Analysis of rubber accelerators by HPLC

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ANALYSIS OF RUBBER ACCELERATORS

BY HPLC

by

Rosmahani Che Isa

A Doctoral Thesis
Submitted in partial fulfilment of the requirements
for the award of
Doctor of Philosophy of Loughborough University
2009

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\[ \text{Sym}[10\%] = \frac{t_{10\%}}{f_{10\%}} \quad (2.3) \]

\[ \alpha = \frac{k_{n+1}}{k_n} \quad (2.4) \]

\[ R_{n,n+1} = 1.18 \left( \frac{(t_{n+1} - t_n)}{(w_{h_n} + w_{h,n+1})} \right) \quad (2.5) \]

\[ \ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (2.6) \]

\[ \Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (2.7) \]

\[ RR = \left( \frac{a_r}{x_0} + b_p \right) \times 100\% \quad (2.8) \]

\[ \text{LOD} = \frac{3xS_{x,y}}{m} \quad (2.9) \]

\[ \text{LOQ} = \frac{10xS_{x,y}}{m} \quad (2.10) \]

\[ M^{2+} + M(\text{DTC})_2 \rightleftharpoons 2M(\text{DTC})^* \quad (3.1) \]

\[ 2\text{NaOH}(aq) + \text{ZnSO}_4(aq) \rightarrow \text{Na}_2\text{SO}_4(aq) + \text{Zn(OH)}_2(s) \quad (4.1) \]

\[ R_2\text{NCS}_2^- + H^+ \rightleftharpoons R_2\text{NCS}_2H^+ \quad (\text{fast}) \quad (4.2) \]

\[ R_2\text{NCS}_2H + H^+ \rightleftharpoons R_2\text{NH}_2^+ + \text{CS}_2 \quad (\text{slow}) \quad (4.3) \]

\[ \frac{R_2\text{NCS}_2^- + H^+}{a} \rightleftharpoons \frac{R_2\text{NCS}_2H}{b} \quad (4.4) \]

\[ \frac{R_2\text{NCS}_2H}{k} \rightleftharpoons \frac{R_2\text{NH} + \text{CS}_2 + H^+}{k_1} \quad (4.5) \]
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<td>$R_2NH + CS_2 + H^+ \leftrightarrow R_2NH_2^+ + CS_2$</td>
<td>110</td>
</tr>
<tr>
<td>$CuR_2 + Cu^{2+} \leftrightarrow 2CuR^+$</td>
<td>125</td>
</tr>
<tr>
<td>$Ni^{2+} + 2Zn(DTC)_2 \rightarrow 2Zn(DTC)^+ + Ni(DTC)_2$</td>
<td>127</td>
</tr>
<tr>
<td>$L_1 - Ni - L_1 + L_2 - Ni - L_2 \leftrightarrow 2L_1 - Ni - L_2$</td>
<td>131</td>
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<tr>
<td>$Zn^{2+} + R_2NCS_2^- \leftrightarrow (R_2NCS_2 - Zn)^+$</td>
<td>148</td>
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<tr>
<td>$(R_2NCS_2 - Zn)^+ + R_2NCS_2^- \leftrightarrow (R_2NCS_2 - Zn - S_2CNR_2)_2$</td>
<td>148</td>
</tr>
<tr>
<td>$Zn(DTC)_2 + Ni^{2+} \rightarrow Zn^{2+} + Ni(DTC)_2$</td>
<td>156</td>
</tr>
<tr>
<td>$Zn(DTC)_2 \leftrightarrow Zn(DTC)^+ + DTC^-$</td>
<td>185</td>
</tr>
<tr>
<td>$Ni^{2+} + DTC^- \rightarrow Ni(DTC)^+$</td>
<td>185</td>
</tr>
<tr>
<td>$Ni(DTC)^+ + DTC^- \rightarrow Ni(DTC)_2$</td>
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</tr>
<tr>
<td>$Zn(DTC)_2 + Ni^{2+} \rightarrow Ni(DTC)^+ + Zn(DTC)^+$</td>
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</tr>
<tr>
<td>$Ni(DEC)_2 + Ni(DBC)_2 \leftrightarrow 2Ni(DEC)(DBC)$</td>
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</tr>
<tr>
<td>$K = \frac{[Ni(DEC)(DBC)]^2}{[Ni(DEC)_2][Ni(DBC)_2]}$</td>
<td>186</td>
</tr>
<tr>
<td>$A = abc$</td>
<td>189</td>
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</table>
List of Abbreviations and Symbols

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADEC</td>
<td>ammonium diethyldithiocarbamate</td>
</tr>
<tr>
<td>APDC</td>
<td>ammonium pyrrolidinedithiocarbamate</td>
</tr>
<tr>
<td>BEC</td>
<td>dibenzylthiocarbamate</td>
</tr>
<tr>
<td>BR</td>
<td>butadiene rubber</td>
</tr>
<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>cobalt (II) chloride</td>
</tr>
<tr>
<td>CoDBC</td>
<td>cobalt dibutyldithiocarbamate</td>
</tr>
<tr>
<td>CoDEC</td>
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</tr>
<tr>
<td>CoDMC</td>
<td>cobalt dimethyldithiocarbamate</td>
</tr>
<tr>
<td>CoDTC</td>
<td>cobalt dithiocarbamate</td>
</tr>
<tr>
<td>CR</td>
<td>chloroprene rubber</td>
</tr>
<tr>
<td>CuBEC</td>
<td>copper dibenzylthiocarbamate</td>
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<td>CuDBC</td>
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<tr>
<td>CuDEC</td>
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<tr>
<td>CuDMC</td>
<td>copper dimethyldithiocarbamate</td>
</tr>
<tr>
<td>CuDTC</td>
<td>copper dithiocarbamate</td>
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<td>DAD</td>
<td>diode array detector</td>
</tr>
<tr>
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<td>dibutylthiocarbamate</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
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<td>DEC</td>
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<tr>
<td>DTC</td>
<td>dithiocarbamate</td>
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<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
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<tr>
<td>EPDM</td>
<td>ethylene-propylene-diene terpolymer</td>
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<tr>
<td>EC</td>
<td>electrochemical detection</td>
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<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>Gemini ODS</td>
<td>Gemini C₁₈ column</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>synthetic polysisoprene</td>
</tr>
<tr>
<td>IIR</td>
<td>isobutylene-isoprene / butyl rubber</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>limit of quantitation</td>
</tr>
<tr>
<td>MBT</td>
<td>2-mercaptobenzothiazole</td>
</tr>
<tr>
<td>NH₃</td>
<td>ammonia</td>
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<tr>
<td>NiBEC</td>
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<td>NiCl₂</td>
<td>nickel (II) chloride</td>
</tr>
<tr>
<td>NiDBC</td>
<td>nickel dibutylthiocarbamate</td>
</tr>
<tr>
<td>NiDEC</td>
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</tr>
<tr>
<td>NiDMC</td>
<td>nickel dimethyldithiocarbamate</td>
</tr>
<tr>
<td>NiDTC</td>
<td>nickel dithiocarbamate</td>
</tr>
<tr>
<td>NiPD</td>
<td>nickel pentamethylenedithiocarbamate</td>
</tr>
<tr>
<td>NiSO₄</td>
<td>nickel (II) sulfate</td>
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### List of Abbreviations and Symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>NP-HPLC</td>
<td>normal phase HPLC</td>
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<tr>
<td>NBR</td>
<td>nitrile butadiene rubber</td>
</tr>
<tr>
<td>NR</td>
<td>natural rubber</td>
</tr>
<tr>
<td>NRL</td>
<td>natural rubber latex</td>
</tr>
<tr>
<td>ODS</td>
<td>octadecyl silane (C18)</td>
</tr>
<tr>
<td>PD</td>
<td>pentamethylenedithiocarbamate</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>reversed-phase HPLC</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
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<tr>
<td>SBR</td>
<td>styrene butadiene rubber</td>
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<tr>
<td>SR</td>
<td>synthetic rubber</td>
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<tr>
<td>TARRC</td>
<td>Tun Abdul Razak Research Centre</td>
</tr>
<tr>
<td>TETD</td>
<td>tetraethylthiuram disulfide</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMTD</td>
<td>tetramethylthiuram disulfide</td>
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<td>Ultrasphere-ODS</td>
<td>Beckman-Ultrasphere ODS column</td>
</tr>
<tr>
<td>XBridge C8</td>
<td>XBridge C8 column</td>
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<tr>
<td>XBridge ODS</td>
<td>XBridge C18 column</td>
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<tr>
<td>XTerra ODS</td>
<td>XTerra MS-C18 column</td>
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<tr>
<td>ZBEC</td>
<td>zinc dibenzyldithiocarbamate</td>
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<tr>
<td>ZDEC</td>
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<tr>
<td>ZDTC</td>
<td>zinc dithiocarbamate</td>
</tr>
<tr>
<td>ZnSO4</td>
<td>zinc (II) sulfate</td>
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<tr>
<td>Zorbax Cyano</td>
<td>Zorbax Cyano column</td>
</tr>
<tr>
<td>ZPD</td>
<td>zinc pentamethylenedithiocarbamate</td>
</tr>
<tr>
<td>Zr-PBD</td>
<td>ZirChrom-polybutadiene coated column</td>
</tr>
</tbody>
</table>

### Symbols

- $\alpha$: selectivity
- $\Delta G^\circ$: Gibbs free energy
- $\Delta H^\circ$: standard enthalpy
- $\Delta S^\circ$: standard entropy
- $H$: plate height
- $k$: retention factor
- $K$: equilibrium constant
- $N$: number of theoretical plates in the column (efficiency)
- $N/m$: efficiency per column length (meter)
- $R$: resolution
- $t_R$: retention time of solute
- $t_0$: dead volume marker
- $w_h$: peak width at half-height
- $Sym$: peak asymmetry
Analysis of rubber accelerators by HPLC

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Abstract

The analysis of rubber accelerators particularly zinc dithiocarbamates (ZDTCs) was carried out by high performance liquid chromatography (HPLC) to develop an improved analytical method for the determination of rubber accelerators. Further understanding of their chemical and chromatographic behaviour has been established by investigating a few conventional as well as the latest reported methods, which include the pre-column derivatisation of ZDTCs to cobalt (III) complexes, direct determination by the addition of other protective agent and pre-column derivatisation of ZDTCs to copper (III) complexes. An improved method called on-column derivatisation to nickel (II) complexes was proposed and optimized, which include the effect of nickel salt concentration added in the mobile phase, pH of mobile phase, type of organic solvent and their composition, temperature, flow rate and the ZDTC mixture composition on the production of mixed ligand complexes. It was found that 0.01% Ni (II) without pH alteration in 70% methanol-water at 1 ml/min and gradient temperature of 40 to 70°C on XBridge C8 (4.6 x 50 mm, 3.5 µm) column were the most suitable conditions for the separation of the zinc dithiocarbamates currently found in the rubber samples. The simpler and direct method of on-column derivatisation was applied to 6 rubber samples and compared with the method of pre-column derivatisation to nickel (II) as well as the previously recommended method of pre-column derivatisation to copper (II) complexes. It was found that the method proposed in this study gave the most reliable results with better sensitivity (Limit of detections and quantitations were between 0.0001 to 0.0002% and 0.0003 to 0.0007% respectively).
The aim of this study is to acquire a better understanding of the analysis of rubber accelerators. The investigation assessed several different methods available to determine rubber accelerators by HPLC and proposed an improved analytical method, which could be used for the determination of rubber accelerators and related sulfur compounds, that will lead to economic and environmental benefits.

Research in recent years [1 - 12] has alarmed users that the use of rubber products may cause the development of type IV allergy (contact dermatitis) as well as the possibility of contact urticaria, as the chemicals added to rubber have become widely recognised as contact sensitizers to animals as well as humans. The latest reports [13 - 20] have also shown that rubber chemicals (mostly thiurams and carbamates), are one of the principal allergen groups. Almost all rubber products, including both natural and synthetic rubber materials, contain accelerators and of which, the most commonly used are the dithiocarbamates.

Although there have been many studies [21, 22] on dithiocarbamates in other applications, up till now not much research has been carried out on the HPLC analysis of dithiocarbamates which have been used as rubber accelerators. The conventional method uses pre-column derivatisation either with cobalt ions [4, 23, 24] or copper ions [25], and requires a lot of organic solvent and possibly causes loss of analytes during the process. Furthermore, the use of cobalt (II) ions produces many additional peaks when two or more zinc dithiocarbamates are present, which complicates the determination [23]. Therefore, it is important and relevant to further investigate the analysis of rubber accelerators by HPLC in order to develop an improved method.
1.1. Rubber

Rubber was historically known as caoutchouc, which derived from the Indian word "caa-o-chu" meaning "weeping tree" [26]. This material was polyisoprene recovered from the sap of *Hevea Brasiliensis*, and is currently referred to as natural rubber (NR). The industry often used the term rubber for the raw polymer and the uncured compound. The polymer often has an elastic property and is also deformable, but not always. Rubber also sometimes called an elastomer when referring to the vulcanized material. [27] Elastomers are the basis for all rubber products and include both natural and synthetic materials.

As described by Hofmann [26] and Ciesielski [27], the early stages of rubber technology began well over 2000 years ago among the Aztecs and Mayas of South America, who used rubber for shoe soles, coated fabrics, and play balls. The use and price of natural rubber later exploded in 1910, when the motor car truly arrived, which needed flexible tyres. Around this time, chemists were also actively searching for rubbery materials which could be manufactured artificially, and the Russians prepared the first known synthetic rubber (SR) called polybutadiene. In the 1930s, the commercial production of a synthetic rubber called Buna-S (styrene butadiene copolymer) began in German. Since then and with the outbreak of Second World War that caused the disruption of supplies, more synthetic rubbers were developed, including styrene butadiene rubber (SBR), chloroprene rubber (CR), nitrile butadiene rubber (NBR) and a synthetic analogue of NR, polyisoprene (IR). A significant recent addition is a class of materials called thermoplastic elastomers. Today, about two thirds of rubber is produced synthetically. However, the new awareness of our environment gives the NR the added advantage of being seen as a renewable resource, because the raw material for most synthetic elastomers is still petroleum oil.
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1.1.1. Natural rubber (NR)

Natural rubber is a milky sap called latex, produced by the rubber tree, consisting of water, rubber component (polyisoprene - Fig. 1.1), and small quantities of other substances, such as proteins, resins, ash and sugars. The coagulated latex is then processed and dried. The latex is also used directly in industry for items like medical gloves and condoms. [26, 27, 28]

![Fig. 1.1. Structure of natural rubber.](image)

The rubber component of NR consists of more than 99% of linear cis-1,4-polyisoprene (Fig. 1.1) [26, 28]. NR is available in many grades related to its dirt content and precise method of production. The most popular are ribbed smoked sheets (RSS), Standard Malaysian Rubber (SMR) and Standard Indonesian Rubber (SIR) [27]. They are also special grades which are produced by fulfilling specific requirements, such as SMR CV (constant viscosity) [27], Superior Processing Rubber (SP/PA Grades - blends of normal and partially crosslinked latex), Deproteinated Natural Rubber (DP-NR), Epoxidized Natural Rubber (ENR) and Thermoplastic NR (blend of NR and polypropylene) [26].

Because of its exceptional chemical and physical properties, NR is a very flexible raw material. Initially, NR was used for all rubber products. However, due to the rising specialization and improvements of SR grades, NR has been replaced in many applications, particularly for most technical products with requirements for heat and swelling resistance. For some rubber products where special chemical and physical properties are needed, NR is still preferred. For example, NR, which has a very good abrasion resistance, is still the important choice for slurry pump liners, impellers and tank lining. It also has a very good dynamic mechanical properties and therefore is used in tires, rubber springs and vibration mounts. NR vulcanizates have a fairly low heat build up and thus have constantly been great importance for producing...
truck tires. Another significant and leading application of NR is in the production of thin-walled soft products, with a high strength like balloons, surgical gloves or other sanitary rubber products. This is because of the strain crystallization and self-reinforcing properties found in NR. Its high elasticity and low hysteresis also have made it an important material for producing suspension components and bumpers. [26, 27]

1.1.2. Synthetic rubber (SR)

Initially, only the rubber materials that were to be similar to NR were considered as SR, and generally, these materials are acquired through the homo- or copolymerization of conjugated dienes. Nowadays, the numbers of SR types has grown to be very large and they can be classified into different grades. This is as a result of the use of more and more mono-olefins and other monomers for the synthesis of saturated rubbers, which can be crosslinked through polymerization, polycondensation and polyaddition reactions. However, rubbers produced from diene monomers are still the most important and widely used, while saturated rubbers have become specialty products, like the special grades of NR. Examples of the main SR types, include polybutadiene (butadiene rubber-BR), styrene-butadiene rubber (SBR), nitrile rubber (acrylonitrile-butadiene rubber-NBR), polychloroprene (chloroprene rubber-CR), polyisoprene (synthetic isoprene rubber-IR), butyl rubber (isobutylene-isoprene rubber-IIR) and ethylene-propylene-diene terpolymer (EPDM). [26]

There are three reaction steps in a polymerization: initiation, propagation, and termination. The basic requirement for the polymerization process of SR is the availability of double bonds in a monomer (Table 1.1). Under the influence of a suitable external agent and the reactivity of the double bond, it can break in two ways: either one electron goes to each atom creating two free radicals (homolysis - equation 1.1), which is the initiation of a free radical polymerization,
Chapter 1: Introduction

or the pair of electrons stays with one or the other of the two atoms (heterolysis – equation 1.2), leading to ions.

\[
\begin{align*}
\text{\text{\input{math}}}
\end{align*}
\]

or the pair of electrons stays with one or the other of the two atoms (heterolysis – equation 1.2), leading to ions.

\[
\begin{align*}
\text{\text{\input{math}}}
\end{align*}
\]

Table 1.1
Monomers for the main SR [26]

<table>
<thead>
<tr>
<th>Monomers</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-butadiene</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \text{CH}:=\text{CH}_2 )</td>
</tr>
<tr>
<td>styrene (vinylbenzene)</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \benzene )</td>
</tr>
<tr>
<td>acrylonitrile</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \text{C}:=\text{N} )</td>
</tr>
<tr>
<td>chloroprene</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \text{CH}:=\text{CH}_2 )</td>
</tr>
<tr>
<td>isoprene</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \text{CH}:=\text{CH}_2 )</td>
</tr>
<tr>
<td>isobutylene</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \text{CH}_3 )</td>
</tr>
<tr>
<td>ethylene</td>
<td>( \text{H}_2\text{C}:=\text{CH}_2 )</td>
</tr>
<tr>
<td>propylene</td>
<td>( \text{H}_2\text{C}:=\text{CH} ) ( \text{CH}_3 )</td>
</tr>
</tbody>
</table>

The second route is the starting point of an ionic addition polymerization, where, depending on the nature of the catalyst used, the reaction proceeds as cationic, anionic, or coordination polymerization. The polymerization can be in a homogeneous phase (bulk, solution polymerization) or in a heterogeneous phase (emulsion polymerization). The free radical polymerization is usually conducted in a heterogeneous phase, and the ionic
polymerization in a homogeneous phase. [26]

Polyadditions and polycondensations on the other hand only play a small role in the production of SR. In these reactions, multifunctional compounds react to form macromolecules, and if the reagents are coupled by simple addition, the process is referred to as polyaddition. On the other hand, if the functional groups in the chain form by elimination of water, hydrogen chloride, or other small molecules, one is dealing with a polycondensation reaction. [26]

Methods of production and some major uses of the most important SR are summarized in Table 1.2.

Table 1.2
Method of production and major uses of main SR (+ = major; (+) = occasional use) [26]

<table>
<thead>
<tr>
<th>Method of production and uses</th>
<th>BR</th>
<th>SBR</th>
<th>NBR</th>
<th>CR</th>
<th>IR</th>
<th>HIR</th>
<th>EPDM</th>
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<td><strong>Method of production</strong></td>
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<td>Solution polymerization</td>
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<td>Emulsion polymerization</td>
<td>+</td>
<td>+</td>
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<td></td>
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<tr>
<td>Coordination polymerization</td>
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<td></td>
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<td>+</td>
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<td>Cationic polymerization</td>
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<td>Passenger car tyres – tread</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passenger car tyres – carcass</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td></td>
<td>(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truck tyres – tread</td>
<td>+</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Truck tyres – carcass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Conveyor belts</td>
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<td>(+)</td>
<td>+</td>
<td>(+)</td>
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<tr>
<td>V-belts</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
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<td>Fuel hose</td>
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<tr>
<td>Milking machine hose</td>
<td>+</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
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<tr>
<td>Heating and cooling hose</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>Oil and grease resistant hose</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>Chemical resistant hose</td>
<td>+</td>
<td></td>
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Chapter 1: Introduction

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>(+)</th>
</tr>
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<tbody>
<tr>
<td>Shaft seals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat resistant seals</td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>Oil resistant seals</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Other seals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food and pharmaceutical products</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatty foods</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nipples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
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<td>+</td>
</tr>
<tr>
<td>Sanitary rubber products and balloons</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rubberized fabrics</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gloves</td>
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<td>+</td>
</tr>
<tr>
<td>Cables</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Shoes and soling</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Latex products</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1.1.3. Rubber compounding

By itself, raw rubber is used in limited applications and further processing is required. Rubber compounding is the process of mixing the raw rubber with compounding ingredients, which will satisfy the requirements of processing of the rubber and of the product's required physical properties. Fig. 1.2 illustrates the basic principles of rubber processing technology and shows where the mixing or compounding step takes place.

Fig. 1.2. Flow diagram illustrating basic principles of rubber processing technology, where mastication is a softening process, moulding, extrusion and calendering are the shaping processes, and building is a process in which the various components of the composite are brought together. [29]

The compounding ingredients include a wide variety of substances and can be divided into six categories:
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1. Polymers or raw rubbers
2. Vulcanization chemicals
3. Protective agents
4. Fillers
5. Plasticizers
6. Miscellaneous materials

Polymers or the raw rubber can either be an individual polymer of NR or any SR or blends of them. Vulcanization chemicals include cross-linking agents, accelerators, metal oxides, activators and vulcanization inhibitors or retarder. Sulfur is the classical cross-linking agent for unsaturated rubbers like NR, SBR, NBR, BR, and EPDM. Rubbers can also be cross-linked with peroxides, electron beams or radiation. However, these methods are not widely used. Accelerators are used to increase both the rate of sulfur cross-linking in a rubber compound and the cross-linking density. More details on accelerated vulcanization and rubber accelerators are discussed in the next sections. Metal oxides on the other hand, are required in the rubber compounding to develop the full potential of accelerators. The main metal oxide used is zinc oxide. Together with metal oxides, stearic acid is needed as an activator for the accelerator systems, and together with the accelerator, they speed up the vulcanization process. Inhibitors or retarders are used to prevent premature vulcanization or scorching. The primary examples of inhibitors are based on phthalimide sulfenamides. [26, 27]

Protective agents are used to obtain resistance from attacks by oxygen, ozone or thermal degradation. They include nonstaining antioxidants, staining antioxidants, antiozonants and waxes. Examples of the most useful and significant protective agents are aromatic amines, such as the p-phenylenediamine derivatives (PPDs). [26, 30] PPDs not only protect rubber products from ozone degradation but also improve resistance to fatigue and degradation from oxygen, heat and metal ions. On the other hand, fillers, such as carbon black, clays or silicas are added into the rubber compound to achieve properties like stiffness (hardness), abrasion resistance, tear
strength and tensile strength. [26, 27, 30]

Plasticizers, sometime called softeners, are oily and slippery materials used to improve the deformability of a polymeric material. At low levels they help the dispersion of fillers and at higher levels they can reduce the viscosity of uncured compound and reduce the vulcanization stiffness. The most significant are petroleum oils that can be categories into paraffinic, naphthenic, and aromatic compounds. Animal and vegetable oils are also used as softeners or processing aids. [26, 27] Other authors, however, categorised all of these oils as processing aids [30]. Other materials which have been used in rubber compounds include resins, factices (vulcanized vegetable oils), processing aids, chemical peptizers and short fibres. Their functions include acting as tackifiers, facilitating the processing, as oxidation catalysts or radical acceptors and to further improve the material properties. [26, 27, 30]

1.1.4. Accelerated vulcanization

Vulcanization is the main process in the rubber technology that links the raw material and the finished product as a crosslinked molecular network (Fig. 1.3). Methods of vulcanization that are currently available include, sulfur, peroxide, resin, moisture, urethane, metal oxide, radiation, high temperature and dynamic crosslinking [31, 32]. Rubber vulcanization by sulfur is the most widely used curing technique, but sulfur vulcanization without any accelerators takes several hours and is no longer of commercial importance [32].

According to a review on the recent developments in the vulcanization of elastomers [32], the discovery of the acceleration of vulcanization was the second most important step after the discovery of vulcanization itself. The addition of a small amount of accelerators can speed up and increase the efficiency of the vulcanization process [34, 35], or enable it to be carried out at a lower temperatures. At the same time, better physical properties and aging resistance are usually achieved [35]. Moreover, the addition of
accelerators has reduced the proportion of sulfur required from 8-10 parts to 1-3 parts, which consequently gives much better oxidation resistance [32]. An accelerator can also be used for the sulfurless vulcanization of some chloro-rubbers, for example chloroprene rubber and chlorosulfonated polyethylene [34].

There are three general reaction steps in accelerated-sulfur vulcanization: Firstly, the accelerator reacts with sulfur to give monomeric polysulfides of the structure Ac-S_x-Ac, where Ac is an organic radical derived from the accelerator and S_x stand for multiple sulfur links. Then, the monomeric polysulfides interact with rubber to form polymeric polysulfides, e.g. rubber-S_x-Ac, and lastly, rubber polysulfides react, either directly or through an intermediate, to give crosslinked, rubber-S_x-rubber. Fig. 1.4 shows an example of the reaction scheme of natural rubber accelerated-sulfur vulcanization using a benzothiazole derivative as an accelerator. [33]
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Fig. 1.4. A reaction scheme of natural rubber accelerated-sulfur vulcanization using a benzothiazole derivative as an accelerator. [33]

1.2. Rubber accelerators

The accelerators that are currently used on a large scale in the sulfur vulcanization process, include thiazoles, sulfenamides, thiurams, dithiocarbamates, xanthates and amines. They are categorized into four main groups depending on their action: ultra, strong, medium-strong and weak accelerators [35]. The most significant accelerators are the dithiocarbamates, thiuram disulfides, thiazoles, sulfenamides and guanidines [34]. Dithiocarbamates and thiuram disulfides are examples of ultra-accelerators, which make the reaction very rapid (Fig. 1.5). Thiazoles and sulfenamides on the other hand are strong accelerators, which are also known as semi-ultra-accelerators. Lastly, guanidines are one of the medium-strong accelerators. Table 1.3 shows some of the commercially available accelerators from these groups. Weak accelerators seem to be less important than the other types of accelerators. This might be because they are only used for special effects,
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such as for the activation of other accelerators. Weak accelerators include aliphatic amines, hexamethylenetetramine, thiocarbanilide, anhydroformaldehyde-p-toluidine, and some inorganic oxides and sulfides [35].

The dithiocarbamates (DTCs) are the most widely used, especially in the manufacturing of latex-dipped products due to their outstanding vulcanization time-temperature profile [25, 32] (Fig. 1.5). Consequently, in the current study, the analysis of rubber accelerators will focus on the DTC group. However, Coran [33] warned that there was a need to cut down the use of accelerators based on secondary amines, especially dithiocarbamate accelerators, because they can react with nitrogen oxides to form suspected carcinogenic nitrosamines.

The selection of the accelerator normally depends on the vulcanization activities and product properties required. Ranges of accelerators are available, and occasionally a mixture of primary and secondary accelerators is employed [34].

Fig. 1.5. Typical cure curve with different accelerator systems: A) Dithiocarbamates; B) Thiurams; C) Thiazoles; D) Sulfenamides. [32]
Table 1.3
Commercially available accelerators from the most significant groups [36]

<table>
<thead>
<tr>
<th>Type</th>
<th>Chemical Name</th>
<th>Trade Name</th>
<th>Other Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dithiocarbarnates (DTCs)</td>
<td>Zinc dimethyl-DTC</td>
<td>Methasan®</td>
<td>Agricultural fungicide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methyl Zimate®</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Naftocit® Di 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zinc diethyl-DTC</td>
<td>Ethyl Zimate®</td>
<td>Heat stabilizer for polyethylene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perkacit® ZDEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anchor® ZDEC</td>
<td></td>
</tr>
<tr>
<td>Thiuram Disulfides (TDs)</td>
<td>Tetramethyl-TD</td>
<td>Akrochem® TMTD</td>
<td>Vulcanizing agent, fungicide, seed disinfectant, animal repellent, antiseptic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methyl Tuads®</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Thurad®</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetraethyl-TD</td>
<td>Akrochem TETD</td>
<td>Fungicide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ekaland TETD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perkacit® TETD</td>
<td></td>
</tr>
<tr>
<td>Thiazoles (Ts)</td>
<td>2-Mercaptopreno-T (MBT)</td>
<td>Captax®</td>
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<tr>
<td></td>
<td></td>
<td>Rotax®</td>
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<td></td>
<td>Thiotax®</td>
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<tr>
<td></td>
<td>Zinc-MBT</td>
<td>Bantox®</td>
<td>Latex foam curing systems</td>
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<tr>
<td></td>
<td></td>
<td>Octocure ZMBT-50</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Zetax®</td>
<td></td>
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<tr>
<td>Sulfenamides</td>
<td>Butyl 2-benzothiazole sulfenamide</td>
<td>Delac® NS</td>
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<td>Santocure® NS</td>
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<tr>
<td></td>
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<td>Vanax® NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thiocarbamyl sulfenamide</td>
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<tr>
<td>Guanidines</td>
<td>Di-o-tolyl guanidine</td>
<td>Akrochem® DOTG</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Anchor® DOTG</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Ekaland DOTG</td>
<td></td>
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<tr>
<td></td>
<td>Diphenylguanidine</td>
<td>Akrochem® DPG</td>
<td>Primary standard for acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DPG</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Naftocit® DPG</td>
<td></td>
</tr>
</tbody>
</table>

1.2.1. Importance of analysis

As well as the analysis of accelerators as part of the blend and process control, the residue analysis of many accelerants is important because of their effects on consumers.
1.2.1.1. Rubber products and allergy type IV

Almost all rubber products, including natural rubber (NR) and synthetic rubber (SR), contain accelerators. Most of these rubber products are also in contact with the human body. Examples are household or recreational rubber products, clothing and footwear products, cosmetic and healthcare products and many more. More specifically, there are rubber gloves, rubber cosmetic applicators like eye shadow sponge, rubber shoes, condoms, rubber threads etc. These products are also in contact with parts of the body, which include hands, face, feet and genitals. Research in recent years [1 - 12] has alarmed users that the use of these products may cause the development of type IV allergy (contact dermatitis) as well as the possibility of contact urticaria, as some of the chemicals added to rubber have been well known as contact sensitizers to animals as well as humans. Allergy type IV is a delayed sensitivity response after repeated contact. It usually occurs 24 to 48 hours after contact and includes erythema, itching, oedema, cracking of the skin and red swollen rashes that appear only on skin areas that have been in contact with the products. Type IV allergy can also arise 8 hours to 5 days after exposure. Contact urticaria on the other hand is a gradual onset reaction which generally includes dryness, scaling, cracking and erythema. [37]

Natural rubber latex (NRL) gloves are usually considered as the source of both type IV allergy and contact urticaria as most allergy cases reported [2, 4, 8 - 11, 38] are related to the use of these gloves. However, contact urticaria due to gloves is mainly connected to the immediate-type (type I) allergy, to NRL proteins, and rarely due to rubber chemicals. Brehler and Sedlmayr [38], who studied the clinical relevancy of contact urticaria and rubber chemicals on 75 latex-allergy patients, also found out that it is very rare. However, they suggested that contact urticaria due to rubber chemicals should also be studied in patients with contact urticaria due to synthetic rubber gloves as well as in patients with contact urticaria due to NRL gloves but no latex allergy. Other than NRL gloves, rubber shoes have also been reported to be a source of sensitization [3, 5, 7]. Synthetic rubber products,
which contain a higher amount of more active accelerators [34] are more likely to give a higher possibility of allergic reaction to the users. However, only a few studies on allergenicity of rubber chemicals have been done on synthetic rubber products [39, 40].

A few recent reports [13 - 20] showed that rubber chemicals are still one of the main significant allergen groups, which have been associated with irritant dermatitis and allergic contact dermatitis (allergy type IV). For example, Martin et al. [13] found that 'black henna' tattoos might be a hidden source of natural rubber latex allergy when a 14-year-old girl showed positive reaction to thiurams, which was suspected to be added as rubber additives in the tacky transfer applied prior to the black henna tattooing. Ten years' experience of patch testing with a shoe series (chemicals used in leather tanning, rubber processing and/or adhesives) on 230 patients by Holden and Gawkrodger [14] also showed that rubber chemicals were still one of the top allergens in patients with foot dermatitis. In another study by Clayton and Wilkinson [15], contact dermatoses in healthcare workers in a UK centre between 1996 and 2003 showed a reduction in the frequency of type I allergy due to NRL proteins. They also found that type IV allergy to fragrance mix (13%) was the most frequent cause of allergic contact dermatitis. However, thiuram mix (8%) and carba mix (4%) were still the most frequent indicators of type IV allergy caused by the chemical additives in rubber gloves. In the most recent 2 years, many more reports [16 - 20] appeared to show that both thiuram and carba mix are still the most common allergens amongst the North American contact dermatitis group [16], construction workers [17], Hispanic population [20], and health care workers [18, 19].

1.2.1.2. Most important sensitisers

Amongst the various chemicals added into the rubber products, dithiocarbamates, thiurams and mercaptobenzothiazole groups are the most commonly reported as contact allergens, while guanidines and several antioxidants have rarely been reported as sensitisers [41]. As mentioned
before, these chemicals are part of the most widely used rubber accelerators although some of these chemicals are also added as preservatives, vulcanizers, retarders, antioxidants etc. However, these chemicals may react with other compounds, such as zinc oxide, which for example may convert thirurams to zinc dithiocarbamates. Unexpected compounds may then be present in the products, although the manufacturer did not add it directly and thus did not put it in the label [42]. A recent study by Bergendorff et al [43] about the chemical changes in common allergens during vulcanisation, confirmed that thirurams disulfides rarely appeared in the final products, although they may have been used as additives. They also confirmed that thirurams were often converted to dithiocarbamates or to products with mercaptobenzothiazole structures, if these have been used together with thirurams.

In a study by Knudsen and Menne [44], thirurams were found to be the most important sensitisers after zinc diethyldithiocarbamate, which was the most commonly reported contact sensitiser amongst the dithiocarbamates in rubber gloves. The authors also found that almost all patients who reacted to carbamates also reacted to thirurams. This observation was discussed as it is either a cross reactivity or parallel sensitisation of thirurams and carbamates. In 1998 a report by Schnuch [45] showed that there was also a significant increase in the frequency of sensitisation to thiruram mix from 1992 to 1995.

However, a recent survey by Depree and colleagues [46] in USA of the levels of rubber accelerators in both latex and nitrile gloves, that were commercially available 'off-the-shelf', showed no thirurams. The rubber accelerators found were mostly 2-mercaptobenzothiazole (MBT), zinc diethyldithiocarbamate (ZDEC) and zinc dibutylthiocarbamate (ZDBC), either alone or in multiple combinations and ranged from "not detectable" to 7.35 mg/g. Another observation was that the amount of accelerators found in powder-free gloves was less than in powdered gloves from every manufacturer. The authors suggested that it could probably be attributed to differences in the manufacturing process between the two types of gloves. The latest survey by Bergendorff and colleagues [47], who studied
disposable medical gloves used in southern Sweden, found that, of 19 gloves, 10 contained ZDEC (0.070-3.5 mg/g), 3 contained zinc pentamethylene-dithiocarbamate (ZPD) (1.0-4.3 mg/g), 4 contained ZDBC (0.9-1.1 mg/g) and another 2 contained MBT (0.005-0.008 mg/g). On the other hand, the thiurams, the most commonly reported cause of rubber chemicals allergy contact dermatitis were not found in any of the gloves in these surveys.

1.2.1.3. Methods of controlling sensitisation

A lot of reliable scientific evidence has been produced showing that the chemicals present in the rubber products caused a threat for sensitisation against these chemicals, thus, a method or way of controlling the levels of these chemicals in rubber products is needed. In theory, the possibility of sensitisation can be eliminated by a complete replacement with non-sensitisers and/or a significant reduction in exposure.

Substitution of the traditional vulcanisation process by a new technology, such as irradiation, may reduce the demand for rubber accelerators. The replacement of strong allergens with weaker allergens may also be used, as butylated compounds are believed to be weaker sensitisers than methyl, ethyl and pentamethylene derivatives [42]. A ranking of the allergenicity potency of the 15 most commonly used rubber chemicals reported by De Jong et al. [12], confirm this concept. The proposed chemicals of choice for use in the production of rubber products would be tetrabutylthiuram disulphide (TBTD) for the thiurams; zinc dibutylthiocarbamate (ZDBC) for the carbamates; and zinc mercaptobenzothiazole (ZMBT) for the benzothiazoles.

However, this may not completely prevent sensitisation by rubber accelerators as the same chemicals may be used in the rubber or latex production. In addition, rubber products may contain reactive chemicals that can react with each other and produced new allergenic chemicals [43], which then cause the same problems. Furthermore, the sensitisation and elicitation thresholds for the rubber chemicals are not known [42]. Therefore, the
identification and determination of the most significant sensitisers are still important.

