Green Chemistry in the Design of Safer Anaerobic Adhesives

By

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Thesis Submitted for the Degree of Doctor of Philosophy

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work, and that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _____ Date: _____

Adam Porter

ID No.: 57623321

For my Parents,

Cyril and Nuala

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Abbreviations

APD: 1-Acetyl-2-Phenylhydrazene

APH: 1-Acetyl-2-Phenylhydrazine

Bis A: Bisphenol A

BMIM: 1-Butyl-3-methylimidazolium

BS: Saccharin (Benzoic Sulfimide)

CHP: Cumene Hydroperoxide

CMR: Carcinogenic, Mutagenic, Reprotoxic

DBP: *N*,*N*-Dibutylpyrrolidinium

DE*p*T: Diethyl-*p*-Toluidine

DM*m*T: Dimethyl-*m*-Toluidine

DM*o*T: Dimethyl-*o*-Toluidine

DM*p*T: Dimethyl-*p*-Toluidine

DMP: *N*,*N*-Dimethylpyrrolidinium

EDTA: Ethylenediaminetetraacetic Acid

EMIM: 1-Ethyl-3-methylimidazolium

EPA: Environmental Protection Agency

EPI: Estimation Programs Interface

GE: General Electric

HEMA: (Hydroxyethyl)methacrylate

HPMA: *N*-(2-Hydroxypropyl)methacrylamide

IBOMA: Isobornyl Methacrylate

ISO: International Organisation for Standardisation

MITI: Ministry of International Trade and Industry

MMA: Methyl Methacrylate

MMIM: 1,3-Dimethylimidazolium

MSDS: Material Safety Data Sheet

NBP: *N*-Butylpyridinium

NMP: *N*-Methylpyridinium

NTf_2^-: Bistriflimide

OECD: Organisation for Economic Co-operation and Development

OMIM: 1-Octyl-3-methylimidazolium

PEGMA: Polyethylene Glycol Dimethacrylate

PM16: Premix 16

PM17: Premix 17

SRC: Syracuse Research Corporation

TMBH: 1,1,3,3-Tetramethylbutyl Hydroperoxide

TM*p*T: *N*,*N*,*N*-Trimethyl-*p*-toluidinium

THQ: 1,2,3,4-Tetrahydroquinoline

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Abstract

New cure accelerators for anaerobic adhesives have been designed to offer less harmful alternatives to current industry standard accelerators. Accelerators in current use are comprised of compounds such as acetylphenylhydrazine (APH), diethyl-*p*-toluidine (DE*p*T), tetrahydroquinoline (THQ) and their derivatives, all of which can be classified as being acutely toxic or harmful to the user. Further to this, there is no consensus as to their mode of action within the curing of the adhesive and how they affect the radically initiated polymerisation mechanism.

Safer alternative cure accelerators have been designed and added insight as to their mechanistic role gained through the conception, synthesis, and screening of "generations" of accelerators and structurally similar compounds for key properties such as anti-microbial toxicity, cytotoxicity, biodegradability, eco-toxicity, and cure activity. With the analysis of each generation, more information was gathered and the structure activity relationship for these properties further elucidated. This greatly aided the design of each succeeding generation of synthetic targets in order to maximise their accelerator activity whilst reducing their risk to the user and the environment.

This approach not only yielded new ionic liquid based cure accelerators but also opened up new directions for their future development and design by identifying classes of compounds not previously used for this purpose. These saccharinate, acesulfamate, and phthalimate based compounds have been proven to possess activity equal to current industry benchmarks whilst being of low cytotoxicity and anti-microbial activity. Furthermore, these compounds offer significant room in their scope for derivitisation and development as custom designed cure catalysts.

Chapter 1:

Introduction to Adhesives

1.1 A Brief History of Adhesion

In its most fundamental sense, an adhesive is defined as any substance capable of holding materials together though surface attachment.

The first examples of adhesion can be traced as far back as the late Palaeolithic era where hunter-gatherers fashioned axes by using bark-tar to bond stone to pieces of wood.[1] Plant products such as these tars and tree resins were the basis for early bonding products with no evidence of the use of animal glues until c. 2000 B.C. which is also when we see the first recordings of the use of adhesives in literature. The Romans and Greeks further developed these glues using fish and other animal sources, as well as natural products such as egg whites, to form long lasting adhesives. It is also around this time that the use of cements and mortar became more common in addition to tar and beeswax as waterproofing agents and sealants.[2]

The development of glues and adhesives surged from the late 17th century through the industrial revolution forming the basis for the industry. This period saw the first commercial manufacturing facilities established as well as the first British and US patents for glue issued. Although these and many other developments greatly advanced the now established adhesive industry, they were all based on plant and animal sources. The first semi-synthetic polymer was created by Alexander Parkes[3] when he marketed a less nitrated form of nitrocellulose as "synthetic ivory" but not until the first fully synthetic commercial polymer was produced in the early 20th century did adhesives begin to develop into how we see them today.

Bakelite was the trade name given to the phenol-formaldehyde based resin marketed by Leo Baekeland in 1907. Initially used as a mouldable plastic, it was first applied as an adhesive in 1912. Urea-formaldehyde resins soon followed along with other synthetic polymers, their production catalysed by World War II, and in these intervening years the vast majority of adhesive development has taken place despite the long and storied history.[3]

To detail the discovery and development of each adhesive class and their importance would be beyond the scope of this study but a selected chronology of their discovery is shown in Table 1.1.

Table 1.1: Development of Selected Adhesives

Year	Adhesive
1940	Polyurethane, Nitrile-phenolic, Acrylic
1950	Epoxies, Cyanoacrylates, Anaerobics
1960	Polyimide, Polybenzimidazole
1970	Second-Generation Acrylics
1980	Foamable Hot-Set Adhesives
1990	Polyurethane Modified Epoxy, UV Curable
2000	Water-Based Adhesives, Solvent-Free Adhesives

It is clear from this illustration just how big of a leap adhesives have made in their development and technology over the past century. This development has been historically linked to the requirements of industries, such as aerospace, where it is of great benefit to replace mechanically fastened joints, as well as to the advances which have been made in organic and polymer chemistry.

1.2 Anaerobic Adhesives

This research project is specifically focused on anaerobic adhesives and, as the name suggests, these are adhesives which cure in the absence of air but remain liquid in its presence. They were developed by General Electric[4] in the 1940's and consisted of a methacrylate monomer mixed with an organic peroxide at elevated temperature. Crucially, this mixture required pure oxygen to be continuously bubbled through to prevent polymerisation from taking place. This instability and the awkward handling conditions required ultimately led to GE discontinuing the product only for Loctite, now Henkel-Loctite, to acquire the license and develop a more stable product in the early 1950's.[5] This mainly centred on developing packaging which allowed for air penetration and so removed the need for a constant flow to be artificially provided.

Until their main patents on the technology began to expire in the 1970's, Loctite maintained firm control on the industry. Nowadays, many multi-national companies produce anaerobic adhesive products including Henkel-Loctite, Eastman, and Threebond amongst others.[6–8] These adhesives are mainly utilised as threadlockers,

their role to keep components such as nuts and bolts from loosening with the vibration of machinery. For this use their penetrating ability allows them to be applied before or after assembly. Due to their curing mechanism, they are only suited to bonding close fitting materials but as they have been developed and their properties modified they have found further applications as "retainers" for cylindrical joints, and as sealants for a wide array of metal joints as well as gasketting. They are now applied across a broad range of industries from aeroplane assembly[9] to dentistry[10,11] with their properties and applications still being explored.

The bond formed is resistant to a wide range of solvents including salt water as well as a broad temperature range from -54°C to upwards of 232°C.[12] Curing can be accelerated by increasing the temperature but it is generally preferred to do so through the addition of catalytic compounds or surface pre-treatment of the substrate. Pre-treatment may involve the removal of the inactive metal-oxide layer by methods of abrasion or by applying a primer containing a reducing agent or initiating catalyst such as a copper salt. Anaerobic adhesives are applied across a wide range of materials however, for mechanistic reasons which will be explained in a later section, they are most commonly used to bond metals. The curing occurs through a redox initiated radical polymerisation where a hydroperoxide is used as the radical source through its degradation. The presence of metal ions greatly affects this cure rate but even so there is a broad range of activity depending on the metal in question with the adhesive being virtually inactive when used with materials such as glass or ceramics[13] (Table 1.2).

Table 1.2: Material Activity of Anaerobic Adhesives[13]

Very Active	Active	Inactive	Very Inactive
Brass	Aluminium	Anodize aluminium	Ceramics
Copper	Bronze	Cadmium	Composites
Manganese	Iron	Chrome finished	Glass
	Kovar	Gold	Plastics
	Monel®	Inconel	Paint-coated parts
	Nickel	Magnesium	Rubber
	Steel	Plated parts	
		Galvanised steel	
		Stainless steel	
		Titanium	
		Zinc	

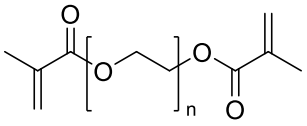
1.2.1 Formulation and Cure Accelerators

A typical formulation of an anaerobic adhesive will contain monomer(s), a hydroperoxide, cure inducers/accelerators, along with stabilisers and performance modifiers (Table 1.3).

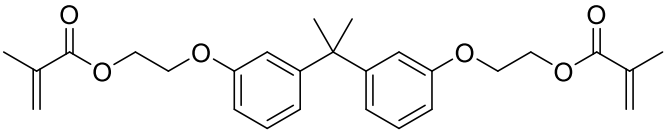
Table 1.3: Typical Composition of an Anaerobic Adhesive

Component	Purpose	Example
Monomer	Provides the adhesion	Polyethylene glycol dimethacrylate
Cure Initiator	Initiates the polymerisation	Cumene hydroperoxide (CHP)
Co-Initiator/Accelerator	Takes part in polymerisation initiation	Saccharin (benzoic sulfimide - BS)
Cure Accelerator	Reduces curing time	Dimethyl-*p*-toluidine
Radical Scavenger	Stabilises the formulation	Benzoquinone
Performance Modifiers	Tailor the properties of the adhesive	Plasticizers, thickeners etc.

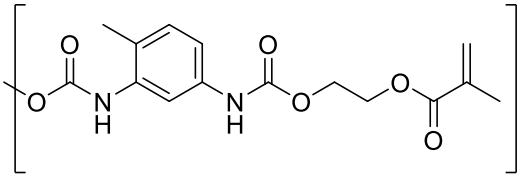
Multi-methacrylate functionalised monomers are commonly chosen for this class of adhesive as the carbon-carbon double bond provides a readily available site at which radical polymerisation can occur. The first monomers used were polyethylene glycol methacrylates of varying length[4], and while they are still used widely in anaerobic products, they are now supplemented by other monomeric species in order to modify their bond properties and applications. Methacrylate derivatives used include hydroxyethyl-, lauryl-, isobornyl, and hydroxypropylmethacrylamide amongst many others as well acrylic acid derivatives used in the bonding of smoother surfaces.[14] Bisphenol A (Bis A) is the basis for many epoxy resins which are commonly thermally cured and these moieties are introduced in the form of compounds such as ethoxylated bis a dimethacrylate to impart greater thermal resistance on the adhesive bond[15]. Diallyl bisphenol A's can be used to improve the shear strength[16] of the bond while the strength and toughness can be altered through the introduction of urethane linkages[17], most commonly by the reaction of toluene diisocyanate with hydroxyalkylmethacrylates (**3**) (Figure 1.1).



Polyethyleneglycol dimethacrylate (**1**)

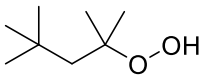


Ethoxylated Bisphenol A Dimethacrylate (**2**)

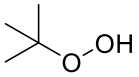


Toluenediisocyante-Hydroxyethylmethacrylate (**3**)

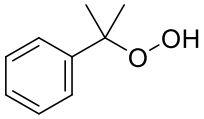
Figure 1.1: Examples of Monomers used in Anaerobic Adhesives



1,1,3,3-Tetramethylbutyl Hydroperoxide (**4**)



t-Butylhydroperoxide (**5**)



Cumene Hydroperoxide (**6**)

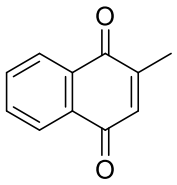
Figure 1.2: Examples of Initiators used in Anaerobic Adhesives

For any adhesion to occur, anaerobic adhesive formulations contain a hydroperoxide which, through its decomposition, acts as the source of the radical required in order for polymerisation initiation to take place. Many different hydroperoxides can be used for this purpose with the main requirements being that they are reasonably stable under aerobic conditions and that the activation energy for their decomposition can be achieved as part of a reduction process where a transition metal is oxidised. Cumene hydroperoxide (**6**) is commonly used example but many others have been utilised successfully such as t-butyl-, and tetramethylbutyl hydroperoxide (TMBH) (Figure 1.2).

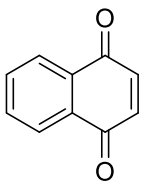
Although peroxides in general are inherently instable and will usually degrade to varying extents depending upon conditions, they must be stable enough to allow for application of the adhesive before curing begins and in the case of commercialised products – to allow for an acceptable shelf-life. A certain amount of radical decomposition will occur no matter what measures are taken but so long as this rate is not unmanageable, its effect can be mitigated by the process where molecular oxygen reacts to retard these radical reactions. In fact, this is the reason why this class of adhesive can be stored as a single mixture and still have a reasonable shelf life in that once they are packaged while in the presence of oxygen, and as long as this packaging allows for a certain level of air penetration, then these radicals will preferentially react with oxygen. For this purpose, low-density polyethylene

containers are used. The radicals then formed will not possess the activation energy necessary to initialise polymerisation, and in the case that they do, or if a radical does not react with a hydroperoxide radical then a certain amount of radical stabiliser/scavenger such as menadione (**7**) or napthoquinone (**8**) is included in the formulation to ensure the stability of the product.[18] While these components are included, they are not in a large enough concentration to hamper the initiation phase of the polymerisation once the adhesive is applied to the substrate and oxygen removed.

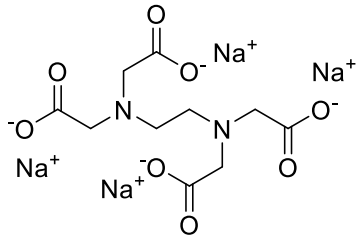
In order to mitigate the effect of any transition metal ion contamination, a small amount of EDTA (**9**) or other metal chelator is also included in the formulation to further stabilise and lengthen shelf-life.[19]



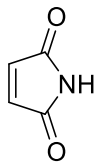
Menadione (**7**)



Napthoquinone (**8**)



EDTA Tetra Sodium Salt (**9**)



Maleimide (**10**)

Figure 1.3: Examples of Stabilisers/Inhibitors and Modifiers used in Anaerobic Adhesives

Other components of the formulation may be present in small amounts to modify the cured or uncured properties of the adhesive. The viscosity and rheology of the adhesive may need to be altered in order to make the composition more fit for purpose. If the adhesive is intended as a threadlocker then it would be advantageous to have the viscosity lowered so as to allow for as much penetration of the threaded joint as possible, on the other hand if it is intended to be used for bonding smooth,

suspended parts then a high viscosity product would be desired so as to maximise contact. These properties can be designed for by the use of thickening agents such as polystyrene or thixotropic agents.[20] Examples of methods used to alter the thermal resistance and strength of the adhesive bond are given above when discussing monomers. Maleimides (**10**) may further add to the thermal resistance[21] while plasticisers such as polyester may be used to lower the bond strength and reduce the permanency of the adhesion.[22] Calcium carbonate can be used as filler to add to the sealant effect of the product while dyes may be included so as to aid in identification and brand recognition.[23]

The remaining significant components present in anaerobic adhesives are the co-initiator/cure accelerators and it is these compounds which are the subject of this research project.

Despite much attention, there is still no consensus as to the specific involvement these components play in the overall curing mechanism of the adhesive and how exactly they expedite the polymerisation process.

The first generation of cure accelerators for commercial anaerobic adhesives relied on simple tertiary amines such as tributylamine to hasten the curing process.[24] These were superseded by aromatic amines such as aniline and nowadays, accelerator compositions are typically comprised of a weak acid such as saccharin (BS) (**13**), accompanied by an aromatic amine e.g. diethyl-*p*-toluidine (DE*p*T) (**11**).[25,26] Both of these compounds displayed cure accelerator activity on their own, but when used in tandem they proved to form an extremely effective curing composition. These compounds and their pairing represented a major step forward in the application of anaerobic adhesives as they greatly decreased the cure time required and by doing so broadened their range of applications.

Along with anilines/toluidines, established cure accelerators to have been developed include many phenylhydrazines (**16**)[27], tetrahydroquinolines (**15**)[28], enamines[29], and benzoxazines.[30] These variations of aromatic amines have enjoyed varying degrees of success and hint at different possible modes of action but crucially none of the compounds thus far have been able to display the desired properties of a commercial anaerobic cure accelerator i.e. to possess excellent cure activity whilst posing no risk to the user or the environment.

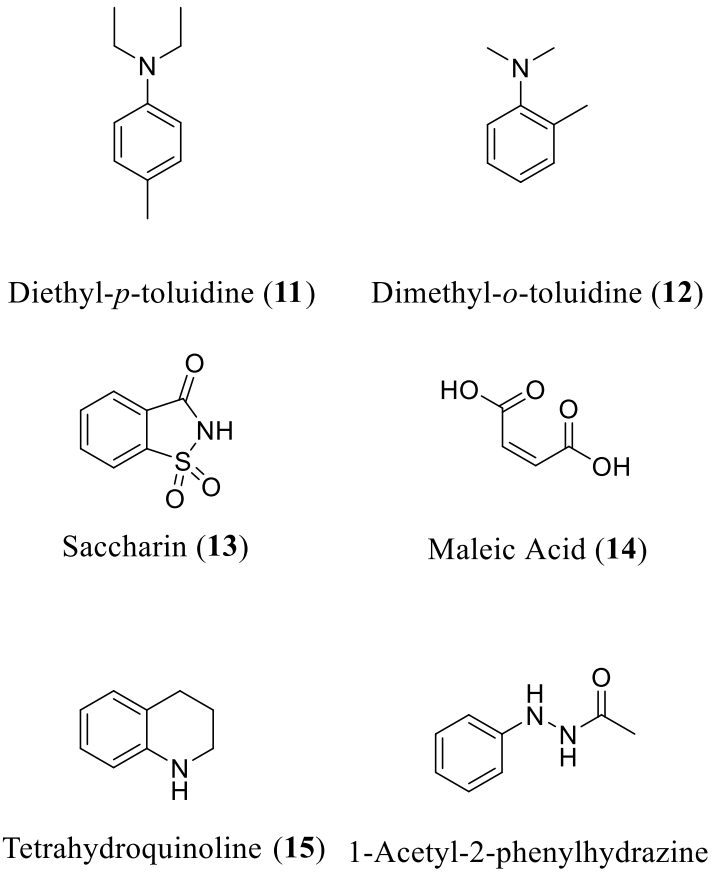


Figure 1.4: Examples of Cure Initiators/Accelerators used in Anaerobic Adhesives

Acetylphenylhydrazine (APH) (**16**) and DE*p*T/DM*o*T (**11**, **12**) represent some of the most effective cure accelerators available in terms of cure time and strength. However, they are also proven to be acutely toxic towards human health[31,32] and this places a restriction on not only the amounts which may be incorporated into formulations, but also the variety of applications for which the adhesive may then be used (Table 1.4).

Any adhesive prepared with components such as these must be labelled accordingly to warn the end-user of the associated risk. For a commercial product, this automatically reduces the size of market to which the product may be sold as well as giving pause to the remaining target consumers.

Table 1.4: Acute Toxicity of Some Accelerator Compounds

Compound	LD_{50}
Dimethyl-*p*-toluidine	Intraperitoneal – Mouse - 212 mg/kg[32]
Dimethyl-*o*-toluidine	Intraperitoneal – Mouse - 338 mg/kg[33]
1-Acetyl-2-phenylhydrazine	Oral – Mouse – 270 mg/kg[31]
Indoline	Intraperitoneal – Mouse - >238 mg/kg[34]

For this reason there is substantial interest in the possibility of designing cure accelerators which would be non-toxic and completely "label free" while still possessing cure activity on par with the established compounds in use. This would result in a much safer product for the end user and, perhaps just as importantly, remove the restrictions on how much of these chemicals can be used as part of formulations in order to conform with industry guidelines & regulations, and therefore create a wider range of applications.

1.3 The Curing Mechanism of Anaerobic Adhesives

In order to be able to design new, safer cure accelerators, it would be of great benefit to establish their mechanism of action with respect to how they interact with and affect the overall curing process. This, however, is not straight forward with studies showing that depending on the accelerator used, and the substrate to which they are applied, varying possibilities and evidence as to the mechanism are apparent.

Since anaerobic adhesives became commercially available in the 1950's, there has been much speculation as to the specific role each component plays in the curing mechanism observed and the resulting adhesion.

Loctite maintained a firm hold on the industry until their main patents on the technology began to expire in the 1970's when this opened up a lot of anaerobic "knowledge" to the rest of the adhesives industry and allowed them to begin developing their own versions and products. Given how this technology was discovered and how it has evolved primarily within industry as opposed to academic research, the majority of published material in the area exists in the form of patent literature and a lot of the nuances and expertise, one can assume, has gone

unpublished in favour of the value placed on “in-house” knowledge by many major companies. As a result, the bulk of research into the understanding of this mechanism, and particularly the role of cure accelerators within it, did not take place until the 1990’s but even then despite this increased attention, there is still no consensus as to the exact curing mechanism and how the polymerisation process can be modified and accelerated.

In its most simplistic form, the adhesion process can be thought of to occur through a redox initiated radical polymerisation process. Since this process is an overall radical mechanism, the methacrylate C-C double bond provides the site at which this polymerisation occurs.

In order for this to take place, the radical must first be generated in order to initialise polymerisation. The hydroperoxide present acts as the source of this initiator through its decomposition and one of the mechanisms by which this can occur is through a redox process whereby the transition metal, present on the substrate surface, acts as the reducing agent.

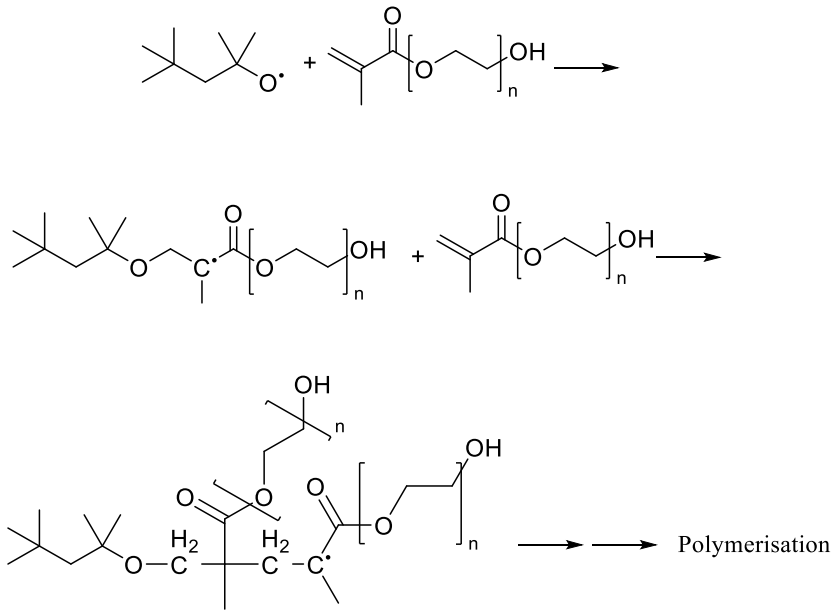
$$M^n + R'OOH \longrightarrow M^{n+1} + RO^{\cdot} + OH^-$$

This radical is then able to react with the methacrylate carbon-carbon double bond of the monomer initialising polymerisation.

As the name suggests, an absence of oxygen from the adhesion environment will promote curing. This is due to oxygen’s ability to retard the process, either by reacting with the peroxide radical formed resulting in a radical unable to initialise polymerisation, or by oxidising the metal surface and preventing hydroperoxide decomposition. In saying this however, certain metals such as iron are still able to decompose hydroperoxides even when in an elevated oxidation state.

$$Fe^{3+} + R'OOH \longrightarrow Fe^{2+} + ROO^{\cdot} + H^+$$

The radical formed in this case though is not as reactive as that of the radical formed from a metal in a lower oxidation state and is unable to reach the activation energy required to initiate polymerisation. Thus, despite the fact that various metals, of varying oxidation state, are able to decompose hydroperoxides, the lower the oxidation state of the metal substrate to be bonded, the more anaerobic adhesion will be promoted.



Scheme 1.1: Radical Initiated Polymerisation

The methacrylate radical formed in the reaction between the peroxide radical and the methacrylate monomer is then free to react with other methacrylate functionalities and the reaction is thus propagated. The end result of this polymerisation is a strong adhesive bond which is resistant to both temperature change and solvent contact.

As mentioned previously, anaerobic adhesives were first commercialised in the 1950's and in these first products "simple" tertiary amines such as tributylamine were employed as cure accelerators. Without such compounds, and in the absence of any heat, these systems would have taken a prolonged time to cure and as a result would have been impractical in terms of commercial application. It was thought that these cure promoters were able to affect the cure time by forming a complex with the hydroperoxide which in turn made the oxygen-oxygen bond more susceptible to decomposition and radical formation, thus accelerating the initiation phase of polymerisation. However, as several studies have shown in the period since, there are many possibilities as to how these compounds are able to participate in the curing process. These studies have not resulted in a definitive consensus as to their exact mechanism of action but they have hinted at the possibility that they may take part in

different ways, with various accelerators emphasising some mechanisms more than others even though the net curing rate could end up the same.

One of the issues with past studies into the source of accelerator activity or any attempt to elucidate their activity in a "QSAR-like" manner has been that the same few accelerator systems are analysed in a number of cases. This can be partly attributed to a lack of variation of the accelerators present in commercial products but when there is a situation such as this where there are several possibilities as to the mode of action, many of which could occur simultaneously only to differing extents, then there is a resulting bias inherently present in any conclusions reached i.e. the outcome will reflect the most favoured mode of action of that system and therefore no insight as to the possibilities and levels to which other modes of action affect the curing rate are gained. This is something which has been overlooked and by not giving enough attention to other accelerator systems, a "true" general mode of action for their activity cannot be described, only a likely mechanism of action for an individual system.

The curing system which has been the focus of this attention is one mainly comprised of triethylene glycol dimethacrylate, cumene hydroperoxide (CHP), and a cure accelerator system comprising of either both dimethyl-*p*-toluidine & saccharin or acetylphenylhydrazine (APH) & saccharin (BS). Another important factor to take into account when critically analysing data reported is the choice of metal substrate. This will be explained in more detail later, but different adhesive formulations perform better depending on the choice of metal to be bonded illustrating that this factor too plays an important role in the curing process and the major mechanism through which polymerisation takes place i.e. a formulation which bonds copper very well may not do so with iron and vice versa.

Y. Okamoto, in a two-part study of the curing mechanism of anaerobic adhesives, was one of the first to allude to the possibility that any cure acceleration could be as a result of a complex formed between saccharin and the amine present as opposed to either compound taking part in an unreacted state.[35,36] Up to this point there was still much ambiguity as to the exact curing mechanism of anaerobic adhesives, and even more concerning the role of cure accelerators within it. Using methyl methacrylate (MMA) as the polymer, and CHP, BS, and DM*p*T as the curing system, temperature

and kinetic studies were carried out in order to elucidate the mechanism. With the rate of polymerisation increasing with temperature, and taking into account the apparent low activation energy also measured, the reaction was believed to occur through a redox radical polymerisation process as opposed to a thermally initiated one. The kinetic measurements carried out showed that the rate of reaction depended on close to equal concentrations of saccharin and DM*p*T and, quite surprisingly, occurred regardless of whether CHP was present or not. This indicated that the acceleration of the polymerisation reaction could be due to an interaction between saccharin and the amine. Okamoto proposed that this was through a charge transfer complex formed between BS and DM*p*T, the formation of which was believed to be illustrated by the deep purple colour formed upon addition of one to the other.

