube and the detector, which are separated using electronic shutter and aperture grids. Ionisation sources for stand-alone systems and early commercial devices have predominantly used $^{63}$Ni to generate gas phase analyte ions. However, advances in IMS and hyphenation with MS has led to ESI and other ionisation sources becoming more common. The function of the shutter grid is to prevent ions passing from the ionisation source into the drift region until a ‘packet’ of ions can be introduced in a single step. This is achieved by applying a potential to the shutter grid to prevent the passage of ions until required. When the potential on the shutter grid is turned off a discrete package of ions can pass into the drift cell.

The drift cell is usually 4-20 cm in length and has a series of stacked ion ring guides encapsulating the ion path that have an applied electric field gradient ($E$). The applied gradient is typically 10-200 V/cm depending on the buffer gas pressure and causes the directional movement of ions through the drift region towards the detector. The drift cell is held either at atmospheric pressure or at a reduced pressure (typically 1-5 mbar) and has an opposing flow of buffer gas (N$_2$, He, CO$_2$). The forward momentum of ions generated by the electric field will be inhibited due to collisions with the drift gas molecules resulting in a directional diffusion of ions through the drift tube. The number of interactions of an analyte ion with the drift gas is dependent upon the CCS of the ion. The velocity of an ion through the drift tube ($v$) is therefore a result of the gas phase mobility of the ion ($K$) under the applied electric field ($E$), Equation 1.13:

$$v = KE$$  

Equation 1.13

Under low electric field conditions the velocity of an ions is directly proportional to the electric field and is dictated by a proportionality constant ($A$). The IMS separation of ions occurs as a result of variation in their drift times, which is directly related to ion velocity and is therefore a function of ion mobility. The mobility of an ion when present at atmospheric or reduced pressure and exposed to an electric field and buffer gas is dependent upon the reduced mass ($\mu$), charge ($q$) and collisional cross-section ($\Omega_D$) of a analyte ion. This can be expressed using the Mason-Schamp equation, Equation 1.14:

$$K = \left(\frac{3q}{16N}\right)\left(\frac{2\pi}{\mu kT}\right)^{0.5} \frac{1}{\Omega_D}$$  

Equation 1.14
Where $K$ is the ion mobility ($\text{cm}^2\text{v}^{-1}\text{s}^{-1}$), $N$ is the number density of the drift gas, $q$ is the ion charge, $\mu$ is the reduced mass of an ion which refers to the collisional mass of two bodies i.e. the ion and the drift gas, $k$ is the Boltzmann constant, $T$ is the ion temperature ($K$) and $\Omega_D$ is the collisional cross section of the ion clusters.

Several experimental parameters need to be considered with regard to their effect on the mobility of an ion including the effect of buffer gas composition, temperature and pressure. The reduced mobility of an ion ($K_0$) normalises the data for temperature and pressure, enabling the generation of comparative data from different IMS systems.

$$K_0 = \left(\frac{d}{tE}\right)\left(\frac{p}{760}\right)\left(\frac{273}{T}\right)$$

Equation 1.15

Where $d$ is the length of the drift region (cm), $t$ is the drift time of an ions (s), $E$ is the electric field gradient, $p$ is the pressure (torr) and $T$ is the temperature (K).

Under constant temperature and pressure the mobility of an ion is primarily dictated by the CCS ($\Omega_D$) and ion charge ($q$). This enables structural elucidation measurements to be conducted and the mobility separation of isobaric ions.

Following IMS separation the ions pass through the aperture grid and enter the detection zone. For standalone devices this is usually a Faraday plate. However, hyphenation of IMS with MS, typically ToF or quadrupole mass analyses, results in a mass separation of the mobility separated ions prior to detection, which can provide an additional level for the analytical interrogation of samples.

**Travelling Wave IMS (TWIMS)**

In TWIMS, the separation of ions is still dependent upon the mobility of an ion ($K$) when exposed to an electric field gradient and a buffer gas. However, unlike DT-IMS, which applies a continuous electric field gradient over the entire length of the drift cell, TWIMS uses a series of stacked ring ion guides to generate a continuous sequence of symmetric potential waves that propel ions through the ion mobility region.\textsuperscript{148} TWIMS was developed by the mass spectrometric company Waters and released as part of an IM-MS hyphenated system in 2004.\textsuperscript{149} The RF-only stacked ring ion guide used in TWIMS consists of a series of ring electrodes through which
the ion beam is passed (Figure 1.18a).\textsuperscript{149} Opposing phases of an RF voltage are applied to adjacent electrode rings to create an ‘ion pipe’. The ion pipe describes the potential distribution across the stacked ring ion guide where an area of low potential gradient is observed through the centre that rises steeply near the walls. In the z-direction (along the length of the stacked ring ion guide) the ion pipe has a series of undulations that can effectively trap ions and impede forward progress through the device (Figure 1.18b).\textsuperscript{149} Propagation of ions is achieved using a super-imposed DC voltage on the RF electrode that is held for a defined period of time before being sequentially applied to the adjacent electrode, continuing along the length of the TWIMS cell. This functions to generate a moving electric field termed a ‘wave’ on which ions present in the gas phase ‘surf’ (Figure 1.18c).\textsuperscript{149}
Figure 1.18: The mechanism of the travelling wave in TWIMS showing a) a schematic of the stacked ring ion guides, b) a side-on view of the ion pipe highlighting the increased potential near the outer walls of the device and the presence of undulations along the length of the TWIMS cell and c) movement of the electric wave using a superimposed DC voltage to propel ions.  

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Separation of ions based upon mobility is achieved using a TWIMS drift cell containing a buffer gas. The presence of buffer gas molecules within the stacked ring ion guides leads to collisions with ions ‘surfing’ the electric wave. The lower the mobility of an ion, i.e. the larger the CCS, the increased number of collision between buffer gas molecules will occur causing the ion to roll over the electric wave and into the undulation behind (Figure 1.19). This increases the time taken for the ion to pass through the stacked ring ion guides and hence enables mobility separation.

![Figure 1.19: Schematic diagram for IMS separation in TWIMS.](image)

The rate at which an ion passes through the TWIMS cell is not only determined by the CCS, temperature and pressure, as shown in DT-IMS, but also the wave height (V) and velocity (m/s). An advantage of the TWIMS based IMS method compared to DT-IMS is that the sensitivity of the mass spectrometer is not comprised by the duty cycle of the IMS.

The commercialisation of the TWIMS device in the Waters Synapt HDMS instrument, a Q-TWIMS-TOF, resulted in a rapid increase in IMS availability. The instrument has a range of different atmospheric ionisation sources, such as ESI, nano-ESI, APPI
and MALDI that can be used for the generation of gaseous phase ions and enable hyphenation with liquid chromatography. Hyphenation of TWIMS with MS will be discussed in more detail in Section 1.4.4.

The separation of ions in TWIMS-MS has been used to enhance analytical resolution, for example the separation of isobaric species\textsuperscript{150} and complex biological samples.\textsuperscript{151} The collision-based separation mechanism of TWIMS and DT-IMS, shows enhanced resolution for larger ions, such as proteins, resulting from an increase in the effect of conformational changes on CCS. As a result TWIMS-MS has been widely applied to the area of proteomics, metabolomics and lipidomics, and to study conformational changes of ions present in biological processes. Calculation of CCS in TWIMS requires the use of calibration standards to account for the additional kinetic energy experienced by the ions as a result of the electrical wave. Such compounds include tetralkylammonium halides (TAAHs)\textsuperscript{152} and peptides.\textsuperscript{153} Although this increases the experimental stages for CCS measurements in TWIMS, the calculated CCS measurements show reasonable levels of accuracy for compound identification and structural elucidation.\textsuperscript{154–157}

1.3.3 Field asymmetric waveform ion mobility spectrometry (FAIMS)

High-field separation of gas phase ions is typically termed DMS or FAIMS, but has also been called ion drift non-linearity spectrometry, radio-frequency ion mobility spectrometry and field ion spectrometry in the past.\textsuperscript{158} The technique is based upon the fundamental work carried out by Buryakov and colleagues, first published in English in 1993.\textsuperscript{159} In FAIMS, ions are passed between two electrodes, which are planar electrodes or concentric cylinders, in the presence of a carrier gas (typically N\textsubscript{2}) under atmospheric pressure, where they are exposed to an RF asymmetric high field waveform. Separation of ions occurs as a result of differences in the ions mobility under the high and low field proportion of the applied waveform.

In the presence of a low electric field gradient, as used in DT-IMS and TWIMS, the mobility of a gaseous phase ion, $K$, is described as a compound-dependant proportionality constant that is independent of the electric field strength. However this functionality of an ion only holds true at low field strengths, where the electric field strength to buffer gas density ratio ($E/N$) is less than or equal to approximately 2 Townsend (Td), where 1 Td = $10^{-17}$ V cm$^2$. When exposed to higher field strengths (300 Td) the mobility of an ion will become dependent upon the field strength as a function of $E/N$. Under these conditions the mobility of an ion can be expressed as\textsuperscript{159}:
Equation 1.16

\[ K_h(E) = K_0[1 + f(E)] \]

Where \( K_0 \) is the mobility of an ion at high field, \( K_0 \) is the mobility of an ion at zero field, \( E \) is the electric field strength and \( f(E) \) describes the ion mobility as a function of \( E \).

Equation 1.16 describes changes in ion mobility at high electric field strengths and accounts for the three types of ion behaviour observed, type A, B and C. Figure 1.20\textsuperscript{160} provides a graphical representation of changes in ion behaviour when exposed to increasing electric field strength.

![Figure 1.20: Hypothetical dependence on ion mobility when exposed to increasing electric field strength for three different types of ions.\textsuperscript{160}](image)

Type A ion behaviour shows an increase in ion mobility with increasing field strength while type C ions show a decrease in mobility as the field strength increases. The behaviour of type B ions is more complex, exhibiting an initial increase in mobility followed by a decrease as the field strength is raised further. Ion behaviour is not an inherent functionality of a specific ion, but is believed to be dependent on interaction of the ion structure, functionality, collisional-cross section and instrumental parameters.\textsuperscript{158} This ion behaviour is described using an alpha coefficient (\( \alpha \)). When subjected to a high electric field under constant pressure the mobility of an ion can be described using equation 1.17\textsuperscript{141}:

\[ K_h(E) = K_0[1 + \alpha \left( \frac{E}{N} \right)] \]

Equation 1.17
Where $K_h$ is the mobility of an ion at high field, $K_0$ is the mobility of an ion at zero field, $E/N$ is the electric field strength and $\alpha$ is the high field mobility coefficient of an ion.

Separation in FAIMS utilises the changes in $K_h$ when ions are exposed to alternating high and low electric field strengths to separate ions in space. Although different FAIMS configurations have been reported, the mechanistic principals for ion separation remain constant. In planar FAIMS, an asymmetric waveform, called the dispersion voltage (DV) of dispersion field (DF), is applied to one or both of two planar electrodes (Figure 1.21).\textsuperscript{160} The waveform consists of a high-field portion of duration $t$ followed by a low field portion of opposing polarity for $t_1$ such that\textsuperscript{158}:

\[ (E)_h t + (E)_1 t_1 = 0 \quad \text{Equation 1.18} \]

Where $(E)_h$ is the high field portion and $(E)_1$ represents the low field portion of opposing polarity.

Differing ratios of $(E)_h t$ and $(E)_1 t_1$ have been tested, although most systems report the use of a 2:1 ratio as an optimum DF.\textsuperscript{159,160} Ions enter the FAIMS device using a carrier gas where they are subjected to the DF. Displacement of an ion's trajectory through the FAIMS device will be dependent upon the relationship between $K_h$ and $K$ and can be described by equations 1.19 and 1.20.

\[ d_h = K_h (E)_h t \quad \text{Equation 1.19} \]

\[ d_1 = K (E)_1 t_1 \quad \text{Equation 1.20} \]

Where $d_h$ and $d_1$ are the drift distances of an ion in the high field and low field respectively, $K_h$ and $K$ are an ion's mobility under high and low field strengths, $(E)_h$ describes the high field electric field strength and $(E)_1$ is a measure of the low field electric field strength and $t$ and $t_1$ refers to the time spent in both the high field and low field portion.

The influence of the DF on ion trajectory is demonstrated in Figure 1.21. If $K_h \neq K$ the waveform will cause a net migration of the ion to one of the metallic plates, where
it will become neutralised and not progress through the device towards the detector. As the relationship between $K_h/K$ is not only influenced by $(E/N)$, but also the $\alpha$ coefficient, different ions will have different $d_h$ and $d_1$ values. Transmission of ion through the device is achieved using a superimposed DC voltage termed the compensation voltage (CV) or compensation field (CF) that corrects for the drift in ion trajectory as a result of the DF. The correct CF is dependent upon the DF applied, temperature, pressure, gas flow rate and analyte concentration.\textsuperscript{161} If the assumption that different ions will exhibit different $K_h/K$ relationships then the CV required for ion transmission should be compound dependant, thus enabling the selective transmission of target ions through the device.

\textbf{Figure 1.21: Schematic diagram of ion motion in a FAIMS device as a function of the carrier gas flow and the applied asymmetric waveform.}\textsuperscript{160}

There are several mechanistic theories used to describe changes in ion mobility under high and low electric field strength that include clustering and declustering processes, conformation switching and dipole alignment. The presence of small neutral molecules in the carrier gas, such as water, as a result of the atmospheric environment or solvent doping, can form weak non-covalent associations with gas phase ions under low electric field strengths, $(E)_1$. These interactions will cause clustering of the neutral molecules with the ion, increasing the both the CCS and the ion mass. When exposed to the high electric field $(E)_h$ an increase in ion temperature will result in the declustering of the neutral molecules, reducing the CCS and mass, increasing ion velocity and resulting in an enhanced mobility (type A ions)
as shown in Figure 1.20. This effect was first observed for the analysis of organophosphorous compounds when exposed to different concentrations of moisture in the carrier gas. In more recent studies the clustering declustering process has been studied using tetraalkylammonium halide ions and DNA adducts. The clustering and declustering process can be used advantageously to influence the mobility of ions when exposed to the asymmetric waveform through the introduction of polar dopant species, typically solvents. The addition of 2-propanol (at 1.5 %) to the N₂ carrier gas for the analysis of compound mixture containing 70 different analyte species was found to influence the ion clustering behaviour in both a field dependant manner and in relation to α, resulting in an overall increase in peak capacity (Figure 1.22). This method has been applied to the analysis or a range of analytes including explosives, proteins, isomeric species and phytohormones.

Figure 1.22: Separation of a 70 compound mixture using a) N₂ transport gas and b) N₂ + 1.5% 2-propanol transport gas.

Conformational changes in ion structure and dipole alignment can also impact $K_h/K$. Conformational changes can occur as a result of ion heating when exposed to the
high electric field strength. This effect is the predominant process observed for proteins and peptides where unfolding of the structure can increase the CCS for an ion, resulting in a reduced mobility as the electric field strength increases (type C ions) as shown in Figure 1.20. The alignment of dipoles under the different field strengths can causes changes in directional CCS. Work carried out by Shvartsburg suggests that the orientation of an ions permanent dipole is fixed when exposed to \((E)_{i}\), but is able to rotate freely in the gas phase when intermediate or low electric fields are applied to the device. This causes a shift in the directional CCS of the ion and can result in changes in mobility having a major effect on FAIMS separation parameters. However, the effect is only likely to be observed in molecules exceeding 30000 Da.\(^{170}\)

Commercial FAIMS devices include both planar (Sionex, Owlstone) and cylindrical (Thermo) configurations. All FAIMS experimentation presented in this thesis was carried out using the Owlstone miniaturised chip-based FAIMS device. The chip-based FAIMS consists of multiple planar electrode channels, each with a 100 µm gap and an electrode length of 700 µm to which a 2:1 DF waveform is applied at a 27 MHz frequency (Figure 1.23). Miniaturisation of the FAIMS device enables the generation of higher electric field strengths compared to other systems, resulting from the smaller gap between electrodes. This has the potential for increased differences to be achieved between high and low field ion mobility for enhanced separation. The reduction in dwell time can also reduce ion dispersion effects within the device and allow faster scan times.

Miniaturized FAIMS has been applied to the analysis of a wide range of analytes including proteins,\(^{171,172}\) biological samples\(^{173,174}\) and pharmaceutical impurities.\(^{175}\) Authors report the selective transmission of differential mobility-selected ions reduced spectral complexity through removal of matrix chemical noise to enhance the qualitative and quantitative capabilities of the method. The application of FAIMS-MS to the analysis of oils has been demonstrated for the characterization of naphthenic acids and the study of crude oil mixtures.\(^{37,38}\) FAIMS was used to separate naphthenic acid structural isomers enabling accurate elemental composition and structural elucidation and simplify the mass spectral response generated from highly complex crude oil.
Figure 1.23: Schematic diagram of Owlstone miniaturised chip-based FAIMS device.

- Total area of chip: 4 mm$^2$
- Electrode gap: 0.1 mm x 0.7 mm (16 electrode pairs)
1.4 Mass analysers

Exposure of gas phase ions to magnetic and electric fields can bring about ion separation based upon mass-to-charge ratio ($m/z$). J.J. Thomson reported the first successful $m/z$ measurement in 1913 during the discovery and separation of neon isotopes,\textsuperscript{176} work that was further developed by F.W. Aston.\textsuperscript{177} The findings of Thomson and Aston prompted Dempster and Neir to construct and improve the first focusing magnetic mass spectrometers, capable of the separation of ion isotopes using the physical and chemical properties of $m/z$.\textsuperscript{178,179} Since the development of the magnetic sector a wide range of different mass analysers have been established that use static or dynamic electric and/or magnetic fields to induce ion separation. Variation in mass analysers results from the manner in which the magnetic and electric fields are applied, which dictates the principals of separation.\textsuperscript{58} Paul applied alternating electric fields to investigate ion stability, both in terms of trajectory and resonance, which resulted in the generation of quadrupoles and ion traps.\textsuperscript{180} The time-of-flight (ToF) mass analyser, which measures the time taken for an ion to traverse a field free flight tube utilising the relationship between $m/z$ and ion velocity, was first suggested by Stephens in 1946.\textsuperscript{58} In more recent years work has focused on the resonance behaviour of ions and the application of a Fourier transform (FT) to very accurately determine $m/z$. Such systems include FTICR-MS, first described by Comisarow and Marshall in 1974,\textsuperscript{181} where ions are excited to their unique cyclotron frequency and resulting trajectory that can be measured, and the Orbitrap mass spectrometer, which is a novel concept by Makarov that uses FT to measure the unique oscillations of ions around a central electrode under the influence of an electrostatic field.\textsuperscript{182}

The key performance characteristics of mass analysers relate to the mass range, analytical speed, transmission, mass accuracy and resolution.\textsuperscript{58} Hyphenation of difference mass analysers is commonly observed in commercial hybrid instruments, such as the Waters Synapt HDMS Q-TOF, which aim to improve the versatility of the mass spectrometer. This thesis will focus on the principals of quadrupole, ToF and Orbitrap mass spectrometers in relation to the instruments used.

1.4.1 Quadrupole

The first quadrupole mass analyser was described by Paul and Steinwedel in 1953.\textsuperscript{183} and uses an oscillating electric field to separate ions based upon differences in the stable trajectory through four metallic rods. A quadrupole device consists of four
perfectly parallel cylindrical, or hyperbolic, rods with a small central channel to enable the passage of ions. The opposing metal rods are electronically connected and have an applied alternating RF frequency \(V \cos(\omega t)\) that oscillates between positive and negative voltages. A constant DC voltage \((U)\) is superimposed over the RF voltage. The parallel rod pairs are out of phase so that one pair has an applied potential of \(\Phi_0 = (U + V \cos(\omega t))\) at the same time as the other has the applied voltage \(-\Phi_0 = -(U + V \cos(\omega t))\), where \(\Phi_0\) is the potential applied to the rods, \(V\) is the ‘zero-to-peak’ amplitude of the RF field, \(\omega\) is the angular RF frequency (radians/s) and \(t\) is time (s). A schematic of a quadrupole device is given in Figure 1.24.\textsuperscript{58}

![Schematic diagram of a quadrupole mass analyser](image)

**Figure 1.24: Schematic diagram of a quadrupole mass analyser\textsuperscript{58}**

Ions are accelerated into the quadrupole device where they will pass through the central channel. The total electric field resulting from the oscillating RF and superimposed DC voltages applied to the parallel rods will cause the trajectory of the ions to deviate in both the x and y directions, although motion in the z axis is maintained. Ions will be attracted to rods of opposing potential therefore as \(\Phi_0\) resonates between the positive and negative potentials the ions trajectory will deviate between x and y motions. This can be expressed using the equation:
The forward motion of an ion will be maintained as long as the ion trajectory under the total electric field remains stable, i.e. the values of \( x \) and \( y \) never reach \( r_0 \). The stability of an ion in a quadrupole can be determined using the Mathieu equation:

\[
\frac{d^2u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0
\]

Equation 1.22

Where \( u \) stands for either \( x \) or \( y \), the terms \( a \) and \( q \) related to the DC and RF potential respectively and \( \xi \) can be defined as:

\[
\xi = \frac{\omega t}{2} \quad \text{therefore} \quad \xi^2 = \frac{\omega^2 t^2}{4}
\]

Equation 1.23

Stability in the \( x \) and \( y \) parameters is determined by the \( a \) and \( q \) terms in the Mathieu equation:

\[
a_u = a_x = -a_y = \frac{-8zeU}{m\omega^2 r_0^2}
\]

\[
q_u = q_x = -q_y = \frac{4zeV}{m\omega^2 r_0^2}
\]

Equation 1.24

Where \( ze \) is the charge of the ion and \( m \) is the mass of the ion. For any given quadrupole, \( r_0 \) is constant and \( \omega = 2\pi v \) is maintained constant, therefore \( U \) and \( V \) are the variables. The stability areas of ions with different masses can be expressed as a function of \( U \) and \( V \) (Figure 1.25). Operating the quadrupole as a ratio of \( U/V \) generates a total electric field for which ions of particular masses have a stable trajectory. Maintaining this ratio enables the successive detection of ions with different masses. Modifying the \( U/V \) ratio will alter the operating line, changing the resolution. If \( U=0 \) then the working points of the ions will be determined by \( V \) alone and the will transmit all ions above a low mass unit. Increasing \( U \) in relation to \( V \) will move operating line of the quadrupole so that there is an increase in instrument resolution as we move towards the edge of the stability zone for particular ions. The typical operation of a quadrupole will scan both \( U \) and \( V \) so that ions at different masses will have stable trajectories at different times under the changing \( U/V \) ratio,
as depicted by the operating line shown in Figure 1.25\textsuperscript{58} This is carried out at a uniform velocity over the entire mass range so that the quadrupole acts as a sequential mass filter. Quadrupoles are described as low resolution mass analysers, typically working to a unit resolution.

![Figure 1.25: Stability diagram of ions in a quadrupole as a function of $U$ and $V$ for ions of different masses ($m_1<m_2<m_3$). Changing $U$ linearity as a function of $V$ creates a straight operating line that allows us to observe ions successively.\textsuperscript{58}]

1.4.2 Time-of-flight (ToF)
The concept of measuring an ions time-of-flight through a known distance under vacuum pressure was first described by Stephens in 1946, but it was not until 1955 that the first commercial linear ToF instrument was developed by Wiley and McLaren.\textsuperscript{184} The pulsed nature of ToF operation made the mass analyser well suited to laser ionisation techniques such as MALDI, and as such advances in ionisation were reflected in ToF instrumental development.\textsuperscript{58} To date there are three ToF configurations, spiral, linear and reflectron. Linear and reflectron ToF systems will be discussed in more detail.

**Linear ToF**
The first linear ToF design, which was commercialised in 1955,\textsuperscript{184} separated ions using the principal "that a population of ions moving in the same direction and having a distribution of masses but a constant kinetic energy, with have a corresponding distribution of velocities in which velocity is inversely proportional to the square root
of m/z'. The instrument consisted of four regions; a pulsed ionisation source, an acceleration region, a flight tube and a detector (Figure 1.26).\(^{185}\)

![Schematic diagram of a linear ToF\(^{185}\)](image)

**Figure 1.26: Schematic diagram of a linear ToF\(^{185}\)**

Ions leave the source region in packets, either achieved through a pulsed ionisation source such as a plasma or the use of a transient application of required potential on the source focusing lenses. The ions are accelerated into the flight tube using a potential difference generated in the acceleration region to propel ions in a forward motion. Under the influence on an electric field the potential energy of an ion \((E_p)\) can be described as:

\[
E_p = qU
\]  
**Equation 1.25**

Where \(q\) is the charge of the ion \((q = ze)\) and \(U\) is the electric potential difference \((V_s)\).

Upon acceleration into the drift tube the potential energy of an ion is converted to kinetic energy:

\[
E_k = \frac{mv^2}{2}
\]  
**Equation 1.26**

The velocity of an ion can therefore be expressed as:

\[
v = \left(\frac{2zeV_s}{m}\right)^{\frac{1}{2}}
\]  
**Equation 1.27**

After the initial acceleration event the velocity of an ion remains constant therefore the time taken to traverse the flight tube and reach the detector \((t)\) would be:
Where $L$ is the length of the flight tube. Substituting equation 1.27 into 1.28 for $v$ we get:

$$t^2 = m/z \left( \frac{L^2}{2eV_x} \right)$$  \hspace{1cm} \text{Equation 1.29}$$

The equation shows that, with all other experimental parameters being kept constant, the $m/z$ of an ion has a linear relationship to the time of flight, and therefore the smaller the ion (of equal charge) the quicker it will pass through the flight tube and reach the detector. The measurement of $t$ in a ToF instrument is used to determine $m/z$:

$$\left( \frac{m}{z} \right)^{1/2} = \left( \frac{\sqrt{2eV_x}}{L} \right) t$$  \hspace{1cm} \text{Equation 1.30}$$

Theoretically there is no upper limit to the $m/z$ for a ToF instrument; therefore it is suitable for hyphenation with softer ionisation methods, such as MALDI, for the analysis of large biomolecules.\textsuperscript{185} In addition, the technique has good transmission values to enable the analysis of low sample concentrations.\textsuperscript{58}

**Reflectron ToF**

The mass resolution in a ToF instrument is derived from the relationship between $m/z$ and flight time:

$$\left( \frac{m}{z} \right)^{1/2} = \left( \frac{\sqrt{2eV_x}}{L} \right) t$$  \hspace{1cm} \text{Equation 1.31}$$

$$\frac{1}{2} \frac{dm}{dt} = \left( \frac{2eV_x}{L^2} \right) 2t \, dt$$  \hspace{1cm} \text{Equation 1.32}$$

$$\frac{m}{dm} = t/2 \, dt$$  \hspace{1cm} \text{Equation 1.33}$$
Therefore ToF resolution \((R)\) can be expressed:

\[
R = \frac{m}{\Delta m} = \frac{t}{\Delta t} \approx \frac{L}{2\Delta x}
\]  

Equation 1.34

Where \(\Delta m\) and \(\Delta t\) are difference in mass and flight time respectively for two adjacent peaks, \(L\) is the flight tube length and \(\Delta x\) is the thickness of the ion packet. Increasing mass resolution can therefore be achieved by increasing the flight tube length. However, this can result in poor sensitivity due to ion losses. In addition the spread of initial velocities resulting from the thickness of the ion packet, which is exaggerated over increased flight lengths, is a limiting factor for ToF resolution. In 1973 Mamyrin presented a “non-magnetic time of flight mass spectrometer” which used a mass-reflectron to focus the ion packets on the basis of energy, dramatically improving mass resolution and enabling the use of longer flight times\(^{186}\) The instrument operates using the same fundamentals as the linear ToF, but has a series of equally spaced electrodes connected through a network of resistors at the end of the flight tube that functions to deflect ions back along the flight path toward a detector that is positioned adjacent to the acceleration region (Figure 1.27).\(^{58}\)

![Figure 1.27: Schematic diagram of a reflectron ToF\(^{58}\)](image)

The reflectron corrects for the spread of kinetic energies of ions of the same \(m/z\) leaving the source. The relative positioning of ions within the ion packet upon entry into the acceleration region can result in slight differences in \(E_k\). If we take two ions of the same \(m/z\) but one with a higher \(E_k\) (A: filled circles in Figure 1.27) than the other (B: open circles in Figure 1.27) we can see that ion A will penetrate further into
the reflectron than B as a result of the increased $E_k$. Although the slight variation in $E_k$ between the two ions is retained, ion A had to travel further through the reflectron, which increases its total flight path. Upon deflection back through the flight tube the variation in flight length corrects for differences in $E_k$, ensuring both ions reach the detector simultaneously. The penetration distance of an ion into the reflectron ($d$), with charge $q$ and kinetic energy $E_k$, can be described by:

$$d = \frac{E_k}{qE} = \frac{qV_r}{q V_r / D} = \frac{V_r D}{V_r}$$

Equation 1.35

Where $V_r$ is the potential of the reflectron, and its length is $D$, the electric field in the reflectron can be expressed as $E = V_r / D$.