1.3. Dithiocarbamates and related sulfur compounds

Dithiocarbamates are the sulfur analogs of carbamates, and were reported as a group of chemical complexes in the early years of organo-sulfur chemistry. Sodium diethyldithiocarbamate, in particular, has established its useful role in the field of inorganic analysis because of the solubility of its metal salts. In the field of rubber chemistry, the dithiocarbamates have been used as vulcanization accelerators and antioxidants, and rubber chemists have also contributed significantly to the knowledge of the behaviour of the dithiocarbamates. The biological activity of the dithiocarbamates, on the other hand, has found a more practical application in the field of medicine and agriculture. In medicine, they were first used in experiments on the control of various dermatophytes, for example for the treatment of scabies. They were also used for the treatment of chronic alcoholism. As in the field of agriculture, a large volume of biological literature has appeared showing the dithiocarbamate's biological activities, such as insecticidal, acaricidal and fungicidal activities [21]. Recently, Szolar [22] has further extended our knowledge on the application of dithiocarbamates, by reviewing the analysis of dithiocarbamates in both environmental and pharmaceutical applications.

Mercaptobenzothiazole is also a widely used accelerator for sulfur vulcanization of rubbers [34]. 2-Mercaptobenzothiazole, especially, has also been used in many other industries as a corrosion inhibitor, flotation agent, lubricant and additive to metalworking fluids as well as plastics [48].

1.3.1. Preparation

N-Substituted dithiocarbamic acids, are usually prepared by the reaction
of carbon disulfide and an amine in alcoholic or aqueous solution, where in practice, an alkali metal hydroxide is used to form the salt [21]:

\[ RNH_2 + CS_2 + NaOH \rightarrow RNHCSSNa + H_2O \]  

(1.3)

Heavy metal salts, many of which are coloured, are further prepared by adding a metal chloride, sulfate etc. solution to a solution of an ammonium or alkali metal salt of the dithiocarbamic acid [21] as equation 1.4 below:

\[ 2RNHCSSNa + MCl_2 \rightarrow RNHCS - M - SSCHNR + 2NaCl \]  

(1.4)

where M stands for metal. However, zinc dialkyldithiocarbamates used as rubber accelerators are usually prepared from the reaction between zinc oxide, dialkylamine and carbon disulfide. Thiuram disulfides, on the other hand are formed by oxidation of alkyl- or dialkyl-dithiocarbamic acids (usually water soluble salts) with a variety of mild oxidation agents, such as iodine, hydrogen peroxide, ammonium persulfate or potassium ferricyanide. However, their commercial production is usually accomplished by passing chlorine through an aqueous solution of a dithiocarbamate. The heterocyclic compound of 2-benzothiazolethiol, commonly known as 2-mercaptobenzothiazole, is prepared industrially by reacting aniline, carbon disulfide and sulfur at elevated pressure and temperature. [49]

1.3.2. Structure and properties

There are three main groups of dithiocarbamates, the dithiocarbamate salts, dithiocarbamate esters and thiuram disulfides [50]. Dithiocarbamate salts and thiuram disulfides are usually the most widely used in the rubber industry. Table 1.4 lists a number of selected rubber accelerators of dithiocarbamate salts, thiuram disulfides and mercaptobenzothiazole with their structures and properties.

All the rubber accelerators listed in Table 1.4 are practically insoluble in water. They are more soluble in organic solvents, such as chloroform, carbon tetrachloride, dichloromethane, acetone and ethyl ether [4, 5, 23, 24, 49, 51 - 56]. The chlorinated solvents are the most commonly used because of the
higher solubility in these solvents. These solvents are also used for extraction, particularly, dichloromethane and chloroform, as they are very volatile, and therefore provide an added bonus when reducing the volume of the extraction solvent. Acetone has also often been a solvent of choice as it is always viewed as a "universal" solvent. [57]

The general properties of metal dithiocarbamate complexes have been well known for an extended time. However, only a few authors have discussed the strengths of different complexes. A study cited by Thorn and Ludwig [21] showed that the complexes of the metals with diethyldithiocarbamate increased in stability as follows: Mn, AsIII, Zn, SnII, FeII, Cd, Pb, Bi, CoII, Ni, CuII, Ag, HgII. Therefore, the metal in the dithiocarbamate complexes, such as zinc or cobalt, will be displaced by any other metal to the right of it in the series, such as nickel or copper. Other authors quoted by Thorn and Ludwig have also observed the same exchange reaction between the less stable and the more stable complexes. One of them also detected that the copper complex exists in two forms in equilibrium:

\[ CuR_2 + Cu^{2+} \leftrightarrow 2CuR^+ \]  \hspace{1cm} (1.5)

Comparable equilibria with the other metals, however, were not seen.

In developing a liquid chromatographic method for the determination of dithiocarbamate accelerators, Kaniwa [23] found that, multivalent ions, like, cobalt (III) or nickel (II) ions produced mixed ligand complexes.
## Table 1.4
Structure and properties of selected rubber accelerators

<table>
<thead>
<tr>
<th>Accelerators</th>
<th>Structure</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc dimethyldithiocarbamate (ZDMC)</td>
<td><img src="image" alt="Structure" /></td>
<td>MW: 305.8 g/mol, MP: 248-257 °C</td>
</tr>
<tr>
<td>C₆H₁₂N₂S₄Zn (Ziram)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc diethylidithiocarbamate (ZDEC)</td>
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<td>MW: 361.93 g/mol, MP: 178-181 °C</td>
</tr>
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<td>C₁₀H₂₀N₂S₄Zn (Ethyl Ziram)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc pentamethylene-dithiocarbamate (ZPD)</td>
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<td>MW: 385.93 g/mol, MP: 220 °C</td>
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<tr>
<td>C₁₂H₂₀N₂S₄Zn</td>
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<td></td>
</tr>
<tr>
<td>Zinc dibutylidithiocarbamate (ZDBC)</td>
<td><img src="image" alt="Structure" /></td>
<td>MW: 474.12 g/mol, MP: 104 °C</td>
</tr>
<tr>
<td>C₁₈H₃₈N₂S₄Zn</td>
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<td></td>
</tr>
<tr>
<td>Zinc dibenzyldithiocarbamate (ZBEC)</td>
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<tr>
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<td>Tetramethylthiuram disulfide (TMTD)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td><img src="image" alt="Structure" /></td>
<td>MW: 167.25 g/mol, MP: 177-181 °C</td>
</tr>
<tr>
<td>C₇H₈NS₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MW = molecular weight; MP = melting point
where \( L_1 \) and \( L_2 \) stand for ligand 1 and 2, and \( M \) stand for metals. Therefore, a mixture of two or more different complexes will give extra mixed ligand peaks. This phenomenon was earlier proposed by Liska et al. [52] in their study of the chromatographic properties of nickel(II) bisdialkyldithiocarbamate complexes.

Dithiocarbamates and their derivatives show high-intensity absorption in the ultraviolet region. Zinc diethyldithiocarbamate for example, has an intense band at 260 nm [21].

Many of the heavy metal salts of dithiocarbamic acids absorb in the visible region. As cited by Thorn and Ludwig, cupric dimethyldithiocarbamate, for example, has an absorption band at 425 nm. Later, Chilton [58] documented the ultraviolet spectra of the cobalt, copper and nickel salts as diethyldithiocarbamate complexes in carbon tetrachloride. Kress [59] also recorded the spectra for zinc diethyldithiocarbamate.

Again as reported by Thorn and Ludwig, tetramethylthiuram monosulfide shows strong ultraviolet bands in the regions of 275 – 290 nm and 245 – 260 nm. However, mercaptobenzothiazole showed different spectrum at 332 nm with a weaker band at 265 nm.

1.3.3. Decomposition

The decomposition of sulfur compounds especially dithiocarbamates and thiuram disulfides have long been known. In the presence of acid, dithiocarbamates are converted to dithiocarbamic acids, which break down into amines and carbon disulfide as follows:

\[
R_2NCSSH \xrightarrow{\text{acid}} R_2NH + CS_2
\]

(1.7)

This breakdown process has been used as the starting point for the quantitative analysis of dithiocarbamates [60]. However, little investigation other than of a qualitative nature was done until 1953 [21]. In that year,
Martin [61] found that there is a relationship between the pH of the buffer and the extractability of copper diethylidithiocarbamate. The decomposition of dithiocarbamates in acid was also believed to follow a first-order reaction rate. Later, Ravindranath and Patel [62] investigated the kinetics of the decomposition of some dialkyldithiocarbamates in the presence of oxygen, carbon dioxide and their mixture as a function of pH and time. Besides acid decomposition, dithiocarbamates, particularly derivatives like thiuram disulfides, also decomposed thermally [21].

1.3.4. Previous analysis methods for dithiocarbamates and related sulfur compounds

As a result of the large number of applications, the analysis of dithiocarbamates and their related compounds are becoming more important in all areas. Large numbers of methods for detection and determination are available, including titrimetric, spectroscopic, electroanalytic as well as chromatographic methods. The older methods are often based on the determination of the acid decomposition products of the compounds, for example, carbon disulfide, and hydrogen sulfide. The method developed may also be for only qualitative analysis, like chemical detection and some of the titrimetric determinations. Although titrimetry can also be used for quantitative analysis, other techniques, such as spectroscopy, capillary electrophoresis, chromatography and some other available methods are preferred. Other techniques of determination are either by the determination of metal chelates of dithiocarbamates, direct determination or chelating with some other functional reagents. Chromatographic methods are now becoming the most common and popular approach. Simple and quick chromatographic methods are by thin layer and paper chromatography. However, these techniques are not suitable for quantitation analysis. Gas chromatographic techniques on the other hand, face problems with the low volatility and poor thermal stability of the compounds. Liquid chromatography remains the most accepted analytical methods for the analysis of dithiocarbamates, with reversed-phase and metal chelation being the most popular techniques [50].
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The identification and quantification of rubber accelerators especially in vulcanizate materials, such as natural rubber (NR), natural rubber latex (NRL) and synthetic rubber (SR) products are complicated by the many reactions involved in the overall process of vulcanization [35]. Some of the reactions were listed by Hummel [35]:

"Guanidines are converted in part into the arylamines and ammonia.

Ammonium dithiocarbamate derivatives react with zinc oxide (ZnO) to form zinc dithiocarbamates.

The thiuram disulfides react with zinc oxide, again with formation of zinc dithiocarbamates.

2-Mercaptobenzothiazole and 2,2'-dithiobisbenzothiazole exist in an equilibrium with each other that is established during vulcanization, irrespective of the starting material. In mixes containing ZnO, zinc 2-mercaptobenzothiazole is formed.

2-Benzothiazolesulfenamides decompose mainly into 2-mercaptobenzothizole and amines."

Thus, to avoid too much reliance on the chemistry of the vulcanization process, Hummel [35] has proposed the identification of the accelerators on the basis of their reaction products formed by acid hydrolysis. This method is based on a traditional method for the analysis of dithiocarbamates and had been adopted earlier [63, 64].

However, other authors have also described possible methods for the determination of accelerators by either chemical detection [35], spectrometric [35, 59, 65 - 69] or chromatographic [4, 5, 23, 25, 35, 46, 47, 56, 65, 66, 70 - 81] techniques either on the basis of the residual content or extractable amount. More details on the previous analysis methods for dithiocarbamates and related compounds are given in sections 1.3.4.2 to 1.3.4.8.

Analysis of any compound consists of three main steps: sample preparation, sample analysis, and data handling and interpretation, which
usually called the ‘Analysis Cycle’. Some samples can be directly analysed, however, the majority need some preparation, which depends on the physical requirements of the instrument, the scope of the measurement, and the complexity of the sample. One of the major preparation steps is sample extraction (section 1.3.4.1), which is becoming more significant with the trend to study more complex samples and samples with essential components at lower and lower levels. [82]

### 1.3.4.1. Extraction of rubber accelerators

The concentration of rubber accelerators needs to be determined for several reasons. Firstly, the correct amount of accelerators is essential for the functioning of the rubber or latex in its intended use. In addition, as discussed earlier, some accelerators are known to be a contact sensitizer to some groups of people. Therefore, the level of residue or extractable amount of these accelerators must be controlled. Thus, the manufacturers as well as the regulatory authorities and users need to be able to determine the levels of accelerator in natural rubber, latex or synthetic rubber especially in the end products.

The additives, including accelerators are commonly totally extracted from the rubber before analysis, and this can be done either by dissolution of the rubber and all other components followed by re-precipitation of the rubber, or solid/liquid extraction. The most common method used in extracting the accelerators from rubber is by liquid/solid extraction. The traditional methods of solid/liquid extraction include, Soxhlet extraction, boiling under reflux, and sonication, where boiling under reflux is the most commonly used method. The other new alternative methods of extraction are by high pressure solid/liquid extraction. These techniques comprise supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE). In general, no specific method is suitable for all situations, and each method has merits and demerits. Thus, the selection is controlled by the conditions available to the user and the exact purpose. [82]
A range of methods have been proposed for the chemical identification of rubber accelerators from the group of dithiocarbamates and its related compounds. These methods permit the identification of groups of accelerators or of an individual accelerator before processing and vulcanization, and also in extracts from vulcanized material. [35]

Acidic accelerators, like dithiocarbamates and mercaptobenzothiazole, can first be separated from other groups by dissolving the matrix in aqueous ammonia or methanolic sodium hydroxide. A study of the pure compound is usually made by dissolving it in chloroform. One of the detection methods for dithiocarbamates, thiurams and benzothiazole, quoted by Hummel [35] is the reaction of the chloroform solution of these compounds with copper oleate, to give a dark brown precipitate or colour due to the formation of a copper dithiocarbamate. Dithiocarbamates produced after the reaction of thiuram derivatives during the vulcanization process or on reduction of 2-mercaptobenzothiazole also give the same reaction and colour. The yellow-green solution of copper oleate in chloroform also changes its colour to brown after the addition of tetramethylthiuram disulfide solutions in chloroform. A similar colour of brown-red or dark brown is also produced on the addition of 2-mercaptobenzothiazole (MBT) to copper oleate in chloroform. On the other hand, an alkaline alcoholic MBT solution produced a yellow precipitate on addition of a few drops of a solution of bismuth nitrate in strong nitric acid [35].

Hummel also has described in detail an acid hydrolysis method for accelerators from vulcanized material and the detection of the degradation products from the total cleavage of the dithiocarbamates, thiurams, benzothiazoles, and thiazolesulfenamides. The degradation products, usually amines and carbon disulfides are then identified qualitatively by chemical detection or quantitatively by titrimetric (section 1.3.4.3), spectroscopic (section 1.3.4.4), capillary electrophoretic (section 1.3.4.5) or chromatographic (section 1.3.4.6) methods as described in the following sections.
1.3.4.3. Titrimetric determination

A classical method for the determination of dithiocarbamates is based on the determination of the degradation products of the acid decomposition of dithiocarbamate compounds. Dithiocarbamate is initially decomposed by an acid to carbon disulfide (equation 1.7). Then, the carbon disulfide is absorbed into alcoholic potassium hydroxide to form a xanthate [63] (equation 1.8),

\[
CS_2 + KOH + CH_3OH \rightarrow CH_3OCSSK + H_2O
\]  (1.8)

which is followed by quantitative redox determination of xanthate by titration with iodine (equation 1.9):

\[
2CH_3OCSSK + I_2 \rightarrow CH_3OC(S)SSC(S)OCH_3 + 2KI
\]  (1.9)

In analyzing the dithiocarbamates as vulcanization accelerators, the initial method developed by Callan and Stratford [60] was based on the above equations of iodimetric determination. However, the method was not reliable, as starch did not show the usual colour change at the end point even after dilution with water.

Later, Linch [83] modified the iodimetric method by titrating the alcoholic solution of dithiocarbamates until the development of a yellow colour, and suggested the use of starch as an external indicator for confirmation of the end point. However, the error was ± 2% which is not fully acceptable.

On the same year, Clark et al. [63] also developed a method for the analysis of the dithiocarbamate fungicides based on the same principle. However, the authors modified the earlier method by washing the carbon disulfide with a lead acetate solution to remove hydrogen sulfide and sulfur dioxide arising from sulfide and thiosulfide impurities in the sample. They believed that the reason for the failure of the other methods of dithiocarbamate determination was the presence of impurities.

Using the same acid method and a non-aqueous solvent, Greenhow and Spencer [84] developed a method for the determination of xanthates by a non-aqueous iodimetric procedure, where the titrametric end point was marked by a rise in temperature resulting from the polymerization of ethyl
vinyl ether. A year later, they demonstrated the suitability of this method for the determination of dithiocarbamates in non-aqueous solutions [85]. This method was believed to be rapid and simple compared to the conventional aqueous iodimetric titration. However, it needed calibration graphs as the reaction stoichiometries are not exact.

Although the acid method was known to be accurate, it was time-consuming and tedious, and offered Shankaranarayana and Patel [86] an opportunity to develop a new method. This method was based on the direct titration of dithiocarbamate with iodine.

\[
\begin{align*}
2{\text{R}}_{2}{\text{NCSSK}} + {\text{I}}_2 & \rightarrow R_2NC(S)SS(S)CNR_2 + 2KI \\
\text{Dithiocarbamate} & \quad \text{Thiuram-disulfide}
\end{align*}
\] (1.10)

Although Linch [83] has earlier developed a similar method, the main source of error lies in detecting the end point by starch, in the presence of thiuram disulfide, formed as a result of the oxidation. Removal of interfering thiuram disulfide by a non-aqueous solvent during the titration made the estimation of water-soluble dithiocarbamate by iodimetric method successful.

Other new alternative ways of titrimetry are also available, such as catalytic thermometric titration [87], titration with electrogenerated iodine [88] or hypobromite [89], by metal complexation [90, 91] as well as using stable oxidants of N-bromoimides as the reagents [92].

1.3.4.4. Spectroscopic determination

A lot of research has been carried out to either study dithiocarbamates or their applications with spectroscopic determination. The main spectroscopic methods are based on the determination of metal complexes either after acid decomposition or by direct complexation.

Using the similar procedure of acid decomposition described for titrimetric determination, Clarke \textit{et al.} [63] developed a micro method with a different way of absorbing and measuring the carbon disulfide. They added reagents, like triethanolamine, diethylamine and cupric acetate in ethanol to
the absorber, which then absorbed carbon disulfide and formed cupric diethyldithiocarbamate. The relative amount of carbon disulfide absorbed was then measured spectrophotometrically by the intensity of the yellow colour produced by the cupric diethyldithiocarbamate complex. At the same time, Lowen [64] further improved the method for the determination of trace amounts of dithiocarbamates fungicides, like ferbam, ziram, zineb and nabam in food crops using a similar colorimetric technique.

A more recent method based on the acid decomposition of dithiocarbamates to carbon disulfide has also been introduced by Caldas et al. [93] using a vertical disulfide reaction system. This new carbon disulfide reaction system was believed to be sturdier than the old system, had an uncomplicated assembly, and permitted a higher sample throughput.

The direct colorimetric determination of metal dithiocarbamate complexes was initially proposed by Callan and Henderson in 1929 [94] for the determination of copper by the reaction with sodium diethyldithiocarbamate. Afterwards, Holland and Ritchie [95] noted that other metals like cobalt and nickel also gave coloured complexes with dithiocarbamate. Using the same principle, Domar et al. [96] then developed a method of quantification of thiuram disulfide by the reaction with cuprous iodide and quantification using a photometer. Using the spectroscopic determination, these authors were amongst the earliest method developers for the quantitation of dithiocarbamates and related compounds by metal complexation.

Besides UV spectrophotometric methods, infrared (IR) spectroscopic [97, 98], atomic spectroscopic with different sources and detectors [99 - 102], Fourier transform infrared spectroscopic (FTIR) [67 - 69] and X-ray spectroscopy [103] have also been used.

IR spectroscopic determination of accelerators has been reported in various publications, however, Hummel [35] described a summary of the IR spectrum characteristics of some accelerators, and a collection of IR spectra of rubber chemicals has also been presented. In other applications, such as pesticides, Susi and Rector [97] showed that it was also possible to
quantitate the dithiocarbamates and related compounds using IR spectroscopy. Then, in 1983, IR spectroscopy was also used by Saundararajan and Subbaiyan [98] in their study of the correlation between the extraction constants of thallium (I) dithiocarbamates and the IR frequencies of C-N bonds.

Atomic spectroscopic methods for the metal component of the complexes have been used with flame [99, 100], furnace [100], and plasma [102, 104 - 106] sources and also electrothermal atomisation [101]. All of these methods had used the dithiocarbamates as a chelating agent to concentrate the trace metal ions from natural water [99, 104], sea water [100, 106], and urine [102].

Other reagents have also been used as complexing agents, such as 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) [107, 108], 2,2'-bipyridyl [109], 9-(4-carboxyphenyl)-2,3,7-trihydroxyl-6-luorone [110] and sodium molybdate [111]. All of these reagents produced coloured complexes with the metal component of dithiocarbamates which were then appropriate for spectrophotometric determination. The most recent paper was published by Sharma et al. [111], who used sodium molybdate as a chelating agent to develop an improved spectrophotometric method. They used a derivative spectrophotometric technique, in which the fourth derivative was found to give improved sensitivity and less interference for the determination of thiram (tetramethylthiuram disulfide).

A method based upon Fourier transform infrared spectroscopy (FTIR) was also recently developed by Temel and colleagues [69]. They have used this analytical procedure in their study to determine residual accelerators in vulcanized natural rubber products with the aim of selecting the appropriate accelerators for the vulcanization of NRL, which would reduce the problem of skin sensitivity. This study showed that quantitation of the vulcanization accelerators in the solvent extracts of NRL films was rapid and did not require a pre-treatment of the extracts. Although the method was claimed to be rapid and simple, the application of this method to real samples has not been made. The quantitation of a specific accelerator in an extract of rubber products would be much difficult as the matrix in the extracts of rubber
products usually contain more than one chemical additive.

1.3.4.5. Capillary electrophoretic determination

About a decade ago, capillary electrophoresis (CE) was a fairly new analytical technique with the key advantage of fast low volume analysis compared to liquid chromatography. CE coupled with diode array detection (DAD) was employed by Lee and co-workers [112] in the simultaneous determination of dithiocarbamate compounds of butyl-, diethyl-, dimethyl-, octyl-, and pyrolidine-1-dithiocarbamates. They found that only three dithiocarbamates were separated but the other two, butyl- and diethyldithiocarbamates, clearly overlapped.

Afterwards, Malik and co-workers [113], studied dithiocarbamates by using a CE method. They showed that CE was also capable of determining the ferbam (ferric dimethyldithiocarbamate) by converting Fe (III) dithiocarbamate into Fe (III)-ethylenediaminetetraacetic acid (Fe (III)-EDTA). They also found that CE could be used to determine ferbam without any interference from the other metal ions present in the same sample.

In other applications, Rudnev et al. [114] have furthermore used CE in their effort to determine traces of Cd, Cu, Hg, Ni and Pb as soluble dithiocarbamate complexes. When lower concentrations of metals were present in the samples, CE with small capillary dimensions faced serious limitations. However, they proposed of using solid phase extraction (SPE), prior to the analysis to concentrate the target ions. Unfortunately, the off-line SPE-CE combination had the disadvantage of sample losses for dilute analyte solutions.

CE with a chemiluminescence detector was also been introduced by Tsukagoshi et al. [115] in their study of metal ions. They used emetine dithiocarbamate chelated with metals and tris (2, 2'-bipyridine)-ruthenium (II) ion as a chemiluminescence reagent. However, they found that the separation of metals, such as Cu (II), Ni (II), and Co (II) complexes, was
rather difficult by CE, and the percentage of acetonitrile in the buffer as well as pH of the buffer had to be optimized to 20% acetonitrile and pH at 6-7 to successfully separate the emetine metal dithiocarbamate complexes.

1.3.4.6. Chromatographic determination

Since the application of chromatographic methods first appeared for the determination of sulfur accelerators, these techniques have been the most widely used method chosen by the analyst, as they have advantages over the other methods. The chromatographic techniques can be categorized into three main types, thin layer chromatography, gas chromatography and lastly high performance liquid chromatography, that has been the method of choice.

1.3.4.6.1. Thin layer chromatography

The determination of dithiocarbamates and their related compounds by thin layer chromatographic (TLC) procedures were one of the earliest methods after paper chromatography. Fishbein and Fawkes [116], for example, applied this technique for the separation and identification of the metallic fungicidal derivatives of ethylenebis(dithiocarbamic acid) and their degradation products ethylenethiourea (ETU), ethylenethiuram monosulfide (ETM), carbon disulfide, sulfur, and ethylenethiuram disulfide-polymer (ETD-polymer).

Later, Czegledi-Janko [117] used this technique in the study of metallic ethylenebis(dithiocarbamates) and their degradation products. The author also investigated these compounds in vitro at several different temperatures and relative humidities. In this study, the author confirmed that maneb decomposed more readily than zineb, in a matter of hours and definitely within a day. Although the method developed in this study was better than the previous published method, it was only able to qualitatively determine ethylenebisdithiocarbamates and their decomposition products.
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Devani et al. [118] used the same TLC technique in their attempt to separate a mixture of isothiocyanates and derivatives of thioureas. Using chloroform-ethyl acetate (10:1) as the solvent system and an ethanolic solution of 2,3-dichloro-1,4-naphthoquinone as the detection spray, they showed that the separation of isothiocyanate and thioureas was possible.

A more recent study of thallium (I) dithiocarbamate compounds by Soundararajan and Subbaiyan [98] used TLC to correlate the extraction constant and showed that TLC can also be used as a useful guide for an initial estimation of the possible chromatographic migration order of other metal dithiocarbamates.

1.3.4.6.2. Gas chromatography

Gas chromatography (GC) is another method used for the determination of sulfur compounds and appeared at nearly the same time as thin layer chromatography. The uses of GC for the determination of dithiocarbamates and their related compounds have been for a wide range of applications, including fungicide compounds [119], tracing Antabuse in human blood [120], tracing heavy metals in biological samples [121, 122] as well as tracing the release chemical residues in rubber products [73].

The determination of dithiocarbamates by GC was initially based on the determination of carbon disulfide [120, 123, 124] evolved after acid decomposition of dithiocarbamate compounds. Later, some other decomposition products, like hydrogen sulfide [124], ethylene thiourea [125] and esters [73] have also been used. The possibility of the direct determination of metal chelates by GC was first pointed out by Masaryk et al. in 1975 [126] and they showed an example of the GC of nickel and zinc diethyldithiocarbamates on the non-polar stationary phase SE-30 at 250°C. A few years later, Krupcik et al. [127] analyzed the influence of different GC supports coated with SE-30 on nickel and zinc dithiocarbamate compounds. In that study, it was found that Cu (II), Co (II) and Cd (II) dialkyldithiocarbamates decomposed during the analysis under the stated
conditions. They were using glass capillary columns coated with polydimethylsiloxane stationary phases (OV-100 and SE-30), but found difficulties when the compounds absorbed to the glass capillary wall. However, Chromosorb G AW DMCS was found to be the most suitable support for dithiocarbamates. Later, Drasch and co-workers [121] also used this technique coupled with mass spectrometry in their quantitative analysis for the detection of cadmium in biological samples.

Various detectors have been used in the determination of dithiocarbamates by GC, including the flame ionization detector (FID), mass spectrometry (MS), flame photometric detector (FPD) and electron capture detector (ECD). Amongst these, FID [4, 5, 123, 124] and MS [4, 5, 73, 122, 128 - 130] have been the preferred methods. Alternatively, Sauter [120] and Friedrichs [131] used FPD in their study. ECD on the other hand, was used by Dubey [132], Zena and co-workers [133], and Dubey et al. used it together with nitrogen phosphorus detection. Microwave-induced plasma emission spectrometry (MIPES) detector has been used by Lobinski and colleagues [134] in their study of organotin compounds in environmental samples extracted as diethyl dithiocarbamates. GC with nitrogen sensitive detector (TID) was also used by Drasch et al. [121]. Sauter and Wartburg [120] in their quantitative analysis of disulfiram and its metabolites in human blood used FPD and a headspace technique. Subsequently, Royer and colleagues [119] developed an automated headspace GC for the determination of dithiocarbamate fungicide residues in vegetable matrixes. In that study they found that using automated headspace gave them better results compared to the manual headspace technique, with some additional advantages of less reagent volume used, less sample preparation time and enabling more samples to be analysed in a day.

1.3.4.6.3. High performance liquid chromatography

The determination of sulfur compounds, especially dithiocarbamates and their derivatives, by high performance liquid chromatography (HPLC) is well
established. Papers published in recent years [78, 146, 153] have used this technique because the method is known to be more sensitive and time effective. The analysis of sulfur compounds by HPLC has been developed both in normal and reversed-phase system for various applications. These include the determination of rubber additives in waste water and rubber products as well as in latex products. Dithiocarbamates have also been used extensively for metal ion analysis either by investigating the metal chelates or for tracing metals in various samples. Furthermore, the determination of dithiocarbamates has been used in the area of agriculture, including fungicides, fertilizers and pesticides. Sometimes, dithiocarbamates were used as an analytical reagent.

Both normal (Table 1.5) and reversed-phase (Table 1.6) HPLC have been used in the determination of the dithiocarbamate compounds with various types of columns, eluents, and detectors. UV detection was found to be the most cited detector.

<table>
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<th>HPLC conditions</th>
<th>Limit of detection</th>
<th>Ref.</th>
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<td>Detector</td>
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<td>Ethanol-2,2,4-</td>
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<td></td>
<td>SI 60</td>
<td>trimethylpentane</td>
<td>(1:9)</td>
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<td>LiChrosorb</td>
<td>Benzene</td>
<td>UV-330 nm</td>
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<tr>
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<td>SI 60</td>
<td></td>
<td></td>
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<tr>
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<td>Corasil &amp; μ-</td>
<td>Toluene</td>
<td>UV-270 nm</td>
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<td>Porasil</td>
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<td>Chloroform-</td>
<td>UV-325 nm</td>
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<td>SI 60</td>
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<td>and 280 nm</td>
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<table>
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<th>HPLC conditions</th>
<th>Limit of detection</th>
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<td>Water-acetonitrile (7.3)</td>
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<td>Fungicidal DTC</td>
<td>Nucleosil RP-18</td>
<td>Water-acetonitrile (3.2)</td>
<td>UV - 272 nm</td>
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<tr>
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<td>Acetonitrile-methanol-water (37:33:30)</td>
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<td>10%, 5%, 1% Acetonitrile-water</td>
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<td>Acetonitrile- acetate buffer (70:30)</td>
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<td>C18 μBondapak, Spherisorb, Radpak</td>
<td>Various solvent mixture</td>
</tr>
<tr>
<td>MBT</td>
<td>C18 μBondapak</td>
<td>Acetonitrile-water (30:70)</td>
<td>UV</td>
</tr>
<tr>
<td>Zinc DTC (Co (III) DTC)</td>
<td>Nucleosil 5C18</td>
<td>Methanol-water</td>
<td>UV - 320 nm</td>
</tr>
<tr>
<td>MBT mix</td>
<td>Lichrospher RP 100 C18</td>
<td>40% THF in 0.1M sodium acetate</td>
<td>UV - 300 nm</td>
</tr>
<tr>
<td>MBT and MBTS</td>
<td>Spheri-10 RP-18</td>
<td>Acetonitrile-water-acetic acid (300:700:0.3)</td>
<td>UV - 272 nm, 328 nm</td>
</tr>
<tr>
<td>MBT and related</td>
<td>Lichrospher RP 100 C18</td>
<td>35-45% THF in 0.1M sodium acetate</td>
<td>UV - 300 nm</td>
</tr>
<tr>
<td>Thiamin mix</td>
<td>Supelcosil LC-18-DB</td>
<td>Acetonitrile-water-THF (99.99.2)</td>
<td>UV-280 nm</td>
</tr>
<tr>
<td>ZDEC and ZDBC</td>
<td>Supelco Discovery C18</td>
<td>85-100% Acetonitrile-water</td>
<td>UV - 260 nm 5 and 10 µg/mL</td>
</tr>
</tbody>
</table>
Normal phase HPLC (NP-HPLC) was the earliest method developed for the determination of sulfur compounds by HPLC. Since a lot of applications used sulfur compounds like dithiocarbamates, thiuram disulfides and mercaptobenzothiazoles, there have been a lot of concern about their residues in the waste water. For that reason, Cox [135], was among the pioneers for the determination of sulfur compounds by HPLC and developed an HPLC method to determine 2-mercaptobenzothiazole in waste water systems. About 10 [154] and 20 years [155] later, other authors have determined the same compounds in both industrial and public waste waters.

Heizmann and Ballschmiter [136] on the other hand used dialkyldithiocarbamates as a chelating agent in their efforts to separate metals by HPLC. They found that the metal dialkyldithiocarbamates could be separated well with some variations in solvent rate, column length or gradient eluent. However, some of the metal dialkyldithiocarbamates showed tailing effects. For the same application of tracing metals, O’Laughlin and O’Brien [137] used HPLC in the separation of metal chelates with two different types of column (Corasil and μ-Porasil) and an eluent of toluene. In this study, they found that the μ-Porasil column gave the best separation.

In 1979, Liska and co-workers [51] systematically studied metal dithiocarbamate complexes by HPLC. They used nickel (II) bisdialkyldithiocarbamates as a representative of this group of compound. Using a LiChrosorb SI 60 column and the best eluent mixture of chloroform-cyclohexane, they found that there was a relationship between eluotropic strength (ε°) of the mobile phase and the retention factor of the nickel (II) bisdialkyldithiocarbamate complexes. On that basis, they separated a mixture of nickel (II) bisdialkyldithiocarbamate complexes. However, an extra peak was always found if two complexes with different ligands were injected together. As a result, they proposed equation 1.6, given earlier. It was suggested that the type of substituents on the nitrogen atom, solvent, and temperature are responsible for the rate of formation of the mixed-ligand dithiocarbamate complexes and their strength in solution. It was also found
that mixed-ligand complexes are unstable at temperatures higher than 35°C and thus were suggested to be the reason for the non-existence of mixed-ligand complexes at higher temperatures reported earlier. Liska et al. [52] also verified the existence of this mixed-ligand complex by two-dimensional thin layer chromatography, determination of the molecular weight and elemental analysis.

Later, Lehotay, co-workers [138] used this technique in their separation and identification of Cu (II), Co (II), Zn (II) and Pb (II) bisdialkyldithiocarbamate complexes. Using the same type of column and various types and mixtures of organic solvent, they found that chloroform and dichloromethane with less polar organic solvents were the most suitable solvents. In their study of mixed complexes, however, they found that bisdialkyldithiocarbamate complexes of Cu (II) and Co (II) did not give extra mixed-ligand complexes as did nickel (II). Furthermore, ligand exchange between different metal complexes was also illustrated, for example between nickel (II) and copper (II) as equation 1.11 below:

$$L_1 - Ni - L_1 + L_2 - Cu - L_2 \leftrightarrow L_2 - Ni - L_2 + L_1 - Cu - L_1$$

(1.11)

It was realized that the analysis of the metal complexes in environment samples can be complicated because ligand-exchange reactions can take place between many of them.

However, in 1980, Edward-Inatimi and Dalziel [139] used dithiocarbamates as one of the chelating agent in their investigation of the multielement analysis using NP-HPLC. Moriyasu, Hashimoto and Endo [55] have further studied the stability of the dithiocarbamate compounds. They found that DTC derived from primary amines in general had a strong reducing property, so that the metal chelates for Ag (I), Hg (II), and Cu (II) decomposed readily, whereas Pd (II), Ni (II) and Co (II) were stable.

Again in 1983, Lehotay et al. [140] added more data for the ligand exchange reactions of Ni (II) bisDTC on NP-HPLC. They studied the influence of solvents on the equilibrium and rate constants of the ligand exchange reactions of this metal complex. It was found that the solvent has
no significant effect on the equilibrium constant value of the following reaction:

\[ L_1 - M - L_1 + L_2 - M - L_2 \overset{k_1}{\leftrightarrow} 2L_2 - M - L_2 \]

(1.12)

However, the reaction rate constant (e.g. \( k_1 \)) was dependent on the solvent and its polarity. The interaction between the molecules of the solvent and the complex has also been suggested to have an important effect on the reaction rate.

Edward-Inatimi [156] on the other hand used diethylidithiocarbamate as the chelating agent in the determination of trace metals in industrial waste water, standard kale and standard fish meal with preliminary solvent extraction to concentrate the sample.

In the area of agriculture, fungicide dithiocarbamates, like mancozeb have been reported to accumulate 2-imidazoline. Thus, Newsom and Panopio [141] have developed an HPLC method for the determination of this compound in foods.

1.3.4.6.3.2. Reversed-phase HPLC (RP-HPLC)

The determination of dithiocarbamates by reversed-phase HPLC (RP-HPLC) mainly appeared after NP-HPLC methods had been developed. RP-HPLC has become the most popular method as it has a lot of advantages. Some of the merits are its versatility, the special stationary phase chemistry that meets the needs of a variety of samples, and the mobile phases with simple mixture of aqueous solvents, which has the advantage that water is cheap, environmental friendly and easily available [157].

The use of RP-HPLC in the determination of dithiocarbamates compounds have been in a wide range of applications. As with NP-HPLC, RP-HPLC has been used in fungicide analysis, trace metal analysis as well as rubber analysis.