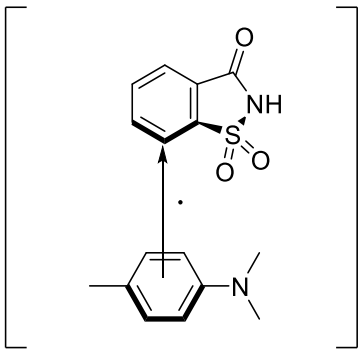


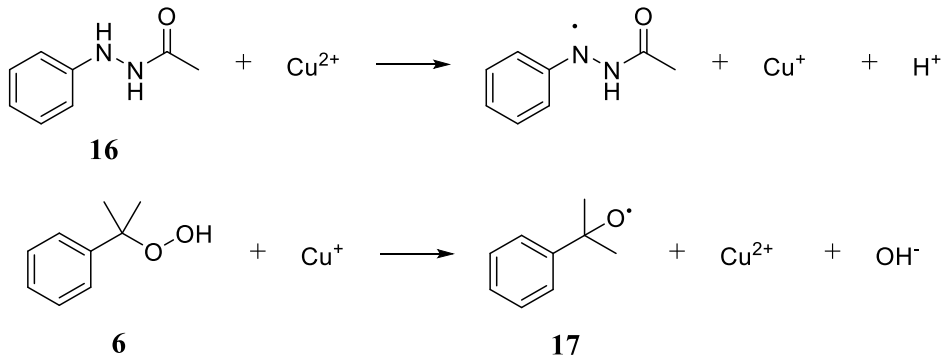
Figure 1.5: Charge Transfer Complex between DM*p*T and Saccharin

The formation of such a complex was supported by measurements carried out which showed that the curing time increased when electron donating groups were added to BS, or when the slightly electron donating effect of the methyl group on DM*p*T was removed by replacing DM*p*T with *N*,*N*-dimethylaniline, or when the cyclised derivatives tetrahydroquinoline (THQ) or indoline were used.

The second part of this study by Okamoto was conducted using APH instead of DM*p*T as part of the curing system.[36] However, this was conducted with one significant difference, because the induction period when using APH was perceived to be longer than when that of a curing system in which DM*p*T is used, a small amount of Cu^{2+} catalyst was also added. This alludes to an important factor which was mentioned earlier, and summarised in Table 1.2, in that depending on the metal

substrate, or in this case the metal ion present, certain curing systems will perform better than others and this can make studies markedly less comparable.

Again, as with DM*p*T, the rate of polymerisation was faster as the temperature increased. The activation energy measured was lower than that of the system containing DM*p*T, however this can most likely be explained by the presence of the Cu^{2+} species. The reaction was found to occur regardless of BS concentration and this time was found to proceed faster with a molar ratio of 1 for APH-CHP concentration. It was postulated that the mechanism thus proceeds by APH acting as a reducing agent with respect to the metal substrate forming Cu^{+} which is then able to decompose the CHP (**6**) present to form a cumyloxy radical (**17**) which in turn initiates polymerisation (Scheme 1.2). The BS, in this system, was proposed to act as an acid catalyst protonating the CHP which would in turn lead to its decomposition into a cumyl cation and hydrogen peroxide which could act as another source of radical formation.



Scheme 1.2: Reduction of Copper by APH and Decomposition of CHP

The effect transition metals have on the curing activity of various anaerobic adhesive systems has been studied.[37–39] Combined, these studies point to copper being the most active substrate followed by iron with metals such as zinc being completely inactive. Aluminium also possesses some activity but this is due to the small amount of copper commonly contained in aluminium alloys.

The difference in activity that various transition metals display towards different adhesive systems could possibly mean that each promotes a different curing

mechanism and thus the substrate possibly may not act as a constant factor in their study of the curing mechanism, only another variable.

Beaunez, Helary, and Sauvet published a two-part study where they investigated the effect of metal substrate on adhesive curing.[38,39] The first study deals with copper curing systems where they use an initiator/accelerator system consisting of CHP, BS, DM*p*T, and crucially, copper saccharinate. In order to study the polymerisation reaction under representative practical adhesive conditions, a metal ion source was required, and, given the potential likelihood that metal salts of saccharin are formed under practical conditions, copper saccharinate was chosen. Using dilatometry, the kinetics of the reaction were studied with respect to each component and they concluded that an equilibrium of copper in its +1 and +2 oxidation states is reached in no small part due to CHP's ability to not only oxidise Cu^{+} to form reactive radicals but also reduce Cu^{2+} and thus keep regenerating the oxidising agent. In addition to this, they propose that the role of the amine accelerator is to complex metal ions and form a more reactive hydroperoxide decomposition reagent.

The second part of this study was conducted in a similar manner but with the substitution of copper saccharinate for the iron salt. This study yielded several key differences in the curing process observed between the two substrates. CHP is able to reduce Cu^{2+} and therefore regenerate the Cu^{+} oxidising agent, this is not observed for Fe^{3+} but importantly DM*p*T is able to carry out this regeneration of iron in a lower ground state despite this not being observed for copper.

George, Touyeras, Grohens, and Vebrel (1997) also carried out an investigation of the role of the metal substrate in the cure process.[37] They arbitrarily chose a commercial adhesive which consisted of a saccharin-DM*p*T accelerating system and applied the formulation to steel, aluminium, and copper substrates to study their effect. Using infrared microscopy, in reflection mode, the adhesive was applied to the substrate surface and the change in intensity of the peaks relating to the C=C and C=O bond of the methacrylate monomer were used as a measure of the progression of the curing. This resulted in a degree of curing which illustrated copper>steel>aluminium where copper was nearly 2.5 times more active than aluminium. This could potentially be as a result of copper being more susceptible to forming metal complexes with the saccharin and/or DM*p*T which are then able to decompose the hydroperoxide to form a radical species.

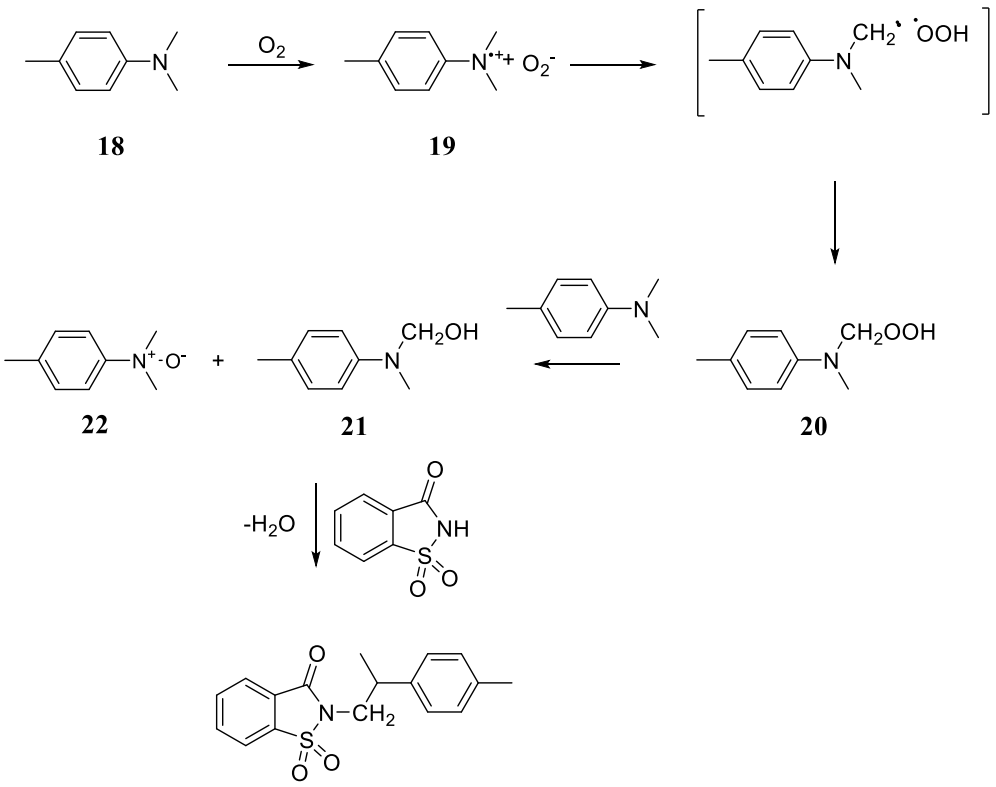
Raftery et al. used polarography in an attempt to study this change in oxidation state of iron and copper in the presence of the cure components.[40] The accelerators APH, 1-acetyl-2-phenyldiazene (APD), and THQ were each paired with BS or maleic acid, and CHP in order to study their individual effect on the redox state of the metal. The group was found that, in the presence of only the amine, there was little metal ion level detected. However, upon addition of either BS or maleic acid, the concentration of each metal in their various oxidation states increased greatly. This is thought to be as a result these compounds acting as an acidic species and liberating metal ions from the substrate surface. In the case of saccharin systems this resulted in more metal ions existing in their lower oxidation state than for the respective maleic acid based systems. Raftery and co-workers was proposed that this is due to maleic acid protonation of the amine hampering their ability to reduce the metal. Furthermore, it was observed that upon addition of CHP, the concentration of each metal in their reduced state decreased, highlighting their role in the oxidative decomposition of the hydroperoxide.

Indeed a separate study conducted by the same research group highlighted the complexity of the factors affecting hydroperoxide decomposition as part of an anaerobic adhesive.[41] Formulations containing various amine accelerators, BS or maleic acid, and one of several hydroperoxides were added to solutions of iron, copper, and cobalt salts of varying oxidation state. Generally, lower oxidation states of each metal decomposed the hydroperoxides to a greater extent while the higher oxidation states relied on the presence of the amine accelerators. Interestingly, in the case of metals in their lowest oxidation states, saccharin and maleic acted to inhibit the decomposition rate and promoted the rate in the case of higher ox. state metals. This could point to saccharin acting to alter the redox potential of the metals by forming a complex and not to protonate the hydroperoxide as proposed elsewhere. Another outcome of this study was the effect the acid-metal ratio had on the cure time. There is no real trend for this factor across the systems however and, practically speaking from an application standpoint, this will change with the area of metal being bonded and the type of species present. This has also been observed for other types of adhesives before.[42]

Up to this point, we have only considered the effect of each component in isolation on the curing process and at most have considered possible ionic bonding

interactions and salt formation reactions between the components. However, it is possible that each of these formulation components may in fact react to form new compounds capable of altering the cure composition in their own right.

Wellmann and Brockmann were the first to investigate the possibility of the formation of cure altering species *in situ*.[43] They used a system consisting of DM*p*T, BS, and CHP and carried out reactions under both anaerobic and aerobic conditions to explore the possible products formed. Under anaerobic conditions, DM*p*T may be oxidised in the presence of CHP (**6**) to form the *N*-oxide (**22**) and cumene alcohol. However, this process is supressed under aerobic conditions i.e. adhesive storage conditions. Nevertheless, the possible reactions of this *N*-oxide with other species were considered leading to the isolation of aminal (**23**) as the product of the reaction with saccharin. Based on reactions of other metal salts with amine-*N*-oxides, a radical mechanism for its formation was proposed. However, because any radical formed is likely to react with the methacrylate present in an adhesive, this radical will be consumed before the *N*-oxide or aminal can be formed. A pathway for the aerobic formation of the N-oxide and aminal was thus formulated instead (Scheme 1.3).



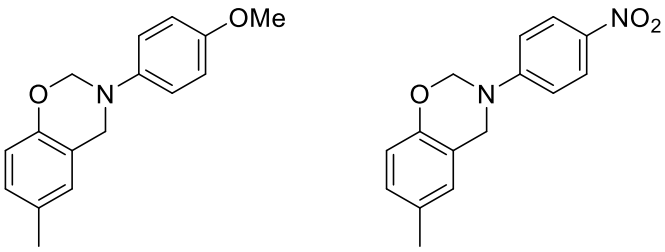
Scheme 1.3: Aerobic Formation of Aminal[43]

DM*p*T undergoes oxidation in the presence of air to form a radical cation (**19**), which can be isolated using TEMPO to prove formation. This cation reacts with air to form the hydroperoxide (**20**) which reacts with another DM*p*T (**18**) molecule to form the alcohol (**21**) and *N*-oxide (**22**). The aminoalcohol finally reacts with saccharin to form aminal (**23**).

Practically speaking, these species are only of interest if once formed they are able to affect the curing of the adhesive. The *N*-oxide was found to have no bearing on the cure time measured however aminal was found to greatly alter it. This was thought to be as a result of its ability to form metal complexes and also reduce copper ions. Other studies have further illustrated the positive effect aminal has on the cure time of copper based systems when added.[44]

Away from studying the cure mechanism itself, efforts in recent times have been made to create new compounds capable of speeding up the curing process. This has involved creating alternative amine cure accelerators, alternatives to saccharin and maleic acid, as well as attempts to form a single combined compound capable of carrying out the role of both.

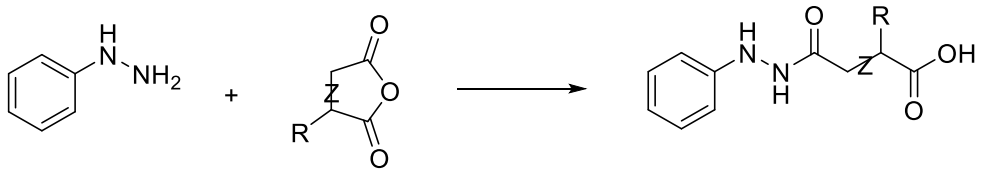
Henkel have produced a series of accelerators based on the THQ and indoline scaffold which retain the cure activity of the parent molecule.[8] Further to this they have designed and patented morpholine and benzoxazine based compounds capable of comparable activity to that of a 50:50 mixture of DE*p*T/DM*o*T.[29,30] The set of derivatives prepared further displayed the electron environment of the aromatic ring as first shown by Okamoto[35] in that when electron withdrawing groups were added, activity decreased, and likewise substitution with electron donating groups showed an increase in cure activity (Figure 1.6).



Active Inactive

Figure 1.6: Effect on Cure Activity of EWG and EDG

In an effort to combine saccharin and the accelerator functionality into one molecule, acid functionalised derivatives of APH were synthesised.[45] If the activity of saccharin is due to its acidic nature, then conceivably combining this property with an accelerator could form a compound capable to replacing both. With this in mind, Klemarczyk et al. reacted phenylhydrazine with cyclic anhydrides to create analogues of APH with acid functionality (Scheme 1.4).



24 Z = C=C, R = H
25 Z = C=C, R = CH_3
26 Z = C-C, R = H

Scheme 1.4: Reaction of Phenylhydrazine with Cyclic Anhydrides

Other efforts have been made to find alternatives to saccharin while searching for new cure accelerators. Instead of concentrating on its acidity however, other compounds containing sulphonamide moieties were examined. These were based on various derivatives of *p*-toluenesulfonamide and were found to possess similar cure activity (Figure 1.7).[46]

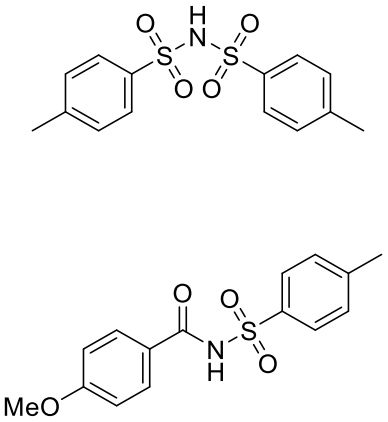


Figure 1.7: *p*-Toluenesulfonamide based Cure Accelerators

From these studies and the species involved, there is potential for many possible cure accelerating mechanisms to take place. Considering the number of variables, it is difficult to determine which, if any, is the primary means of catalysis and perhaps it is in fact a result of a balance between several pathways occurring at once with certain systems displaying slight preferences for some over others.

1.4 The Necessity of Green Chemistry

Traditionally, the means of reducing the risk or hazard posed by potentially dangerous substances was to reduce the level of exposure to the substance in question by making adjustments to the work environment such as handling, storage, and disposal. However, while such practices are not without merit they have no impact on the primary source of the exposure in the first place, the chemical.

This is where green chemistry aims to make a difference by advocating the design of chemicals and synthetic pathways which minimise the use and formation of hazardous substances in the first place. Sheldon stated "Green chemistry efficiently utilises (preferable renewable) raw materials, eliminates waste and avoids the use of toxic and/hazardous reagents and solvents in the manufacture and application of chemical products."[47] Paul Anastas along with John Warner developed the twelve principles of green chemistry expressly outlining what should be considered when creating a new process or chemical and what issues should be addressed in order to make existing processes "greener".[48]

1. **Prevention**

   It is better to prevent waste than to treat or clean up waste after it has been created.

2. **Atom Economy**

   Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

3. **Less Hazardous Chemical Syntheses**

   Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. **Designing Safer Chemicals**

   Chemical products should be designed to effect their desired function while minimizing their toxicity.

5. **Safer Solvents and Auxiliaries**

   The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

6. **Design for Energy Efficiency**

   Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. **Use of Renewable Feedstocks**

   A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. **Reduce Derivatives**

   Unnecessary derivatisation (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. **Catalysis**

   Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

10. **Design for Degradation**

    Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. **Real-time analysis for Pollution Prevention**

    Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

12. **Inherently Safer Chemistry for Accident Prevention**

    Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

If we consider these as a general rule of thumb when designing new chemical products, and then combine them with the "twelve principles of green engineering", also developed by Anastas with Julie Zimmerman[49], which encourage the consideration of factors such as the production line and life-cycle of the product,

then the outcome is a product which poses as low a risk as possible to the user and the environment all the way through production, use, and disposal.

There is an ever increasing need for new, safer cure accelerators for anaerobic adhesives. As we learn more about chemicals and their metabolism, hazard and risk labelling can change over time. When this happens, it alters the amounts and way in which they can be used and by continuing to include them in adhesive and sealant formulations, the labelling of the end product must also be changed to reflect their inclusion. This makes the product less attractive to the end user and the market to which is offered. Without coming up with safer alternatives, the only way to mitigate this effect is to alter the product formulation such that the harmful components only comprise such an amount which means their risks do not fully need to be disclosed on the product label. However, to do so and still retain the same performance requires redevelopment of the complete formulation, not to mention ignoring the problem at hand.

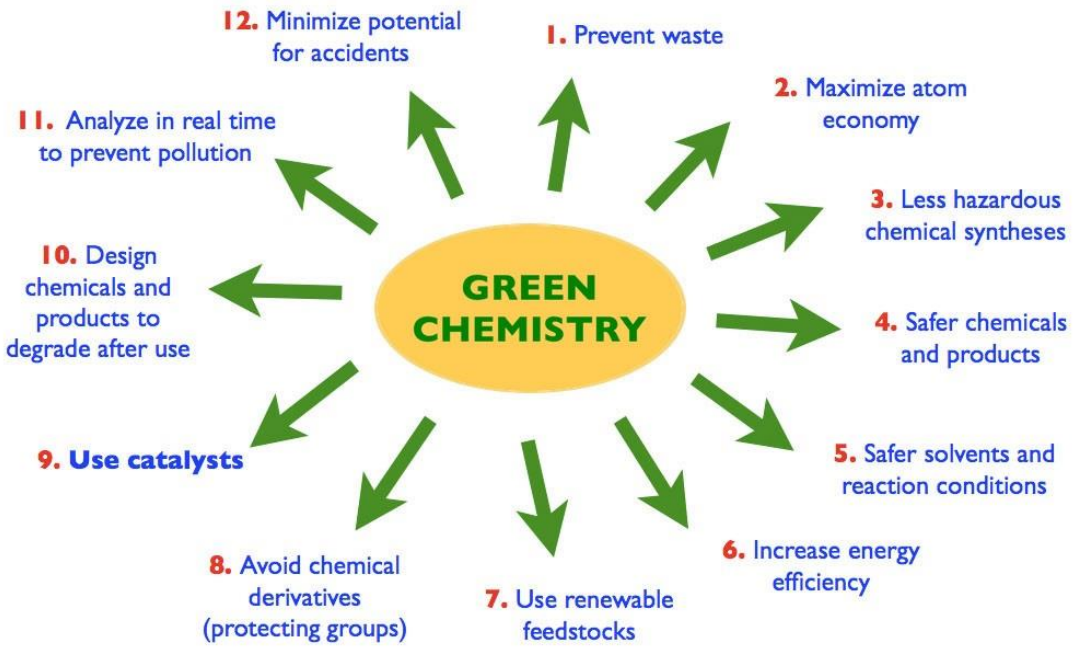


Figure 1.8: Principles of Green Chemistry[48]

If the new accelerators were to be designed using the principles of green chemistry as outlined above, then this would mean any such problems with hazard classification would inherently be accounted for and dealt with in the design stage as

the compound would have been created with the minimisation of its potential to pose risk in mind.

Henkel are devoted to creating safer anaerobic adhesive products. In 2008 they carried out a consumer survey which revealed a demand for safer anaerobic products. As a result of this, in 2009 they launched the first two anaerobic adhesives to have a "white" MSDS sheet no containing any declarable CMR's or hazardous components, Loctite 2400 and Loctite 2700, and, in 2011 they launched a further three products with the same health and safety standards. These products have proved to be a success, largely without eating into the traditional market sales of anaerobic adhesives. While this represents a milestone in creating more innocuous anaerobic adhesives, work still needs to be done to replace harmful components and truly remove their risk.

The aim of this research project is to not only design novel cure catalysts capable of replacing those established in the industry while considering these safety principles, but further to this, the environmental fate of the accelerators designed along with some of those which have already been established is to be evaluated through biodegradation and eco-toxicity screening. This represents the responsibility within green chemistry to ensure that an effort is made to make sure that any advances and breakthroughs made also encapsulate safety and environmental awareness as a design feature and do not treat it as a secondary concern to the primary challenge at hand.

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Chapter 2:

First Generation Cure Accelerators

2.1 Aim

The overall aim of this research project was to design anaerobic adhesive cure accelerators which are not only of comparable cure activity to those established in industry, but which also do not have any of the associated safety risks for the user, and are readily biodegradable and environmentally friendly.

In order to design these new, low toxicity cure accelerators however, the characteristics of these compounds which lead to their accelerator activity and those that lead to their toxicity first had to be identified.

The approach taken to achieve this was to identify a primary set of compounds which were of varying accelerator activity, then establish their toxicity and biodegradation. This would be compiled from reported data in the literature and additional screening as part of this PhD project. Firstly, this would allow for benchmarks to be set in terms of the level of activity/toxicity of existing accelerators against which future compounds developed could be compared. Further to this, the evaluation of such compounds would also allow for conclusions to be drawn in terms of the structural characteristics which emphasise either toxicity or cure activity and therefore aid the design and development of new accelerators.

Then by using these characteristics elucidated to develop new accelerators and evaluate their performance in the same areas, the more information about the structural-activity relationship of accelerator activity could be gathered and the design further refined.

2.2 Criteria of First Generation Accelerators

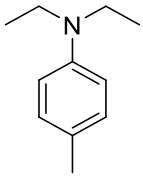
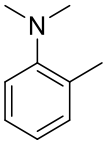
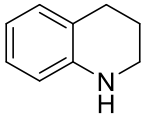
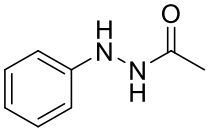
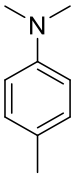
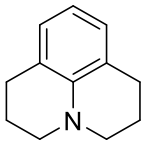
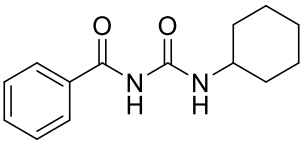
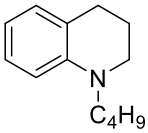
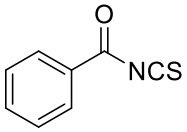
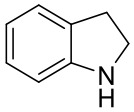
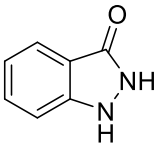
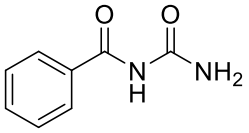
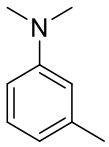
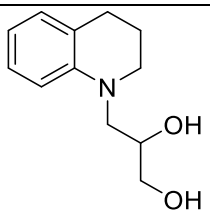
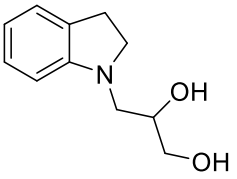
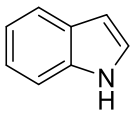
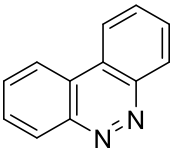
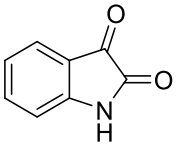
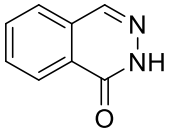
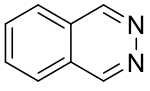
As explained above, with the first generation of anaerobic adhesive cure accelerators, the aim was to establish a primary set of correlation data against which future results could be contrasted and compared. Thus, this set of compounds was purposefully chosen to consist of those which were all of either – known toxicity, known accelerator activity, known biodegradation & eco-toxicity, or a mixture of these characteristics. This would, in theory, allow us to make estimations of the likely toxicity and/or activity of future compounds tested where one or both of these

properties was unknown and determine the positive/negative effects which the structural differences lead to.

Also, by breaking down the overall design of new cure accelerators into these desired properties, a concise way of illustrating the properties of each compound evaluated and where they needed to be improved is provided. Namely these were: mammalian toxicity, anti-bacterial activity, anti-fungal activity, accelerator activity, formulation solubility, biodegradability, and aquatic toxicity.

The structures of the compounds included in the first generation analysis are shown in Table 2.1 and in the subsequent sections their performance in the areas listed above is described.

Table 2.1: First Generation Accelerators

Structures of First Generation Accelerators		
 Diethyl-*p*-toluidine (**11**)	 Dimethyl-*o*-toluidine (**12**)	 Tetrahydroquinoline (**15**)
 Acetylphenylhydrazine (**16**)	 Dimethyl-*p*-toluidine (**18**)	 Julolidine (**27**)
 Benzoyl cyclohexylthiourea (**28**)	 *N*-Butyl THQ (**29**)	 Benzoylisothiocyanate (**30**)
 Indoline (**31**)	 Indazolinone (**32**)	 Benzoylurea (**33**)
 Dimethyl-*m*-toluidine (**34**)	 THQ "Diol" (**35**)	 Indoline "Diol" (**36**)
 Indole (**37**)	 Benzo[c]cinnoline (**38**)	 Isatin (**39**)
 Phthalazone (**40**)	 Phthalazine (**41**)	

2.3 Anti-Microbial Results & Discussion

In order to assess the toxicity of accelerator compounds, it would be of a great benefit if the need for screening methods such as mammalian testing could be modelled for by creating a correlation between their mammalian toxicity and a more accessible method of screening.

With this in mind, anti-microbial screening was chosen whereby it was proposed that by screening accelerator compounds of known mammalian toxicity against several bacterial and fungal strains, and then comparing these two sets of values, a correlation could be drawn between the two properties. This would serve to create a much more cost-effective, quicker method of evaluating the likely mammalian toxicity of any accelerator candidates during the design stage. Ultimately, where lead compounds are then established and possess the other desired properties of an accelerator, further toxicity screening can be carried out to further determine their toxicity and risk profile.

Anti-microbial screening was carried out at Charles University, Prague by Dr.Marcel Spulak as per Section 7.3 and 7.4. The anti-bacterial and anti-fungal activity of the first generation accelerator compounds is tabulated and discussed below.

2.3.1 Anti-Bacterial Test Results

Each compound (**11-12, 15-16, 18, 27-34)** was tested against eight different strains of bacteria listed below and where the minimum inhibitory concentration (MIC) value for each strain is reported as >2000 µM, no activity was observed within the limits of the test. For all compounds where activity was observed, the MIC is highlighted in bold font.

In the case of compounds **14-20**, the maximum concentration tested was 1000 µM due to limited solubility in the media used for screening.

The full name of each strain is also provided after the results (Table 2.5).