The development of the reflectron has been revolutionary for enhancing ToF resolution. However, it does impart an upper mass limit for reflectron ToF mass analysers.

1.4.3 Orbitrap

The Orbitrap mass analyser was designed by Makarov in 2000\textsuperscript{182} using fundamentals from the Kingdon trap\textsuperscript{187} and is a new technique for the separation of ions as a function of $m/z$. The instrument was later commercialised by Thermo Fisher Scientific in 2005. The Orbitrap functions as an electrostatic ion trap that uses Fourier transform to convert the oscillating frequencies of ions into mass spectral data. The device consists of two outer cup shaped electrodes that face each other to form a barrel shape. The two electrodes are isolated by a hair-thin gap and secured by a central ring made of a dielectric.\textsuperscript{188} Inside the barrel electrode is a central spindle shaped electrode that runs through the length of the mass analyser (Figure 1.28).\textsuperscript{58}
A DC voltage is applied between the two axially symmetric electrodes to create a linear electric field along the axis.\textsuperscript{186,189} The electric potential within the trap can be defined by:

\[ U(r, z) = \frac{k}{2} \left( z^2 - \frac{r^2}{z^2} \right) + \frac{k}{2} (R_m)^2 \ln \left( \frac{r}{R_m} \right) + c \]

Equation 1.36

Where \( r \) and \( z \) are cylindrical coordinates, \( C \) is a constant, \( k \) is field curvature and \( R_m \) is the characteristic radius.

Packets of ions are injected tangentially into the area between the inner and outer electrodes through an interslice in the outer electrode offset from \( z=0 \). The introduction of ions occurs just after the voltage to the inner electrode has been turned on (50-90 \( \mu \)s) but before it reaches its final value. This causes the ions to experience a monotonic increase in electric field strength within the Orbitrap, which pulls the ion cloud to the central electrode and prevents initial collisions with the outer electrode.\textsuperscript{189} The radial electric field causes the ions trajectory to bend towards the inner electrode while the tangential velocity creates an opposing centrifugal force. Under the correct field strength these combined forces will cause the ion to spiral around the inner electrode. At the same time the spindle shape of the inner electrode will create an axial electric field that pushes ions to the widest part of the trap and initiates harmonic oscillations of ions along the \( z \)-axis.\textsuperscript{188} The axial frequency of ion
oscillation is independent of the initial ion velocities and occurs solely as a function of an ions $m/z$:

$$\omega = \sqrt{\frac{q}{m}}k$$

Equation 1.37

Where $q=ze$.

As a result, ions with the same $m/z$ can remain in phase along the z axis for thousands of oscillations. The broadband current on the outer electrode induced by the oscillating frequencies generates a unique waveform for each $m/z$ that is converted into a mass spectrum by FT.

The independence of the oscillating frequency to the initial ion energy and the ability for radial oscillations to be maintained in phase results in the very high mass resolution of the Orbitrap compared to alternative mass analysers (Table 1.9).

1.4.4 Hyphenated instruments

The hyphenation of different mass analysers within mass spectrometric instruments enables an enhanced range of operation for the user. Different mass analysers have different functional properties, Table 1.9, which can be combined to improve the key performance characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Quadrupole</th>
<th>Reflectron ToF</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass limit</td>
<td>4,000 Th</td>
<td>10,000 Th</td>
<td>50,000 Th</td>
</tr>
<tr>
<td>Resolution</td>
<td>2,000 FWHH</td>
<td>20,000 FWHH</td>
<td>100,000-400,000 FWHHH</td>
</tr>
<tr>
<td>Accuracy</td>
<td>100 ppm</td>
<td>10 ppm</td>
<td>&lt; 5 ppm</td>
</tr>
<tr>
<td>Ion sampling</td>
<td>Continuous</td>
<td>Pulsed</td>
<td>Pulsed</td>
</tr>
<tr>
<td>Pressure</td>
<td>$10^{-5}$ Torr</td>
<td>$10^{-6}$ Torr</td>
<td>$10^{-10}$ Torr</td>
</tr>
</tbody>
</table>

Table 1.9 shows the key performance characteristics (mass range limit, transmission, mass accuracy and resolution) demonstrating an overall improvement from quadrupole<Reflectron ToF<Orbitrap. Orbitrap mass analysers enable the continuous introduction of ions from the source, which is beneficial for techniques...
such as ESI, and can be used both as a mass filter before accurate mass analysis and for tandem mass spectrometry with collision induced dissociation (CID). Although the performance of the Orbitrap exceeds the reflectron ToF the price of the Orbitrap can limit its availability.

In this PhD, two hyphenated mass spectrometers were used; a Waters Synapt HDMS Q-ToF (with a TWIMS cell) and a Thermo Orbitrap Q Exactive Plus, a Q-Orbitrap. Figure 1.29 shows a schematic diagram of the Waters Synapt HDMS instrument.

![Figure 1.29: Schematic diagram of the Waters Synapt Q-TWIMS-ToF instrument](image)

Ions generated at the analyte source, primarily through atmospheric ionisation methods such as ESI, pass into the instrument where they travel through the z-spray source block and the first ion guide, which focuses ions into a beam, before acceleration into the mass spectrometer. At each stage of the mass spectrometer, turbo molecular pumps are present to reduce the internal pressure and create the vacuum environment necessary for both quadrupole and ToF mass analysis. In MS mode the quadrupole will only have an applied RF voltage so that it functions to transmit all ions through the device where they enter the reflectron ToF region. In this design the ions will be subjected to an orthogonal acceleration in the ToF using a pusher electrode in the flight tube. Ions passing through the instrument will be
accumulated into packets in the pusher region due to their forward trajectories before a pulsed voltage is applied to accelerate ions down the flight tube. The orthogonal nature of the acceleration process functions to reduce the spread of initial energies of the ions. The quadrupole can be used in RF and DC mode to pre-select ions before ToF analysis. This function can simplify collision induced dissociation studies (CID), where fragmentation can be induced in either the trap or transfer regions using a flow of collision gas at increased eV. Finally the instrument contains a TWIMS cell for separation of ions based upon their mobility. The versatility of the instrument has seen its use in a wide range of in both qualitative and quantitative applications, including small molecule analysis\(^{191,192}\) and in proteomic and metabolomics studies.\(^{190,193}\)

The other hybrid instrument used in this work was a Q-Orbitrap mass spectrometer (Figure 1.30).

![Figure 1.30: Schematic diagram of an Orbitrap Q Exactive Plus instrument (Q-Orbitrap)](image)

Ions enter the mass spectrometer through the inlet capillary where they are focused using the RF lens. The quadrupole can either act as an ion transfer tube or enable mass selection of precursor ions. Subsequently ions can either pass into the higher-energy collisional dissociation (HDC) cell where CID can be carried out, or collected in the c-trap. Packets of ions collected in the c-trap are injected into the Orbitrap for
high resolution mass analysis. Hypenation of the quadrupole with the Orbitrap has
been shown to not only enable pre-selection of ions for simplification of product ion
data, but also improve robustness of the instrument through efficient removal of
unwanted ions. 

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1.5 References


CHAPTER TWO

The Development of Desorption Electrospray Ionisation Sources for the Analysis of Additives used in Lubricant Oils and Petroleum Processing
2.1 Introduction

The term ambient ionisation is used to describe a range of ionisation methods, capable of the direct analysis of samples with no sample preparation or modification, carried out under atmospheric conditions. The increasing need for high-throughput analyses and in situ analyte detection has resulted in the development of over 25 different ambient ionisation methods. The majority of ambient ionisation techniques are based on three different principals of ionisation: spray, chemical and laser as discussed in Chapter 1, Section 1.2. However, one notable difference between the array of techniques is the configuration of the ionisation sources. The development of sources in-house is commonplace in the field and can impart flexibility within the design to target specific samples or analytes that would normally require extensive sample preparation for analysis by MS. Since its development in 2004, DESI has been at the forefront of ambient ionisation. In DESI, an electrospray generated solvent plume is targeted towards a sample surface to desorb and ionise molecular analytes for analysis by MS (Chapter 1, Section 1.2.2). The ‘traditional’ DESI source configuration consists of an electrospray nebuliser positioned at an angle of ~ 55° relative to the sample surface and the mass spectrometer inlet, with the tip-sample and sample-MS inlet distances within 5 mm. Optimisation of the DESI source geometry is dependent upon the surface material, electrospray solvent and target analytes for efficient desorption and ionisation (Chapter 1, Section 1.2.2). A range of modifications to the typical DESI source configuration have been reported in the literature to overcome problems associated with the standard geometry, including geometry-independent DESI, non-proximate DESI for the analysis large or immobile samples and TM-DESI for high through put analysis of liquid samples or solid extracts. Tailoring the DESI geometry when developing a source in-house can be used to ensure the design meets the practical requirements of the sample and the mass spectrometer.

Traditional mass spectrometric techniques for the analysis of oil and petroleum samples have relied upon high-resolution instruments, such as FTICR-MS, hyphenated with chromatographic separations and ESI. These methods are well established and provide detailed compositional data for samples. However, they cannot generate in situ localisation data relating to the distribution of molecular compounds on a surface. Ambient ionisation techniques such as ambient sonic-spray ionisation, DART, PS and thin layer chromatography spray-mass spectrometry have been used for the analysis of oils and additives, but no technique has shown the direct analysis of compounds from the native state surface. Whilst little has been
reported in the literature showing the application of DESI in the field of petroleomics and additive analysis, the use of DESI for the targeted studies of analytes present in complex matrices, such as biological tissues\textsuperscript{13} and cosmetic formulations,\textsuperscript{14} highlights the potential of the technique. Furthermore DESI-MS imaging experiments show the successful mapping of compounds from a range of surface materials and matrices\textsuperscript{15–17} that could be applied to the investigation of oil and oil additive deposition.

Advances in post-ionisation separation methods, such as IMS and FAIMS, can overcome some of the limitations of ambient ionisation for the analysis of complex oil and petroleomic samples. The mass spectrographic information generated for such samples without sample pre-treatment or separation is restricted by the mass resolution of the instruments. IMS and FAIMS can be used as post ionisation methods to separate ions and increase the analytical window of the DESI-MS method (Chapter 1, Section 1.3). The use of IMS and FAIMS for the analysis of petroleomic based samples is the subject of increasing interest.\textsuperscript{18,19} Hyphenation of DESI with IMS-MS and FAIMS-MS has been shown for the targeted studies of pharmaceutical,\textsuperscript{20–22} forensic,\textsuperscript{23} proteomic\textsuperscript{24} and biological samples.\textsuperscript{25} The range of DESI-MS experiments reported in the literature show the versatility of the technique, suitability for surface imaging, hyphenation with IMS and the capability for complex matrix analysis, showing the potential of the technique for oil and petroleomic studies.
2.2 Aims and Objectives

- Design, develop and construct DESI sources for the rapid analysis of compounds, including lubricant additives, directly from a range of surface materials with no/minimal sample preparation.

- Hyphenation of DESI with IMS-MS and MS/MS.

- The interrogation of surface-active lubricant oil additives directly from metal surfaces by DESI-MS.

- The direct analysis of engine components by DESI-MS.
2.3. Experimental

2.3.1. Reagents and Chemicals

Methanol, acetonitrile and water were purchased from Fisher Scientific (Loughborough, UK). Toluene, hexane and formic acid were purchased from Sigma Aldrich (Gillingham, UK). All solvents were HPLC grade.

A range of commercially available lubricant oil additives was used for source development and testing. The antioxidant additives; N-phenyl-1-napthylamine (1) and octyl (4-hydroxy-3,5-di-tert-butylphenyl)propionate) (2) and the friction modifier/corrosion inhibitor oleamide (3) were supplied by Castrol (Pangbourne, UK). The corrosion inhibitor additives compounds benzyldimethylidodecylammonium chloride (4a), benzyldimethyltetradecylammonium chloride (4b) and benzyldimethylhexadecylammonium chloride (4c) were purchased from Sigma Aldrich (Gillingham, UK) at 99%, 97% and cationic detergent grade respectively. The structures of the lubricant oil additives are shown in Figure 2.1. A sample of commercial lubricating oil and a group 1 treated base oil were supplied by Castrol Ltd (Pangbourne, UK).

The inter-laboratory VAMAS experiment investigating DESI-MS precision was carried out using a sample of Rhodamine B and double sided tape that were supplied by the National Physics Laboratory (NPL, Teddington, UK) and required no further sample preparation prior to analysis.
Figure 2.1: Structures of the lubricant oil additives used for DESI-MS studies. Compound 1: N-phenyl-1-naphthylamine, Compound 2: octyl (4-hydroxy-3,5-di-tert-butylphenyl)propionate), Compound 3: oleamide, Compound 4a: benzyldimethyldodecylammonium chloride, Compound 4b: benzyldimethyltetradecylammonium chloride and Compound 4c benzyldimethylhexadecylammonium chloride.

2.3.2. DESI Source
The in-house development of DESI sources was a multi-stage process that is summarised in Table 2.1 and will be described in detail in the Results and Discussion section of this chapter. The DESI sources were designed to fit to the existing Waters Synapt HDMS mass spectrometer inlet with no modification to the outer housing of the mass spectrometer. Unless otherwise stated the source geometry within the DESI source was: electrospray nebuliser angle ~ 55°, capillary tip-sample distance ~ 3-5 mm, sample-mass spectrometer inlet distance ~ 1-3 mm and capillary tip-mass spectrometer inlet distance ~ 3-5 mm.

The non-proximate DESI cone system was designed in-house, to replace the standard cone system on the z-spray source block, and constructed by a specialist-engineering firm (JRE Precision, Loughborough, UK). The modified cone was made
from a stainless steel unit that had the same internal dimensions as the Waters cone gas nozzle (M946549CD-1). A Swagelok 1/8\textsuperscript{th} to 1/16\textsuperscript{th} inch reducer fitting was welded to the tip of the modified cone to enable the attachment of 1/16\textsuperscript{th} inch (O.D) stainless steel ion transfer tubing (Thames Restek, Saunderton, UK) in pre-cut lengths of 5-20 cm. The DESI stage hardware (source version 1.3) was designed and constructed partly in-house and partly by an engineering firm (Sileby Fabrics, Sileby, UK) using 3 mm stainless steel sheeting. The electronic sample stage manipulator (sourced from a Waters LCT MALDI source) and the electrospray nebuliser (sourced from an Applied Biosystems Mariner Workstation) was secured to DESI source version 1.3 using a 3 mm stainless steel platform and an in-house constructed mount that enabled height and angle manipulation of the electrospray nebuliser. Control of the electronic sample stage manipulator was carried out using National Aperture Inc. (NAI) motion controller MC-4SA connected to a National Instruments controller board (NI-73) located in a designated computer. The NI-73 controller board enabled communication between the sample stage manipulator and both National Instruments Measurement & Automation Explorer (NI-MAX) and National Instruments LabView software packages for automated movement in the x and y dimensions. Movement in the z direction was manual.
Table 2.1: Overview of DESI source development designs for Waters Synapt HDMS instrument.

<table>
<thead>
<tr>
<th>Version</th>
<th>Electrospray nebuliser</th>
<th>Electrospray nebuliser gas supply and voltage</th>
<th>Sample stage</th>
<th>MS inlet system</th>
<th>Experimental applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Waters ESI nebuliser (part number M956357DC1)</td>
<td>N$_2$ and voltage controlled using Waters Synapt HDMS instrument.</td>
<td>Freestanding manual x,y,z manipulator constructed in house.</td>
<td>Standard Waters cone system.</td>
<td>DESI-MS, DESI-IM-MS and DESI-MS/MS screening of lubricant additives</td>
</tr>
<tr>
<td>1.2</td>
<td>Waters ESI nebuliser (part number M956357DC1)</td>
<td>N$_2$ and voltage controlled using Waters Synapt HDMS instrument.</td>
<td>Freestanding manual x,y,z manipulator constructed in house.</td>
<td>Non-proximate DESI cone system.</td>
<td>VAMAS experiment Analysis of metal surfaces Quantitative analysis of a lubricant additive</td>
</tr>
<tr>
<td>1.3</td>
<td>Applied Biosystems Mariner electrospray nebuliser (part number 014368)</td>
<td>N$_2$ sourced from lab gas supply and controlled using a gas regulator. Voltage from external power supply (Brandenburg voltage supplier).</td>
<td>Automated x,y manipulator (Waters LCT MALDI) and manual z manipulator fitted to DESI source.</td>
<td>Non-proximate DESI cone system.</td>
<td>Analysis of test samples Effect of solvent composition on DESI response Analysis of formulated oil Analysis of crude oil</td>
</tr>
</tbody>
</table>

2.3.3 Sample preparation and target surfaces

For screening studies and source development the lubricant oil additives were dissolved in a solvent solution (MeOH,H$_2$O and toluene mixtures) at a concentration of 1-2 mg/mL. An aliquot of the solution (1-10 μL) was deposited onto a target surface and left for ~ 30 sec to allow the solvent to evaporate, so that the additive
was present at 1-10 μg on spot, prior to analysis by DESI-MS. A range of surface materials were investigated including; glass, PTFE, filter paper (Whatman Type 1) and metal coupons (cold rolled stainless steel, Grade 1008 1010, polished). A spiked oil sample was prepared by dissolving compound 1 in hexane (2 mg/mL) and mixing this solution 1:1 with commercial lubricating oil (supplied by Castrol) for analysis by DESI-MS and DESI-MS/MS.

The effect of solubility on DESI-MS response and optimisation of ESI solvent composition was investigated for the analysis of the corrosion inhibitor additive compounds 4a, 4b and 4c. The compounds were dissolved in 1:1 MeOH:H₂O to give stock solutions of 9.15 mg/mL, 9.9 mg/mL and 10.65 mg/mL for 4a, 4b and 4c respectfully. A 2 μL aliquot of the stock solution was deposited onto a cleaned stainless steel metal coupon using a spotting template so that the metal coupon had sample spots of each targeted additive present on the surface in equimolar amounts (4a at 18.3 μg/spot, 4b at 19.8 μg/spot and 4c at 21.3 μg/spot). The target surface was left for ~ 1 min for the stock solvent to evaporate prior to analysis by DESI-MS. Determination of the solubility of the target additives in the electrospray solvent composition was assessed using the saturation point method. The corrosion inhibitor additives were dissolved in 200 μL of solvent (8:2 toluene:MeOH, ACN, MeOH, H₂O, 1:1 MeOH:toluene, 1:1 MeOH:ACN 1:1 MeOH:H₂O) in a water bath at 28 °C (just above room temperature) until the point of saturation. The solutions were centrifuged and 100 μL of the top layer was extracted and deposited into a pre-weighed vial and left overnight for solvent evaporation. The vial was re-weighed and the concentration of additive in 100 μL solvent was calculated using the difference in weight. The corrosion inhibitor standards were subsequently dissolved in 1:1 MeOH:toluene (9.15-10.65 mg/mL) and spiked into a group 1 treated base oil (100 μL additive solution + 900 μL oil). The solvent was left to evaporate before 2 μL of the spiked oil was deposited onto a metal coupon for analysis by DESI-MS.

Three test samples were analysed by DESI-MS using source version 1.3 in order to determine the potential of the method for direct lubricant additive detection. A sample of corrosion inhibitor additive in 1:1 MeOH:H₂O was manually deposited onto a metal coupon over three areas to give a total of ~ 0.5 μg additive on surface (sample 1). A wear test coupon that had undergone automated wear analysis in the presence of formulated lubricant deposition followed by a solvent wash (sample 2) was supplied by Castrol. No information was provided as to the composition of the lubricant formulation or the solvent wash. The sample was stored in a plastic bag. An engine
valve (sample 3) used in a combustion test was also supplied by Castrol for analysis by DESI-MS.

2.3.4 Instrumental parameters
The screening of additives, surfaces and the spiked oil sample was carried out using DESI source version 1.1 with the instrumental parameters set to: electrospray nebuliser voltage; +/- 3 kV, cone voltage; 20 V, source temperature; 120 °C, desolvation gas; 100 L/Hr at room temperature, cone gas; 30 L/Hr, trap gas; 1.5 mL/min. The electrospray phase flow rate was dependent upon the target surface interrogated, but was in the range of 2-20 μL/min. IMS separations of the target antioxidant 1 were achieved using N₂ gas at a flow rate of 24 mL/min, with a wave velocity of 300 m/s and a wave height of 4.5-20 V ramped over 200 bins. Fragmentation was induced in the trap CID cell using a collision energy of 22 eV (ESI: 25 eV). The ESI instrumental parameters for the comparative IMS and MS/MS analysis of 1 were capillary voltage; +3 kV, cone voltage; 20 V, source temperature; 120 °C, desolvation gas; 300 °C at 300 L/Hr, cone gas; 30 L/Hr, trap gas; 1.5 mL/min and flow rate of 5 μL/min.

The experimental conditions used in the NPL VAMAS experiment for the analysis of Rhodamine B and a sample of tape were in accordance with the recommended parameters found in the VAMAS protocol. The source was: DESI source version 1.2 fitted with a 10 cm ion transfer tube with a 5° bend at the tip; electrospray to surface angle, 50°; electrospray to surface distance, 1 mm and electrospray to sniffer distance, 3 mm. The other instrumental parameters were: capillary voltage, 5 kV; nebuliser gas flow 75 L/Hr; electrospray solvent composition, 90:10 acetonitrile:water + 0.1 % formic acid at a flow rate of 2 μL/min.

ESI optimisation and investigation into the effect of solubility on DESI-MS response was carried out using DESI source version 1.3 fitted with a 5 cm ion transfer tube with PTFE sleeve and automated sample stage manipulation using the LabView code described in Section 2.4.1. Each sample of corrosion inhibitor additive deposited on the metal coupon was analysed in the positive ion mode using 7 different electrospray solvent compositions (8:2 toluene:MeOH, ACN, MeOH, H₂O, 1:1 MeOH:toluene, 1:1 MeOH:ACN 1:1 MeOH:H₂O). The instrumental parameters were: electrospray nebuliser voltage; + 2 kV, nebuliser gas pressure; 40 psi, cone voltage; 20 V, source temperature; 120 °C. The acquisition was started 1 min prior to movement of the sample under the electrospray plume in the DESI source, where surface interrogation was carried out for a further 3 minutes. The sample was not
moved during analysis to ensure depletion of the additive from the surface. Three replicates were carried out for each sample. Oil/additive mixtures deposited onto metal coupons were analysed by DESI-MS using an electrospray solvent composition of 1:1 MeOH:ACN.

The DESI-MS analysis of the three test samples (samples 1, 2 and 3) was carried out using DESI source version 1.3. The samples were positioned in the DESI source and movement was controlled using the automated sample stage manipulator. For imaging studies the rate of sample movement was 200 counts/sec. The height of the sample stage was controlled manually to ensure the standard DESI configuration within the source was maintained even with the changing topography. The instrumental parameters used for the analysis of all three samples were: electrospray nebuliser voltage; +2 kV, nebuliser gas pressure; 40 psi, cone voltage; 20 V, source temperature; 120 °C, electrospray flow rate; 8 µL/min. The electrospray solvent was 1:1 MeOH:ACN (sample 1), 1:1 MeOH:toluene + 0.1 % formic acid (sample 2) and 8:2 toluene:MeOH, ACN, MeOH, H₂O, 1:1 MeOH:ACN, 1:1 MeOH:H₂O, 1:1 MeOH:toluene (sample 3).
2.4 Results and Discussion

2.4.1 DESI source development

The development of DESI sources for the analysis of lubricant additives directly from a range of surface materials and topographies had to meet several key requirements:

1. Allow hyphenation of DESI with IMS-MS and MS/MS.
2. A single unit design that secured to the existing fittings on the mass spectrometer with no modification to the mass spectrometer housing.
3. Capable of repeatable source configurations that could be maintained over time.
4. Capable of analysing a wide range of samples, not restricted to the analysis of fixed surface shapes.
5. Analysis of different surface materials including the analysis of metal surfaces and tribological components.
6. Sample movement to be controlled remotely to improve accuracy and precision.
7. Control of the source to be carried out away from the electrospray, minimising operator exposure to solvent spray and high voltages.

DESI source version 1.1: DESI-MS, DESI-IM-MS and DESI-MS/MS

The Waters Synapt HDMS instrument was selected for DESI source development to enable hyphenation with MS, IMS and MS/MS. The ESI source housing was removed from the front of the mass spectrometer, to expose the z-spray source block and the mass spectrometer inlet, and the Waters electrospray nebuliser probe was extracted. The electrospray probe was mounted at ~ 55 ° in line with the mass spectrometer inlet orifice using a laboratory clamp stand positioned on a table in front of the instrument. A manual x, y, z sample stage manipulator, with fine movement in the x and y axis and crude movement control in the z axis, was used to position the target surface under the electrospray probe in the DESI source (version 1.1), horizontal to the mass spectrometer inlet. The positioning of the ESI probe and the DESI source configuration was consistent with the ‘traditional’ DESI source set-up reported in the literature. Version 1.1 of the DESI source is shown in Figure 2.2.
Figure 2.2: Prototype DESI source (version 1.1) for the Synapt HDMS instrument showing a) a schematic for the source configuration, b) a close up-view of the DESI source constructed using the Waters Synapt ESI probe and a manual sample stage manipulator.
Preliminary DESI-MS studies were carried using DESI source version 1.1 to assess the capabilities of DESI as an ambient ionisation technique for the direct and rapid interrogation of target lubricant oil additives present on a surface and the potential for hyphenation with IMS and MS/MS. The results were compared to ESI-MS.