For example, Hashimoto [158] has used HPLC with cation-exchange
column and ammonium sulfate aqueous solution as the mobile phase in the analysis of mixture of thioureas and thioacetamide by first separating the mixture by salting-out chromatography. Later, Smith et al. [142] have proposed the use of transition-metal salts as ion-pair reagents in their study for the determination of fungicide diethylthiocarbamate compounds. Using this technique, they were able to simultaneously study the fungicide dithiocarbamate and its degradation products. For the same application, Gustafsson and Thompson [143] have also used RP-HPLC in their effort to develop a specific method for the determination of thiram, salts of alkylenebis(dithiocarbamic acids), and N,N-dimethyldithiocarbamic acid. These dithiocarbamate compounds were converted to water-soluble sodium salts with an alkaline EDTA solution and analyzed by HPLC. Later, the same technique was also reported for the determination of dithiocarbamate fungicides in vegetable foodstuffs [144]. In 1984, Brandtetterova et al. [145] developed a simultaneous HPLC separation method for ferbam and its degradation products. The best separation conditions were used in their study of the ferbam thermal decomposition process. Recently, Sanagi and colleagues [146] investigated the possibility of using high temperature HPLC in the determination of triazole fungicides using a carbon-clad zirconia column. Using only small amounts of organic modifier and temperatures of 100 °C to 150 °C, they were able to separate some of the triazole compounds.

Smith and Yankey [147] used dithiocarbamates as chelating agents by adding them to the mobile phase for the analysis of trace metal ions. They found that diethylthiocarbamates was the most suitable compound to be complexed with the metals compared to pyrrolidine dithiocarbamates. Pyrrolidine dithiocarbamates were found to be suitable only for cobalt ions. At the same time, Bond and Wallace [148] developed a similar method for the determination of copper by RP-HPLC with electrochemical detection. In this paper, they suggested two different ways of metal chelation. One was by in-situ metal complexation, where sample solutions were injected into the column and formed metal complexes with the dithiocarbamate salts incorporated in the eluent. This method had the advantage of greater
sensitivity and was direct. However, with electrochemical detection, the background current might increase due to the oxidation of the ligand if the oxidation process was being used. Otherwise, pre-column derivatization could be applied, which needed a sample preparation that might dilute the concentration of the analyte. The authors have also showed that multi-element analysis was possible as they did not find any interference from the other metals.

Later, Hutchin et al. [149] used polymer cartridge radial compression columns in their RP-HPLC study of metal diethyldithiocarbamate complexes. Until then, stainless steel, glass and microbore columns had been used in the determination of dithiocarbamates. However, it was realized that the relatively unstable metal diethyldithiocarbamates of Bi (III), Zn (II), Cd (II) and Pb (II) can easily go through ligand exchange reaction with nickel from the stainless steel components of the HPLC system. They found no peak for the Zn (II) complex and asymmetrical peaks for Pb (II) and Cd (II) complexes even using the radial compression column. Ternary solvent mixtures of methanol-water-acetonitrile gave better peak shapes and resolution compared to the binary solvent mixtures. The column needed to be conditioned by either injecting the concentrated solution of the complexes or by flushing with EDTA, followed by the addition of different concentrations of EDTA to the mobile phase.

High molar absorptivities and strong complexes with metals have made dithiocarbamates the chelating agent of choice and inspired many authors to study multi element analysis using RP-HPLC technique. A study by Drasch et al. [121] for example used RP-HPLC to detect heavy metals from biological material like urine. Their comparison of GLC to the RP-HPLC method showed that the HPLC method was more flexible, less critical and had better reproducibility. Furthermore, the use of a ternary solvent mixture of methanol-water-chloroform and a glass column improved the peak shapes of the metal complexes. Ichinoki and co-workers [150] used hexamethylenedithiocarbamates as the chelating agent in their simultaneous determination of heavy metals in bovine liver and oyster tissue. They also
used solvent extraction to concentrate the sample. In the same year (1984), Bond and Wallace [151] investigated the different methods of complex formation; direct formation in a solvent, liquid-liquid extraction and removal of co-extracted ligand, and pre-column formation using Sep-Pak cartridges. From their study, they suggested the use of pre-column formation of the complex for multi-element determination. Direct formation was believed to be more practical method for sample analysis. However, the possibility of ligand exchange to a more stable metal has limited its use in the multi-element analysis. In-situ formation of dithiocarbamate complexes has also been investigated by the same authors in their studies of the simultaneous and automatic determination of Pb, Cd, Hg, Co, Ni, and Cu with electrochemical and/or spectroscopic detection. They also found that the addition of ion based suppressor column prior to the detection to remove the excess dithiocarbamate ligand can improve the detection limits of some metals [152].

Other authors have also reported the same trace metal analysis [159 - 164]. In contrast to these methods, a method developed by Wang and Whang [165] used 2-mercaptobenzothiazole (MBT) for the determination of inorganic mercury and organomercury in aqueous solutions.

In the area of rubber analysis, Reepmeyer and Juhl [65] have used RP-HPLC as one of the methods in their investigation of the contamination of the digoxin injectable solutions with 2-mercaptobenzothiazole, which was suspected to come from the rubber closures. Later, in developing a better HPLC method for the determination of dithiocarbamates in rubber products, Kaniwa [23] showed that zinc dithiocarbamates were easily converted into cobalt (III) complexes when shaken with cobalt (II) chloride. They also found that mixed-ligand complexes were formed when two or more kinds of zinc dithiocarbamates were mixed together. As shown before, a mixture of two multivalent ions like Zn (II) with different ligands will produced an extra peak of a mixed-ligand complex. However, in this study, two or more extra peaks of mixed-ligand were formed depending on the number of different ligands present. For example, a mixture of two cobalt (III) dithiocarbamates produced 4 different complexes. These findings have made the determination of
mixtures of dithiocarbamates in rubber sample more complicated.

In developing a method for the stability study of the mercaptobenzothiazole compounds, Hansson and Agrup [72] used HPLC. However, they found that there was a cross-reaction between the compounds in the mixture and therefore suggested that the single substance of mercaptobenzothiazole should be used in the tests. In the same year Gaind and Jedrzejczak [71] used HPLC in their attempt to develop a simple and rapid method for the determination of 2-mercaptobenzothiazole and mercaptobenzothiazole disulfide in rubber closures. They also have used mass spectrometry for the confirmation of the structure of the impurities. A few years later, Hansson et al. [70] again used HPLC to monitor the quantity of mercaptobenzothiazole in their study for the optimization of the extraction technique. The earlier findings concerning the stability of mercapto mix [72] gave a good reason for Bergendorff and Hansson [153] to study the stability of thiuram mix. In this study, they used HPLC in the analysis of all of the thiuram compounds and the newly produced compounds. Although the new compounds produced reduced the concentration of the original compounds, the results of patch testing (skin allergy test) showed no differences.

More recently, Depree et al. [78] proposed a simple and direct method for the determination of zinc dialkyldithiocarbamates in latex condoms by HPLC. In this study, they used an alternate zinc dialkyldithiocarbamate as a protecting agent from the transmetalation reactions. They also proposed a screening assay by treatment of the sample extract with cobalt chloride and measuring the UV absorption at 320 nm. In their later study [46], they have also made modifications to the determination of approximate total sulfur-containing accelerator content (screening assay) by introducing a simple, inexpensive screening method, which combined extraction and the determination of the total accelerator content.

In 2006, Bergendorff et al. [47] also used HPLC for the analysis of zinc dithiocarbamates (ZDTCs), which employed polyetheretherketone (PEEK) lined chromatographic components with a mobile phase containing saturated zinc ions. The same method was first reported by Mathieu et al. [56] in 2000
and was used by Bergendorff et al. [79] in 2005 in their study of the influence of solvent, extraction time and the procedure for extraction of haptens from solid products. Although this method claimed to have solved the problem of ligand substitution reactions between the zinc salts of dithiocarbamates and nickel from the stainless steel components of the chromatographic systems, a few authors [25, 78] implied that this method was expensive to implement.

The most recent method developed for the analysis of dithiocarbamates was by Abraham and colleagues [25]. This HPLC method was developed for the determination of ZDEC that was released into artificial sweat from natural rubber latex vulcanizates. ZDEC was quantitated as its copper complex by reacting it with copper (II) sulfate. The separation was achieved on a Symmetry C18 (Waters) column using 90% (v/v) acetone-water mixture as the mobile phase. They also made a comparison between the complexation of ZDEC with cobalt (II) chloride and copper (II) sulfate, and showed that copper (II) sulfate was a better complexing agent as the percentage of recovery of ZDEC using cobalt (II) chloride was only 36%. They suggested that the low recovery could be attributed to the slow substitution rate of cobalt (II) cations compared with that of copper (II) cations. The limit of detection (LOD) and limit of quantitation (LOQ) for this method were found to be 0.25 and 0.86 μg/ml, respectively. The same method has also been used in their earlier studies on the release of dithiocarbamates into sweat from NRL surgical gloves [80], and into artificial sweat from latex vulcanizates [81].

1.3.4.7. Other analysis methods

Several other techniques are also available for the determination of dithiocarbamate compounds, including polarographic methods [166 - 168], neutron activation analysis (NAA) [169], voltammetric methods [170], bioenzymic sensors [171, 172], and immunoassays [173].

Polarography was used by Halls et al. [166] in their investigation of the behaviour of monoalkyl- and dialkyldithiocarbamates and several other metal complexes used as pesticides. Later, other authors [167, 168] also used this
technique in their study of the determination of amines and amino acids. Chan et al. [167] studied primary and secondary amines by changing them to dithiocarbamate salts and measurement using differential-pulse polarography. A year later, Shiu and co-workers [168] reported the same technique in their determination of amino acids. However, in this study, they used carbon disulfide and triethylamine to change the amino acids to the dithiocarbamate compounds and detected them using the same technique of polarography.

Lo and Lee [170] alternatively used an anodic stripping voltammetric method in their study of the determination of mercury in water and biological samples. Diethyldithiocarbamates were used as the chelating agent to concentrate the mercury from the samples together with Au (III) as a back extraction agent. The two step extraction, not only preconcentrated the sample but also functioned to coat a gold film on a glassy carbon electrode for the following differential pulse anodic stripping voltammetry (DPASV).

Neutron activation analysis was used by Mok and Wai [169] in their study for the determination of molybdenum in seawater. In this study, they used perrrolidinedithiocarbamate and diethyldithiocarbamate in chloroform to concentrate the metals. Using a specific pH that so other metals were not extracted, the solvent extraction of molybdenum as dithiocarbamate complexes became a rapid and efficient method as well as eliminating the interferences of other metals and the sample matrix.

A biosensor method developed by Noguer and Marty [171, 172] used the inhibition of aldehyde dehydrogenase. They successfully used this method for the detection of maneb used as a pesticide. This method has an advantage of not using any organic solvent or other chemical reagents that might be harmful to humans and the environment.

Queffelec and colleagues [173] used an enzyme linked immunosorbent assay (ELISA) to determine thiram in lettuce. This method has been shown to be sufficiently sensitive to detect the maximum permitted residue levels of thiram. The method has advantage of a simple extraction step, little or no cross reaction and less analysis time.
1.3.4.8. Comparison of analysis methods

The traditional method for the determination of dithiocarbamates is by acid treatment. This method is accurate but time consuming and tedious [86]. Titrimetric methods for example, which are mostly based on the acid decomposition, have a problem of detecting the true end point. It is also time consuming and has a low sensitivity [113]. Although the problems faced by this method have been solved by Shankaranarayana and Patel [86], it is still not a popular method. Alternatively, spectroscopic determination has become one of the preferred methods as it can be direct and rapid. However, this method suffers from interference of the other metal ions and generally cannot distinguish amongst the dithiocarbamates [112]. Capillary electrophoresis uses a low volume of sample and a shorter analysis time, however, some dithiocarbamate compounds overlapped and it is only suitable for water soluble dithiocarbamates [112]. Other methods are also available for the determination of dithiocarbamates, however, chromatographic methods are now the most popular and the method of choice, especially high performance liquid chromatography.

Paper and thin layer chromatography were the earliest methods in chromatography. However, these two methods are only suitable for qualitative determination. Gas chromatography on the other hand is limited because of the low volatility and the low thermal stability of most of these sulfur compounds. High performance liquid chromatography nevertheless appeared to be more suitable for the determination of these compounds. Although HPLC is known to be more sensitive and time efficient, its use of a lot of organic solvent has been a concern. Therefore, there is a need to either reduce the amount of organic solvent used or change to a complete by green solvent like water. The conventional RP-HPLC with pre-column derivatisation method furthermore might dilute the concentration of the analyte and raise the possibility of losing some of the analyte during the process. For that reason, a new improved method is needed.
1.4. Present study

Determination of sulfur compounds by RP-HPLC is already established, however, a thorough understanding of their chromatographic behaviour has not been established especially in the area of rubber analysis. Therefore, one of the objectives of this study is to further investigate and understand the chromatographic as well as the chemical behaviour of some selected rubber accelerators of sulfur compounds by using RP-HPLC by evaluating some of the reported methods [4, 23, 25, 78].

Although a direct injection method of zinc dialkyldithiocarbamate [78] has been developed, many authors [136, 149, 174, 175] found the zinc (II) complexes to be unstable and that ligand exchange reactions might occur between zinc (II) ions and nickel from the stainless steel components of the HPLC system. This reaction might either cause there to be no peaks or a poor peak shape for zinc dithiocarbamates. However, it is of interest to try the method of direct injection of ZDTCs again using ordinary chromatographic systems but with addition of zinc cations as the protective agent. The conventional method of using cobalt (III) ions on the other hand, produces many extra peaks when two or more zinc dithiocarbamates are mixed together. For that reason, it is relevant to find an alternative stable compound by either using pre-column derivatisation, in which the complex is formed before the injection [23] or online metal conversion (on-column derivatisation), in which the metal ions were included in the mobile phase to form the complexes [142, 151].

Although the HPLC method is known to be more sensitive and time effective compared to the other available methods, this technique uses a lot of solvent during the sample preparation as well as during the separation of the analytes [111], and some organic solvents are well known as toxic and harmful to humans, aquatic lives and environment. Therefore, there is a need to either reduce the amount of solvent used or use alternative non-toxic, cheaper and easily available solvent like water. The use of high temperature HPLC in the determination of triazole fungicides using carbon-clad zirconia stationary phase, [146] has also inspired the author to study the possibility of
using temperature as one of the parameters with a suitable column for the
determination of the rubber accelerators.

Another objective of the study is to evaluate the effect of some other
parameters using the proposed method of on-column derivatisation. This
method will be compared with the conventional pre-column derivatisation
method and lastly to assess the potential of this new method for real samples
of rubber products.
CHAPTER 2 : EXPERIMENTAL

2.1. Materials and reagents

2.1.1. Solvents

Water used throughout this study was purified by an Elga Maxima Ultrapure Water purification system (Elga LabWater Ltd., Marlow, UK) at 18.2 MΩ. HPLC-grade dichloromethane (DCM), acetonitrile (ACN), methanol (MeOH), acetone and tetrahydrofuran (THF) were supplied by Fisher-Scientific (Loughborough, UK). All other chemicals were of analytical reagent grade unless otherwise stated supplied by the same supplier. All solvents used for HPLC were prepared by v/v ratio and degassed by sonication for 10 minutes at ambient temperature.

2.1.2. Chemicals

Zinc diethyldithiocarbamate-98% (ZDEC), zinc dibenzyldithiocarbamate (ZBEC), ammonium diethyldithiocarbamate (ADEC), uracil and acenaphthene were obtained from Sigma Aldrich (Steinheim, Germany). Ammonium pyrrolidinedithiocarbamate (APDC) was obtained from Sigma Aldrich (USA). Zinc dimethyldithiocarbamate (ZDMC), zinc dibutylldithiocarbamate (ZDBC), tetramethylthiuram disulfide (TMTD), tetraethylthiuram disulfide (TETD) and 2-mercaptobenzothiazole (MBT) were obtained from Tun Abdul Razak Research Center (TARRC – Heford, UK). Zinc pentamethylenedithiocarbamate (ZPD) was supplied by Robinson Brothers Ltd. (West Bromwich, UK). Nickel diethyldithiocarbamate (NiDEC) and nickel dibutylldithiocarbamate (NiDBC) were obtained from TCI Europe (Zwijndrecht, Belgium).

Cobalt (II) chloride hexahydrate (CoCl₂.6H₂O), nickel (II) sulfate hexahydrate (NiSO₄.6H₂O), nickel (II) chloride hexahydrate (NiCl₂.6H₂O) and
zinc (II) sulfate heptahydrate (ZnSO$_4$.7H$_2$O) were obtained from Fisons Scientific Apparatus (Loughborough, UK). Copper (II) sulfate pentahydrate (CuSO$_4$.5H$_2$O) was supplied by Alfa Aesar (Lancaster, UK).

Unless otherwise stated, all stock solutions of dithiocarbamate compounds, thiurams and mercaptobenzothiazole were prepared in dichloromethane. Uracil, which was used as a dead volume marker was prepared at either 100 µg/ml or 25 µg/ml in mobile phase mixture of the specific study. A compound or mixture used to check the performance of the column was either 100 µg/ml of toluene or a mixture of 1 µg/ml acetone and 100 µg/ml acenaphthene in 70% acetonitrile-water. Cobalt chloride (CoCl$_2$) and nickel sulfate (NiSO$_4$) used for the conventional and pre-column derivatisation methods were prepared as 1 mM solutions in water. Copper (II) sulfate solution (5%) used for the determination of ZDTC by pre-column derivatisation to copper (II) complexes was prepared in 1.6% aqueous ammonia solution. Zinc (II) sulfate and nickel (II) sulfate each at 0.1% were prepared in deionised water. All other preparations of the reagents are as described in the methods and discussions.

2.1.3. Samples of rubber products

Six samples of rubber products were supplied by Tun Abdul Razak Research Centre (TARRC - Hertford, UK). Table 2.1 shows details of the samples used for the determination of rubber accelerators. All samples were extracted using the Soxhlet method, where they were firstly cut into small pieces of about 1 cm strips and accurately weighed at 1 to 3 grams. Samples were extracted with 100 ml of DCM at about 60°C for about 16 hours (overnight). The extracted solutions were then reduced to dryness at 60°C using rotary evaporator. The residues were then re-dissolved with 20 ml of DCM and were devided into three parts for the determination of zinc dithiocarbamates using the methods described in section 2.5.
Chapter 2: Experimental

Table 2.1
Samples description, identification and weight used for extraction

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of sample</th>
<th>Sample ID</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Purple nitrile gloves</td>
<td>1-1</td>
<td>1.5920</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-2</td>
<td>1.6272</td>
</tr>
<tr>
<td>2.</td>
<td>Blue nitrile gloves A</td>
<td>2-1</td>
<td>1.1397</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2</td>
<td>2.7135</td>
</tr>
<tr>
<td>3.</td>
<td>Blue nitrile gloves B</td>
<td>3-1</td>
<td>1.5679</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-2</td>
<td>1.6892</td>
</tr>
<tr>
<td>4.</td>
<td>Natural rubber latex gloves A</td>
<td>4-1</td>
<td>2.1180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-2</td>
<td>1.9127</td>
</tr>
<tr>
<td>5.</td>
<td>Natural rubber latex gloves B</td>
<td>5-1</td>
<td>1.7303</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-2</td>
<td>1.7540</td>
</tr>
<tr>
<td>6.</td>
<td>Multi coloured balloon - red</td>
<td>6-1</td>
<td>2.7006</td>
</tr>
<tr>
<td></td>
<td>- pink</td>
<td>6-2</td>
<td>2.6390</td>
</tr>
</tbody>
</table>

2.2. Instrumentation

HPLC analysis were mainly performed on a system 1 (Fig. 2.1) consisting of a Jasco PU-980 Intelligent HPLC Pump (Jasco, Tokyo, Japan), a Rheodyne 7161 manual injector with 10 μl sample loop (Rheodyne, California, USA), a Philips PU 4500 gas chromatography oven (Philips/Pye Unicam, Cambridge, UK), a variable wavelength absorbance detector (Applied Biosystems 757, California, USA) and JCL 6000 Chromatography Data System Version 5.06 (Jones Chromatography, Lakewood, CO, USA). Later data for HPLC system 1 were collected using Clarity Chromatography System Version 2.4.1.77 (DataApex, Prague, Czech Republic). Calibration and determination of ZDTCs in rubber products on the other hand were performed on Agilent/HP 1100 series liquid chromatography (LC) with 10 μl injection volume, diode array detector (DAD) (Agilent Technologies, CA, USA) and separate column oven (Shimadzu CTO-6A, Shimadzu Corporation, Kyoto, Japan) (system 2 - Fig. 2.2) with Agilent ChemStation (Rev. A.10.02) software for data analysis.

The separations were performed on a number of columns, which included: Beckman-Ultrasphere ODS (Ultrasphere ODS - 4.6 x 250 mm, 5
µm - San Ramon, CA, USA), ZirChrom-polybutadiene (Zr-PBD - 4.6 x 150 mm, 3 µm - ZirChrom Separations Inc., USA), Zorbax Cyano (Zorbax Cyano - 4.6 x 250 mm, 5 µm - Dupont, USA), XTerra MS-C$_{18}$ (XTerra ODS - 4.6 x 150 mm, 3.5 µm), XBridge C$_{8}$ (XBridge C8 - 4.6 x 50 mm, 3.5 µm), XBridge C$_{18}$ (XBridge ODS - 4.6 x 150 mm, 3.5 µm - Waters, Ireland) and Gemini C$_{18}$ (Gemini ODS - 2.0 x 150 mm, 5 µm - Phenomenex, USA). Regular checking of the column performance was carried out using a testing mixture consisting of acetone and acenaphthene, but if prolonged elution of HPLC system with pure solvents and sometimes with series of solvents as suggested by column manufacturer for cleaning and regeneration steps did not remove all the trace amounts of metal complexes from the column, a fresh column was used for some of the studies.

The quantitation of cobalt, nickel, zinc and copper dithiocarbamate complexes, thiram and mercaptobenzothiazole were carried out at 320, 330, 260, 435, 275 and 254 nm, respectively. All measurements were carried out at least in duplicate. Results for calibration and method validation were the mean of six replicate injections. Other HPLC conditions are as described in the methods and discussions.
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Fig. 2.1. High performance liquid chromatography (HPLC) system 1.

Fig. 2.2. HPLC system 2 - Agilent/HP 1100 series LC with DAD and separated fan oven.
2.3. Initial investigation

2.3.1. Preliminary study and pre-column derivatisation to cobalt (III) complexes

Standard solutions of the ZDTCs, thiurams and MBT used in the preliminary studies were prepared at 100 or 50 µg/ml by dilution of stock solution with dichloromethane. Then, 10 ml of zinc dithiocarbamate complexes aliquots were converted to cobalt (III) complexes by shaking with 5 ml of 1 mM CoCl₂ for 30 minutes. The green dichloromethane solutions of cobalt (III) dithiocarbamates complexes were then separated on Ultrasphere ODS column using mobile phase of 90% methanol-water (v/v) at 1 ml/min and detected at 320 nm. Room temperature was used unless otherwise stated. The mobile phase of 90% methanol-water with 1% CoCl₂ (w/v) was prepared by dissolving 1 mg of CoCl₂ to 100 ml of mobile phase mixture.

2.3.2. Direct injection by addition of an alternate zinc dithiocarbamate as a protecting agent

Standard solutions used for the direct injection method as described by Depree et al. [78] were prepared in acetonitrile. For direct injection of ZDEC, 600 µl of the alternate zinc dithiocarbamate (ZDMC at 480 µg/ml) was taken down to dryness under constant pressure of nitrogen stream at ambient temperature. Then, 600 µl of the analyte of interest (ZDEC at 120 µg/ml) were added. The mixture of zinc dithiocarbamates (ZDTCs) was then directly injected to the HPLC system by firstly conditioned the system by injecting 2 times concentrated ZDMC. The same procedure was followed for determination of ZDMC with ZDEC as a protecting agent.

2.3.3. Pre-column derivatisation to copper (II) complexes

Preliminary studies of pre-column derivatisation to convert ZDTCs to copper (II) complexes were carried out using standard solutions of 100 µg/ml
individual ZDTC or 33 μg/ml each of ZDTC mixture (ZPD, ZDBC and ZBEC) dissolved in two different solvent mixtures, either acetone-1.6% aqueous ammonia solution (1:1) with 10 mmol copper (II) or 70% acetone-water with 10 mmol copper (II).

2.4. Direct injection and derivatisation of zinc dithiocarbamates

2.4.1. Direct injection of zinc dithiocarbamates

Direct injection of zinc dithiocarbamates was carried out either without or with conditioning the HPLC system as described in the discussion. The mobile phase used for the conditioning of the HPLC system contained 1% or 0.1% of Zn (II) by dissolving 1 g of Zn (II) relative to anhydrous zinc salt (ZnSO₄·7H₂O) formula weight (287.56) into 100 ml and 1 litre of mobile phase mixture respectively. For the study on the effect of pH, the pH of water used for preparing the mobile phase mixture before and after adding the metal salts was monitored and adjusted to the desired pH using 1% acetic acid for pH 3.62 and 35% ammonium hydroxide for pH 11.00.

2.4.2. Derivatisation of zinc dithiocarbamates

2.4.2.1. Pre-column derivatisation to nickel (II) complexes

Otherwise stated, pre-column derivatisation was carried out by mixing zinc dithiocarbamates diluted in DCM with an equal volume of 0.1% Ni (II) diluted in methanol, which subsequently produced nickel dithiocarbamates in 50% DCM-methanol mixture.

2.4.2.2. In-line derivatisation method

In-line derivatisation method was carried out by injecting the metal salts for conditioning just before injecting the analyte. For example, two 40 μl of
0.1% Ni (II) solution in methanol were injected about two to three minutes before injecting 10 µl of 100 µg/ml of ZDBC. All other conditions and variations are as described in the discussions.

2.4.2.3. On-column derivatisation method

The on-column derivatisation was carried out by directly injecting the zinc dithiocarbamates into the system consisting of a mobile phase containing metal salts. Mobile phase with 0.01% Ni (II) for example, was prepared by adding 0.1 g of Ni relative to anhydrous nickel salt (NiSO$_4$.7H$_2$O) formula weight (280.88), which was about 0.4785 g into 1 litre of methanol-water mixture. All other conditions are as described in the discussion.

2.4.3. Confirmation of the origin of the mixed ligand complexes

Stock solutions of ammonium diethyldithiocarbamate (ADEC) and ammonium pyrrolidinedithiocarbamate (APDC) were prepared by dissolving 10 mg of each compound in 10 ml of deionised water. Aliquots of 100 µg/ml of each dithiocarbamate were prepared by diluting stock solutions with the same solvent. ADEC and APDC were separated by on-column derivatisation to the respective metal dithiocarbamate using 75% methanol-water with addition of 0.01% zinc (II) or nickel (II) at 1 ml/min and 40°C on Ultrasphere ODS column. The ZDEC or ZPDC and NiDEC or NiPDC were then detected at 260 and 330 nm, respectively.

2.5. Determination of zinc dithiocarbamates in rubber products

2.5.1. Using pre-column derivatisation to copper (II) complexes

Determination of ZDTCs in rubber products using pre-column derivatisation to copper (II) complexes was carried out according to the in-house method of TARRC [176], where 6 ml of standard or sample solution
dissolved in DCM were vigorously shaken with 2 ml of 5% aqueous ammoniated copper (II) sulfate solution for at least one minute. After about 5 minutes, the bottom layer was transferred to another test tube. 3 ml of this solution was reduced using nitrogen stream and then re-dissolved with 3 ml of acetone. The re-dissolved sample solutions were filtered with 0.2 μm Nylon Syringe Filter (Whatman, Maidstone, UK).

Series of standard solutions used for calibration and method validation were also prepared in DCM and went through the same pre-column derivatisation to copper complexes as above. ZDEC standard solution series were 0.40, 0.79, 1.59, 3.18, 6.36, 12.72, 25.43, 50.86 and 101.73 μg/ml. ZDBC standard solution series were 0.44, 0.87, 1.75, 3.49, 6.99, 13.98, 27.96, 55.91 and 111.83 μg/ml. A mixture of ZDEC and ZDBC standard solution series were also prepared with the same concentrations.

2.5.2. Using pre-column derivatisation to nickel (II) complexes

Pre-column derivatisation of ZDTCs to nickel (II) complexes was carried out by taking 6 ml of standard or sample solutions dissolved in DCM and vigorously shaking with 2 ml of 1% aqueous nickel (II) chloride solution for at least one minute. After about 5 minutes, the lower layer was transferred to another test tube. 3 ml of this solution was reduced using nitrogen stream and then re-dissolved with 3 ml of methanol. The re-dissolved sample solutions were filtered with 0.2 μm Nylon Syringe Filter.

2.5.3. Using on-column derivatisation to nickel (II) complexes

Determination of ZDTCs in rubber products using the on-column derivatisation method to nickel (II) complexes was carried out by reducing 3 ml of standard or sample solutions dissolved in DCM to dryness using nitrogen stream and re-dissolving in 3 ml of methanol. The re-dissolved sample solutions were filtered with 0.2 μm Nylon Syringe Filter.
2.5.4. Recovery study for the pre- and on-column derivatisation

A recovery study was carried out using pure reference samples of NiDEC and NiDBC. Standard solution series of NiDEC, NiDBC and mixtures of the two were prepared by diluting the stock solutions of the nickel dithiocarbamates (NiDTCs) in methanol. Aliquots of NiDEC prepared were 1.63, 3.25, 6.5, 13, 26 and 52 µg/ml while NiDBC were 1.57, 3.13, 6.26, 12.53, 25.05, 50.1 µg/ml. Aliquots for the mixture of NiDEC and NiDBC had the same concentrations. Separations of the reference standard solutions of nickel (II) complexes were carried out using XBridge C8 column, 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and detection at 330 nm. The recovery study was only carried out for pre-column derivatisation and on-column derivatisation of ZDEC, ZDBC and mixtures of the two as only nickel (II) diethyldithiocarbamate (NiDEC) and nickel (II) dibutyldithiocarbamate (NiDBC) complexes were available as standards. Standard solutions of ZDTCs underwent pre-column and on-column derivatisation and were then compared with the standard reference series.

The observed concentration \( (x_p) \) was calculated based on the calibration functions of the references. The \( x_p \) data were then plotted against the original concentration \( (x_0) \), which gave the recovery function, \( x_p = b_p(x_0) + a_p \), where \( a_p \) and \( b_p \) are the y-intercept and slope of the recovery function.

2.6. Calculations for the determination of analytical parameters

The retention factors \( (k) \) of all the compounds studied were calculated using the following equation:

\[
k = \frac{t_R - t_0}{t_0}
\]

where, \( t_R \) is the retention time of the analyte and \( t_0 \) is the dead volume marker (uracil).

The column efficiency was calculated by data system as \( N/m \), where \( N \) is the number of theoretical plates in the column calculated using
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\[ N = 5.54 \left( \frac{t_R}{w_H} \right)^2 \] (2.2)

and \( L \) is the column length in metre, \( w_H \) is the peak width at half-height. The plate height \((H)\) was calculated as \( L/N \), where \( L \) is the column length in mm.

The peak asymmetry was calculated as equation 2.3 below:

\[ \text{Sym}[10\%] = \frac{t_{10\%}}{f_{10\%}} \] (2.3)

where, \( t_{10\%} \) is the width of the peak at the tail and \( f_{10\%} \) is the width of the peak at the front, both measured at 10\% of the height of the peak. The peak symmetry using Clarity Chromatography System was at 50\% of the height of the peak and denoted as \( \text{Sym} [50\%] \).

The selectivity was calculated by the following equation:

\[ \alpha = \frac{k_{n+1}}{k_n} \] (2.4)

which, \( k \) is the retention factor for the analytes, and \( n \) is the number of the analyte.

The resolution was calculated by the equation 2.5 below:

\[ R_{n,n+1} = 1.18 \frac{(t_{n+1} - t_n)}{(w_{n} + w_{n+1})} \] (2.5)

where, \( t \) is the retention time of the analyte and \( w_n \) is the width of the analyte peak at half of the peak height. [177]

The thermodynamic parameters, standard enthalpy \((\Delta H^o)\), standard entropy \((\Delta S^o)\) and Gibbs free energy \((\Delta G^o)\) of the transfer of compounds from the mobile phase to the stationary phase at the mean of the temperature range were calculated using the equation 2.6 and 2.7 below:

\[ \ln k = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \phi \] (2.6)

\[ \Delta G^o = \Delta H^o - T\Delta S^o \] (2.7)

where \( R \) is the gas constant, \( T \) is the absolute temperature and \( \phi \) is the
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phase ratio of the column. The $\Delta H^\circ$ and $\Delta S^\circ$ values were calculated using the slope and intercept of the linear Van't Hoff plot using equation 2.6. [178]

The parameters, such as selectivity, linearity, precision, accuracy and sensitivity, for the method validation step were determined by analyzing replicate determinations of ZDTCs using the appropriate method.

Selectivity was calculated using equation 2.4 for a mixture of ZDEC and ZDBC. Linearity of the method was demonstrated using the calibration curve found for each method.

The precision of the method was calculated as the coefficient of variance for each standard concentration used for calibration.

The accuracy of the method was calculated as recovery rate (RR) using the recovery method (section 2.5.4), which was calculated using the following equation:

$$RR = \left(\frac{a_p}{x_0} + b_p\right) \times 100\%$$  \hspace{1cm} (2.8)

where $a_p$ and $b_p$ are the y-intercept and slope of the recovery function and $x_0$ is the original concentration of the dithiocarbamates before pre- or on-column derivatisation to nickel complexes.

Sensitivity of the method can be expressed as a Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the compounds using the specified method. LOD and LOQ were calculated using equation 2.9 and 2.10 below:

$$LOD = \frac{3xS_{x/y}}{m}$$  \hspace{1cm} (2.9)

$$LOQ = \frac{10xS_{x/y}}{m}$$  \hspace{1cm} (2.10)

where $S_{x/y}$ is the standard regression error of the calibration curve and $m$ is the slope of the calibration curve. [177]
3.0. Introduction

A preliminary study of rubber accelerators was first carried out using the conventional RP-HPLC method developed by Kaniwa and colleagues [4, 23] in order to further understand their chemistry and their chromatographic behavior. The conditions of the HPLC system used in this study was only a guideline for further investigation and some modifications have been made in order to fit the objective of the study. Later, a method of direct injection of zinc dialkyldithiocarbamates developed by Depree et al. [78] was also studied. A few studies using pre-column derivatisation methods were initially tried for various metal complexes. Then, further studies on the pre-column derivatisation to cobalt (III) complexes were carried out. Pre-column derivatisation techniques to form copper complexes as used by Abraham et al. [25] and Materials Characterisation Unit of Tun Abdul Razak Research Centre (TARRC) [176] was also explored.

3.1. Preliminary study

A preliminary study on rubber accelerators was carried out by following the RP-HPLC method developed by Kaniwa and colleagues [4, 23] in which the dithiocarbamates are converted to their cobalt complexes pre-column with some manipulations in order to further understand their chromatographic behaviour. However, TETD, TMTD and MBT, were injected directly into the system.

Using this method, the initial chromatographic characteristics of a few selected rubber accelerators, which were zinc dimethyldithiocarbamate (ZDMC), zinc diethyldithiocarbamate (ZDEC), zinc dibutyldithiocarbamate (ZDBC), tetramethylthiuram disulfide (TMTD), tetraethylthiuram disulfide (TETD) and 2-mercaptobenzothiazole (MBT) were established. However, it
was found that pure dichloromethane (DCM) was not a suitable solvent for dilution of some of the rubber accelerators (ZDMC and TMTD) as it gave broad fronting peaks.

One reason for this is probably because of the immiscibility of DCM with water in the mobile phase. DCM is also a very strong solvent, which then flushed out some of the compounds earlier than it should and thus produced a fronting peak. On the other hand, TMTD and TETD diluted in the mobile phase mixture of 90% methanol-water, showed a better peak shape, higher peak height (Fig. 3.1), and subsequently gave higher efficiencies (number of theoretical plates) compared to dissolution in DCM alone (Table 3.1 and Table 3.2).

![Fig. 3.1. Chromatogram of tetramethylthiuram disulfide (TMTD) diluted in mobile phase of 90% methanol-water with flow rate at 1 ml/min, ambient temperature, 275 nm on Ultrasphere ODS column.](image)

Table 3.1
Comparison of peak height and plate number between TMTD dissolved in DCM and TMTD re-diluted in mobile phase mixture

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TMTD in DCM</th>
<th>TMTD diluted with mobile phase (MP) (12:88 of DCM:MP)</th>
<th>% increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak height (mAU)</td>
<td>24436</td>
<td>32470</td>
<td>33</td>
</tr>
<tr>
<td>Plate number (N)</td>
<td>9069</td>
<td>33816</td>
<td>273</td>
</tr>
</tbody>
</table>

HPLC conditions: 90% methanol-water at 1 ml/min, ambient temperature, 275 nm on Ultrasphere ODS column.

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Table 3.2
Comparison of peak height and plate number between TETD dissolved in DCM and TETD re-diluted in mobile phase mixture

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TETD in DCM</th>
<th>TETD diluted with mobile phase (MP) (12:88 of DCM:MP)</th>
<th>% increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak height (mAU)</td>
<td>20294</td>
<td>25509</td>
<td>26</td>
</tr>
<tr>
<td>Plate number (N)</td>
<td>24062</td>
<td>33279</td>
<td>38</td>
</tr>
</tbody>
</table>

HPLC conditions: 90% methanol-water at 1 ml/min, ambient temperature, 275 nm on Ultrasphere ODS column.