Table 2.2: Anti-Bacterial Screening of Compounds 11-12, 15-16, 18, 27-28

Bacterial Strain	Time (h)	Bacterial Response – MIC IC_{95} (µM)						
		11	**12**	**15**	**16 (APH)**	**18**	**27**	**28**
SA	24	>2000	>2000	>2000	>2000	>2000	**500**	>2000
	48	>2000	>2000	>2000	>2000	>2000	**500**	>2000
MRSA	24	>2000	>2000	>2000	>2000	>2000	**1000**	>2000
	48	>2000	>2000	>2000	**1000**	>2000	**1000**	>2000
SE	24	>2000	>2000	>2000	**1000**	>2000	**1000**	>2000
	48	>2000	>2000	>2000	**1000**	>2000	**1000**	>2000
EF	24	>2000	>2000	>2000	**1000**	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
EC	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
KP	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
KP-E	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
PA	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000

Table 2.3: Anti-Bacterial Screening of Compounds 29-34

Bacterial Strain	Time (h)	Bacterial Response – MIC IC_{95} (µM)					
		29	**30**	**31**	**32**	**33**	**34**
SA	24	>2000	**250**	>2000	>2000	>2000	>2000
	48	>2000	**250**	>2000	>2000	>2000	>2000
MRSA	24	>2000	**250**	>2000	>2000	>2000	>2000
	48	>2000	**250**	>2000	>2000	>2000	>2000
SE	24	>2000	**250**	>2000	>2000	>2000	>2000
	48	>2000	**1000**	>2000	>2000	>2000	>2000
EF	24	>2000	**250**	>2000	>2000	>2000	>2000
	48	>2000	**1000**	>2000	>2000	>2000	>2000
EC	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
KP	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
KP-E	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
PA	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000

Table 2.4: Anti-Bacterial Screening of Compounds 35-41

Bacterial Strain	Time (h)	Compound – MIC IC_{95} (µM)						
		35	**36**	**37**	**38**	**39**	**40**	**41**
SA	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
MRSA	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
SE	24	>1000	>1000	>1000	**250**	**1000**	>1000	>1000
	48	>1000	>1000	>1000	**250**	**1000**	>1000	>1000
EF	24	>1000	>1000	>1000	**1000**	**1000**	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
EC	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
KP	24	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	48	>1000	>1000	>1000	>1000	>1000	>1000	>1000
KP-E	24	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	48	>1000	>1000	>1000	>1000	>1000	>1000	>1000
PA	24	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	48	>1000	>1000	>1000	>1000	>1000	>1000	>1000

Table 2.5: Abbreviations of Each Bacterial Strain

Bacteria Code	Full Name	Strain	Gram Positive/Negative
SA	Staphylococcus aureus	ATCC 6538	Gram +
MRSA	Staphylococcus aureus (Methicillin Resistant)	HK5996/08	Gram +
SE	Staphylococcus epidermidis	HK6966/08	Gram +
EF	Enterococcus sp.	HK14365/08	Gram +
EC	Escherichia coli	ATCC 8739	Gram -
KP	Klebsiella Pnuemoniae	HK11750/08	Gram -
KP-E	Klebsiella Pnuemoniae (ESBL Positive)	HK14368/08	Gram -
PA	Pseudomonas Aeruginosa	ATCC 9027	Gram -

2.3.2 Anti-Fungal Test Results

Each compound was tested against twelve different strains of fungi listed below and where the minimum inhibitory concentration (MIC) value for each strain is reported as >2000 µM, no activity was observed within the limits of the test. For all compounds where activity was observed, the MIC is highlighted in bold font.
In the case of compounds **14-20**, the maximum concentration tested was 1000 µM.

The abbreviation and information of each strain is detailed in Table 2.9.

Table 2.6: Anti-Fungal Screening of Compounds 11-12, 15-16, 18, 27-28

Fungal Strain	Time (h)	Fungal Response – MIC IC_{80}/IC_{50}[a] (µM)						
		11	12	15	16 (APH)	18	27	28
CA1	24	>2000	>2000	>2000	**31.25**	>2000	**1000**	**1000**
	48	>2000	>2000	>2000	**125**	>2000	**1000**	**2000**
CA2	24	>2000	>2000	>2000	**7.81**	>2000	**1000**	**2000**
	48	>2000	>2000	>2000	**31.25**	>2000	**1000**	>2000
CP	24	>2000	>2000	>2000	**15.62**	>2000	**1000**	>2000
	48	>2000	>2000	>2000	**125**	>2000	**1000**	>2000
CK1	24	>2000	>2000	>2000	**3.9**	>2000	**2000**	>2000
	48	>2000	>2000	>2000	**7.81**	>2000	**2000**	>2000
CK2	24	>2000	>2000	>2000	**3.9**	>2000	**1000**	>2000
	48	>2000	>2000	>2000	**7.81**	>2000	**1000**	>2000
CT	24	>2000	>2000	>2000	**125**	>2000	**2000**	>2000
	48	>2000	>2000	>2000	**250**	>2000	**2000**	>2000
CG	24	>2000	>2000	>2000	**125**	>2000	**2000**	>2000
	48	>2000	>2000	>2000	**1000**	>2000	**2000**	>2000
CL	24	>2000	>2000	>2000	**15.62**	>2000	**2000**	>2000
	48	>2000	>2000	>2000	**62.5**	>2000	**2000**	>2000
TA	24	>2000	>2000	>2000	>2000	>2000	**2000**	**125**
	48	>2000	>2000	>2000	>2000	>2000	>2000	**250**
AF	24	>2000	>2000	>2000	>2000	>2000	**1000**	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
AC	24	>2000	>2000	>2000	**250**	>2000	**1000**	**65.5**
	48	>2000	>2000	>2000	>2000	>2000	>2000	**125**
TM	24	>2000	>2000	>2000	**3.9**	>2000	**250**	**31.25**
	48	>2000	>2000	>2000	**3.9**	>2000	**500**	**31.25**

[a] IC_{50} values were assessed for AF, AC, and TM. For all other fungi strains IC_{80} values were obtained

Table 2.7: Anti-Fungal Screening of Compounds 29-34

Fungal Strain	Time (h)	Fungal Response – MIC $IC_{80}/IC_{50}{}^{a}$ (µM)					
		29	**30**	**31**	**32**	**33**	**34**
CA1	24	>2000	**250**	>2000	>2000	>2000	>2000
	48	>2000	**500**	>2000	>2000	>2000	>2000
CA2	24	>2000	**2000**	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
CP	24	>2000	**250**	>2000	>2000	>2000	>2000
	48	>2000	**250**	>2000	>2000	>2000	>2000
CK1	24	>2000	**1000**	>2000	>2000	>2000	>2000
	48	>2000	**2000**	>2000	>2000	>2000	>2000
CK2	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
CT	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
CG	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
CL	24	>2000	**125**	>2000	>2000	>2000	>2000
	48	>2000	**250**	>2000	>2000	>2000	>2000
TA	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
AF	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
AC	24	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000
TM	24	>2000	**125**	>2000	**1000**	**250**	**125**
	48	>2000	**250**	>2000	**1000**	**250**	**250**

[a] IC_{50} values were assessed for AF, AC, and TM. For all other fungi strains IC_{80} values were obtained

Table 2.8: Anti-Fungal Screening of Compounds 35-41

Fungal Strain	Time (h)	Fungal Response – MIC IC_{80}/IC_{50}[a] (µM)						
		35	**36**	**37**	**38**	**39**	**40**	**41**
CA1	24	>1000	>1000	>1000	**500**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
CA2	24	>1000	>1000	>1000	**500**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**500**	>1000	>1000	>1000
CP	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
CK1	24	>1000	>1000	>1000	**500**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**500**	>1000	>1000	>1000
CK2	24	>1000	>1000	>1000	**500**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**500**	>1000	>1000	>1000
CT	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
CG	24	>1000	>1000	>1000	**250**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**500**	>1000	>1000	>1000
CL	24	>1000	>1000	>1000	**500**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**500**	>1000	>1000	>1000
TA	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
AF	24	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**1000**	>1000	>1000	>1000
AC	24	>1000	>1000	>1000	**500**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**500**	>1000	>1000	>1000
TM	24	>1000	>1000	>1000	**250**	>1000	>1000	>1000
	48	>1000	>1000	>1000	**250**	>1000	>1000	>1000

[a] IC_{50} values were assessed for AF, AC, and TM. For all other fungi strains IC_{80} values were obtained

Table 2.9: Fungal Strain Abbreviations

Fungal Code	Full Name	Strain	Fungi Type
CA1	Candida albicans	ATCC 44859	Can be grown as either yeast or filamentous(ATCC)
CA2	Candida albicans	ATCC 90028	Can be grown as either yeast or filamentous(ATCC)
CP	Candida parapsilosis	ATCC 22019	Yeast (ATCC)
CK1	Candida krusei	ATCC 6258	Yeast
CK2	Candida krusei	E28	Yeast
CT	Candida tropicalis	156	Yeast
CG	Candida glabrata	20/I	Yeast
CL	Candida lusitaniae	2446/I	Yeast
TA	Trichosporon asahii	1188	Yeast
AF	Aspergillus fumigatus	231	Filamentous
AC	Absidia corymbifera	272	Filamentous
TM	Trichophyton mentagrophytes	445	Filamentous

Using this first set of anti-microbial data, a complete correlation between anti-microbial activity and mammalian toxicity was difficult to establish. This is due to an inconsistency between which compounds the screening is identifying as active towards microorganisms and which compounds we already know to be toxic towards mammals. As can be seen, many of the compounds appear to be inactive towards all bacterial and fungal strains. Nevertheless, there are still several important outcomes illustrated by this study.

Acetylphenylhydrazine (APH) (**16**) showed a high level of toxicity towards ten of the twelve fungal strains tested and moderate toxicity towards several of the strains of bacteria. This result is an example of the premise which it was hoped the study as a whole would clearly demonstrate i.e. APH is already known from other sources to be acutely toxic towards mammals, and the anti-microbial data obtained, particularly the anti-fungal data, is in agreement with this. Thus, this displays how anti-microbial testing could be used as an indicator of the toxicity of accelerator compounds towards mammals.

The relationship between anti-fungal activity and mammalian toxicity does not come as a surprise given the cellular structure similarity of mammals and fungi. Both are eukaryotic and share similarities in how they synthesis proteins, replicate DNA, and the sterol make-up of their cell membranes. Thus, if the compound tested interferes with processes such as these in the fungal strains, they may also behave similarly towards mammalian cells.

However, other results obtained contradicted this theory. Three of the four toluidine based accelerators tested (**11**, 1**2**, **18**) showed no toxicity towards any of the bacterial or fungal strains tested with the fourth (**34** – Dimethyl-*m*-toluidine) exhibiting toxicity to only some of the strains of fungi. It would be expected that, given what is already known about the toxicity of these compounds and toluidines generally, that all four of these accelerators would exhibit higher toxicity.

For the most part, the compounds tested showed little or no toxicity towards any of the selected microbial strains. Benzocinnoline (**38**) displayed activity towards all of the fungal strains and many of the bacterial strains tested against and of the others that did, julolidine (**27**) and benzoyl isothiocyanate (**30**) exhibited moderate levels of toxicity towards a portion of both the bacterial and fungal strains with benzoyl cyclohexylthiourea (**28**) showing toxicity towards some of the fungal strains only.

With regards to the significance of the anti-fungal data, one should note that the strain, TM (Trichophyton mentagrophytes), is particularly sensitive and so any of the compounds which appear as toxic towards only this strain of fungi may actually not be particularly active. Furthermore, any which do not show any toxicity towards this strain could be tentatively thought of as being quite inactive. The sensitivity of the TM strain also allows us to construct a loose trend for increasing toxicity. For instance, the compounds which do not show any activity towards TM are of lower

toxicity than those which show only activity towards the strain, followed finally by compounds which possess toxicity towards TM as well as other strains:

11-12, 15, 18, 29, 31 < **32-34** < **16, 27-28**

Looking purely at this set of toxicological data, bicyclic compounds such as those based on tetrahydroquinoline (THQ) and indoline scaffolds appear to be least active and would show the most promise as a direction of development. This is in agreement with how anaerobic accelerators have been developed over the years in that despite now knowing that THQ and its analogues are harmful, they were seen as safer alternatives to the continued use of toluidines and APH.

When considering this study as a whole, even though APH was shown to be the most active of all the compounds tested and therefore in agreement with the original theory, a concrete trend between anti-fungal activity and mammalian toxicity cannot be constructed, at least until further testing is carried out and a larger dataset compiled.

2.4 Biodegradation & Eco-Toxicity Study

Fifteen of the first generation accelerator compounds were submitted for biodegradation and eco-toxicity evaluation at IQAC, Barcelona by Dr Teresa Garcia. Their biodegradation was evaluated using the CO_2 headspace test (OECD Test No. 310) under which conditions each compound must degrade by 60% over 28 days in order to be considered readily biodegradable (Section 7.1).
The aquatic toxicity of the compounds was evaluated using both microtox as well as against *daphnia magna (Section 7.2).*

2.4.1 Aquatic Toxicity

Each of the fifteen compounds were submitted for both Microtox and *Daphnia magna* aquatic toxicity evaluation and the results are tabulated below.

Under OECD standards, of the fifteen compounds analysed using *Daphnia Magna*, seven were classified as ‘acute toxicity II’ (Toxic to aquatic life) and seven as ‘acute

toxic III' (harmful to aquatic life) with the EC_{50} of the remaining sample (Benzoylurea - **33**) unable to be determined due to its low solubility.

Comparing the compounds within the group, certain derivitisations have shown to have no effect whereas others have been shown to have quite a positive impact on the toxicity observed. Concerning the THQ's tested, julolidine (**27**) and n-Butyl THQ (**29**) show little deviation from the parent molecule THQ (**15**) whereas when the THQ "diol" derivative (**35**) is tested we see a sizeable decrease in the toxicity observed. The positive effect of this derivitisation is further illustrated by the same conversion being applied to indoline (**31**) whose toxicity has also been favourably altered (**36**). This is an example of the low log$P_{o/w}$ "rule" for designing less aquatic toxic compounds. This "rule" states that the more water soluble a chemical is, the less bioavailable and by extension less toxic it is to aquatic life as the chemical must have high enough lipid solubility to cross the cell membrane. In this case the addition of the diol functionality makes both compounds more water soluble and therefore less harmful than their similar analogues. A similar trend involving the ΔE (HOMO-LUMO) of compounds exists whereby the higher this value is, the less likely it is to be toxic towards aquatic life.

Unfortunately experimentally determined Log$P_{o/w}$ values could not be found for the majority of the compounds and so a broader analysis of the aquatic toxicity of these compounds and their water solubility was not able to be made. We can nevertheless make estimations when designing derivatives as to whether they are likely to be more or less water soluble and thus design for more benign compounds.

Concerning the Microtox results, they largely did not deviate from the same trends seen in the Daphnia magna assay apart from the value determined for acetylphenylhydrazine. The daphnia magna test generated an EC_{50} value of 12 mg/L whereas the Microtox test returned an EC_{50} of 104 mg/L. While these tests are not directly comparable and the difference likely due to the greater sensitivity of the molecule to one than the other, this is not uncommon as of the many eco-toxicity assays which exist, no one is able to evaluate every class of chemical compound correctly and so rather the information gained from each such be pooled in order to gain a larger perspective on the toxicity of a compound.

Table 2.10: Microtox Evaluation of Compounds 11-12, 15-16, 18, 27-36

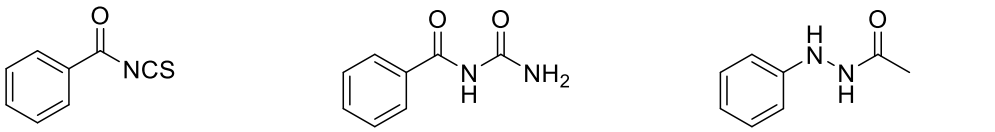
Compound	MICROTOX (30 min)		EC_{50} concentration range (mg/L)
	EC_{50} mg/L	IC95%	
Diethyl-p-toluidine (11)	5.8	4.3-7.7	1 to 10
Dimethyl-p-toluidine (18)	4.5	2.6-8.1	
Tetrahydroquinoline (15)	7.3	6.7-7.9	
Julolidine (27)	5.4	3.2-9.2	
Benzoyl cyclohexylthiourea (28)	1.7	1.3-2.1	
n-Butyl THQ (29)	6.7	3.8-12	
Dimethyl-o-toluidine (12)	2.3	2.0-2.6	
Benzoyl isothiocyanate (30)	3.0	1.9-4.9	
Indoline (31)	24	22-27	10 to 100
Dimethyl-m-toluidine (34)	19	16-24	
Indoline Diol (36)	69	43-110	
THQ Diol (35)	23	16-34	
Indazolinone (32)	>100	-	>100
Benzoylurea (33)	>100	-	
APH (16)	104	84-129	

Table 2.11: Daphnia Magna Screening of Compounds 11-12, 15-16, 18, 27-36

Compound	*Daphnia magna* (48 h)		EC_{50} concentration range (mg/L)	Hazard Classes (OECD, 2001)
	EC_{50} mg/L	IC95%		
Diethyl-p-toluidine (11)	5.6	4.9-6.5	1 to 10	Acute toxicity II (toxic to aquatic life)
THQ (15)	1.8	1.4-2.3		
Julolidine (27)	1.6	0.07-6.2		
Benzoyl cyclohexylthiourea (28)	1.1	0.97-1.2		
n-Butyl THQ (29)	1.0	0.81-1.2		
Benzoyl isothiocyanate (30)	5.9	5.1-7.9		
Indoline (31)	2.9	1.5-5.1		
Dimethyl-*p*-toluidine (18)	13	9.8-21	10 to 100	Acute toxicity III (harmful to aquatic life) -
Dimethyl-*m*-toluidine (34)	50	43-67		
Indazolinone (32)	13	12-13		
Dimethyl-*o*-toluidine (12)	29	24-39		
APH (16)	12	7.9-16		
Indoline Diol (36)	31	26-40		
THQ Diol (35)	27	26-40		
Benzoylurea (33)	>50	-	-	-

2.4.2 Biodegradation Results

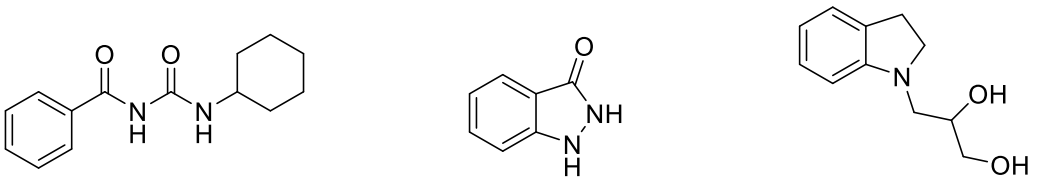
Of the fifteen compounds tested (**11-12**, **15-16**, **18**, **27-36**), benzoyl isothiocyanate (**30**), benzoylurea (**33**), and APH (**16**) reached the 60% pass threshold of the CO_2 headspace test and could be classified as readily biodegradable. Due to the experimental procedure, the set of fifteen compounds was split into two batches for analysis with two separate controls run. For this reason the results are split into two separate tables when reported.



Benzoyl Isothiocyanate 85% Benzoylurea 91% Acetylphenylhydrazine 74%

Figure 2.1: Readily Biodegradable First Generation Accelerators

Of the remaining twelve compounds, Benzoyl cyclohexylthiourea (**28**) and Indazolinone (**32**) showed significant biodegradation over the 28 day period (33-41%) but not enough to pass the CO_2 headspace test with compounds **35** and **36** showing little biodegradation and the remaining compounds showing no biodegradation.



Benzoylcyclohexylthiourea 33% Indazolinone 41% Indoline "Diol" 13%

Figure 2.2: First Generation Accelerators which Displayed Some Biodegradation

When comparing the readily biodegradable compounds (**16**, **30**, and **33)** it can be seen that sites which are susceptible to enzymatic hydrolysis as well as the enzymatic insertion of oxygen into the molecule are key when it comes to the design of a less persistent organic compound. This is the main biodegradability feature of the three compounds shown in Figure 2.1 and forms the main basis for their high activity along with the remaining phenyl ring fragments, particularly where an aniline-like group is a conceivable metabolite, still offering quite a straightforward site for the oxygen inserting oxygenase enzymes.[1]

These features can be better highlighted when **16**, **30**, and **33** are compared to those in Figure 2.2 which displayed some level of biodegradation and then to those which showed little to none. The majority of these contain bicyclic heterocycle fragments which structurally on their own are quite resistant to biodegradation as can be seen from the results of tetrahydroquinoline (**15**), 1%, and indoline (**31**), 1%. This is most likely due to the lack of positions across which cleavage or oxygen insertion can occur along with the electronegativity of the nitrogen making oxygen insertion of the aromatic ring less likely due to the electrophilic nature of this process.

Therefore if derivatives of these structures were to at some point arrive at this base fragment or another similarly persistent fragment in their biodegradation pathways then the process would slow down dramatically. This can perhaps be seen when the results of the indoline diol compound (**36**) are compared to that of indoline. We can see that after 28 days 12% of the compound had biodegraded compared to 1% of indoline. The difference in activity is possibly due to the diol substitution at the nitrogen position offering a good site for metabolism to begin. However, the still relatively low level of biodegradation of the indoline diol could be due to the pathway reaching an indoline-like structure and so slowing dramatically as we can see in the final seven day period the total biodegradation only increases by 1%. The same point can be illustrated by the comparison of THQ (**15**) and tetrahydroquinoline diol (**35**) which biodegraded by 1% and 8% respectively over the 28 days.

Further comparisons of THQ and similar structures illustrate some negative and ineffective ways of altering the biodegradability of a molecule. In the case of julolidine (**27**), where a third ring fused to both existing rings is added, and *N*-butyl THQ (**29**), where a butyl chain is added, the total biodegradation has decreased to

less than 1% in both cases. A general “rule of thumb” in the design of biodegradable molecules is that the more rings that are added to a molecule the more persistent the compound becomes and this indeed holds true. However, these same “rules” also show that adding unsubstituted alkyl chains should decrease a molecule’s persistence which has not been evident in this study although this factor is only thought to have an impact if the chain length is at least $\geq$4 carbons.

The toluidine compounds selected for testing also demonstrated poor biodegradability. This is most likely due to the disubstitution of the aromatic ring and the presence of the nitrogen atom which makes the oxygen insertion of the aromatic ring less likely as the oxygenase enzymes utilise an electrophilic form of oxygen in order to achieve this.

Table 2.12: Biodegradation (First Batch)

Compound	Biodegradation (%)				Inhibition (%)
	6 Days	13 Days	21 Days	28 Days	
SDS	78	85	93	93±0.7	
Diethyl-p-toluidine (11)	0	0	0	1±0.7	0
Dimethyl-p-toluidine (18)	0	0	0	0±0.5	0
Tetrahydroquinoline (15)	0	0	0	1±0.7	0
Julolidine (27)	0	0	0	0±0.8	11
Benzoyl cyclohexylthiourea (28)	3	1	35	33±1.3	0
n-Butyl THQ (29)	0	0	0	0±1	15
Dimethyl-o-toluidine (12)	1	0	0	1±0.9	0
Benzoyl isothiocyanate (30)	74	88	87	85±1.7	0
Indoline (31)	0	4	0	1±0.9	0
Indazolinone (32)	32	41	48	41±2.3	0

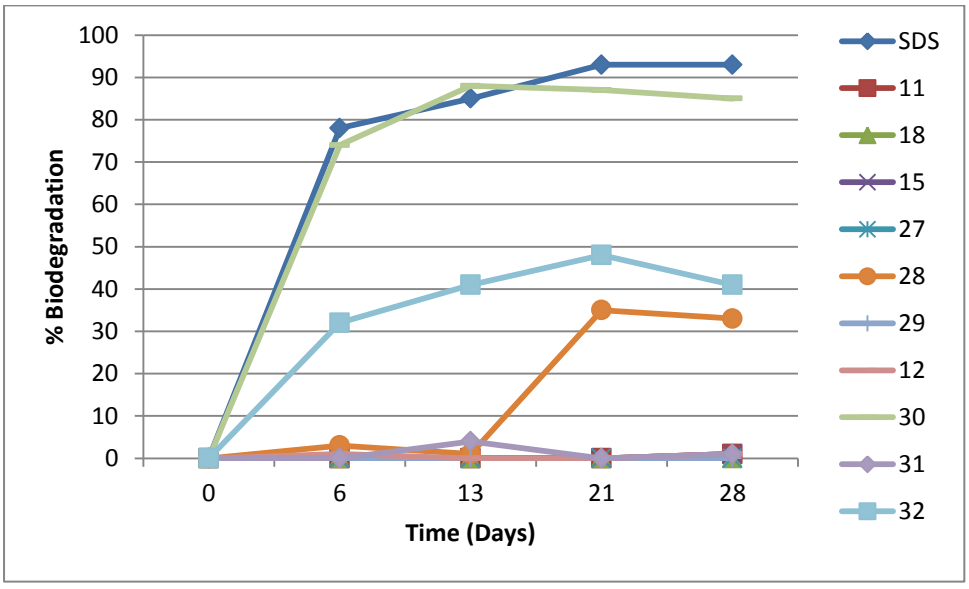
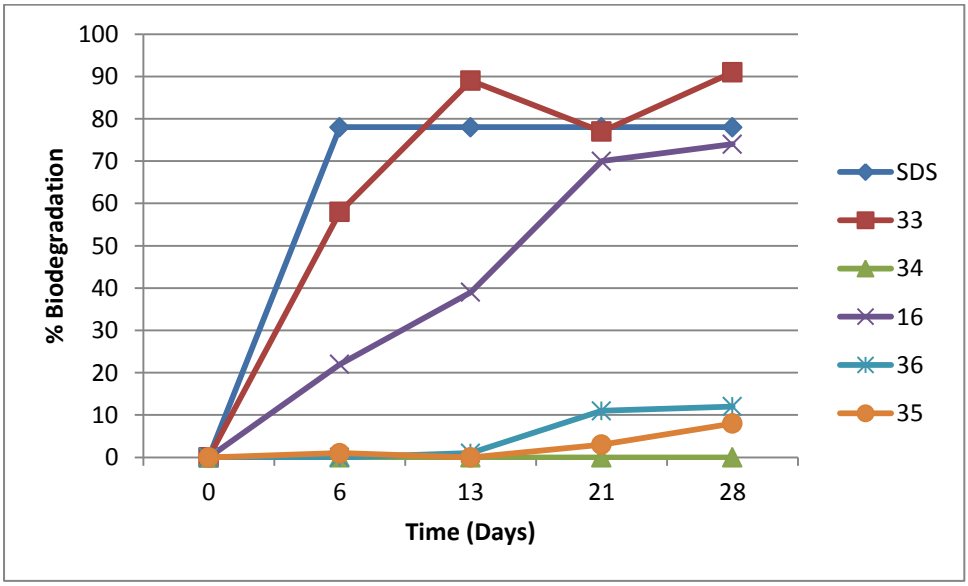


Table 2.13: Biodegradation (Second Batch)

Compound	Biodegradation (%)				Inhibition (%)
	6 Days	13 Days	21 Days	28 Days	
SDS	78	87	95	91±0.6	
Benzoylurea (33)	58	89	77	91±7.1	0
Dimethyl-m-toluidine (34)	0	0	0	0±0.5	0
Acetylphenylhydrazine (16)	22	39	70	74±4.5	0
Indoline Diol (36)	0	1	11	12±5.5	0
THQ Diol (35)	1	0	3	8±3.1	0



2.5 Prediction of Biodegradability of Cure Accelerators & –like Compounds

Given the considerable amount of time and cost required to carry out biodegradation screening, it would be extremely advantageous if existing predictive models could be successfully used for the prediction of the biodegradation of accelerator compounds. Likewise, in order to better aid the design of non-persistent accelerators, a survey of existing experimental biodegradation data on accelerator-like compounds was conducted. This was done with the aim of providing a base set of physical data with which to direct the initial design process as, due to the length of time required, the experimental testing of only accelerator molecules to construct this data set would take a prohibitive amount of time.