All additives were successfully desorbed and ionised by DESI-MS from the inert surface materials selected, generating mass spectra containing molecular ion peaks. The two antioxidant additives, 1 and 2, were chosen to provide exemplar data. The structures of the antioxidant additives are given in Figure 2.1. The analytes were deposited on glass, PTFE and filter paper surfaces (10 µL of a 1 mg/mL solution in MeOH) and analysed by DESI-MS using an electrospray phase of 95:5 MeOH:H₂O (+ 0.1 % formic acid for positive ion mode) in the positive and negative ion modes respectively. Figure 2.3 shows the positive ion mode DESI-MS analysis of 1 and Figure 2.4 shows the negative ion mode DESI-MS analysis of 2 from the different surface materials. The molecular ion peaks of 1, [M+H]⁺ = m/z 220.11, and 2, [M-H]⁻ = m/z 389.30, were clearly observed above the background noise in all mass spectra when 10 µg was deposited on the surface. The responses for the molecular ion peaks of 1 and 2 were lower when analysed from the glass surface compared to the PTFE and filter paper, which was consistent for all the additives tested. Sample to sample variation in ion intensity will often result from inhomogeneous sample distribution on the surface. However, the reduced sensitivity with the glass could result from a range of additional factors. Deposition of the sample on the glass surface in a MeOH solvent had a larger spread compared to the PTFE and filter paper due to the reduced surface tension of the sample droplet at the surface interface. The larger sample droplet means the concentration of the additive at a given spot on the glass surface will be less than the other target materials, which is reflected in the molecular ion intensity. A high affinity of the additives to the glass surface or the application of un-optimised instrumental parameters could also reduce the desorption/ionisation efficiency of the DESI process also resulting in a lowered sensitivity. The instrumental parameters were kept consistent throughout the experiment to enable comparative data to be generated. However it was noticed that an electrospray phase solvent flow rate of 15 µL/min could have a “washing” effect when applied to smooth surface materials, such as the glass microscope slide. The “washing” effect occurred when the solvent flow rate and desolvation gas flows where too high for the target surface material, causing the solvent puddle generated on the surface during DESI to be washed off before analyte extraction and the formation of analyte-containing secondary droplets, reducing the efficiency of the
ionisation process. The PTFE and filter paper generated similar mass spectral responses and were therefore selected as target surfaces for further experimentation.

Figure 2.3: DESI-MS analysis of 10 µg compound 1 deposited on a) glass, b) PTFE and c) filter paper surfaces and analysed in the positive ion mode using an electrospray phase of 95:5 MeOH:H₂O + 0.1 % formic acid.
Figure 2.4: DESI-MS analysis of 10 µg compound 2 deposited on a) glass, b) PTFE and c) filter paper surfaces and analysed in the negative ion mode using an electrospray phase of 95:5 MeOH:H₂O.

Hyphenation of ambient ionisation techniques, such as DESI, with IMS and MS/MS can help to confirm the presence of target analytes in mixtures without the use of chromatographic separations. Direct ionisation methods enable a more rapid analysis of samples with no additional sample preparation, but do not allow for the extraction of analytes from the background matrix prior to analysis by mass.
spectrometry. The use of IMS for targeted analyte detection can improve mass spectral response through removal of chemical interferences, indicate the presence of unresolved species using drift profile shape distribution and provide an additional identifiable characteristic feature using drift time. The fragmentation of ions, primarily through CID, is an established method used for ion identification and structural characterisation. Tandem mass spectrometry is used routinely alongside chromatography and accurate mass measurements to identify both unknown ions and targeted species within samples. IMS and MS/MS therefore can both be combined with DESI to improve confidence in species identification when analysing complex mixtures such as formulated oils.

The two antioxidant additives, 1 and 2, were selected to assess the capability of DESI-IM-MS and DESI-MS/MS using the in-house constructed DESI source version 1.1. The results for the analysis of antioxidant 1 are shown in Figures 2.5-2.8. ESI was compared with DESI to ensure no change in fragmentation or drift profiles were observed resulting from the DESI ionisation mechanism. A sample of 1 (1mg/mL in MeOH) was deposited onto a PTFE surface (10 µL) for DESI analysis using an electrospray phase solvent composition of 95:5 MeOH:H₂O + 0.1 % formic acid (20 µL/min) and further diluted for direct infusion ESI (1/100 dilution). The results from the ESI-MS and ESI-IM-MS analysis of 1 are shown in Figure 2.5. The ESI-MS mass spectrum shows a strong molecular ion response for [M+H]⁺ for 1 and other ions arising from this technical grade sample. The insert in Figure 2.5b shows the TWIMS mobility selected ion response of the protonated molecular ion, [M+H]⁺, for 1. A symmetric distribution is observed with a peak drift time of 59 bins or 2.7 ms, indicating the absence of isomeric impurities. Extraction of the mass spectrum from bins 57-61, or 2.6-2.7 ms (Figure 2.5b) shows removal of some of the chemical background noise by an ion mobility separation of the gas phase ions.

The analysis was repeated by DESI-IM-MS (Figure 2.6). Compound 1 was the base peak in the DESI-MS mass spectrum, however, the overall sensitivity was lower than that observed for ESI-MS. This is an accumulative result of the desorption/ionisation process in the DESI mechanism, where ions need to be extracted from a surface material, and the variability in ion response due to continuous sample movement and depletion when compared to ESI. The poor spray stability and reduced sensitivity can make running IMS and MS/MS analyses, which require a continuous input of analyte ions over a several second time frame, difficult. An enhanced level of background chemical noise was also observed for DESI compared to ESI resulting from the
exposed nature of the ionisation source to the laboratory environment and the presence of potential contaminants on the PTFE that may not have been removed following washing and storage of the target surface. Deposition of 10 µg additive on spot can generate a sufficiently stable ion current from the desorption and ionisation of the compound from the surface to enable an ion mobility separation to be carried out. The drift profile for the selected ion response of the protonated molecular ion (Figure 2.6b insert) was consistent in both peak bin response (60 bins) and profile with the ESI-IM-MS data. The drift profile for both ionisation techniques was approximately 20 bins, or 0.9 ms, wide at the base of the peak and symmetrical in shape confirming the application of DESI for the direct analysis of the additive did not affect the post ionisation ion mobility separation. Extraction of the DESI mass spectrum from a 4 bin window (57-61 bins) again showed an improved response for the [M+H]^+ ion of 1 (Figure 2.6b). Removal of the chemical background noise improved the target analyte signal:noise from 140:1 to 176:1.
Figure 2.5: Analysis of antioxidant additive 1 by a) ESI-MS (60 scans averaged) and b) ESI-IM-MS (4 scans averaged). The insert shows the TWIMS mobility selected ion response for the molecular ion of 1.
Figure 2.6: Analysis of antioxidant additive 1 by a) DESI-MS (60 scans averaged) and b) DESI-IM-MS (4 scans averaged). The insert shows the TWIMS mobility selected ion response for the molecular ion of 1.

Tandem mass spectrometry was carried out on the protonated molecular ion of 1 using the ESI and DESI ionisation sources. Isolation of the [M+H]^+ ion was achieved by selecting m/z 219.5 in the resolving quadrupole. The m/z 219.5 was selected because it provided the cleanest selection of the precursor ion. Fragmentation was induced in the collision cell using CID before analysis of the product ions in the ToF. The trap collision energy was increased from 6 eV to 25 eV for ESI and 22 eV for DESI. The use of a lower collision energy for DESI was due to the reduced sensitivity of the ionisation technique and poor ion current stability. The higher collision energy
in DESI resulted in a very poor signal that made determining fragmentation patterns difficult. The product ion spectra, showing the precursor ion (m/z 220) and the product ions, are shown in Figure 2.7.

The product ion spectra in Figure 2.7 are consistent for both ESI and DESI, showing DESI did not affect the MS/MS spectrum of the target analyte. The relative intensity of the product ion at m/z 92 was slightly higher in ESI compared to DESI, which could result from the lower collision energy used in the analysis.
A proof of principal experiment was carried out using a sample of antioxidant 1 spiked into a commercial lubricating oil (supplied by Castrol) and analysed by DESI-MS/MS to determine if the analytical method could confirm the identity of lubricating additives present in a lubricating base oil matrix. The spiked oil sample was deposited onto PTFE (10 µL) and analysed using DESI source version 1.1 with an electrospray solvent of 95:5 MeOH:H₂O + 0.1 % formic acid at a flow rate of 20 µL/min. Isolation of the precursor ion was carried out using the resolving quadrupole set to m/z 219.5 and fragmentation induced using a trap collision energy of 22 eV. Three replicate samples were analysed in the same acquisition. Figure 2.8 shows the SIR for the protonated molecular ion of antioxidant 1 for the 3 replicate samples and the associated MS and MS/MS mass spectra. No carryover was observed between each replicate sample. A high level of chemical noise resulting from the lubricating base oil and other chemical additives used in the commercial formulation can be observed in the DESI-MS mass spectrum (Figure 1.8b). However, a strong response for the [M+H]⁺ ion of 1 can be observed at m/z 220. The product ion mass spectrum of m/z 220 from the DESI-MS/MS analysis of the spiked oil sample (Figure 2.8c) can be compared to that observed from the analysis of the standard sample of 1 (Figure 2.8b). The product ions generated and their relative intensities in both the standard and spiked samples closely match confirming the absence of unresolved or isobaric species at the same m/z to the protonated molecular ion species of 1 in the oil sample. These results highlight the potential of the technique for the direct and rapid detection of lubricant additives from a commercial product with no sample preparation.
Figure 2.8: The DESI-MS and DESI-MS/MS analysis of a commercial lubricating oil spiked with antioxidant 1 showing a) the SIR for the [M+H]$^+$ ion of 1 for 3 replicate samples analysed in the same acquisition, b) the resulting mass spectrum and c) the MS/MS product ion spectrum for the CID fragmentation of m/z 220.
DESI source version 1.2

The DESI source version 1.1 enabled the successful desorption and ionisation of several commercial additive standards from inert surface materials to be demonstrated. However, the Waters cone gas nozzle limited the DESI sampling area that could be used on the target surface to the outer edge closest to the orifice, reducing the area for which surface interrogation was possible. To overcome this problem a new mass spectrometer inlet system was designed and constructed to incorporate an ion transfer tube, or a ‘sniffer’, for non-proximate DESI-MS analyses. Figure 2.9 shows photographs of the standard mass spectrometer inlet system, highlighting the cone gas nozzle and the sampling cone. The inlet consists of two stainless steel cones that secure to the z-spray source block. The cone gas nozzle acts to control the flow of cone gas (N\textsubscript{2}) between the two cones and to protect the inner sampling cone. The inner sampling cone has a small orifice (0.3 mm) that maintains the pressure within the source block and guides ions into the mass spectrometer. Both cones are in contact with the source block and therefore have the same applied voltage and temperature.

Figure 2.9: Mass spectrometer inlet system attached to the z-spray source block showing a) the cone gas nozzle and b) a rear view of the sampling cone fitted inside the cone gas nozzle.
The modified cone system design with an ion transfer tube is based on the two-cone inlet system. Extension of the sampling site away from the front of the mass spectrometer was achieved using a custom built cone that replaces the cone gas nozzle of the Synapt HDMS z-spray interface, a schematic diagram is shown in Figure 2.10. The non-proximate DESI cone was fitted with a Swagelok adaptor that enables 1/16\(^{\text{th}}\) stainless steel tubing of various lengths to be fitted to the mass spectrometer inlet, which acts as an ion transfer tube. The standard inner sampling cone on the Waters Synapt HDMS instrument fits within the custom made cone to maintain the vacuum of the mass spectrometer. A photograph of the modified cone system is shown in Figure 2.11. Pre-cut stainless steel with an external diameter of 1/16\(^{\text{th}}\) " and internal diameter of 0.04" in 5, 10 and 20 cm lengths were purchased to act as ion transfer tubing and secured to the Swagelok fitting of the modified outer cone using a graphite ferrule. The voltage applied to the z-spray source block was transferred along the length of the ion transfer tubing. However, the conductivity of heat along the tube was not highly effective and the temperature at the tip was approximately room temperature.

**Figure 2.10: Schematic of the custom built non-proximate DESI cone system**
The ability to extend the sampling point of the DESI-MS away from the source block and instrument housing reduced the structural constraints of the source design and enabled the potential for larger objects to be sampled. Figure 2.12 shows DESI source version 1.1 fitted with the modified cone and ion transfer tubing (version 1.2). A 15 cm ion transfer tube is connected to the modified outer cone to extend the sampling point away from the source block and instrument housing for the DESI-MS analysis of larger objects (Figure 2.12a). In Figure 2.12b, a 5 cm ion transfer tube is shown with a protective PTFE sleeve on the tip for the analysis of metal sample surfaces. The PTFE sleeve prevents the ion transfer tube coming into direct contact with the metal target, which stops the transfer of the source block voltage onto the conductive surface and reduces the risk of arcing between the capillary tip and the ion transfer tube.
Figure 2.12: DESI source version 1.2 with in-house designed non-proximate DESI cone system fitted with a) a 15 cm ion transfer tube to extend the sampling point away from the instrument housing and b) a 5 cm ion transfer tube with protective PTFE sleeve for the analysis of metal surfaces.
An overall increase in sensitivity was observed when using the non-proximate DESI cone system compared to the Waters cone system for DESI-MS studies with 0-15 cm ion transfer tubing in place. The 20 cm stainless steel tube did not permit effective ion transfer to the mass spectrometer due to vibration at the tip as a combined result of the nebuliser gas flow and instability of the stainless steel tubing with the increasing length, reducing the collection efficiency. Figure 2.13 shows the mass spectra (60 scans averaged) for the DESI-MS analysis of 2 deposited onto a filter paper surface (10 µg in MeOH) and analysed using an electrospray phase of 95:5 MeOH:H₂O at 10 µL/min with the standard Waters cone inlet (Figure 2.13a) and the DESI cone with a 5 cm extender (Figure 2.13b). The data for all ion transfer tubing lengths is shown in Figure APP 1.1. The increase in sensitivity for all ions is attributed to the closer proximity between the mass spectrometer inlet and sample surface achieved with the modified DESI cone, reducing the transfer distance required for the analyte containing secondary droplets formed during the splashing phase of the DESI mechanism. The deprotonated molecular ion of 2, [M-H]⁻, can be clearly seen in the mass spectra showing the successful transmission of the target analyte ion through the stainless steel tubing, even when the tubing was at room temperature. An increase in background ion response is also seen with the addition of the modified cone and ion transfer tubing which could result from contamination occurring directly from the stainless steel tubing, preferential transmission or unknown interaction of ions as they pass through the ion transfer tube. The source of the ions requires further investigation to enable confident identification.
The modified DESI cone fitted with the 5 cm ion transfer tube and PTFE sleeve enabled the direct interrogation of metal surfaces with reduced safety concerns compared to the standard Waters cone system. Lubricant oil additives are used within tribological systems that consist primarily of metal components located in close proximity, and function at the point of interaction on the surface. The application of DESI as a direct surface analysis technique was therefore required to determine additive composition on metal targets with no sample preparation. The corrosion inhibitor/friction modifier additive (3), described as a surface-active compound, was deposited onto filter paper and metal (earthed) surfaces for analysis by DESI-MS to test the capabilities of the technique. The structure of compound 3 is provided in Figure 2.1. The standard was dissolved in MeOH (2 mg/mL) and deposited (2 µL) onto the target surfaces to give 4 µg additive on spot. The sample spot size on the metal surface was in the range of 4 to 10 mm in width, which was larger than the filter paper due to the reduction in surface tension of the sample droplet on the target surface. DESI-MS analysis was carried out in the positive ion mode with an electrospray phase of MeOH + 0.1 % formic acid at a flow rate of 20 µL/min for filter
paper and 5 μL/min for metal. A reduced solvent flow rate was applied for the analysis of the metal coupons to limit the volume of solvent present on the surface during the wetting phase of the DESI mechanism. This has two functions, to prevent any ‘washing’ of the surface and reduce the potential risk of arcing between the high voltages applied to the electrospray tip and the conductive metal surface. Figure 2.14a shows the SIR for the [M+H]^+ ion of 3 deposited on the metal surface as the sample spot is passed under the electrospray solvent flow. The 2D DESI-MS image of 3 on the surface is shown in Figure 2.14a. The fluctuation in the DESI ion profile of the protonated molecular ion of 3 when the sample was passed under the electrospray is due to inhomogeneous disposition of the sample on the surface, sample depletion and movement of the surface under the electrospray tip. The two peaks in the profile correspond to accumulation of the sample at the edge of the sample spot, known as the coffee-ring effect where the concentration of the sample is greater at the outer edge of the deposited spot following solvent evaporation. No change in the mass spectra was observed when comparing an inert surface material (filter paper) to the conductive stainless steel (Figure 2.14b and 2.14c). Application of a voltage onto the target metal surface was investigated to see if an improved response could be achieved. The applied voltage in the range of 0-1500 V was the same polarity as the voltage on the electrospray probe, positive in this case, to encourage repulsion of analyte ions away from the surface and toward the mass spectrometer inlet. No variation in the sensitivity of the DESI-MS analysis was recorded with the application of a voltage, refer to the data shown in Figure APP 1.2, and sparks between the electrospray capillary tip and the surface was observed when the applied voltage was increased above 1500 V. As a result the metal surfaces were earthed for all subsequent analyses.
Figure 2.14: DESI-MS analysis of 3 using DESI source version 1.2 with a 5 cm ion transfer tube and PTFE sleeve for the interrogation of metal surfaces showing a) the SIR for the [M+H]* ion of 3 as the sample spot was passed under the electrospray and the DESI-MS mass spectra from the analysis of 3 deposited on b) filter paper and c) stainless steel.
DESI source version 1.3

DESI source version 1.2 enabled the successful desorption, ionisation and transfer of ions generated from sources remote into the mass spectrometer inlet for all targeted additives when deposited onto inert and conductive surfaces. The design was used for the analysis of a range of target analytes throughout the project, including the screening of multiple lubricant additives and the quantitative study of an antioxidant, whilst further hardware developments for the final DESI source were being undertaken. The problem with the design of DESI source version 1.2 was a lack of reproducibility resulting from variations in the mounting of the electrospray probe and sample stage. Both the electrospray probe and the manual sample stage manipulator were located on a table in front of the mass spectrometer housing, with the probe secured to by clamp stand. The use of the standard Waters electrospray probe (used for all ESI experiments) and the manner in which DESI source version 1.2 was located adjacent to the mass spectrometer required the complete dismantling of the source when not in use. The source configuration was therefore manually determined at the start of each experimental day leading to variations in the DESI source geometry, which affected the ionisation/desorption efficiency and sensitivity of the technique.

The problems associated with the daily construction of DESI source version 1.2 were apparent during participation in an intra-laboratory study conducted by the National Physics Laboratory (NPL) investigating DESI-MS intensity repeatability and consistency.\textsuperscript{27} The project, called VAMAS (Versailles Projects on Advanced Materials and Standards), was developed to assist in "supporting world trade in products dependent on advanced materials technologies, through International collaborative projects aimed at providing the technical basis for harmonized measurements, testing, specifications, and standards". The study was designed to determine the intra and inter-day intensity repeatability of DESI measurements obtained from both home-built and commercial sources using two standard materials; Rhodamine B and a sample of adhesive tape. A total of 20 laboratories participated in the study, which included 13 home-built and 7 commercial DESI sources. The standard materials and an experimental protocol were provided by NPL highlighting the important instrumental parameters and source geometry required for the investigation. The raw mass spectral data was sent to NPL for processing.
A summary of the experimental procedure is given below and illustrated in Figure 2.15:

1. DESI-MS analysis of Rhodamine B to determine absolute intensity repeatability involving the sequential analysis of 55 sample spots and 3 blanks.
2. DESI-MS analysis of a sample of adhesive tape to determine relative intensity repeatability (of 3 mass groups). This aimed to look at the intra-day repeatability and the intra-day consistency over an 8 day period.

Due to the demand for the Synapt instrument from other users and the problems with accurately reproducing the geometry of DESI source version 1.2 day-to-day, we were only able to generate results for the inter-day intensity repeatability study. The collective results from the study were published in Analytical Chemistry (E. Gurdak, F. M. Green, P. D. Rakowska, M. P. Seah, T. L. Salter, and I. S. Gilmore, Anal. Chem., 2014, 9603–9611), where Loughborough University is identified as Respondee 6 (R6). The absolute intensity repeatability (% RSD) from the analysis of 55 sample spots of Rhodamine B from each laboratory was used to calculate an average absolute repeatability of 49 % (Figure 2.16). The absolute repeatability...
determined for DESI source version 1.2 was 52% (R6), which is slightly higher than the average, but still consistent with the data obtained by the different participants. A large variation in the repeatability of the absolute Rhodamine B intensity was observed between laboratories (~10-140%), which is attributed to the efficiency of the DESI mechanism and changes in the atmospheric environment. Investigation into the variation between the individual sampling points showed three distinct trends, illustrated in Figure 2.17 where ion intensity is plotted against sample spot. The top graph shows variation in ion intensity consistent with movement of the sample under the electrospray tip, suggesting that the sample surface was not positioned in a horizontal and level manner with regard to the sprayer tip/sniffer. The middle graph shows some variation in the ion intensity that has a general increase or decrease over the course of the analysis, and the lower graph shows little variation, but with several large increases in ion response for some analytical spots that could be considered outliers. Loughborough was classified in the middle graph grouping (Figure 2.17), which had the best repeatability results. NPLs processing of the raw data generated results showing a general increase in analyte intensity, which could be attributed to the sniffer tube coming into contact with the sample surface leading to some levels of contamination. Due to the problems with the source construction and instrument demand it was not possible to participate in the inter-repeatability experiment on the adhesive tape over the required 8 days.
Figure 2.16: Absolute intensity repeatability for the inter-laboratory DESI-MS study of Rhodamine B showing the % RSD response for 55 sample spots analysed consecutively. Loughborough University can be identified as R6.\textsuperscript{27}
Participation in the VAMAS study showed that, with regard to absolute intensity repeatability, the DESI source design functioned with similar precision to alternative DESI sources, some of which were commercially developed. The current source design (1.2) however was not capable of repeatable source configurations and therefore not robust enough to maintain an acceptable level of intra-day repeatability.

DESI source version 1.3 aimed to improve repeatability and maintain inter-day DESI source geometry. The source design was based on a stainless steel support that secured directly to the mass spectrometer source block using the existing mountings for the ESI source housing on the instrument. This was fitted with an electrospray nebuliser and automated sample stage manipulator that were secured in place so that the whole unit could be removed from the mass spectrometer inlet, with no change in source geometry, when not in use. The electrospray nebuliser was mounted on the fixed cylindrical height and angle manipulator. Both the sample stage manipulator and the electrospray probe stand were secured to a base plate that had
horizontal movement relative to the mass spectrometer inlet to enable the incorporation of different lengths of ion transfer tubing and reduce any potential structural hindrances of the source. Having the electrospray probe holder and the sample stage manipulator fitted to the same base plate ensure that the distance and angle between the electrospray tip and sample could be maintained even when relocating the sampling point away from the mass spectrometer source block. A prototype of source version 1.3 was constructed in-house to ensure it was fit for purpose (Figure 2.18), which was used as a template for the final source. The final source is shown in Figure 2.19 (front view) and Figure 2.20 (top view). An external power supply (Brandenburg) was used to provide a voltage to the DESI sprayer taken from an Applied Biosystems Mariner mass spectrometer in the range of 0-2.5 kV. The nebuliser gas (N₂) was sourced directly from the laboratory’s nitrogen generator using a low purity nitrogen line and controlled using an external gas regulator.

Figure 2.18: In-house constructed prototype DESI source version 1.3
Figure 2.19: Front on view of the DESI source version 1.3
The incorporation of a remotely operated sample stage manipulator for x and y axis movement in DESI source version 1.3 had improved precision and accuracy of sample movement and positioning compared to the manual manipulator. In addition, movement of the sample could be controlled remotely reducing operator exposure to the solvent plume of the electrospray and the high voltages at the capillary tip during a DESI-MS analysis. The sample stage manipulator was constructed to have two key functions: an accurate and precise movement of the sample under the DESI electrospray plume for sample interrogation and a crude movement of the sample stage manipulator in a horizontal motion relative to the inlet of the mass spectrometer to enable variable lengths of ion transfer tubing to be attached to the non-proximate DESI cone system. Figure 2.21 provides a schematic for the sample stage manipulator.
Figure 2.21: Sample stage manipulator for DESI source version 1.3. a) shows a side view and b) shows a top down view.
Movement in the x and y dimensions was controlled using either NI-MAX or NI LabView software packages. The LabView software enabled greater automation of sample stage movement compared to NI-MAX, but the requirement for computer code meant writing individual motion pathways for uninform sample topographies would be time consuming. A motion pattern for the analysis of 6 sample spots on a standard metal coupon used for the screening of lubricant additives and replicate studies was written using LabView. A spotting template (Figure 2.22a) was developed to allow up to 10 µL of sample to be deposited reproducibility onto the metal coupon for each analytical target, with designated blank areas. The presence of six sample spots on one metal coupon enabled replicate analyses to be conducted without the need to remove the sample from the DESI source or even stop the electrospray flow, reducing potential sources of variation.

Figure 2.22: Spotting template for the analysis of 6 replicate samples deposited onto a standard metal coupon using LabView coding.

The LabView code and user interface corresponded to the specific sample deposition areas in the spotting template. The user was able to either target individual sample spots directly or run a series analysis that moved the sample stage in a serpentine pattern whereby all six sample spots and two blank areas on the metal surface were sequentially positioned under the electrospray probe for DESI analysis. Analysis of the metal coupon started with the edge closest to the mass spectrometer inlet and moved backwards along the surface to reduce any sample contamination resulting from the splashing phase of the DESI mechanism. A hold time for the analysis of the sample spot prior to movement of the sample stage could be selected in the use
interface. Figure 2.23a shows the user interface designed. Details of the LabView coding can be found in the appendix (APP 1).

![LabView user interface](image)

**Figure 2.23:** LabView user interface to control sample stage movement on DESI source 1.3 for the analysis of targeted sample spots deposited on a surface using the spotting template shown in Figure 2.22.

### 2.4.2 Effect of solvent composition and solubility on the DESI-MS response of corrosion inhibitors

DESI-MS is an alternative desorption/ionisation technique compared to the standard surface analysis techniques currently used by the studentship industrial partners, such as atomic force microscopy (AFM); SIMS and scanning electron microscopy (SEM). In DESI compounds are desorbed using an electrospray flow of charged solvent to extract analytes from the surface through solid-liquid extraction processes before secondary droplets are generated and analysed by the mass spectrometer. DESI-MS has the potential to generate alternative molecular information regarding the presence of surface-active lubricant additives that can supplement standard analytical techniques. However, in DESI the analyte response can be influenced by
both ionisation efficiency and solubility/dissolution of the target analyte in the electrospray solvent composition. The influence of solubility in the DESI mechanism was therefore investigated using a series of corrosion inhibitor additives, 4a, 4b and 4c, that have the same quaternary amine chemical functionality but differ in hydrocarbon chain length (Figure 2.1). These quaternary amines already have a positive charge and so will not have to undergo protonation, ensuring changes in DESI-MS response result only from solubility/dissolution differences. Equimolar stock solutions of the additives in 1:1 MeOH:H₂O were deposited onto cleaned metal coupons and analysed using 7 different electrospray phase solvent compositions. The solvent compositions were 8:2 toluene:MeOH, ACN, MeOH, H₂O, 1:1 MeOH:toluene, 1:1 MeOH:ACN, 1:1 MeOH:H₂O. A flow rate of 8 μL/min was found to be suitable for all solvent compositions and enabled full depletion of the target analyte from the surface within 3 minutes. Figure 2.24 shows the SIR of the M⁺ ion of 4c for six replicate analyses and two blank analyses. All samples were deposited onto the same metal coupon and the electrospray (1:1 MeOH:H₂O) was maintained at a constant flow rate throughout the analysis. The experiment was carried out within one acquisition using automated sample stage movement. Each peak in the SIR corresponds to movement of the sample stage so that a new sample spot was positioned under the electrospray for DESI-MS analysis, which is followed by depletion of the sample from the surface.