ZDMC and TMTD also may have the same interaction with the stationary phase under these conditions as they eluted about the same time as the solvent peak, but other accelerators have a stronger interaction. Yet, all of the accelerators studied were found to dissolve in DCM and the solubility of ZDEC in pure DCM was the highest compared to the solubility in pure acetone and acetonitrile. DCM, acetone, acetonitrile and methanol were used for this study as they were the most widely used solvents for the dissolution of rubber accelerators. Dissolution of dithiocarbamates and thiurams in pure methanol, however, was not successful as most of them did not dissolve. However, as dilution of dithiocarbamates and thiurams in pure DCM produced fronting peaks, a study of the dilution of the compound in a mixture of DCM with mobile phase solvent (1:1) was carried out. It was found that the solubility of the compounds was comparable to the solubility in pure DCM. Therefore, the preparation of the working standard solution used DCM-mobile phase mixture (1:1) or DCM-methanol (1:1) as a solvent in order to prevent solvent peak fronting and co-elution of the analyte with the solvent peak.

Quantitative chromatographic characteristics of the selected rubber accelerators using the RP-HPLC method as described by Kaniwa and co-workers [4, 23] was carried out (Table 3.3). Based on this table, ZDBC seemed to take a very long time to elute even with 90% methanol-water mobile phase. All the other compounds on the other hand eluted very quickly with retention factors less than 1. It was also found that there was a linear relationship between \( \log k \) and the number of carbon atoms in the alkyl substituents of zinc dialkylthiocarbamates (ZDMC, ZDEC and ZDBC). The
same relationship was also observed by Liska et al. [51] in their HPLC study on nickel (II) bisdialkyldithiocarbamates. Reducing the organic solvent in the mobile phase for all of the compounds except ZDBC is therefore possible. However, for ZDBC or other retained compounds like dibutylidithiocarbamate complexes, changing the column type to a more polar stationary phase or increasing the organic percentage in the mobile phase would then decrease the retention of the complexes. Using less organic solvent at higher temperatures might also be one of the solutions for these compounds. For this reason, the study on the effect of temperature in Chapter 5 will use more retained complexes like ZDBC as the analytes.

Table 3.3
Retentions of ZDBC, ZDEC, ZDMC, TETD, TMTD and MBT on Ultrasphere ODS column

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Retention time (t_R, min)</th>
<th>Retention factor (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDBC</td>
<td>66.88</td>
<td>26.30</td>
</tr>
<tr>
<td>ZDEC</td>
<td>4.42</td>
<td>0.80</td>
</tr>
<tr>
<td>ZDMC</td>
<td>3.23</td>
<td>0.32</td>
</tr>
<tr>
<td>TETD</td>
<td>3.80</td>
<td>0.55</td>
</tr>
<tr>
<td>TMTD</td>
<td>2.88</td>
<td>0.18</td>
</tr>
<tr>
<td>MBT</td>
<td>2.87</td>
<td>0.17</td>
</tr>
</tbody>
</table>

HPLC conditions: 90% methanol-water, 1 ml/min, room temperature and UV detection of 320 nm for cobalt complexes, 275 nm for thiurams and 254 nm for MBT on Beckman Ultrasphere ODS column (4.6 x 250 mm, 5 μm). ZDBC, ZDEC and ZDMC were detected as their respective cobalt complexes.

The quantitations of thiurams and mercaptobenzothiazoles using reported method [4, 23] were pretty direct. However, the quantitation of zinc dithiocarbamates required sample preparation, which includes liquid-liquid extraction that was time consuming, tedious and used a lot of solvent. Therefore, an attempt to use and develop more direct and simple method was carried out.

3.2. Direct injection by addition of alternate ZDTC as a protecting agent

Recently, Depree et al. [78] have developed a rapid and direct method for assaying ZDTC in latex condoms by adding an alternative ZDTC to the sample and mobile phase by pre-injection as a protective agent. Therefore, it
was of interest to try and investigate this method. However, a study in our lab in contrast found four peaks instead of two as reported (Fig. 3.2). The possible reason for this phenomenon has been discussed by several authors. Lehotay et al. [138] for example, suggested that a multivalent ion like zinc (II) produced mixed ligand complexes when two or more ZDTC with different ligands are mixed together as in the equation 1.6. This means that a mixture of two different ligands of ZDTC will always produced one extra peak. However, another extra peak appeared which means that probably the peaks were actually cobalt (III) complexes as residual cobalt ions might still be in the column/tubing as the same system was previously used for the separation of cobalt (III) complexes. The cobalt (III) complexes were also known to be more stable than zinc (II) complexes [21, 179] and thus would be able to replace the zinc complexes rapidly. A mixture of two cobalt (III) complexes with different ligands was previously shown to produce four peaks [23]. Therefore, the mixed ligand complexes found as in Fig. 3.2 might be the complexes of cobalt (III).

The peak heights of mixed ligand 1 and 2 as in Fig. 3.2 were also found to consecutively decrease for the second and third injection of ZDTC mixture (ZDEC and ZDMC). These results suggested that zinc (II) complexes are not stable and other metal ions present in the system may react with some of the ligands. Conditioning the HPLC system with an injection of a concentrated solution of ZDMC into the system just before the analytical separation gave corresponding results to the 1st injection. However, there were still four peaks appeared instead of two. Therefore, direct injection of ZDTC might be possible, provided that the concentration of zinc (II) ions is high enough to protect the dithiocarbamate from metal exchange of zinc ion to other more stable metal DTC complex. Nevertheless, it is of interest to further investigate the effect of adding zinc (II) salt into the mobile phase mixture, and thus is discussed in the next chapter.
Chapter 3: Initial Investigation

3.3. Pre-column derivatisation of zinc dithiocarbamates

At this point, since direct injection of zinc dithiocarbamates was not successful, further studies using pre-column derivatisation methods to form more stable metal complexes were carried out. An initial study of the derivatisation technique was tried for different metal complexes. Further studies using pre-column derivatisation to cobalt (III) complexes were also carried out with additional examination on the effect of the addition of cobalt (II) salt into the mobile phase and the effect of temperature. The latest method reported by Abraham et al. [25] was also investigated and reported in section 3.3.3.

3.3.1. Derivatisation to various metal complexes

An initial study of the derivatisation behaviour of different metal ion complexes was examined by comparing cobalt (II) and nickel (II) ions. Cobalt (II) ions are resistant to oxidation, except in the presence of an amine;
dithiocarbamate compounds contain an amine-like nitrogen and thus can be easily converted to more stable cobalt (III) complexes by oxidation even by oxygen in the air [180]. Therefore, during the metal conversion of zinc dialkylthiocarbamates (ZDTC) to cobalt dialkylthiocarbamates (CoDTC), cobalt (II) was converted to the cobalt (III) complex by oxidation, during the shaking process of the ZDTC with the cobalt (II) chloride solution. An obvious colour change of cobalt (II) chloride solution from pink to green also suggested that the cobalt complexes were changing from cobalt (II) to cobalt (III). A few authors quoted by Smith et al. [181] also reported the same finding, and that mass spectrum of the extracted complex agreed with the formation of a cobalt (III) dithiocarbamate complex. However, this reaction is fairly slow [25]. As a result, more time was needed in order to convert cobalt (II) to cobalt (III) complexes and incomplete conversion of ZDTC to CoDTC might also happen. Kaniwa [23] found that shaking time for the full conversion of zinc to cobalt complexes took more than 10 min. In current study, nickel (II) ions reacted faster than cobalt for the same concentration of diethylthiocarbamate and also the same duration of reaction.

Further studies of the pre-column derivatisation of ZDBC to Co (III), Ni (II) and Cu (II) complexes at 250 nm faced problems of interferences from the other competitive metals as all the metal complexes studied produced peaks at this particular UV wavelength. However, the addition of ZDBC complexes into a mixture of metals containing Co (II), Ni (II) and Cu (II) ions showed that, the Co (III) complexes produced were the most stable complexes with little interferences from Cu (II) ions, and no Zn (II) and Ni (II) complexes were formed (Fig. 3.3).
Chapter 3: Initial Investigation

Fig. 3.3. Chromatogram of ZDBC after its addition to a mixture of metals (Co(II), Ni(II) and Cu(II)) using 95% methanol-water at 1 ml/min, 40°C and 250 nm on Ultrasphere ODS column. CuDBC$_1$ and CuDBC$_2$ were the equilibrium forms of CuDBC complexes. No peak was detected for Zn (II) and Ni (II) complexes.

The same study was also repeated at their unique UV wavelengths (320 nm for cobalt, 330 nm for nickel and 435 nm for copper complexes) (Fig. 3.4). However, it was difficult to make a quantitative comparison between all of the metals studied as the competitive metals were still interfered. On the other hand, Ni (II) complexes were found to give the highest peak height and area with considerably higher column efficiencies and low tailing. Moreover, a second injection of NiDBC was found to give higher peak height, peak area as well as higher column efficiency for NiDBC complexes and lower for other competitive metal complexes, which suggest that the effect from the other metal can be reduced by increasing the concentration of the metal of interest in the system. Nickel dithiocarbamate complexes were also reported to be thermodynamically stable, while with the other metal complexes like copper and zinc dithiocarbamates, the mono complexes were in equilibrium with the metal ion and bis complex as equation 3.1 below:

$$M^{2+} + M(DTC)_2 \leftrightarrow K \rightarrow 2M(DTC)^+$$

(3.1)

where $M = \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+}, \text{Pb}^{2+}$ and $\text{Hg}^{2+}$ [179]. Hence, it was decided to use nickel salt for the derivatisation of zinc dialkyldithiocarbamates studied in the next chapters.
3.3.2. Derivatisation to cobalt (III) complexes

Additional studies of the pre-column derivatisation of zinc dialkyldithiocarbamates to cobalt (III) complexes were carried out to further understand the chemistry and chromatographic behaviour of zinc dithiocarbamates. The conversion of zinc dithiocarbamates to cobalt (III) complexes was chosen as it was reported [180] that cobalt (III) complexes were so stable that no exchange reactions with other metal ions occurred.

However, our initial separation of cobalt (III) complexes formed from the reaction of zinc (II) diethyldithiocarbamate (ZDEC) with cobalt (II) chloride at ambient temperature (15°C) was found to have a ghost peak at 5.3 min, which was also found to increase after a few injections. A metal exchange reaction was suspected to be taking place between the cobalt complexes and nickel from the stainless steel components of the chromatographic system. Nickel diethyldithiocarbamate complexes have already been shown to be more stable than the zinc and cobalt complexes [21]. Thus, if nickel is
present in the chromatographic system, it might displace both zinc and cobalt from its complexes. Various previous studies [136, 149, 174, 175] have reported no peaks for zinc dithiocarbamates by using conventional mobile phases and stainless steel columns and tubing. Referring to Fig. 3.4, nickel complexes also give a maximum absorption at the wavelength used to detect cobalt complexes (320 nm). Detection at 370 nm, which was specific for cobalt complexes found only one peak. The addition of 1% w/v of cobalt (II) chloride to the mobile phase mixture served as a protecting agent showed one sharp peak of cobalt (III) diethylidithiocarbamate (CoDEC - Fig. 3.5). The separation of cobalt (III) diethylidithiocarbamate using the original mixture of 90% methanol-water, but at 40°C also gave only one peak but it was a tailing peak. Again, addition of 1% CoCl₂ to the mobile phase successfully improved the peak shape.

The separation of cobalt (III) dibutyldithiocarbamate (CoDBC) complex on the other hand gave a very long retention time (more than 60 min at ambient temperature and more than 40 min at 40°C) using 90% methanol-water as mobile phase. Although using 90% methanol-water with 1% CoCl₂ has reduced the retention time about half at 40°C, the peak has produced a little shoulder (Fig. 3.6). Therefore, changing the column type or increasing the percentage of organic solvent in the mobile phase might decrease the retention time of CoDBC. Kaniwa [23] has already shown that higher percentage of organic solvent (95% methanol) decreased the retention time for CoDBC to less than 20 minutes. However, because the aim of this study is to decrease or omit the use of organic solvent, changing the column type to a shorter side chain would be a preferred solution. Using less organic solvent at a higher temperature may also give the same effect. Further study on pre-column derivatisation techniques was carried out and discussed in the next chapter.
Fig. 3.5. Chromatogram of cobalt (III) diethylthiocarbamate using 90% methanol-water with 1% CoCl$_2$ at 1 ml/min, ambient temperature (15°C) and 320 nm on Ultrasphere ODS column.

Fig. 3.6. Chromatograms of cobalt (III) dibutylthiocarbamate using 90% methanol-water with 1% CoCl$_2$ at 1 ml/min, 40°C and 320 nm on Ultrasphere ODS column.

Earlier authors [23, 51] have found mixed ligand complexes when two or more than two kinds of zinc dithiocarbamates ligands were present and then converted to cobalt (III) complexes. In this study, the same results were found for a mixture of ZDEC and ZDBC after converted to cobalt (III) complexes (Fig. 3.7).
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Fig. 3.7. Chromatogram of ZDEC and ZDBC mixture after conversion to cobalt (III) complexes using 90% methanol-water at 1 ml/min, 40°C and detected at 320 nm on Ultrasphere ODS column.

A better resolution of cobalt dithiocarbamates (CoDTC) mixture was produced if 1% of cobalt chloride was added into the mobile phase. Unfortunately, the peak shape of the fourth peak was broad and tailing. It was probably because of the solvent (DCM) effect as diluting the sample mixture with mobile phase gave better peak shape (Fig. 3.8). Increasing the column temperature to 60°C was also found to produce better peak shapes. Further study on the effect of addition of metal salts into the mobile phase and the effect of temperature for pre-column derivatisation of ZDTCs to cobalt (III) complexes are discussed in the next sections.
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Fig. 3.8. Chromatogram of ZDEC and ZDBC mixture after conversion to cobalt (III) complexes using 90% methanol-water with 1% CoCl₂ at 1 ml/min, 40°C and detected at 320 nm on Ultrasphere ODS column. The ZDTC mixture was diluted with mobile phase.

3.3.2.1. Effect of the addition of metal salts into mobile phase mixture

As mentioned earlier, the addition of 1% CoCl₂ (w/v) into the mobile phase mixture not only reduced the effect from the other metals possibly present in the HPLC system but also improved the peak shapes which subsequently increased the separation efficiencies. Furthermore, the addition of metal salts into the mobile phase has also reduced the retention time of the metal complexes (Table 3.4). Previous authors [181] have also discovered different retention properties in their studies of using transition-metal salts as ion-pair reagents for the determination of dithiocarbamates and their derivative fungicides.

A recent study by Flieger and colleagues [182] on the chromatographic characteristics of organic hydrocarbons has further verified the effect of adding additives like zinc and nickel sulfates to the mobile phase on the retention, efficiency and selectivity of the analytes. The authors suggested that the influence of the salts could be attributed to the dissolution of the analytes, which mainly due to the sulfate anions, or selective complexation.
with particular component by metal cations. In that particular study, they also found significant changes in efficiency with the increase of salt concentration. Another study by Reta and Carr [183] explained more about the effect of divalent metals, where they can actually block the silanol effects, like adding an amine buffer to the mobile phase. Although they were not as strong as amine additives, they still decreased both the retention time and asymmetry factor compared to their values using a conventional mobile phase mixture. They also found that as the cation's atomic radius increased, the retention time decreased, which was explained as a result of a solubility equilibrium-like reaction between the cations and the ionized silanol groups. Higher concentrations of salts in the mobile phase were also found to decrease the retention time. This behaviour might be because of the residual unblocked silanol groups or the salting-in effect [184].

Table 3.4
Effect of the addition of metal salts into the mobile phase mixture for pre-column derivatised CoDEC and CoDBC at room temperature (RT) and 40°C

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Temperature (°C)</th>
<th>Retention time (tR, min)</th>
<th>Percent reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>90% methanol-water</td>
<td>90% methanol-water with 1% CoCl₂</td>
</tr>
<tr>
<td>CoDEC</td>
<td>RT</td>
<td>4.42</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4.18</td>
<td>3.88</td>
</tr>
<tr>
<td>CoDBC</td>
<td>RT</td>
<td>66.88</td>
<td>46.40</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>44.25</td>
<td>31.27</td>
</tr>
</tbody>
</table>

HPLC conditions: Ultrasphere ODS column using mobile phase at 1 ml/min and detected at 320 nm.

The same effect was also found for the mixture of CoDEC and CoDBC at both temperatures. However, the addition of metal salts into the mobile phase was found to give a more significant effect on retention for a more retained complex like CoDBC. The addition of 1% CoCl₂ into the mobile phase reduced the retention time of CoDBC to almost the same value as the effect of increasing the column temperature from ambient to 40°C without adding the metal salts. Therefore, the addition of metal salts into the mobile phase mixture not only protects the effect from the other metals probably present in the HPLC system, but also decreases the retention especially for more non-polar compounds.
3.3.2.2. Effect of temperature

As shown in Table 3.4, increasing the temperature from room temperature (RT - 25 ± 5°C) to 40°C does not have an appreciable effect for CoDEC. Further study of the effect of temperature on CoDEC up to 60°C (Fig. 3.9) has also been investigated. As expected, the retention factor of CoDEC was reduced by up to 32% from room temperature to 60°C.

Fig. 3.9. Effect of temperature on CoDEC after pre-column derivatisation using 90% methanol-water with 1% CoCl₂ at flow rate of 1ml/min on Ultrasphere ODS column and detected at 320 nm.

Moreover, increasing the oven temperature, which subsequently increased the temperature of the eluent as well as the column, increased the peak heights of both CoDEC (Fig. 3.10 peak 1) and CoDBC (Fig. 3.10 peak 4), reduced the retention time particularly for CoDBC, and reduced the ghost peak (Fig. 3.10 peak 5: probably the effect from the other metals present in the HPLC system) which appeared in the separation of CoDEC and CoDBC mixture at room temperature (Fig. 3.10-a).
Fig. 3.10. Chromatograms of CoDEC and CoDBC mixture using 90% methanol-water at (a) room temperature, (b) 40°C and 90% methanol-water with 1% CoCl₂ at (c) 40°C and (d) 60°C at a flow rate of 1 ml/min on Ultrasphere ODS column detected at 320 nm. Peak 1:Co(DEC)₃, 2:Co(DEC)₂DBC, 3:CoDEC(DBC)₂, 4:Co(DBC)₃ and 5:ghost peak.
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Increasing the oven temperature to the maximum possible for the silica based columns like Ultrasphere ODS, and with the addition of metal salts into the mobile phase has improved the peak shapes (which means increased separation efficiency or theoretical plate number) of all peaks of the CoDEC and CoDBC mixture along with reduced retentions (Fig. 3.10-d). However, at the same temperature of 40°C, the CoDBC peak (peak 4) in the separation of CoDEC and CoDBC mixture using mobile phase with a metal salt was found to be distorted (Fig. 3.10-c) and thus the efficiency (N) has dropped more than 80% compared to the peak using mobile phase without metal salt. Although the silanol effect has been reduced due to the addition of cobalt salt, the presence of chloride anions might still affect the later cobalt (III) complexes [185], and therefore produced distorted peaks. On the other hand, using the same mobile phase mixture with metal salt but at higher temperature of 60°C not only reduced the retention time, but also increased the efficiency of all the peaks including the CoDBC peak (Fig. 3.10-d).

The effect of temperature was also analyzed for one of the thiuram disulfide compounds (TMTD). However, the increase of temperature did not markedly affect the peak height or the number of theoretical plates of TMTD. At 60°C, the retention only decreased by about 7% and the peak height and plate number increased by only 21% and 10%, respectively, compare to the room temperature which was about 24°C.

3.3.3. Derivatisation to copper (II) complexes

The latest method for the determination of ZDTC as reported by Abraham et. al [25] used pre-column derivatisation of zinc dithiocarbamates to the more stable copper dithiocarbamate complexes. Although, in the present work it was found that copper complexes have the possibility of having two forms at equilibrium, the use of different type of solvent might give a different behaviour, as the present work used methanol-water mixture and Abraham et. al [25] used acetone-water mixture. Thus, it was of interest to try using acetone-water as the mobile phase mixture because they did not report
As expected, separation of the individual ZDTCs using acetone-water mixture as the mobile phase only produced one peak in each case. However, dissolving ZDTC in different mixtures containing copper produced different chromatograms. Separation of individual complex of ZDBC which had been dissolved in 50% acetone-aqueous ammonia (NH₃) (1.6%) containing Cu (II), so that it was derivatised to CuDBC, was found to produce a better peak shape compared to the ZDBC dissolved in mobile phase mixture of 70% acetone-water with Cu (II).

Mixture of 3 ZDTCs (ZPD, ZDBC and ZBEC) prepared in the first manner was also different from the second. As shown in Fig. 3.11-a, the mixture dissolved in 50% acetone-aqueous NH₃ containing copper produced 3 major peaks of CuPD, CuDBC and mixed ligand complexes of CuPD-DBC. Moreover, very little copper dibenzyldithiocarbamate (CuBEC) was found. Two more peaks of mixed ligand complexes, probably as a result of the equilibration of the copper complexes were also detected. From these chromatograms, it can be concluded that copper complexes with dibutylthiocarbamate ligands were more stable than any other complexes in acetone-aqueous NH₃ solution.

On the other hand, the separation of the same mixture dissolved in 70% acetone-water (Fig. 3.11-b) which was similar to the mobile phase showed a different behaviour. Again, mixed ligand complexes were still present, however, it seemed that all compounds were equally stable, and therefore, the equilibrium of the three compounds produced about an equal amount of each compound including the mixed ligand complexes.
Chapter 3: Initial Investigation

Fig. 3.11. Chromatograms of 3 ZDTC mixture (ZPD, ZDBC, ZBEC) dissolved in: (a) 50% acetone-aqueous NH₃ with Cu (II) and (b) 70% acetone-water with Cu (II), separated using mobile phase of 70% acetone-water at 1 ml/min, ambient temperature and 435 nm on XBridge C8 column. Where 1: CuPD, 2: CuPD-DBC, 3: CuPD-BEC, 4: CuDBC, 5: CuDBC-BEC and 6: CuBEC.

From this experiment, it can be concluded that even a little difference in the solvent used will make the copper complexes behave differently. This is in a good agreement with the findings reported by Lehotay et. al [140], who investigated the influence of polar and non-polar solvents on the equilibrium
and rate constants of the ligand exchange reactions of some bis(dialkyldithiocarbamate) complexes of Ni (II). Although the equilibrium constant was not significantly dependent on the solvent used, the rate constant was strongly dependent, which indicates different reaction mechanisms for different solvents. However, as both produced mixed ligand complexes for the mixture of dithiocarbamates introduced, therefore, quantitation of this kind of mixture will still be difficult.

As a result, this method may be suitable for individual compounds of ZDTC, but not for a mixture of ZDTCs as they have the same problem of mixed ligand complex formation as for the cobalt complexes. However, as the mobile phase used in this study was an isocratic flow of 70% acetone-water, it might be different when a gradient flow of the same mixture is used. Therefore, a trial on the same method but using gradient elution of mobile phase was carried out. The mobile phase mixture was initially at 45% acetone-water and gradually increased up to 80% acetone-water in 15 minutes. It was then increased up to 100% acetone in another 15 minutes and was kept constant for another 5 minutes. The preparation of copper complexes was also a little bit different. The mixture of zinc dithiocarbamates, diluted in DCM, was converted into copper dithiocarbamates using liquid-liquid extraction. Then, copper dithiocarbamate complexes in DCM were reduced to dryness and re-dissolved with pure acetone. The same method was also used by TARRC as their in-house method [176]. Fig. 3.12 shows an example of a chromatogram for standard solution of ZDEC and ZDBC mixture using this method.

From Fig. 3.12, it was found that the separation of copper complexes was much more efficient and no mixed ligand complexes were found. However, the baseline became noisier and gradually drifted towards the end of the separation. This is probably because of the changes in the percentage of organic solvent, which was an increase in the acetone concentration. Either the use of gradient elution or the manner of derivatisation also caused the disappearance of the mixed ligand complexes as found earlier. Thus, this method was chose to be one of the methods for the determination of rubber
accelerators in rubber products as discussed in Chapter 6. The possibility of on-column derivatisation of ZDTCs to copper complexes using methanol-water mixture was also investigated and discussed in Chapter 4.

Fig. 3.12. Chromatogram of a mixture of ZDEC and ZDBC after pre-column derivatisation to CuDEC and CuDBC using gradient elution (0 min: 45% acetone-water, 15 min: 80% acetone-water, 30-35 min: 100% acetone) at 1 ml/min, 40°C and 435 nm on XBridge ODS column.

3.4. Summary

Evaluation of a few previously reported methods [4, 23, 78] showed that zinc dithiocarbamates were very unstable compounds. Therefore, direct determination of the compound was rather difficult as interference from other metals still occurred, and probably requires a higher concentration of protecting complexes or need prior conversion to a more stable metal complex. Further study on a similar concept but with the addition of zinc salts into the mobile phase was carried out and discussed in the next chapter.

A study on the derivatisation behaviour of zinc dithiocarbamates to other stable metal complexes (cobalt (III), nickel (II) and copper (II)) showed that nickel (II) ions reacted faster, had higher molar absorptivity and thermodynamically stable complexes. Therefore, nickel (II) ion was decided
to be used for further studies.

Additional study on the pre-column derivatisation to cobalt (III) complexes showed that the complexes appear to have interference from other metal. The addition of cobalt salt into the mobile phase had reduced the interference. However, the appearance of two extra peaks of mixed ligand complexes for the mixture of two zinc dithiocarbamates makes the quantitation of the ZDTCs rather difficult. Initial study on the effect of temperature using this particular method showed the possibility of using temperature as one of the parameter, which improved the peak shape, efficiency and reduced the analysis time.

Although Ni (II) ions has already been chosen to be used for further studies discussed in the next chapters, a recent study [25] that used pre-column derivatisation of ZDTC to copper (II) complexes and acetone-water mixture as their mobile phase led us to also investigate this method. As expected, the same problem of mixed ligand complexes was found if sample was diluted in the mobile phase mixture and used an isocratic mobile phase. However, the mixed ligand complexes disappeared when sample was diluted in 50% acetone-ammoniated solution and used liquid-liquid extraction and gradient elution of mobile phase. This method was then selected to be one of the methods for the determination of ZDTCs in rubber products as reported in Chapter 6.
CHAPTER 4 : DIRECT INJECTION AND DERIVATISATION OF ZINC DITHIOCARBAMATES

4.0. Introduction

In the previous chapter, a study of the direct injection of zinc dithiocarbamates by the pre-injection of an alternate zinc dithiocarbamate as described by Depree et al. [78] was not successful. Therefore, a further study of a similar concept in which the addition of zinc salts into the mobile phase was carried out and is discussed in this chapter. This method was initially tried using a ZirChrom-polybutadiene column, but was also not successful, and therefore, continued on the Ultrasphere ODS column. The effect of pH was also examined using the Ultrasphere ODS column for acidic and no pH control conditions, while using an XBridge C8 column for basic conditions.

As the pre-column derivatisation of zinc dithiocarbamates to nickel complexes was faster than to cobalt complexes, and produced the highest peak height and peak area with good efficiency and peak shape, it was decided to use nickel ions for further studies on pre-column derivatisation method. Nickel ion was also used for the other two newly proposed methods of in-line and on-column derivatisation methods. Using a pre-column derivatisation method, five different columns, which include Zr-PBD, Zorbax Cyano, Gemini ODS, XTerra ODS as well as Ultrasphere ODS columns, were investigated. A stability study of the derivatisation of zinc dithiocarbamates to nickel dithiocarbamates with time was also carried out over 8 days.

In-line derivatisation method, in which the metal was injected just before the analyte was tried for a few metals, and nickel was again found to be a better metal complex and thus was tried and compared with the pre-column derivatisation method. In addition, the effect of metal salt concentration injected into the system just before injecting the sample was also studied for
The third method studied was on-column derivatisation, where zinc dithiocarbamates were directly injected into the mobile phase containing a constant amount of metal salts. In this chapter, an initial study of on-column derivatisation to nickel complexes was carried out, that compared this method with the pre-column derivatisation method. Another study of on-column derivatisation that converts zinc dithiocarbamates to copper (II) complexes was also explored.

During all those studies, the chromatographic characteristics of uracil used as the dead volume marker were found to be changing over time. Therefore, further studies and discussion were also made. Besides that, a further investigation was also carried out to determine the origin of the mixed ligand complexes produced after derivatisation of zinc dithiocarbamates mixture to nickel dithiocarbamates.

4.1. Direct injection of zinc dithiocarbamates

4.1.1. Using ZirChrom-polybutadiene column

An initial study of the direct injection of zinc dialkyldithiocarbamates began with direct injection of ZDBC on ZirChrom-polybutadiene (polybutadiene coated zirconia: Zr-PBD) column, known as one of the more chemical and thermally stable columns [186 - 188]. The column was selected because of its ability to stand higher pH and temperature for later studies. However, similarly to the results found earlier (section 3.3.2), an extra peak was found. This extra peak might be interference from other metals that are probably present in the system and could bind with the dithiocarbamate ligands.

Conditioning the HPLC system with a pre-injection of a concentrated Zn (II) solution was not successful as an extra peak still appeared (Fig. 4.1-a).
Fig. 4.1. Chromatograms of ZDBC by direct injection and conditioning the HPLC system with (a) 2 pre-injection of 10 µl of 1 mg/ml Zn (II) and (b) addition of 1% Zn (II) to mobile phase using 95% methanol-water at 1ml/min, room temperature and 290 nm on Zr-PBD column.

The injection of zinc solution just before the injection of sample was meant as a protective agent from the effect of other possible metals. However, the amount of zinc ions might not be sufficient to protect the interference from other metals. Therefore, increasing the concentration of Zn (II) ions in the HPLC system might work, and thus the addition of 1% Zn (II) into the mobile
Chapter 4: Direct Injection and Derivatisation of Zinc Dithiocarbamates

phase was carried out. Mathieu et al. [56] have used a similar technique previously (addition of zinc (II) ions into the mobile phase); however, they used a metal free column. As shown in Fig. 4.1-b, a single peak was found but the efficiency and peak shape were quite poor.

Raising the temperature to 40°C still gave very low peak heights and poor column efficiency (Table 4.1-a). The peak was also tailing (poor asymmetry), which means that some other reactions or interference may still be present or the ZDBC complexes are not stable enough to be separated using this particular HPLC system. Direct injection of ZDBC with pre-injection of Ni (II) on the other hand was found to give a better peak height and column efficiency (Table 4.1-b). Both peak height and column efficiency were also increased as the amount of Ni (II) ions injected increased (Table 4.1-c). However, the tailing has also increased even at the higher concentration of Ni (II).

Table 4.1
Peak height, column efficiency per meter (N/m) and peak asymmetry (Sym [10%]) of ZDBC by direct injection at different conditions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>UV wavelength</th>
<th>Peak height (mAU)</th>
<th>N/m</th>
<th>Sym [10%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Addition of 1% Zn (II) in MP</td>
<td>290 nm</td>
<td>3870</td>
<td>4318</td>
<td>2.88</td>
</tr>
<tr>
<td>(b) Pre-injection of 80 μl 1mg/ml Ni (II)</td>
<td>330 nm</td>
<td>5197</td>
<td>5081</td>
<td>3.14</td>
</tr>
<tr>
<td>(c) Pre-injection of 120 μl 1mg/ml Ni (II)</td>
<td>330 nm</td>
<td>8993</td>
<td>6038</td>
<td>3.56</td>
</tr>
</tbody>
</table>

HPLC conditions: 95% methanol-water at 1 ml/min and 40°C on Zr-PBD column.

The tailing effect can have multiple causes. One is probably because of a poorly packed or deteriorated column. Extra column volumes, column overloaded, chemical tailing (incompatibility of sample with stationary and/or mobile phase) and time constant too high may also produce these unfavourable situations [177]. In the case of the separation of ZDBC as discussed above, it is probably the incompatibility of the sample with the stationary phase as dithiocarbamate compounds like ZDBC are able to chelate with other stable metals, which could have chelated to the zirconium (IV) surface sites [189] of the zirconia based columns. It was also reported that zirconia can interact with the analytes by ligand exchange because it is a
Chapter 4: Direct Injection and Derivatisation of Zinc Dithiocarbamates

Lewis acid [177].

After the separation of ZDBC, a test to monitor the performance of the Zr-PBD column using toluene showed that the column has either deteriorated or degraded as the column efficiency was low \((N/m = 5905)\) with a tailing peak \((\text{Sym } [10\%] = 2.04)\).

Therefore, it was concluded that the zirconia based column was not a suitable column for the separation of dithiocarbamates complexes even though it has the advantages of stability over a wide range of eluent pH and at elevated temperatures. The same approach of direct injection was then continued on Ultrasphere ODS column.

4.1.2. Using Ultrasphere ODS column

Analysis of ZDTCs on the Ultrasphere ODS column started with direct injection without conditioning the HPLC system. However, most of the zinc chelates exchanged to the nickel complexes and were therefore not detected at the wavelength used for zinc dithiocarbamates (280 nm) but were found as NiDTCs complexes at the maximum wavelength for nickel dithiocarbamates (330 nm). A closer look at a wavelength of 260 nm, where both zinc and nickel complexes can be detected found a split peak of zinc and nickel dithiocarbamates (Fig. 4.2-a).

As with the Zr-PBD column, the addition of Zn (II) ions into the mobile phase mixture reduced the interference from nickel (Fig. 4.2-b). However, the ZPD and ZDBC peaks had a very low column efficiency and very high tailing or fronting. The ZBEC peak on the other hand had a considerably high tailing even with high column efficiency (Table 4.2 and Fig. 4.3).
Fig. 4.2. Chromatograms of zinc pentamethylene dithiocarbamate (ZPD) by direct injection (a) without conditioning at 260 nm and (b) with the addition of 0.1% Zn (II) into the mobile phase mixture at 280 nm using 95% methanol-water at 1 ml/min, 40°C on Ultrasphere ODS column.
Chapter 4: Direct Injection and Derivatisation of Zinc Dithiocarbamates

Table 4.2
Column efficiencies ($N/m$) and peak asymmetry ($Sym\ [10\%]$) for separation of ZDTCs

<table>
<thead>
<tr>
<th>Compound</th>
<th>$N/m$</th>
<th>$Sym\ [10%]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZPD</td>
<td>6193</td>
<td>5.43</td>
</tr>
<tr>
<td>ZDBC</td>
<td>5843</td>
<td>0.27</td>
</tr>
<tr>
<td>ZBEC</td>
<td>13359</td>
<td>2.95</td>
</tr>
</tbody>
</table>

HPLC conditions: 95% methanol-water with 0.1% Zn (II) at 1 ml/min, 40°C, 280 nm on Ultrasphere ODS column and HPLC system 1 with JCL 6000 Chromatography Data System.

The same experiment was repeated to find the reason for the fronting of the ZDBC peak. As shown in Fig. 4.4, direct injection of ZDTCs with the addition of zinc (II) ions into the mobile phase was still tailing as before. However, the peak shape of ZDBC compound was not fronting anymore and the efficiency of the peak was about double the other peaks (Table 4.3). The fronting in the previous chromatogram of ZDBC (Fig. 4.3) was probably due to an overloaded sample.
Chapter 4: Direct Injection and Derivatisation of Zinc Dithiocarbamates

Fig. 4.4. Overlay chromatograms of ZPO, ZOBC and ZBEC by direct injection using 95% methanol-water with 0.1% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column and HPLC system 1 with Clarity Chromatography System.

Table 4.3
Column efficiencies (N/m) and peak asymmetry (Sym [10%]) for the separation of ZOTCs

<table>
<thead>
<tr>
<th>Compound</th>
<th>N/m</th>
<th>Sym [10%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZPD</td>
<td>7446</td>
<td>3.66</td>
</tr>
<tr>
<td>ZDBC</td>
<td>13260</td>
<td>2.53</td>
</tr>
<tr>
<td>ZBEC</td>
<td>7610</td>
<td>3.66</td>
</tr>
</tbody>
</table>

HPLC conditions: 95% methanol-water with 0.1% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column and HPLC system 1 with Clarity Chromatography System.

The separation of a mixture of ZPD, ZDBC and ZBEC using Zn (II) in the mobile phase furthermore found only three peaks (but ZDBC and ZBEC partially overlapped) (Fig. 4.5), whereas using nickel (II) in the mobile phase (on-column derivatisation technique) the mixture of these three compounds produced three extra peaks, which at the moment were assumed to be the mixed ligand complexes (Fig. 4.6). The separation of five ZOTCs (Fig. 4.7) was found to give the worst situation as ten extra peaks of mixed ligand complexes appeared. As a result, the quantitation of a ZDTC mixture would be very difficult.
Chapter 4: Direct Injection and Derivatisation of Zinc Dithiocarbamates

![Chromatogram of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) by direct injection using 95% methanol-water with 0.1% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.](image1)

**Fig. 4.5.** Chromatogram of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) by direct injection using 95% methanol-water with 0.1% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.

![Chromatogram of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) after on-column derivatisation to NiDTCs using 95% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column, where 1: Ni(L1)2, 2: NiL1L2, 3: NiL1L3, 4: Ni(L2)2, 5: Ni L1L3, 6: Ni(L3)2 which L1 is pentamethylene-, L2 is dibutyl- and L3 is dibenzyl-dithiocarbamates.](image2)

**Fig. 4.6.** Chromatogram of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) after on-column derivatisation to NiDTCs using 95% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column, where 1: Ni(L1)2, 2: NiL1L2, 3: NiL1L3, 4: Ni(L2)2, 5: Ni L1L3, 6: Ni(L3)2 which L1 is pentamethylene-, L2 is dibutyl- and L3 is dibenzyl-dithiocarbamates.
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Fig. 4.7. Chromatogram of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) after on-column derivatisation to their respective NiDTCs using 95% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column, where 1:Ni(L1)2, 2:NiL1L2, 3:NiL1L3, 4: Ni(L2)2, 5:Ni L2L3, 6:Ni(L3)2, 7:NiL1L4, 8:NiL2L4, 9:NiL1L5, 10:NiL3L4, 11:NiL2L5, 12:NiL3L5, 13:Ni(L4)2, 14:NiL4L5 and 15:Ni(L5)2 which L1 is dimethyl-, L2 is diethyl-, L3 is pentamethylene-, L4 is dibutyl- and L5 is dibenzyl-dithiocarbamates.