A number of prediction models exist which have been developed based on various approaches. Group contribution models have been designed from the approach that the overall biodegradation of a compound is determined by the contribution of its different chemical fragments and the frequency at which they occur. These fragments are defined on the basis of existing biodegradation evidence and are used with a training set of compounds to develop and tune the model.

'Chemometric' and artificial intelligence models on the other hand have been developed with various statistical models at their core of determining molecular descriptors of importance. This can result in the descriptors having no bearing on observed biodegradation processes but these models can be combined with sets of transformation rules in order to predict not just the extent of biodegradability but also the pathway through which degradation occurs.

2.5.1 Software Information

The chosen model for this study was the group contribution model BIOWIN. BIOWIN is a set of predictive models for the biodegradation of chemicals which forms part of the Estimation Programs Interface (EPI) Suite developed by the United States Environmental Protection Agency's office of Pollution Prevention & Toxics and the Syracuse Research Corporation. The software consists of seven models which have been developed using different means and with different chemical classes and tests in mind.

- **BIOWIN Models 1 & 2**[2,3] are linear and non-linear regression models respectively which were developed using weight of evidence evaluations of 295 compounds resulting in the determination of 36 biodegradability relevant chemical fragments. These 36 fragments along with the frequency at which they occur are combined with a chemical's molecular weight to determine how quickly a compound biodegrades. For the training set of compounds (295 – of which 186 degrade fast and 109 degrade slowly), both models were able to predict their biodegradability with >90% accuracy.
- **BIOWIN Models 3 & 4**[4] are ultimate and primary biodegradation models respectively which were developed using the same 36 fragments determined by the same 295 compound training set for models 1 & 2. In order to semi-quantitatively predict the time for biodegradation, a panel of 17 experts were surveyed by the EPA to predict the length of time, hours->months, it would take a series of 200 compounds to degrade in a typical aquatic environment. The predictions were averaged to gain a single number which was combined with the 36 fragments and molecular weight parameters to form the models which both predict the fate of the 200 compound training set with >80% accuracy.
- **BIOWIN Models 5 & 6**[5] are also linear and non-linear regression models respectively which were developed for predicting the performance of chemicals in the Ministry of International Trade and Industry (MITI)-1 test. This is a test which is carried out under Japan's chemical substance control law and is one of the six "Organization for Economic Co-operation and Development" (OECD) approved tests for ready biodegradability. The dataset of 884 compounds was split into a 589 compound training set and a 295 compound validation set. The relevant chemical fragments were determined in much the same manner as for models 1 & 2 with 42 being determined and this combined with a chemical's molecular weight is used to predict the ready biodegradability with both models able to predict the outcome of the validation set with >80% accuracy.
- **BioWin Model 7**[6] is an anaerobic model which predicts the degradation of a compound under the ''serum bottle'' test conditions. 169 compounds were used in its development with all incorporated into both training and

validation stages. A total of 37 fragments were identified and molecular weight is not included as a parameter in its calculation. The model correctly predicted 90% of the compounds with Musson et al.[7] carrying out further independent evaluation.

2.5.2 Testing

In order to carry out this study, compounds of interest were chosen from each of the tetrahydroquinoline, indoline, benzoxazine, and toluidine classes of accelerator. Beginning with the base accelerator structures for each class, changes were made which could be perceived to either enhance or diminish their persistence. In order to assess what changes the software recognised to be positive or negative, each of these structures were input into the BIOWIN prediction program and their performance in each of the seven models recorded. For BIOWIN models 1-2 & 5-7, this performance is either positive or negative result but for models 3 and 4 a timeline for the likely degradation is output.

These results were then compiled and trends constructed in order to determine which structural changes were likely to increase or decrease the likelihood of a compound to be predicted as readily biodegradable. One could then potentially use these trends to aid the design of less persistent accelerators.

In order to determine the accuracy of predictions for anaerobic accelerators, the software was also used to predict the fate of the fifteen "first generation" accelerators tested experimentally.

A list of all structures evaluated and their results can be found in Appendix A.

2.5.3 Results

The results, with respect to what changes the models consider to strengthen/weaken a compound's biodegradability, were largely expected given what we know about the principles of biodegradation.

- The introduction of oxygen atoms, whether in the form of carbonyl, carboxyl, ether functionalities etc. greatly improve the biodegradation due to the increased propensity for hydrolysis to occur.
- Likewise, the reduction in bioavailability caused by the addition of groups such as sulfonated moieties is recognised. The addition of amine and nitro groups also has a negative impact upon biodegradation.
- The less aromatic or aliphatic rings in a structure, the better the performance, and in the case of THQ/Indoline derivatives, substitution of the aromatic ring improves the biodegradation greater than substitution of the aliphatic heterocycle.

These points are illustrated below in the results of the best performing compounds from each class.

Tetrahydroquinolines

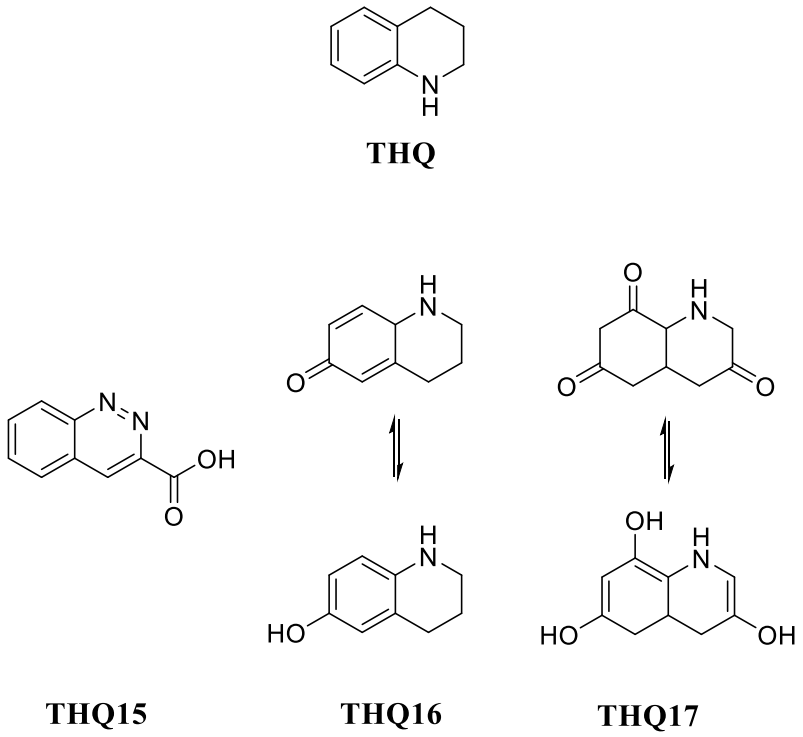


Figure 2.3: Best Performing THQ Based Compounds

Indolines

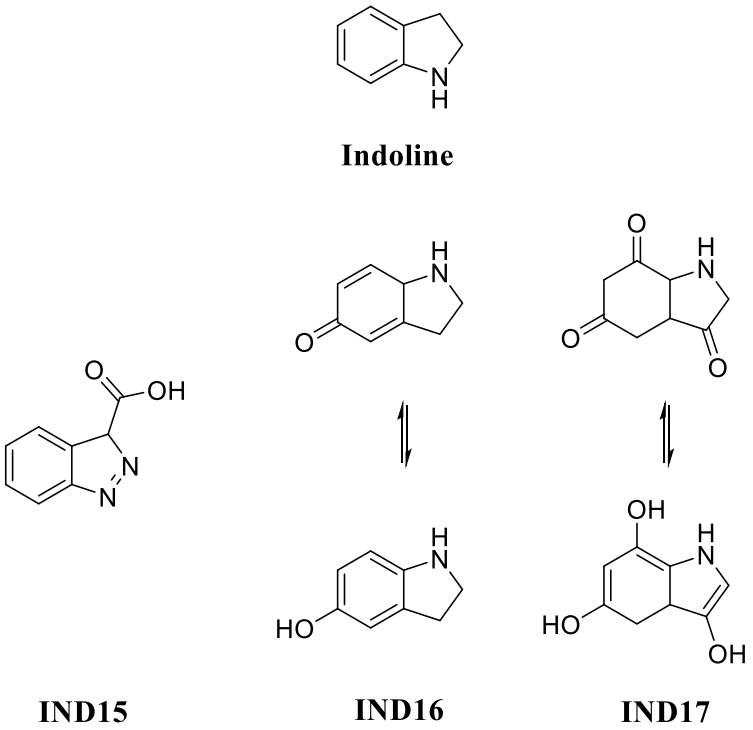


Figure 2.4: Best Performing Indoline Compounds

Benzoxazines

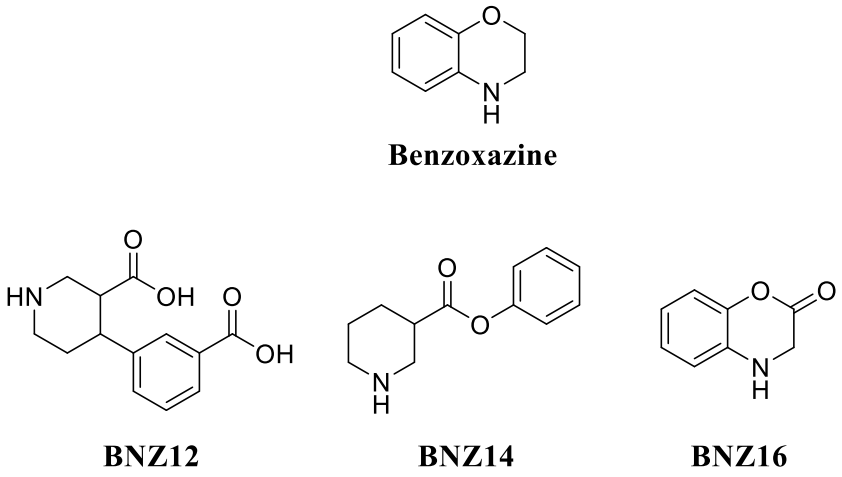


Figure 2.5: Best Performing Benzoxazines

The tetrahydroquinolines best illustrate the points made above where the addition of oxygen atoms along with the substitution of the aromatic ring system results in greatly reduced environmental persistence. As expected given their similarity, the derivitisations made to the indoline compounds followed much the same trend as those for the THQ's. Strictly speaking benzoxazines **BNZ12** and **BNZ14** do not belong to the class but as changes were made to the base structure in order to study the effects on biodegradation, they became less similar.

Unfortunately whilst these examples represent the best performing compounds from a biodegradation standpoint, this does not necessarily mean they will also possess anaerobic accelerator activity. Not every derivitisation made to the base structures could be considered positive in terms of retaining accelerator activity and so it was expected that some would not be useful as accelerators. Examples of such changes are the removal of the aromaticity of a compound such as THQ and Indoline **17**, the movement of the nitrogen atom too far from the aromatic centre of the compound such as in **BNZ12**. Thus, if we also take the likelihood of accelerator activity into account, then the best lead compounds to rise from this short study would be THQ **15** and benzoxazine **16** as they best retain the structural characteristics of an accelerator whilst still yielding promising predicted biodegradation results.

It should also be pointed out that the design trends predicted to enhance biodegradability come as no surprise. These are largely the same "rules of thumb" which would already be incorporated into the design and evaluation of possible biodegradable compounds and so there is little necessity for prediction software to aid in their identification. Ultimately the applicability of the predictions made will be determined by the comparison with the physical data already generated.

2.5.4 Comparison of CO_2 Headspace Results with BIOWIN Predictions

Using the BioWin program included in the EPI software suite, the biodegradation of the same fifteen compounds discussed in Section 1.3 was predicted. In order to evaluate the accuracy of the prediction software for structures of these types the results are compared below.

Table 2.14: Comparison of Predicted and Experimental Biodegradation Results

Compound	BIOWIN Prediction Models								CO_2 Headspace Test Result (%)
	1	2	3	4	5	6	7	RB Prediction	
11	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No	1±0.7
18	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No	0±0.5
15	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No	1±0.7
27	BF	DNBF	W-M	W	NRD	NRD	DNBF	No	0±0.8
28	BF	BF	W-M	D-W	NRD	NRD	DNBF	No	33±1.3
29	BF	BF	W-M	D-W	NRD	NRD	DNBF	No	0±1
12	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No	1±0.9
30	BF	BF	W	D-W	NRD	NRD	BF	No	85±1.7
31	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No	1±0.9
32	BF	BF	W	D-W	NRD	NRD	DNBF	No	41±2.3
33	BF	BF	W	D-W	NRD	NRD	BF	No	91±0.7.1
34	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No	0±0.5
16	BF	BF	W	D-W	NRD	NRD	DNBF	No	74±4.5
36	BF	BF	W	D-W	NRD	NRD	DNBF	No	12±5.5
35	BF	BF	W-M	D-W	NRD	NRD	DNBF	No	8±3.1

Table 2.15: Abbreviations of BIOWIN Prediction Output Results

Abbreviation	Explanation	Abbreviation	Explanation
BF	Biodegrades fast	DNBF	Does not biodegrade fast
NRD	Not readily degradable	D	Days
W	Weeks	M	Months

Although none of the compounds (**16**, **30**, **33**) which passed the CO_2 headspace test (Tables 2.12 and 2.13) and can be considered readily biodegradable, were predicted as degradable by all of the BioWin model tests,, certain loose trends can still be seen.

The two compounds (**30**, **33**) which showed the highest levels of biodegradation in the CO_2 headspace test were the only two compounds to pass the BioWin model 7 prediction, although this is an anaerobic model, BioWin may still suggest that if a compound is likely to be readily biodegradable it will be predicted so by this model. Also any compound (**16**, **28-30**, **32-33**, **35-36**) which showed a biodegradation level of greater than 1% was predicted to biodegrade fast by BioWin model 2 which suggests that any compound which passes this test will degrade to some extent but is not guaranteed to degrade to a level where it can be considered to be readily biodegradable. Likewise this group of compounds is the most likely to have their ultimate degradation predicted to be weeks by BioWin model 3.
It should also be pointed out where the only the disagreement between the predicted and the actual results was in failures of BioWin models 5 & 6 (as is the case with the readily biodegradable compounds **16**, **30**, **33**) and that this is not entirely unexpected as the MITI-1 test is often regarded as one of the hardest tests to gain a pass result in and so its prediction models are equally tough to pass given they were constructed using physical MITI-1 test results.

It is important to note that these correlations are loose at best and, from this set of data points concrete trends between the BioWin prediction software and the CO_2 headspace test cannot be constructed. It is evidence to suggest that the BioWin set of predictive models is not accurate enough to predict the fate of anaerobic cure accelerators and like compounds.

The main limiting factor of a group prediction model such as BioWin is the training set of compounds used to develop it. If there are not enough compounds of sufficient similarity to the compounds which the model is being used to predict, then one possibility is that the "rules" the model uses simply do not transfer completely from one class to the other and so the result may not be 100% accurate. Each model's level of accuracy is also affected by the number of positive and negative "real" results within the training set. If there is a large favouring of one over the other then the predictions which the model makes will reflect this, i.e. when a compound is input that the model does not completely recognise or is uncertain of then it will wrongly make a positive or negative prediction by default.

2.5.5 Existing Data

Whilst carrying out this predictive study, an analysis of existing biodegradation databases was carried out to assess the level of existing biodegradation data relating to accelerator and accelerator-like compounds.

This was carried out by surveying the Syracuse Research Corporation's environmental fate database, BIODEG.[8] This is a database of experimental biodegradation data in which the fate of each compound is recorded in terms of likely time to degrade as well as an attached confidence factor of that result which is based on the number of tests carried out, availability of concurring data from other sources, and the source itself.

This database was examined for not only existing anaerobic accelerators but also molecules of similar structure whose environmental fate could aid the design of biodegradable cure accelerators. The results are tabulated in Appendix B and results are highlighted where there is a high confidence in the degradative process proceeding either fast or slow, as well as the results of any current accelerators.

The data largely agrees with the trends displayed by the biodegradation study of the first generation accelerators in Section 1.3. For example it further illustrates the slow degradation of aniline/toluidine compounds where there is substitution at the nitrogen position. When this substitution is removed the degradation process speeds up, but unfortunately this substitution is also crucial in terms of maximising cure activity for these compounds.

2.6 First Generation Analysis Outcomes

Although the evaluation of this first set of accelerators has not entirely been successful in building the correlations which were hoped, there are still a lot of encouraging outcomes. The anti-microbial tests did not match up completely with the known mammalian toxicity data, however, the anti-fungal data did show possible signs that incorporation into the estimation of mammalian toxicity is possible. This can be built upon with the screening of further sets of compounds but it appears that at least anti-fungal data may be used as a primary method of eliminating lead compounds and then with the addition of cytotoxicity or other toxicological evaluation methods, the viability of the remaining compounds could be assessed. By building up a broader set of screening data using non-mammalian assays, a correlation between mammalian and non-mammalian toxicity may then be able to be constructed. This approach could then also lead to more concrete assertions as to the source of toxicity of accelerator structures and how best to alleviate this whilst retaining cure activity.

The biodegradation screening carried out determined that three of the fifteen compounds (**16**, **30**, **33**) passed the 60% threshold for readily biodegradability. This illustrated the "rules" of designing less persistent chemicals in effect which is an important factor that should not be overlooked as it reinforces the logic behind the premise on which this study hopes to design safer anaerobic accelerators. The biodegradation data readily displayed the positive effect of introducing structural components which are susceptible to hydrolysis and oxygen insertion while illustrating the drawbacks of modifications such as short chain alkyl substitution. This can also be observed in the eco-toxicity study carried out where the respective guidelines for the design of chemicals which are non-toxic to aquatic organisms can be seen quite clearly.

Where the predicted biodegradation results did not agree in most cases with the actual results obtained, this is potentially due to a lack of a training set of data large enough for accelerator compounds to create an accurate software prediction. This opens up the possibility that biodegradation results obtained during this project may ultimately be submitted for the further development of predictive models. The results obtained across these initial studies and the key outcomes can be used to determine possible candidates for the next generation of accelerator compounds to be

tested. For instance, the structural subunits of APH lend it to being aquatically non-hazardous as well as biodegradable and so alternative cure accelerators should retain this property for favourable comparison. However, since we already know of the mammalian toxicity of APH[9], and the subsequent classification as "acutely toxic (category 3)" under GHS criteria, APH is not a viable, safe accelerator. Likewise we can see with the THQ and indoline derivatives how the changes made to the base molecule have improved their biodegradability and eco-toxicity and can therefore be used in the selection of more environmentally friendly accelerator compounds. Whilst structural changes such as these may appear insignificant, in the overall design of a novel safer cure accelerator, the establishment of primary parameters to aid the incorporation of biodegradability and reduce eco-toxicity are crucial, particularly at an early phase such as this.

2.7 References

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Chapter 3:

Second Generation Cure Accelerators

3.1 Screening & Evaluation of Second Generation Accelerators

Based on the screening results of the first generation accelerators examined in Chapter 2, fused bicyclic ring structures clearly pose a problem in terms of their persistence in the environment. Despite their favourable cure activity, compounds such as THQ and indoline are not considered environmentally friendly due to their classification as "toxic to aquatic life" and their poor biodegradability. They do however represent progress in terms of having replaced APH and DE*p*T amongst others in certain adhesives to create safer products for the user.

They still possess some safety drawbacks, but if a group of analogous derivatives were to be synthesised whereby the environmental issues were addressed whilst retaining the cure activity, then this would result in an accelerator which is a step closer to be being inherently safer and environmentally benign.

To identify molecules which could potentially address these issues, a survey of naturally occurring nitrogen heterocycles was carried out. If a naturally produced group of compounds were to be identified as cure accelerators, then they could possibly be intrinsically of no threat to the environment.

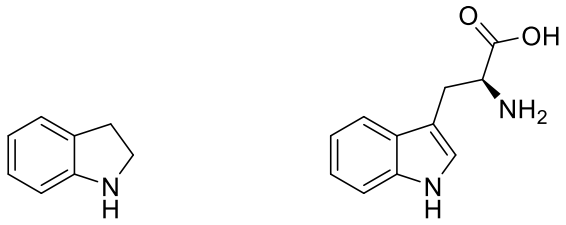
3.2 Natural Sources of Accelerator-like Compounds

Were a naturally occurring compound to be identified as an active accelerator, this could prove to be extremely advantageous. Given that this project is being carried out with "green" chemistry ethics in mind, identifying natural sources of accelerators or the building blocks required to synthesise them would fulfil a number of the "twelve principles of green chemistry"[1]. Any suitable natural product identified could represent a renewable feed stock which is possibly of low toxicity, both mammalian and to the wider environment, as well as being inherently degradable.

To this end, naturally occurring nitrogen heterocycles were explored with a shortlist drawn up of any which displayed reasonable structural resemblance to existing anaerobic adhesive accelerators and were therefore likely to possess activity.'

3.2.1 Tryptophan

Of the 21 common eukaryotic amino acids, there is one, tryptophan, which bears a striking resemblance to the structure of some known anaerobic accelerators. When the structure of tryptophan is examined, it can be seen quite clearly that it is very similar to the indoline accelerators (**31**, **36**) which were tested as part of the first generation accelerator study discussed in chapter two.



Indoline L-Tryptophan

Figure 3.1: Comparison of Indoline and Tryptophan Structures

Judging the molecule based on the same criteria as introduced in chapter two: anti-microbial toxicity, eco-toxicity, and biodegradability, tryptophan compares quite well to some of the established accelerators.

In terms of mammalian toxicity L-tryptophan has an oral LD_{50} of 16000 mg/kg (rat - oral)[2] which, while not being directly comparable, is considerably less harmful in the context of other commonly used accelerators, acetylphenylhydrazine (LD_{50} oral – mouse – 270 mg/kg)[3], and its parent molecule indole (LD_{50} Oral – rat 1000 mg/kg).[4] This results in L-tryptophan being available "label-free" as per GHS chemical hazard identification and would represent a significant improvement in the risk associated with handling anaerobic adhesive.

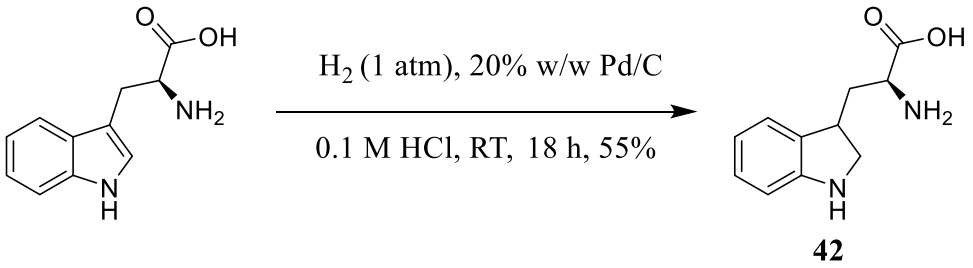
Tryptophan as a molecule does not persist in the environment due to it being susceptible to photolysis by sunlight.[5,6] These favourable properties coupled with the fact that there are established methods of its production on an industrial scale[7] and readily available feed stocks make it an attractive lead target.

Be that as it may, when evaluating the potential for accelerator activity in the molecule there is one significant drawback to the application of tryptophan.

Indoline is a compound which has already been proven to be active as an accelerator,[8] but its parent molecule, indole, has very little activity even though the two compounds only differ by the presence of an unsaturated carbon-carbon bond. If we compare tryptophan to indole we can see that it can be thought of as a substituted indole where this bond is present and so to truly evaluate possible activity, this bond should be reduced. Doing so results in the compound 2,3-dihydrotryptophan which, in theory allows for maximised accelerator activity whilst potentially retaining the benefits of using an amino acid based source. From a green chemistry perspective, this transformation is satisfactory given that 100% atom economy is attained and the reactions is carried out under mild, aqueous conditions using a reusable catalyst.

3.2.1.1 Dihydrotryptophan

The preparation of dihydrotryptophan (**42**) was achieved through the hydrogenation of L-tryptophan as outlined by Daly et al.[9] Under the reaction conditions outlined in scheme 1 below, a yield of 55% was achieved.



42

Scheme 3.1: Synthesis of Dihydrotryptophan

The reason for this lower than expected yield is thought to be a degradation of the product or starting material in the reaction solvent, 1 M hydrochloric acid, as upon analysis of the aggregate it does not appear to be solely unreacted starting material, but a decomposition product. In fact, the photo-oxidation of tryptophan in solution has been shown to occur under room conditions[5] as well as at low pH.[6] Unfortunately, the reaction must be carried out under these acidic conditions in order to completely dissolve the tryptophan starting material due to its low solubility in the reaction solvent. The reaction then must also be allowed to progress for this length of

time in order to ensure the optimum reaction of the starting material and balance between product formation and decomposition with the monitoring of the reaction by methods such as TLC difficult due to the marginal polarity difference between the starting material and product.

Once enough material was successfully synthesised, anaerobic cure accelerator testing was carried out. This involved the incorporation of dihydrotryptophan into a test formulation of an anaerobic adhesive as outlined below in Table 3.1.

Table 3.1: Test Formulation of an Anaerobic Adhesive

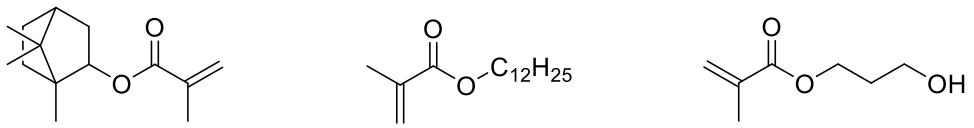
Component	**% Weight**
Polyethylene glycol dimethacrylate	95.5
Saccharin	0.5
TMBH	2
Premix 16	0.5
Premix 17	0.5
Dihydrotryptophan	1

In order to do so the monomer and both premixes 16 & 17 are mixed for 10 minutes before the dihydrotryptophan (**42**) accelerator is added. This solution was allowed stir at room temperature for two hours, however, after doing so no dissolution of the accelerator had occurred. The mixture was therefore heated to 45°C whilst stirring for 45 minutes after which the dihydrotryptophan appeared to have gone into solution. The saccharin was then added before subsequently adding the TMBH hydroperoxide after cooling to room temperature.

A fixture time test was set up to evaluate the shear strength by affixing two pieces of grit-blasted mild steel together using the adhesive formulation such that they overlapped. In addition to this, black oxide nuts and bolts were thread-locked and sealed with the mixture in order to test the torque strength of the bond after 24 hours. A stability test was also carried out by leaving a sample of the formulation at 82^{o}C in an oil bath overnight.

The strength of the lap shear specimens were tested by attaching a 3 kg weight to one of the steel pieces and measuring the time taken for the bond to fail. The joint failed almost immediately illustrating that no curing of the adhesive and bond formation had taken place. When the stability test solution was analysed after being left overnight, the accelerator had precipitated out of solution. This failure of the formulation stability rendered both the fixture time and torque strength tests null and void and so they were not completed. The absence of any observed curing was most likely due to the precipitation of the dihydrotryptophan upon cooling of the formulation after bond preparation.

In an effort to study the solubility properties of dihydrotryptophan and its feasibility as a cure accelerator, it's solubility in several other monomers was examined. These were lauryl methacrylate, isobornyl methacrylate (IBOMA), and hydroxypropyl methacrylate (HPMA).



Isobornyl methacrylate Lauryl methacrylate Hydroxypropyl methacrylate

Figure 3.2: Structures of Selected Monomers

Unfortunately, dihydrotryptophan did not dissolve in any of these monomers but it was noted that when the pH was lowered, dissolution occurred. For this purpose acrylic acid was used given its use within certain adhesive formulations. It was attempted to prepare another test formulation using PEGMA as the monomer, however for every 1 g of PEGMA used, 1 mL of acrylic acid was required to dissolve the 1% w/w of dihydrotryptophan present. This would not accurately represent any anaerobic adhesive formulation and thus it was recommended that no testing be carried out using this mixture as the results gathered would not offer any comparability to other anaerobic systems.