![Figure 2.24: SIR for the DESI-MS analysis of 4c (m/z 360.36) using an electrospray phase of 1:1 MeOH:H₂O at 8 μL/min. Six replicate samples were analysed in the one acquisition to show sample depletion profiles from the surface.](image-url)
The sample depletion profile and the peak area were investigated to determine changes in DESI response with the different solvent compositions. A similar depletion profile for the three target additives was observed when analysed using the different electrospray solvent compositions. Figure 2.25 shows the depletion profiles for 4c analysed using the electrospray phase 8:2 toluene:MeOH, ACN, MeOH and H₂O. A blank area of metal coupon was analysed for 1 minute (60 scans) before movement of the sample spot under the electrospray. The sample spot was not moved once positioned under the electrospray to enable depletion of the additive. All profiles display a rapid increase in DESI response following introduction of the sample under the electrospray that reduces as the sample is depleted. The rapid peak in response relates to desorption of the bulk of the additive from the sample spot during the solid-liquid extraction of the DESI-mechanism. The rate of extraction will reduce as depletion of the sample from the surface occurs creating a tail in the profile. This effect will result from a reduced dissolution rate and depletion of the upper layers of additive in the sample spot. The corrosion inhibitor additives are surface active compounds, meaning they function by binding to the metal surface, therefore, as the upper layers of the sample spot are removed the interaction of the additive with the surface may increase, reducing the extraction efficiency of the DESI process. A very rapid depletion of the additive from the sample surface was observed with the MeOH electrospray phase, with the SIR for the M⁺ ion returning to baseline within 1.4 minutes. The sensitivity of the DESI-MS method was lower for the 8:2 toluene:MeOH and H₂O solvent compositions (Figure 2.25a and 2.25d) compared to the ACN and MeOH (Figure 2.25b and 2.25c), and an increased amount of tailing is present. This result is associated with the poor extraction of the additive from the surface and problems with generating an electrospray. The high boiling point of water and the hydrophobicity of toluene are not conducive for the formation of an electrospray plume for DESI-MS, reducing the efficiency of the ionisation technique.
Figure 2.25: SIR showing the depletion profiles for the M’ ion of the corrosion inhibitor compound 4c deposited on a metal coupon and analysed using an electrospray solvent of a) 8:2 toluene:MeOH, b) ACN, c) MeOH and d) H2O. A blank analysis was carried out for the first 60 scans before movement of the sample under the electrospray plume.

The peak area under the SIR depletion profile for the additives was calculated for each electrospray solvent composition and compared to in-house determined solubility data. Figure 2.26 shows the mean peak area for the 3 replicate analyses. Increasing the polarity of the solvent generally reduced the overall DESI-MS response of the targeted corrosion inhibitors, which may be a function of the hydrocarbon chain in the molecular structure. The MeOH and ACN are the best in terms of DESI-MS sensitivity, possibly because they are the best electrospray solvents. In the presence of ACN and MeOH the sensitivity of the DESI-MS response followed a 4a>4b>4c trend, showing the smaller the hydrocarbon chain the more efficient the extraction of the compound from the surface. However this trend was not
apparent with the toluene:MeO solvent, which showed weaker responses with similar peak areas for 4a, 4b and 4c.

A poor correlation between the DESI-MS response and the in-house generated solubility data was observed. The poor correlation between response and solubility is because DESI response is determined not only by solubility but also other factors including activity at the liquid/solid and liquid/air interfaces and the rate of dissolution of analytes into the electrospray solvent. The rate of dissolution is described by the Noyes-Whitney equation:

\[
\frac{dm}{dt} = A \frac{D}{d} (C_s - C_b)
\]

Equation 2.1

Where \( m \) is the mass of dissolved material, \( t \) is time, \( A \) is the surface area of interface between dissolving analyte and solvent, \( D \) is the diffusion co-efficient, \( d \) is the thickness of boundary layer of solvent at the surface of the dissolving solvent, \( C_s \) is the mass concentration of substance on surface and \( C_b \) is the mass concentration of substance in bulk of solvent.

The solubility for each analyte in a particular solvent composition was determined through the use of the saturation point method, where the compound was dissolved in a known quantity of solvent until the solution became fully saturated. An aliquot of saturated solution was extracted and the solvent left to evaporate before weighing the remaining compound so that the solubility (g/mL) could be calculated. Using the saturation point method of generating solubility data means the rate of dissolution, calculated using equation 2.1, will not influence the result as \( t \) is not a limiting factor for the concentration ratio of \( C_s \) and \( C_b \) to reach equilibrium. However, in DESI, the rate of transfer of molecules from the analyte surface into the solvent film formed in the wetting stage of the mechanism depends on the compound-dependent diffusion coefficients of target analytes (D) and the concentration gradient \( (C_s - C_b) \) at the surface boundary layer. The DESI solvent flow will determine the rate at which solvent is added to the surface solvent film and causes mixing on the surface, both of which will impact the concentration gradient facilitating the transfer of target analytes. The spray temperature affects the diffusion coefficient and solubility of the analytes and hence in DESI the extraction efficiency for different compounds.
The optimum electrospray solvent for the DESI-MS analysis of the corrosion inhibitor additives was 1:1 MeOH:ACN because of the better reproducibility compared to ACN alone (% RSDs = 31 % for 1:1 MeOH:ACN and 52 % for ACN for n=3. Refer to Figure APP 1.3 for additional information). Spiked oil samples containing equimolar amounts of the corrosion inhibitor additives were analysed using the optimized electrospray solvent composition. Extraction of the additives from within the oil matrix by DESI directly from the surface was achieved with no apparent loss in sensitivity. The depletion profiles of the additives were similar when present on the surface as a standard and in the base oil (Figure 2.27).

Figure 2.26: Graph showing the mean DESI-MS response (SIR peak area) for the analysis of equimolar amounts of the corrosion inhibitor additives 4a, 4b and 4c deposited onto a metal coupon and analysed using different electrospray solvents. The in-house determined solubility of the compounds in the solvent compositions at 28 °C has also been plotted.
Figure 2.27: SIR showing the depletion profiles of the corrosion inhibitor additive 4c deposited as a standard and spiked into an oil matrix onto a metal coupon and analysed by DESI-MS using an electrospray solvent of 1:1 MeOH:ACN. A blank analysis was carried out for the first 60 scans before movement of the sample under the electrospray.
2.4.3. DESI-MS analysis of test samples
The development of the DESI source version 1.3 was intended for the direct analysis of lubricant additives present on tribological components with no sample preparation. The nature of DESI as an ionisation technique enables in situ analyte analysis and therefore has the potential for localization of additives on sample surfaces, which can provide information regarding additive deposition and functionality. Three test samples were selected to assess the capabilities of the DESI-MS method. Test sample 1, prepared in house, contained ~ 0.5 µg of the corrosion inhibitor additive 4c deposited onto a metal coupon in three distinct areas. The sample was then moved along the x-axis at a rate of 200 counts/s while a continuous DESI-MS analysis of the surface was carried out. The sample was moved 1000 counts (equivalent to ~1.5 mm) in the y-axis and the analysis repeated. The optimised electrospray solvent (1:1 MeOH:ACN) determined in section 2.4.2 was used for the desorption of 4c. The aim of the experiment was to determine the capability of the DESI-MS method to image additive deposition. Figure 2.28 shows the distribution of 4c on the surface and an intensity map for the M+ ion of 4c determined by DESI-MS. The intensity map shows the x and y dimension of the metal coupon and the DESI-MS response of the M+ ion of 4c as a colour heat map. A strong correlation between the DESI-MS intensity map and the deposition of the sample on the surface can be observed. The ‘gaps’ in the intensity map are due to movement of the sample in the y axis. Between each DESI-MS analysis of the sample in the x direction the sample was moved ~ 1.5 mm in the y direction, which resulted in a space between the analytical lines that was not interrogated by DESI-MS. This range could be improved by using a smaller movement in the y dimension to remove the gaps in the DESI intensity map and a smaller DESI spot size. However, the results show the potential for DESI-MS in imaging targeted lubricant additives present on metal surfaces.
Figure 2.28: a) Deposition of corrosion inhibitor 4c on a metal coupon for additive imaging analysis by DESI-MS and b) DESI-MS intensity map for the $M^+$ ion of 4c.
The DESI-MS imaging experiment was repeated using a wear test coupon supplied by Castrol (sample 2). In a wear test a range of additives/lubricant formulations are deposited onto a metal surface before it is subjected to accelerated wear using secondary metal surface. DESI-MS generates molecular information of compounds present on the surface that can be used to monitor changes in additive composition during this process, such as chemical breakdown or secondary reactions. The wear coupon was washed using solvents and stored in a plastic bag before analysis by DESI-MS. Figure 2.29a shows the wear coupon located under the electrospray nebuliser and ion transfer tube in DESI source version 1.3. The sample was analysed along the x-axis in a continuous motion so that both the surrounding area and the area subjected to wear were sampled to detect changes in chemical composition. The analytical lines from the DESI-MS analysis of the sample can be seen in the image. Between each analytical line, the sample was moved 100 counts in the y-direction to ensure there was no un-sampled area. No change in the chemical composition of desorbed analytes between the un-treated and worn surface could be detected by the DESI-MS method as shown in Figure APP 1.4. However this is likely to be due to the washing and storage of the sample which removed all traces of additives from the surface that could undergo desorption and ionisation by DESI.

Sample 3 was an engine valve that had been subjected to a combustion test before DESI-MS analysis. The underside of the engine valve, which came into contact with lubricating oil, was analysed using a range of different electrospray solvent compositions: 8:2 toluene:MeOH, ACN, MeOH, H₂O, 1:1 MeOH:toluene, 1:1 MeOH:ACN, 1:1 MeOH:H₂O. A blank metal surface was analysed before the engine valve was positioned under the electrospray and interrogated by DESI. The sample was not moved during the analysis. This was repeated for each of the electrospray solvents. Figure 2.29b shows a picture of the valve inside the DESI source. The red dots indicate the different sampling positions for the electrospray solvent compositions. The use of H₂O as the electrospray solvent extracted an unidentified ion at m/z 441.33 from the surface. Figure 2.30 shows the mass spectrum from the analysis of sample 3 using H₂O as a solvent and the SIR for m/z 441.33 showing the depletion of the ion from the surface. The other solvent compositions were unsuccessful in desorbing and ionising compounds from the engine valve surface and showed no change in mass spectral response relative to the blank. This could be due to combustion of the lubricant additives causing thermal degradation of the
compounds present on the surface to below the limit of detection of the DESI-MS method.

![Figure 2.29: Picture of a) the wear sample (sample 2) and b) the engine valve (sample 3) for DESI-MS analysis.](image)

Figure 2.29: Picture of a) the wear sample (sample 2) and b) the engine valve (sample 3) for DESI-MS analysis.

![Figure 2.30: a) SIR for the m/z 441.33 ion desorbed from the surface of the engine valve (sample 3) by DESI-MS using H₂O as the electrospray solvent and b) the corresponding mass spectrum. A blank area of metal was analysed for the first 90 scans before movement of the valve under the electrospray.](image)

Figure 2.30: a) SIR for the m/z 441.33 ion desorbed from the surface of the engine valve (sample 3) by DESI-MS using H₂O as the electrospray solvent and b) the corresponding mass spectrum. A blank area of metal was analysed for the first 90 scans before movement of the valve under the electrospray.
2.5. Conclusions

The design and construction of DESI sources suitable for the direct analysis of oil additives and petroleomic samples was developed throughout the project. The successful desorption and ionisation of target analytes from a range of surface materials as standards and in the presence of an oil matrix has been demonstrated. Hyphenation of DESI with IMS and MS/MS has been shown to improve confidence with target analyte identification. Construction of the non-proximate DESI cone system and DESI source version 1.3 enabled the direct analysis of tribological components and overcame the repeatability problems associated with previous source designs, highlighted by participation in the NPL VAMAS experiment. Investigation into the effect of electrospray solvent composition on the sensitivity of the DESI-MS method for the analysis of a series of surface active corrosion inhibitor additives has been carried out. Solvent composition was found to affect the sensitivity and depletion profiles of the additives, but a poor correlation between additive response and solubility was observed. The results show that DESI-MS is capable for the direct analysis of petroleomic samples and has the potential for in situ additive deposition imaging experiments.
2.6 References


CHAPTER THREE

The Quantitative Surface Analysis of an Antioxidant Additive in a Lubricant oil Matrix using Desorption Electrospray Ionisation Mass Spectrometry.
3.1 Introduction

The chemical and physical environment found within tribological systems, especially engines, is subject to rapidly changing and often extreme conditions. The continuous movement of counterpart components, that are often located in close proximity to each other, generates friction and therefore heat. The elevated temperatures and high pressures, in combination with the presence of atmospheric oxygen, create an ideal environment for oxidation reactions to occur. The use of hydrocarbon based lubricants, which are susceptible to oxidation, within this environment can result in chemical degradation of the product.

Oxidation reactions of hydrocarbons happen in a three-step process; initiation, propagation and termination (Figure 3.1).\(^1\) The hydrocarbon is first attacked by either an atmospheric oxygen or a nitrogen peroxide which causes the formation of hydroperoxides (ROOH) and radicals (ROO· and R·). In the propagation stage the hydroperoxides breakdown, either on their own or in the presence of metal ions, to generate alkoxy (RO·) and peroxy radicals. The radical ions will readily react with additional hydrocarbons in the lubricant matrix to generate more radicals and oxygen containing species, such as carboxylic acids.\(^2\) Termination of the oxidation reactions occurs when the radicals either react with each other or with an oxidation inhibitor (InH).

The rate of oxidation within tribological systems will be impacted by a range of different factors including temperature, oxygen concentration and the presence or absence nitrogen oxides and metal ions.\(^2\) The oxidation process will have multiple effects on the formulation of the lubricant, which will affect its physical and chemical properties. The formation of oxygenated species such as carboxylic acids will reduce the pH of the oil, which can contribute towards corrosion within the system. Additionally the breakdown of the long hydrocarbon chains into smaller species will change the viscosity of the lubricant impacting how it is able to interact with the surface at the point of contact between two moving counterparts.\(^3\) When the lubricant is subjected to oxidation over time the viscosity of the formulation will increase leading to the formation of sludge.
Antioxidant additives are incorporated into lubricant formulations to help reduce the effects of oxidation on the bulk of the product. Antioxidants are commonly sulphur containing compounds, sterically hindered phenols or aromatic amines (Figure 3.2) that act in a sacrificial manner to break down the hydroperoxide species and react with the radicals to bring about termination of the oxidation process. There are two key mechanisms for the termination of oxidation by antioxidant species; primary antioxidants, such as phenols, are preferentially oxidised over the hydrocarbons to form stable radicals whereas secondary antioxidants, such as the sulphur containing compounds, will reduce hydroperoxides into less reactive alcohols.\textsuperscript{2,4} Commercial lubricants will all contain antioxidant additives to help prolong the life-time of the product.

\begin{align*}
RH + O_2 \rightarrow & \text{ROOH} \\
RH + O_2 \rightarrow & \text{ROO} \cdot + H \cdot \\
RH + \cdot NO_x \rightarrow & R \cdot + \text{HNO}_x
\end{align*}

\begin{align*}
\text{ROOH} \rightarrow & \text{RO} \cdot + \cdot OH \\
\text{ROOH} + Fe^{+2} \rightarrow & \text{RO} \cdot + OH^- + Fe^{3+} \\
\text{ROOH} + Fe^{+3} \rightarrow & \text{ROO} \cdot + Fe^{+2} + H^+ \\
\text{ROO} \cdot + RH \rightarrow & \text{ROOH} + R \cdot \\
\text{ROO} \rightarrow & R \cdot + O_2 \\
\text{RO} \cdot + RH \rightarrow & \text{ROH} + R \cdot \\
\text{OH} \cdot + RH \rightarrow & H_2O + R \cdot \\
\text{ROO} \cdot + \text{ROO} \rightarrow & RR + 2O_2 \\
\text{ROO} \cdot + \text{InH} \rightarrow & \text{ROOH} + \text{In} \cdot \\
\text{RO} \cdot + \text{InH} \rightarrow & \text{ROH} + \text{In} \cdot \\
R \cdot + \text{InH} \rightarrow & \text{RH} + \text{In} \cdot
\end{align*}

\textbf{Figure 3.1: Process of hydrocarbon oxidation, where In is an inert product used to form an inhibitor radical and terminate oxidation} \textsuperscript{2}
Figure 3.2: Structures of some common lubricant antioxidants

Analysis of lubricant oxidation can be carried out using different wet chemical tests that look at viscosity increase, total acid number (TAN) and physical changes in the product as oxidation progresses. While these tests are vital for assessing the levels of oxidation they do not directly measure the concentration of the antioxidant additive, but rather look at oxidation rate. Typically fourier transform infra-red (FTIR) is used to look at changes in antioxidant concentration over time.\(^1\) The application of mass spectrometry for the analysis of lubricant additives, including antioxidants, is becoming more routine. The mass spectrometric analysis of lubricant antioxidants has been demonstrated using techniques such GC-MS,\(^5\) LC-MS,\(^6\) ESI-MS,\(^7\) MALDI-MS,\(^8\) TLC-spray MS\(^9\) and ASAP-MS.\(^{10}\) These techniques have been shown to enable antioxidant detection and quantification.\(^5,6,9\) However, they can be time consuming and require sample preparation.

The DESI-MS approach enables antioxidants to be analysed directly from the active surface within the tribological system without having to remove the lubricant or extract out the antioxidant. The use of DESI-MS in quantitative analyses remains relatively unexplored, but has been reported for the analysis of complex matrices such as biological fluids,\(^11,12\) pharmaceuticals,\(^13\) foodstuffs,\(^14,15\) polymers,\(^16\) and cosmetic formulations,\(^17\) showing the potential of the technique. However, the application of DESI-MS to either the qualitative or quantitative analysis of a commercially available lubricant antioxidant directly from a lubricant oil matrix had not been previously reported.
3.2 Aims and Objectives

- Direct detection of a commercially available antioxidant additive present in a lubricant oil matrix with no sample preparation or extraction procedures using DESI-MS.

- Synthesis of a suitable internal standard to enable the quantitative analysis of the antioxidant additive in the lubricant oil.

- Evaluation of the quantitative capabilities of DESI-MS for the rapid and direct analysis of an antioxidant additive in the presence of a complex lubricant oil matrix with and without the use of an in-house synthesised internal standard.
3.3 Experimental

3.3.1 Reagents and chemicals

Methanol and water were purchased from Fisher Scientific (Loughborough, UK), hexane was purchased from Sigma Aldrich (Gillingham, UK). All solvents were HPLC grade. The base oil matrix (group one treated base oil) and an antioxidant additive octyl (4-hydroxy-3,5-di-tert-butylphenyl)propionate (2) were supplied by Castrol (Pangbourne, UK) for the analysis. Ethylene glycol monopentyl ether and concentrated sulphuric acid were purchased from Sigma Aldrich (Gillingham, UK) and 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid was purchased from Alfa Aesar (Heysham, UK) for the in-house synthesis of the internal standard.

3.3.2 DESI MS equipment and experimental conditions

The DESI-MS analysis was conducted on a Waters Synapt HDMS quadrupole-time-of-flight (Q-TOF) mass spectrometer (Waters, Manchester, UK) which consists of a quadrupole, trap, T-wave ion mobility and transfer stacked-ring ion guide regions and a time-of-flight mass analyser. The version of the DESI-MS source used for the quantitative analysis of 2 in a lubricant oil matrix was a prototype design developed during the DESI source construction (version 1.2), described in detail in Chapter 2, section 2.4.1. The standard ESI source housing for the Synapt HDMS was removed and the instrument was equipped with the custom-built outer cone. The standard capillary of the Synapt HDMS instrument was extracted from the ESI source and positioned on the DESI source using a clamp stand to generate the electrospray plume. The sample was mounted on a manual x,y,z sample stage manipulator and positioned under the capillary tip and ion inlet tube. An image of the DESI source used for this analysis is shown Figure 3.3.

The electrospray capillary was positioned at an angle of approximately 45 ° relative to the sample surface with an ESI tip to sample distance of ~ 3 mm. The sample was positioned horizontally to the mass spectrometer inlet with an inlet to sample distance of ~ 1 mm. Each sample was analysed in negative ion mode using an electrospray solvent of 95:5 MeOH:H\textsubscript{2}O at a flow rate of 20 µL/min. The instrumental parameters were: capillary voltage, -3 kV; sampling cone, 20 V; source temperature, 120°; desolvation gas (N\textsubscript{2}), 100 L/Hr; and trap collision energy 6 eV.
Structural confirmation of 2 in the oil matrix was carried out using DESI-MS/MS. Isolation of the precursor ion was achieved in the quadrupole and fragmentation was induced in the trap region using a trap collision energy of 35 eV. The observed product ion spectrum was compared to the spectrum obtained for a standard sample of 2 spotted in methanol on filter paper (Figure 3.7b and c).
3.3.3 Synthesis of internal standard (2a)
The internal standard 2-(pentyloxy)ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (2a) was synthesised via a Fischer esterification reaction. Ethylene glycol monopentyl ether (100 µL) and 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid (47 mg) were mixed in a HPLC vial and concentrated H₂SO₄ (1 µL) was added as a catalyst. A pierced lid was fixed onto the vial to enable water to escape from the reaction mixture as steam, and the sample vortexed. The reaction vial was then heated to 105 °C for 16 hours.

3.3.4 Sample preparation
Stock solutions were prepared by dissolving known weights of 2 (0.5-40 mg) in 1 mL hexane and spiking in 10 µL of 2a to give a nominal concentration of 6.6 mg/mL 2a. An aliquot of each standard solution (100 µL) was mixed with the base oil (400 µL). The resulting oil (10 µL) was spotted onto a filter paper surface to give deposited amounts of additive in the range of 1-80 µg of 2 per spot. The filter paper was secured onto a glass slide for support and positioned under the electrospray capillary on the sample stage. The sample was traversed under the electrospray in a horizontal motion perpendicular to the mass spectrometer inlet using the x,y,z-manipulator and data was acquired for a total of 1.5 minutes. A blank analysis of a filter paper surface without any sample was conducted before and after each sample to check for carryover. Six replicate analyses were conducted at each concentration of 2. The sensitivity of the method was determined by calculating the LOD from the absolute response of 2.
3.4 Results and discussion

3.4.1 DESI-MS analysis of 2 and 2a

The analysis of a commercial lubricant antioxidant additive, octyl (4-hydroxy-3,5-di-tert-butylphenyl)propionate (antioxidant compound 2), in a complex base oil matrix by DESI-MS was carried out in negative ion mode to generate the deprotonated molecules ([M-H]) of the target analytes, using 2-(pentyloxy)ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (2a) as an internal standard. The structures of 2 and 2a are shown in Figure 3.4. The modified DESI ion source (Figure 3.3) was found to have improved sensitivity compared to the standard spectrometer configuration, because the custom-built outer cone enables a closer proximity of the mass spectrometer inlet to the sample surface.

![Figure 3.4: Structures of octyl (4-hydroxy-3,5-di-tert-butylphenyl) propionate (2), a commercial antioxidant additive, and 2-(pentyloxy)ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (2a), an in-house synthesised internal standard.](image)

Successful desorption and ionisation of the antioxidant and internal standard was achieved for samples when deposited on a filter paper surface as standards, mixtures and in the presence of a lubricant oil matrix. The characterization of base oils and the detection of additives has been reported using both polar and non-polar electrospray phases with differing detection capabilities. The use of a polar electrospray phase, 95:5 MeOH:H₂O, and a negative mode analysis for the detection of the additive 2 in an oil matrix using DESI-MS was found to generate a mass spectrum that had little chemical noise resulting from the base oil matrix (Figure 3.5). This result is a combination of factors including the solubility of the target analytes in both the electrospray solvent and the oil droplet. Compounds 2 and 2a contain two distinct regions characteristic of lubricant additives, a polar head group and a non-
polar hydrocarbon chain, which have different chemical properties and functionalities. The polar head group contains the elements necessary to bind radical species and terminate the oxidation process, while the hydrocarbon tail enables solubility within the lubricant oil. For this study the lubricant oil matrix used was a group 1 treated base oil, derived from crude oil and formulated from a mixture of hydrocarbons. Group1 base oils used in lubricant formulations are defined as containing <90 % saturates, >10 % aromatics and > 300 ppm sulphur. The hydrocarbon based lubricating oil is not very soluble in the polar solvents used for the DESI-MS analysis. However, the presence of the polar head group on the additive and internal standard will enable solubility in the MeOH:H₂O electrospray phase. During the wetting stage of the DESI mechanism the polar electrospray phase will create a solvent droplet on top of the oil spot, from which the more polar antioxidant and internal standard will be extracted out of the oil and into this wetted area, leaving the insoluble bulk of the oil matrix on the surface. The solubility of the compounds in the oil droplet will therefore determine their extraction into the DESI electrospray phase. The two compounds have a relatively small hydrophobic region so are therefore likely to be located towards the outer edge of the droplet, rather than within the bulk of the oil, which will increase the rate of extraction into the electrospray phase. This preferential extraction of the target analytes reduces the complexity of the mass spectrum observed without the need for any sample preparation prior to analysis.

Figure 3.5 shows the DESI-MS mass spectrum obtained from the desorption of a spot containing 2 (10 µg) and the internal standard 2a (13 µg) in a base oil matrix deposited on a filter paper surface. The deprotonated molecule of the additive at m/z 389 (observed m/z 389.3062, calculated m/z 389.3056, 1.5 ppm error) and the internal standard (m/z 391) can be clearly distinguished from the chemical noise resulting from the oil matrix. An ion with m/z 387 was also observed in the mass spectrum, present in the standard solution of 2 and is tentatively assigned to the deprotonated molecule of the dehydro species of compound 2 (2'); a related product that is formed through the loss of two hydrogen atoms (observed m/z 387.2896, calculated m/z 387.2899, 0.8 ppm error).
Figure 3.5: Mass spectrum of the deprotonated molecular ion peaks of 2 (10 µg on spot) and 2a spiked into an oil matrix, spotted onto filter paper and analysed using DESI-MS in the negative ion mode. Compound 2’ is the dehydro version of 2, present in the standard solution of 2 used in the study.

3.4.2 DESI-MS/MS
Tandem mass spectrometry was used to confirm the identity of 2 at m/z 389 when spiked into the lubricant oil matrix. DESI-MS/MS analyses were conducted for a standard sample of 2 in MeOH deposited on a filter paper surface and for a sample of 2 spiked into the base oil matrix and spotted onto the surface. The [M-H]⁻ ion of 2 generated was isolated by the quadrupole and fragmentation induced in the trap ion guide section of the T-wave region using CID. The product ion spectra of the m/z 389.3 precursor ion of 2 observed when 2 was spiked into the base oil was compared to the spectra of the standard sample of 2, to confirm the absence of interfering ions resulting from the oil matrix.

The presence of 2’, the dehydro version of 2, in the standard sample of 2 lead to some difficulty when interpreting the MS/MS data. Due to the large isolation window of the quadrupole in the Synapt HDMS instrument, when used in the standard MS/MS mode, both species were transmitted into the collision cell to undergo
fragmentation. Figure 3.6a shows the ions transmitted through the quadrupole when using the instrument in MS/MS mode and selecting an isolation window of $m/z$ 389.3. The exact width of the isolation window is unknown and a default parameter of the instrument. Although the observed relative abundance of the deprotonated molecular ion of 2' was approximately 8% of that of 2, fragmentation of 2' was more easily induced using CID. The presence of 2' in the standard solution of 2, which was used to spike the lubricant oil matrix, resulted in the product ion spectrum being dominated by ions arising from the fragmentation of 2' (Figure 3.7a). Changing the isolation window of the quadrupole manually to $m/z$ 390.3 to ensure the [M-H]$^-$ ion of 2' was filtered out (Figure 3.6b) altered the observed MS/MS spectrum. Inducing fragmentation of the $m/z$ 389 precursor ion produced from the DESI-MS/MS analysis of 2 as a standard, using the isolation window $m/z$ 390.3 and CID (35 eV), generated the product ion spectra shown in Figure 3.7b. The product ion spectra for 2 when present in the lubricant oil matrix (Figure 3.7c) closely matched that of the standard confirming that the $m/z$ 389.3 ion observed in the spiked oil samples was the [M-H]$^-$ species of 2 and not a contaminant resulting from the oil matrix.