Therefore, it was concluded that the direct injection of zinc dithiocarbamates is possible with the addition of zinc cations into the mobile phase and would probably be a better method compared to the derivatisation method with nickel. However, the resolution of the peaks and the peak shapes need to be improved. Thus, the effect of pH of the mobile phase at a lower percentage of organic solvent was examined.

4.1.3. Effect of pH on the direct injection of zinc dithiocarbamates

Direct injection of zinc dithiocarbamates was not very efficient as these compounds are very unstable. It was also reported that pH has an important role in the stability of these compounds [21]. Therefore, a study on the effect of pH was carried out of the direct injection of zinc dithiocarbamates using three different conditions: acidic, neutral (no pH control) and basic. Because of the pH limitation of the Ultrasphere ODS column, a study at higher pH value (basic) was carried out using XBridge C8 column, a more pH stable column.
During the preparation of the mobile phase, the pH of the de-ionized water (7.34) was found to decrease when metal salts were added. The addition of a zinc salt was found to give a lower pH value (5.67) compared to the nickel salt (6.73). Adjusting the pH of the mobile phase that contained zinc salts to an acidic condition was not a problem. However, adjusting the pH to basic conditions caused difficulties as the addition of a base ended up with the mobile phase containing either a white precipitation or metal crystallization. The addition of 10mM sodium hydroxide to adjust the pH of the mobile phase to 12.00 produced a white jelly-like precipitate of zinc (II) hydroxide as equation 4.1 below [190]:

\[
2\text{NaOH}(aq) + \text{ZnSO}_4(aq) \rightarrow \text{Na}_2\text{SO}_4(aq) + \text{Zn(OH)}_2(s)
\]  

(4.1)

The original pH value of water with zinc salt was 5.67 and the precipitation appeared above pH 6.00. It was reported that adding an excess of sodium hydroxide would re-dissolved the zinc hydroxide precipitate [190]. However, we did not find that the precipitate re-dissolved even up to the pH 12.00. Addition of 35% ammonium hydroxide (ammonia solution) to adjust the pH of the same solution of water with zinc salts also produced a white precipitate of zinc (II) hydroxide at around pH 10.00. Adding more ammonia solution up to pH 11.00 then re-dissolved the precipitate. However, the precipitation re-appeared as the zinc solution was mixed with the methanol. The ammonia solution was then added again to adjust the pH back to 11.00 and the precipitate re-dissolved.

As shown in Fig. 4.8 and Fig. 4.9, both zinc dimethyldithiocarbamate (ZDMC) and zinc diethyldithiocarbamate (ZDEC) decomposed and produced extra peaks at pH 3.62. It confirmed the well known characteristic of dithiocarbamates, that they can be easily decomposed to amines and carbon disulfide by the presence of acid [21]. The same author also quoted that two reactions considered to be involved when acid is added to dithiocarbamate solutions:

\[
R_2\text{NCS}_2^- + H^+ \leftrightarrow R_2\text{NCS}_2H \quad \text{(fast)}
\]  

(4.2)

\[
R_2\text{NCS}_2H + H^+ \leftrightarrow R_2\text{NH}_2^+ + \text{CS}_2 \quad \text{(slow)}
\]  

(4.3)
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Fig. 4.8. Chromatogram of ZDMC by direct injection using 75% methanol-water with 0.01% Zn (II) at pH 3.62 at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.

Fig. 4.9. Chromatogram of ZDEC by direct injection using 75% methanol-water with 0.01% Zn (II) at pH 3.62, 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.
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A small stepwise increase in pKₐ values of the dialkyldithiocarbamic acids was also observed as the size of the alkyl group increased (Table 4.4).

<table>
<thead>
<tr>
<th>Alkyl group (R)</th>
<th>pKₐ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>3.66</td>
</tr>
<tr>
<td>Ethyl</td>
<td>4.04</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>4.79</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>5.19</td>
</tr>
</tbody>
</table>

Table 4.4: pKₐ values of different dialkyldithiocarbamic acids [21]

Zuman and Zahradnik [191] on the other hand explained the acid decomposition of dithiocarbamate by a proton addition according to the following equilibrium reactions:

\[
R_2NCS_2^- + H^+ \rightleftharpoons R_2NCS_2H \quad (4.4)
\]

\[
R_2NCS_2H \rightleftharpoons R_2NH + CS_2 + H^+ \quad (4.5)
\]

\[
R_2NH + CS_2 + H^+ \rightleftharpoons R_2NH_2^+ + CS_2 \quad (4.6)
\]

Structure \( R_2NCS_2H \) is considered to be the reactive form of the dithiocarbamic acid in acid solution. This is then split into the amine and \( CS_2 \) with reaction rate constant \( k \) as in equation 4.5. The equilibrium C is shifted towards protonated amine so that \( k_z \) (the reaction rate of reversed reaction as in equation 4.5) is insignificant. They also explained that at a pH value which is the same or less than the pKₐ value of the equilibrium A (4.4), then the irreversible decomposition of the dithiocarbamic acid will occur. The pH value for acidic separation was 3.62, which was less than the pKₐ values for both dimethyl- and diethyl-dithiocarbamic acids (Table 4.4), thus the decomposition of the dithiocarbamic acids become irreversible and produced the peaks as shown in Fig. 4.8 and Fig. 4.9.

Using the original mixture of 75% methanol-water with 0.01% Zn (II) without any pH alteration (pH 5.67), both compounds became more stable. This can be seen from the Fig. 4.10-a and Fig. 4.11-a where the extra peaks produced for both ZDMC and ZDEC compounds are reduced. Zuman and
Zahradnik [191] again explained that "at pH values equal to or greater than the pKₐ of the amine, the equilibrium C (4.6) is shifted towards the free base R₂NH. This form, with a lone pair of electrons on the nitrogen, reacts with the Lewis acid CS₂ to give the condensation reaction products and the corresponding reaction rate k₂. The equilibrium A is shifted towards the anion R₂NCS₂⁻ so that the reverse reaction with rate constant k is insignificant.” However, as the pKₐ value of the amine is higher than the pH value used in this condition, then the equilibria as in equation 4.4, 4.5 and 4.6 still exists.

![Chromatograms of ZDMC](image-url)

Fig. 4.10. Chromatograms of ZDMC by direct injection using 75% methanol-water with 0.01% Zn (II) at (a) pH 5.67 and (b) pH 6.00 at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.
The same experiment was repeated using new distilled water with pH 6.00 after adding the same amount of zinc salt. As shown in Fig. 4.10-b and Fig. 4.11-b, the peak of ZDMC becomes better but ZDEC retention became shorter. This study again demonstrates that a little difference in pH values make a relatively big changes in the stability of zinc dithiocarbamate complexes.

Separation of a ZPD, ZDBC and ZBEC mixture (Fig. 4.12) using a mobile
phase at acidic condition again showed the same problem as for ZDMC and ZDEC (Fig. 4.8 and Fig. 4.9) before. On the other hand, the separation of mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) using mobile phase without alteration in the pH value (Fig. 4.13-a) produced only the original peaks of the ZDTC mixture. The ZBEC compound however was not detected in both studies. However, the same experiment made while the pH of water with zinc salts was 6.00 showed a worse peak shape. This is probably because of the precipitation of zinc hydroxide as equation 4.1 which was previously found to happen above pH 6.00. Thus, direct injection of a mixture of zinc dithiocarbamates at this condition is possible as long as the pH of the eluent stays below 6.00 and above their pKₐ values. Direct injection for an individual compound, however, is still not practical. Again, as shown in Fig. 4.14, the ZDTC compounds were not sufficiently stable to be injected directly without conditioning or without adding zinc salts into the mobile phase as the pH value of 7.34 was higher than the previous separation.

Fig. 4.12. Chromatogram of a mixture of three ZOTCs (ZPO, ZOBe and ZBEC) by direct injection using 75% methanol-water with 0.01% Zn (II) at pH 3.62, 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column. No peak of ZBEC was detected.
Fig. 4.13. Chromatograms of a mixture of five ZOTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) by direct injection using 75% methanol-water with 0.01% Zn (II) at (a) pH 5.67 and (b) pH 6.00 at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column. No peak of ZBEC was detected in both conditions studied.
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Fig. 4.14. Chromatogram of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) by direct injection without conditioning using 75% methanol-water (pH 7.34) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.

The separation of the ZDTC mixture by direct injection on XBridge C8 column at pH 11.00 (Fig. 4.15) once more showed the instability of these compounds. Therefore, it can be concluded that even at higher pH value, the separation of ZDTC mixture by direct injection was not efficient. Thus, the derivatisation of this compound to a more stable complex will probably be the best method.

Fig. 4.15. Chromatogram of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) by direct injection using 75% methanol-water with 0.01% Zn (II) at pH 11.00, 1 ml/min, RT and 260 nm on XBridge C8 column.
4.2. Derivatisation of zinc dithiocarbamates with metal ions

Derivatisation of zinc dithiocarbamates was carried out using three different methods; (1.) Pre-column derivatisation method, (2.) In-line derivatisation method and (3.) On-column derivatisation method. Both in-line and on-column derivatisation methods were carried out in the HPLC system.

All the three methods go through a derivatisation step, which is actually a metal exchange reaction between metal ions and their dithiocarbamate complexes. As reported by Sachinidis and Grant [179], two different routes for the reaction were detected, which comprise dissociation of dithiocarbamate ligand from the metal complex followed by substitution at the new metal ion, and/or direct electrophilic attack by the metal ion on the dithiocarbamate complex. Derivatisation of zinc dithiocarbamates, however, must use a metal which give a more stable complex for the reaction to take place. As discussed in the previous chapter under section 3.3.1, it was decided to use nickel for the metal exchange reaction (derivatisation). Therefore, nickel salts were used for all derivatisation methods.

4.2.1. Pre-column derivatisation method

4.2.1.1. Comparison of five different columns

In order to carry out the separation of ZDTCs using high temperature to increase efficiency and the rate of exchange, a thermally stable column is needed. The column that has been used in the most of the previous studies (Ultrasphere ODS column) is not stable at higher temperatures, thus there is a need to find another thermally stable column in order to further this study.

Comparison of five different columns (Ultrasphere ODS, XTerra ODS, Zorbax Cyano, Zr-PBD and Gemini ODS) was made by comparing the chromatographic characteristics of the individual ZPD, ZDBC and ZBEC after pre-column derivatisation to their respective nickel complexes. Fig. 4.16 shows the comparison of the retention factors for nickel
pentamethylenedithiocarbamate (NiPD), nickel dibutylthiocarbamate (NiDBC) and nickel dibenzylthiocarbamate (NiBEC) for the different columns. It was also found that the peaks were unresolved for Zorbax Cyano, while the Zr-PBD columns gave different peak order compared to Ultrasphere ODS column.

Amongst the columns studied, Zorbax Cyano was the shortest chain bonded phase. Thus, as expected, the compounds eluted faster than the others. A lower percentage of organic solvent would probably resolve the compounds, and therefore reduce the amount of solvent used. Zorbax Cyano columns, however, was not as stable as other columns (Zr-PBD, XTerra ODS and Gemini ODS) at extreme pH and high temperature. Therefore, no further studies were carried out using Zorbax Cyano column. Zr-PBD on the other hand had a different selectivity for the pentamethylenedithiocarbamate complexes, and thus gave different peak order compared to the ODS columns. This agrees with the reported statement that Zr-PBD column may give different selectivity for some compound [189].

![Fig. 4.16. Comparison of retention factors (k) of NiPD, NiDBC and NiBEC on different type of columns (Beckman Ultrasphere ODS - 4.6 x 250 mm, Zorbax Cyano - 4.6 x 250 mm, ZirChrom-polybutadiene - 4.6 x 150 mm, Gemini C18 - 2.0 x 150 mm) using 95% methanol-water at 1 ml/min, 10 μl injection, 40°C and 330 nm. Gemini ODS column used 0.2 ml/min and 2 μl injection.](image)
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Fig. 4.17. Chromatograms of ZDTCs after pre-column derivatisation to (a) NiPD, (b) NiDBC and (c) NiBEC on XTerra ODS column using 95% methanol-water at 1 ml/min, 40°C and 330 nm.
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On the other hand, very small distorted peaks were detected on XTerra ODS column for the nickel dithiocarbamates (Fig. 4.17). A few attempts were tried on this particular column but the same chromatograms were found for all of the three compounds studied. A few authors [23, 149, 192] acknowledge that some metal dithiocarbamate complexes are more stable than the others. Thus, more stable metals present in the chromatographic system maybe are able to chelate with the dithiocarbamate ligands via a ligand exchange reaction.

Chelators have also been used to check for the residual metals present in the column packing [193, 194]. Chelator peaks that tailed or irreversibly absorb onto the column packing indicated the present of residual metals, and this is probably the reason for what was happening in the XTerra ODS column. However, the column might have contaminated or deteriorated, which could give the same results. Therefore, no other quantitative results are reported for this column. The Gemini ODS column used in this study was a narrow bore column. Therefore, a lower flow rate and injection volume was used, and it was able to separate the three compounds with similar retention factors to the Ultrasphere ODS column. Comparing the chromatographic characteristics of NiPD, NiDBC and NiBEC (Table 4.5) showed that the Ultrasphere ODS column was the preferred column with the highest column efficiencies, the best peak shapes and the highest resolution values. An example of a chromatogram for the separation of a mixture of NiDTCs on the Ultrasphere ODS column is shown in Fig. 4.18.

As shown in Fig. 4.16 before, a mixture of NiDTCs was unresolved on Zorbax Cyano column (Fig. 4.19-a). The major contribution for the low resolution values on this particular column was the retention factors at the condition used. Using lower percentage of organic modifiers in the mobile phase mixture would increase the retention and thus the resolution. However, the tailing effect was quite high, which was possibly caused by the same reasons as in XTerra ODS column. A closer look at one of the compounds showed the tailing effect, which probably caused by the shoulder found after the main peak (Fig. 4.19-b).
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Table 4.5
Retention factors \((k)\), efficiency \((N)\), tailing effect \((\text{Sym }[10\%])\), selectivity \((\alpha)\) and resolution \((R)\) of NiDTCs on four different columns

<table>
<thead>
<tr>
<th>Compound</th>
<th>Column</th>
<th>(k)</th>
<th>(N)</th>
<th>(\text{Sym }[10%])</th>
<th>(\alpha_{1,2})</th>
<th>(\alpha_{2,3})</th>
<th>(R_{1,2})</th>
<th>(R_{2,3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiPD</td>
<td>Ultrasphere ODS</td>
<td>0.53</td>
<td>8828</td>
<td>1.26</td>
<td>3.80</td>
<td>1.46</td>
<td>16.79</td>
<td>7.10</td>
</tr>
<tr>
<td></td>
<td>Zorbax Cyano</td>
<td>0.15</td>
<td>3821</td>
<td>2.08</td>
<td>4.82</td>
<td>1.70</td>
<td>0.81</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Zr-PBD</td>
<td>0.49</td>
<td>238</td>
<td>3.75</td>
<td>3.08</td>
<td>4.35</td>
<td>1.05</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>Gemini ODS*</td>
<td>0.35</td>
<td>976</td>
<td>1.79</td>
<td>4.17</td>
<td>1.60</td>
<td>5.81</td>
<td>3.98</td>
</tr>
<tr>
<td>NiDBC</td>
<td>Ultrasphere ODS</td>
<td>2.01</td>
<td>11616</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zorbax Cyano</td>
<td>0.02</td>
<td>2601</td>
<td>1.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zr-PBD</td>
<td>0.16</td>
<td>354</td>
<td>3.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemini ODS*</td>
<td>1.47</td>
<td>2192</td>
<td>1.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NiBEC</td>
<td>Ultrasphere ODS</td>
<td>2.92</td>
<td>11473</td>
<td>0.96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zorbax Cyano</td>
<td>0.09</td>
<td>2339</td>
<td>1.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zr-PBD</td>
<td>2.13</td>
<td>169</td>
<td>3.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemini ODS*</td>
<td>2.35</td>
<td>3313</td>
<td>1.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HPLC conditions: 95% methanol-water at 1 ml/min, 10 μl injection, 40°C and 330 nm where 1, 2 and 3 are peak no. in order of elution.

*This column was used at flow rate of 0.2 ml/min and 2 μl injection.

Fig. 4.18. Chromatogram of a mixture of three NiDTCs (NiPD, NiDBC and NiBEC) on Ultrasphere ODS column using 95% methanol-water at 1ml/min, 40°C and 330 nm.
As discussed before (section 4.1), Zr-PBD column was not a suitable column for this study as the dithiocarbamate ligands might have chelated to the surface zirconia sites. Therefore, no further discussion is made.

The separation of pre-column derivatised ZDTCs as their respective nickel complexes on Gemini ODS column was found to give the nearest chromatographic characteristics to the Ultrasphere ODS column (Fig. 4.18 and Fig. 4.20). The peaks however had low column efficiencies and higher
peak tailing. As the internal diameter of this column is very small, a lower injection volume would likely give higher efficiencies. A narrow bore column is also very sensitive to the dead volumes, and thus, minimizing the dead volume will also improve the column efficiency and peak shape. Yet, it was decided that Gemini ODS column or similar thermally stable hybrid column would be used for a further study of the ZDTC complexes at higher temperatures (section 5.4 and 5.5) because it gave the nearest chromatographic characteristics to the Ultrasphere ODS column. A narrow bore column like Gemini ODS will also save the amount of solvent and sample used as lower flow rate and small sample injection can be used.

![Overlay chromatograms of ZDTCs (ZPD, ZDBC and ZBEC) after pre-column derivatisation to NiDTCs on Gemini ODS column using 95% methanol-water at 0.2 ml/min, 40°C and 330 nm.](image)

An XBridge C8 column was also chosen for further studies (section 4.2.3.2) as it is another thermally stable column with a short alkyl bonded chain.

### 4.2.1.2. Stability study of derivatisation of ZDTCs to NiDTCs

During comparison of the columns using pre-column derivatisation, it was found that the standard solutions of derivatised ZDTCs (i.e. NiDTCs) either
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oxidized or decomposed with time. It was also found that the NiPD solutions became cloudy and finally produced a dark green precipitate or crystals. Therefore, it was of interest to study the stability of the nickel dithiocarbamate compounds with time. The chromatographic characteristics of each compound were evaluated for 8 days up to the disappearance of the solution colour.

During this study, the system back pressure of Ultrasphere ODS column was found to increase with time, and as the back pressure increased, the retention factors also increased. Among the three compounds studied, NiBEC was found to have the highest retention change. The tailing of each compound were also found to increase.

However, it did not affect the efficiency of all the compounds as much. The peak heights also showed no significant change except for the NiPD. The peak areas on the other hand, drastically reduced on the fifth day for all complexes. As NiPD was found to be the most unstable compound, a more detailed stability study was also carried out for this particular compound over 5 days. It was concluded that NiPD was not a sufficiently stable compound as the peak height and area already reduced significantly after day 1. However, as the stock solutions used in this study were already more than one month old, the same study was repeated using freshly prepared stock solutions, and as shown in Fig. 4.21, pre-derivatised NiPD could be considered stable even up to five days after it was first prepared.

As the pre-column derivatisation technique is time consuming and used a lot more solvent during the liquid-liquid extraction, simpler methods other than direct injection of zinc dithiocarbamates were investigated. They were in-line and on-column derivatisation methods, where both methods used a technique of adding metal salts into the HPLC system as discussed in next sections. Both methods were also compared to the pre-column derivatisation method under the same HPLC conditions.
4.2.2. In-line derivatisation method

In-line derivatisation of zinc dithiocarbamates to other metal complexes was tried using ZDBC with pre-injections of an aqueous solution of Ni (II) 1 min before zinc complex. This method gave much better efficiency and peak shape compared to the direct injection of ZDBC. However, a test made to replicate the in-line derivatisation reaction in the test tube (1 part ZDBC in methanol: 1 part NiSO$_4$ in water) found that the solution became cloudy which probably means that the NiDBC produced was not soluble in the final solution of 50% methanol-water. On the other hand, a clear solution was produced when the same test was carried out using NiSO$_4$ solution dissolved in methanol. Yet, the solution became cloudy again if the amount of water was increased in the solution. Therefore, the separation of this compound was tolerable with 95% methanol-water used in some of the studies, but it may have some problems if lower percentages of organic solvent are used. This problem, however, might be solved if a higher temperature is used.

Other metals like Co (II) and Cu (II) were also studied for the in-line derivatisation of ZDBC. Direct injection of Zn (II) DTCs complexes has already shown that they were not stable even with the addition of zinc (II)
ions, which were meant to reduce the interference from other metals. As discussed in section 3.1, Co (II) was oxidized to the more stable Co (III) complexes, when Co (II) ions react with dithiocarbamates. The appearance of two extra peaks for the mixture of two cobalt complexes indicates that oxidation of the central metal ion occurs with formation of 1:3 complexes [55]. Other authors have also reported the same observation [23, 181, 195]. However, as discussed in section 3.3.1, the formation of Co (III) complexes was slower than Ni (II) complexes. A recent report by Abraham and colleagues [25] also showed that complexation of cobalt (II) chloride with dithiocarbamates was slow compared to copper (II) sulfate. It was suggested that it could be attributed to a slower substitution rate of cobalt (II) cations compared with that of copper (II) cations. Our study of in-line derivatisation of ZDBC to CoDBC and NiDBC found that the dibutyl dithiocarbamates have been partially converted to the other metal complexes. However, a repeat injection for in-line derivatisation of these two metal complexes was found to give higher peak height, column efficiencies and better peak shapes. For example, the in-line derivatisation of ZDBC to NiDBC showed that repeat injection gave about 26% higher peak height, 14% higher column efficiency and about 40% less tailing than the first injection. These phenomena suggested that a higher concentration of metal ions in the HPLC system might give better results. A study of in-line derivatisation of ZDBC to CuDBC on the other hand was found to give the best results in terms of column efficiency and peak shape. However, CuDBC was also found to give two peaks (Fig. 4.22) which might be the results of an equilibrium of two forms of CuDBC as suggested by some authors referred by Thorn and Ludwig [21] as equation 4.7 below:

\[ CuR_2 + Cu^{2+} \leftrightarrow 2CuR^+ \]  

(4.7)

where R is the dithiocarbamate ligand.
Although Cu (II) complexes were found to give the best column efficiency and peak shape, the problems of the presence of two forms in equilibrium suggested that Ni (II) might be the best metal to be used for in-line derivatisation. Furthermore, Ni (II) complexes were also found to have the highest peak height and peak area amongst the metals studied using the same concentration of ZDBC. Ni (II) would also be a suitable metal for complex formation as it has only a single oxidation state of +2 in dithiocarbamate complexes [175, 192, 195, 196].

After a few trials of in-line derivatisation of ZDBC to NiDBC, it was found that in-line derivatisation was possible as long as the concentrations of Ni (II) ions were high enough to protect from the effect of the other metals. Therefore, a study of an effect of metal concentration was carried out and reported in the next section.

4.2.2.1. Comparison between pre-column and in-line derivatisation methods

After cleaning the column to remove absorbed metals by washing with mobile phase containing EDTA, a comparison of in-line and pre-column
derivatisation of ZDBC to NiDBC has been carried out (Table 4.6).

Table 4.6
Comparison of peak height and peak area of in-line and pre-column derivatisation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Peak height (mAU)</th>
<th>Peak area (mAU s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-line derivatisation*</td>
<td>6461</td>
<td>71778</td>
</tr>
<tr>
<td>Pre-column derivatisation**</td>
<td>7469</td>
<td>83654</td>
</tr>
</tbody>
</table>

*HPLC conditions: 0.5 μg ZDBC to NiDBC using 5 μg Ni (II) using 95% methanol-water at 1 ml/min, 40°C, 330 nm on Ultrasphere ODS column.

**As per in-line derivatisation with prior complex formation.

From Table 4.6, it can be concluded that even at a 10 times concentration of Ni (II) compared to the concentration of analytes, in-line derivatisation still did not give 100% conversion of ZDBC to NiDBC. In-line derivatisation under these conditions was found to produce about 13.5% and 14.2% lower peak height and area respectively compared to the pre-column derivatisation. Therefore, a study of the effect of metal concentration was carried out for the in-line derivatisation of ZDBC to NiDBC using Ultrasphere ODS column.

Two series of metal concentration were studied, which one ranged from 1.25 to 10 μg and the other from 10 to 80 μg of Ni (II). From the first series (Fig. 4.23), it was found that at the lowest concentration studied (1.25 μg – Fig. 4.23-a), two broad extra peaks appeared which was probably caused by the co-elution of the interfering compounds that reacted with the dithiocarbamates. It might also be because of the incomplete reaction between Ni²⁺ and ZDTC as Sachinidis and Grant [179] reported that when ZDTC was in excess over Ni²⁺, the stoichiometry of the reaction was as equation 4.8 below:

\[ \text{Ni}^{2+} + 2\text{Zn(DTC)}_2 \rightarrow 2\text{Zn(DTC)}^+ + \text{Ni(DTC)}_2 \]  

Therefore, two extra peaks of ZDTC and its mono complex appeared. These two extra peaks, however, largely disappeared as a higher concentration of Ni (II) was injected. It was also found that the higher the metal concentration, the better the peak shape and the higher the peak height with about the same efficiency (Fig. 4.24).
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Fig. 4.23. Chromatograms of ZDBC after in-line derivatisation to NiDBC at different masses of Ni (II): (a) 1.25 µg, (b) 2.5 µg, (c) 5 µg and (d) 10 µg using 95% methanol-water at 1ml/min, 40°C and 330 nm on Ultrasphere ODS column.
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In the second series of metal concentration studied, an extra peak of an interfering compound re-appeared at 20 μg, which then also affected the peak height as well as the separation efficiency. Both the peak height and efficiency however went up again for the higher concentration of Ni (II) (Fig. 4.25).
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The peak height was highest at 80 µg of Ni (II) injected. However, even at this concentration of metal, the peak height and peak area of NiDBC were only 70% and 95% respectively compared to the pre-column derivatisation method. Therefore, a study on the effect of higher concentration of nickel ions by the addition of nickel salt into the mobile phase mixture was carried out. The method was called on-column derivatisation as reported in the next section.

Although ZDBC was not fully derivatised to its respective nickel complexes, the response of ZDBC as its NiDBC complex using in-line derivatisation was found to be linear (Fig. 4.26).

4.2.3. On-column derivatisation method

As the in-line derivatisation was incomplete, it was of interest to try the other technique termed as on-column derivatisation. As discussed in sections 3.3.1 and 4.2.2, nickel (II) was found to be the most suitable metal amongst the four metals (Zn, Co, Ni and Cu) studied. Therefore, it was decided to add nickel salt into the mobile phase mixture for the on-column derivatisation
method. An initial study for the on-column derivatisation method to nickel (II) complexes was carried out, that compare the current method with the pre-column derivatisation method. A brief study of on-column derivatisation to copper (II) complexes was also explored as a comparison.

4.2.3.1. On-column derivatisation to nickel (II) complexes

In the chromatograms of a mixture for ZPD, ZDBC and ZBEC after on-column derivatisation to their nickel complexes (Fig. 4.27), three extra peaks were observed. A similar observation was reported by Liska and co-workers [51] for their separation of mixture of nickel (II) bis-dialkyldithiocarbamate complexes using normal phase HPLC. They suggested that this systematic occurrence of extra peaks mean that equilibration readily take place between the original symmetrical complexes, and suggested the following equation for the mixture of two complexes:

\[ L_1 - Ni - L_1 + L_2 - Ni - L_2 \leftrightarrow 2L_1 - Ni - L_2 \] (4.9)

Other authors [23, 55, 195] have also reported the same occurrence of mixed ligand formation. In another paper, Liska et al. [52] further verified their earlier assumption that this third compound was a mixed ligand complex by two-dimensional thin-layer chromatography, determination of molecular weight and elemental analysis. However, they also discovered that these complexes were not stable after isolation and re-equilibrate immediately in chloroform solution, generating the ternary equilibrium mixture of the mixed-ligand complex and the two original symmetrical complexes. In the determination of organic substances by metal chelate derivatisation, Moriyasu et al. [55] suggested that the appearance of the mixed-ligand complexes as in equation 4.9 is because it is stable enough to be eluted without being subjected to disproportionation during the course of chromatographic run. As confirmation, the separation of NiDTCs complexes by the on-column derivatisation of ZDTCs mixture was repeatable with identical chromatograms (Fig. 4.27).
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Fig. 4.27. Chromatogram of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) after on-column derivatisation to NiDTCs using 95% methanol-water with 0.01% Ni (II) at 1ml/min, 40°C and 330 nm on Ultrasphere ODS column. Peak 1 is Ni(L)$_2$ (NiPD), 2 is NiL-L$_2$, 3 is NiL$_3$, 4 is Ni(L)$_2$L$_2$ (NiDBC), 5 is NiL$_2$L$_3$ and 6 is Ni(L)$_3$L$_2$ (NiBEC), where L$_1$ is pentamethylene-dithiocarbamate (PD), L$_2$ is dibutyl-dithiocarbamate (DBC), and L$_3$ is dibenzyldithiocarbamate (BEC).

Separation of the same NiDTCs mixture by a different technique was also carried out by first pre-column derivatisation of the individual ZDTCs complexes to their respective nickel complexes, and then, mixing them together just before the injection. By this technique, a different phenomenon was found, where only the original complexes were initially discovered for the first injection, and then on re-injections (at about twenty minutes apart), the three extra peaks appeared and continuously increased in the peak height (Fig. 4.28). Thus, the equilibration was slow and the peaks of mixed ligand complexes were small compared to the same peaks using earlier technique. This is probably because the nickel complexes were possibly stable enough not to go for the forward reaction in the first instant but finally would go to equilibrium as in equation 4.9, whereas the zinc complexes readily broke down and in the presence of Ni$^{2+}$ a random derivatisation occurred. The same phenomenon was also reported by Moriyasu and Hashimoto [197], where they found that the equilibrium was achieved after 60 minutes of mixing.
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Fig. 4.28. Chromatograms of a mixture of three NiDTCs (NiPD, NiDBC and NiBEC) for three different injection after pre-column derivatisation using 95% methanol-water at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column.
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The differences found in here also confirm that nickel complexes are more stable than zinc [21, 149, 179]. Moreover, the conversion of ZDTCs to NiDTCs was so quick, no disproportionation of the mixed ligand complexes was found.

Using the same zinc dithiocarbamate mixture, and then derivatised either pre- or on-column to nickel complexes, both method were compared. As shown in Fig. 4.29 and Table 4.7, the peak heights and peak areas of the on-column derivatisation method were lower compared to the pre-column derivatisation method. The efficiencies using on-column derivatisation were also found about one third lower than the pre-column derivatisation. However, the on-column derivatisation with addition of metal salt into the mobile phase increased the values of calculated retention factor for all the nickel complexes, although the retention times were very similar. This was due to the changes in the retention of uracil used as marker for the dead volume (discussed further in section 4.3) as well as a reduction in the silanol effect for uracil as discussed in section 3.3.2.1.

On the other hand, the differences in the peak heights were found to decrease with increasing retention. A possible reason of lower peak heights, peak areas as well as column efficiencies for the on-column derivatisation was probably the difference in the solvent used for the preparation of the mixture. In this particular study, the final mixture for pre-column derivatisation was in 50% dichloromethane-methanol, whereas the mixture for on-column derivatisation was in 100% dichloromethane. The use of 100% DCM would have broadened the peaks as DCM is a stronger eluent than the mobile phase mixture.
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Fig. 4.29. Chromatograms of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) after (a) pre-column and (b) on-column derivatisation to NiDTCs using (a) 95% methanol-water and (b) 95% methanol-water with 0.01% Ni(II) at 1mL/min, 40°C and 330 nm on Ultrasphere ODS column. Peak identifications are as in Fig. 4.27.
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Table 4.7
Comparison of peak areas, peak heights, retention factors ($k$) and efficiencies ($N$) between pre- and on-column derivatisation methods

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>$t_R$ (min)</th>
<th>Peak area (mAU.s)</th>
<th>Peak height (mAU)</th>
<th>$k$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-column derivatisation ($t_o = 2.88$ min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.67</td>
<td>58612</td>
<td>7899</td>
<td>0.27</td>
<td>7277</td>
</tr>
<tr>
<td>2</td>
<td>4.97</td>
<td>83772</td>
<td>9829</td>
<td>0.72</td>
<td>8778</td>
</tr>
<tr>
<td>3</td>
<td>5.58</td>
<td>70658</td>
<td>7232</td>
<td>0.94</td>
<td>8678</td>
</tr>
<tr>
<td>4</td>
<td>7.40</td>
<td>32948</td>
<td>2915</td>
<td>1.57</td>
<td>10717</td>
</tr>
<tr>
<td>5</td>
<td>8.45</td>
<td>54380</td>
<td>4213</td>
<td>1.93</td>
<td>10711</td>
</tr>
<tr>
<td>6</td>
<td>9.72</td>
<td>23766</td>
<td>1635</td>
<td>2.38</td>
<td>10985</td>
</tr>
<tr>
<td>On-column derivatisation ($t_o = 2.60$ min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.65</td>
<td>51743</td>
<td>5615</td>
<td>0.40</td>
<td>4070</td>
</tr>
<tr>
<td>2</td>
<td>4.96</td>
<td>79512</td>
<td>7520</td>
<td>0.91</td>
<td>5426</td>
</tr>
<tr>
<td>3</td>
<td>5.58</td>
<td>75025</td>
<td>6123</td>
<td>1.14</td>
<td>5319</td>
</tr>
<tr>
<td>4</td>
<td>7.42</td>
<td>32717</td>
<td>2421</td>
<td>1.86</td>
<td>7406</td>
</tr>
<tr>
<td>5</td>
<td>8.49</td>
<td>59269</td>
<td>3843</td>
<td>2.26</td>
<td>7483</td>
</tr>
<tr>
<td>6</td>
<td>9.79</td>
<td>26263</td>
<td>1557</td>
<td>2.76</td>
<td>7984</td>
</tr>
</tbody>
</table>

HPLC conditions: 95% methanol-water (pre-column) and 95% methanol-water with 0.01% Ni (II) (on-column) at 1ml/min, 40°C and 330 nm on Ultrasphere ODS column. Peak identifications are as in Fig. 4.27.

The same study of a comparison between pre- and on-column derivatisation was also carried out at 90% methanol-water, and the final mixtures for both methods were in the same solvent mixture of 50% dichloromethane-methanol. As shown in Fig. 4.30 and Table 4.8, it was obvious that the on-column derivatisation technique was better compared to the pre-column derivatisation as it gave higher peak heights and peak areas with about similar values for other parameters (except retention factors, which would be effected by dead volume).
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Fig. 4.30. Chromatograms of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) after (a) pre-column and (b) on-column derivatisation to NiDTCs using (a) 90% methanol-water and (b) 90% methanol-water with 0.01% Ni(II) at 1ml/min, 40°C and 330 nm on Ultrasphere ODS column. Peak identifications are as in Fig. 4.27.
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Table 4.8
Comparison of retention times ($t_R$), peak areas, peak heights, retention factors ($k$) and efficiencies ($N$) between pre- and on-column derivatisation methods

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>$t_R$ (min)</th>
<th>Peak area (mAU.s)</th>
<th>Peak height (mAU)</th>
<th>$k$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-column derivatisation ($t_0 = 3.61$ min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.39</td>
<td>982</td>
<td>122</td>
<td>0.21</td>
<td>7844</td>
</tr>
<tr>
<td>2</td>
<td>7.36</td>
<td>1319</td>
<td>112</td>
<td>1.04</td>
<td>10242</td>
</tr>
<tr>
<td>3</td>
<td>8.79</td>
<td>1015</td>
<td>74</td>
<td>1.43</td>
<td>10576</td>
</tr>
<tr>
<td>4</td>
<td>14.33</td>
<td>443</td>
<td>21</td>
<td>2.97</td>
<td>12358</td>
</tr>
<tr>
<td>5</td>
<td>17.43</td>
<td>679</td>
<td>28</td>
<td>3.82</td>
<td>12748</td>
</tr>
<tr>
<td>6</td>
<td>21.38</td>
<td>269</td>
<td>9</td>
<td>4.92</td>
<td>13695</td>
</tr>
<tr>
<td>On-column derivatisation ($t_0 = 2.44$ min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.41</td>
<td>1681</td>
<td>197</td>
<td>0.81</td>
<td>7638</td>
</tr>
<tr>
<td>2</td>
<td>7.46</td>
<td>2358</td>
<td>196</td>
<td>2.06</td>
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</tr>
<tr>
<td>3</td>
<td>8.92</td>
<td>2008</td>
<td>144</td>
<td>2.66</td>
<td>10328</td>
</tr>
<tr>
<td>4</td>
<td>14.66</td>
<td>933</td>
<td>45</td>
<td>5.02</td>
<td>12746</td>
</tr>
<tr>
<td>5</td>
<td>17.86</td>
<td>1479</td>
<td>59</td>
<td>6.33</td>
<td>12830</td>
</tr>
<tr>
<td>6</td>
<td>21.94</td>
<td>640</td>
<td>22</td>
<td>8.00</td>
<td>13432</td>
</tr>
</tbody>
</table>

HPLC conditions: 90% methanol-water (pre-column) and 90% methanol-water with 0.01% Ni (II) (on-column) at 1ml/min, 40°C and 330 nm on Ultrasphere ODS column. Peak identifications are as in Fig. 4.27.