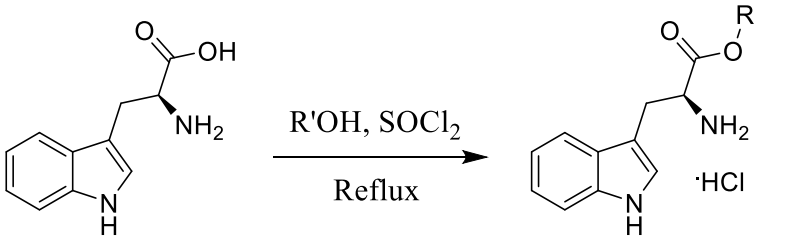
This occurrence represents a simple yet challenging aspect of this research project. A novel cure accelerator can be designed to be both of low toxicity and

environmentally friendly, and the time spent in order to synthesise and purify the compound efficiently. However, unless the molecule proves to be soluble in a desired monomer and anaerobic adhesive formulation, the work will ultimately prove fruitless as its curing activity cannot be evaluated.

It was proposed that the preparation of a more lipophilic derivative of dihydrotryptophan may solve this solubility issue, for example at either nitrogen position. This however would require protection group chemistry in order to ensure that substitution only took place at the intended position and not at both nitrogen positions.

In order to try and circumvent this multi-step synthesis and keep in line with the green chemistry approach of this project, a more straight forward solution was sought. The most facile way of making more lipophilic dihydrotryptophan analogues would be to synthesise ester derivatives. Their solubility in the PEGMA monomer could then be determined. This offered two synthetic routes: to reduce the tryptophan double bond and then carry out esterification directly on dihydrotryptophan, or to synthesise the tryptophan ester before reduction of the unsaturated bond.

To determine which order of synthesis is the most efficient, the synthesis of dihydrotryptophan ethyl ester was attempted. Both methods were evaluated and it was determined that the most efficient way to synthesise dihydrotryptophan esters was to synthesise the corresponding tryptophan first followed by hydrogenation to reduce the double bond.



43 R = CH_3, 83%
44 R = C_2H_5, 72%
45 R = C_4H_9, 76%
46 R = C_8H_{17}, 69%

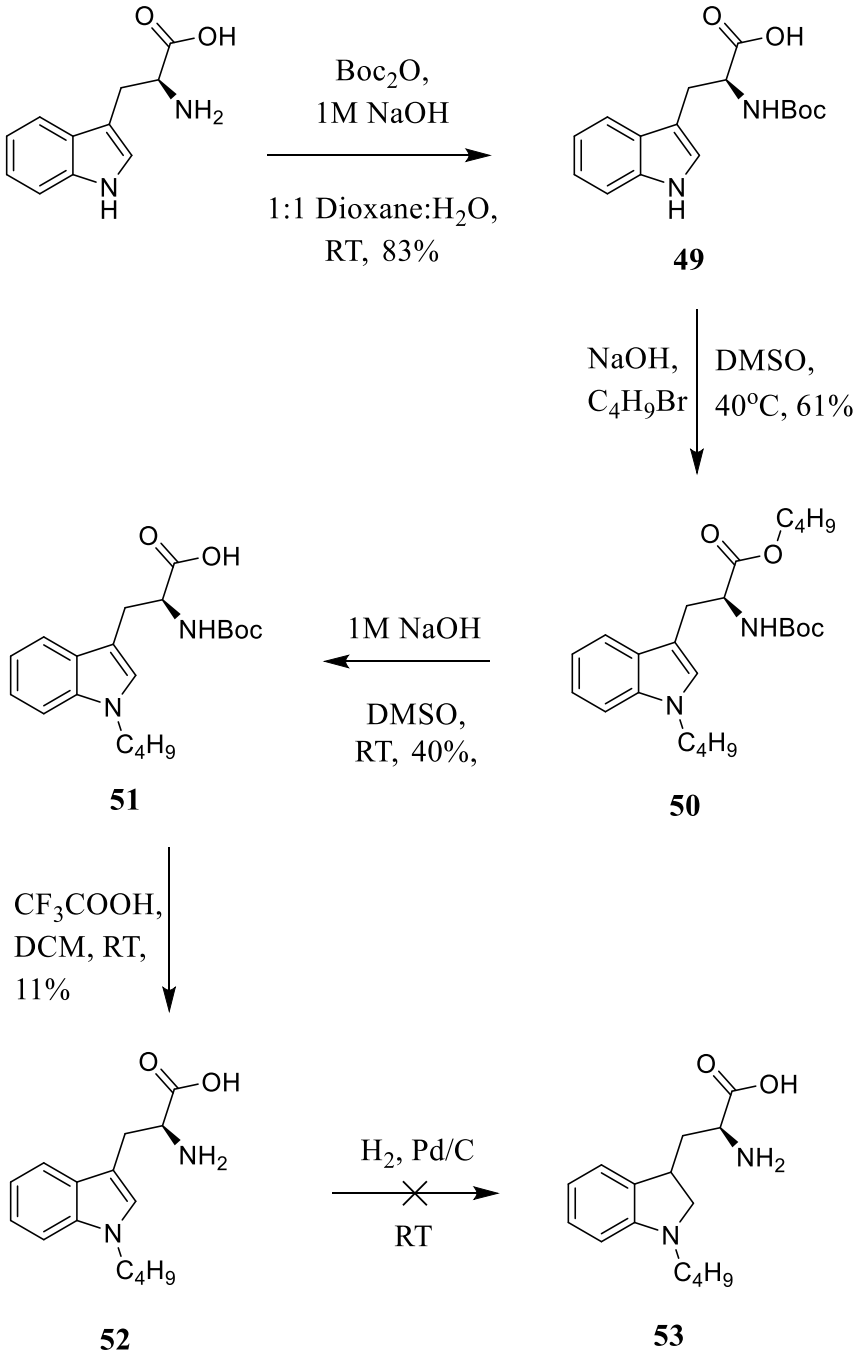
Scheme 3.2: Esterification of L-Tryptophan

Using a modified procedure of that outlined by Kir'yanova et al[10], the methyl (**43**), ethyl (**44**), butyl (**45**), and octyl ester (**46**) hydrochloride salts of L-tryptophan were synthesised. This offered a range of lipophilic compounds with which to assess the solubility of dihydrotryptophan. The reduction of the double bond between positions two and three was then attempted as before under slightly modified conditions.
Under these conditions the methyl (**47**) and butyl (**48**) derivatives of dihydrotryptophan were synthesised successfully, however the octyl ester showed no evidence of even partial reduction. The difference in activity displayed by the long chain derivative was thought to possibly be as result of the long alkyl chain restricting interaction between the C-C double bond and the catalyst surface.

The methyl and butyl esters were tested for their solubility at 1% w/w in the PEGMA monomer to determine whether to prepare a full adhesive formulation to carry out accelerator evaluation or not. They too were also found to be insoluble both at room temperature and when heated to 50°C. Dihydrotryptophan and its methyl and butyl esters were also tested for their solubility in hydroxyethyl methacrylate (HEMA) in addition to HPMA as before but with the same outcome.

With these results in hand, and knowing that the design "rules-of-thumb" for anti-microbial toxicity state that the effects worsen as unsubstituted carbon chains grow beyond four carbon atoms in length[11], the decision was made to move on to the next set of dihydrotryptophan targets.
Derivitisation at either nitrogen position represented the next most facile position at which to attempt to modify the physical properties of dihydrotryptophan analogues.

The chosen targets were N^{α}-alkyl- as well as 1-alkyl tryptophan derivatives. As is illustrated by Scheme 3.3, their synthesis also involves the preparation of several intermediates which could also prove to be of interest in terms of studying their solubility effects and potentially consolidate the synthesis of several targets. As before, it was decided to first synthesise the corresponding tryptophan derivative before attempting reduction of the double bond to yield the dihydrotryptophan.



Scheme 3.3: Synthesis of 1-Butyl Tryptophan

The preparation of 1-butyl tryptophan (**52**) was achieved through the boc-protection of L-tryptophan followed by bromo-alkylation after which the ester and boc-protecting groups were cleaved.

However, as was the case with Tryptophan octyl ester, no reduction of the double bond was observed under the same reaction conditions. Again, this could possibly be due to steric hindrance around the C-C double bond preventing interaction between

52 and the catalyst surface. Although dealing with a shorter carbon chain in this instance, substitution at either nitrogen position means that the effect of any shielding towards the unsaturated bond could be amplified.

Many reaction conditions were tested in an attempt to establish a method of reducing tryptophan derivatives to their corresponding dihydrotryptophan. This included the variation of the reaction solvent, temperature, catalyst, time, and pH. As well as these direct catalysed hydrogenations, several other reductive methods were carried out in an attempt to achieve tryptophan reduction (Table 3.2).

Table 3.2: Hydrogenation Conditions

Entry	Catalyst	Catalyst Loading	pH	Temperature (^oC)	Solvent	H Source
1	10% Pd/C	20% w/w	2	RT	THF	H_2
2	10% Pd/C	10% w/w	2	RT	THF	H_2
3	10% Pd/C	20% w/w	7	RT	THF	H_2
4	10% Pd/C	20% w/w	11	RT	THF	H_2
5	10% Pd/C	20% w/w	2	50	THF	H_2
6	10% Pd/C	20% w/w	2	RT	MeOH	H_2
7	10% Pd/C	20% w/w	2	RT	EtOAc	H_2
8	10% Pd/C	20% w/w	2	RT	EtOH	H_2
9	20% Pd/C	20% w/w	2	RT	THF	H_2
10	Pt/C	10% w/w	2	RT	THF	H_2
11	PtO_2	2% w/w	2	RT	THF	H_2
12	PtO_2	5% w/w	2	RT	THF	H_2
13	PtO_2	10% w/w	2	RT	THF	H_2
14	Wilkinson's Cat.	10%w/w	2	RT	THF	H_2
15	$Rh(nbd)_2BF_4$ - Taniaphos	5% w/w	7	RT	THF	H_2
Transfer Hydrogenation						
16	-	-	-	50	TFA	Triethylsilane
17	10% Pd/C	20% w/w	-	50	TFA	Triethylsilane

These included transfer hydrogenation with triethylsilane-trifluoroacetic acid as carried out by Lanzilotti *et al* using substituted indoles (Table 3.2 - Entry 16)[12] as well as a modified method which incorporated 10% Pd/C as a catalyst for the reaction (Table 3.2 - Entry 17).

Reductive alkylation of the indole nitrogen using sodium borohydride combined with the desired carboxylic acid has been proven to simultaneously reduce the indole bond whilst also alkylating the nitrogen[13,14] and thus was attempted but to no avail.

Apart from using palladium and platinum based catalysts, others based on rhodium were used such as Wilkinson's catalyst as well as a bis(norbornadiene)rhodium tetrafluoroborate-Taniaphos ligand system which had previously been demonstrated within the research group to be capable of reducing the benzene ring and double bond of methyl α-acetamido cinnamate under room conditions.[15]

Unfortunately these alternative methods of double bond reductions did not prove to be successful in the case of tryptophan derivatives. Other more aggressive methods of double bond reduction such as using hydrazine as a source of diazene were considered however they bring with them considerable risk and, ultimately it was decided to cease development of dihydrotryptophan as a cure accelerator. Considerable time and resources had already been devoted in an attempt to get to a stage where dihydrotryptophan could be primarily evaluated for any accelerator activity, and so without the guarantee of it actually being cure active, the expenditure of further resources would have been implausible.

The single reaction parameter that was not explored, but which may have been likely to have a significant effect on bond reduction was the pressure at which the process was carried out. By increasing the pressure under which the hydrogen gas and the respective tryptophan derivatives were placed, it may have been possible to convert them to their corresponding dihydrotryptophan. However, the capability to do so does not currently exist within the research centre and thus was not pursued for the reasons outlined above.

3.2.2 Purine Bases

Another series of naturally occurring compounds which were considered when identifying possible natural sources as candidates for anaerobic adhesive accelerators were the purine bases. Along with its derivatives, purine is the most widely occurring nitrogen heterocycle in nature, and, coupled with the pyrimidines which would also be of interest, they make up the two groups of nucleotide bases forming DNA and RNA.

Purine Pyrimidine

Figure 3.3: Structures of Purine and Pyrimidine

As can be seen in Figure 3.3, the structure of purine is not that dissimilar to the indoline and THQ classes of accelerators which have been discussed previously. What separates it, and makes it such an interesting target however, is the presence of multiple nitrogen centres. This could mean there is a reasonable possibility that purine and its derivatives may indeed possess accelerator activity. Furthermore, there is the added possibility that the extra centres could lead to increased activity, particularly when compared in equal mole quantities to established accelerators. If indeed several of the nitrogen centres were to be active in the cure acceleration process, then conceivably a lower amount of material could be capable of the same activity as some established compounds making for a more efficient formulation.

From an environmental stand point, several purine derivatives have also been shown to be non-persistent in the environment. Caffeine, theophylline, and theobromine are all readily biodegradable.[16]

It must be noted that depending on the particular compound chosen, purines can be expensive but if they indeed were proven to possess multi active cure accelerator sites then the amount required for formulation could be minimal compared to the amount currently used for existing accelerators.

3.2.2.1 The Development of Purines as Possible Cure Accelerators

Of purine and its derivatives, caffeine was chosen for preliminary solubility testing to determine the viability of any possible application as anaerobic adhesive cure accelerators. Under room conditions caffeine was not soluble in PEGMA at 1% w/w but with sonication at 40^{o}C, dissolution in the monomer occurred and when left at RT for several days no precipitation from solution evident. Although not completely unsuitable, this meant more suitable compounds with which to evaluate the activity of the purine class were considered and a shortlist of those likely to exhibit a variety of activity was drawn up.

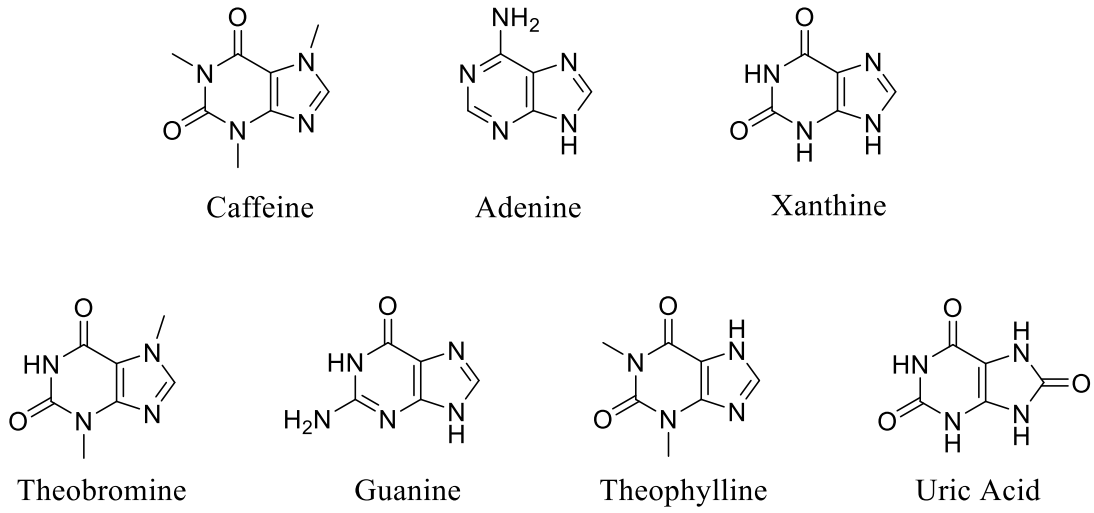
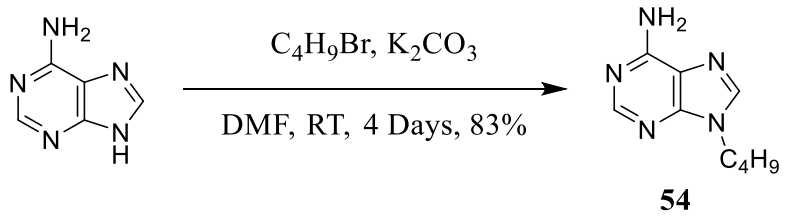


Figure 3.4: Structures of Selected Purines

Adenine, Guanine, and Xanthine were chosen for further investigation of purines as accelerators but unfortunately they too proved to be insoluble in PEGMA at 1% w/w.

Thus, as with tryptophan, methods of derivitisation so as to increase their solubility were examined. Beginning with adenine, alkylation of the nitrogen position was identified as the most straight forward method of creating analogues of the structure. 9-Butyl-Adenine (**54**) was synthesised as per the method described by Lambertucci et al[17] in 83% yield (Scheme 3.4).



54

Scheme 3.4: Synthesis of 9-Butyl Adenine

9-Butyl-Adenine (**54**) proved to be soluble in PEGMA when tested and thus an adhesive formulation was prepared using **54** as the cure accelerator. During this process saccharin is added after the cure accelerator has been dissolved fully and, curiously, when done so, caused precipitation of a white solid which despite stirring would not re-dissolve. When isolated, this solid was identified as 9-butyl-adenine by NMR spectroscopy and indicated that a form of competing solubility in PEGMA may be occurring between **54** and saccharin. This was investigated further by attempting preparation of another adhesive formulation but this time reversing the order in which the two components were added. After saccharin was dissolved fully, 9-butyl adenine was added but no dissolution occurred. It was postulated that this problem may be due to a charge complex formation or hydrogen bond interactions between the two molecules.

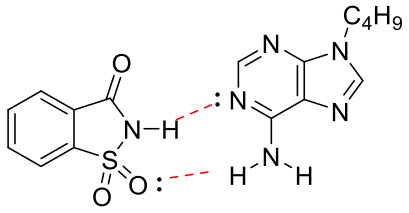
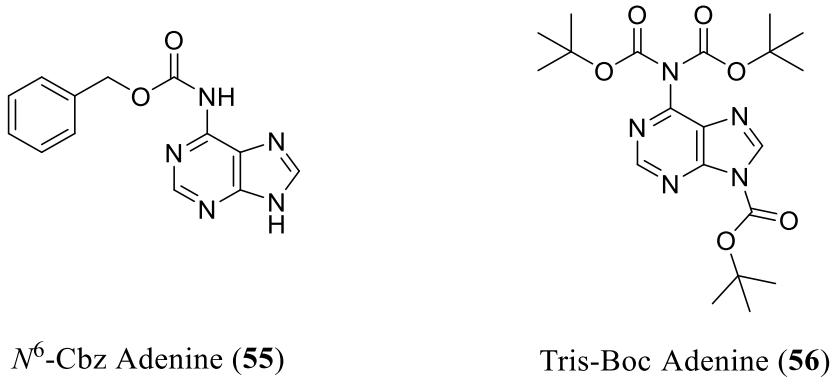


Figure 3.5: Possible H-Bonding Interactions between Saccharin and 9-Butyl Adenine

In order to try and prevent interactions such as these, the synthesis of adenine derivatives whereby bulky groups would serve to block possible H-bonding sites as well as hinder any form of charge transfer between the aromatic rings of saccharin and adenine was performed. These derivatives were chosen to be based on the use of amine protecting groups due to their inherent purpose as methods of dissuading

interaction as well as their necessity in the synthesis of further more complex derivatives should they be required.

Using benzyl chloroformate, N^6-cbz-adenine (**55**) was prepared in 41% yield[18] and the tris-boc (**56**) protected analogue was prepared as per Dey & Garner.[19]



N^6-Cbz Adenine (**55**) Tris-Boc Adenine (**56**)

Figure 3.6: Structures of Tris-Boc Adenine and N^6-Cbz Adenine

Cbz protected adenine proved insoluble in the monomer and so could not be tested to evaluate its cure activity but the boc protected derivative did prove to be so and an adhesive formulation was prepared for testing which will be discussed in Section 3.7.

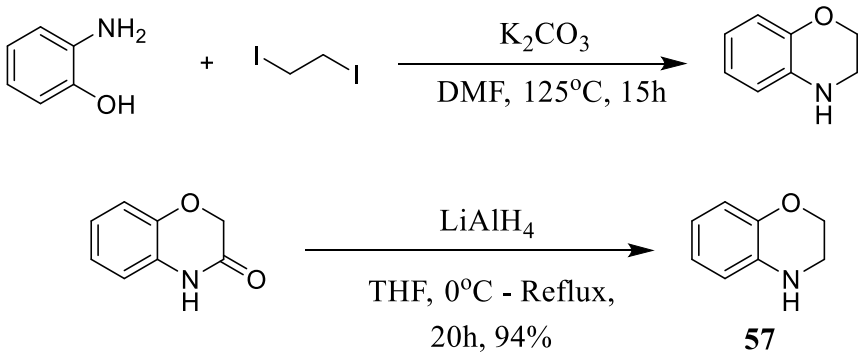
Regarding xanthine and guanine, the preparation of their derivatives was not pursued due to the prohibitive amount of synthetic steps and protecting group chemistry required. Further to this, with the outcome of the tryptophan investigation in mind, the amount of time devoted to developing compounds for primary evaluation had to be considered.

3.3 Benzomorpholine

Benzomorpholine and analagous compounds have already been illustrated to be of interest when developing anaerobic cure accelerators.[8,20] At the beginning of the project they were outlined as possible compounds of interest in terms of exploring the potential of their activity. Following the results of the screening of the "first generation" accelerators in chapter two, the interest in exploring their activity and properties became of more significance.

Specifically, this was in relation to the performance of THQ (**15**), indoline (**31**), and their derivatives (**29**, **35**-**36**), particularly in the biodegradation evaluation as well as the eco-toxicity screening. All compounds were found to be persistent and the derivatives where there was substitution present at the N position (**35**, **36**) were found to degrade slightly more than their parent compound. This suggests that this lack of degradation may be due to the persistence of the bicyclic ring structure. Thus, it was postulated that by introducing another heteroatom, such as oxygen, into the heterocyclic ring another "handle" for the biodegradation of the compound by microorganisms could be created whilst also retaining the cure activity. Morpholine has been shown to be readily biodegradable[21] and so by introducing this fragment into the accelerator structure, degradation may possibly be directed through this, possibly to an aniline-like molecule which we also know to be inherently degradable. Likewise in terms of the toxicity displayed in the microtox and *daphnia magna* screenings, it was proposed that by introducing an oxygen atom the lipophilicity of the compounds would be reduced. This would reduce their ability to cross the membrane of any organisms and reduce their overall harm to any water borne entities.

Two main routes to its synthesis were identified, the simultaneous alkylation and ring formation using 2-aminophenol combined with a dihaloethane[22] or reduction of the corresponding benzoxazinone[23] to form benzomorpholine (Scheme 3.5).

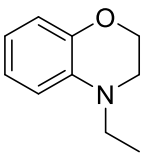


Scheme 3.5: Possible Synthetic Routes to Benzomorpholine

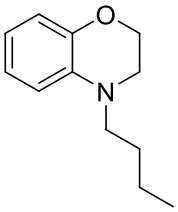
The alkylation method was evaluated first due to the ready availability of the required materials. Despite successful product formation, due to the lengthy time required to isolate and purify the benzomorpholine, the reduction and removal of the carbonyl group path was evaluated.

Despite the structural similarity, the benzoxazinone above is not a target cure accelerator due to the position of the carbon oxygen double bond next to the nitrogen centre of the molecule. It is believed that the presence of the carbonyl group alpha to the nitrogen affects the distribution of the electron density, specifically the lone pair on the nitrogen, and weakens any activity which would otherwise be present (Personal Communication, Henkel).

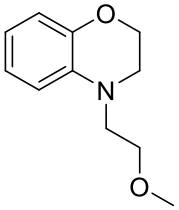
When evaluating potential benzomorpholine derivatives for preparation, several factors were evaluated. Firstly the nitrogen centre stood out as both the most facile position at which to derivatise, but also potentially where the largest effects on accelerator activity could be displayed. When determining the derivatives themselves, none were chosen which would potentially break the “rules” of designing green chemicals such as adding long alkyl chains, but also given the structural similarities to benzomorpholine, the decision was made to include derivatives which were analogous to those synthesised for THQ and indoline as this would allow us to compare and contrast the classes of molecules more directly.



N-Ethyl
Benzomorpholine (**58**)



N-Butyl
Benzomorpholine (**59**)

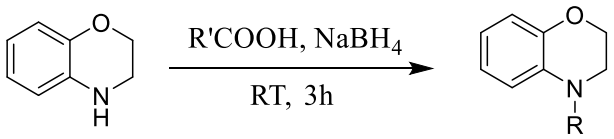


N-Methoxyethyl
Benzomorpholine (**60**)

Figure 3.7: Benzomorpholine Target Derivatives

Short chain alkyl chain derivatives were thus chosen and, depending on their performance in the various screenings, a larger set of targets could be drawn up. The *N*-methoxyethyl- derivative (**60**) was chosen due to the possibility that the ether group may enhance any positive effects in the environmental fate and toxicity studies whilst also having analogous activity to the butyl derivative in an isostere-like manner.

Firstly, benzomorpholine (**57**) was isolated in 94% yield by reduction through stirring benzoxazine-3-one with 2.5 equivalents of $LiAlH_4$ in THF at reflux. Myrboh & Mizar prepared *N*-substituted benzomorpholines through reductive alkylation using sodium borohydride and the respective acid.[22]



58 R = C_2H_5, 47%
59 R = C_4H_9, 61%
60 R = $C_2H_5OCH_3$, 65%

Scheme 3.6: Synthesis of *N*-Substituted Benzomorpholines

To a flask containing both benzomorpholine and the acid, ten equivalents of $NaBH_4$ were added whilst cooling before being allowed to warm to room temperature and stirred for three to four hours. *N*-ethyl- (**58**), *N*-butyl- (**59**), and *N*-methoxyethyl (**60**) benzomorpholine were synthesised by this method in 47%, 61%, and 65% yields respectively.

In terms of accelerator evaluation, each of the derivatives prepared proved soluble in PEGMA at the required 1% w/w level for formulation. Thus, these compounds were tested in order to evaluate their cure activity, anti-microbial toxicity, biodegradability, and eco-toxicity.

3.4 Anti-Microbial Evaluation of Compounds Prepared

All of the dihydrotryptophan and benzomorpholine derivatives synthesised in the previous sections were submitted for screening of their anti-bacterial and anti-fungal activity. It was decided not to submit any of the purines for this testing due to the inability to fully evaluate them as possible cure accelerators coupled with the amount of information which is already known about their toxicity and effects.

Anti-microbial screening was carried out at Charles University, Prague by Dr.Marcel Spulak and the anti-bacterial and anti-fungal activity of the first generation accelerator compounds is tabulated and discussed below.

3.4.1 Anti-Bacterial Test Results

As in Chapter 2, for each of the eight strains tested against, where the MIC recorded is >2000 μM no activity was observed within the parameters of the test.

Note: Due to dissolution problems in the media, dihydrotryptophan could only be tested to a max concentration of 500 μM.

Table 3.3: Anti-bacterial Activity of Second Generation Compounds

Bacterial Strain	Time (h)	Compound - MIC/IC_{95} (µM)							
		42	**47**	**48**	**57**	**58**	**59**	**60**	**61**
SA	24	**500**	>2000	**1000**	**2000**	>2000	>2000	>2000	>2000
	48	**500**	>2000	**1000**	**2000**	>2000	>2000	>2000	>2000
MRSA	24	>500	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
SE	24	**250**	>2000	**1000**	**2000**	**2000**	>2000	>2000	>2000
	48	**250**	>2000	**1000**	**2000**	**2000**	>2000	>2000	>2000
EF	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
EC	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
KP	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
KP-E	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
PA	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000

3.4.2 Anti-Fungal Test Results

As in Chapter 2, for each of the twelve strains tested against, where the MIC recorded is >2000 µM no activity was observed within the parameters of the test.

Note: Due to dissolution problems in the media, dihydrotryptophan could only be tested to a max concentration of 500 µM. Thus unless otherwise stated, results for each strain were >500 µM.