Figure 3.6: DESI-MS spectra showing the quadrupole isolation window at a) $m/z$ 389.3 and b) $m/z$ 390.3 for the analysis of 2.
3.4.3 Quantitative DESI-MS analysis of 2

The quantitative determination of the antioxidant 2 spiked into the oil matrix was carried out by DESI-MS using the relative mass spectral response of 2, at concentrations in the range 1-80 μg/spot, to the internal standard 2a (13 μg/spot). This corresponds to a concentration range of additive in oil of 0.1-8 mg/mL, with the internal standard present at 1.3 mg/mL. For each concentration of 2, in the presence of 2a and the oil matrix, six replicate sample spots were deposited onto filter paper and analysed by DESI-MS in the same acquisition run. Blank areas of the surface were analysed before and after each sample analysis, which demonstrated the
absence of background interference and sample-to-sample carry over. Figure 3.8 shows the SIR for the deprotonated molecular species of 2 and 2a, when 2 was present at 10 µg on spot. A very rapid response for both 2 and 2a was observed following introduction of the sample under the electrospray solvent flow, followed by a reduction in analyte response as the sample was depleted from the surface. The sample spot was then moved under the electrospray so that a new area could be interrogated. This process was repeated for the 1.5 minute acquisition time, covering a cross section of the spot, which was a representation of the whole sample deposited. The highly fluctuating ion current observed was a result of the continuous depletion and movement of the sample under the electrospray phase, inhomogeneous sample deposition on the surface and variations in the positioning and rate of movement of the sample under the electrospray during the analysis, which can impact the absolute response of an analyte (Figure 3.8a). This was particularly apparent when spotting and traversing of the sample surface was carried out manually as was the case in this experiment. The use of a suitable internal standard has been shown to help to overcome this problem in DESI quantitation. The response for both the target analyte and the internal standard are both influenced by fluctuation in ion current resulting from the DESI ionisation mechanism, sample deposition and analysis rate, therefore the internal standard can be used to account for some of this variation. Investigations into the suitability of different internal standards for quantitative surface analysis using DESI have been reported,\[29\] and key properties include similarity in the solubility of the internal standard and target analyte in the spotting solutions and the electrospray solvent. Additionally, similar proton affinities for the analyte and internal standard will reduce any potential ion suppression effects. In this study 2a, an analogue of 2, was synthesised and used as the internal standard to minimise structural and chemical differences between the analyte and internal standard. The internal standard retains the functionality around the aromatic ring present in the target analyte, with the substitution of CH$_2$ for an oxygen in the hydrocarbon chain (Figure 3.4). This structural modification is considered to have minimal impact on the physical and chemical properties of the molecule, such as the proton affinity.
Figure 3.8: The selected ion responses of a) 2 and b) 2a for the DESI-MS analysis of 6 replicate sample spots deposited onto a filter paper surface (10 μg 2 and 27 μg 2a in an oil matrix).

The calibration graphs for the absolute response of 2 and the relative response of 2/2a are shown in Figure 3.9. The calibration plot was produced by accumulating one minute of DESI-MS data for each sample and extracting the mass spectrum. The absolute response of 2 refers to the mean mass spectral intensity of the [M-H]\(^{-}\) ion of 2 derived from the six replicates at each concentration of 2 in oil. The relative response of 2/2a was calculated using a ratio of the [M-H]\(^{-}\) mass spectral intensities of 2 and 2a. Both calibration plots show linearity over the concentration range investigated, with R\(^{2}\) values of 0.989 and 0.994 for the absolute response of 2 and the relative response of 2/2a respectively. This demonstrates that the DESI ion source constructed in-house and used for the analysis, which was a prototype design with a manual sample stage manipulator, was capable of the quantitative assessment of the selected additive in an oil matrix. The use of the internal standard, however, not only improved the linearity but also the precision of the technique. The
error bars plotted on the calibration lines in Figure 3.9 are +/- two standard deviations. There is a large overlap between the error bars at the different concentrations of 2 in oil when plotting the absolute response of 2, showing the variability in the mass spectral intensity of the [M-H] of 2 between replicates. This is a result of DESI ion fluctuation, inhomogenous sample deposition and the manual movement of the sample under the electrospray probe, which can all affect the rate of sample depletion from the surface. There is a noticeable improvement in the precision of the technique when using the internal standard. Table 3.1 provides intra-day repeatability data for the six replicate analyses using the absolute response of 2 and the relative response of 2/2a. The % relative standard deviation (% RSD) for the 6 replicates when using an internal standard was in the range of 3-14 %, and without 15-44 %, with mean % RSD values of 3.23 % and 6.37 % respectively. An F-test has been carried out to determine if the difference in the precision of data obtained with and without the use of an internal standard is significant. The absolute response of 2 and relative response of 2/2a are in different units therefore it is not possible to perform a direct F-test on the standard deviations obtained. To overcome this, the amount of 2 on spot has been calculated using the respective calibration plots, at each concentration of 2 in the lubricant oil matrix for all 6 replicates, to generate data with the unit µg on spot. The standard deviations have been used to determine an F value for each concentration of 2 in oil using the following equation:

\[ F = \frac{s_1^2}{s_2^2} \]

Equation 3.1

Where s_1 and s_2 are the standard deviations for the amount of 2 on spot calculated using the absolute response of 2 and the relative response of 2/2a. Table 3.2 shows the calculated F values at each concentration of 2 in oil. The critical F_{5,5} value for a one-sided F test using P=0.05 is 5.05, all calculated F values exceeded this showing there is a significant difference in the precision of the DESI-MS technique with the use of an internal standard.

The calculated % RSD values for the quantitative assessment of a lubricant additive in an oil matrix is consistent with other reported values for quantitative DESI-MS, which fall within the range of 1-40 %. % RSD values for the quantitative assessment of target analytes within complex matrices by DESI-MS in recent literature include 1-17 % for polymer additives in a polymer matrix,^{16} 9-27 % for cosmetic ingredients in
authentic formulations,\textsuperscript{17} 4-17\% for pharmaceuticals in plasma\textsuperscript{11} and 10-31\% for alkaloids in plant tissue.\textsuperscript{19}

![Graph showing calibration plots for DESI-MS analysis.](image)

Figure 3.9: Calibration plots for the DESI-MS analysis of 2 in an oil matrix showing a) the absolute DESI-MS response of 2 and b) the relative response of 2/2a. The error bars plotted are +/- two standard deviations.
Table 3.1: Precision data for the quantitative determination of additive 2 in an oil matrix by DESI-MS both with and without the use of an internal standard (2a).

<table>
<thead>
<tr>
<th>Amount of compound 2 on spot (µg)</th>
<th>% RSD</th>
<th>Mean absolute response of compound 2</th>
<th>Mean relative response of compound 2/2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>17.4</td>
<td>13.8</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>14.7</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>43.8</td>
<td>5.9</td>
</tr>
<tr>
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<td></td>
<td>18.7</td>
<td>7.1</td>
</tr>
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<td>5.9</td>
</tr>
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</tr>
<tr>
<td>80</td>
<td></td>
<td>26.1</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 3.2: F-test data for the statistical analysis of precision data for the quantitative analysis of 2 with and without the use of an internal standard (2a).

$F_{\text{crit}}$ at $P = 0.05$ is 5.05.

<table>
<thead>
<tr>
<th>Amount of compound 2 on spot (µg)</th>
<th>Standard deviation for calculated amount of compound 2 on spot</th>
<th>$F_{\text{calc}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute response of compound 2</td>
<td>Relative response of compound 2/2a</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>0.1</td>
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<tr>
<td>4</td>
<td>4.0</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>0.8</td>
</tr>
<tr>
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<td>7.5</td>
<td>1.3</td>
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<tr>
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<td>14.0</td>
<td>1.4</td>
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<tr>
<td>60</td>
<td>13.0</td>
<td>3.2</td>
</tr>
<tr>
<td>80</td>
<td>25.0</td>
<td>5.3</td>
</tr>
</tbody>
</table>
The linearity of $2'$ was also investigated to confirm that the $m/z$ 387 response was associated with $2'$ in the standard sample of $2$ and not the oil matrix. Figure 3.10 shows the calibration plot for the relative response of $2'/2a$ generated in the same manner as for the plot of $2/2a$. A linear response of $2'/2a$ was observed, $R^2 = 0.991$, with % RSD values in the range of 9-32 % for the concentration range of $2$ investigated. The amount of oil deposited onto the filter paper surface was constant throughout the experiment, therefore the linear increase in the response of $2'$ as the concentration of $2$ spiked into the oil increased resulted from $2'$ being in the standard.

![Figure 3.10: Calibration plot for the DESI-MS analysis of $2'$, a related compound to $2$, showing the relative response of $2'/2a$ at different concentrations of $2$. The error bars plotted are +/- two standard deviations.](image)

The concentration of additives in commercial lubricant formulations is typically in the range of 0.1-5 % w/v. Traditional methods for the quantitative determination of antioxidant additives used in lubricants include HPLC-MS and GC-MS, where reported LODs are in the range of 0.2-100 ng/mL. $^{5,6,20,21}$ An ambient ionisation method called thin-layer chromatography-spray mass spectrometry has reported an
LOD at 20 mg/L for the semi-quantitative analysis of antioxidant additives in lubricant base oils,\textsuperscript{3} which corresponds to 0.002 % (w/v) of additive in oil.

The LOD for the DESI-MS method for the antioxidant additive 2 reported was calculated as the blank response of 2 plus three standard deviations of the blank using the absolute mass spectral response of 2, obtained from a section of the spot on the surface which was then related to the total spot size. The LOD was determined to be 11 ng/mm\textsuperscript{2} additive on spot, which relates to less than 0.7 μg ablated from the surface during the acquisition. This corresponds to < 0.03 % w/v additive in oil or 0.3 mg/mL. Although the LOD calculated is higher than that obtained by the more traditional methods used for the analysis of lubricant antioxidants, the DESI-MS method is sensitive enough to detect and quantify commercial additives in a native oil matrix with no sample preparation or time consuming chromatographic separations required.
3.5 Conclusions

The application of DESI-MS to the quantitative surface analysis of a lubricant antioxidant additive in a complex oil lubricant matrix has been demonstrated with good linearity and repeatability when using an internal standard ($R^2 > 0.99, \text{RSD} = 3\text{-}14\%$). Modification of the electrospray source on the Waters Synapt HDMS enabled the development of a robust DESI-MS system capable of DESI-MS analysis of surfaces. In the absence of an internal standard there is a correlation between the absolute DESI-MS response and the analyte concentration but with poor precision. The use of an internal standard minimized variations between DESI-MS runs caused by inhomogeneous sample distribution on the surface and other factors affecting the use of DESI-MS in quantitative measurements. The LOD for the additive in the oil lubricant was suitable for the typical levels of additive concentrations found in commercial lubricant products. The reported DESI-MS procedure has the potential for the quantitative determination of sub microgram quantities of compounds deposited on a surface in the presence of a complex oil lubricant matrix with the appropriate choice of internal standard.
3.7 References


CHAPTER FOUR
Electrospray Ionisation and Desorption Electrospray Ionisation Combined with High Field Asymmetric Waveform Ion Mobility Spectrometry-Mass Spectrometry for the Direct Analysis of Oil Additives used in Petroleum Processing.
4.1. Introduction

Chemical additives are blended into a wide range of chemical feedstocks and products to enhance application performance and mitigate adverse properties of the fluid. Such additives include detergents and dispersants, antioxidants and friction modifiers. A group of additives, described as surface active compounds, act as corrosion inhibitors by binding to the metal surface forming a protective layer between the metal and the fluids within the system, reducing the rate of oxidative corrosion. One class of corrosion inhibitors are oil-soluble quaternary amine complexes that are used in a wide range of petrochemical products and procedures.

The mass spectrometric analysis of lubricant additives from surfaces can provide information regarding the age, composition and degradation state of the formulation. A wide range of atmospheric pressure ionisation techniques, including atmospheric pressure photoionisation, matrix assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI) have been employed for analytical studies of oil samples. Direct ambient ionisation techniques allow for the rapid native state interrogation of samples with minimal sample pre-treatment. This can increase sample throughput and reduce the requirement for sample preparation prior to analysis. The analysis of oil samples by direct ambient ionisation-mass spectrometry has been demonstrated using easy ambient sonic-spray ionisation and venturi easy ambient sonic-spray ionisation for characterization studies and paper spray ionisation hyphenated with miniaturized mass spectrometry for in-situ additive detection. Desorption electrospray ionisation (DESI) is an ionisation method that uses an electrospray-generated solvent spray directed towards a target surface to desorb and ionize molecular analytes. This enables the rapid in situ analysis of compounds from a sample surface with little or no sample preparation. DESI-mass spectrometry (MS) has been used for the analysis of molecules present on a variety of surface materials such as polymers, paper, glass and metal. We have previously reported the application of DESI-MS to the quantitative determination of an oil antioxidant additive.

Chromatographic separation prior to MS is typically used for petrochemical analyses to simplify the data generated from the complexity of oil samples and liquid chromatography, combined with ESI and mass spectrometry, is a powerful method for the quantitative determination of additives in oils. Ultra-high resolution and accurate mass instrumentation, such as Fourier transform-ion cyclotron resonance-
mass spectrometry are widely used for characterization studies,\textsuperscript{21,22} although the complex spectra and high levels of chemical noise resulting from the oil matrix can mask the responses of additives. Alternatively, multi-stage sample preparation techniques can be used to fractionate or extract the additives from the oil prior to analysis.\textsuperscript{20} However, this is often time consuming and is not always suitable with direct ambient ionisation techniques such as DESI. In addition, the \textit{in situ} nature of DESI has the potential to determine the location of additives, such as the surface-active corrosion inhibitors, on tribological components to determine the additive activity and distribution.

Ion mobility spectrometry (IMS) and high-field asymmetric waveform ion mobility spectrometry (FAIMS), also known as differential mobility spectrometry, can be used to separate ions rapidly in the gas phase.\textsuperscript{23,24} In drift tube IMS, ions are separated in the presence of a weak electric field on the basis of collision cross section (CCS), which is related to the size and shape of the ion. In FAIMS, ion transmission is determined by differences in ion mobility in the presence of alternating low and high electric fields, which is dependent on the CCS and chemical characteristics of the ions. Hyphenation of IMS or FAIMS with MS therefore provides a rapid post ionisation separation of gaseous ions by differential ion mobility and mass-to-charge ratio, making the combined technique suitable for use with ambient ionisation methods such as DESI.\textsuperscript{25-27} The use of IMS with MS for the analysis of oils has been reported for the study of chemically related compounds within oils, oil characterization and petroleomics.\textsuperscript{28-31} FAIMS has been applied to the analysis of a wide range of analytes including proteins,\textsuperscript{32,33} biological samples\textsuperscript{34,35} and pharmaceutical impurities\textsuperscript{36} for both qualitative and quantitative approaches. The application of FAIMS-MS to the analysis of oils has been demonstrated for the characterization of naphthenic acids and the study of crude oil mixtures.\textsuperscript{37,38} FAIMS was used to separate naphthenic acid structural isomers enabling accurate elemental composition and structural elucidation and simplify the mass spectral response generated from highly complex crude oil.

The hyphenation of DESI with IMS-MS has been used for the direct analysis of native surface substrates, with little or no sample preparation, showing improved sensitivity for targeted analytes compared to DESI-MS alone.\textsuperscript{25-27} The use of DESI-FAIMS-MS has been reported for the analysis of counterfeit pharmaceuticals and the imaging of biological tissues\textsuperscript{39,40}, but the combined technique has not been applied to petroleum
samples. In this chapter we demonstrate the hyphenation of ESI and DESI with FAIMS-MS using a miniaturised FAIMS device for the targeted analysis of commercially available surface active corrosion inhibitors in the presence of an oil matrix. The corrosion inhibitors were analysed in solution by ESI and directly from steel surfaces using DESI ambient ionization.
4.2. Aims and Objectives

- Analysis of corrosion inhibitor additives used in petroleum processing and present in an oil matrix by ESI and DESI-MS with no sample pre-treatment.

- Hyphenation of DESI with FAIMS-MS.

- Evaluation of FAIMS as a post ESI/DESI ionisation separation technique for the analysis of corrosion inhibitors in a lubricating oil matrix.
4.3. Experimental

4.3.1 Reagents and chemicals

Methanol (HPLC grade) was purchased from Fisher Scientific (Loughborough, UK) and toluene (HPLC grade) was purchased from Sigma Aldrich (Gillingham, UK). The quaternary amine corrosion inhibitor standards; benzyldimethyldodecylammonium chloride (4a), benzyldimethyltetradecylammonium chloride (4b) and benzyldimethylhexadecylammonium chloride (4c) were purchased from Sigma Aldrich (Gillingham, UK) and were 99%, 97% and cationic detergent grade respectively. The structures are shown in Figure 4.1. A group 1 base oil was supplied by Castrol (Pangbourne, UK) for the analysis.

![Chemical Structure](image_url)

\[ n = 10 \text{ (4a)}, 12 \text{ (4b)} \text{ and } 14 \text{ (4c)} \]

**Figure 4.1: Structures of the benzyldimethylalkylammonium surface active corrosion inhibitor oil additives.**

4.3.2. Sample Preparation

The additive standards were prepared as equimolar mixtures in 50:50 MeOH:toluene. The additives were directly infused into the ESI source at a concentration of 183 ng/mL (4a), 198 ng/mL (4b) and 213 ng/mL (4c). For DESI analyses the additives were present in solution at a concentration of 183 µg/mL (4a), 198 µg/mL (4b), 213 µg/mL (4c), which corresponds to 1.83 µg (4a), 1.98 µg (4b) and 2.13 µg (4c) on spot (10 µL spot).

The oil/additive mixture was prepared by making stock solutions of the corrosion inhibitor additives: 37 µg/mL (4a), 40 µg/mL (4b) and 43 µg/mL (4c) in 50:50 MeOH:toluene. The stock solutions (10 µL) were spiked into 100 mg of group 1 base oil and the solvent left to evaporate to yield an oil/additive mixture with the additives present in the oil matrix at ~0.0004% w/w (equivalent to 4 ppm). The oil/additive mixture was diluted 1/200 in 50:50 MeOH:toluene for the direct infusion ESI analysis of the sample, giving a final concentration of 1.9 ng/mL (4a), 2 ng/mL (4b), 2.1 ng/mL (4c).
(4c) and 0.5 mg/mL group 1 base oil. The oil/additive mixture (5 mg) was deposited onto an earthed steel coupon (cold rolled, Grade 1008 1010, polished) for DESI-FAIMS-MS analyses so that the additives were present on the surface at 19, 20 and 22 ng on spot, corresponding to 0.33 ng/mm², 0.35 ng/mm² and 0.39 ng/mm² for a typical 57 mm² oil spot, for compounds 4a, 4b and 4c respectively. An oil blank was prepared for DESI-FAIMS-MS analysis by depositing 5 mg of unspiked oil on the metal surface.

4.3.3. Instrumental Parameters
The analysis of the corrosion inhibitor and oil samples was carried out using an Agilent 6230 time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, USA) fitted with either a modified JetStream ESI source or an in-house constructed DESI source, which is described in detail below. The mass spectrometer was operated in positive ion mode. A prototype miniaturised, chip-based FAIMS device (Owlstone Limited, Cambridge, UK) was located between the spray shield and the transfer inlet capillary of the mass spectrometer as shown in Figure 2. Nitrogen gas (99.5 % purity) was used for all gas flows including the carrier gas for the FAIMS chip. The samples were introduced into the source using direct infusion (10 µL/min) for ESI-MS and analyzed using the following experimental conditions: drying gas, 10 L/min at 100 °C; sheath gas, 12 L/min at 150 °C; nebuliser gas, 30 psig; capillary voltage, 3.5 kV; nozzle voltage, 2 kV; fragmentor voltage, 175 V.

4.3.4 DESI source construction and instrumental parameters
An in-house constructed DESI source was fitted to the inlet region of the Agilent 6230 TOF mass spectrometer to enable hyphenation of DESI with FAIMS-MS. The Agilent JetStream ESI source housing was removed from the mass spectrometer and the electrospray nebuliser was extracted. The nebuliser was then mounted in the ion source region of the instrument at an angle of ~ 55 ° to the DESI target surface, so that the tip was ~ 5 mm from the mass spectrometer inlet and ~ 2 mm from the target surface. An external power supply (Brandenburg voltage supply) provided ESI voltages in the range of 0 - 2500 V. Figure 4.2 shows a schematic of the ESI and DESI source configurations for FAIMS-MS analyses. A photograph of the in-house constructed DESI source is shown in Figure 4.3. The additive/oil samples were deposited directly onto steel sample coupons (10 µL for the standard mixture and 5 µL for the oil additive mixture) and the solvent left to evaporate (~30 seconds). The target surface was mounted on an automated x,y manipulator, with manual z-axis
control, secured to a platform attached to the front of the mass spectrometer housing, so that the sample was positioned under the tip of the nebuliser at the mass spectrometer inlet. Movement of the sample under the nebuliser was controlled using NI MAX and LabView software packages. Sample spots were analyzed in positive ion mode using the following experimental conditions: drying gas, 7 L/min at 150 °C; nebuliser gas, 30 psig; nebuliser voltage, 1.5 kV; capillary voltage, 3.5 kV; fragmentor voltage, 175 V; electrospray flow of 50:50 MeOH:toluene at 5 µL/min.

Figure 4.2: Schematic diagram of the TOF-MS interfaced with the miniaturized chip-based FAIMS using (a) the standard ESI source configuration and (b) the in-house constructed DESI source.
4.3.5 FAIMS instrumental parameters

The prototype miniaturized chip-based FAIMS device (Owlstone Ltd., Cambridge), located at the mass spectrometer inlet, has been described in detail elsewhere\textsuperscript{32,24} and consists of multiple planar electrode channels, each with a 100 µm gap and an electrode length of 700 µm. An asymmetric waveform dispersion field (DF) was supplied to the device through the modified source housing. The DF (in the range of 190-320 Td) was applied to the FAIMS chip using an approximate low to high field ratio of 2:1 at a 27 MHz frequency.

Optimum FAIMS conditions for the selective transmission of the corrosion inhibitors were determined by conducting a compensation field (CF) scans from -2 to 5 Td CF at a sweep rate of 0.5 Td/sec, at DFs in the range 190 - 320 Td at 10 Td intervals.
The CF voltages (DF 250 Td) for optimum transmission of the additives were determined to be 1.80 Td for ESI and 1.55 Td for DESI.
4.4 Results and discussion

The analysis of benzyldimethylalkylammonium surface active corrosion inhibitor oil additives (Figure 4.1; 4a-4c) was carried out using a time-of-flight mass spectrometer fitted with a miniaturized FAIMS device using both ESI and DESI as ionisation sources. The samples were prepared as mixtures, with and without an oil matrix, to evaluate the potential of FAIMS for the targeted analysis of the surface active compounds in a complex oil matrix.

4.4.1 ESI-FAIMS-MS studies of oil additives

An equimolar mixture of the additives was initially analysed by ESI combined with MS, which generated strong responses at \( m/z \) 304.30 (4a), \( m/z \) 332.33 (4b) and \( m/z \) 360.36 (4c), as shown in Figure 4.4.

![Figure 4.4: ESI-MS analysis of a mixture of corrosion inhibitor additive standards at 183 ng/mL (4a), 198 ng/mL (4b) and 213 ng/mL (4c).](image)

The corrosion inhibitors were spiked into an oil matrix to investigate the ESI-MS analyte response without a FAIMS separation. An unspiked oil mass spectrum showing the absence of the additive ions is provided in Figure APP 1.5. The resulting mass spectrum from the spiked oil sample (Figure 4.5a) shows the chemical profile resulting from the oil matrix generated by ESI. A typical mass spectral response for an oil based sample in the mass range \( m/z \) 200-500, is observed. The ion at \( m/z \) 360.36 is assigned to the \( M^+ \) ion of the additive compound 4c, but the additive ions
for compounds 4a and 4b are difficult to distinguish from the chemical background resulting from the matrix using ESI-MS alone.

The application of FAIMS ion selection to the analysis of complex mixtures has been shown to improve the relative analyte responses through the selective transmission of target ions and removal of background chemical noise. The oil/additive mixture was therefore analyzed by ESI-FAIMS-MS to optimize the parameters for the FAIMS-selected transmission of the additive ions. The FAIMS transmission characteristics of the corrosion inhibitor additives were investigated by stepping the dispersion field (DF) from 190-320 Td (at 10 Td intervals) and scanning the compensation field (CF) from -2 to 5 Td at each DF. The three additive ions had similar CF spectra (Figure 4.6), with maximum transmission CFs of 1.68 Td, 1.75 Td and 1.80 Td for compound 4a, 4b and 4c ions respectively at a DF of 250 Td. FAIMS separation is based on differences in ion mobility at low and high electric fields resulting from the interactions of ions with the FAIMS buffer gas and with water and other small neutral molecules present at trace levels in the FAIMS device, as well as other factors such as temperature, ion structure and conformation. The three inhibitors are all quaternary amines, which would be expected to have similar FAIMS characteristics and CFs for maximum transmission, with the alkyl chain length making a smaller contribution to FAIMS transmission. In contrast, other compounds present in the oil with different functionality and chain length may have maximum transmission at higher or lower CFs, allowing selectivity in the transmission of the additive ions. This is illustrated for two ions from the oil matrix, at m/z 331 and m/z 381, also shown in the CF spectrum (Figure 4.6), which have different CFs for optimum transmission. This results in the filtering effect of the FAIMS-selected transmission and the suppression of matrix ion responses.

A CF of 1.80 Td was chosen as the optimum for the FAIMS-selected simultaneous transmission of all three additives. Under these conditions the use of FAIMS resulted in a reduction in the response associated with the oil matrix and a relative enhancement in the compound 4a-4c ion responses (Figure 4.5b), which enabled the additive ions to be clearly observed in the mass spectrum, with the compound 4c ion as the base peak. This improved both the selectivity of the technique and also the sensitivity, for example there is a S/N improvement of 2.6 for corrosion inhibitor 4c ion compared to ESI-MS alone. The accurate mass of the M+ ion of compound 4c (m/z 360.3619 ion) is within 3.1 ppm of the expected mass and the accurate masses
of the $M^+$ ions of compound 4a ($m/z$ 304.2997, 2.38 ppm) and 4b ($m/z$ 332.3309, 2.48 ppm) are also close to the expected values.

Figure 4.5: Analysis of an oil/additive mixture (additives present at 4 ppm) using (a) ESI-MS and (b) ESI-FAIMS-MS (DF 250; CF 1.8 Td).
4.4.2 DESI-FAIMS-MS studies of oil additives

The equimolar mixture of corrosion inhibitor additives was spotted onto a metal surface and analyzed by DESI using the in-house constructed ion source. The resulting mass spectrum (Figure 4.7) provides the same ions as ESI (Figure 4.4), demonstrating that DESI could be used to successfully desorb and ionize these compounds from a metal surface.
The oil/additive mixture was then deposited onto a metal surface for analysis by DESI-MS and DESI-FAIMS-MS. The sensitivity of the DESI-MS analysis of the oil/additive mixture without FAIMS selection using the in-house constructed DESI source (Figure 4.8) was significantly lower than that observed by ESI and did not show the characteristic oil profile, which may be a result of differences between the ESI and DESI ionisation processes.