This was probably because the on-column derivatisation method used the mobile phase with a constant amount of added nickel throughout the separation, where the effect from other interfering metals perhaps has been reduced. The differences between peak heights and peak areas for both methods were also found to be more apparent for the later complexes.

The addition of nickel salt into the mobile phase mixture not only affects the analytes but also the uracil used for calculating the dead volume. As shown in Fig. 4.31 and Table 4.9, the addition of nickel salt has reduced the retention as well as the tailing of the uracil, which then reduced the peak width, increased the peak height and subsequently increased the efficiency.

The presence of metal ions in the mobile phase has probably changed the chemistry of uracil as shown by Ghosh and co-workers [198] who reported that uracil can actually chelate with the divalent metal ions like Ni (II) acting like a bidentate ligand. Further studies on the effect of metals on the void volume marker (uracil) were carried out and are discussed as in section 4.3.
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Table 4.9
Chromatographic values of uracil using different mobile phase mixture

<table>
<thead>
<tr>
<th>Mobile phase mixture</th>
<th>$t_R$ (min)</th>
<th>Peak area (mAU.s)</th>
<th>Peak height (mAU)</th>
<th>$w_h$ (min)</th>
<th>Sym [50%]</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% methanol-water</td>
<td>3.61</td>
<td>871</td>
<td>20</td>
<td>0.61</td>
<td>7.69</td>
<td>194</td>
</tr>
<tr>
<td>90% methanol-water with 0.01% Ni (II)</td>
<td>2.44</td>
<td>900</td>
<td>119</td>
<td>0.12</td>
<td>1.41</td>
<td>2417</td>
</tr>
</tbody>
</table>

HPLC conditions: 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column.

4.2.3.2. On-column derivatisation to copper (II) complexes

The separation of individual compound of ZPD after on-column derivatisation to CuPD produces an extra peak of probably its derivative or equilibrium complex (Fig. 4.32-a). The very non-polar compounds like ZDBC and ZBEC on the other hand produced only one peak with a very broad shape (Fig. 4.32-b&c), and if injected together were unresolved (Fig. 4.33). It superficially seems that on-column derivatisation of zinc dithiocarbamates to copper dithiocarbamates might be better than to nickel dithiocarbamates as no mixed ligand complexes were produced.
Fig. 4.32. Chromatograms of (a) ZPD, (b) ZDBC and (c) ZBEC after on-column derivatisation to CuDTCs using 70% methanol-water with 0.01% Cu (II) at 1 ml/min, 40°C, 435 nm on XBridge C8 column.
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Fig. 4.33. Chromatograms of a mixture of three ZDTCs (ZPD, ZDBC and ZBEC) after on-column derivatisation to CuDTCs using 70% methanol-water with 0.01% Cu (II) at 1 ml/min, 40°C, 435 nm on XBridge C8 column.

However, the peaks of CuDBC and CuBEC had very low column efficiencies and produced very broad peak shapes. The same observation was found for ZDMC, ZDEC and mixture of 5 dithiocarbamates. Although the on-column derivatisation of ZDTCs to CuDTCs is possible, the CuSO₄ salt was difficult to dissolve in either methanol-water or acetone-water mixture. Therefore, if this method is to be used, it might give a problem later on as copper salts might precipitate in the system. On-column derivatisation of ZDTCs to CuDTCs might be more difficult or problematic because copper (II) dithiocarbamate salts are less water soluble compared to nickel (II) or the original zinc (II) dithiocarbamate salts [21]. The column performance after studying this method was also found to be very low and produced a higher back pressure, suggesting precipitation might have caused the blockage. Therefore, this method is quite challenging although it is still possible with some further optimization. So far, on-column derivatisation of ZDTCs to nickel complexes is a preferred method and further studies to optimize the on-column derivatisation method were carried out and discussed in the next chapter. Both the method of pre-column and optimized on-column derivatisation to nickel complexes will thus be used for the determination of
rubber accelerators in rubber products (Chapter 6).

4.3. Effect of metal ions in the eluent on the void volume marker

As mentioned in section 4.2.3.1, the addition of the metals into the mobile phase not only affects the analytes but also the retention and extra peak shape of the uracil used for calculating the dead volume marker. Many experiments and theoretical studies [198, 199] have shown that metals can bind to the nucleic acids, which includes uracil. However, in this particular study, a more thorough investigation was carried out to find out the effects of the metals on the void volume marker (uracil).

In general, the addition of metal salts in the mobile phase has gradually affected the chemistry of the stationary phase surfaces. As shown in Fig. 4.34, with time the peak shapes of the uracil become more tailing. Table 4.10 also showed that the retention time of uracil gradually increased with time and after a number of experiments with addition of metal salts into the mobile phase. As an excess of metal ions were actually introduced into the system, they probably being adsorbed into the column as in Fig. 4.35. Strongly electronegative metal ions (e.g. nickel (II) or zinc (II)) in the silica matrix is acknowledged to have an influence to boost the acidity of surrounding silanols, which then increased the possibility of ion-exchange interactions [200]. The silanol group also has a tendency of self ionisation when a nearby silicon atom is substituted by a metal ion (Fig. 4.36).

Furthermore, analytes with Lewis-base properties would easily interact with the metal ions present in the silica surface layer [200]. It appeared as in Fig. 4.34 that, although the absorbed metal ions are possible to be removed during the regeneration process, it also created an increased amount of silanol activity. Uracil is also reported to interact with the divalent metal ions [198]. Therefore, both the silanols and the presence of metals in the column result in severe peak tailing as shown in the latest chromatogram of uracil (210307) in Fig. 4.34.
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Fig. 4.34. Chromatograms of 100 µg/ml uracil (except 210307 – 50 µg/ml) using 95% methanol-water at 1 ml/min, 40°C and 254 nm on Ultrasphere ODS column taken on (a) 08/03/2006, (b) 31/05/2006, (c) 08/06/2006 and (d) 21/03/2007, where a few series of in between experiments with addition of metal salts into the mobile phase were carried out.
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Table 4.10
Retention time of 100 µg/ml uracil (except 210307 – 50 µg/ml) on different dates after a series of in between experiments with addition of metal salts into the mobile phase

<table>
<thead>
<tr>
<th>Date</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 March 2006</td>
<td>2.37</td>
</tr>
<tr>
<td>31 May 2006</td>
<td>2.50</td>
</tr>
<tr>
<td>8 June 2006</td>
<td>3.05</td>
</tr>
<tr>
<td>21 March 2007</td>
<td>4.48</td>
</tr>
</tbody>
</table>

HPLC conditions: 95% methanol-water at 1 ml/min, 40°C and 254 nm on Ultrasphere ODS column.

Fig. 4.35. The effect of metal ions in the silica matrix and in the silica surface layer [200].

Fig. 4.36. The stabilizing effect of metals on ionized silanols [201].
On the other hand, using mobile phase containing metal ions produced better peak shapes for uracil, which is not only different for different metals but also for different concentrations. As shown in Fig. 4.37, both the peak shapes and retention times of uracil were different using different mobile phase mixtures. The retention time of uracil was reduced from 2.88 min using 95% methanol-water to 2.60 and 2.48 min using 95% methanol-water with 0.01% Ni (II) and 0.01% Zn (II), respectively. The addition of metal salts into the mobile phase has significantly improved the peak shape as well as reducing the retention time of uracil. This is probably due to the excess amount of metal ions present in the mobile phase, which eventually protect against the effect from surface silanol and metal reactions.

As using uracil might give a problem in the calculation of a true retention factor, two other analytes were analyzed for the possibility of using them as dead volume marker. They were thiourea and sodium nitrate. However, as shown in Fig. 4.38 and Fig. 4.39, both thiourea and sodium nitrate were not suitable for the condition used in this method. Therefore, uracil will still be used for the determination of $t_0$ as it is more suitable for this method. Although it might give different values for different conditions, the same percentage of nickel (II) salt in the mobile phase will be used for other studies except for the effect of nickel (II) salt concentration study. Therefore, the use of uracil for dead volume marker is still possible, and thus will not affect the retention study of the dithiocarbamate complexes.
Fig. 4.37. Chromatograms of 100 µg/ml of uracil using (a) 95% methanol-water, (b) 95% methanol-water with 0.01% Ni (II) and (c) 95% methanol-water with 0.01% Zn (II) at 1 ml/min, 40°C and 254 nm on Ultrasphere ODS column.
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Fig. 4.38. Chromatogram of 50 µg/ml thiourea using 95% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 254 nm on Ultrasphere ODS column.

Fig. 4.39. Chromatogram of 1 mg/ml sodium nitrate using 90% methanol-water at 1 ml/min, RT and 254 nm on Ultrasphere ODS column.
4.4. Confirmation of the origin of the mixed ligand complexes

As mentioned in section 4.2.3, a mixture of zinc dithiocarbamates going through on-column derivatisation to nickel dithiocarbamates produced extra peaks of mixed ligand complexes. Therefore, a further investigation was carried out on whether the mixed ligand complexes produced were from the reaction of nickel dithiocarbamates or from the reaction of mixing the zinc dithiocarbamates themselves. Zinc or nickel dithiocarbamates were prepared by derivatising ammonium dithiocarbamates on-column, which was either added with zinc salts or nickel salts into the mobile phase.

The reaction of zinc salts with dithiocarbamates as suggested by Goksøyr [202] was as equation 4.10 and 4.11 below:

\[
\begin{align*}
\text{Zn}^{2+} + R_2\text{NCS}_2^{-} & \rightleftharpoons k (R_2\text{NCS}_2 - \text{Zn})^+ \quad (4.10) \\
(R_2\text{NCS}_2 - \text{Zn})^+ + R_2\text{NCS}_2^{-} & \rightleftharpoons k R_2\text{NCS}_2 - \text{Zn} - S_{2\text{CNR}}_2 \quad (4.11)
\end{align*}
\]

As shown in Fig. 4.40, zinc pyrrolidinedithiocarbamate (ZPDC) was hydrophobic and not a stable complex as the ZPDC peak was broad, tailing and with the presence of a few derivatives. Zinc diethyldithiocarbamate (ZDEC) on the other hand was less hydrophobic and more stable as only one peak appeared at an earlier time (Fig. 4.41).

As in Fig. 4.42, a mixture of APDC and ADEC which was going through an on-column derivatisation to their respective ZPDC and ZDEC only produced the original peaks of the mixture. However, ZPDC produced was not stable and the peak was still broad and tailing as before.

On the other hand, as in Fig. 4.43, NiPDC was found to be more stable and therefore only one peak appeared. Where as NiDEC was less stable and produced a mixed ligand complex peak of NiPDC-DEC (Fig. 4.44). The interference from the NiPDC was probably because of the pyrrolidinedithiocarbamate (PDC) ligands being absorbed into the column surface from the previous run of APDC as the second injection of ADEC (Fig. 4.44-b) showed lower amount of the interference compound.
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Fig. 4.40. Chromatogram of APDC after on-column derivatisation to ZPDC using 75% methanol-water with 0.01% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.

Fig. 4.41. Chromatogram of ADEC after on-column derivatisation to ZDEC using 75% methanol-water with 0.01% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column.
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Fig. 4.42. Chromatograms of a mixture of APDC and ADEC after on-column derivatisation to their respective ZPDC and ZDEC using 75% methanol-water with 0.01% Zn (II) at 1 ml/min, 40°C and 260 nm on Ultrasphere ODS column. (a) Original chromatogram and (b) close-up.

Fig. 4.43. Chromatogram of APDC after on-column derivatisation to NiPDC using 75% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column.
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Fig. 4.44. Chromatograms of ADEC after on-column derivatisation to NiDEC using 75% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column. (a) 1st injection and (b) 2nd injection.

Separation of a mixture of APDC and ADEC after on-column derivatisation to their respective NiPDC and NiDEC complexes (Fig. 4.45) showed that an extra peak of the mixed ligand complex of NiPDC-DEC occurred. As stated earlier, NiDEC was not as stable as NiPDC which then being converted more to the mixed ligand complex of NiPDC-DEC compared to the original compound of NiDEC. Previous authors [23, 52, 55, 195] have reported the same phenomena, where when two complexes with different alkyl groups were injected together on the column, three peaks were always
obtained. This fact was thought to happen for all divalent metal ions. However, as shown earlier, a mixture of zinc complexes only gave the original peak of the mixture. Zinc complexes do not have ligand exchange like nickel complexes because the 3d orbital for zinc are full and therefore the electrons in d-orbital are antibonding with respect to the ligands [180]. Therefore, no ligand exchange was also involved for a mixture of zinc dithiocarbamate complexes.

![Chromatogram](image)

**Fig. 4.45.** Chromatogram of a mixture of APDC and ADEC after on-column derivatisation to their respective NiPDC and NiDEC using 75% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column.

### 4.5. Summary

Further studies on the method of direct injection by the addition of zinc salts into the mobile phase demonstrated that the separation of ZDTCs by this method was not successful. An initial study on Zr-PDB column showed that the column was not suitable for the separation of dithiocarbamate complexes, as they might have chelated to the zirconium surface sites of the column. Furthermore, the same study on the Ultrasphere ODS column gave poor resolution and peak shapes. The study on the effect of pH of the mobile phase also confirmed that dithiocarbamate complexes decomposed under
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acidic conditions, while were much more stable at higher pH values. However, under these conditions, some compounds were stable while the others were not. A study at higher pH value (pH 11.00) on the XBridge C8 column also showed that direct injection of zinc dithiocarbamates was not efficient. Thus, the derivatisation of dithiocarbamate complexes to the more stable metal complexes might still be the best method, and hence was used for further studies.

Three derivatisation methods were investigated, which include pre-column derivatisation, in-line derivatisation and on-column derivatisation. Pre-column derivatisation of zinc dithiocarbamates to nickel complexes was used to compare the chromatographic characteristics of five different columns (Ultrasphere aDS, XTerra aDS, Zorbax Cyano, Zr-PBD, and Gemini ODS). It was found that the Ultrasphere ODS column gave the highest column efficiencies, the best peak shapes and the highest resolution values, while the Gemini ODS column produced the nearest chromatographic characteristics to the Ultrasphere ODS column, and therefore was chosen to be used for further studies. An XBridge C8 column was also chosen for further studies, as it is another thermally stable column with a short alkyl bonded chain. During this experiment, the derivatised ZDTCs (NiDTCs) were found to be unstable after day one of preparation. However, a repeat study using new stock solutions showed that they were actually stable even up to 5 days.

The comparison between the in-line derivatisation method, which was a direct injection of zinc dithiocarbamate complexes after pre-injection of metal salts to condition the HPLC system, with pre-column derivatisation method showed an incomplete metal conversion even at the highest nickel salt concentration studied (80 µg). Therefore, a study by an addition of metal salt into the mobile phase at a higher and constant concentration over the entire system was proposed (on-column derivatisation).

On-column derivatisation of zinc dithiocarbamates to two different metal complexes (nickel (II) and copper (II)) were carried out. An initial study of the on-column derivatisation to nickel complexes showed that this method was
the better method compared to the pre-column derivatisation method as it gave higher peak heights and peak areas with similar values for other parameters. A brief study on the same method to copper complexes showed that the method was not efficient as it gave very broad peak shapes. Furthermore, the lower solubility of copper sulfate salts as well as copper dithiocarbamate complexes in the mobile phase gave poor column performance and produced higher back-pressure. Therefore, on-column derivatisation to nickel complexes is still a preferred method and further studies to optimize this method are discussed in the next chapter.

During the experiments on the derivatisation of ZDTCs, the chromatographic characteristics of uracil used for dead volume marker were found to change over time. In general, the addition of metal salts into the mobile phase has gradually affected the chemistry of the stationary phase surfaces, which make the peak shapes of uracil became more tailing. It was probably because of the higher silanol activities created after using metals in those studies and the interaction of uracil with the metal ions present in the column. On the other hand, the addition of metal salts into the mobile phase has significantly improved the peak shape as well as reducing the retention time possibly to a more consistent value of $t_0$. As no other analytes were found to be suitable for this study, uracil will still be used for further studies, as the same percentage of nickel (II) ions in the mobile phase produced the same retention time of $t_0$, and therefore will not affect the retention study made for the dithiocarbamate complexes.

Finally, the investigation on the origin of the mixed ligand complexes found after separating a mixture of derivatised ZDTCs (NiDTCs) showed that the ligand exchange occurred after on-column derivatisation of ZDTCs mixture to nickel complexes was carried out but not on the original mixture of ZDTC complexes.
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CHAPTER 5: ON-COLUMN DERIVATISATION OF ZINC DITHIOCARBAMATES

5.0. Introduction

In the previous chapter, on-column derivatisation was found to be the best method and therefore was further optimized in this chapter. The studies included the effect of nickel salt concentration in the mobile phase, pH of mobile phase, type of organic solvent and their proportion, temperature, flow rate and finally the mixture composition on the production of mixed ligand complexes.

The effect of nickel salt concentrations added to the mobile phase was investigated in the range of 0.001% to 0.05% on Gemini ODS column. Then, using the selected nickel concentration, two different pH values were studied, where one was at pH 6.73 using Ultrasphere ODS column and the other was pH 11.00 using XBridge C8 column. The effect of the type of organic solvent used in the mobile phase, include acetone, tetrahydrofuran, acetonitrile and methanol was examined. As methanol was found to be the best solvent for this method of on-column derivatisation of zinc dithiocarbamates, it was then chosen for a further study of the composition at 90, 70, 50 and 30% methanol with the temperature increased gradually from 40°C up to 140°C. A wider range of temperature (40 – 80°C) at a specific methanol composition of 70% was also studied on ZDBC and a mixture of ZDEC and ZDBC. The effect of four different flow rates range between 0.5 to 2.0 ml/min at two selected temperatures (40 and 70°C) was also studied. The final study on the optimization of on-column derivatisation method was on the effect of ZDTC mixture composition to the production of mixed ligand complexes using two series of mixture composition. Based on these results, the calculations of the equilibrium constant (K) and back calculation of the original mixture composition were carried out.
5.1. Effect of nickel salt concentrations

As shown in Fig. 5.1, increasing the metal concentration from 0.001\% to 0.05\% Ni (II) in the mobile phase has reduced the retention of uracil to the expected dead volume, which is about 2.5 min for this particular condition using the Gemini ODS column. Increasing the metal concentration also changed the chromatographic properties of the NiDBC, which was derivatised on-column from ZDBC. The first and most pronounced effect was to the retention of the NiDBC. It was found that as the metal concentration increased, the retention of this compound also gradually increased (Fig. 5.2).

From the graph of retention factor ($k$) versus log of the nickel salts concentration (Fig. 5.3), the relationship was found to be linear. This is probably because the metal concentration increased, and therefore the equilibrium 5.1 [179] is moved further to complex formation, and hence the observed retention is increased.

$$Zn(DTC)_2 + Ni^{2+} \rightarrow Zn^{2+} + Ni(DTC)_2$$  \hspace{1cm} (5.1)
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Fig. 5.2. Effect of nickel salt concentration added in the mobile phase on ZDBC (100 µg/ml) after on-column derivatisation to NiDBC using 90% methanol-water at 0.2 ml/min, 40°C and 330 nm on Gemini ODS column.

![Graph of absorbance over time](image)

Fig. 5.3. Graph of retention factor \( (k) \) of ZDBC after on-column derivatisation to NiDBC versus log nickel salt concentration (%) using 90% methanol-water at 0.2 ml/min, 40°C and 330 nm on Gemini ODS column.

\[
y = 0.1105\ln(x) + 2.9123 \\
R^2 = 0.9827
\]

Other chromatographic parameters, however, were not significantly changed except at the highest concentration of nickel ions (0.05%) where the percentage differences of peak area and efficiency were 5 and 11%, respectively, higher compared to the lowest metal concentration (Table 5.1).
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Therefore, a reasonable amount of metal to choose for further study was 0.01% as 0.05% was more difficult to dissolve and might give a problem of metal salt crystallization.

Table 5.1
Effect of nickel salt concentration on the chromatographic characteristics of ZDBC after on-column derivatisation to NiDBC

<table>
<thead>
<tr>
<th>Nickel salt conc. (%)</th>
<th>Area (mAU.s)</th>
<th>Height (mAU)</th>
<th>Sym [%50%]</th>
<th>%Δ</th>
<th>N</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>9153</td>
<td>343</td>
<td>1.30</td>
<td>-</td>
<td>2438</td>
<td>-</td>
</tr>
<tr>
<td>0.005</td>
<td>9120</td>
<td>330</td>
<td>1.30</td>
<td>-4</td>
<td>2349</td>
<td>-4</td>
</tr>
<tr>
<td>0.01</td>
<td>9108</td>
<td>321</td>
<td>1.29</td>
<td>-6</td>
<td>2523</td>
<td>3</td>
</tr>
<tr>
<td>0.05</td>
<td>9603</td>
<td>336</td>
<td>1.29</td>
<td>-2</td>
<td>2710</td>
<td>11</td>
</tr>
</tbody>
</table>

HPLC conditions: 90% methanol-water at 0.2 ml/min, 40°C and 330 nm on Gemini ODS column. %Δ was compared against the lowest nickel concentration (0.001%).

5.2. Effect of pH of mobile phase

As acid will readily decompose dithiocarbamates, no acidic conditions were used. As happened to the zinc solution (section 4.1.3), adding nickel salts into the mobile phase reduced the pH of the solution from 7.40 to 6.73. However it was higher compared to the value after adding zinc salts. On the other hand, the separation of a mixture of 5 ZOTC compounds at this pH on the Ultrasphere ODS column produced a few extra peaks, which believed to be the mixed-ligand complexes (Fig. 5.4). These peaks, however, were very stable compounds as they showed really sharp peaks. Moreover, they were all very well separated.

As an Ultrasphere ODS column is not stable at high pH (11.00), the XBridge C8 column was again used for studying the effect of basic conditions on the on-column derivatisation of ZDTCs to NiDTCs. The pH value was adjusted to 11.00 using 35% ammonium hydroxide. As shown in Fig. 5.5, the nickel dithiocarbamates became unstable and behaved like the original zinc dithiocarbamates.
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Fig. 5.4. Chromatogram of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) after on-column derivatisation to NiDTCs using 75% methanol-water with 0.01% Ni (II) (pH 6.73) at 1 ml/min, 40°C and 330 nm on Ultrasphere ODS column. Peak identifications are as in Fig. 4.7. Note that the last 3 peaks in that chromatogram were not detected by this system.

Fig. 5.5. Chromatogram of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) after on-column derivatisation to NiDTCs using 75% methanol-water with 0.01% Ni (II) (pH 11.00) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.
This is probably because of the presence of ammonia in the system, as quoted by Thorn and Ludwig that Johnson and Hall [21] has found that the ammonia has a strong effect on the stability of the nickel complex, actually weakening the bonds of the dithiocarbamate units to the nickel. This is a result of a competition between the nickel ion and ammonium cation for a dithiocarbamate ligand to form nickel or ammonium dithiocarbamate. Nickel could also complex with ammonia competing with dithiocarbamate. Therefore, for the on-column derivatisation to nickel complexes, it was decided not to alter the pH of the mobile phase.

5.3. Effect of type of organic solvent

Four different solvents normally used for the reversed-phase HPLC, acetone, tetrahydrofuran, acetonitrile and methanol, were selected for the study of the on-column derivatisation of ZDTCs to their respective NiDTCs. The mobile phase mixture was prepared at 50% of each solvent with water and 0.01% Ni (II), and used to study the on-column derivatisation of ZDMC, ZDEC, ZPD and a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC).

5.3.1. Acetone

Flushing the system with 50% acetone-water with 0.01% Ni (II) showed a problem of the baseline as the UV cut off for acetone is 330 nm. At 254 nm the absorbance was over the maximum. Therefore, the retention time of uracil (dead volume marker) cannot be analyzed and thus the retention factor of the nickel dithiocarbamates was difficult to measure.

As shown in Fig. 5.6, Fig. 5.7 and Fig. 5.8 the retention time of nickel dimethyldithiocarbamate (NiDMC), NiDEC and NiPD were very short even at 50% organic solvent-water mixture. However, between 1 to 1.5 min in all chromatograms, there was a form of a "system peak", which possibly caused
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by solvent/eluent incompatibility. Furthermore, no peaks were detected for ZDBC and ZBEC. On the other hand, the chromatogram (Fig. 5.9) of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) after on-column derivatisation to their respective nickel dithiocarbamates showed better separation. However, the resolution of the peaks was still poor. These mean that acetone was not a suitable mobile phase solvent for the determination of ZDTCs by the on-column derivatisation to nickel complexes.

![Chromatogram of ZDMC after on-column derivatisation to NiDMC using 50% acetone-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.](image_url)
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Fig. 5.7. Chromatogram of ZDEC after on-column derivatisation to NiDEC using 50% acetone-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.

Fig. 5.8. Chromatogram of ZPD after on-column derivatisation to NiPD using 50% acetone-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.
5.3.2. Tetrahydrofuran

Using the same percentage of tetrahydrofuran (THF), it was found that it was the strongest solvent as all of the compounds came out at almost the same time and very near to the solvent peak (Fig. 5.10). A study on ZDMC using a lower percentage of THF (20%) showed that the complexes were not stable in this solvent mixture (Fig. 5.11). The use of the dichloromethane (DCM) together with THF (1:1) might also cause the peak splitting, as DCM is a strong solvent. Separations on a mixture of five ZDTCs using this solvent at both compositions were also not successful as no peak was detected and therefore no chromatogram showed here.
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![Graph showing chromatograms of ZDTCs and ZOMC after on-column derivatisation to NiDTCs and NiOMC using THF-water solutions.](image)

**Fig. 5.10.** Chromatograms of ZDTCs after on-column derivatisation to NiDTCs using 50% THF-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column. ZDTCs were diluted in 50% THF-DCM.

**Fig. 5.11.** Chromatograms of ZOMC after on-column derivatisation to NiOMC using 20% THF-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column. ZOMC was diluted in 50% THF-DCM.
5.3.3. Acetonitrile

At 50% acetonitrile-water, the NiDTCs gave similar peaks to those found using acetone-water mixture. Only NiDMC, NiDEC and NiPD were detected by the system but not the NiDBC and NiBEC. All the three compounds also had shoulders (Fig. 5.12, Fig. 5.13 and Fig. 5.14), while a separation on a mixture of five ZDTCs (Fig. 5.15) showed only a few compounds with broad fronting peak shapes. Therefore, no further study was carried out for this solvent as well.

![Fig. 5.12. Chromatogram of ZDMC after on-column derivatisation to NiDMC using 50% acetonitrile-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.](image)
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Fig. 5.13. Chromatogram of ZDEC after on-column derivatisation to NiDEC using 50% acetonitrile-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.

Fig. 5.14. Chromatogram of ZPD after on-column derivatisation to NiPD using 50% acetonitrile-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.
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Fig. 5.15. Chromatogram of a mixture of five ZDTCs (ZDMC, ZDEC, ZPD, ZDBC and ZBEC) after on-column derivatisation to NiDTCs using 50% acetonitrile-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.

5.3.4. Methanol

In contrast to all the problems found when ZDTCs were derivatised and separated using acetone, THF or acetonitrile, methanol gave the best peak shapes and separation. Fig. 5.16, Fig. 5.17 and Fig. 5.18 shown below are the chromatograms of NiDMC, NiDEC and mixture of 5 NiDTCs. From the mixture of 5 compounds, only 6 compounds were detected. Three were NiDMC, NiDEC and NiPD, and the others were mixed ligand complexes of NiDMC-DEC, NiDMC-PD and NiDEC-PD.

It can be concluded that methanol was the best solvent for the on-column derivatisation of ZDTCs to NiDTCs. Previous authors [23, 174, 175] also preferred methanol-water mixture as their mobile phase for the separation of metal dithiocarbamate complexes. Thus, methanol will be used for all our further experiments. However, other solvents may also give better separation with further optimizations.
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Fig. 5.16. Chromatogram of ZDMC after on-column derivatisation to NiDMC using 50% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.

Fig. 5.17. Chromatogram of ZDEC after on-column derivatisation to NiDEC using 50% methanol-water with 0.01% Ni (II) at 1 ml/min, 40°C and 330 nm on XBridge C8 column.
5.4. Effect of methanol composition and temperature

Since methanol gave the best separation for the on-column derivatization of zinc dithiocarbamates, further study on the effect of its percentage and temperature was carried out. Four different percentages of methanol composition were investigated at three different temperatures each. The temperature range studied at 90, 70, 50 and 30% methanol-water with 0.01% Ni (II) were 40-60°C, 60-80°C, 80-100°C and 110-140°C respectively. Because NiDBC and NiBEC are highly retained compounds, the temperature range has been increased gradually as the percent of methanol decreased. Fig. 5.19 is a van't Hoff plot demonstrating the effect of temperature on the retention of ZDTC compounds after on-column derivatisation to NiDTCs at different methanol composition over the range of 40 to 140°C.

In general, as the methanol composition was reduced, the retention of all the compounds increased. The $k$ values also have greater decline over the
temperature range studied as the methanol content decreased. At 90% methanol, the resolution between NiDBC and NiBEC was quite low and kept decreasing as the temperature increased. At lower methanol composition of 70%, these values increased to more reasonable values but again the resolution between NiDBC and NiBEC reduced to less than 1 as the temperature increased to the highest temperature in the range (80°C). It seems that 70°C is the most reasonable temperature for 70% methanol. The resolution between NiPD and NiDBC however stayed quite high even at the highest possible temperature studies (80°C). As NiDBC and NiBEC were not detected at 50 and 30% methanol, their results were neglected at these conditions. NiPD was also not detected at 100°C using 30% methanol-water, and therefore no result of this compound at this particular condition was reported. From these results, it seem that 70% methanol-water was the best mobile phase composition for this method, and thus was chosen for further studies in the next section.

Fig. 5.19. Van't Hoff plots for ZPD, ZDBC and ZBEC after on-column derivatisation to NiDTCs using 90, 70, 50 and 30% methanol-water with 0.01% Ni (II) at 1 ml/min, 40-60°C for 90%, 60-80°C for 70%, 80-100°C for 50%, 110-140°C for 30% and 330 nm on XBridge C8 column. Note that only NiPD was detected using 50 and 30% methanol, and NiPD was not detected at 100°C using 30%.
5.5. Effect of temperature using 70% methanol-water with 0.01% Ni (II)

As 70% methanol in the mobile phase was found to be the best conditions for the separation of the selected compounds using the on-column derivatisation method, a further study on a wider range of temperature at this condition was carried out. The separation of ZDBC and a mixture of ZDEC and ZDBC were carried out at a temperature range of 40°C to 80°C and 40°C to 70°C respectively. An individual study of ZDBC and a study of a mixture of ZDEC and ZDBC were selected as these compounds were reported to be in the samples to be studied later. Fig. 5.20 and Fig. 5.21 show the overlay chromatograms of both studies. The range of temperature made for the study on the mixture of ZDEC and ZDBC was only up to 70°C, as at higher temperatures, the peaks showed a sign of a poor column, which was later replaced. At 80°C for example, double peaks were found for the dithiocarbamate compounds as well as the test mixtures used in checking the column performance.

Fig. 5.20. Overlay chromatograms of ZDBC after on-column derivatisation to NiDBC at different temperatures using 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column.
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Fig. 5.21. Overlay chromatograms of a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs at different temperatures using 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column.

Although only a small range of temperatures were studied, the use of temperature could be of benefit to the analysis of rubber accelerators specifically the highly retained zinc dithiocarbamates as higher temperatures allows a reduction of the organic content in the mobile phase, provides faster separation which sometimes without loss of efficiency and could also improve the selectivity, detectability and peak shape [203, 204].

5.5.1. Effect on retention

The van't Hoff plots presenting the effect of temperature from 40 to 80°C on the retention of selected ZDTC compounds by on-column derivatisation to NiDTCs were shown in Fig. 5.22 for ZDBC and Fig. 5.23 for a mixture of ZDEC and ZDBC. In general, these plots showed a normal behaviour as they were quite linear and have positive slopes. The slope, intercept and $R^2$ values of these plots are as in Table 5.2. Referring to the $R^2$ values for the relationship of the retention factors of both study however, suggested that it may not be a linear relationship. A quadratic correlation though only gave moderate correlation with $R^2$ values ranged between 0.9721 to 0.9969, but as
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Fig. 5.22. Van't Hoff plots for ZDBC after on-column derivatisation to NiDBC using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 40-80°C and 330 nm on XBridge C8 column.

Fig. 5.23. Van't Hoff plots for a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 40-70°C and 330 nm on XBridge C8 column.
Table 5.2
Regression parameters of Van't Hoff plots in Fig. 5.22* and Fig. 5.23**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>Intercept</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiDBC*</td>
<td>3568.3</td>
<td>-8.4355</td>
<td>0.9948</td>
</tr>
<tr>
<td>NiDEC**</td>
<td>2315.2</td>
<td>-7.4249</td>
<td>0.9494</td>
</tr>
<tr>
<td>NiDEC-DBC**</td>
<td>2824.1</td>
<td>-7.5317</td>
<td>0.9670</td>
</tr>
<tr>
<td>NiDBC**</td>
<td>3783.8</td>
<td>-9.1051</td>
<td>0.9953</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column. X axis=1/T (K⁻¹).

shown in Fig. 5.24 and Fig. 5.25, the data could be fitted with two linear plots where there were slight jumps between 50 to 60°C. The regression parameters for these plots were as in Table 5.3. These small deviations from linearity might indicate a phase transition occurrence. For C18 silica based stationary phases, this occurrence which appears in the 20 – 50°C range is the consequence of change in the molecular structure of the stationary phase [205, 206].

In an extensive review about phase transition by Wheeler et al. [207], they suggested that observation of phase transition is related almost to every chromatographic variable; bonded phase alkyl chain length, chain density, type of bonding reaction (monomeric or polymeric), mobile phase solvent(s) and the choice of solutes. A more recent study by Guillarme et al. [203] however, showed that this behaviour is totally independent on the nature of the solute but is dependent on both types of stationary and mobile phases. The same occurrence of phase transition has too been reported on other ethylene bridged hybrid (BEH) phases like C18 bonded [208] at about 97°C and phenyl bonded at about 125°C [209]. Wheeler et al. [207] also reported that the presence of methanol demonstrated a different type of phase transition around 50-60°C, which were attributed to an elimination of methanol from the hydrocarbon network due to solvation. Although no real conclusion could be made as the study was only carried out at a limited temperature range, it should be noted that this might be one of the reason of the phase transition found in this study.
Fig. 5.24. Van't Hoff plots with different relationship for ZDBC after on-column derivatisation to NiDBC using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 40-80°C and 330 nm on XBridge C8 column.

Fig. 5.25. Van't Hoff plots different relationship for a mixture of ZDEC and ZDBC after on-column derivatisation to NiTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 40-70°C and 330 nm on XBridge C8 column.
Table 5.3
Regression parameters of Van't Hoff plots in Fig. 5.24* and Fig. 5.25**

<table>
<thead>
<tr>
<th>Compound</th>
<th>40-50°C</th>
<th></th>
<th></th>
<th>60-80/70°C</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Intercept</td>
<td>R²</td>
<td>Slope</td>
<td>Intercept</td>
<td>R²</td>
</tr>
<tr>
<td>NiDBC*</td>
<td>3443.3</td>
<td>-8.0244</td>
<td>1</td>
<td>3034.5</td>
<td>-6.8913</td>
<td>0.9990</td>
</tr>
<tr>
<td>NiDEC**</td>
<td>2710.8</td>
<td>-8.6565</td>
<td>1</td>
<td>618.1</td>
<td>-2.4174</td>
<td>1</td>
</tr>
<tr>
<td>NiDEC-DBC**</td>
<td>2402.0</td>
<td>-6.1833</td>
<td>1</td>
<td>1283.0</td>
<td>-2.9945</td>
<td>1</td>
</tr>
<tr>
<td>NiDBC**</td>
<td>3405.4</td>
<td>-7.9038</td>
<td>1</td>
<td>3118.9</td>
<td>-7.1504</td>
<td>1</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column. X axis=1/T (K⁻¹).

Effect of temperature on retention factor (k), selectivity (α) and resolution (R) of ZDEC and ZDBC mixture were as detailed in Table 5.4. As expected, all of the values decreased as the temperature increased. ZDEC showed a better separation at 40°C while ZDBC still had high retention as high as 6.95 even at 70°C. However, as the XBridge C8 column used in this study was already in poor condition, 70°C is proposed to be used for the current study on the determination of ZDTCs in rubber products (Chapter 6). The selectivity and resolution of ZDEC and ZDBC mixture was also higher than 1 even at 70°C. Thus an isocratic temperature of 70°C can also be used for the separation of a mixture of these compounds.

Table 5.4
Effect of temperature on retention factor (k), selectivity (α) and resolution (R) of ZDEC and ZDBC mixture

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>k₁</th>
<th>k₂</th>
<th>k₃</th>
<th>α₁₂</th>
<th>α₂₃</th>
<th>R₁₂</th>
<th>R₂₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1.00</td>
<td>4.43</td>
<td>19.51</td>
<td>4.43</td>
<td>4.41</td>
<td>8.77</td>
<td>15.78</td>
</tr>
<tr>
<td>50</td>
<td>0.77</td>
<td>3.49</td>
<td>13.94</td>
<td>4.56</td>
<td>3.99</td>
<td>7.60</td>
<td>14.80</td>
</tr>
<tr>
<td>60</td>
<td>0.55</td>
<td>2.36</td>
<td>9.13</td>
<td>4.28</td>
<td>3.88</td>
<td>5.85</td>
<td>12.94</td>
</tr>
<tr>
<td>70</td>
<td>0.54</td>
<td>2.11</td>
<td>6.95</td>
<td>3.60</td>
<td>3.30</td>
<td>4.87</td>
<td>10.57</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column. These values are for the separation of a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs (1: NiDEC, 2: NiDEC-DBC and 3: NiDBC).