Table 3.4: Anti-Fungal Activity of Second Generation Compounds

Fungal Strain	Time (h)	Fungal Response – MIC IC_{80}/IC_{50}[a] (µM)							
		42	**47**	**48**	**57**	**58**	**59**	**60**	**61**
CA1	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
CA2	24	>500	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
CP	24	**500**	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
	48	**500**	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
CK1	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
CK2	24	>500	>2000	>2000	>2000	**2000**	**1000**	**2000**	>2000
	48	>500	>2000	>2000	>2000	**2000**	>2000	>2000	>2000
CT	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
CG	24	>500	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	**2000**	>2000	>2000	>2000	>2000	>2000
CL	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
TA	24	>500	>2000	>2000	>2000	>2000	>2000	**2000**	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
AF	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
AC	24	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>500	>2000	>2000	>2000	>2000	>2000	>2000	>2000
TM	24	>500	>2000	>2000	>2000	>2000	**1000**	**2000**	>2000
	48	>500	>2000	>2000	>2000	>2000	**1000**	**2000**	>2000

[a] IC_{50} values were assessed for AF, AC, and TM. For all other fungi strains IC_{80} values were obtained

Unlike the results generated for the first generation accelerators discussed in chapter two, there is very little existing anti-microbial or mammalian toxicological information for this set of compounds to compare and contrast with. What is known is discussed below and we can draw comparisons within the two sets of data, particularly where there is structural similarity of the compounds.

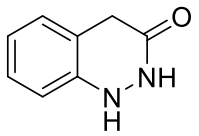
Beginning with the dihydrotryptophans tested (**42, 47, 48**), we can see that the three molecules display different inhibition activity of some of the gram positive strains. Dihydrotryptophan (**42**) appears to be the most active of the group, especially when we compare the structure to the methyl ester (**47**) which displays no activity. It is reasonable to suggest that this inhibition may be due to the ionisation of the acid group. This could potentially facilitate uptake of the molecule and increase interaction with the cell membrane disrupting cell activity. Likewise, if we compare the methyl ester to the inhibition activity of the butyl ester (**48**), we can see that this trend is reversed. There is some inhibition at 2000 μM to three of the gram positive strains screened against and this is a representation of a general "rule of thumb" when designing low toxicity compounds alluded to earlier; unsubstituted alkyl chains will increase anti-microbial inhibition when ≥4 carbons in length. This can be explained somewhat by the associated increase in lipophilicity of such derivitisations making it more facile for the compound to cross the organism membrane. This same trend of dihydrotryptophan < -methyl ester > -butyl ester also exists when we look at the anti-fungal data and is possibly as a result of similar processes occurring. Whilst none of these compounds could be evaluated in terms of their cure accelerator activity, they still illustrated important design trends for inactive anti-microbial compounds.

Of the benzomorpholines examined (**57-60**), we can see that both the parent molecule (**57**) and the ethyl derivative (**58**) display activity towards two and one of the bacterial strains respectively with neither the butyl (**59**) nor the methoxyethyl (**60**) derivatives showing any selectivity. This activity however is at the limit of detection of the screening and so no real conclusions can be taken from this difference other than perhaps the possibility that it is due to molecule size or conformation affecting any cellular interaction.

On the other hand, the anti-fungal data displays the opposite trend. As the chain length increases, so does the selectivity of inhibition towards certain fungal strains.

The same caution should be noted as was with the bacterial results in that the active concentration is at the upper limit of detection for the screening and as a result, no major definitive conclusion as to how to direct the design of other accelerator compounds should be based on these points alone.

It was hoped that the benzomorpholines synthesised would exhibit the same inactivity displayed by THQ (**15**) and indoline (**31**) towards microbial organisms whilst performing better in other areas. However, in comparison to THQ and indoline, they display an increase in activity in so far as the inhibition at high concentrations of certain bacterial and fungal strains. Whilst not ideal, this activity is only observed at higher concentrations and so does not definitively prohibit the development of benzomorpholines should they display positive results in the other areas screened.



3-Cinnolinone (**61**)

In the case of **61**, structurally it can be thought of as a cyclised version of APH (**16**) which was screened as part of the "first generation accelerators". APH was determined to possess activity to several of the bacterial and particularly fungal strains screened against. This toxicity towards fungi was somewhat expected given its mammalian toxicity but with this cyclised derivative this activity has been removed. This may represent the formation of a safer molecule which does not present the same level of risk associated with APH and displays the potential for removal of anti-microbial activity through incorporation of chain-like substitution as a closed ring instead.

3.5 Environmental Toxicity of Compounds Prepared

Table 3.5: Microtox Results of Second Generation Compounds

Compound	**Microtox (30 min)**		**EC_{50} concentration range (mg/L)**
	EC_{50} mg/L	**IC95%**	
Dihydrotryptophan (42)	28	14-55	10 to 100
Dihydrotryptophan Methyl Ester (47)	43	22-83	
Dihydrotryptophan Butyl Ester (48)	49	41-58	
Benzomorpholine (57)	49	38-62	
***N*-Ethyl Benzomorpholine (58)**	11	8.8-14	
***N*-Butyl Benzomorpholine (59)**	12	8.6-16	
***N*-Methoxyethyl Benzomorpholine (60)**	20	14-29	

Table 3.6: Daphnia Magna Screening Results of Second Generation Compounds

Compound	***Daphnia magna* (48 h)**		**EC_{50} concentration range (mg/L)**	**Hazard Classes (OECD, 2001)**
	EC_{50} mg/L	**IC95%**		
Benzomorpholine (57)	8.9	5.7-11	1 to 10	Acute toxicity II (toxic to aquatic life)
***N*-Ethyl Benzomorpholine (58)**	7.7	4.7-9.9		
***N*-Butyl Benzomorpholine (59)**	4.9	4.5-5.5		
***N*-Methoxyethyl Benzomorpholine (60)**	13	8.3-15	10 to 100	Acute toxicity III (harmful to aquatic life)
Dihydrotryptophan (42)	>100	-	>100	-
Dihydrotryptophan Methyl Ester (47)	>100	-		
Dihydrotryptophan Butyl Ester (48)	>100	-		

The eco-toxicity of the set of compounds was evaluated using the microtox assay as well as against *daphnia magna*. Classification by OECD standards results in labelling benzomorpholine (**57**) along with its ethyl (**58**) and butyl (**59**) derivative as "acute toxicity II – toxic to aquatic life" with the methoxyethyl (**60**) analogue classed as harmful. The trend within these four compounds confirms that the replacement of the butyl substitution with the methoxyethyl derivative does indeed result in a decrease in the aquatic toxicity due to the increase in water solubility. The hypothesis was that changes such as these, as well as the introduction of an oxygen atom into the structure, would result in a decrease in the toxicity compared with THQ (**15**) and indoline (**31**) based compounds. Whilst there was a marginal change, this was not significant enough to change the toxicity classification. In fact, the overall toxicity of indoline and THQ was altered most positively by way of the "diol" derivatives (**35**, **36**) synthesised. This suggests that, in terms of reducing aquatic toxicity, introduction of groups to increase the water solubility in more "free" positions as opposed to having them "locked" within a cyclic structure is favoured.

There was a larger degree of contradiction between the results of the microtox and daphnia magna screenings concerning this set of compounds. This can be illustrated with the dihydrotryptophans (**42**, **47**, **48**) tested; in the latter test their EC_{50} was deemed to be >100 mg/L and so requires no toxicity classification, however the microtox assay places them within the 10-100 mg/L range. While this is a significant difference, one should highlight that no single eco-toxicity assay is accurate for every class of chemical compound.

3.6 Biodegradability of the Compounds Prepared

Table 3.7: Biodegradation of Second Generation Compounds

Compound	**Biodegradation (%)**				**Inhibition (%)**
	6 Days	**13 Days**	**21 Days**	**28 Days**	
SDS	80	93	95	97±3.6	
Dihydrotryptophan (42)	1	2	3	5±0.3	0
Dihydrotryptophan Methyl Ester (47)	0	0	0	0±0.5	0
Dihydrotryptophan Butyl Ester (48)	0	0	0	0±1.9	0
Benzomorpholine (57)	0	0	1	0±1.2	11
***N*-Ethyl Benzomorpholine (58)**	0	0	0	0±2.4	0
***N*-Butyl Benzomorpholine (59)**	0	0	2	0±1.4	15
***N*-Methoxyethyl Benzomorpholine (60)**	1	1	3	1±2.3	0

The results of the biodegradation screening did not show any of the dihydrotryptophan (**42**, **47**, **48**) or benzomorpholine (**57**-**60**) compounds to be readily biodegradable.

The dihydrotryptophans proved to be quite persistent with the methyl (**47**) or butyl ester (**48**) derivatives showing little to no impact on improving this level. However, as was discussed in section 3.2.1, tryptophan itself readily undergoes photo-oxidation and so does not persist in the environment. Thus, it is possible that if a similar evaluation were to be carried out using dihydrotryptophan, this compound may also readily degrade abiotically when irradiated with sunlight and would not pose as a pollution risk.

It had been postulated that the introduction of the oxygen atom into the heterocyclic ring as in benzomorpholine would result in a THQ/indoline-like compound which has a much more positive environmental fate. Unfortunately this has not proven to be the case with no discernable level of degradation occurring for any of the derivatives. This set of results along with those relevant from Chapter two serve to illustrate that fused bicyclic ring structures are persistent by nature and if readily biodegradable analogues are to be found, significant alteration to the structure will have to be carried out. This represents a significant challenge when we take into account the fact that any derivitisation that does take place will in turn affect the cure activity and toxicity of the compound. Therefore there is a careful balance to be struck between the desired properties as if we design a compound solely to fulfil the requirements of one, then it is at the risk of comprising the performance in other areas.

3.7 Cure Accelerator Evaluation of Compounds Prepared

As described in section 3.2.1, the primary means of evaluating any cure accelerator was to perform a fixture time test. This involved the preparation of an adhesive formulation as per Table 3.9.

Table 3.8: Formulation Parameters of Anaerobic Adhesives Prepared

Component	% Weight
Polyethylene glycol dimethacrylate	95.5
Saccharin	0.5
Trigonox TMBH-L	2
Premix 16	0.5
Premix 17	0.5
Accelerator	1

As was noted earlier, none of the dihydrotryptophans prepared were soluble in PEGMA at 1% w/w and so were not tested, as was also the case with all of the purines except for tris-boc adenine (**56**). The only complete class which could be evaluated was benzomorpholine (**57**) and the three derivatives prepared (**58-60**).

The fixture time test involves the application of the adhesive on two pieces of grit-blasted mild steel such that they are bonded across a half-inch overlap. The substrate is grit-blasted prior to testing in order to remove any oxidized metal layer present which would otherwise inhibit the redox curing mechanism.

A series of these bonds are prepared and allowed to cure. At 30 minute intervals, the strength of a bond is tested by attaching a 3 kg weight to one of the steel pieces and measuring the length of time for the bond to fail. If no failure occurs after five minutes the bond is deemed to have set and reached the test strength specification. Once a 30 minute window cure time has been established, e.g. a bond failed after 30 minutes for a particular formulation but passed after 60 minutes, then further bonds are prepared until this window is narrowed until the cure time is known within 5-10 minutes, i.e. after further tests the bond was found to cure between 40-45 minutes. However, if the cure time was found to be too long such that the test compound was not of interest as a cure accelerator, no further consolidation of the cure time was carried out. As a reference and a control, a formulation using APH (**16**) as an accelerator was also tested.

The results of the benzomorpholines and tris-boc adenine were as follows:

Table 3.9:Fixture Times of Formulations Tested

Accelerator	Cure Time (Minutes)
Benzomorpholine (**57**)	35-40
N-Ethyl Benzomorpholine (**58**)	40-45
N-Butyl Benzomorpholine (**59**)	40-45
N-Methoxyethyl Benzomorpholine (**60**)	40-45
Tris-boc Adenine (**56**)	120-150
APH (**16**)	0-5

For an accelerator to be considered of interest a cure time of <30 minutes is required, and to be of pertinent developmental interest the cure time should be approaching that of APH.

As can be seen, the adenine derivative (**56**) is of poor cure acceleration activity. This however may not be exactly due to adenine or the purine class as a whole inherently being of poor activity but possibly due to the boc substitution present. In Chapter 1, possible curing mechanisms and interactions of the accelerator component were described and some of these such as the formation of a charge-transfer complex could be influenced by the accelerator molecule size and shape. If bulky groups were to be present as part of an accelerator, such as the three boc groups in this example, then they may hinder the formation of any possible charge transfer complex and therefore inhibit any accelerator activity which would otherwise have been present. Unfortunately, as previously discussed other less bulky purines synthesised (**54-55**) could not be evaluated due to their solubility properties.

The benzomorpholines tested did possess interesting activity. One can see that the cure process time has been shortened significantly compared to tris-boc adenine, but it still is not of the level of activity exhibited by APH. One can also see that activity has decreased with the substitution of the nitrogen position. This derivitisation affects several of the properties of the benzomorpholine molecule which may in turn affect the cure activity. Firstly the overall shape and size of the molecule is altered and for reasons which were alluded to when discussing the activity of the adenine molecule, this could be of significant consequence. The position of the nitrogen lone pair of electrons is also altered; they may possibly be of importance to activity and whether they interact with the aromatic ring or not, the ability to do so would be affected by any substitution. Accelerators can be thought of as weak bases, and their interaction or ability to form salt complexes with the weak acid saccharin is of interest in terms of a possible curing mechanism. The protonation of the nitrogen position would be affected by any substitution and therefore may inhibit activity.

The decrease in activity with substitution may also be due to drop in the mole amount of accelerator added. Formulations are prepared based on their gram amount of accelerator. This does not take into account the difference in molecular mass of accelerators and so an accelerator with a large molecular mass will result in a lower concentration of accelerator molecules present compared to one of a lower molecular

mass. This could have a significant effect if we are to think of cure accelerators as playing a catalyst-like role in the process and therefore any change in mole amount will alter the loading and any potential impact.

3.8 Second Generation Analysis Outcomes

From the compounds screened and the attempts made to synthesise new targets, several key outcomes in how to design new accelerators have been determined.

From a structure property standpoint, through the synthesis of benzomorpholine and its derivatives (**57**-**60**), efforts were made to enhance the biodegradability and decrease the environmental toxicity or fused bicyclic nitrogen heterocycles. It had been postulated that the introduction of an oxygen atom into the saturated ring would aid the improvement of both of these properties. However, from the biodegradation and eco-toxicity screening carried out it is clear that this has little impact on the persistence and potential harm of these compounds. Therefore, if possible, the further addition of saturated rings to any aromatic target molecule should be avoided. However, the cyclisation of APH (**61**) was observed to result in more favourable anti-microbial results. APH we know to be acutely toxic to mammalian life and this was exhibited in its anti-fungal activity discussed in Chapter 2; **61** resulted in an elimination of this activity towards micro-organisms and illustrated the potential advantages which can be gained through cyclisation.

From the exploration of tryptophan and the purines as possible natural sources of accelerators, the solubility of certain classes of nitrogen heterocycles in PEGMA monomer is an issue. As a result of this, the solubility of classes of compounds should be treated as a key property used when evaluating their possible applicability as candidates for catalyst development. Otherwise, a significant amount of time and resources may be expended in an effort to simply make derivatives which are soluble enough just to preliminarily assess their activity. Ideally, these same resources and time could be better spent furthering the actual curing activity of other compounds and develop their properties.

Apart from extending the time required to fundamentally assess the viability of compounds, this also serves to add a sometimes lengthy pathway to the synthesis of

the target molecule, before any steps (if needed) are even added to enhance its cure, toxicity, or environmental properties.

In order to avoid these issues, the solubility of any class of compounds should be evaluated in detail prior to any study taking place. Doing so will allow for a more comprehensive structure-activity study of the molecules and how to alter their framework to better their cure performance, toxicity profile, and environmental fate.

3.9 References

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Chapter 4:

Imidazolium Cure Accelerators

4.1 Imidazoles – Cure Accelerator Structure-Activity Study

In the previous studies carried out there had been an emphasis on designing and developing nitrogen heterocycle based cure accelerators with a view to moving to more natural and sustainable sources whilst enhancing their biodegradability and lowering their toxicity profile. However, due to the increased attention to these characteristics, and due to the solubility issues encountered whilst attempting to improve them, not as much information was elucidated about the cure accelerator structure-property relationship.

In an effort to learn more about the possible source of the catalytic activity, the focus was switched to be able to more efficiently gain an insight into the factors which affect it. In order to achieve this, it would be preferential to study a class of compounds which were already likely to possess activity so that, as was the case in previous studies, significant time would not be spent developing the physical properties required to be able to evaluate the level activity present, much less improve upon it. In addition, it would be preferable to study a class of compound which also offers a broad scope for derivitisation so that a wider understanding of substituent effects could be realised.

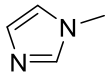
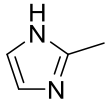
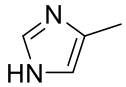
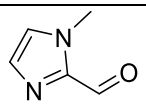
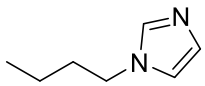
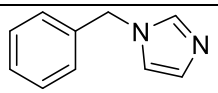
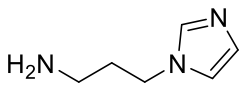
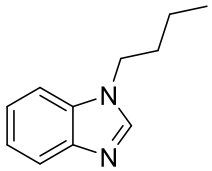
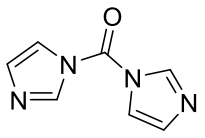
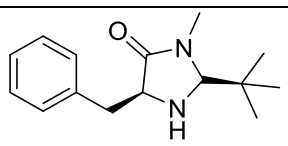
Imidazoles are one such class of heterocycle which fulfil these prerequisite conditions. They have already been utilised as cure catalysts in other types of adhesive systems such as epoxy resins[1,2] and have been included in anaerobic systems to modify the properties of the overall bond.[3] It would therefore not be unreasonable to expect that they would also be capable of modifying the cure time for anaerobics given their functional group and structural similarity to established accelerators. Moreover, the two nitrogen centres offer a facile handle with which to create derivatives and examine the effect on cure time.

4.2 Determining Suitability as a Test Case

The suitability of imidazoles for investigation was assessed through first establishing a set of derivatives which displayed a satisfactory variation in structure and then determining their solubility in PEGMA at formulation level, 1% w/w. This would allow for the preparation of test adhesive formulations for cure analysis and, as can be seen in Table 4.1, the range of imidazoles selected all displayed solubility in the monomer. Next, three compounds were selected from the list and evaluated to determine whether or not imidazoles would possess cure activity, particularly that of

a level which would indicate if they would be a viable test case for a structure-activity investigation. If the activity present proved to be too low and completely incomparable to existing catalysts capable of practical application, then the class would be deemed unsuitable for investigation.

Table 4.1: Solubility of Imidazole Compounds in PEGMA

Compound	Structure	Soluble
Imidazole (**62**)		✓
1-Methylimidazole (**63**)		✓
2-Methylimidazole (**64**)		✓
4-Methylimidazole (**65**)		✓
1-Methyl-2-imidazolecarboxaldehyde (**66**)		✓
1-Butylimidazole (**67**)		✓
1-Benzylimidazole (**68**)		✓
1-(3-Aminopropyl)-imidazole (**69**)		✓
N-Butyl-1*H*-benzimidazole (**70**)		✓
1,1-Carbonyldiimidazole (**71**)		✓
(2*S*,5*S*)-(-)-2-*tert*-Butyl-3-methyl-5-benzyl-4-imidazolidinone (**72**)		✓

Using a formulation prepared to the specification outlined in Table 4.2, the compounds were evaluated using a fixture time test. This was carried out through bonding two pieces of grit-blasted mild steel across a half inch overlap. The time taken for this bond to reach a cure strength capable of supporting a 3 kg weight for five minutes was then recorded and used as an indication of the catalyst performance. To do so, a series of bonds were initially prepared in tandem and were tested at 30 minute intervals. Once a preliminary 30 minute cure window had been established, further series' of bonds were then prepared and tested until a five minute cure time window was achieved. To be considered of possible practical interest, an accelerator should lead to a cure time in this evaluation test of <30 minutes and for comparative purposes, as well as to act as a control, the cure time of acetylphenylhydrazine was also measured. If the initial 30 minute window established was deemed to be too long, then no further refinement of the cure time as carried out.

Table 4.2: Test Formulation Parameters

Component	% Weight
Polyethylene glycol dimethacrylate	95.5
Saccharin	0.5
Trigonox TMBH-L	2
PM 16	0.5
PM 17	0.5
Accelerator	1

Table 4.3: Fixture Time Results of Selected Imidazoles

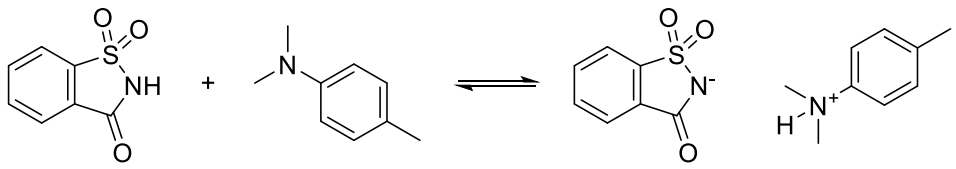
Compound	Cure Time (minutes)
Imidazole	10-15
1-Methylimidazole	15-20
2-Methylimidazole	15-20
APH	0-5

4.3 Selecting Compounds for Investigation

In both preliminary evaluation screenings the imidazoles examined indicated that the class of compound would be suitable as a test case for a structure-activity investigation into anaerobic cure accelerator activity. Not only do they possess adequate activity comparable to catalysts capable of commercial application, but they also may be derivitised without the loss of monomer solubility. This allows for the examination of the effects of various structural changes on activity without investing significant time in the design and synthesis of compounds which ultimately may not be evaluated as in previous studies.

Thus, in addition to the compounds examined as part of the preliminary study in Table 4.1, a set of imidazole compounds of possible significance with regards accelerator structure-activity were drawn up. This was done so based on their readily availability as well as structural variety.

In Chapter 1, possible curing mechanisms as well as possible active cure accelerator compounds and their role were described. One such possibility was the formation of salts within the product formulation which then proceed to act as the active accelerator molecule as opposed to any of the respective neutral formulation components.[4] These salts are formed as a result of interactions occurring between acidic species, such as saccharin, and alkaline species, such as the amine cure accelerators present. The very fact that the catalytic compounds currently used are all largely alkaline in nature gives encouragement to the possibility that the accelerating component could indeed be a salt. Scheme 4.1 shows the salt formed between dimethyl-*p*-toluidine and saccharin for example.



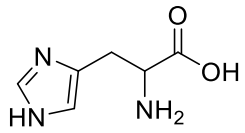
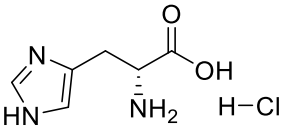
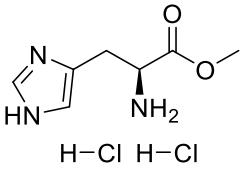
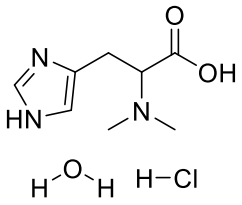
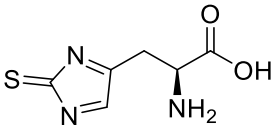
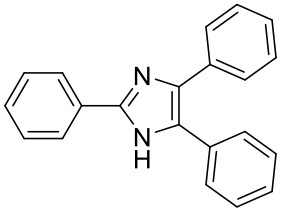
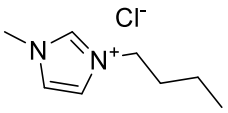
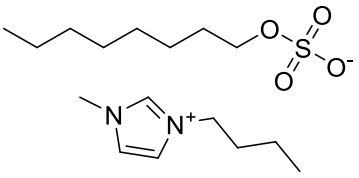
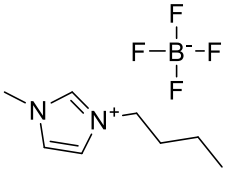
Scheme 4.1: Formation of DM*p*T-Saccharin Salt

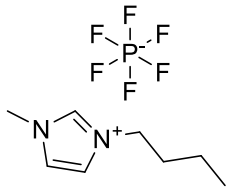
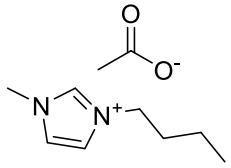
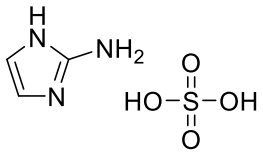
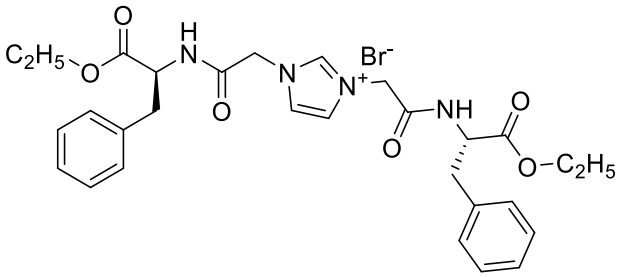
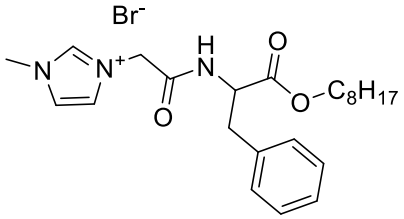
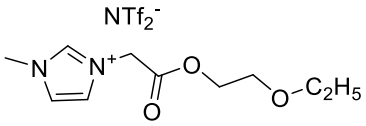
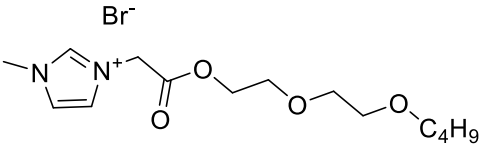
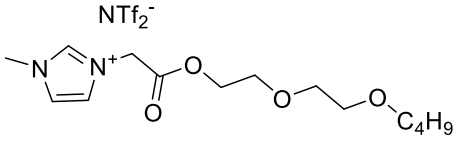
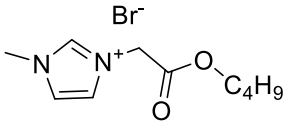
By examining the imidazole family, this represents an excellent opportunity to study the activity of salt compounds as cure accelerators. Imidazolium salts can be synthesised by protonation or quarternarisation of the N-3 position and have been applied across a range of areas, particularly as ionic liquids.[5] They may also be used as a source of carbenes[6] which could potentially play a unique role in the mechanism of the radical polymerisation process. One of their applications has been use as catalysts for a host of reaction types, importantly including polymerisation reactions.

One of the expertise of the research group is the design and synthesis of novel green nitrogen heterocycle based ionic liquids. This provides access to a vast amount of knowledge regarding the alteration of charged heterocycle structures to improve their toxicity and biodegradability as well as to the methodology required to make and library of novel compounds.

Although the emphasis of this study was to be centred on the cure properties of accelerators, consideration was still given to the potential toxicity and environmental impact of imidazole based compounds. Histidine may be considered a substituted imidazole and as an amino acid offers a sustainable, potentially safer alternative. For these reasons it would extremely favourable if this amino acid were to possess accelerator activity comparable to that of the unsubstituted parent compound. To this end a selection of histidine based compounds as well as imidazolium ionic liquids were preliminarily assessed as before by evaluating their solubility in PEGMA at 1% w/w (Table 4.4).