**Figure 4.7:** DESI-MS analysis of a mixture of corrosion inhibitor additive standards present at 1.83, 1.98 and 2.13 µg on spot for compounds 4a, 4b and 4c respectively.

**Figure 4.8:** DESI-MS analysis of an oil/additive mixture (additives present at 4 ppm).
The DESI process, which was discussed in Chapter 1, Section 1.2.2, is based upon a ‘solvent spray’ mechanism, which has features in common with ESI. In ESI, the sample is dissolved in a solvent and is passed through a high voltage capillary to produce an electrospray plume of charged solvent droplets containing analyte ions. In DESI, solvent from the electrospray flow forms a thin liquid film on the surface into which the analytes are extracted, before momentum transfer events generate progeny analyte-containing droplets. The processes leading to the generation of gaseous phase analyte ions from charged droplets for analysis by mass spectrometry, such as the charge residue and ion evaporation models, are expected to be the same for both techniques, therefore the differences between ESI and DESI spectra are determined by the extraction of analytes from the surface and the formation of analyte containing droplets. The extraction/desorption processes in DESI are complex and depend on a variety of different factors including surface activity at the liquid-solid and liquid-air interfaces, and the solubility of the components of the sample in the electrospray solvent (Section 1.2.2). The electrospray solvent composition used in this study (1:1 methanol:toluene) is likely to be a key contributing factor in the extraction of the oil matrix and imparts selectivity to the DESI spectrum. The solubility of the oil matrix in the electrospray solvent is necessary for both ESI and DESI. However, the efficiency and rate of transfer of molecules from the bulk of the oil droplet into the solvent film, not applicable in ESI, will also affect the DESI response and is determined by factors such as chemical composition, electrospray flow rate, temperature and the diffusion of molecules in the oil and solvent film.

The source geometry may also have an impact upon the generation of ions from the oil matrix. A study into the characteristics and mechanistic features of DESI has shown that the spray impact angle and the collection angle at the mass spectrometer inlet affect the ionisation efficiency and selectivity. In addition, it has been reported that the optimum spray impact angle and spray position is compound dependent (Figure 1.10, Section 1.2.2). It has been suggested that the differences in spray geometry result in changes in the ionisation mechanism, leading to the preferential ionisation of certain compound types. The difference in chemical structure and ionisation efficiency between the components of the oil matrix compounds and the quaternary amine corrosion inhibitor compounds could result in different geometric requirements, with the spray angle and distance used in these experiments more suited to the target additives rather than the matrix.
The contributions from these factors to the efficiency of the DESI mechanism for different molecules may explain the differences between the ESI and DESI spectra. However, a full understanding of the various contributions requires further work to observe the effect of DESI experimental parameters on specific compound classes present in oils.

The reduced chemical noise observed from the oil matrix is a potential advantage of DESI for the targeted additive analysis compared to the ESI, but despite the selectivity of DESI, the additive ions could not be confidently distinguished from the oil matrix (Figure 4.9a). The analysis was repeated using the in-house constructed DESI source positioned just in front of the FAIMS chip, which was located adjacent to the inlet capillary of the mass spectrometer (Figures 4.2b, 4.3). The sample platform was held in line with the mass spectrometer inlet, with the electrospray plume directed at the sample and angled towards the inlet capillary, so that the flow of desorbed ions was directed towards the FAIMS chip. The transient nature of the DESI response prevented the running of a full DF and CF scan to optimize the FAIMS parameters. The optimum DF (250 Td) determined in the ESI-FAIMS-MS analysis of the oil/additive mixture was therefore used for the DESI-FAIMS-MS analysis and a CF sweep of -2 to 5 Td (at 0.5 Td/sec) was carried out. Transmission of all three additives was achieved at a CF of 1.55 Td and all further experimentation was carried out in static mode, in which the DF and CF voltages were fixed (DF 250 Td, CF 1.55 Td). The optimum CF at a DF of 150 Td for the transmission of the additives was lower for DESI (1.55 Td) compared to ESI (1.80 Td). This is attributed to the lower temperature of the FAIMS chip using the open DESI source, which was at ambient temperature, whereas the closed ESI source was heated by the sheath gas. The source temperature influences the FAIMS chip temperature resulting in the lower CF for ion transmission with DESI. The influence of source temperature on the FAIMS transmission of target analyte ions has been demonstrated using ESI-FAIMS-MS by changing the drying gas temperature, which will determine the temperature within the chip. Figure 4.10 shows the SIR for the M⁺ ion of compound 4c analysed by ESI-FAIMS-MS using a drying gas temperature of 100 °C and 75 °C. As the temperature of the FAIMS chip is reduced the optimum transmission CF for the analyte ion is shifted to a lower Td. The same effect is observed when the ESI source housing is removed for DESI, exposing the chip to the ambient environment.

Analysis of the oil/additive mixture by DESI-FAIMS-MS generated an approximately 10 fold enhancement in the additive responses relative to the oil matrix ions as a
result of FAIMS-selected transmission (compound 4a, S:N 10; compound 4b, S:N 12; compound 4c, S:N 16) as shown in Figure 4.9b. The DESI-FAIMS-MS analysis of the unspiked base oil showed no responses for the corrosion inhibitor ions (Figure APP 1.5). The DESI-FAIMS-MS method is therefore demonstrated to allow the additive ions, undetectable without FAIMS selection, to be detected in the presence of the oil matrix present on a metal sample surface at low levels.

Figure 4.9: Analysis of an oil/additive mixture (5 mg oil with additives present at 19, 20 and 22 ng on spot for compounds 4a, 4b and 4c respectively corresponding to 4 ppm additive in oil) deposited on a metal surface using (a) DESI-MS and (b) DESI-FAIMS-MS (DF 250; CF 1.55 Td).
Figure 4.10: SIRs for the ESI-FAIMS-MS analysis of compound 4c using a drying gas temperature of 100 °C and 75 °C
4.5. Conclusions

This study demonstrates the application of FAIMS-MS, combined with ESI and DESI ionisation, for the targeted analysis of additives present at low levels in an oil matrix, using a series of surface active corrosion inhibitors as model compounds. FAIMS selection of target ions improved the sensitivity of ESI and DESI through enhanced analyte transmission and a reduction in the chemical noise resulting from the oil matrix. The analysis of the oil/additive mixture on a metal surface, replicating real life samples, is the first hyphenation of DESI with FAIMS-MS for the direct analysis of oil additives without sample preparation. A reduction in the oil matrix response was observed with DESI, compared to ESI, which is believed to result from mechanistic differences between the two ionisation techniques. The reduced matrix response highlights an additional advantage of DESI for targeted additive analysis. FAIMS is well suited to direct ambient ionisation techniques, such as DESI, where pre-concentration of analytes in complex samples is not always possible. The FAIMS-selected transmission of the additive ions provided a rapid post-ionisation sample clean up method to enhance the additive responses to a quantifiable level. The approach has potential for wider application to targeted and non-targeted analysis of oils and additives and for the imaging of tribological components to determine additive deposition and activity.
4.6. References


CHAPTER FIVE

Evaluation of Different Ionisation Sources for the Mass Spectrometric Analysis of Oil Additives
5.1 Introduction

A range of different techniques have been reported for the analysis of oil additives including scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDX), thermogravimetric analyses and mass spectrometry (MS).\textsuperscript{1–5} The use of mass spectrometry can generate highly detailed information regarding the chemical composition of lubricants and enable quantification of additives. Mass spectrometry is typically hyphenated with chromatography techniques, such as supercritical fluid chromatography,\textsuperscript{6,7} gas chromatography (GC),\textsuperscript{8} and liquid chromatography/electrospray ionisation (LC/ESI)\textsuperscript{9} to separate the additives from each other and the base oil matrix. However, these techniques are often time consuming and may require sample preparation, such as derivatization, prior to analysis.\textsuperscript{10,11} Additionally, removal of the sample from within the tribological system and from the surface at which they function is necessary, which can result in the loss of information that would be generated by the direct analysis of additives desorbed from surfaces. Ambient ionisation enables the direct analysis of samples by mass spectrometry with minimal, or no, sample preparation. Unlike other mass spectrometry ionisation methods that require the sample to be present in either a liquid or gaseous state, ambient ionisation techniques allow native state sample interrogation. A range of different ambient ionisation techniques had been applied to the analysis of lubricants and lubricant additives including matrix assisted laser desorption ionisation (MALDI),\textsuperscript{12,13} atmospheric solids analysis probe (ASAP),\textsuperscript{14} and desorption electrospray ionisation (DESI).\textsuperscript{15}

Direct analysis in real time (DART) is an ambient ionisation method that uses a heated flow of metastable nitrogen (N) or helium (He) gas to desorb and ionise target analytes directly from a surface.\textsuperscript{16} An electrical discharge from a needle electrode is used to create a plasma of nitrogen or helium that contains metastable species. This is directed to a sample deposited on a surface where ionisation of target compounds occurs primarily through Penning ionisation to yield gas phase analyte ions. Desorption of target analytes from the surface in DART is facilitated through both thermal desorption, as a result of the heated gas flow, and by energy transfer from the metastable atoms and molecules to the surface. The mechanism of DART has been discussed in more detail in Chapter 1, Section 1.2.3. The DART ionisation technique has been used to desorb molecules from a wide range of surfaces.\textsuperscript{17,18} The application of DART to target analyte determination has been reported for forensic, food and environmental samples,\textsuperscript{19–22} and the analysis of self-assembled monolayers.\textsuperscript{23,24} Hyphenation of DART with high performance thin layer
chromatography for the qualitative determination of the lubricant additive ZnDTP has been demonstrated. However, the application of DART to the direct qualitative and quantitative analysis lubricant additives directly from surfaces with no sample preparation has not been previously studied.

The direct analysis of target analytes from surfaces can be carried out using both DART and DESI. However, the desorption and ionisation mechanisms of the two techniques are very different. The mechanism in DART uses a plasma to induce chemical ionisation that is facilitated by thermal desorption. In contrast, DESI is an electrospray based ionisation technique that uses a flow of charged solvent droplets to extract analyte molecules from the surface through a “droplet pick-up mechanism”. The ionisation techniques have been used individually to detect a wide range of target analytes, and in a complementary manner to enable ions of a diverse chemical nature to be generated.

We report the application of DART-MS to the analysis of commercially available lubricant oil additives present on range of surface materials, both with and without an oil matrix. The influence of surface material and He gas temperature for qualitative targeted studies is discussed. The quantitative capabilities of DART-MS have been evaluated for the determination of the antioxidant additive in an oil matrix, which was carried out by DESI-MS and reported in Chapter 3. An untargeted study for the ESI, DESI and DART-MS analysis of a fully formulated lubricant oil is also presented to evaluate differences in the observed mass spectra using different experimental conditions.
5.2 Aims and Objectives

- Assess the suitability of DART for the direct analysis of compounds deposited on surfaces with minimal or no sample preparation in both a qualitative and quantitative manner.

- Evaluate the effect of surface material, DART gas temperature and the presence of a matrix on analyte response for the DART-MS analysis of targeted lubricant and oil additives.

- Investigate differences between the observed mass spectral response for the ESI, DESI and DART analysis of a fully formulated lubricant oil.
5.3 Experimental

5.3.1 Reagents, Chemicals and Materials

Cyclohexane, methanol, water (all HPLC grade) and concentrated sulphuric acid were purchased from Fisher Scientific (Loughborough, UK). Toluene and tetrahydrofuran (THF) were purchased from Sigma Aldrich (Gillingham, UK). The antioxidant additive octyl (4-hydroxy-3,5-di-tert-butylphenyl)propionate (2), a lubricating base oil matrix (group one treated base oil) and a fully formulated lubricating oil (Sample 1) were supplied by Castrol (Pangbourne, UK) for the analysis. Ethylene glycol monopentyl ether was purchased from Sigma Aldrich (Gillingham, UK) and 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid was purchased from Alfa Aesar (Heysham, UK) for the synthesis of 2-(pentyloxy)ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (2a). A series of structurally related quaternary amine corrosion inhibitor additives; benzyldimethyldodecylammonium chloride (4a), benzyldimethyltetradecylammonium chloride (4b) and benzyldimethylhexadecylammonium chloride (4c) were purchased from Sigma Aldrich (Gillingham, UK) and were 99%, 97% and cationic detergent grade respectively. The additive oleamide (3, ≥99.9 % purity) was also purchased from Sigma Aldrich (Gillingham, UK). The structures of all oil additives are shown in Figure 5.1. Filter paper (Whatman 541), glass and metal (steel, cold rolled, Grade 1008-1010, polished) surfaces were selected for analysis.

5.3.2 Synthesis of 2-(pentyloxy)ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (2a)

2-(pentyloxy)ethyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (2a), a related compound to 2, was synthesised via a Fischer esterification reaction as described previously. Ethylene glycol monopentyl ether (150 µL) and 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid (71.4 mg) were mixed in a HPLC vial and concentrated H$_2$SO$_4$ (~ 1 µL) was added as a catalyst. A pierced lid was fixed onto the vial to enable water to escape from the reaction mixture as steam, and the sample vortexed. The reaction vial was then heated to 100 °C for 6 hours.
5.3.3 Sample Preparation

Qualitative studies

Optimisation of the DART source and the investigation into the effect of surface material and He gas temperature on analyte response was evaluated using aliquots (10 µL) of ~2 mg/mL solutions of 2, 2a, 4a-4c, deposited onto the filter paper, glass or steel surface to give ~ 20 µg additive on spot. For the qualitative analysis of 2, a mixture of 2 (10 mg/mL) and 2a (nominal concentration of 13.4 mg/mL) was prepared and then diluted 1/5 in either cyclohexane or the base oil to give final concentrations of 2 mg/mL 2 and 2.68 mg/mL 2a. Stock solutions of 4a-4c were prepared in 1:1 methanol:water so that the additives were present at 1.8 mg/mL (4a), 2 mg/mL (4b) and 2.1 mg/mL (4c) in solution. To spike 4a-4c in the base oil matrix stock solutions of the additives in 1:1 methanol:toluene were prepared so that the additives were present at 180 mg/mL (4a), 200 mg/mL (4b) and 210 mg/mL (4c) before 10 µL of each solution was spiked into 930 µL base oil to give additive concentrations of 1.8-2 mg/mL in oil. Compound 3 was dissolved in THF (1 mg/mL) before deposition onto the steel surface and left to air dry. The sample of 3 on the steel surface was subsequently exposed to several solvent washes using cyclohexane, MeOH and toluene in which the surface was washed with the solvent before excess solvent was removed using a Kimwipe. Sample analysis by DART-MS was carried out between each wash. The fully formulated lubricant oil (sample 1) was analysed by ESI, DESI and DART. For direct infusion ESI-MS the sample was dissolved in 8:2
toluene:MeOH (10 µg/mL). For DART and DESI studies the sample was deposited neat onto a filter paper surface and analysed using He gas in the range of 100-300 °C (DART) and with an electrospray solvent composition of H₂O, 6:4 H₂O:MeOH and 6:4 toluene:MeOH (DESI).

**Quantitative studies**
Stock solutions of 2 were prepared by dissolving known weights (0.5-40 mg) in 1 mL cyclohexane and spiking in 10 µL of a solution of 2a in cyclohexane to give a concentration of 6.7 mg/mL 2a. An aliquot of each standard solution containing 2 and 2a (100 µL) was added to the base oil (400 µL), so that the additive was present in the oil at concentrations in the range 0.1-8 mg/mL. The spiked oil (10 µL) was spotted onto a filter paper surface to give deposited amounts of additive in the range of 1-80 µg of 2 per spot.

**5.3.4 Equipment and instrumental parameters**

**DART-MS**
A commercially available DART source (IonSense, MA, USA) was used for the analysis. The DART source was positioned 2.25 cm away from the mass spectrometer inlet at a 45° angle to enable interrogation of surfaces, Figure 5.2. The sample surface was positioned under the DART source, so that it was located ~ 1 mm below the mass spectrometer inlet and 5 mm below the tip of the DART source. A gas temperature (He) of 200 °C was found to be the optimum temperature for the desorption and ionisation of 2. However, for the analysis of 4a-4c the He gas temperature was varied in the range of 50-300 °C and maintained at 300 °C for the analysis of 3. The gas flow is determined by the gas temperature and therefore is not a parameter that could be set independently. The DART source was hyphenated with an Orbitrap Q Exactive Plus mass spectrometer (Thermo, MA, USA), operated in both the negative ion (2 and 2a) and positive ion (4a-4c) modes. The mass spectrometer instrumental parameters were: capillary temperature 250 °C, spray voltage 1.5 kV, scan range m/z 133-1000, resolution 140,000 and ACG target 1e6. For all experiments data were acquired for 1.5-2.5 minutes before inserting the sample into the DART source. For the quantitative study of 2, six replicates of each concentration of 2 in oil were analysed. Data was acquired for 2 minutes for each sample and the intensities of the deprotonated molecules of 2 and 2a used to calculate their relative response.
DESI-MS
DES1-MS analysis of the fully formulated lubricant oil (sample 1) was carried out using DES1 source version 1.3 (described in detail in Chapter 2, Section 2.4.1) fitted to a Waters Synapt HDMS instrument. The source was fitted with a 5 cm ion transfer tube and the configuration was electrospray tip-sample angle of ~ 55 ° and distance of ~ 3 mm, sample-MS inlet distance ~ 1 mm, nebuliser voltage 2.5 kV, nebuliser gas 80 psig, with an electrospray solvent flow rate of 10 µL/min. The mass spectrometer was operated in the positive ion mode using a cone voltage of 20 V, source temperature 120 °C, cone gas 30 L/Hr and trap gas 1.5 mL/min.

ESI-MS
ESI-MS analysis of sample 1 was carried out on the Thermo Orbitrap Q Exactive Plus mass spectrometer for high resolution mass analysis of the sample. The sample was directly infused into the mass spectrometer using a syringe pump at 10 µL/min.
The mass spectrometer instrumental parameters were: capillary temperature 250 °C, spray voltage 1.5 kV, scan range $m/z$ 133-1000, resolution 140,000 and ACG target 1e6.
5.4 Results and Discussion

5.4.1 Qualitative DART-MS for the targeted analysis of lubricant additives

The application of DART-MS to the direct analysis of a commercially available lubricant antioxidant additive (2), a series of corrosion inhibitors (4a-4c) and a friction modifier additive (3), deposited on a range of different surfaces as solvent standards and in an oil matrix, has been studied. The effects of surface material, matrix effects and DART gas temperature on the desorption profiles and molecular ion responses of the target analytes were evaluated. The DART source was positioned 2.25 cm away from the mass spectrometer inlet at an approximate angle of 45° to enable the direct analysis of surfaces. The samples were mounted on a platform located within the DART source to reduce variation in ion response that could result from changes in sample positioning and enable rapid sample throughput.

Antioxidant additive

The successful desorption and ionisation of the antioxidant additive 2 deposited on filter paper, glass and metal surfaces using DART-MS in the negative ion mode with a He gas temperature of 200 ° is shown in Figure 5.3. For each analysis, a 2 minute blank of the surface away from the sample spot was acquired before the introduction of the sample into the DART source. The desorption profile of the deprotonated molecular ion of 2 ([M-H], m/z 389) was monitored for ~ 18 minutes before the sample was removed. The desorption profile of the [M-H]⁻ ion of 2 was influenced by the target surface material. Analysed from the filter paper and glass surfaces, a strong response for the [M-H]⁻ ion of 2 was observed immediately after the sample was placed under the heated flow of metastable He gas, shown in Figure 5.3a and b. This initial response, resulting from the bulk desorption of 2 from the surface, generated the maximum response for the target analyte within a few seconds, and continued interrogation of the surface resulted in a steady depletion of the sample over the 18 minutes investigated. The desorption profile of the [M-H]⁻ ion of 2 deposited on the metal surface and analysed by DART-MS shows a different trend to the filter paper and glass surfaces (Figure 5.3c). The initial increase in response for [M-H]⁻ of 2 is more gradual, with the maximum peak intensity observed approximately 1.5 minutes after sample introduction into the DART source. The depletion of the sample from the metal surface resulted in the response for the [M-H]⁻ ion falling to 10% maximum intensity within 10 minutes of the first signs of sample depletion. The difference in desorption profile is likely to be a consequence of the thermal conductivity of the metal surface. Exposure of the metal surface to the heated gas flow of the DART source causes an increase in surface temperature. However,
conductivity of heat away from the sample spot on the metal surface may result in a lower rate of heating and reduced thermal desorption of 2 from the surface in the early part of the analysis. For all surface materials removal of the sample from the DART source resulted in the response of [M-H]⁻ of 2 returning to baseline levels within a few seconds.

Figure 5.3: Desorption profiles for the [M-H]⁻ ion (m/z 389) of the additive 3 deposited on a) filter paper, b) glass and c) steel surfaces and analysed by DART-MS. The corresponding mass spectra extracted from 2-10 minutes of the extracted ion chromatogram for d) filter paper, e) glass and f) steel surfaces are shown.

The effect of a mixture of two compounds and then that mixture spiked into an oil matrix on the DART-MS response of 2 was investigated by depositing a mixture of 2 and 2a onto filter paper and metal surfaces in cyclohexane, air drying and monitoring the desorption profiles for the [M-H]⁻ ions. Figure 5.4 shows an example of the depletion profiles of the of [M-H]⁻ ions of 2 and 2a deposited onto filter paper and analysed by DART-MS. The [M-H]⁻ ions for 2 and 2a showed the same depletion profiles as a result of the two compounds being chemically and structurally related and therefore having similar ionisation efficiencies and volatilities.
Figure 5.4: Desorption profiles for the [M-H]⁻ ions of a) antioxidant 2 (m/z 389) and b) compound 2a (m/z 391) deposited as a mixture on filter paper and analysed by DART-MS.

The mixture of 2 and 2a was then spiked into a base lubricating oil to investigate the potential of DART-MS for the direct analysis of lubricating oil additives without extraction of the additives from the oil matrix. Typically additive analysis is carried out using methanol extraction, before analysis by ESI or LC-MS, but this requires sample preparation steps that can be time consuming. DART offers the ability to rapidly analyse a sample deposited on a surface with no sample preparation, increasing sample throughput. Compounds 2 and 2a were both successfully desorbed and ionised by DART-MS in the negative ion mode in the presence of the oil matrix when deposited on filter paper and steel surfaces as shown in Figure 5.5. However, the presence of the oil matrix did effect the desorption profiles of the two analytes compared to desorption in the absence of the oil matrix. The oil matrix reduced the depletion rate of 2 and 2a from the surface and the analyte response was observed over a prolonged period of time. In addition, a small delay in the initial response for the additive was noted after the sample was placed into the DART source when in the presence of an oil matrix compared to the profile without the oil (Figure 5.3a and c). This is most likely to be a result of the thermal desorption of the oil matrix, which due to differences in volatility of the oil compared to the target analytes reduces the desorption rate for the additive. The resulting mass spectrum (Figure 5.5c), shows the deprotonated molecules for 2 and 2a as the most intense ions, with very little
chemical background resulting from the oil matrix because of preferential desorption and ionisation of the target compounds over the base oil during the DART process. This can be advantageous in reducing the complexity of the spectrum observed and improve selectivity for the target analytes when applying the DART technique to the direct analysis of additives present in a complex oil matrix.

**Figure 5.5:** DART-MS analysis of 2 and 2a in a lubricating base oil showing the SIR for the [M-H]⁺ ion of antioxidant 2 (m/z 389), deposited on a) filter paper and b) a steel surface and c) the resulting mass spectrum.

**Corrosion Inhibitors**

The series of corrosion inhibitor standards (4a-4c), that maintain the same functionality but differ in the length of the hydrocarbon R group, were deposited individually and as a mixture on filter paper, glass and steel surfaces for analysis by DART-MS. At He gas temperatures below 200 °C desorption and ionisation of the molecular ion of the quaternary ammonium compounds by DART was not achieved. However, strong responses at m/z 214, m/z 242 and m/z 270 for samples of 4a, 4b and 4c respectively were observed during the DART-MS analysis of the standards from all surface materials investigated (Figure 5.6). The observed ions correspond to the free protonated alkylamines for 4a ([CH₃(CH₃)₁₁N(CH₃)₂⁺H⁺]), 4b ([CH₃(CH₂)₁₃N(CH₃)₂⁺H⁺]) and 4c ([CH₃(CH₂)₁₅N(CH₃)₂⁺H⁺]) respectively, resulting from the common loss of the benzyl group (C₆H₅CH₂). The presence of the free
amine unreacted synthetic precursor could make a small contribution to the intensity of these protonated amines. However, the origin of these ions is most likely to be as a result of thermal degradation of the quaternary amine followed by gas phase protonation during the DART ionisation process. The free amine species were undetected using ESI and DESI ionisation methods (Section 4.4.1, Figure 4.4 and Section 4.4.2, Figure 4.7). Thermal breakdown of quaternary amines resulting in the loss of R groups attached the nitrogen is well documented. An ion at \( m/z \) 136 assigned to \([C_6H_5CH_2N(CH_3)_2+H]^+\) was observed in the mass spectra of 4a-4c as a common thermal decomposition product of the quaternary amine species. Ions were also observed at \( m/z \) 290, 318 and 346 in the mass spectra of 4a-4c (Figure 5.7), 14u lower than the expected mass for the M⁺ ions, the mass difference assigned to CH₂ by accurate mass measurement. The ions correspond to the loss of a methyl group followed by a subsequent protonation to generate \([4a-CH_3+H]^+\), \([4b-CH_3+H]^+\) and \([4c-CH_3+H]^+\) species. Similar fragmentation has been observed using techniques such as direct exposure chemical ionisation and field desorption for the analysis of quaternary ammonium salts. 

Increasing the He temperature of the DART gas enabled the detection of the corrosion inhibitor molecular ion species (M⁺) as weak peaks at \( m/z \) 304, 332, and 360 for 4a, 4b and 4c respectively, confirmed by accurate mass measurement, showing their successful thermal desorption from the surface. Molecular ion species were observed at He gas temperatures of ≥200 °C for glass (Figure 5.7) and ≥300 °C for steel (Figure 5.8). The molecular ion responses for the corrosion inhibitor additives are lower when desorbed from steel compared to glass which is likely to be due to differences in the thermal conductivity of the surface. The thermal desorption and subsequent mass analysis of intact quaternary amines is often difficult due to their poor volatility, but detection of quaternary ammonium salts by thermal desorption alone has been previously reported.
Figure 5.6: DART-MS analysis of corrosion inhibitor additives 4a-4c deposited as a mixture on a) filter paper, b) glass and c) steel surfaces using He gas temperatures < 200 °C.
Figure 5.7: DART-MS analysis of the corrosion inhibitor additive mixture deposited on a glass surface and analysed using a He temperature of 200 °C.