Using the equation 2.7 and 2.8, the thermodynamic properties of the compounds studied were calculated (Table 5.5 and Table 5.6), where the values in Table 5.5 are based on Fig. 5.22 and Fig. 5.23 where only one linear line was used, while the values in Table 5.6 are based on Fig. 5.24 and Fig. 5.25 with two linear plots.
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Table 5.5
Standard enthalpy ($\Delta H^*$), entropy ($\Delta S^*$) and Gibbs free energy ($\Delta G^*$) for separation of ZDBC and a mixture of ZDEC and ZDBC after on-column derivatisation to NiOTCs

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta H^*$ (kJ/mol)</th>
<th>$\Delta S^*$ (kJ/K-mol)</th>
<th>$\Delta G^*$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiDBC</td>
<td>-29.67</td>
<td>-0.07</td>
<td>-6.30</td>
</tr>
<tr>
<td>NiDEC</td>
<td>-19.25</td>
<td>-0.06</td>
<td>1.01</td>
</tr>
<tr>
<td>NiDEC-DBC</td>
<td>-23.48</td>
<td>-0.06</td>
<td>-2.93</td>
</tr>
<tr>
<td>NiDBC</td>
<td>-31.46</td>
<td>-0.08</td>
<td>-6.62</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column. Values are based on Fig. 5.22 and Fig. 5.23.

Table 5.6
Standard enthalpy ($\Delta H^*$), entropy ($\Delta S^*$) and Gibbs free energy ($\Delta G^*$) for separation of ZDBC and a mixture of ZDEC and ZDBC after on-column derivatisation to NiOTCs

<table>
<thead>
<tr>
<th>Compound</th>
<th>40-50°C</th>
<th>60-80/70°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta H^*$ (kJ/mol)</td>
<td>$\Delta S^*$ (kJ/K-mol)</td>
</tr>
<tr>
<td>NiDBC</td>
<td>-28.63</td>
<td>-0.07</td>
</tr>
<tr>
<td>NiDEC</td>
<td>-22.54</td>
<td>-0.07</td>
</tr>
<tr>
<td>NiDEC-DBC</td>
<td>-19.97</td>
<td>-0.05</td>
</tr>
<tr>
<td>NiDBC</td>
<td>-28.31</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column. Values are based on Fig. 5.24 and Fig. 5.25.

The values of $\Delta H^*$ and $\Delta S^*$ are negative for both individual studies of ZDBC as well as for a mixture of this compound with ZDEC. This again showed that the retention process was exothermic and had a positive adsorption of the compounds on the stationary phase. Larger negative enthalpies were also observed as the compounds have higher molecular weight, but this was only true for the higher temperature range and if linearity was assumed for the whole range of temperature. When a closer look was made as in Fig. 5.24, Fig. 5.25 and Table 5.6, the changes in the enthalpies in the two regions from low temperature of 40-50°C to higher temperature of 60-70/80°C showed a different pattern. Different values in the enthalpies at these two temperature range also showed that there was a marked change in the nature of the column. The changes also dependant on the solutes as different changes were observed for different compounds studied. NiDEC, which is the lowest molecular weight amongst the three showed the highest
changes from -22.54 kJ/mol to -5.14 kJ/mol. NiDBC on the other hand showed the lowest changes with a similar changes either by itself or in the mixture. Not only the original compounds of dithiocarbamate changed, but also the newly produced mixed ligand complexes of NiDEC-DBC, which changed about half of the lower temperature range value.

5.5.2. Effect on efficiency and peak shape

As shown in Fig. 5.26 no marked changes in the peak areas and efficiencies were observed up to 70°C as the temperature increased. However, the efficiency of ZDBC reduced a little bit when temperature was increased from 70 to 80°C. On the other hand, the asymmetry of ZDBC peak gradually increased as the temperature increased from 50 to 80°C.

![Graph showing temperature vs. efficiency, peak area, and asymmetry for ZDBC.](image)

Fig. 5.26. Effect of temperature on efficiency (N), peak area and asymmetry (Sym [50%]) for ZDBC after on-column derivatisation to NiDBC using 70% methanol-water with 0.01% Ni (II) between 40 to 80°C at 1 ml/min and 330 nm on XBridge C8 column.

A study on the effect of the temperature on a mixture of ZDEC and ZDBC (Fig. 5.27) showed only small variation on the peak areas, peak efficiencies as well as peak asymmetries for ZDEC and ZDBC peaks. However, all the three parameters changed as the temperature increased for the newly produced mixed ligand complexes.
Fig. 5.27. Effect of temperature on efficiency (N), peak area and asymmetry (Sym [50%]) for a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs (a: NiDEC, b: NiDEC-DBC and c: NiDBC) using 70% methanol-water with 0.01% Ni (II) between 40 to 80°C at 1 ml/min and 330 nm on XBridge C8 column.
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This occurrence might mean that the dithiocarbamate complexes, especially the mixed ligand complexes are not thermally stable complexes, as Liodakis et. al [210] found that subambient temperature was advantageous as it assists the stability of the solute. Ichinoki and colleagues [211] also found that dithiocarbamate chelates were more stable at lower temperatures, although, they preferred high temperatures as it gave better column efficiency, detection limit and reproducibility. However, a few authors [204, 212] emphasized that many compounds considered thermally labile, like carbamates do not degrade when being analysed at higher temperatures because of the solvated state of the molecules. Even if the degradation occurs, the amount of breakdown depends particularly on the nature and quality of the packing material together with the time spent at high temperature and the actual temperature. Therefore, using thermally stable columns and with the right equipment, the effect of temperature on the analysis of dithiocarbamates should be further investigate.

5.6. Effect of flow rate and temperature

The effect of flow rate on the retention of ZDEC and ZDBC mixture after on-column derivatisation to their nickel complexes with the production of mixed-ligand complexes of NiDEC-DBC were studied at four different flow rates (0.5, 1.0, 1.5 and 2.0 ml/min) and two different temperatures (40 and 70°C). At 40°C the flow rates studied were only up to 1.5 ml/min as at 2.0 ml/min the back pressure was too high and over the maximum pressure limit of the pump (350 bar).

Table 5.7 shows the effect of flow rate and temperature on retention factor \((k)\), selectivity \((\alpha)\) and resolution \((R)\), while Fig. 5.28 and Fig. 5.29 show the overlay chromatograms of ZDTC mixture (ZDEC and ZDBC) after on-column derivatisation to NiDEC, NiDBC and the products of the two compound which is a mixed-ligand complex of NiDEC-DBC at different flow rates.

As expected, the values of \(\alpha\) remained almost constant with the different
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flow rate, but decreased with the increase in the column temperature. A slight decreased in the $k$ values was also observed as the flow rate increased. A more significant decrease in the $k$ values on the other hand was found when the temperature increased. The decreases in $k$ values were more apparent for the more retained complexes like NiDBC. This then gives an advantage of shortening the analysis time as there is possibility of using a higher flow rate at this higher temperature because of the reduced back pressure.

Table 5.7
Effect of flow rate and temperature on retention factor ($k$), selectivity ($\alpha$) and resolution ($R$)

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>$F$ (ml/min)</th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$k_3$</th>
<th>$\alpha_{12}$</th>
<th>$\alpha_{23}$</th>
<th>$R_{12}$</th>
<th>$R_{23}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.5</td>
<td>1.02</td>
<td>4.87</td>
<td>21.63</td>
<td>4.77</td>
<td>4.44</td>
<td>7.77</td>
<td>11.43</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.00</td>
<td>4.79</td>
<td>21.32</td>
<td>4.79</td>
<td>4.45</td>
<td>7.23</td>
<td>10.36</td>
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<tr>
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<td>1.5</td>
<td>1.00</td>
<td>4.77</td>
<td>21.14</td>
<td>4.77</td>
<td>4.44</td>
<td>6.25</td>
<td>8.72</td>
</tr>
<tr>
<td>70</td>
<td>0.5</td>
<td>0.62</td>
<td>2.32</td>
<td>8.07</td>
<td>3.77</td>
<td>3.48</td>
<td>4.19</td>
<td>6.94</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.60</td>
<td>2.29</td>
<td>7.99</td>
<td>3.82</td>
<td>3.49</td>
<td>4.30</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.60</td>
<td>2.28</td>
<td>7.94</td>
<td>3.83</td>
<td>3.48</td>
<td>4.24</td>
<td>7.37</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.59</td>
<td>2.27</td>
<td>7.91</td>
<td>3.85</td>
<td>3.48</td>
<td>3.64</td>
<td>5.96</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) and 330 nm on XBridge C8 column. These values are for injections of ZDEC and ZDBC mixture after on-column derivatisation to NiDTCs which produced 1: NiDEC, 2: NiDEC-DBC and 3: NiDBC.

The changes in flow rate and temperature also affect the $R$ values. However, the $R$ values showed a different pattern between different temperatures. At 40°C, the $R$ values decreased with the increased in the flow rate. On the other hand, at 70°C, the $R$ values increased when flow rate increased from 0.5 to 1.0 ml/min but dropped back when the flow rates increased to 1.5 and 2.0 ml/min. This is probably because an increased in the flow can also increase the pressure in the column, and thus change the retention factor [213]. However, these changes are not really significant, and their values are still acceptable [177]. It should be noted that at 70°C, the ZDEC/NiDEC peak shapes were much poorer especially for 0.5 ml/min due to the longitudinal diffusion effect that caused the peak broadening, which becomes significantly higher with increasing temperature at low linear velocities [214].
Fig. 5.28. Overlay chromatograms of a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs where (1) NiDEC, (2) NiDEC-DBC and (3) NiDBC at different flow rates using 70% methanol-water with 0.01% Ni (II) at 40°C and 330 nm on XBridge C8 column.

Fig. 5.29. Overlay chromatograms of a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs at different flow rates using 70% methanol-water with 0.01% Ni (II) at 70°C and 330 nm on XBridge C8 column. Peak identifications as in Fig. 5.28.

The Van Deemter curve which relates the plate height (H) with the flow rate is shown in Fig. 5.30, Fig. 5.31 and Fig. 5.32. The optimum flow rate for all compounds at 70°C was about 1 ml/min. The curves at 40°C however are considerably steeper and didn't show a clear optimum flow rate even at 0.5
ml/min. These results suggest that there is a somewhat larger mass transfer term in the van Deemter equation which would reasonably result from slow interaction kinetics of these compounds at lower temperature. Normally, as the temperature increases, one would expect an increase in the column efficiency [177, 204]. However, in this study, the column efficiencies for the two earlier compounds at 70°C were found to be poorer compared to the values at 40 °C. Only NiDBC separated at 1.5 ml/min gave higher column efficiency compared to the same flow at 40°C. These were probably because of the fact that the two earlier peaks (NiDEC and NiDEC-DBC), which were the less retained compounds were already close to the dead volume. Thus, these two earlier compounds were sensitive to the column temperature, and thus were seriously affected by the flow rate. The tailing and broad peak shapes found for these two compounds (Fig. 5.29) at 0.5 ml/min suggesting the disproportionation of the mixed ligand complex (NiDEC-DBC) in the column [197]. However, this problem can be eliminated by using lower temperature. Unfortunately, if one wishes to use 0.5 ml/min and 40°C, it will give very high retention of NiDBC. Therefore, 1 ml/min of flow rate is a reasonable condition and will be used with a gradient temperature between 40 to 70°C as the best temperature for NiDEC, NiDEC-DBC and the more retained compounds like NiDBC.

![Fig. 5.30. Van Deemter curve of plate height (H) against flow rate of NiDEC at 40 and 70°C using 70% methanol-water with 0.01% Ni (II) and at 330 nm on XBridge C8 column.](image-url)
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Fig. 5.31. Van Deemter curve of plate height ($H$) against flow rate of NiDEC-DBC at 40 and 70°C using 70% methanol-water with 0.01% Ni (II) and at 330 nm on XBridge C8 column.

Fig. 5.32. Van Deemter curve of plate height ($H$) against flow rate of NiDBC at 40 and 70°C using 70% methanol-water with 0.01% Ni (II) and at 330 nm on XBridge C8 column.
5.7. Effect of ZDTC mixture composition on the production of mixed ligand complexes of NiDTC

In order to be able to use the chromatograms of the mixture to determine the composition of the original ZDTCs, the effect of ZDTC mixture composition on the production of mixed ligand complexes of NiDTC has been investigated, where two ZDTCs (ZDEC and ZDBC) were mixed and then underwent on-column derivatisation to NiDTCs. Two series of studies have been carried out (series A and B). In the A series different concentrations of ZDBC ranged from 6.25 to 100 µg/ml were mixed with a constant ZDEC concentration, at 25 µg/ml, while in the B series different concentrations of ZDEC (6.25 to 100 µg/ml) were mixed with constant ZDBC (25 µg/ml).

During the on-column derivatisation process or the metal exchange reaction between ZDTC and Ni (II), two different routes of the reaction have been reported [179]. One was the dissociation of the dithiocarbamate ligand from the zinc complex followed by the substitution of the nickel ion (equations 5.2 – 5.4), and the second was a direct electrophilic attack by the nickel ion on the dithiocarbamate complex (equation 5.5). Below are the proposed reaction schemes:

First route:

\[
\begin{align*}
Zn(\text{DTC})_2 & \leftrightarrow Zn(\text{DTC})^+ + DTC^- \\
Ni^{2+} + DTC^- & \rightarrow Ni(\text{DTC})^+ \\
Ni(\text{DTC})^+ + DTC^- & \rightarrow Ni(\text{DTC})_2
\end{align*}
\]

Second route:

\[
Zn(\text{DTC})_2 + Ni^{2+} \rightarrow Ni(\text{DTC})^+ + Zn(\text{DTC})^+
\] (5.5)

The relative importance of both routes is determined by the stability of Zn(DTC)_2 and its rate of dissociation, and by the substitution lability and electrophilic nature of Ni^{2+} [179]. As the amount of nickel ions present in the mobile phase was in excess (0.01% or 1.7 mM), therefore, the reaction would be expected to go to completion as in equation 5.1.

With an excess of Ni^{2+}, the primary mechanism of the exchange of ligand
between ZDTC or Zn(DTC)$_2$ and Ni$^{2+}$ involved rapid equilibrium dissociation of DTC$^-$ from Zn(DTC)$_2$ (equation 5.2) and build up of Ni(DTC)$^+$ (equation 5.3), which then slowly reacts further to form Ni(DTC)$_2$ (equation 5.4) [179].

In this study, a mixture of two ZDTC compounds, each with different ligand (diethyl- and dibutyl-dithiocarbamates) was injected, and both went through the same metal exchange reaction as shown in equation 5.2 and 5.3. The presence of two different ligands, therefore, will create possibility that the second ligand will react with a nickel mono-complex of the first ligand as in equation 5.4 and produced a ternary complex of mixed ligands as in equation 5.6. The equilibrium constant will be determined as in equation 5.7.

$$Ni(DEC)_2 + Ni(DBC)_2 \leftrightarrow 2Ni(DEC)(DBC) \quad (5.6)$$

$$K = \frac{[Ni(DEC)(DBC)]^2}{[Ni(DEC)]_2[NI(DBC)]_2} \quad (5.7)$$

As shown in Fig. 5.33 and Fig. 5.34, when the concentration of either ZDEC or ZDBC was increased, the other compounds in the mixture also changed, which suggested that equilibration of the mixture took place. Fig. 5.35 and Fig. 5.36 further illustrate the effect of the mixture concentration to the response of all three nickel complexes produced after the on-column derivatisation process. From these two graphs, it shows that the responses of both NiDEC and NiDBC were nearly linear with the increase of their original ZDTC concentrations. The responses for the other compounds, where ZDTC concentration had been kept constant (either NiDEC or NiDBC) and the newly produced (NiDEC-DBC) on the other hand, respectively were reduced or increased to balance the equilibrium as in equation 5.6 and 5.7.
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Fig. 5.33. Overlay chromatograms of a mixture of ZDEC and ZDBC after on-column derivatisation to nickel complexes at different ZDBC concentrations (6.25 to 100 μg/ml) and ZDEC at 25 μg/ml using 70% methanol-water with 0.01% Ni (II) at 1ml/min, 60°C and 330 nm on XBridge C8 column.

Fig. 5.34. Overlay chromatograms of a mixture of ZDEC and ZDBC after on-column derivatisation to nickel complexes at different ZDEC concentrations (6.25 to 100 μg/ml) and ZDBC at 25 μg/ml using 70% methanol-water with 0.01% Ni (II) at 1ml/min, 60°C and 330 nm on XBridge C8 column.
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Fig. 5.35. Effect of ZDBC concentration (6.25 to 100 µg/ml) with ZDEC at 25 µg/ml on the response of each nickel complexes produced after on-column derivatisation using 70% methanol-water with 0.01% Ni (II) at 1ml/min, 60°C and 330 nm on XBridge C8 column.

Fig. 5.36. Effect of ZDEC concentration (6.25 to 100 µg/ml) with ZDBC at 25 µg/ml to the response of each nickel complexes produced after on-column derivatisation using 70% methanol-water with 0.01% Ni (II) at 1ml/min, 60°C and 330 nm on XBridge C8 column.
From this study, the equilibrium concentration of NiDEC and NiDBC at each level were calculated using the calibration curves of the individual compounds of ZDEC and ZDBC after on-column derivatisation to their respective nickel complexes. The concentration of mixed ligand complex of NiDEC-DBC was then calculated using the Beer Lambert Law equation as below:

$$A = abc$$

where $A$ is the absorbance of the peak, $c$ is the molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$), $b$ is the path length of the sample (assumed as 1 cm), and $c$ is the concentration of the compound in solution ($\text{mol L}^{-1}$). The molar absorptivity for NiDEC-DBC (23693048 ± 419 L mol\(^{-1}\) cm\(^{-1}\)) was calculated as the average of NiDEC (23427203 ± 551 L mol\(^{-1}\) cm\(^{-1}\)) and NiDBC (23958894 ± 651 L mol\(^{-1}\) cm\(^{-1}\)) values. Using those equilibrium concentrations and equation 5.7, the equilibrium constant for each mixture were calculated. The results are shown in Table 5.8 and Table 5.9, where the average $K$ value at this particular condition was found to be 2.92 ± 0.72 at 95% confidence level. These values are in a very good agreement with the reported data of 2.81 ± 0.07 by Lehotay et. al [140].

### Table 5.8
Calculation of the equilibrium constant ($K$) of each point in the A series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared concentration</th>
<th>Equilibrium concentration</th>
<th>$K$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu g/ml$</td>
<td>mM</td>
<td>$\mu g/ml$</td>
</tr>
<tr>
<td>A1 NiDEC</td>
<td>25.00</td>
<td>0.0704</td>
<td>7.00</td>
</tr>
<tr>
<td>NiDEC-DBC</td>
<td></td>
<td></td>
<td>36.47</td>
</tr>
<tr>
<td>NiDBC</td>
<td>100.00</td>
<td>0.2139</td>
<td>72.99</td>
</tr>
<tr>
<td>A2 NiDEC</td>
<td>25.00</td>
<td>0.0704</td>
<td>10.40</td>
</tr>
<tr>
<td>NiDEC-DBC</td>
<td></td>
<td></td>
<td>30.04</td>
</tr>
<tr>
<td>NiDBC</td>
<td>50.00</td>
<td>0.1070</td>
<td>30.62</td>
</tr>
<tr>
<td>A3 NiDEC</td>
<td>25.00</td>
<td>0.0704</td>
<td>14.76</td>
</tr>
<tr>
<td>NiDEC-DBC</td>
<td></td>
<td></td>
<td>21.99</td>
</tr>
<tr>
<td>NiDBC</td>
<td>25.00</td>
<td>0.0535</td>
<td>10.91</td>
</tr>
</tbody>
</table>
Chapter 5: On-column Derivatisation of Zinc Dithiocarbamates

Table 5.9
Calculation of the equilibrium constant (K) of each point in the B series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared concentration</th>
<th>Equilibrium concentration</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>mM</td>
<td>µg/ml</td>
</tr>
<tr>
<td>B1</td>
<td>NiDEC</td>
<td>100.00</td>
<td>0.2815</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>0.0535</td>
</tr>
<tr>
<td>B2</td>
<td>NiDEC</td>
<td>50.00</td>
<td>0.1408</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>0.0535</td>
</tr>
<tr>
<td>B3</td>
<td>NiDEC</td>
<td>25.00</td>
<td>0.0704</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>0.0535</td>
</tr>
<tr>
<td>B4</td>
<td>NiDEC</td>
<td>12.50</td>
<td>0.0352</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>0.0535</td>
</tr>
<tr>
<td>B5</td>
<td>NiDEC</td>
<td>6.25</td>
<td>0.0176</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>0.0535</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 60°C and 330 nm on XBridge C8 column.

A closer look at the K values at each point for A series, however, showed that it increased as the concentration of NiDBC decreased. The values for B series on the other hand, found to be close to the average value except for the second mixture. The reason of the differences in the K values in the A
series, however, was still not clear.

In order to be able to use this method to estimate the original concentrations in the mixture of an unknown sample solution, a back calculation of the original concentration of the mixtures using the average K value of 2.92 was carried out as shown in Table 5.10 and Table 5.11. In these tables, the observed or equilibrium concentrations of NiDEC and NiDBC were the values calculated using the calibration curves of their individual compound. Then, assuming equation 5.6 is true and K value was equal to 2.92, the concentration of mixed ligand complex (NiDEC-DBC) was calculated. This method avoided the necessity to assume an extinction coefficient for the mixed ligand complex. The original concentration of NiDEC ([A₀]) and NiDBC ([B₀]) were then calculated as [A₀]=[A]+[C]/2 and [B₀]=[B]+[C]/2, where [A], [B] and [C] were the observed equilibrium concentrations of NiDEC, NiDBC and NiDEC-DBC respectively.

Table 5.10
Back calculation of the original concentration of NiDEC and NiDBC of A series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared concentration</th>
<th>Observed [calculated] concentration</th>
<th>Calculated original concentration ± SD</th>
<th>Absolute error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>µg/ml</td>
<td>µg/ml</td>
<td>µg/ml</td>
</tr>
<tr>
<td>A1</td>
<td>NiDEC 25.00</td>
<td>7.00</td>
<td>23.81 ± 0.09</td>
<td>1.19</td>
</tr>
<tr>
<td>A1</td>
<td>NiDEC-DBC -</td>
<td>[38.95]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A1</td>
<td>NiDBC 100.00</td>
<td>72.99</td>
<td>95.12 ± 0.90</td>
<td>4.88</td>
</tr>
<tr>
<td>A2</td>
<td>NiDEC 25.00</td>
<td>10.40</td>
<td>23.69 ± 0.14</td>
<td>1.31</td>
</tr>
<tr>
<td>A2</td>
<td>NiDEC-DBC -</td>
<td>[30.77]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A2</td>
<td>NiDBC 50.00</td>
<td>30.62</td>
<td>48.10 ± 0.35</td>
<td>1.90</td>
</tr>
<tr>
<td>A3</td>
<td>NiDEC 25.00</td>
<td>14.76</td>
<td>24.20 ± 0.26</td>
<td>0.80</td>
</tr>
<tr>
<td>A3</td>
<td>NiDEC-DBC -</td>
<td>[21.87]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A3</td>
<td>NiDBC 25.00</td>
<td>10.91</td>
<td>23.33 ± 0.16</td>
<td>1.67</td>
</tr>
<tr>
<td>A4</td>
<td>NiDEC 25.00</td>
<td>17.99</td>
<td>23.62 ± 0.17</td>
<td>1.38</td>
</tr>
<tr>
<td>A4</td>
<td>NiDEC-DBC -</td>
<td>[13.05]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A4</td>
<td>NiDBC 12.50</td>
<td>3.20</td>
<td>10.61 ± 0.74</td>
<td>1.89</td>
</tr>
<tr>
<td>A5</td>
<td>NiDEC 25.00</td>
<td>21.05</td>
<td>24.42 ± 0.46</td>
<td>0.58</td>
</tr>
<tr>
<td>A5</td>
<td>NiDEC-DBC -</td>
<td>[7.79]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A5</td>
<td>NiDBC 6.25</td>
<td>0.97</td>
<td>5.40 ± 0.05</td>
<td>0.85</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 60°C and 330 nm on XBridge C8 column. K = 2.92.
Chapter 5: On-column Derivatisation of Zinc Dithiocarbamates

Table 5.11
Back calculation of the original concentration of NiDEC and NiDBC of B series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared concentration</th>
<th>Observed concentration</th>
<th>Calculated original concentration ± SD</th>
<th>Absolute error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>µg/ml</td>
<td>µg/ml</td>
<td>µg/ml</td>
</tr>
<tr>
<td>B1</td>
<td>NiDEC</td>
<td>100.00</td>
<td>93.57</td>
<td>107.55 ± 0.75</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>[32.36]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>3.77</td>
<td>22.15 ± 0.27</td>
</tr>
<tr>
<td>B2</td>
<td>NiDEC</td>
<td>50.00</td>
<td>42.25</td>
<td>54.64 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>[26.69]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>6.56</td>
<td>22.86 ± 1.21</td>
</tr>
<tr>
<td>B3</td>
<td>NiDEC</td>
<td>25.00</td>
<td>16.60</td>
<td>26.71 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>[23.40]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>11.10</td>
<td>24.39 ± 0.53</td>
</tr>
<tr>
<td>B4</td>
<td>NiDEC</td>
<td>12.50</td>
<td>5.82</td>
<td>12.91 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>[16.43]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>15.62</td>
<td>24.95 ± 0.32</td>
</tr>
<tr>
<td>B5</td>
<td>NiDEC</td>
<td>6.25</td>
<td>1.27</td>
<td>4.90 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>NiDEC-DBC</td>
<td>-</td>
<td>[8.39]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NiDBC</td>
<td>25.00</td>
<td>18.65</td>
<td>23.42 ± 0.53</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 60°C and 330 nm on XBridge C8 column. K = 2.92.

Based on the results in these tables, the absolute errors found to be in between 0.05 and 7.55 µg/ml. The highest deviation being found on the highest concentrations suggesting the relative error would be similar in each case, which probably in the range of the experimental error.

From this experiment, it can be concluded that the determination of zinc dithiocarbamates using the method of on-column derivatisation for the zinc dithiocarbamate mixture to nickel complexes is possible with the assumption that the equation 5.6 and 5.7 are true. The determination of a mixture of more than two compounds might be more complicated. However, by assuming the extinction coefficient it could be possible to estimate a reasonable value even in more complex mixtures. As most rubber samples only contained a maximum of two zinc dithiocarbamates, thus this method is already applicable.
5.8. Summary

Further studies to optimize the on-column derivatisation method were carried out, which included the effect of nickel salt concentration, pH of the mobile phase, type of organic solvent, methanol composition, temperature, flow rate and ZDTC mixture composition.

Increasing the metal concentration from 0.001% to 0.05% of Ni (II) in the mobile phase has gradually increased the retention time of the dithiocarbamate complexes but had not much effect on other chromatographic parameters. The relationship between retention factor ($k$) and log nickel salt concentration was also linear. This is probably because of higher metal concentrations had pushed the metal conversion between zinc and nickel further to completion, and hence increase the retention. Since 0.05% of nickel were found to be more difficult to dissolve and could therefore give a problem on usage, 0.01% of Ni (II) was selected for further studies although 0.05% showed better results.

A study on the effect of pH of mobile phase using the Ultrasphere ODS column without alteration of the pH showed that the separation was very stable. However, the same study at higher pH value (pH 11.00) using a high-pH stable column, XBridge C8, showed that nickel dithiocarbamates became unstable. This was probably because of the presence of ammonia as previous authors [21] reported that ammonia has a strong effect on the stability of the nickel complexes. Therefore, it was decided not to alter the pH of the mobile phase.

As for the study on the effect of type of organic solvent used for mobile phase mixture, it was shown that methanol-water mixture gave the best peak shapes and separation for the on-column derivatisation of ZDTCs to NiDTCs. A further study on the effect of methanol composition found that 70% methanol-water with 0.01% Ni (II) is probably the best composition for this method as it was the lowest possible composition that can detect the highly retained nickel complexes (NiDBC and NiBEC) at the reasonable resolution.

The study on the effect of temperature at a wider range was carried out
at the selected conditions only up to 80°C as the condition of the XBridge C8 column was already poor. In this study, a relationship between log k and temperature (van’t Hoff plot) showed slight deviation from linearity about 50-60°C, which indicated a transition phase phenomenon that was probably related to almost every chromatographic variable. Increasing the temperature though has little effect to the efficiency of ZDEC and ZDBC but showed a reduction in the mixed ligand complex of NiDEC-DBC. However, a study on the effect of temperature on the analysis of dithiocarbamates should be further investigated as no real conclusion can be made. Nevertheless, 70°C was proposed to be used for the determination of rubber accelerators in rubber products in the next chapter, as under these condition nickel complexes had lower retention with acceptable other chromatographic characteristics.

A study on the effect of flow rate, which was carried out at four different flow rates and two different temperatures showed a slight reduction in the retention factor values as the flow rate increased. However, a more significant reduction on the retention factors was found as the temperature increased, which more apparent for a more retained complex like ZDBC. The optimum flow rate at 70°C was about 1 ml/min while at 40°C no clear value was found. However, the column efficiencies of most compounds at 70°C were poorer compared to the value at 40°C. This was probably because of the fact that the two earlier peaks (NiDEC and NiDEC-DBC), which were the less retained compounds were already closed to the dead volume. However, 1 ml/min was found to be the reasonable flow rate for this method. Thus, the recommended conditions for the determination of ZDTCs in rubber products by the on-column derivatisation method were; 70% methanol-water with 0.01% Ni (II) at 1 ml/min, temperature gradient from 40-70°C or isocratic temperature of 70°C, UV detection of 330 nm on XBridge C8 or any other similar column. Because of time and equipment limit, an isocratic temperature of 70°C on XBridge C8 column will be used for the determination of ZDTCs in rubber products (Chapter 6).

The final study in this chapter was on the effect of ZDTC mixture
concentration on the mixed ligand complex formation. The results showed that as the concentration of ZDEC or ZDBC increased the response for all nickel compounds including the mixed ligand complex of NiDEC-DBC were also changed, which suggested that an equilibrium reaction took place. The equilibrium constant (K) was calculated and found to be about 2.92 ± 0.72 at 95% confidence level. A back calculation of the original concentration of the mixture was also carried out using the calculated K value. The absolute errors compared to the prepared amount were found to be in between 0.05 to 7.55 μg/ml (0.20 to 21.66%). Hence, it can be concluded that the determination of a mixture of zinc dithiocarbamates using on-column derivatisation method to nickel (II) complexes is still possible even though there is an extra peak of the mixed ligand complex. Although a mixture of more than two types of complexes might be more complicated, this study showed that the method was applicable to the real samples as the rubber samples usually have a maximum of two zinc dithiocarbamates.
CHAPTER 6 : DETERMINATION OF ZINC DITHIOCARBAMATES IN RUBBER PRODUCTS

6.0. Introduction

Using the optimized conditions found for the on-column derivatisation of zinc dithiocarbamates to nickel dithiocarbamates, the determination of ZDTCs in a series of rubber products was carried out and compared with the pre-column derivatisation to copper (II) and nickel (II) complexes.

Initially, all the three methods were compared based on the criteria of selectivity, linearity, precision, accuracy, and sensitivity. The methods were then used to quantitate the amount of ZDTCs in six rubber products, which include nitrile gloves (Sample 1 – 3), natural rubber latex gloves (Sample 4 and 5), and multicoloured balloon (Sample 6). All samples except sample 6 had already been quantified by TARRC [176] using the method of pre-column derivatisation to copper (II) complexes.

Finally, the comparison was presented between the three methods used for the determination of rubber accelerators (pre-column derivatisation to copper (II) complexes, pre-column derivatisation to nickel (II) complexes, and on-column derivatisation to nickel (II) complexes).

6.1. Using pre-column derivatisation to copper (II) complexes

In Chapter 3, a few previously reported methods [23, 25, 78, 176] have been investigated. It was found that the method adopted by TARRC [176] (section 2.5.1), which used a pre-column derivatisation technique to copper complexes gave efficient separation with no mixed ligand complexes of ZDTC mixture. Thus, it was chose to be one of the methods for the determination of ZDTCs in real samples of rubber products.

Using this method, they have claimed that the Limit of Detection (LOD) and Limits of Quantitation (LOQ) for all ZDTCs were 0.0003% and 0.0005%
respectively. The same method using our current system was validated and reported in the next section. The method was then used to quantitate the amount of ZDTCs in rubber products.

6.1.1. Method validation

The method used was validated based on the criteria of selectivity, linearity, precision, accuracy and sensitivity [177]. Amongst nine concentrations of ZDEC, ZDBC and mixtures of the two, which ranged between 0.40 to 101.73 μg/ml for ZDEC and between 0.44 to 111.83 μg/ml for ZDBC, only four and three concentrations, respectively, of the higher levels were detectable using the method. Detection at 435 nm, which was specific for copper dithiocarbamates, makes the method quite selective. However, other compounds like thiuram disulfides might also cause interference to the results as they were reported [21] to react with metals and produced metal dithiocarbamates. Tetraethylthiuram disulfide (TETD), for example, will produce copper diethyldithiocarbamate (CuDEC) when mixed with copper (II) ions. Therefore, if present in the sample extract, they might also interfere during the derivatisation steps and be converted to copper complexes. Moreover, only the two highest concentrations were detected for a mixture of ZDEC and ZDBC. The overall method was linear, based on the calibration graph of ZDEC (Fig. 6.1). The calibration curve for ZDBC (Fig. 6.2), however, gave lower correlation coefficient value. Both calibration curves also showed that the method has a constant systematic negative bias. The deviations were probably because of the un-recovered amounts of dithiocarbamates by copper during the derivatisation steps of ZDTCs to CuDTCs. Table 6.1 shows the results of method validation in terms of accuracy and precision. Good precision of the method were found which ranged from 0.49 to 5.13% for both ZDEC and ZDBC compounds.
Chapter 6: Determination of Zinc Dithiocarbamates in Rubber Products

From the same calibration curves shown in Fig. 6.1 and Fig. 6.2, the values of LOD and LOQ were calculated as per equation 2.9 and 2.10 for ZDEC and ZDBC (Table 6.2). Comparison of the observed LOD and LOQ values (Table 6.2) with the values reported by TARRC [176] as mentioned...
earlier, however, showed a very big difference. It is maybe because of either a difference in the instrument used or the difficulty of the method itself to be performed by anybody else (poor transferability). It was found that the current HPLC system used for the determination of rubber accelerators produced a lower sensitivity compared to the HPLC system used by TARRC.

### Table 6.1
Accuracy and precision data for the determination of ZDEC and ZDBC after pre-column derivatisation to copper complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared ZDTC concentration (µg/ml)</th>
<th>Observed concentration (µg/ml) ± SD (n=6)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDEC/CuDEC</td>
<td>12.72</td>
<td>15.07 ± 0.49</td>
<td>3.24</td>
</tr>
<tr>
<td></td>
<td>25.43</td>
<td>23.35 ± 1.00</td>
<td>4.27</td>
</tr>
<tr>
<td></td>
<td>50.86</td>
<td>49.86 ± 1.84</td>
<td>3.70</td>
</tr>
<tr>
<td></td>
<td>101.73</td>
<td>102.46 ± 3.18</td>
<td>3.10</td>
</tr>
<tr>
<td>ZDBC/CuDBC</td>
<td>27.96</td>
<td>32.28 ± 0.43</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>55.91</td>
<td>49.43 ± 0.24</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>111.83</td>
<td>113.98 ± 5.84</td>
<td>5.13</td>
</tr>
</tbody>
</table>

HPLC conditions: Gradient elution (as in Fig. 3.12) at 1 ml/min, 40°C and 435 nm on XBridge ODS column.

### Table 6.2
Limit of detection (LOD) and limit of quantitation (LOQ) for ZDEC and ZDBC after pre-column derivatisation to copper (II) complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (µg/ml)</th>
<th>(%)</th>
<th>LOQ (µg/ml)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDEC/CuDEC</td>
<td>7.15</td>
<td>0.0007</td>
<td>23.83</td>
<td>0.0024</td>
</tr>
<tr>
<td>ZDBC/CuDBC</td>
<td>24.24</td>
<td>0.0024</td>
<td>80.79</td>
<td>0.0081</td>
</tr>
</tbody>
</table>

HPLC conditions: Gradient elution (as in Fig. 3.12) at 1 ml/min, 40°C and 435 nm on XBridge ODS column.

#### 6.1.2. Quantitation of ZDTCs in rubber products

Quantitation of ZDTCs in rubber products used the method described earlier, where the samples were first extracted using Soxhlet method in DCM (section 2.1.3), and the concentrated sample solution was derivatised to the copper complexes as described in section 2.5.1.

Using the calibration graphs showed earlier (Fig. 6.1 and Fig. 6.2), the
amounts of ZDTCs in a set of 6 rubber samples (as listed in section 2.1.3) were quantitated. The levels of ZDTCs found in each sample are given in Table 6.3. All determinations were carried out in duplicate using two separate rubber product pieces from the same lot of each sample. An example of chromatograms is shown in Fig. 6.3 (Sample 6-2).