Table 4.4: Solubility of Histidine and Ionic Liquid Compounds in PEGMA at 1% w/w

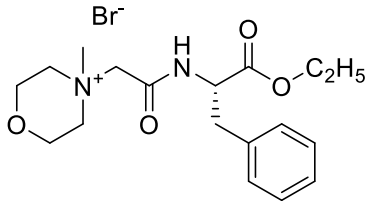
Compound	Structure	Soluble
DL-Histidine (**73**)		×
D-Histidine monohydrochloride (**74**)		×
L-Histidine methyl ester dihydrochloride (**75**)		×
N,*N*-Dimethylhistidine hydrochloride hydrate (**76**)		×
L-2-Thiohistidine (**77**)		×
2,4,5-Triphenylimidazole (**78**)		×
1-Butyl-3-methylimidazolium chloride (**79**)		×
1-Butyl-3-methylimidazolium octyl sulfate (**80**)		✓
1-Butyl-3-methylimidazolium tetrafluoroborate (**81**)		✓

1-Butyl-3-methylimidazolium hexafluorophosphate (**82**)		✓
1-Butyl-3-methylimidazolium Acetate (**83**)		✓
2-Amino imidazole sulfate (**84**)		✗
AJ169 (**85**)		✗
AJ189 (**86**)		✓
MG135 (**87**)		✓
MG168 (**88**)		✗
MG168 NTf_2 (**89**)		✓
HP58 (**90**)		✗

Unfortunately, as with the tryptophan amino acid compounds examined previously, all histidine derivatives proved to be insoluble and so were not tested further. This was the case for both the free base analogues as well as the hydrochloride/hydrate salts and the decision was made not to pursue any derivitisation to increase their formulation solubility until more was known about the potential application of imidazoles as accelerators. The ionic liquids showed much more promise on the other hand. The examples tested were all largely soluble meaning that the presence of any cure activity could be evaluated. Be this as it may, if we are to take a closer look at the solubility, derivatives with a halide counter ion are generally insoluble. The effect of larger anions such as bistriflimide should therefore not be ignored as they appear to be more adept at solvating the molecule in the monomer. This can be seen directly in a comparison of compounds **88** and **89** where the bromide derivative is insoluble yet the bistriflimide soluble, and likewise with the BMIM compounds evaluated. This is an example of how ionic liquids offer an extra dimension when altering the properties of a molecule - the solubility of the compound is increased through replacing the counter-ion whilst not drastically changing the heterocycle scaffold allowing for its cure activity to be evaluated. With neutral compounds it would have been required to directly alter the heterocycle structure to confer solubility and thus the possibility that in the process end up with an unrealistic accelerator molecule.

As well as evaluating some more common place imidazolium ionic liquids such as the 1-butyl-3-methylimidazolium derivatives, several novel compounds were included. Within the research group exists a large library of ionic liquid compounds synthesised as part of other research projects. The availability of these compounds allowed for the ready investigation of the effect of different functionalisations on solubility and then potentially their impact on cure activity (**85-90**). Compounds **85** and **86** can be thought of as phenylalanine ester functionalised imidazoliums and it was postulated that the introduction of further nitrogen heterocycle fragments may have a positive effect on cure activity whilst also being a sustainable means of derivitisation. Compounds **87-89** on the other hand contain ethylene glycol substitution and were included in the evaluation study in order to assess their impact on solubility. If the presence of ethylene glycol chains could be shown to have an enhancing effect on the level of solubility in PEGMA, this could then be used as a method of solubilising and “delivering” heterocyclic fragments designed for

maximum catalytic ability. As a short chain ester, compound **90** acted as a comparison to the BMIM compounds evaluated as well as to **87-89**. Unfortunately, the ethylene glycol functionalization did not turn out to possess any discernable effect on solubility in PEGMA at 1% w/w with the solubility of **87** and **89** attributed to the bistriflimide counterion. Figure 4.1 contains further structural analogues of the soluble compounds **86** and **87**. After they had proven to be soluble the decision was made to evaluate whether similar compounds comprising of a different heterocycle head group or amino acid ester could be evaluated. This included the addition of pyridinium, morpholinium, dimethylaminopyridinium, and cholinium head groups combined with phenylalanine and tyrosine esters of varying chain length. If soluble, this would have allowed for the evaluation and comparison of a wide range of ionic liquids and the determination of effects of factors such as varying nitrogen environment or alkyl chain length/lipophilicity. Given that these compounds have been developed as part of other research projects with similar toxicity and biodegradation goals in mind, the presence of any cure accelerator activity would open up a wide range of anti-microbial and biodegradation data for the development of anaerobic polymerisation accelerators. Unfortunately all compounds proved insoluble at 1% w/w and so could not be included for catalytic activity estimation. This could possibly again be due to the halide counterion but was still somewhat unexpected, particularly in the case of **96** which only differs from **86** by the extension of the ester chain by two carbons.



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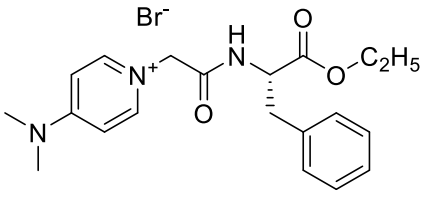

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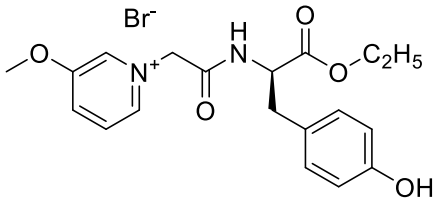

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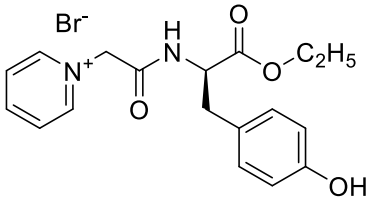

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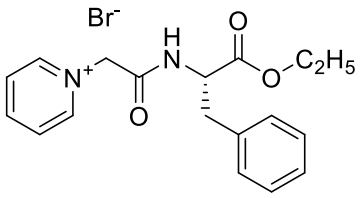

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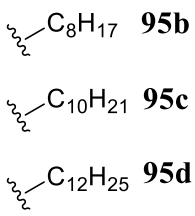
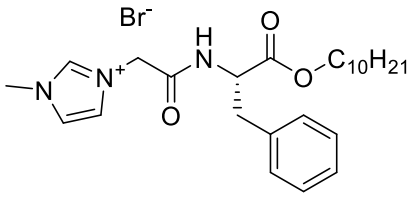


95b **95c** **95d**



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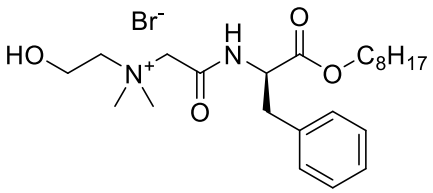

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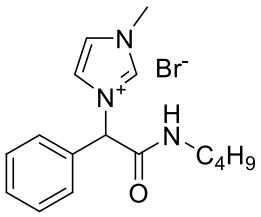

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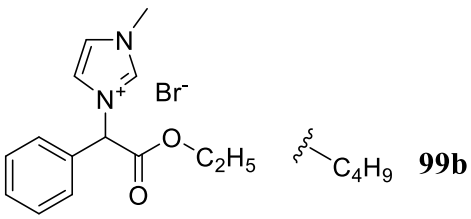

99a **99b**

Figure 4.1: Selected Ionic Liquids from Other Projects which were Insoluble in PEGMA

4.4 Cure Activity Evaluation of Soluble Compounds

The catalytic activity of the imidazoles in Table 4.1 was evaluated along with some of the ionic liquid compounds identified which proved to be soluble in PEGMA. This was carried out using the fixture time test as described in Section 4.1.

The fixture time test offers a good method of evaluating the level of cure activity any catalyst may have and identifying potential lead compounds. If this initial test then results in a compound of interest, i.e. of comparable activity to a commercial cure accelerator such as APH, the molecule may be marked for further development and/or evaluated in a complete series of cure time/strength tests. For the purposes of this screening, the accelerators were added at a level of 1% w/w when preparing the adhesive formulations. This is representative of current anaerobic adhesive products and the amount of accelerator contained within.

Table 4.5: Fixture Time Results of Compounds Analysed

Compound	Cure Time	Molecular Weight (g/mol)
62	10-15	68.08
63	15-20	82.11
64	15-20	82.11
65	15-20	82.11
67	30-40	124.19
68	80-90	158.20
72	60-120	246.35
80	0-5	348.50
86	60-70	480.45
87	80-90	493.39
89	130-140	565.50
1-Acetyl-2-phenylhydrazine	0-5	150.18

As can been from the results in Table 4.5, unsubstituted imidazole (**62**) as well as its methyl derivatives (**63-65**) all performed well in the screening, displaying activity similar to that of APH. This in itself was not hugely surprising given that they have been used as cure catalysts for other adhesive systems to good effect. The performance of 1-butyl-3-methylimidazolium octyl sulfate (**80**) however was a stand-out result performing to the same level as APH which has been used in many commercial adhesive products. Ionic liquids have not been explored in depth as cure catalysts previously but this result represents the possibility that they may be applied as such and opens up a new avenue for the design of cure accelerators. As noted earlier, their nature as being essentially two separate components attracted only by charge offers more freedom to develop and tweak their properties by altering the structural make-up of either the cation or anion to enhance performance in one area while retaining the performance in another by leaving the other untouched. This extra dimension for development possibly makes the charge of combining high cure activity, low toxicity, and biodegradability more achievable given the wide scope of charged compounds which may be combined. Their unique physical properties also represent different possibilities as to how cure accelerators affect the overall radical polymerisation mechanism. For example, ionic liquids have been applied as solvents in many other areas and their activity in this case could be due to enhanced solvation of the transition metal ions at the surface interface making them more accessible to the redox process. Likewise the same effect could be achieved if the transition metal ions were to exchange with the cation of the ionic liquid to form a new complex.

In terms of the effect of further derivitisation on the cure performance, it appears that as the molecule size gets bigger, the more the cure rate slows. However, one cannot definitively say based on these results that the size or conformation of the catalyst directly impacts the curing mechanism. Instead, this serves to magnify an issue which is brought about by the subtle differences between commercial product development and chemical research into novel substances.

The specification of products for purposes of formulation and production are measured in gram amounts in order to be as streamlined and user friendly as possible, particularly in any environment where the person(s) involved may not have a chemistry background. On the other hand, when working with chemicals, and especially where two or more compounds need to be directly compared, the logical

approach is usually to use mole units of material. This was not as pertinent an issue where small molecules were being compared by activity per gram amount used as they were of comparable molecular weight, for example diethyl-*p*-toluidine (163.26 g/mol) and APH (150.18 g/mol), and thus of comparable mole amount. Add to this the fact that as established accelerators, the rest of the product formulation components and the amounts used have been carefully optimised to achieve the most efficient cure performance from them. In the case of this study however, a representative formulation comparable to that of a commercial adhesive was used and each compound of interest was simply "swapped" using the same gram amount across the series without any further optimisation and without any previous knowledge of how that compound may interact with a polymerisation reaction. In order to be able to draw conclusions from a SAR study such as this, there is an inherent need to be able to directly compare different chemical compounds regardless of mass or conformation. This is something which becomes quite difficult where we have molecules which differ greatly in terms of their molecular mass, for example 100 mg of **1** = 1.6 mmol whereas the same amount of **25** = 0.17 mmol. In this example there is a difference of almost one order of magnitude between the two accelerators and, without knowing whether or not the accelerator amount affects the cure rate observed, we cannot compare with any degree of confidence the two results achieved. To this end, a study on the relationship between accelerator amount and activity was carried out.

4.5 The Effect of Accelerator Amount on Cure Activity

In order to examine the effect of catalyst amount on cure activity, several adhesive formulations were prepared as per Table 4.2 differing only in the amount of accelerator added. For this study APH was chosen as the accelerator of interest due to its establishment in the role. To carry out the fixture time evaluations described, 10 g formulations were prepared of which the accelerator comprised 1% w/w or 100 mg. This is equivalent to 0.7 mmol of APH and thus in addition to this amount, samples were prepared using 0.1-0.5 mmol as well as 1.5 mmol of APH. The method used to evaluate the samples was the fixture time test as before with the results presented in Table 4.6.

Table 4.6: Cure Activity of APH at Varying Concentrations

Amount (grams)	Amount (mmol)	Accelerator % w/w of Overall Formulation	Cure Time Range (minutes)
0.015	0.1	0.15	15-25
0.030	0.2	0.30	5-10
0.045	0.3	0.45	5-10
0.060	0.4	0.60	5-10
0.075	0.5	0.75	0-5
0.100	0.7	1	0-5
0.23	1.5	2	0-5

From this data generated one can see that the amount of accelerator added does indeed have an effect on the time required for curing to occur. As the amount of accelerator is increased, the more quickly polymerisation occurs. Although this factor may be reasonably self-evident, it is important to note that no study on the effect of cure accelerator concentration on cure speed for anaerobic adhesives had been published previously. Catalyst loading is no doubt an important reaction condition in any catalytically altered process but the extent to which it interacts varies for each unique catalyst and application. In the case of using APH as the catalyst, this appears to have a low impact but nonetheless indicates that for true comparisons to be drawn between any novel cure accelerators evaluated, the mole amount used must be consistent so as to avoid any erroneous conclusions. It should also be noted that the effect of accelerator loading on cure time was carried out using the fixture time test and not another means of observing the polymerisation time as the shortest cure time may not always result in a strong adhesive bond, and for the purposes of this investigation, bond formation of adequate strength is just as important as the cure time.

4.6 Re-Testing - Concentration Studies

The compounds examined in Section 4.3 were retested at three concentrations, 0.2, 0.7, and 1.5 mmol, using the fixture time test as before. By doing so this allows for the direct comparison of the cure speeds measured and conclusions based on the structural properties of each compound to be drawn.

Table 4.7: Cure Activity of Compounds Analysed at Multiple Concentrations

Compound	**Accelerator Amount – Cure Time (minutes)**		
	0.2 mmol	**0.7 mmol**	**1.5 mmol**
62	120-240	10-15	10-15
63	120-180	20-30	10-20
64	120-180	20-30	0-5
65	120-180	5-10	0-5
67	60-120	30-40	15-20
68	80-90	80-90	10-20
72	-	100-180	60-70
80	0-5	0-5	0-5
83	40-45	0-5	15-20
86	60-70	20-25	15-20
87	80-90	30-35	40-50
89	130-140	20-30	>150
1-Acetyl-2-phenylhydrazine	5-10	0-5	0-5

Analysing each potential cure accelerator at different concentrations yielded several key outcomes. The results suggest that there is indeed a relationship between accelerator size/conformation and activity. A general trend can be constructed from the data whereby as the molecular mass of the catalyst increases, the activity decreases. Depending on how the accelerator affects the curing mechanism this may mean that bulky molecules are not able to fit or interact at the active site. This may be with the metal substrate surface or one of the other components present in the formulation.

The screening also highlighted another issue when evaluating novel cure catalysts. The activity increased with accelerator concentration in almost all of the cases analysed. In the case of compounds **87** and **89** however, we see the shortest cure time is between 0.2-1.5 mmol as at the 1.5 mmol point the cure time increases from that measured using 0.7 mmol, The presence of too many accelerator molecules actually begins to inhibit cure speed and this yields the complication that if there may be a "bell-shaped" relationship for every compound where without analysing activity at many more catalyst loadings there is no way of knowing where along the curve the data points are. For example, the optimum activity concentration of compound **2** may in fact be between 0.7-1.5 mmol despite the fact that the cure speed appears to have increased by raising the concentration. Increasing the catalyst amount can also in some cases lead to solubility issues and therefore may mean some cannot be evaluated at higher concentrations; this solvation issue may somehow be responsible for the "bell-shaped" curves of compounds **87** and **89** observed given that they are two of the larger compounds analysed.

As noted in the analysis at 1% w/w loading, the performance of BMIM octyl sulfate (**80**) is at a level whereby it may be considered a lead compound and of great interest. In order to learn more about this result and the possible source of activity, another BMIM derivative was included for analysis in this screening. BMIM acetate (**83**) was obtained through the anion metathesis of BMIM chloride with silver acetate and differs only in the counterion present but having the added advantage of being considered hazard free. At 0.7 mmol **83** displayed the same activity as the octyl sulfate analogue (**80**) and APH indicating that the BMIM scaffold is capable of significantly altering the cure time. Despite this however, the performance at 0.2 and 1.5 mmol of **83** is lower than APH and **80**. Nonetheless, on a broader scope it is further evidence to support the exploration of ionic liquids as catalysts for redox radical polymerisations.

The level of deviation of activity whilst varying concentration may offer an indication of how reliable or robust an accelerator is. Although there is a change in cure speed, APH largely retains its performance across the concentrations analysed and **80** does not vary at all, whilst we see in other cases that where they perform very well at one concentration, they vary massively when this is altered. This displays that some of the compounds are very sensitive to concentration change and is another way in which the potential of any novel accelerator can be evaluated. Given that

BMIM octyl sulfate (**83**) does not vary at all across the concentration levels tested at, then this is only further evidence to strengthen its viability as a lead compound.

4.7 Conclusions

In the studies described in the previous chapters more priority had been paid to the development of anaerobic accelerators with respect to improving their toxicity and environmental persistence. Not as much information about the source of their catalytic activity had been elucidated as a result but this was partially due to the compounds synthesised not possessing the required physical properties, i.e. solubility in the formulation monomer, and not neglect of the importance of this aspect of the research. This created a necessity for a concerted effort to be made to study a class of compounds which could provide an insight into the cure accelerator's role in the polymerisation mechanism. Suitable test candidates were therefore required to be able to undergo wide structure derivitisation without the loss of solubility in PEGMA so that the effect of structural changes on activity could be investigated. These prerequisites resulted in the selection of imidazoles for investigation as preliminary tests showed widespread solubility of imidazole based compounds in the monomer at 1% w/w as well as promising cure activity. Not only were they suitable, they also offered the opportunity to study other aspects of the cure mechanism through synthesis of charged imidazolium compounds.

The evaluation of this range of imidazoles resulted in several key outcomes. The preparation of the adhesive formulations first had to be altered in order to account for and create comparability between all compounds analysed. It was found that the catalyst loading in the formulation can have a large impact on the rate of curing observed. Generally speaking activity increased with the addition of more catalyst however several results pointed to the possibility that this relationship between activity and concentration may deteriorate at higher catalyst concentrations where the accelerator will eventually begin to inhibit the curing process.

Most importantly, the testing of these compounds resulted in the identification of lead cure accelerator compounds. In the fixture time test, BMIM acetate (**83**) and BMIM octyl sulfate (**80**) yielded the same performance as the commercial accelerator, APH. BMIM octyl sulfate resulted in faster cure times at lower concentration than APH and this extent of activity in the two molecules is thought to

be as a result of the imidazolium cation. The difference in performance between the two compounds suggests that the anion does play a role however, and as pointed out earlier, variation of the counterion present could assist in enhancing the safety and environmental aspects of the compound while the cation retains the accelerator activity. For example, in this scenario BMIM octyl sulfate (**80**) is considered an irritant whereas BMIM acetate (**83**) is label-free.

For these reasons they are deserving of further analysis as cure accelerators and imidazolium salts as well as ionic liquids as a whole should be explored as sources of new anaerobic cure accelerators. Their potential for structural variation allows for a wide range of possibilities with which to optimise their cure activity and explore the mode of action.

4.8 References

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Chapter 5:

Development of Lead Cure Accelerators

5.1 Identifying Lead Cure Accelerators

In the previous chapter the discovery of the use of 1-butyl-3-methylimidazolium (BMIM) based ionic liquids as anaerobic cure accelerators was detailed. Through use of the fixture time test to analyse various imidazoles and imidazolium salts, two compounds which performed at and above the level of the commercially used cure catalyst, APH, were identified. These were the octyl sulfate (**80**) and acetate (**83**) salts of butyl-3-methylimidazolium which displayed optimum performance when - incorporated at a level of 0.7 mmol as part of a 10 g formulation. Given the catalytic efficiency displayed by the molecules, the decision was made to fully evaluate them as potential anaerobic adhesive cure accelerators by carrying out complete lead adhesive formulation testing.

Further to this, the results of the two compounds, (**80**) and (**83**), along with the potential shown by the other ionic liquids analysed, meant that the alkyl imidazoliums as well as nitrogen heterocycle based ionic liquids as a whole merited further development and investigation. Given that anaerobic cure accelerators are all nitrogen containing compounds, the better of which are in addition usually aromatic, the source of the activity displayed was thought to be due to the cation portion of the ionic compound. There is little evidence to support the use of non-nitrogen based compounds as cure accelerators and so, despite the octyl sulfate and acetate analogues possessing differing performance the initial understanding was that the anion would not be cure active. This difference could instead be explained by the octyl sulfate and acetate anions leading to differing physical properties such as solvation. This in turn would affect how each molecule may interact with the other formulation components as well as the overall polymerisation mechanism resulting in contrasting results.

By altering the structure of the imidazolium cation, and by varying the anions used, it was postulated that more could be learned about the structural characteristics behind the cure activity and how the level of performance can be controlled and modified. Alteration of the chemical structure also offered an opportunity to examine the effect on cytotoxicity and anti-microbial toxicity.

5.2 Full Performance Testing of Lead Compounds

In the evaluation tests, BMIM octyl sulfate (**80**) and BMIM acetate (**83**) both performed to a level of cure accelerators already used commercially. In order to examine the extent of this activity and indeed their applicability as possible commercial cure catalysts, a full anaerobic adhesive evaluation was carried out where they were incorporated into the formulation.

These methods involve preparing a formulation using the cure accelerator of interest and then applying it in tests more representative of practical applications to generate an indication of its performance capabilities. Namely, these are tests using nuts and bolts (ISO 10964) which give an indication of their ability to act as thread lockers or sealants, and pins and collars (ISO 10123) where the shear strength of the adhesive is determined.

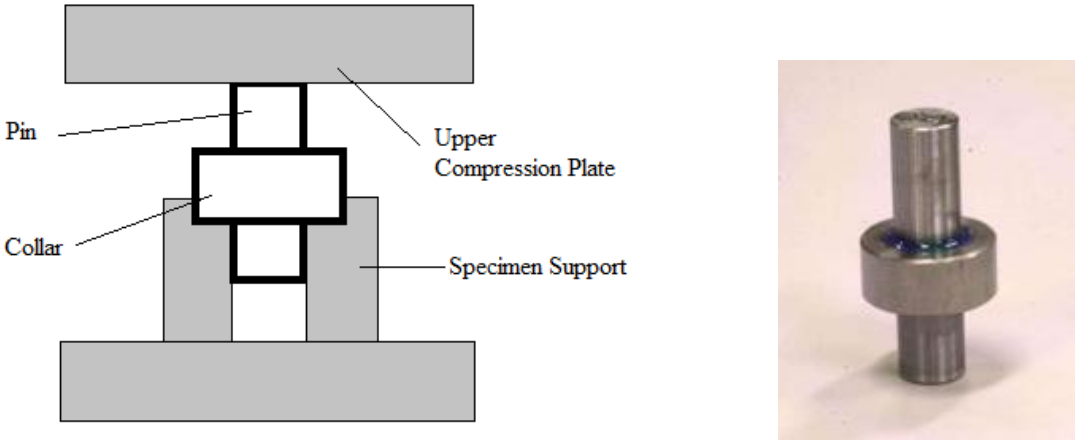


Figure 5.1: Pin and Collar Shear Strength Test Setup

The pin and collar test is comprised of a cylindrical pin and a collar which fits around it but which leaves a small gap between the two surfaces. Both specimens are prepared by going through a degreasing process to remove any residues which may interfere with the adhesive curing and are made of mild steel with a smooth surface. Adhesive is applied to both substrate surfaces such that the gap between the two is bonded and the specimen supported for the duration of the cure. The shear strength of the adhesive bond formed is then measured by placing the test specimen on a

stage where a load is applied to the pin and the force and pressure required to move the pin through the collar measured (Figure 5.1).

The nuts and bolts evaluation is carried out by applying adhesive to the bolt and assembling the nut and bolt joint. After the specified cure time, the test specimen is placed in a torque analyser and the torque required to break the bond formed is measured. The initial break torque is measured along with the prevail torque (180°) and the maximum torque reached. The break torque is the force required to initially break the bond and move the nut, the prevail torque is the force required to achieve continuous movement of the nut with the maximum torque being the maximum resistance reached when rotating the nut. The significance of prevail torque as compared to break torque is that the break torque is the force required to cause an initial break in the adhesive seal or "lock" formed, whereas the prevail torque is the force required to actually move the nut along the threads or, in practical terms, loosen and remove the nut from the bolt. This would be significant in an environment where the bond has to withstand prolonged levels of vibrations which otherwise would cause the nut to move.

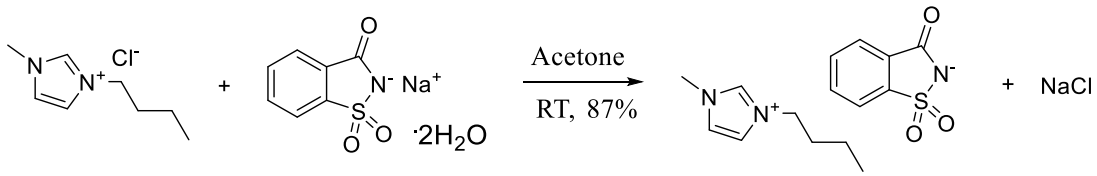
As another measure of the formulations activity, the nuts and bolts test is carried out using test specimens made from more than one grade of metal. The strength of the adhesive is measured using black oxide bolts coupled with mild steel nuts as well as stainless steel nuts and bolts. With stainless steel, the steel has been protected by adding a certain amount of chromium to the alloy. This chromium protects the steel by oxidising to form chromium oxide which forms an inactive layer preventing further oxidation of the either the chromium or iron present. In doing so, this restricts any redox interaction of the metal ion species with the adhesive and so the reaction cannot be initiated and thus no bonding occurs.

The surface of black oxide nuts is much more active in comparison, here the iron has been reacted to form an iron oxide where there are both Fe^{2+} and Fe^{3+} species present, Fe_3O_4 ($(Fe^{2+}(Fe^{3+})_2O_4$) – Magnetite), and as such interacts with and initiates the polymerisation and bond formation much more efficiently.

The pins and collars used for evaluation are made from mild steel. This is an alloy of steel where there is no significant content of chromium or any other element which could lead to surface inactivation and as such the iron molecules present are free to take part in the redox initiation of the radical mechanism.

Given that the anaerobic cure mechanism relies on the activity of the metal surface, a strong bond is much harder to generate with metals which have been treated or protected and so if when analysing such substrates significant bond strengths are achieved, this can be taken as an indication of a very active cure catalyst.
For each test, the formulations were evaluated after one hour and 24 hour cure times with APH used to prepare a formulation to act as a control. Each measurement was carried out in quintuplicate with the average result reported.

In addition to BMIM octyl sulfate (**80**) and BMIM acetate (**83**), BMIM saccharinate[1] (**100**) was prepared for analysis. This compound was prepared[1] through anion metathesis of BMIM chloride (**79**) with sodium saccharinate.H_2O (**101**) and combines the active cation with an anion which is the charged analogue of a compound already widely used in anaerobic adhesives to alter cure time, saccharin.
Since saccharin is already known to be cure active as an initiator/accelerator and is commonly included in many adhesive formulations,[2,3] incorporating it into the accelerator component of the formulation creates the possibility of not only improving upon the good activity displayed by BMIM based compounds, but also combining two formulation components and negating the need for saccharin and the accelerator to be added separately. Having a compound which is able to fulfil more than one function of the adhesion mechanism and reduce the number of formulation constituents would make the process more efficient and possibly more cost effective.
Due to these reasons BMIM saccharinate (**100**) did not undergo preliminary screening using the fixture time test and instead was incorporated into the lead accelerator evaluation along with BMIM octyl sulfate (**80**) and BMIM acetate (**83**).



79 **101** **100**

Scheme 5.1: Formation of BMIM Saccharinate (100)

For the purposes of this evaluation, each accelerator was tested as part of two separate types of formulation (Tables 5.1 & 5.2). The first formulation is the same as that which was prepared when carrying out the fixture time tests described in the previous chapter while the second introduces an additional monomer and replaces some of the stabilisers and hydroperoxide. This second formulation is more akin to a commercial anaerobic adhesive product and as such gives a more accurate indication of the practical application of any novel accelerator evaluated as well as further comparability with the performance of existing commercial products.

Table 5.1: PEGMA Formulation

Component	% Weight
Polyethylene glycol dimethacrylate	95.5
Saccharin	0.5
Trigonox TMBH-L	2
PM 16	0.5
PM 17	0.5
Accelerator	1

Table 5.2: Model Product Formulation

Component	% Weight
Polyethylene glycol dimethacrylate	50
Rigid Resin Oil Cutting Monomer	45.05
PM17	2
Saccharin	1
Paramenthane Hydroperoxide	1
PM40	0.4
Accelerator	0.95

Since the BMIM octyl sulfate (**80**) and BMIM acetate (**83**) both displayed optimum performance at a level of 0.7 mmol per 10 g in the fixture time test (Table 4.7), the decision was made to prepare the PEGMA only formulations using this concentration of accelerator.