Figure 5.8: DART-MS analysis of the corrosion inhibitor additive mixture deposited on a steel surface and analysed using a He gas temperature of 300 °C.
The desorption profiles for the M⁺ ion of 4c and thermal fragment ions associated with the loss of the alkyl, benzyl and methyl groups from glass (Figure 5.9) and metal (Figure 5.10) are shown. The ions show a similar desorption profile to that observed from the DART-MS analysis of the antioxidant additive 2 (Figure 5.3b and c). The desorption profiles of the M⁺ and thermal fragment ions from the glass surface showed an initial increase in response after sample introduction into the DART source at 1.5 minutes which is followed by a steady fall in intensity as the sample is depleted from the surface. The free benzylamine ([C₆H₅CH₂N(CH₃)₂+H]⁺; m/z 136) and alkylamine ([CH₃(CH₂)₁₅N(CH₃)₂+H]⁺; m/z 270) ions show comparable profiles (Figure 5.9a and b). However, a slight delay in initial response is observed for the [4c-CH₃+H]⁺ and [4c]⁺ ions, which is attributed to a reduced initial rate of thermal desorption (Figure 5.9c and d). This is likely to be a surface temperature effect. Desorption from the steel surface showed a delayed initial response for the M⁺ and thermal fragment ions (Figure 5.10) that was also observed for the antioxidant additive (Figure 5.3c) and appears to be a surface material effect. The effect was more pronounced for the [4c-CH₃+H]⁺ and [4c]⁺ ions, with a delay of ~0.5 min, which suggests an increase in surface temperature following introduction of the sample into the DART source helps facilitate the thermal desorption process of the [4c-CH₃+H]⁺ fragment and the molecular ion species.

Figure 5.9: DART-MS desorption profiles for 4c and associated thermal fragments from a glass surface analysed using a He temperature of 200 °C, showing the SIRs for the quaternary amine and the thermal fragments of (a) ([C₆H₅CH₂N(CH₃)₂+H]⁺ (m/z 136), (b) [CH₃(CH₂)₁₅N(CH₃)₂+H]⁺ (m/z 270), (c) [4c-CH₃+H]⁺ (m/z 346) and (d) [4c]⁺ (m/z 360).
Figure 5.10: DART-MS desorption profiles for 4c and associated thermal fragments from a steel surface analysed using a He temperature of 300 °C, showing the SIRs for the quaternary amine and the thermal fragments of (a) \([C_6H_5CH_2N(CH_3)_2+H]^+\) (m/z 136), (b) \([4c-C_6H_5CH_2+H]^+\) (m/z 270), (c) \([4c-CH_3+H]^+\) (m/z 346) and (d) \([4c]^+\) (m/z 360).

The additive mixture was spiked into the base oil matrix and deposited on the steel surface before analysis by DART-MS using a He temperature of 300 °C, Figure 5.11. The presence of the oil matrix caused an elevated background which prevented the \(M^+\) ions from being distinguished from the chemical noise. Weak responses were observed for the [M-CH₃+H]⁺ fragment ions for compounds 4a-4c (Figure 5.11 insert) within the chemical background. However, the base peaks in the mass spectrum correspond to the protonated alkylamines that act as diagnostic fragments for the quaternary amine compounds. The ions are dominant in the mass spectrum and are not obscured by the oil matrix profile enabling the direct identification of corrosion inhibitor additives deposited on a steel surface and in the presence of an oil matrix by DART-MS using these thermal fragments.
Figure 5.11: DART-MS analysis of corrosion inhibitors present in a lubricating oil matrix and deposited onto a steel surface.

**Friction Modifier**

Compound 3 is a surface-active friction modifier used in a range of commercially available lubricant oil additives. The oleamide creates a layer on the surface that reduces friction at the boundary of two moving counterparts to minimise wear. The application of DART-MS to the direct analysis of 3 deposited onto a steel surface resulted in a strong response for the protonated molecule at \( m/z \) 282 (Figure 5.12a) showing the successful desorption and ionisation of the additive from the steel surface. The steel surface was then washed sequentially using cyclohexane, MeOH and toluene with analysis by DART-MS carried out between each wash. The mass spectra following each wash are shown in Figure 5.12. For all spectra the same number of scans have been accumulated to enable comparative data. A slight fall in the overall intensity is observed for the \([\text{M+H}]^+\) for 3 is observed following the wash stages, but other ions such as \( m/z \) 298 and 254, are preferentially removed from the surface, indicating a higher surface activity for 3 on the steel surface. These data
show that DART is suitable for the direct desorption and ionisation of active friction modifier oil additives present on steel and may also provide information on the surface activity following exposure of the sample to different solvents.

Figure 5.12: DART-MS analysis of 3 deposited onto a steel surface. The surface was first analysed by DART-MS (a) before being subjected to a series of solvent washes using (b) cyclohexane, (c) MeOH and (d) toluene with DART-MS analysis carried out between each solvent wash.

5.4.2 Quantitative determination of an antioxidant in lubricating matrix by DART-MS

The application of DESI-MS using an in-house constructed DESI source hyphenated with a Waters Synapt HDMS instrument for the quantitative analysis of the antioxidant additive, 2, present in a base oil matrix and deposited on a filter paper surface was discussed in Chapter 3 and a related publication. Here we present the
evaluation of DART-MS for the quantitative analysis of the antioxidant additive directly from the target surface. The antioxidant additive 2 was spiked into the oil matrix at concentrations in the range 0.1-8 mg/mL and the samples were deposited on to filter paper for analysis by DART-MS. Each acquisition consisted of the analysis of a blank region of the filter paper (2 min) followed by analysis of the area containing 2 and 2a in oil (n=6, each replicate analysed for 2 min). The data for the central 60 scans of the 2 minute sample analysis (total of 120 scans) was accumulated to generate the mass spectra of compounds 2 and 2a in oil. The relative intensities of the [M-H]⁻ ions for 2 and 2a were used to calculate their relative responses. Figure 5.13 illustrates an example of the analysis.

![Figure 5.13: a) Selected ion responses for the [M-H]⁻ ion of 2 present in a lubricating oil, deposited on filter paper (10 µg on spot) and analysed by DART-MS, showing a blank filter paper and six replicate samples. b) The resulting mass spectrum showing ions associated with the deprotonated molecules of 2 and internal standard 2a.](image)

The relative response of 2/2a was plotted against amount of additive deposited on spot to generate a calibration plot (Figure 5.14). Good linearity was observed for the DART-MS analysis of 2 in oil, $R^2 > 0.997$, for the relative responses of 2 and 2a. The addition of 2a, an analogue of 2, as an internal standard helped to minimise variation in relative ion responses that can arise from fluctuation in overall ion current that results from the DART-MS analysis of the surface (Figure 5.13a). The chemical and structural similarities between 2 and 2a, with the difference in the two molecules
being the substitution of oxygen for CH₂ in the hydrocarbon chain, makes 2a a suitable internal standard for the determination of 2, as shown by the closely matching desorption profiles for the two species (Figure 5.4). This use of 2a as an internal standard has been discussed in more detail in Chapter 3, Section 3.4.3. The precision of the technique was assessed by conducting replicate analyses to determine the % relative standard deviation (%RSD). The %RSD for the relative response of 2/2a was 2.6%. The use of an internal standard improved the linearity, %RSD and linear dynamic range of the experiment. Figure 5.15 shows the calibration plot for the absolute response of compound 2. The linearity and %RSD values for the absolute response of compound 2 were $R^2 = 0.991$ and 16.8% but the linear dynamic range ended at approximately 20 µg antioxidant on spot.

![Figure 5.14: Calibration plot for the DART-MS analysis of the antioxidant compound 2 deposited in the presence of an internal standard, compound 2a, and a base oil matrix on a filter paper surface showing the relative response of 2/2a. The error bars are (+/-) 2 standard deviations (n=6).](image)

$$y = 0.0611x + 0.1061$$

$R^2 = 0.9971$
Figure 5.15: Calibration plot for the DART-MS analysis of the antioxidant compound 2 deposited in a base oil matrix on a filter paper surface showing the absolute response of compound 2. The error bars are (+/-) 2 standard deviations (n=6).

The limit of detection (LOD) was calculated as the blank response for 2 plus three standard deviations of the blank using the absolute SIR response of 2. For the DART-MS analysis of 2 in oil the LOD was calculated to be 0.04 µg 2 on spot which is equivalent to 0.04 mg/mL of antioxidant in oil or 0.0004 % (w/w).

The quantitative determination of 2 in oil using 2a as an internal standard has been previously reported using the DESI ionisation technique combined with a Q-TOF mass spectrometer (Chapter 3). Caution should be exercised in comparing the DESI and DART data, which were acquired on different mass spectrometer platforms. However, the two methods both showed good linearity ($R^2 > 0.99$) and precision when using 2a as an internal standard. The %RSD for DESI-MS was 6.4%, which are typical for ambient ionisation methods, but slightly higher than the DART-MS analysis with a %RSD of 2.6%. This indicates that DART-MS is applicable...
to the quantification of additives directly from surfaces at concentrations below the levels (typically 0.1-5 %) expected in commercial formulations.

5.4.3 Comparison of ESI, DESI and DART for the analysis of a whole lubricant sample.

The direct analysis of additives in fully formulated lubricant oil, sample 1, with no sample preparation has been carried out using ESI, DESI and DART ionisation sources. Direct infusion of the sample diluted in 8:2 toluene:MeOH was carried out using a Thermo Orbitrap Q Exactive Plus mass spectrometer operated in the positive ion mode to generate high resolution mass spectra data. The mass spectrum is shown in Figure 5.15. Several dominant peaks are observed at m/z 170, 296, 335, 408 and 422 that are likely to correspond to chemical additives incorporated in the formulation. There is little chemical noise observed in the mass spectrum resulting from the oil matrix because of the synthetic nature of the base oil which is not generally ionisable by ESI.

Figure 5.15: ESI-MS analysis of sample 1, a fully formulated lubricant oil, dissolved in 8:2 toluene:MeOH.
The sample was subsequently deposited neat onto a filter paper surface for direct analysis by DESI-MS and DART-MS. The effect of solvent composition (DESI) and He gas temperature (DART) on mass spectral response for the untargeted analysis of a formulated lubricant oil was investigated. Figure 5.16 shows the mass spectra for the DESI-MS analysis of sample 1 deposited on the filter paper surface and analysed using DESI electrospray solvent compositions of H₂O, 6:4 H₂O:MeOH and 6:4 toluene:MeOH. For both DESI and ESI little chemical background resulting from the oil matrix was observed and no ions were detected above m/z 600. The use of different DESI electrospray solvent compositions had a large influence on the observed ions in the resulting mass spectra due to differences in analyte solubility and dissolution rate during the desorption/ionisation processes of the DESI mechanism. The use of H₂O as the electrospray solvent generated a mass spectrum dominated by ions at m/z 170 and 296 (Figure 5.16a) which were observed in the ESI-MS spectrum (Figure 5.15). The relative response for the ion at m/z 170 reduced as the H₂O content in the DESI electrospray phase reduced to 6:4 H₂O:MeOH (Figure 5.16b) and was no longer observed when using 6:4 toluene:MeOH (Figure 5.15c). The m/z 170 ion was observed in the ESI analysis using 8:2 toluene:MeOH which could be due to the presence of trace amounts of H₂O in the ESI solvents but may suggest that the reduced response in DESI is a result of a slower dissolution rate of the compound in the less polar solvent compositions. The addition of MeOH to the electrospray solvent caused a strong response for the ion at m/z 413 (Figure 5.16b and c) that was not observed in the ESI-MS spectrum or in the DESI spectrum using water as the electrospray solvent.
Figure 5.16: DESI-MS analysis of sample 1 deposited onto a filter paper surface and analysed using DESI electrospray solvent compositions a) H$_2$O, b) 6:4 H$_2$O:MeOH and c) 6:4 toluene:MeOH.
The sample was also analysed by DART-MS using a range of He gas temperatures (100-400 °C) directly from a filter paper surface. The mass spectra are shown in Figure 5.17. Again little chemical background was observed in the DART-MS mass spectra. The dominant ions observed for the ESI-MS analysis of the sample, at m/z 170, 296, 408 and 422 were also observed in the DART-MS spectra. However, the different ions were detected using different temperatures and no single spectrum contained all target ions. The optimum temperature for the DART analysis of a compound will be dependent upon its volatility. Increasing the temperature of the He gas improves the thermal desorption efficiency of the DART ionisation process, but can result in thermal degradation of compounds. As the He gas temperature was increased an overall shift in the mass range of detected ions was observed. At low temperatures (100 °C) the dominant ions were in the m/z range of 100-400 which correspond to analytes that are relatively small in molecular weight and therefore are likely to be more volatile (Figure 1.17a). At higher temperatures (400 °C) ions in the mass range of m/z 400-800 were observed because of the increase in thermal desorption which enables larger species to be detected (Figure 5.17d). The spectra suggest that thermal decomposition of the species at m/z 170 may be occurring at higher temperatures. At a helium temperature of 100 °C the m/z 170 ion was present as the base peak in the mass spectrum. Increasing the helium temperature resulted in a reduced response for the m/z 170 ion until it was no longer detected at 300 °C. Using a temperature of 200 °C enhanced the response for ions at m/z 296 and 408 compared to 100 °C and also generated responses from ions at m/z 560.410 and 686.551. A further increase in helium temperature to 300 °C enabled the detection of ions at m/z 352 and 422 that were not observed previously, but inhibited the detection of ions at m/z 296.237 and 408.347 (Figure 5.17c). The data highlights the importance of helium gas temperature for both targeted and untargeted studies and how the effect of temperature can influence the observed mass spectra.
Figure 5.17: DART-MS analysis of sample 1 deposited on a filter paper surface using a He gas temperature of a) 100 °C, b) 200°C, c) 300 °C and d) 400°C.
5.5 Conclusions
The application of DART-MS to the qualitative analysis of commercially available lubricant and oil additives, including an antioxidant (2), corrosion inhibitors (4a-4c) and a friction modifier (3) has been investigated. The successful desorption and ionisation of all additives has been demonstrated from a range of surface materials including filter paper, glass and metal when deposited as standards, mixtures and in a lubricant base oil matrix. The target surface material has been shown to change the desorption profile of 2, as a result of differences in the thermal desorption temperature profile of the analyte from the surface. The influence of He gas temperature on the desorption and thermal fragmentation of the quaternary amine corrosion inhibitors has been discussed. The thermal fragmentation of the quaternary ammonium salts produces diagnostic ions that can be used to identify the quaternary amine species even when the molecular ions are not observed. This has been demonstrated for the DART-MS analysis of the corrosion inhibitor additives present in an oil matrix and deposited on a steel surface. An analogue of 2, was synthesised and used as an internal standard in the quantitative assessment of DART-MS. The desorption profiles of 2 and 2a from filter paper and metal surfaces followed the same trend indicating the two compounds behave in a similar physical and chemical manner within the DART source, making 2a a suitable internal standard for the quantitative analysis of 2. The application of DART-MS to quantify 2 in a lubricating oil matrix deposited on a filter paper surface has been demonstrated with good linearity and precision ($R^2 > 0.99$ and 2.6% RSD). The LOD for the technique was calculated to be 0.04 µg 2 on surface which corresponds to 0.04 mg/mL additive in oil or 0.0004% w/w. Comparison of DART and DESI for the quantification of compound 2 shows that both techniques are able to detect the additive below typical levels found in commercial formulations with good linearity of the two ionisation methods ($R^2 > 0.99$ for both when using 2a as an internal standard) and satisfactory precision. ESI, DESI and DART ionisation approaches were applied to the untargeted analysis of a formulated lubricant oil, sample 1. The effect of solvent composition (DESI) and He temperature (DART) have been shown to greatly influence the mass spectral response for sample 1. The results show the importance of suitable experimental parameters for untargeted characterisation studies using ambient ionisation approaches.
5.6 References


CHAPTER SIX

Analysis of Crude Oil using Electrospray Ionisation and Desorption Electrospray Ionisation Hyphenated with Mass Spectrometry and High Field Asymmetric Waveform Ion Mobility Spectrometry
6.1 Introduction

The analysis of crude oils is a global challenge driven by the desire to unravel the naturally occurring product at a molecular level. Crude oils form one of the most complex mixtures known and contain a large number of difficult to analyse compounds. However, the characterisation of crudes not only generates a unique chemical fingerprint that can provide geographical information, but can predict the chemical and physical properties of the product. Due to the highly complex nature of the mixture, analysis typically relies upon pre-fraction of the crude into smaller and easier to handle portions based upon differences in solubility and polarity. An example of this is the SARA fractionation method that generates four groups; saturates, aromatics, resins and asphaltenes. Once the crude oil mixture has been fractionated it can be subjected to a range of analytical techniques including wet tests, NMR and, UV-visible and infra-red spectroscopy. Methods for the analysis of crude oils were described in more detail in Chapter 1, Section 1.1.1 The application of mass spectrometry to the analysis of crude oils can generate detailed molecular information based on $m/z$ that can enable identification of individual molecular species. Mass spectrometry for the analysis of crude oil generally relies upon chromatographic separations and/or the application of high resolution mass analysers such as the Orbitrap or Fourier transform-ion cyclotron-mass spectrometry (FT-ICR-MS). The data generated is subsequently processed using specialist software tools that enable easy visualisation of the crude oil composition based upon factors such as the molecular class, Kendrick mass defect and double bond equivalents (DBEs).

The choice of ionisation technique needs to be considered when analysing such complex mixtures, as different ionisation approaches will preferentially ionise specific groups of compounds. The most common ionisation techniques for crude oil are electrospray ionisation (ESI), for the analysis of the more polar species, and atmospheric pressure photo ionisation (APPI) for determination of hydrocarbon content. Although the composition of crude oil is 90 % hydrocarbons, the N, O and S containing species which are readily ionised by ESI are important in determining the physical and chemical nature of the crude. The application of ambient ionisation techniques for the direct analysis of crude oil has been reported using methods such as atmospheric pressure chemical ionisation (APCI), low-temperature plasma, easy ambient sonic spray ionisation (EASI), desorption electrospray ionisation (DESI), nano-DESI, matrix assisted laser desorption ionisation (MALDI), direct analysis in real time (DART) and field desorption/field ionisation (FD/FI).
An advancing area of study is the application of ion mobility spectrometry as a post-ionisation separation approach to create another analytical dimension when using mass spectrometry for crude oil analysis. Unlike wet based chemical fractionation and chromatography, that separate compounds based upon solubility and polarity, separation in ion mobility spectrometry (IMS) is a function of mobility of ions in the gas phase, which is determined by collisional cross section (CCS) amongst other factors. This can be used to separate species from within the same solution based fractions, which can improve the peak capacity and provide a secondary identification parameter for oil components. In addition IMS has the potential for interrogation into structural relationships, such as differences in gas phase conformation or aggregation, of oil components using CCS measurements. Drift tube and travelling wave IMS-MS has been applied to the separation of N, NO, NO\textsubscript{2}, O and O\textsubscript{2} classes of compounds,\textsuperscript{25} isomeric separation\textsuperscript{26} and the analysis of diesel fuels and fuel additives.\textsuperscript{27,28} CCS measurements have been used to generate structural information for chemically related compounds and the investigation into gas phase aggregation.\textsuperscript{29,30,31} The use of complexing reagents to improve IM resolution has also been investigated.\textsuperscript{27} An alternative approach is the use of field asymmetric waveform ion mobility spectrometry (FAIMS) for ion separation. In FAIMS separation occurs as a result of differences in an ions mobility when exposed to high and low field strengths. The mechanism of FAIMS has been described in detail in Chapter 1, Section 1.1.3 Unlike IMS, were there is a significant correlation between \(m/z\) and drift time, high to low field mobility differences are less dependent on \(m/z\) and therefore FAIMS has the potential to separate molecular ions that cannot be separated by IMS. The application of FAIMS to the analysis of crude oils still remains a relatively unexplored area with only two accounts reported in the literature. In 2003 Gabreyelski applied ESI-FAIMS-MS to the characterisation of napthenic acids found in crude oils using a home built FAIMS device hyphenated with a quadrupole and TOF. FAIMS separation of ions enabled the generation of tandem mass spectra that was not previously possible for structural elucidation of naphenic acids present in a mixture.\textsuperscript{32} In 2014 Schrader and colleagues studied the complexity of crude oil mixtures using ESI-FAIMS-MS with the hope of showing how FAIMS can be used to simplify the mass spectral data generated by crude oil analysis. The study was carried out using a Thermo FAIMS device hyphenated to an Orbitrap mass spectrometer for high resolution mass analysis. The results show that FAIMS is capable of separating ions in the crude oil to generate simplified data, and that the orthogonality of FAIMS to \(m/z\) can enable the detection of compounds, such as structural isomers, that could not be achieved otherwise.

This chapter describes an evaluation of the potential of ESI-FAIMS-MS for the analysis of a crude oil mixture without pre-analysis sample preparation or fractionation and an
investigation of the use of DESI-MS for the direct analysis of a crude oil deposited on a surface as an alternation ionisation technique.
6.2 Aims and Objectives

- Analysis of a crude oil using electrospray-high resolution mass spectrometry and specialist software for characterisation studies.

- Evaluation of the potential of FAIMS as a rapid post-ionisation separation method for the analysis of crude oil to determine if FAIMS can be used to simplify mass spectral data obtained for highly complex chemical mixtures.

- Investigation into the potential of DESI-MS for the direct analysis of crude oil deposited on a surface and the effect of electrospray solvent composition.
6.3 Experimental

6.3.1 Reagents and chemicals
Methanol, toluene and formic acid were purchased from Sigma Aldrich (Gillingham, UK). Water was purchased from Fisher Scientific (Loughborough, UK). All solvents were HPLC grade. The crude oil sample, NIST standard reference material 2721, was purchased from the National Institute of Standards and Technology (NIST) and described as a Light-Sour crude oil. Whatman Type 1 filter paper was used in the DESI-MS analysis of the sample.

6.3.2 Sample preparation
The crude oil sample NIST 2721 was dissolved in 6:4 toluene:MeOH + 0.1 % formic acid (~0.5 mg/mL) and directly infused into the ion source at 5-15 µL/min for ESI-MS and ESI-FAIMS-MS analysis. For DESI-MS analysis, an aliquot of crude NIST 2721 (~10 µL) was deposited onto filter paper using a syringe before the sample was placed into the DESI source.

6.3.3 ESI-Orbitrap analysis of NIST 2721
The crude oil sample was analysed by ESI-MS using a Thermo Orbitrap Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Massachusetts, USA). The mass spectrometer was operated in the positive ion mode using the instrumental parameters: sheath gas flow rate 10, capillary temperature 300 °C, spray voltage 4.5 kV, scan range m/z 133-1000, resolution 280,000 and ACG target 1e6. The data was processed using the PetroOrg software package developed by Florida State University in association with Future Fuels Institute and National High Magnetic Field laboratory. The data was internally calibrated using the CₓHᵧN series (2 ppm error, 1 % noise level) before analysis using the following parameters: C 1-100, H 4-200, N 0-2, O 0-5, S 0-2, 5 ppm error, [M+H]⁺.

6.3.4 ESI-ToF MS and ESI-FAIMS-ToF MS analysis of NIST 2721
The crude oil sample was analysed by ESI-MS and ESI-FAIMS-MS in the positive ion mode using an Agilent 6230 TOF mass spectrometer (Agilent Technologies, Santa Clara, USA) fitted with a miniaturised chip-based FAIMS device (Owlstone Ltd, Cambridge, UK). The sample was directly infused into a modified Jetstream ESI source at a flow rate of 15 µL/min. The optimised ESI parameters were drying gas 250 °C at 7 L/min, nebuliser gas 40 psig, sheath gas 250 °C at 12 L/min, capillary voltage 1200 V, nozzle voltage 2000 V and fragmentor voltage 175 V. The FAIMS device, described in detail in Chapter 4 Section 4.3, was operated in both DF/CF scan mode and CF only scan mode. Optimised FAIMS parameters for the separation of crude oil ions were: DF 250 Td, CF scan -2 to 5 Td at a sweep rate of 140 sec with the MS operating at 10 scans/sec.
6.3.5 DESI-ToF MS analysis of NIST 2721

DESI-MS analysis of NIST 2721 crude deposited neat onto a filter paper surface was carried out using a Waters Synapt HDMS spectrometer (Waters, Massachusetts, USA) fitted with the in-house constructed DESI source, version 1.3 (described in detail in Chapter 2) and DESI electrospray solvent compositions of 6:4 toluene:MeOH + 0.1 % formic acid and 6:4 H₂O:MeOH + 0.1 % formic acid. The source was fitted with a 5 cm ion transfer tube and the configuration was electrospray tip-sample angle of ~ 55 ° and distance of ~ 3 mm, sample-MS inlet distance ~ 1 mm, nebuliser voltage 2.5 kV, nebuliser gas 40 psig, with an electrospray phase flow rate of 10 µL/min. The mass spectrometer was operated in the positive ion mode using a cone voltage of 20 V, source temperature 120 °C, cone gas 30 L/Hr and trap gas 1.5 mL/min.
6.4 Results and discussion

6.4.1 ESI-MS analysis of crude oil using high resolution mass spectrometry

The NIST 2721 crude oil standard was analysed by ESI-MS using a Thermo Orbitrap mass spectrometer, set to a mass resolution of 280000, to generate high resolution mass spectral data. The resulting mass spectrum (Figure 6.1) shows an asymmetric profile of ions that starts at ~ m/z 250, with the most intense peak observed at ~ m/z 310, and the response tailing to background levels at ~ m/z 900. This profile is typical for the ESI mass spectral analysis of a crude oil sample. The data was processed using the specialist software PetroOrg that enables class and compound identification. The software package internally calibrates the data before identifying species based upon carbon number, heteroatom class, double bond equivalent (DBE) and Kendrick mass defect. The data showed the crude oil to be N-rich with high levels of sulphur, which is consistent with the NIST standard information. A summary of the crude oil composition based upon heteroatom class is provided in Figure 6.2. The N\textsubscript{1} class was dominant within the crude oil mixture and generated the base peaks observed in the mass spectrum. Of the N\textsubscript{1} species, the observed DBEs were in the range of 4-20, with a DBE of 9 being the most abundant. Analysis of crude oil samples is often presented as DBE vs C no. (Kendrick plots) or Krevelen plots. The plots enable the visualisation of very complex crude oil data that would not be possible using a mass spectrum.\textsuperscript{14,33} An example of a Kendrick plot for the N\textsubscript{1} species for the NIST crude 2721 oil is shown in Figure 6.3.

![Figure 6.1: ESI-MS analysis of the NIST 2721 crude oil using high resolution mass spectrometry.](image)
Figure 6.2: ESI-MS analysis of NIST 2721 crude oil showing a compositional summary based upon heteroatom class generated using PetroOrg software.

Figure 6.3: Kendrick plot (DBE vs C no.) for the N₁ series of the NIST 2721 crude oil sample.
6.4.2 ESI-FAIMS-MS analysis of crude oil

The use of high resolution mass spectrometry has become the gold standard within the petroleum industry. However, the high complexity of the sample can still be problematic and result in the loss of chemical information due to suppression and discrimination effects. FAIMS provides a method for the rapid separation of ions based upon differences in their mobility when exposed to high and low field strengths. The application of FAIMS to the analysis of crude oil mixtures provides a potential opportunity to enhance the information generated by mass spectrometry without any sample preparation.

The NIST 2721 crude oil sample was analysed by ESI-MS and ESI-FAIMS-MS using an Agilent 6230 TOF mass spectrometer fitted with a miniaturised chip based FAIMS device. Figure 6.4a shows the mass spectrum from the initial ESI-MS analysis of the crude oil sample using ESI parameters: drying gas 250 °C at 7 L/min, nebuliser gas 30 psig, sheath gas 250 °C at 12 L/min, capillary 2500 V, nozzle 2000V. The extended tail observed in the mass spectrum from ~ m/z 650-1000 for the crude oil profile indicates the presence of dimer species. Optimisation of the ESI parameters to those in the method section was therefore required to reduce this effect. Decreasing the capillary voltage to 1200 V and increasing the nebuliser gas pressure to 40 psig reduced the formation of dimers (Figure 6.4b) which generated a mass spectrum that showed a similar profile for the crude oil sample to that observed for the ESI-MS analysis using the Thermo Orbitrap mass spectrometer, (Figure 6.1). The optimised ESI parameters are described in the experimental section.
Figure 6.4: ESI-MS analysis of the NIST 2721 crude oil using an Agilent 6230 TOF. The presence of dimers were observed in the initial analysis observed in the mass spectrum as an extended tail in the oil profile (a) which was removed through optimisation of ESI parameters (b).