Table 6.3
Comparison of reported [176] and observed ZDTC values found in rubber samples after Soxhlet extraction in DCM and pre-column derivatisation to copper (II) complexes

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Type of ZDTCs</th>
<th>Reported values [176]</th>
<th>Observed values ± SD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZDEC</td>
<td>0.4400%</td>
<td>0.6008 ± 0.0011%</td>
</tr>
<tr>
<td>2</td>
<td>ZDBC</td>
<td>0.0027%</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>3</td>
<td>ZDEC</td>
<td>0.0550%</td>
<td>0.0281 ± 0.0029%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.2173%</td>
<td>0.1440 ± 0.0177%</td>
</tr>
<tr>
<td>4</td>
<td>ZDEC</td>
<td>0.0100%</td>
<td>0.0190 ± 0.0012%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0021%</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>No residues detected</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6-1</td>
<td>ZDEC</td>
<td>Not measured</td>
<td>0.0808 ± 0.0002%</td>
</tr>
<tr>
<td>6-2</td>
<td>ZDEC</td>
<td>Not measured</td>
<td>0.1137 ± 0.0010%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>Not measured</td>
<td>0.1846 ± 0.0012%</td>
</tr>
</tbody>
</table>

**Gradient program as in Fig. 3.12 at 2 ml/min, 50°C and 435 nm on 5µ ODSB column using Dionex HPLC system and Chromeleon software (version 6.5) for data analysis. [176]

**Same gradient program at 1 ml/min, 40°C and 435 nm on XBridge ODS column using HPLC system 2 (HP 1100 as in Fig. 2.2) and Agilent ChemStation software (Rev. A.10.02) for data analysis. ND = not detected.

Although the ZDTC values found in all rubber sample extracts were somewhat different from the values reported by TARRC [176], the same components were found in both studies. The amount of ZDEC found in sample 1 and sample 4 extract were higher than reported values while the sample 3 extracts were lower. The amount of ZDBC found in sample 2 was lower than the quantitation limit while none was detected in sample 4. However, both methods did not detect any ZDTC in sample 5. The amount of ZDTCs found in sample 6, which was the multicolored balloon was also different for each piece of sample extracted. The only difference in this sample was because they were in different color. One was red and the other was pink. As no details were known about the rubber accelerators present in the sample, the results for each piece of rubber balloon extract were treated...
as an individual sample. Only ZDEC was found in sample 6-1 while both ZDEC and ZDBC were present in sample 6-2. The amount of ZDEC in each piece of sample was also different, where one was 0.0808% and the other was 0.1846%.

6.2. Determination of ZDTCs in rubber products by pre-column derivatisation to nickel (II) complexes

Pre-column derivatisation to nickel (II) complexes (section 2.5.2) was used to be one of the methods used for the determination of ZDTCs in rubber products in order to make the comparison between pre- and on-column derivatisation to the same nickel (II) complexes using real samples. The method was initially validated using the standard solution series as listed in section 2.5.1.
6.2.1. Method validation

This method was as specific as the previous method of pre-column derivatisation to copper (II) complexes, since the derivatisation of ZDTCs was specific to nickel complexes and detection at 330 nm, which was also specific for nickel complexes. Furthermore, the spectrum of each peak was checked with the reference spectrum of nickel complex. The spectra of all the NiDTCs were the same. However, the differences in the retention of each compound could be used to identify the compound. The retention times for NiDEC and NiDBC by pre-column derivatisation method using 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 70°C and 330 nm on Gemini ODS column were 1.51 ± 0.04 and 10.35 ± 0.73 min, respectively. The retention of the mixed ligand complexes of NiDEC-DBC on the other hand was 3.51 ± 0.18 min. An example chromatogram of a mixture of ZDEC and ZDBC after pre-column derivatisation to NiDTCs is given in Fig. 6.4.

Fig. 6.4. Example chromatogram of a mixture of ZDEC and ZDBC after pre-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 µl injection, 70°C and 330 nm on Gemini ODS column.

The calibration graph of both NiDEC (Fig. 6.5) and NiDBC (Fig. 6.6) using Gemini ODS column on HPLC system 2 (HP1100 with DAD-Fig. 2.2) seems to be non-linear curves. Yet, the same method calibrated using an XBridge C8 column on the same HPLC system was linear (Fig. 6.7). The
reason for the non-linearity was probably because the Gemini ODS column was a narrow bore column (2.0 x 150 mm, 5 μm), which might have a proportionally greater metal surface than the XBridge C8 column (4.6 x 50 mm, 3.5 μm). Thus, the interference from the column metal surface might cause the non-linearity of those calibration graphs. However, the calibrations of ZDTCs by this method using both columns seem to give very high negative y-intercept (systematic error). This systematic error was probably because of the loss of some analytes during the derivatisation process as well as the use of the HPLC system 2 (HP1100 with DAD-Fig. 2.2), as this system was new to the method. The same method using the XBridge C8 column on the HPLC system 1 (Fig. 2.1), also gave a linear graph, but with a 10 fold lower systematic error. Thus, there may be interferences from the metal parts of the system, or copper ions that perhaps trapped in the system, as the same system was previously used for the determination of ZDTCs using pre-column derivatisation to copper complexes.

\[
y = 22.457x - 134.16 \\
R^2 = 0.9789
\]

Fig. 6.5. Calibration curve for ZDEC after pre-column derivatisation to NiDEC using 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 μl injection, 70°C and 330 nm on Gemini ODS column.
Fig. 6.6. Calibration curve for ZDBC after pre-column derivatisation to NiDBC using 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 μl injection, 70°C and 330 nm on Gemini column.

The line equation is:

\[ y = 22.879x - 252.9 \]

\[ R^2 = 0.9943 \]

Fig. 6.7. Calibration curves for ZDBC after pre-column derivatisation to NiDBC using 70% methanol-water with 0.01% Ni (II) at 70°C and 330 nm on (a) Gemini ODS column - 0.6 ml/min, 5 μl injection and (b) XBridge C8 column - 1 ml/min, 10 μl injection.

(a) Gemini C18
\[ y = 0.1038x^2 + 9.8627x - 85.066 \]
\[ R^2 = 0.9997 \]

(b) XBridge C8
\[ y = 18.485x - 239.51 \]
\[ R^2 = 0.9993 \]

Table 6.4 shows the results of method validation in terms of accuracy and precision. The accuracy of the method was calculated based on the recovery of the standard series after pre-column derivatisation to nickel complexes using the calibration functions of the reference NiDEC (y =
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31.508x + 17.085) and NiDBC (y = 22.842x + 16.838). Based on these two equations and the responses of the standard series, the observed concentration ($x_p$) was calculated. The data $x_p$ were then plotted against the original concentration ($x_0$), which then gave the recovery function of $x_p = 0.7127x_0 - 4.8001$ for NiDEC and $x_p = 1.0016x_0 - 11.809$ for NiDBC. The negative values of y-intercept again showed that there were constant negative systematic errors as discussed before. The slopes of the recovery function were also not equal to one, which means that there were proportional systematic errors. Negative observed concentrations were also found for the lowest detectable values, which could mean the false positive detection for this particular method. However, good precision of the method was found, where standard deviation and %CV ranged from 0 to 2.78 µg/ml and 0.17 to 9.09% (disregard the negative values), respectively.

Table 6.4  
<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared concentration $x_0$ (µg/ml)</th>
<th>Observed concentration $x_p$ (µg/ml)</th>
<th>± SD (µg/ml, n=6)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDEC/NiDEC</td>
<td>50.87</td>
<td>32.91</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>25.43</td>
<td>10.89</td>
<td>0.14</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>12.72</td>
<td>2.51</td>
<td>0.02</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>6.36</td>
<td>0.22</td>
<td>0.01</td>
<td>4.38</td>
</tr>
<tr>
<td></td>
<td>3.18</td>
<td>[-0.28]</td>
<td>0.00</td>
<td>[-1.65]</td>
</tr>
<tr>
<td>ZDBC/NiDBC</td>
<td>111.83</td>
<td>101.15</td>
<td>2.78</td>
<td>2.74</td>
</tr>
<tr>
<td></td>
<td>27.96</td>
<td>11.77</td>
<td>0.19</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>13.98</td>
<td>1.28</td>
<td>0.12</td>
<td>9.09</td>
</tr>
<tr>
<td></td>
<td>6.99</td>
<td>[-0.42]</td>
<td>0.04</td>
<td>[-8.45]</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (ii) at 0.6 ml/min, 5 µl injection, 70°C and 330 nm on Gemini ODS column.

From the calibration curves shown in Fig. 6.5 and Fig. 6.6, the values of LOD and LOQ were calculated, and shown in Table 6.5. LOD and LOQ were calculated using equations 2.9 and 2.10. Comparison between the observed LOD and LOQ values with the reported values (LOD = 0.0003% and LOQ = 0.0005% for all ZDTCs) [176] showed a big difference, where this current method (pre-column derivatisation to nickel complexes) seems to have lower...
sensitivity for both ZDEC and ZDBC compounds. However, comparing the LOD and LOQ values for ZDBC, it showed that this method was twice as sensitive as the method of pre-column derivatisation to copper complexes (Table 6.2).

Table 6.5
Limit of detection (LOD) and limit of quantitation (LOQ) for ZDEC and ZDBC after pre-column derivatisation to nickel (II) complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>%</td>
</tr>
<tr>
<td>ZDEC/NiDEC</td>
<td>9.86</td>
<td>0.0010</td>
</tr>
<tr>
<td>ZDBC/NiDBC</td>
<td>13.49</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 µl injection, 70°C and 330 nm on Gemini ODS column.

6.2.2. Quantitation of ZDTCs in rubber products

Quantitation of ZDTCs in rubber products using the pre-column derivatisation method to nickel (II) complexes was as described in Chapter 2. Rubber products were first extracted using Soxhlet method in DCM (section 2.1.3). The sample extract solutions were the same solutions used for the determination using pre-column derivatisation to copper (II) complexes. However, the concentrated sample solutions were derivatised to nickel (II) complexes as described in section 2.5.2.

The results for the sample extracts were then quantitated against the calibrations graphs showed earlier (Fig. 6.5 and Fig. 6.6). For the samples that contained two types of ZDTCs, the calculation was carried out as described in section 5.7. Then, the concentration of ZDTCs found in the sample extracts were converted to the percentage composition of ZDTCs per gram of sample. The levels of ZDTCs found in each sample are given in Table 6.6.
Table 6.6
Comparison of reported [176] and observed ZDTC values found in rubber samples after Soxhlet extraction in DCM and pre-column derivatisation to copper (II) complexes (reported) and to nickel (II) complexes (observed)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Type of ZDTCs</th>
<th>Reported values [176]*</th>
<th>Observed values ± SD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZDEC</td>
<td>0.4400%</td>
<td>1.9528 ± 0.0607%</td>
</tr>
<tr>
<td>2</td>
<td>ZDEC</td>
<td>ND</td>
<td>0.0902 ± 0.0014%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0027%</td>
<td>0.1762 ± 0.0766%</td>
</tr>
<tr>
<td>3</td>
<td>ZDEC</td>
<td>0.0550%</td>
<td>0.0444 ± 0.0185%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.2173%</td>
<td>0.0732 ± 0.0024%</td>
</tr>
<tr>
<td>4</td>
<td>ZDEC</td>
<td>0.0100%</td>
<td>0.0592 ± 0.0051%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0021%</td>
<td>0.0381 ± 0.0023%</td>
</tr>
<tr>
<td>5</td>
<td>ZDEC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZPD</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>ZDBC</td>
<td>Not measured</td>
<td>0.0859 ± 0.0118%</td>
</tr>
</tbody>
</table>

*The HPLC conditions are as in Table 6.3.

**HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 µl injection, 70°C and 330 nm on Gemini ODS column. ND = Not detected. NQ = Not quantitated.

All determinations were carried out in duplicate using two separate rubber product pieces from the same lot of each sample. Example chromatograms of the selected samples are shown in Fig. 6.8 (Sample 2), Fig. 6.9 (Sample 5), and Fig. 6.10 (Sample 6). Chromatograms for the other samples were similar correspond to their levels of ZDTCs that were present.

The amount of ZDTCs found in all rubber samples using pre-column derivatisation to nickel (II) complexes were yet again different from the reported values [176]. As observed by the pre-column derivatisation method to copper (II) complexes (section 6.1.2), the amount of ZDTCs found in sample 1 and sample 4 were higher than the reported values, while sample 3 was lower. Although no residues were previously (section 6.1.2) detected in sample 2 and 5 using the pre-column derivatisation method to copper complexes, both ZDEC and ZDBC compounds were detected using this method. The ZPD compound was also detected in sample 5 as a mixed ligand complex between pentamethylene- and dibutyl-dithiocarbamate (NiPD-DBC).
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Fig. 6.8. Chromatogram of sample extract no. 2 containing a mixture of ZDEC and ZDBC after pre-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 μl injection, 70°C and 330 nm on Gemini ODS column.

Fig. 6.9. Chromatogram of sample extract no. 5 containing a mixture of ZDEC, ZPD and ZDBC after pre-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 5 μl injection, 70°C and 330 nm on Gemini ODS column. NiDEC, NiPD and a mixed complex of NiDEC-PD overlapped with each other in the first peak.
However, the amount of ZDTCs found in sample 5 not quantitated as no calibration of ZPD using this method had been carried out, and the equilibrium constant had not been determined for the mixture of both ZDEC and ZDBC with ZPD. The presence of two competing equilibrium could also complicate the calculation. Both pieces of sample 6, on the other hand, only contained ZDBC with an average amount of 0.0859%.

6.3. Determination of ZDTCs in rubber products by on-column derivatisation to nickel (II) complexes

Based on the optimized conditions found in Chapter 5, the determination of rubber accelerators (zinc dithiocarbamates) using on-column derivatisation was carried out. The method was initially validated based on the same criteria as in the previous section.
6.3.1. Method validation

Since the on-column derivatisation method also used nickel complexes, the selectivity of this method with the previous one is the same. The identification of the NiDTC peaks in terms of the retention time, however, was different as this method used different flow rate and column, which was 1 ml/min using XBridge C8 (4.6 x 50 mm) column from 0.6 ml/min using Gemini ODS (2.0 x 150 mm) column. The retention times for NiDEC and NiDBC by the on-column derivatisation method using the XBridge C8 column were 0.99 ± 0.01 min and 4.36 ± 0.12 min respectively. The retention of the mixed ligand complexes of NiDEC-DBC was 1.83 ± 0.03 min (Fig. 6.11). Although ZDMC, ZPD and ZBEC were originally not detected in the samples, the method validation for these compounds was still carried out. Their retention times were 0.73 ± 0.00, 1.05 ± 0.01, and 4.60 ± 0.10 min for ZDMC, ZPD and ZBEC respectively. The overlay of their individual chromatograms is shown in Fig. 6.12. From these results, the quantitation of a mixture of some of the ZDTCs under these particular conditions would be difficult as the retention times of some of ZDTCs are close to each other.

As shown in Fig. 6.13 and Table 6.7, the method was linear for all the dithiocarbamate compounds with relatively low systematic negative errors. The $R^2$ values found for all the calibration curves were between 0.9991 and 0.9998.
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Fig. 6.11. Example chromatogram of a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 10 μl injection, 70°C and 330 nm on XBridge C8 column.

Fig. 6.12. Overlay chromatograms of ZDMC, ZPD and ZBEC after on-column derivatisation to their respective NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 10 μl injection, 70°C and 330 nm on XBridge C8 column.
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Fig. 6.13. Calibration curves for ZDTCs after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.

Table 6.7
Regression parameters of the calibration curves in Fig. 6.13

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>Intercept</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDMC</td>
<td>35.96</td>
<td>-27.07</td>
<td>0.9996</td>
</tr>
<tr>
<td>ZDEC</td>
<td>31.18</td>
<td>-26.09</td>
<td>0.9998</td>
</tr>
<tr>
<td>ZPD</td>
<td>29.21</td>
<td>-13.92</td>
<td>0.9997</td>
</tr>
<tr>
<td>ZDBC</td>
<td>23.11</td>
<td>-21.27</td>
<td>0.9998</td>
</tr>
<tr>
<td>ZBEC</td>
<td>15.63</td>
<td>-21.62</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min and 330 nm on XBridge C8 column.

Compared to the previous method of pre-column derivatisation, this new method of on-column derivatisation to nickel complexes was more accurate and precise (Table 6.8). The same method and calibration function of references to validate the accuracy and precision of the method was used, where the recovery functions of both compounds found to be \( x_p = 0.9291x_0 - 0.572 \) for NiDEC, and \( x_p = 1.0000x_0 - 7E-06 \) for NiDBC. The negative value of the y-intercept showed that there was little negative systematic error in this method. The slope of the recovery function for NiDEC was not equal to one, which meant that the method still had a proportional systematic error. Because of this proportional error, the recovery rate (equation 2.8) for NiDEC/ZDEC compound reduced as the concentration reduced. The
recovery rate reduced from 92.35% to 56.92% when the concentration decreased from 101.73 to 1.59 μg/ml. This is probably because of the high temperature used (70°C), as the ZDEC compound was previously found to separate better at 40°C or lower. However, very good precision of the method was found which ranged from 0.09 to 2.85%. On the other hand, the ZDBC compound was fully recovered. No calculations for the accuracy and precision of the method for ZDMC, ZPD and ZBEC were carried out, as there are no reference nickel complexes for these dithiocarbamates.

Table 6.8
Accuracy and precision data for the determination of ZDEC and ZDBC after pre-column derivatisation to nickel complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Prepared concentration (x_0) (μg/ml)</th>
<th>Observed concentration (x_o) (μg/ml)</th>
<th>± Standard deviation (n=6)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDEC/NiOEC</td>
<td>101.73</td>
<td>92.69</td>
<td>0.44</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>50.87</td>
<td>49.20</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>25.43</td>
<td>23.42</td>
<td>0.04</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>12.72</td>
<td>11.01</td>
<td>0.04</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>6.36</td>
<td>4.86</td>
<td>0.03</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>3.18</td>
<td>1.87</td>
<td>0.02</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>1.59</td>
<td>0.52</td>
<td>0.01</td>
<td>2.85</td>
</tr>
<tr>
<td>ZDBC/NiOBC</td>
<td>111.83</td>
<td>111.63</td>
<td>0.67</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>55.92</td>
<td>56.23</td>
<td>0.42</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>27.96</td>
<td>28.37</td>
<td>0.24</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>13.98</td>
<td>13.65</td>
<td>0.12</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>6.99</td>
<td>6.71</td>
<td>0.09</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>3.49</td>
<td>3.39</td>
<td>0.07</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.94</td>
<td>0.05</td>
<td>2.61</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.

From the calibration curves shown in Fig. 6.13, the values of LOD and LOQ were calculated for all five zinc dithiocarbamates (ZDEC, ZDBC, ZDMC, ZPD and ZBEC) after on-column derivatisation to nickel (II) complexes as shown in Table 6.9. From this table, it shows that the current method of on-column derivatisation is more sensitive than the method of pre-column derivatisation either to copper (section 6.1) or nickel complexes (section 6.2). Furthermore, the same method using the same column on the HPLC system
1 was more sensitive (Fig. 6.14). This is probably because the HPLC system 1 was already saturated with nickel ions when it was used for the method optimization. Another reason would be, because of the difference in the detector used in each system, where, the HPLC system 1 used the conventional UV detector and HPLC system 2 used the diode array detector. In this particular case, the detector in the HPLC system 1 might have been set to a condition that is making it more sensitive.

Table 6.9  
LOD and LOQ for ZDEC and ZDBC after on-column derivatisation to nickel (II) complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD µg/ml</th>
<th>%</th>
<th>LOQ µg/ml</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDMC/NiDMC</td>
<td>1.31</td>
<td>0.0001</td>
<td>4.36</td>
<td>0.0004</td>
</tr>
<tr>
<td>ZDEC/NiDEC</td>
<td>0.90</td>
<td>0.0001</td>
<td>3.02</td>
<td>0.0003</td>
</tr>
<tr>
<td>ZPDK/NiPD</td>
<td>1.16</td>
<td>0.0001</td>
<td>3.86</td>
<td>0.0004</td>
</tr>
<tr>
<td>ZDBC/NiDBC</td>
<td>0.86</td>
<td>0.0001</td>
<td>2.88</td>
<td>0.0003</td>
</tr>
<tr>
<td>ZBEC/NiBEC</td>
<td>1.99</td>
<td>0.0002</td>
<td>6.62</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 10 µl injection, 70°C and 330 nm on XBridge C8 column.

Fig. 6.14. Calibration curve for ZDEC after on-column derivatisation to NiDEC using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column by (a) HPLC system 1 and (b) HPLC system 2.
6.3.2. Quantitation of ZDTCs in rubber products

The quantitation of ZDTCs in the 6 rubber samples (Sample 1 - 6) using the on-column derivatisation method to nickel complexes was carried out as in section 2.5.3. The sample extract solutions were the same solutions used for the previous determination using pre-column derivatisation to copper (II) or nickel (II) complexes, which had been previously extracted using Soxhlet method in DCM (section 2.1.3). All the determinations were carried out in triplicate using two separate rubber product pieces from the same lot of each sample. Table 6.10 shows the levels of ZDTCs found in all the rubber samples. Chromatograms of each sample are shown in Fig. 6.15 (Sample 1), Fig. 6.16 (Sample 2), Fig. 6.17 (Sample 3), Fig. 6.18 (Sample 4), Fig. 6.19 (Sample 5), Fig. 6.20 (Sample 6-1), and Fig. 6.21 (Sample 6-2).

### Table 6.10

Comparison of reported [176] and observed ZDTC values found in rubber samples after Soxhlet extraction in DCM and pre-column derivatisation to copper (II) complexes (reported) and on-column derivatisation to nickel (II) complexes (observed)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Type of ZDTCs</th>
<th>Reported values [176]*</th>
<th>Observed values ± SD**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZDEC</td>
<td>0.4400%</td>
<td>1.6302 ± 0.0164%</td>
</tr>
<tr>
<td>2</td>
<td>ZDEC</td>
<td>ND</td>
<td>0.0264 ± 0.0028%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0027%</td>
<td>0.0532 ± 0.0035%</td>
</tr>
<tr>
<td>3</td>
<td>ZDEC</td>
<td>0.0550%</td>
<td>0.0405 ± 0.0021%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.2173%</td>
<td>0.2972 ± 0.0149%</td>
</tr>
<tr>
<td>4</td>
<td>ZDEC</td>
<td>0.0100%</td>
<td>0.0236 ± 0.0011%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0021%</td>
<td>0.0124 ± 0.0004%</td>
</tr>
<tr>
<td>5</td>
<td>ZDEC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZPD</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6 - 1</td>
<td>ZDEC</td>
<td>Not measured</td>
<td>0.0168 ± 0.0003%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>Not measured</td>
<td>0.1855 ± 0.0024%</td>
</tr>
<tr>
<td>6 - 2</td>
<td>ZDBC</td>
<td>Not measured</td>
<td>0.0339 ± 0.0000%</td>
</tr>
</tbody>
</table>

HPLC conditions: 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column. ND = Not detected and NQ = Not quantitated.

The amounts of ZDTCs found in all the rubber samples using the on-column derivatisation method to nickel complexes were often considerably higher than the reported values [176], which were between non-detectable to 0.4400%, except the ZDEC value for sample 3, which was lower. The higher
values reported by this method could mean that it is more sensitive and/or because of the ability of nickel ions to chelate with both zinc dithiocarbamates as well as any free dithiocarbamate ligands present in the rubber samples. As in section 6.2, both ZDEC and ZDBC were detected in sample 2. However, only a mixed ligand complex of NiPD-DBC was detected in sample 5. This could mean that the dithiocarbamate ligands of pentamethylene (PD) and dibutyl (DBC) were there in sample 5 either as free ligands or as zinc complexes. The appearance of only one peak of mixed ligand complexes also means that the separation using XBridge C8 column (4.6 x 50 mm) was not efficient as the Gemini ODS column (2.0 x 150 mm) in the section 6.2. Gemini ODS column not only was a bonded column with longer chain, but also was a narrow bore with a longer column length. Therefore, by using the XBridge C8 column, the efficiency and resolution of the peaks were lower, which subsequently has reduced the sensitivity of this method. The amount of ZDTCs found in sample 5, however, was not quantitated as no equilibrium constant was calculated for the mixture of both ZPD and ZDBC, which is needed to calculate the amount of distribution of NiPD-DBC. As in the pre-column derivatisation method to copper complexes (section 6.1.2), different pieces of balloon gave different results. Both ZDEC and ZDBC compounds were found in sample 6-1, while only ZDBC compound was found in sample 6-2.
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Fig. 6.15. Chromatogram of sample extract no. 1 containing ZDEC after on-column derivatisation to NiDEC using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.

Fig. 6.16. Chromatogram of sample extract no. 2 containing a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.
Fig. 6.17. Chromatogram of sample extract no. 3 containing a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.

Fig. 6.18. Chromatogram of sample extract no. 4 containing a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.
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Fig. 6.19. Chromatogram of sample extract no. 5 containing a mixture of ZDEC, ZPD and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column. Only the mixed ligand complex of NiPD-DBC was detected using this method.

Fig. 6.20. Chromatogram of sample extract no. 6-1 containing a mixture of ZDEC and ZDBC after on-column derivatisation to NiDTCs using 70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.
6.4. Comparison of the methods used for the determination of rubber products

Three methods chosen for the determination of rubber products were; (1) pre-column derivatisation to copper (II) complexes (section 6.1), (2) pre-column derivatisation to nickel (II) complexes (section 6.2), and (3) on-column derivatisation to nickel (II) complexes (section 6.3).

The method of pre-column derivatisation to copper (II) complexes gave efficient separation and did not produce any mixed ligand complexes. However, the baseline became noisier and gradually drifted towards the end of the separation. Although the same method reported by TARRC [176] had low detection and quantitation limits (Table 6.11), we observed poorer values, which probably because of the lower sensitivity of the system used. Moreover, comparison between the three methods using the same system showed that this particular method was the least sensitive (Table 6.11). This method also often detected fewer amounts of ZDTCs in rubber samples (Table 6.12).
compared to the other two methods, except for ZDBC in sample 3 and ZDTCs in sample 6.

Although the method requires further validation using a better column, pre-column derivatisation to nickel (II) complexes method seems to be more sensitive as it showed lower detection and quantitation limits for ZDBC (Table 6.11) and is able to detect considerably higher amount of ZDTCs in most samples, including sample 2 and 5, which no ZDTC was detected by pre-column derivatisation to copper (II) complexes method (Table 6.12). However, both methods of pre-column derivatisation underwent sample preparation, which was time consuming, tedious and used quite a lot of solvent. Furthermore, the method of pre-column derivatisation to copper complexes used higher amount of organic solvent (up to 100% acetone) for the separation of the complexes. Both methods also confirmed a tendency of losing the analytes during the derivatisation process or decomposition of the metal complexes after producing high systematic negative errors for their calibration curves (section 6.1.1 and 6.2.1).

Comparison of the ZDTC values found in the multicoloured balloon (Table 6.12– sample 6) between the three methods used showed no correlation. Since the amount of ZDTCs in this sample was not previously measured, both pieces (sample 6-1: red colour and sample 6-2: pink colour) were treated as an individual sample. Using the pre-column derivatisation to copper (II) complexes, only ZDEC was found in sample 6-1, while both ZDEC and ZDBC were found using the on-column derivatisation to nickel (II) complexes. On the contrary, both ZDEC and ZDBC were found in sample 6-2 using the earlier method, while only ZDBC was found using the on-column derivatisation method. Then again, only ZDBC was found in both samples (6-1 and 6-2) by using pre-column derivatisation to nickel (II). The reason for the variation in the results of these samples was not known. It might be because of the non-homogeneous distribution of ZDTCs in the sample extract or the interference from other present compound like colour.

On the other hand, the method of on-column derivatisation to nickel complexes was simple and direct, which subsequently save a lot of time,
solvent and energy. This method is also proved to be more accurate (section 6.3.1) and sensitive (Table 6.11), which probably gave the correct amount of ZDTCs in rubber samples (Table 6.12). Therefore, it was concluded that the new method of on-column derivatisation has a real potential to be the most simple and direct method with the advantages of more specific, linear, precise, accurate and sensitive.

Table 6.11
Comparison of LOD and LOQ between the reported method [176] and the three methods used in this study

<table>
<thead>
<tr>
<th>Method</th>
<th>Compound</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μg/ml</td>
<td>%</td>
</tr>
<tr>
<td>TARRC [176]</td>
<td>All ZDTCs</td>
<td>-</td>
<td>0.0003</td>
</tr>
<tr>
<td>Pre-column derivatisation to copper (II)*</td>
<td>ZDEC</td>
<td>7.15</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>24.24</td>
<td>0.0024</td>
</tr>
<tr>
<td>Pre-column derivatisation to nickel (II)**</td>
<td>ZDEC</td>
<td>9.86</td>
<td>0.0010</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>13.49</td>
<td>0.0013</td>
</tr>
<tr>
<td>On-column derivatisation to nickel (II)***</td>
<td>ZDEC</td>
<td>0.90</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.86</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>ZDMC</td>
<td>1.31</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>ZPD</td>
<td>1.16</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>ZBEC</td>
<td>1.99</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

HPLC conditions:
*Gradient elution (0 min: 45% acetone-water, 15 min: 80% acetone-water, 30-35 min: 100% acetone) at 1 ml/min, 40°C and 435 nm on XBridge ODS column.
**70% methanol-water with 0.01% Ni (II) at 0.6 ml/min, 70°C and 330 nm on Gemini ODS column.
***70% methanol-water with 0.01% Ni (II) at 1 ml/min, 70°C and 330 nm on XBridge C8 column.
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Table 6.12
Comparison of the reported [176] and observed ZDTC values found in the rubber samples using derivatisation to metal complexes

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Type of ZDTCs</th>
<th>Reported values by TARRC [176]</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-column derivatisation to copper (II)</td>
<td>Pre-column derivatisation to nickel (II)</td>
</tr>
<tr>
<td>1</td>
<td>ZDEC</td>
<td>0.4400%</td>
<td>0.6008%</td>
</tr>
<tr>
<td>2</td>
<td>ZDEC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0027%</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>3</td>
<td>ZDEC</td>
<td>0.0550%</td>
<td>0.0281%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.2173%</td>
<td>0.1440%</td>
</tr>
<tr>
<td>4</td>
<td>ZDEC</td>
<td>0.0100%</td>
<td>0.0190%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>0.0021%</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ZDEC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZPD</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6 – 1</td>
<td>ZDEC</td>
<td>Not measured</td>
<td>0.0808%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>Not measured</td>
<td>ND</td>
</tr>
<tr>
<td>6 – 2</td>
<td>ZDEC</td>
<td>Not measured</td>
<td>0.1137%</td>
</tr>
<tr>
<td></td>
<td>ZDBC</td>
<td>Not measured</td>
<td>0.1846%</td>
</tr>
</tbody>
</table>

The HPLC conditions are as in Table 6.11. ND = Not detected, NQ = Not quantitated.

6.5. Summary

The determination of ZDTCs in rubber products was compared using the methods of pre-column derivatisation to copper (II) and nickel (II) as well as on-column derivatisation to nickel (II) complexes. The validation of the methods showed that the on-column derivatisation method was far better than the pre-column derivatisation methods. Although all methods studied were specific either to copper (II) or to nickel (II) complexes, the on-column derivatisation method gave more linear calibration curves for all compounds, and was more precise, accurate and sensitive than the pre-column derivatisation methods. However, linearity, accuracy and sensitivity of the pre-column derivatisation method to nickel complexes might still be further improved if one is using better column and HPLC system, as the same method using XBridge C8 column on the HPLC system 1 (Fig. 2.1) gave a
linear graph with a 10 fold lower systematic error.

Quantitation of ZDTCs in a series of rubber products (6 samples), which include nitrile rubber gloves (Sample 1 – 3), natural rubber latex gloves (Sample 4 – 5), and a multicoloured balloon (Sample 6) using both methods showed different results from the reported values [176]. However, they seem to give similar compounds at the same region to each other. The levels of ZDTCs found in those samples ranged between not detected to 0.6008% for ZDEC and to 0.1846% for ZDBC using pre-column derivatisation to copper (II), between not-detected to 1.9528% for ZDEC, 0.0381 to 0.1762% for ZDBC using pre-column derivatisation to nickel (II), and between not-detected to 1.6302% for ZDEC, 0.0124 to 0.2972% for ZDBC using on-column derivatisation to nickel (II). It should be noted that both methods of pre- and on-column derivatisation to nickel complexes also detected new compounds (ZDEC in sample 2 and ZDEC, ZPD and ZDBC in sample 5), which were not detected using the method of pre-column derivatisation to copper complexes either reported by TARRC [176] or observed in our laboratory (section 6.1.2).

Finally, comparison between all the three methods used in the determination of ZDTCs in rubber products (pre-column derivatisation to copper (II) and nickel (II) complexes as well as on-column derivatisation to nickel (II) complexes), showed that the method of on-column derivatisation to nickel complexes gave the results with reliability, adequate precision and accuracy.
7.1. Conclusions

The aim of work was to develop an improved HPLC method for the determination of rubber accelerators.

An evaluation of previously reported methods [4, 23, 25, 78] was carried out to further understand the chemical and chromatographic behaviour of the selected rubber accelerators. Preliminary studies using the methods of pre-column derivatisation to cobalt (III) complexes and direct injections confirmed that zinc dithiocarbamates (ZDTCs) were very unstable [136, 149, 174, 175]. Determination of rubber accelerators from the group of thiurams and mercaptobenzothiazoles was quite direct, but ZDTCs needed sample preparation, which conventionally includes liquid-liquid extraction to convert the ZDTCs to other stable metal complexes (section 3.1). The instability of ZDTCs also makes their direct determination rather difficult and inefficient even with the addition of an alternate ZDTC compound (section 3.2) or zinc salt (section 4.1) that served as protecting agents against the interferences, or altering the pH of the mobile phase. We also confirmed the appearance of many extra peaks for the mixture of cobalt (III) complexes (section 3.3.2), which would complicate the quantification.

The performance of nickel (II) ions, which were found to react more rapidly with dithiocarbamates, gave higher molar absorptivity and produced thermodynamically stable complexes (section 3.3.1) compared to other metal ions (cobalt (II) and copper (II)) has led us to use this ion in most of our studies.

The use of a short column with a short alkyl chain (XBridge C8, 4.6 x 50 mm) and together with the proposed technique of on-column derivatisation has reduced the use of organic solvent tremendously. To use pure water as
the mobile phase for the separation of metal dithiocarbamates may not be possible as they are not soluble in water.

The availability of a thermally stable column like XBridge makes the study on the effect of temperature possible (section 5.4 to 5.6), which have reduced the analysis time and significantly further reduced the quantity of organic solvent used.

Optimization of the on-column derivatisation method for the zinc dithiocarbamates to nickel (II) complexes found that 0.01% Ni (II) with no alteration of pH in 70% methanol-water of mobile phase at a flow rate of 1 ml/min and gradient temperature of 40 to 70°C were the most suitable conditions for the determination of zinc dithiocarbamates on XBridge C8 using HPLC system 2 (Chapter 5). However, because of the time and equipment limitation, isocratic temperature at 70°C was used.

This new method was used to determine the amount of zinc dithiocarbamates in 6 rubber samples and compared with the methods of pre-column derivatisation to copper (II) and nickel (II) complexes. The results showed that the determination of zinc dithiocarbamates using the on-column derivatisation method was more reliable and sensitive (LODs and LOQs were between 0.0001 to 0.0002% and 0.0003 to 0.0007% respectively) (Chapter 6).

In addition to the fact that the on-column derivatisation method was simple and direct, it may also be able to simultaneously detect dithiocarbamates and its degradation products. However, since this method used metal salts in the mobile phase, care need to be taken by regular flushing and cleaning of the system.

7.2. Recommendations for future work

No attempt was made to optimize the on-column derivatisation method using a ternary mobile phase composition. However, this technique might
also improve some of the separations, especially the higher homologues complexes, as Dilli and Tong [175] reported that the ternary system is superior for this separation.

Previous report [45] showed that there was a significant increase in the frequency of sensitization to thiurams, but surveys [46, 47] on the levels of rubber accelerators in rubber gloves showed no thiurams. These, could mean that the sensitizations were actually due to the newly produced substances as suggested by Bergendorff and colleagues [43, 215], or the free dithiocarbamate ligands that were not detected by the analysis method. Therefore, this current method of on-column derivatisation could also be further investigated on the possibility of detecting the free dithiocarbamates with an additional step of short sample preparation. This method could also be used to study the correlation between the level and type of dithiocarbamates with the allergen analysis like skin prick test.

Hybrid columns like XBridge have been reported to be stable at high temperatures. Unfortunately, in this study the column seems to be stable only for a limited time. This could be due to the used of metal salts in the mobile phase. However, further study is needed to prove this assumption.

Copper dithiocarbamates were found to behave differently in different solvents (section 3.3.3), which agreed with the previous finding [140] that different solvents have different reaction mechanism. Since our study focussed on the nickel complexes and similar attempt was not successful, additional study might further suggest the reason of the disappearance of the mixed ligand complex.

The same technique can also be apply to other areas like agriculture for the determination of dithiocarbamate pesticides and fungicides (for example thiram, ziram and zineb), pharmaceutical samples (for example antabuse/thiuram disulfide which was used for treatment of alcoholism in blood, plasma or urine samples), or in the environmental analysis of dithiocarbamates (for example trace analysis of dithiocarbamates in food and water) [22].
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PRESENTATIONS