The application of FAIMS for the rapid separation of crude oil species was then evaluated using the NIST 2721 crude oil. The sample was directly infused into the ESI source using the optimised ESI parameters to generate ions that passed directly through the FAIMS device, which was located at the capillary inlet of the mass spectrometer, before mass analysis in the TOF. DF optimisation was carried out using a DF/CF scan, in which the DF was
increased from 180 to 300 Td in 10 Td steps and at each DF step the CF was scanned from -2 to 5 Td at a rate of 2 s/Td. Increasing the DF field functions to enhance the separation capabilities of the FAIMS device as a greater high to low field mobility difference can be achieved. However, this is often associated with a reduction in ion transmission resulting in a loss of sensitivity. Increasing the DF above 260 Td resulted in a breakdown in the total ion current. This is likely to be due to the highly complex nature of the crude oil sample. The DF was therefore set to 250 Td and the CF scanned from -2 to 5 Td at a rate of 10 s/Td. Figure 6.5 shows the total ion response (TIR) for the CF scan of the crude oil sample. Transmission of ions occurs as a relatively broad peak between 1.5 and 3.5 Td, centred at 2 Td. There is some variation in ion intensity observed in the TIR profile resulting from the transmission of different species, but due to the chemical complexity of the sample individual species are not resolved. Mass spectra generated by the transmission of ions through the FAIMS at selected CFs between 1.5 and 3.5 Td are shown in Figure 6.6a-e. The transmission of different ions is quite clearly visible as shifts in the mass spectral profile of the crude oil sample. The insert in Figure 6.6 shows an overlaid image of the mass spectra generated using when applying a CF of 2.05 Td (black) and 2.30 Td (pink) to the FAIMS device, highlighting the difference in the observed ions when using different separation parameters. However, unlike IMS, in which a strong correlation between drift time and m/z is observed, there is not a similar trend between CF and m/z in FAIMS. The m/z range shifts slightly to a lower mass as the CF is increased from 1.76 to 2.3 Td, but there is significant overlap in the mass ranges. FAIMS transmission of ions at 1.4 and 1.57 Td results in a bimodal distribution covering almost the entire mass range of the ion.

![Figure 6.5: ESI-FAIMS-MS analysis of NIST 2721 crude oil using a DF of 250 Td and a CF sweep of -2 to 5 Td showing the TIR.](image-url)
Figure 6.6: ESI-FAIMS-MS analysis of NIST 2721 crude oil using a DF of 250 Td and a CF sweep of -2 to 5 Td showing the mass spectra extracted at a CF of a) 1.40 Td, b) 1.76 Td, c) 2.05 Td, d) 2.30 Td and e) 2.57 Td. The mass spectra extracted at 2.05 Td (black) and 2.30 Td (pink) have been overlaid in the insert.

Expanding the mass spectra to investigate the transmission of two ions at m/z 356.23 and 356.33, tentatively assigned to the N₁ class with DBEs of 13 and 6 respectively (error < 10 ppm), shows that the relative intensities of the ions change with the CF range (Figure 6.7). The application of different FAIMS parameters could therefore potentially be used for the preferential transmission of compounds from the same class but with different levels of aromaticity. Furthermore, since the FAIMS peak width of a single compound in this mass range is typically <1 Td, an ion with maximum FAIMS transmission at a CF of 1.4 Td (Figure 6.7a) cannot be the same isobaric ion transmitted at CF 2.57 Td (Figure 6.7e). Combining
FAIMS with MS therefore has the potential to separate isobaric ions that cannot be resolved by MS. The FAIMS device can also enhance the relative response of ions previously unresolved in the TOF (Figure 6.8). The mass spectrum in Figure 6.8a shows an ion at m/z 320.23 that has a small partially resolved shoulder. The peak is quite dominant in the mass spectrum and is likely to relate to the N₁ class. Application of a FAIMS separation using a CF of 1.40 Td can filter out a high proportion of the m/z 320.23 response and cause a relative enhancement in the response of the previously unresolved species to improve peak resolution, shown in Figure 6.8b.

Figure 6.7: ESI-FAIMS-MS analysis of NIST 2721 showing the change in the relative responses of two ions when applying different CFs
Figure 6.8: Analysis of the NIST 2721 sample of crude oil using a) ESI-MS and b) ESI-FAIMS-MS with a CF of 1.40 Td.

6.4.3 DESI-MS analysis of crude oil and effect of solvent composition

The application of DESI has received little attention for the analysis of crude oils due to the complexity of the sample. The direct DESI-MS analysis of the NIST 2721 crude oil was therefore investigated. The sample was deposited neat onto filter paper before DESI-MS analysis using a DESI electrospray solvent composition of 6:4 toluene:MeOH + 0.1 % formic acid. The resulting mass spectrum (Figure 6.9) shows a similar profile to that observed using ESI (Figure 6.1 and 6.4b) highlighting the similarities between ESI and DESI, which is a solvent spray based ambient ionisation method. The chemical profile observed in the DESI spectrum shows a more symmetrical shape compared to ESI. The maximum intensity is still centred at ~ m/z 310, but the response tails away at around m/z 600, significantly lower than in the ESI mass spectra. The result shows the successful desorption and ionisation of several crude oil species directly from a surface, but that the DESI-MS method preferentially desorbs lower molecular weight compounds under the conditions used in this analysis.
The variation of solvent composition in DESI has been shown to induce a level of selectivity for analytes as a result of differences in solubility and dissolution rate.\textsuperscript{34} The effect of DESI solvent composition as a tool for the selective desorption of compounds within the crude oil mixture was therefore investigated using a DESI electrospray solvent of 6:4 H\textsubscript{2}O:MeOH + 0.1 \% formic acid. The sample was first analysed by ESI-MS to determine the ESI mass spectral response for a 6:4 H\textsubscript{2}O:MeOH + 0.1 \% formic acid extraction. An aliquot of the NIST 2721 crude oil was placed in a 6:4 H\textsubscript{2}O:MeOH + 0.1 \% formic acid solution, mixed and left to stand to enable dissolution of the soluble species before the 6:4 H\textsubscript{2}O:MeOH + 0.1 \% formic acid solution was extracted and directly infused into the ESI source. The resulting mass spectrum (Figure 6.10a) shows a slight shift in profile towards the lower mass range compared to 6:4 toluene:MeOH + 0.1 \% formic acid (Figure 6.4b). The observed profile is more symmetrical in shape, beginning at ~ $m/z$ 200, but tailing at ~ $m/z$ 400, showing that smaller and more polar compounds in the crude oil mixture are extracted into the water based solvent. The crude oil was subsequently deposited neat onto the filter paper surface for DESI-MS analysis using the 6:4 H\textsubscript{2}O:MeOH + 0.1 \% formic acid DESI electrospray.
solvent composition. Few ions were observed in the resulting DESI-MS spectrum (Figure 6.10b) compared to ESI-MS (Figure 6.10a). These ions are in the same region as that shown in the ESI-MS spectrum using the sample solvent composition. However, the DESI-MS method did not generate a strong oil profile like ESI. In ESI the crude oil sample was given ~10 min for extraction of the target analytes into the water based solvent before analysis. The dynamic nature of the desorption and ionisation of target analytes by DESI is complex, dependent upon many factors including solubility and dissolution rate, meaning extraction of analytes into the solvent film needs to occur at a rapid rate. The water based DESI electrospray solvent was not miscible with the crude oil sample, as shown in Figure 6.9b insert. A droplet of 6:4 H₂O:MeOH + 0.1 % formic acid sits on the surface of a crude oil spot deposited on filter paper, showing that there is little mixing of the sample and the solvent. The lack of miscibility of the water with the crude oil reduces the potential dissolution of soluble analytes into the solvent film. While problems with miscibility were noted for a water based DESI solvent, the technique has potential for the use of different solvent systems that are more suitable to crude oil analysis, such as increasing the MeOH proportion in the toluene:MeOH mixture or incorporating different solvents such as hexane, DCM and IPA.
Figure 6.10: Analysis of NIST 2721 crude oil by a) ESI and b) DESI using a 6:4 \(H_2O:MeOH + 0.1\%\) formic acid solvent.
6.5 Conclusions
The direct analysis of a crude oil sample with no sample preparation or fractionation has been investigated using ESI-MS, ESI-FAIMS-MS and DESI-MS on Orbitrap, TOF and Q-TOF mass spectrometer platforms. The use of high resolution mass spectrometry (Orbitrap) has enabled detailed chemical characterisation of the sample to be carried out. Data analysis using specialist software (PetroOrg) generated class and compound information that can be used as a chemical fingerprint for the sample. The application of FAIMS to the analysis of a complex crude oil mixture has been investigated as a tool for the rapid post-ionisation separation of compounds to both simplify and enhance the obtainable mass spectral data. The application of FAIMS enables the selective transmission of ions through the FAIMS device, which resulted in changes in the mass spectral profiles of the crude oil. This highlights the potential of FAIMS to add an additional level of selectivity and separation to the mass spectrometric analysis of complex crude oil samples. The direct analysis of the crude oil has been investigated using DESI-MS. The successful desorption and ionisation of crude oil components were achieved using a DESI electrospray solvent composition of 6:4 toluene:MeOH + 0.1 % formic acid, whilst selective desorption of compounds was observed using an aqueous methanol electrospray solvent.
6.6 References


CHAPTER SEVEN

Conclusions and Further Work
7.1 Thesis Summary
The application of mass spectrometry hyphenated with ambient ionisation and ion mobility has been investigated for the rapid and direct analysis of lubricant oil additives, formulated oils and crude oil. The results presented in this thesis are summarised in this section and further work is discussed.

7.1.1 Summary of Chapter One
Chapter one introduces the field of petroleomics and brings together a range of mass spectrometric techniques for the study of lubricants and crude oils. Mass spectrometry provides a unique tool for the molecular analysis of complex samples such as oils that can supplement traditional wet chemical methods. The role of ambient ionisation for the rapid and direct analysis of analytes with minimal or no sample preparation is discussed. Ambient ionisation enables the analysis of compounds desorbed directly from surfaces which can improve sample throughput and enable imaging studies to be carried out. Hyphenation with ion mobility, primarily high field asymmetric waveform ion mobility (FAIMS), is shown to enhance selectivity and sensitivity through the selective transmission of target analytes. For targeted studies of compounds in complex mixtures FAIMS can be used to simplify the mass spectral data obtained and enhance signal:noise. The theoretical aspects of several ionisation methods applicable to the thesis, ion mobility spectrometry and mass spectrometry are discussed.

7.1.2 Summary of Chapter Two
Chapter two describes the in-house development of DESI sources hyphenated with a Q-TWIMS-TOF mass spectrometer (Waters Synapt HDMS) for the analysis of oil additives and petroleomic samples. The preliminary studies carried out to assess source performance have also been discussed. The DESI sources, versions 1.1 and 1.2, enabled the successful desorption and ionisation of the selected lubricant oil chemical additives from a range of target surface materials (glass, PTFE, filter paper and steel) in both the positive ion and negative ion modes. DESI-TWIMS-MS reduced the chemical background observed in the mass spectra and enable a relative increase in target analyte signal:noise. DESI-MS/MS was carried out for the analysis of the antioxidant 1 when deposited as a standard and in a lubricant oil matrix. The similarities in the product ion spectra generated for the analysis of the standard and the spiked oil sample show the absence of unresolved or isobaric species in the oil and could be used to confirm the identity of 1. Problems with repeatability of the DESI source geometry of versions 1.1 and 1.2 were highlighted during participation in an inter-laboratory study conducted by National Physics Laboratory (NPL). The DESI-MS analysis of a rhodamine B sample deposited on a glass surface was carried out to determine
absolute intensity repeatability of DESI-MS. Loughborough generated a repeatability of 52 % for 55 replicate analyses, with the average for the 20 participants being 49 %. The concerns associated with poor source repeatability were assessed and overcome with the design and construction of DESI source version 1.3. Version 1.3 consists of a secure platform that attaches to the front of the mass spectrometer, to which a DESI nebuliser and a remotely operated sample stage manipulator are mounted. The design has improved intra-day repeatability of source geometry, the potential for automated sample movement and improved safety features. DESI source version 1.3 was used for several studies including the investigation into the effect of solvent composition on the DESI-MS response of a series of corrosion inhibitor additives. The solvent composition was found to effect the sensitivity of the DESI-MS method and the desorption profiles of the corrosion inhibitors. However, there was a poor correlation between DESI-MS response and in-house generated solubility data highlighting the complexity of DESI desorption and ionisation processes. The optimum solvent composition for the analysis of corrosion inhibitor samples from a steel surface was found to be 1:1 ACN:MeOH. This was used for preliminary imaging studies for additive deposition using the corrosion inhibitor 4c as a model compound. Surface imaging by DESI-MS enabled localisation data of the additive to be generated. The method was applied to the analysis of several samples supplied by Castrol.

There are several areas for further work. Determination of the intra-day repeatability of the DESI source version 1.3 using a model compound should be carried out. The influence of DESI solvent composition on target analyte response was investigated using the corrosion inhibitors, which are all quaternary amines. The next stage of this experiment would be to look at the effect of functional group in conjunction with solvent composition on DESI-MS response using different lubricant oil additives. The key area of progression, in my opinion, centres on the use of DESI-MS as an imaging technique for in-situ additive analysis. Imaging by DESI-MS enables the generation of molecular information of analytes on a surface that cannot be achieved with the current surface analysis techniques, such as XPS or SEM. Preliminary data was generated for 4c showing potential for the method. Further work is to improve the analytical resolution for in-house generated test samples by reducing the spaces between the analytical lines. The method could then be applied to the direct analysis of unwashed tribological components. An additional area of interest would be to monitor the presence, deposition and breakdown of lubricant additives on a surface at a molecular level following exposure to chemical and physical wear.
7.1.3 Summary of Chapter Three

The quantitative DESI-MS analysis of a lubricant oil antioxidant additive in a lubricant base oil matrix and in the presence of an in-house synthesised internal standard is presented. The antioxidant additive and internal standard were spiked into the oil before deposition onto the surface so that the antioxidant was present in the oil in the concentration range of 0.1-8 mg/mL (1-80 µg additive on spot). The use of a 95:5 MeOH:H₂O DESI electrospray solvent composition enabled the selective extraction of the target analytes from the lubricant oil during the DESI process, resulting in a simplified mass spectrum and strong deprotonated molecular responses for the antioxidant and internal standard. DESI-MS/MS was carried out, along with accurate mass measurement, to confirm the identity of [M-H]⁻ ion at m/z 389 observed for the analysis of the antioxidant in oil as the deprotonated molecule of the antioxidant. The limit of detection for the antioxidant additive was calculated to be 0.03 % w/v or 0.03 mg/mL additive in oil. The use of an internal standard for quantitative ambient ionisation studies has been discussed. The intra-day repeatability (%RSD, n=6) for the relative response of the antioxidant and internal standard, a structural analogue of the target analyte, was 3.23 % and for the absolute response of the antioxidant 6.37 %. This result is shown to be a significant improvement.

The experiment was carried out using a prototype version of the in-house constructed DESI source and therefore it would be interesting to investigate the impact of source development on the inter-day repeatability of the DESI-MS method. The linear dynamic range for the quantitative DESI-MS analysis of the antioxidant in a lubricant oil matrix and the inter-day repeatability of the technique could be assessed using the final DESI source design. The quantitative DESI-MS method could then be applied to a wider range of lubricant additives, such as corrosion inhibitors, present in the oil individually or as mixtures. The influence of additive behaviour during the ionisation and desorption process, such as competitive ionisation effects, and the choice of suitable internal standard would need to be considered when investigating different target analytes.

7.1.4 Summary of Chapter Four

The analysis of corrosion inhibitor additives (4a-4c) in the presence and absence of an oil matrix is reported using ESI and DESI, hyphenated with a miniaturized FAIMS device and MS. The target analytes were successfully detected in the oil matrix using ESI and directly from metal surfaces using DESI at levels above 0.0004% w/w in oil. The use of FAIMS improved selectivity for ESI generated analyte ions through reduction in the chemical noise resulting from the oil matrix and enabled optimisation of FAIMS parameters for the selective transmission of the corrosion inhibitor ions. A slight shift in optimum CF for the corrosion
inhibitor additives was observed when using DESI (1.55 Td), compared to ESI (1.80 Td), as a result of changes to the FAIMS chip temperature. In addition a reduction in the oil matrix response was observed with DESI, compared to ESI, which is believed to result from mechanistic differences between the two ionisation techniques, highlighting an additional advantage of DESI for additive analysis. Hyphenation of DESI with FAIMS-MS showed a 10-fold increase in corrosion inhibitor response compared to DESI-MS alone and enabled the detection of the additives at a quantifiable level.

The DESI-FAIMS-MS analysis of corrosion inhibitor additives present in an oil matrix and deposited on a metal surface has been presented using model compounds. The approach has potential for wider application to targeted and non-targeted analysis of oils and additives and for the imaging of tribological components to determine additive deposition and activity. Further work would be to assess the capabilities of the DESI-FAIMS-MS method for a range of different commercially available analytes when present as standards and mixtures in an oil matrix. The DESI-FAIMS-MS method could be applied to the study of additive depletion/degradation following exposure to wear or chemical treatments to generate detailed molecular information that has an additional level of selectivity compared to DESI-MS alone. In addition it would be advantageous to evaluate the DESI-FAIMS-MS method for the imaging of an additive on a target surface which could be applied to the direct analysis of additive deposition on tribological components.

7.1.5 Summary of Chapter Five
In Chapter five, the application of DART-MS to the qualitative analysis of targeted lubricant additives (2, 3 and 4a-4c), and the quantitative analysis of the antioxidant 2 in the presence of an internal standard (2a) and an oil matrix is presented. The effect of ESI, DESI and DART ionisation approaches for the untargeted analysis of a fully formulated lubricant oil is also investigated. All selected additives were successfully desorbed and ionised by DART for high resolution mass analysis. Thermal fragmentation of the molecular ion species of the quaternary amine corrosion inhibitors (4a-4c) was observed, generating characteristic free amine fragment ions. The molecular ions were only detected when using helium gas temperatures of 200 °C for filter paper and 300 °C for steel. The desorption of the additives 2 and 4c from the surfaces were monitored using the SIRs. Both additives showed comparable trends. Desorption from glass and filter paper showed similar profiles. However, when desorbed from steel a reduced initial rate of desorption was observed due to thermal conductivity of the heat away from the sample spot immediately following introduction of the sample into the DART source. The presence of an oil matrix reduced the depletion rate of the 2 from the surface because of differences in the volatility of the additive/oil mixture.
compared to the additive alone. In the DART-MS mass spectrum for the corrosion inhibitor/oil mixture, deposited on steel and analysed using a gas temperature of 300 °C, the molecular ions for 4a-4c were not observed above the chemical noise. However, the thermal fragment ions could be used to in a diagnostic manner for identification of the additive ion. The quantitative assessment of DART-MS for the analysis of 2 showed good linearity and precision (R² > 0.99 and 2.6% RSD). The use of an internal standard improved both the repeatability (%RSD for the absolute response of 2 = 16.8 %) and the linear dynamic range. The LOD was calculated to be 0.04 µg 2 on surface which corresponds to 0.04 mg/mL additive in oil or 0.0004% w/w. ESI, DESI and DART ionisation techniques were applied to the analysis of a fully formulated lubricant oil to investigate the effect of solvent composition (DESI) and temperature (DART). The DESI solvent composition and the DART gas temperature both influenced the observed ions in the resulting mass spectra due to differences in analyte dissolution (DESI) and volatility and thermal stability (DART).

Progression of this work would be to investigate the quantitative capabilities of the DART-MS method for the analysis of additives when present as mixtures in the lubricant oil. Hyphenation with MS/MS could be used, alongside high resolution mass analysis, for structural elucidation of the ion observed in the fully formulated lubricant sample.

7.1.6 Summary of Chapter Six
The direct analysis of crude oil using ESI-MS, ESI-FAIMS-MS and DESI-MS on Orbitrap, ToF and Q-ToF mass spectrometer platforms is investigated. A NIST 2721 crude oil standard was purchased for the study and simply diluted in 6:4 toluene:MeOH + 0.1 % formic acid (ESI) or deposited neat onto filter paper (DESI) for analysis. The use of ESI-high resolution mass spectrometry (Orbitrap) generated complex mass spectrum for the NIST 2721 sample that showed a typical ESI response for a crude oil. The asymmetric profile of ions starts at ~ m/z 250, with the most intense peak observed at ~ m/z 310, and then tails to background levels at ~ m/z 900. Data analysis for characterisation of the sample was carried out using specialist PetroOrg software that can classify the crude oil based upon heteroatom class, DBE and Kendrick’s mass defect. The data generated showed the N₁ class was dominant within the crude oil mixture and generated the base peaks observed in the mass spectrum. The crude oil also had relatively high levels of sulphur, which is consistent with the NIST sample information. Analysis of the NIST 2721 crude was subsequently carried out using a ToF mass spectrometer fitted with an ESI source and a miniaturised chip-based FAIMS device. The use of FAIMS as a rapid post-ionisation separation technique has the potential to simplify the mass spectral response of complex crude oil samples and resolve isobaric species that could not be achieved by MS alone. Mass spectra generated by the
transmission of ions through the FAIMS at selected CFs between 1.5 and 3.5 Td shows visible shifts in the mass spectral profile of the NIST 2721 crude oil and the preferential transmission of individual ions. The application of a FAIMS separation results in the selective transmission of different species when applying different DF/CF field strengths, showing the potential of FAIMS to add an additional level of selectivity and separation to the mass spectrometric analysis of complex crude oil samples. Finally the NIST 2721 sample was analysed by DESI-MS on a Q-ToF instrument to assess the capabilities of DESI for the direct analysis of complex crude oil mixtures. The use of a DESI electrospray solvent composition of 6:4 toluene:MeOH + 0.1 % formic acid enabled the successful desorption and ionisation of many crude oil components within the sample. The DESI-MS mass spectrum showed a similar profile to that observed using ESI, but with a more symmetrical shape. The maximum intensity is still centred at ~ m/z 310, but the response tails away at around m/z 600, significantly lower than in the ESI mass spectra. The effect of DESI solvent composition as a tool for the selective desorption of compounds within the crude oil mixture was investigated using a DESI electrospray solvent of 6:4 H₂O:MeOH + 0.1 % formic acid. Although few ions were observed in the resulting mass spectrum the approach shows the use of DESI-MS for the selective desorption of compounds within the crude oil.

The use of high resolution mass spectrometry for crude oil analysis is often hyphenated with sample fractionation or chromatography prior to mass analysis. FAIMS provides an alternative separation approach that could be used in a complementary manner to the established chromatographic methods or as a rapid post-ionisation separation tool. The results shown in this Chapter highlight the potential of FAIMS to separate species in the crude oil and enhance the mass spectral response. Further work in this area would be to hyphenate FAIMS with ultra high resolution mass spectrometry to improve the resolution of the mass spectral data. This would enable data analysis to be carried out using specialist software for detailed chemical characterisation of the sample that could not be achieved using a ToF mass spectrometer and provide more confidence in species identification.

7.2 Thesis Conclusion
The work presented in this thesis aims to assess the capabilities of different ambient ionisation approaches for the rapid and direct analysis of lubricant oil additives and a crude oil sample with minimal or no sample preparation. The use of ambient ionisation techniques, such as DESI and DART, can enable the desorption and ionisation of chemical additives directly from a surface, to generate detailed molecular data. This approach improves sample through-put and can be used to generate in situ localisation data relating to additive deposition that could not be obtained using LC or ESI. Hyphenation of DESI with post-
ionisation separation methods, such as FAIMS, can incorporate a level of selectivity into the method that is difficult to achieve with ambient ionisation due to a lack of sample preparation. The DESI-FAIMS-MS method was shown to have improved selectivity and sensitivity compared to DESI-MS alone, which can be used to enhance ambient ionisation data for low concentration analytes or for target compounds present in complex matrices. In conclusion, the data shown highlights the potential application for ambient ionisation in the field of petroleomics.
Appendices
APP 1- Additional Figures

Figure APP 1.1: DESI-MS analysis of antioxidant compound 2 deposited on filter paper using the standard Waters cone system (a) and the in-house constructed outer cone with no ion transfer tube (b) and ion transfer tube lengths of 5 cm (c), 10 cm (d) and 20 cm (e).
APP 1.2: DESI-MS analysis of 10 μg Compound 3 deposited on a stainless steel surface with an applied voltage (to the metal coupon) of a) earthed b) 540V, c) 1040V and d) 1540V and analysed using a MeOH electrospray solvent. For each mass spectrum 60 scans have been averaged.
Figure APP 1.3: Graph showing the mean DESI-MS response (SIR peak area) for the analysis of equimolar amounts of corrosion inhibitor additives 4a, 4b and 4c deposited on a metal coupon and analysed using ACN and 50:50 MeOH:ACN electrospray solvent compositions. The error bars plotted show the SIR peak area range (n=3).
Figure APP 1.4: DESI-MS analysis of a wear coupon (sample 2) showing the interrogation of a) the worn area and b) the untreated area using an electrospray solvent composition of 1:1 MeOH:toluene + 0.1 % formic acid. No change in chemical composition of desorbed analytes was detected between the two areas.

Figure APP 1.5: a) ESI-MS and b) DESI-FAIMS-MS analysis of an unspiked group 1 base oil.
APP 2- LabView Coding

The LabView code is comprised of an overall VI and individual Sub VI’s that correspond to each button on the user interface.

APP 2.1 Overall VI Code:
APP 2.2- Sub VI coding

The individual sub VI's are shown as grey boxes on the overall VI. An example of the sub VI coding is shown.
APP 3-Publications and Presentations

APP 3.1 Peer Reviewed Publications


APP 3.2 Conference Presentations and Posters

BMSS Annual Meeting (April 2012)
Presentation of a poster titled “Electrospray ionisation and desorption electrospray ionisation ion mobility-mass spectrometry studies of antioxidants used in commercial lubricants.”

Loughborough Chemistry Research Day (April 2012)
Presentation of a poster titled “Desorption electrospray ionisation mass spectrometry for trace surface analysis.”

Warwick 80/60 Mass Spectrometry Conference (December 2012)
Presentation of a poster titled “The quantitative analysis of an antioxidant additive in a lubricant oil matrix by DESI-MS.” Awarded runner-up in the poster competition.

BMSS Annual Meeting (September 2013)
Presentation of a poster titled “Application of ESI and DESI interfaced with chip based FAIMS-MS for the analysis of a lubricant additive in a complex oil matrix.”

BMSS Annual Meeting (March 2014)
Presentation of a poster titled “The direct analysis of lubricant oil additives using ESI and DESI hyphenated with FAIMS-MS.”
Loughborough Chemistry Research Day (May 2014)
Delivery of an oral presentation titled “Studies of lubricant additives using ambient ionisation-mass spectrometry.”

RSC Separation Science Meeting: Meeting the petrochemical challenge with separation science and mass spectrometry (November 2014)
Delivery of an oral presentation titled “The application of desorption electrospray ionisation hyphenated with ion mobility and mass spectrometry for the analysis of oil additives”

BMSS Annual Meeting (September 2015)
Presentation of a poster titled “Assessment of DART-MS for the qualitative and quantitative analysis of a lubricant oil additive”