No previous analysis had been carried out using the model product formulation (Table 5.2) before and so in order to establish the optimum accelerator concentration required, only one of the accelerators was tested as part of this mixture. BMIM saccharinate was chosen and tested at both the 0.7 mmol per 10 g level as well as the formulation specification 0.95% w/w level with APH used as the control once more.

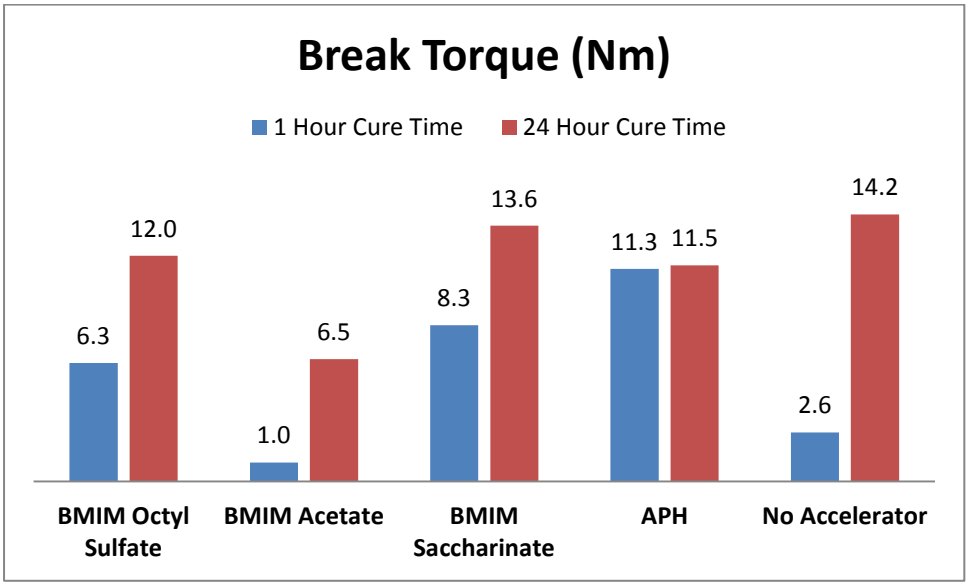
5.2.1 Results of PEGMA Formulations:

Each compound (**80**, **83**, **100**) as part of the PEGMA only formulation (Table 5.1) was tested as a cure accelerator using the nuts & bolts and pins & collar tests as described at the beginning of Section 5.2. After both 1 hour and 24 hour cure times the force required to cause bond failure was measured and this was used an indication of the level of polymerisation which had occurred**.**

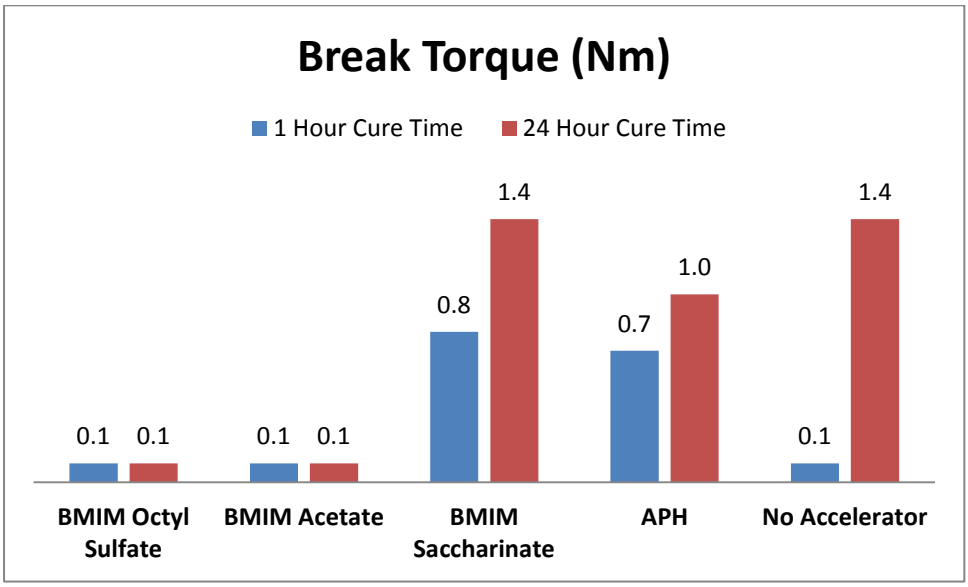
The break torque strength for each compound in both of the nuts and bolts tests is presented below. The complete set of results including the prevail torque (Graph 5.1(b) and 5.2(b)) and maximum torque measurements (Graph 5.1(c) and 5.2(c)) can be found in Appendix C.

Likewise the compressive strength (Graph 5.3(a)) for each compound tested as part of the pins and collars evaluation is presented below with their maximum load (Graph 5.3(b)) found in Appendix C.

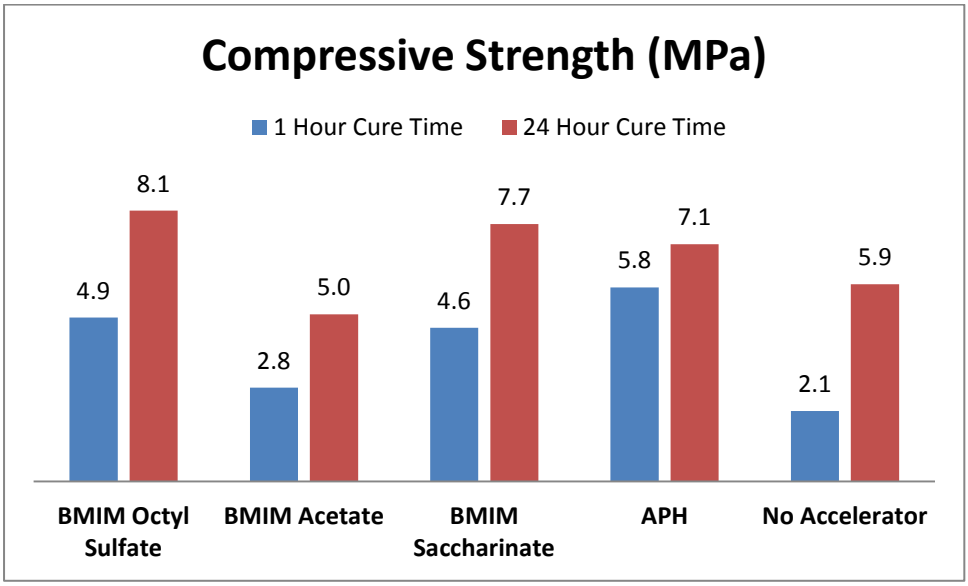
Graph 5.1(a): Black Oxide Bolts with Mild Steel Nuts - Break Torque



Graph 5.2(a): Stainless Steel Nuts and Bolts - Break Torque



Graph 5.3(a): Mild Steel Pins and Collars - Compressive Srength



When analysing the outcomes of the nuts and bolts tests (Graphs 5.1(a, b, c) and 5.2(a, b, c), the results are separated into three measurements; the break torque (Graph 5.1(a)) which indicates the initial force required to cause a break in the adhesive bond, the prevail torque (Appendix C - Graph 5.1(b)) which is the force required to turn the nut 180°, and the maximum torque reached (Appendix C - Graph 5.1(c)).

Where the break torque provides us with the force required to cause the primary failure of the adhesive bond, the prevail torque indicates the ability of the bond to resist movement. This is particularly important where the adhesive is applied as a threadlocker as it will illustrate how much force (due to mechanical vibrations for example) can be resisted before beginning to loosen. For adhesives to be fit for applications such as this it is preferable for this value to be as large as possible and for the cure strength to be achieved in as short a time as possible, i.e. the polymer is able to cure quickly but yet still reach an operational strength. In other types of adhesives factors such as how brittle the bond formed is have to be accounted for as well as the overall strength but in anaerobic adhesives where the bond gap is usually quite small, this is not as large an issue. If we compare the 24 hour break torque of the formulation which contained no accelerator to the others (Graph 5.1(a)), we can see that it actually resulted in the strongest bond. By introducing catalysts in an

attempt to speed up the polymerisation reaction, weakness is inherently introduced into the bonds formed, whereas if the reaction is allowed to proceed at its "natural" rate then it will result in optimum polymerisation and bond strength. Thus, whilst the objective is to accelerate the polymerisation rate, attention must also be paid to how far the strength of the end bond is compromised as compared to the maximum strength achievable.

One of the reasons APH is so widely used as a cure accelerator can be seen from the break torque measured along with its performance in the other tests (Graphs 5.1-3). Whilst APH does not possess the best 24 hour cure time strengths of all the accelerators tested, 11.5 Nm, this strength is very similar to that observed after one hour cure time, 11.3 Nm (Graph 5.1(a)). This is an illustration of the efficiency of the catalyst showing that APH is able to reach its final bond strength in a short period of time and that a prolonged cure time is not required for the adhesive to set.

The one hour break strengths of two of the three BMIM accelerators (**80**, **100**) compare very favourably to APH with 6.3 and 8.3 Nm respectively. Whilst APH performs better, the difference is not hugely significant in the case of BMIM saccharinate and shows that it too is an efficient cure catalyst. The acetate (**83**) derivative on the other hand performed extremely poorly, 1.0 Nm, even worse than the formulation which contained no accelerator at all in fact. Clearly, there is a very different interaction with the formulation occurring compared to the other BMIM based compounds and does not allow for polymerisation to occur. One should note that upon addition of the BMIM acetate to the formulation a colour change was observed which may indicate a possible reaction with the formulation component(s) as no other accelerator displayed this stark colour change. If so, this could prevent the polymerisation mechanism from proceeding as intended.

If we consider the PEGMA formulation results as a whole (Appendix C - Graphs 5.1-3), one can see that BMIM octyl sulfate (**80**) showed very promising cure activity and a distinct ability to affect the polymerisation reaction. However, the most significant result of the series was that of BMIM saccharinate (**100**) which showed activity on a similar scale to APH, for example Graph 5.3(a) where the compressive strengths of the two formulations only differed by 1.2 MPa in favour of APH and BMIM saccharinate resulted in a stronger 24 hour cure bond. In fact, the level of activity displayed by **100** across all of the tests is such that it may be considered as a candidate for commercial application. This represents a major

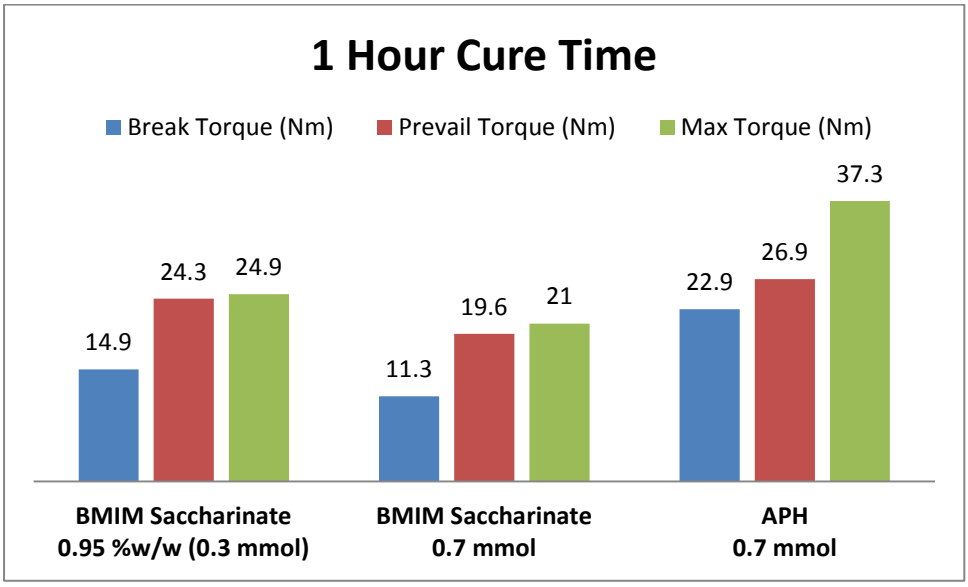
breakthrough in the search for and development of anaerobic cure accelerators to have a molecule which is capable of matching the activity of those for which alternatives are desired. The contrasting activity of the three BMIM based molecules also illustrates that the anion may in fact play a much larger role in the mechanism of action and that activity may not be primarily as a result of the cation as previously thought.

As noted earlier, lack of cure activity when using stainless steel substrate is not a cause for concern due to the inactivity of the metal surface. Tests involving stainless steel substrate are very challenging for anaerobic adhesives and for this reason they are rarely applied on such substrates. We can see that even APH struggles to achieve any significant cure strength after one hour compared to the results of the same tests carried out using black oxide bolts with mild steel nuts (Graph 5.1(a) vs. 5.2 (a)). Instead, when any level of cure at all is observed, this is used as an indication that the compound is indeed capable of affecting the cure mechanism to a significant degree as is evident with BMIM saccharinate.

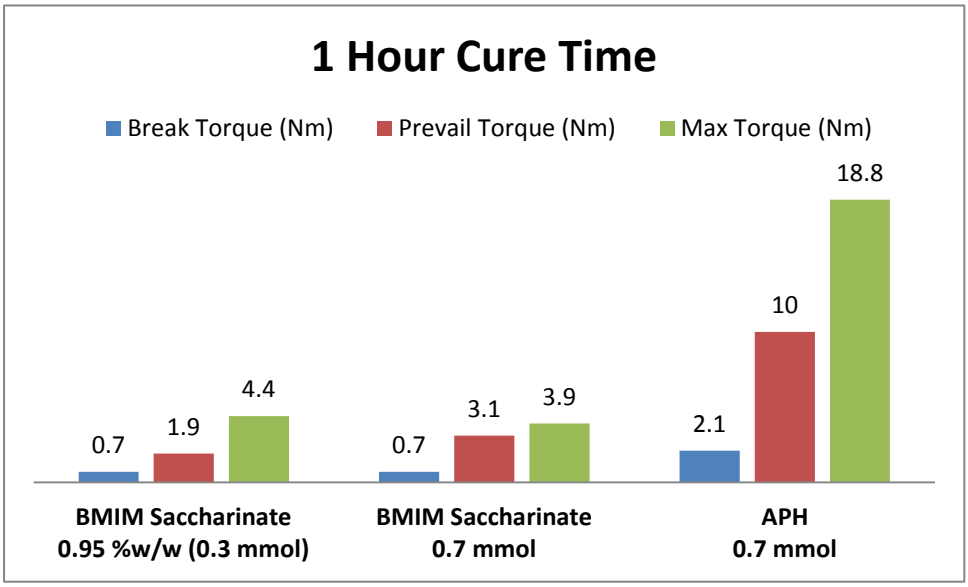
5.2.2 Results of Model Product Formulations

Due to no analysis having been carried out using this formulation previously, the evaluation was only carried out using one hour cure times to assess their suitability to the formulation.

Graph 5.4: Black Oxide Bolts with Mild Steel Nuts

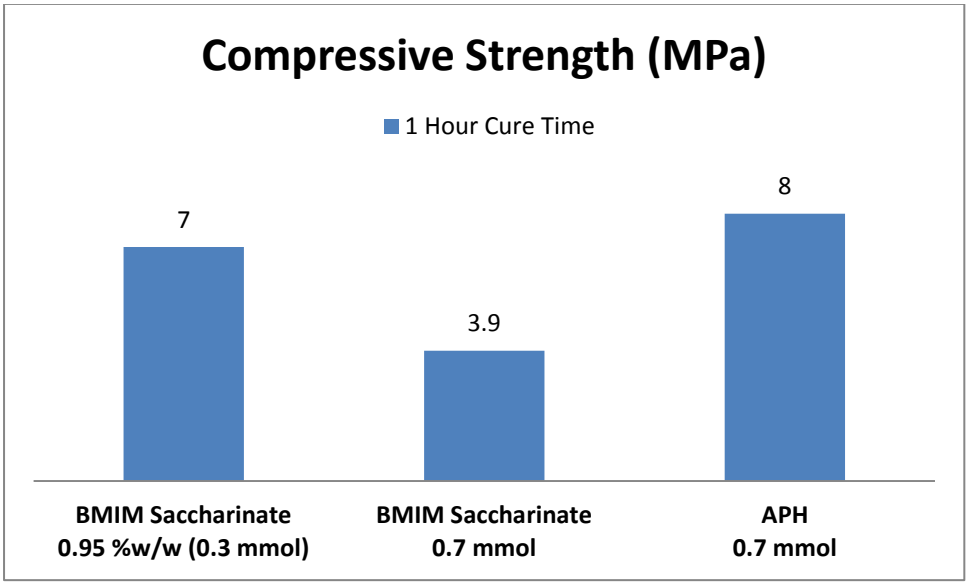


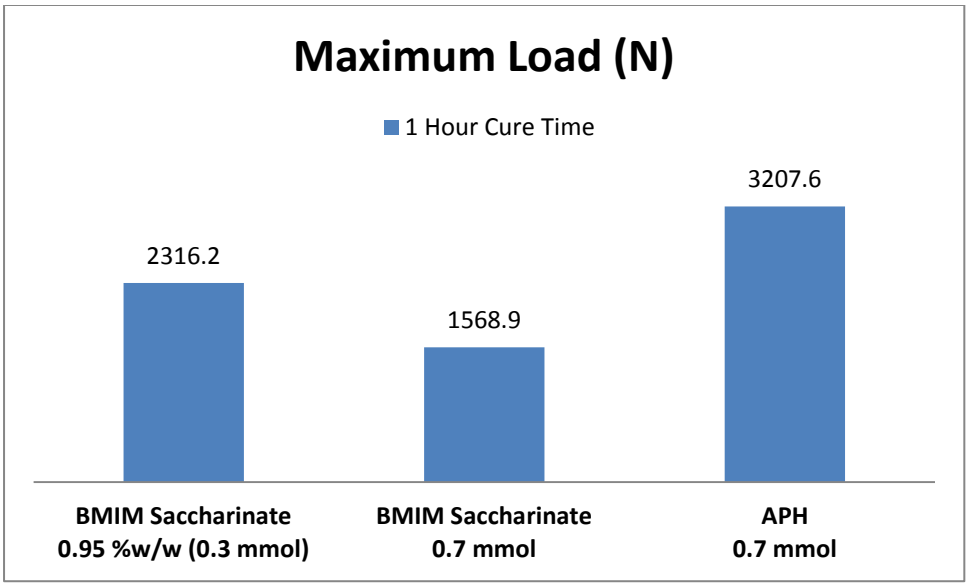
Graph 5.5: Stainless Steel Nuts and Bolts



Graph 5.6: Mild Steel Pins and Collars:

(a) Compressive Strength and (b) Maximum Load





With respect to the model formulations analysed (Graphs 5.4-6), this was a formulation which has been developed by Henkel and is much closer to a complete commercial formulation than the PEGMA only formulation. Since no analysis had been carried out using it before, only one of the BMIM accelerators was selected for analysis to gain an insight of how the presence of an extra monomer would affect the polymerisation interaction, and this was only carried out after one hour cure time.
Due to the excellent performance of BMIM saccharinate (**100**) when used as part of the PEGMA formulation, e.g. Graph 5.1(a) break torque strength, **100** was chosen as the accelerator to be evaluated. The standard amount of accelerator to be used in the formulation is 0.95% w/w but, as illustrated in the previous chapter, following the gram amount will not necessarily result in optimum activity when the accelerator is changed and so in addition to this amount the testing was also performed at 0.7 mmol per 10 g, the optimum PEGMA formulation concentration. Again BMIM saccharinate (**100**) performed comparably to APH as with the previous formulation providing further evidence to support the possible application as a commercial accelerator. Interestingly **100** performed best at a level of 0.95% w/w which is less than half the mole amount added compared to when 0.7 mmol per 10 g is used (Graphs 5.4 and 5.6). This is another example that elevating the catalyst loading excessively can result in poorer acceleration of the polymerisation as was observed in Section 4.6.

Until the results of BMIM saccharinate are evaluated further, a word of caution must be noted. There is the possibility that the increased activity may simply be due to an increased amount of saccharin molecules being available in the formulation. We know saccharin plays an important role in the curing mechanism, either in an uncharged state or possibly as part of a salt formed in the formulation process, and so when introducing a saccharinate compound into the adhesive there is the chance that any increased activity observed is simply as a result of an increased amount of saccharinate species akin to if an increased amount of saccharin had been added. Thus, in order to validate the results, testing using range of concentrations of saccharin needed to be carried out.

5.3 Investigation of the Source of Saccharinate Activity

In order to determine whether the activity of BMIM saccharinate as an accelerator was as a result of the compound or simply due to the addition of extra active saccharin to the formulation, a series of formulations were prepared and tested using varying levels of saccharin.

These formulations included; two formulations prepared using the accelerator only and no saccharin (one using BMIM saccharinate (**I**) and one using APH (**III**) for comparative purposes), and one made up using no accelerator but an amount of saccharin equal to the sum amount of the accelerator and saccharin which would normally be added (**II**).

All three of these formulations were prepared using both the PEGMA and Model formulations as before with the results of the complete BMIM saccharinate formulation included for comparison (**IV**). In the case of the model formulation, the 24 hour cure time tests of BMIM saccharinate which were not carried out before in Section 5.2.2 were done so.

The break torque strength for each compound in both of the nuts and bolts tests is presented below (Graphs 5.7(a), 5.8(a), 5.10(a), and 5.11(a)). The complete set of results including the prevail torque (Graphs 5.7(b), 5.8(b), 5.10(b), and 5.11(b)) and maximum torque (Graphs 5.7(c), 5.8(c), 5.10(c), and 5.11(c)) can be found in Appendix C.

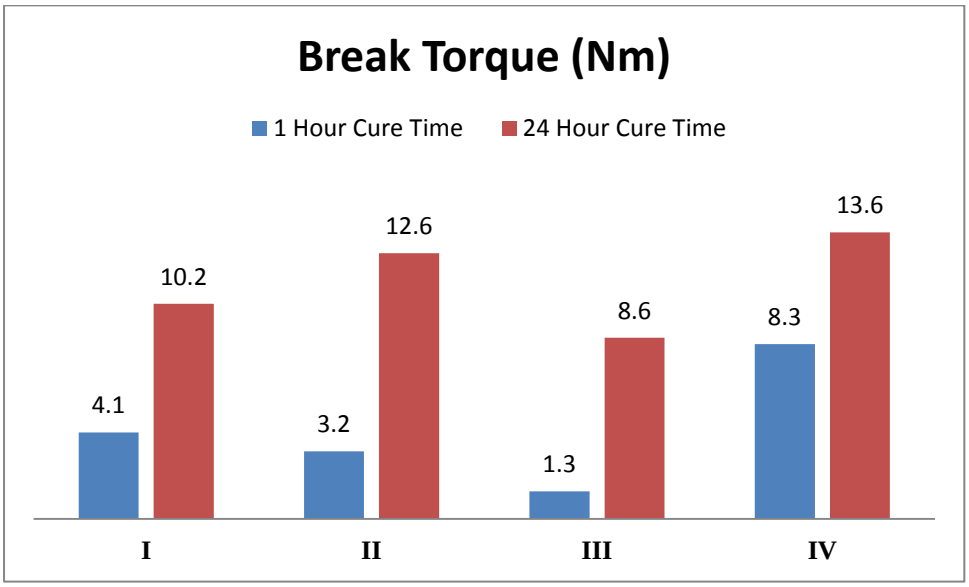
Likewise the compressive strength (Graphs 5.9(a) and 5.12(a)) for each compound tested as part of the pins and collars evaluation is presented below with their maximum load (Graphs 5.9(b) and 5.12(b)) found in Appendix C.

5.3.1 PEGMA Formulations

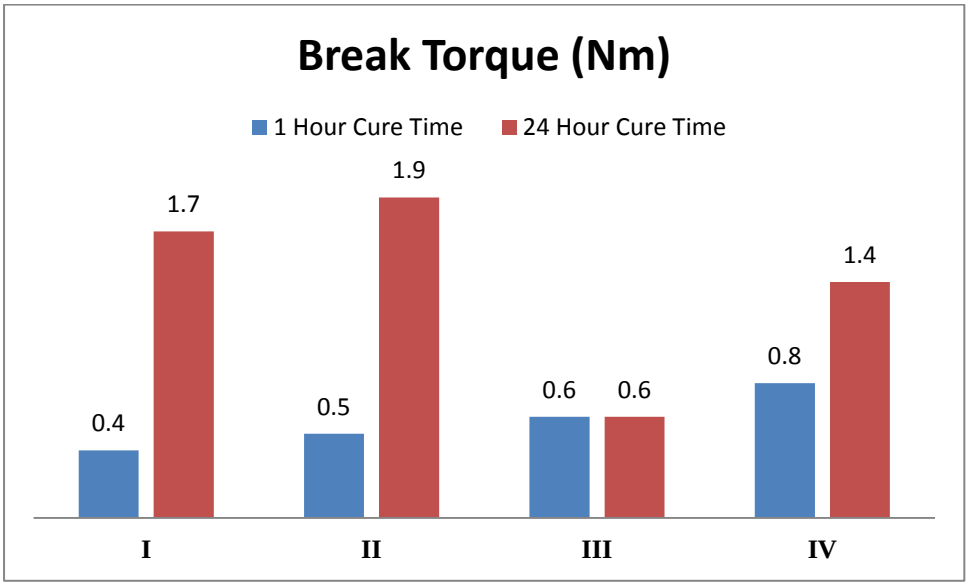
Component	Amount contained in each formulation (g)			
	No Saccharin with BMIM Saccharinate (I)	Extra Saccharin (II)	No Saccharin with APH (III)	BMIM Saccharinate with Saccharin (IV)
PEGMA	9.55	9.55	9.55	9.55
Premix 16	0.05	0.05	0.05	0.05
Premix 17	0.05	0.05	0.05	0.05
Tetramethylbutyl hydroperoxide (TMBH-L)	0.2	0.2	0.2	0.2
Saccharin	-	0.183 (1 mmol)	-	0.05 (0.27 mmol)
Accelerator	0.22 (0.7 mmol)	-	0.105 (0.7 mmol)	0.22 (0.7 mmol)

Note: Mass of saccharin in (II) is equal to mass of IL and saccharin in (IV), however this is not an equimolecular comparison

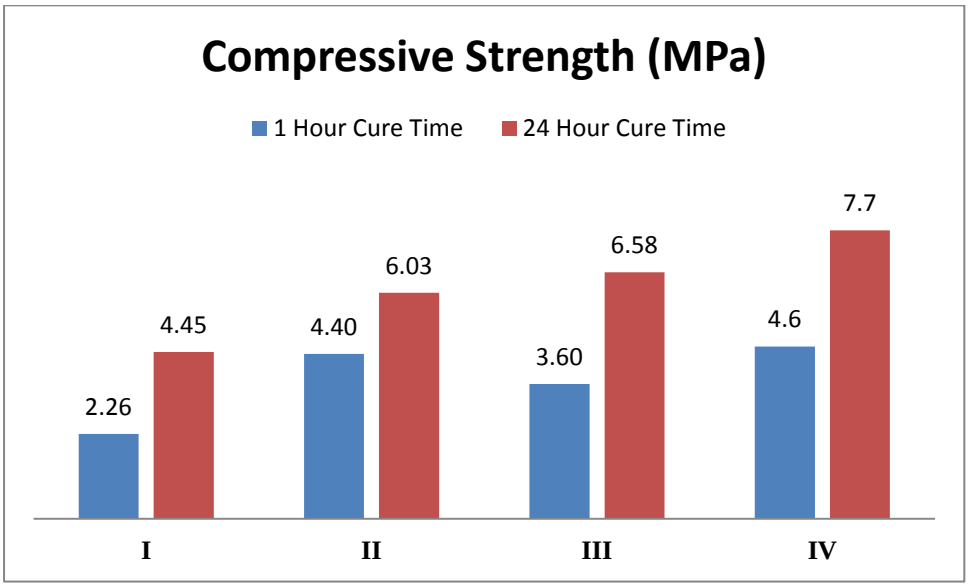
Graph 5.7(a): Black Oxide Bolts with Mild Steel Nuts - Break Torque



Graph 5.8(a): Stainless Steel Nuts and Bolts - Break Torque



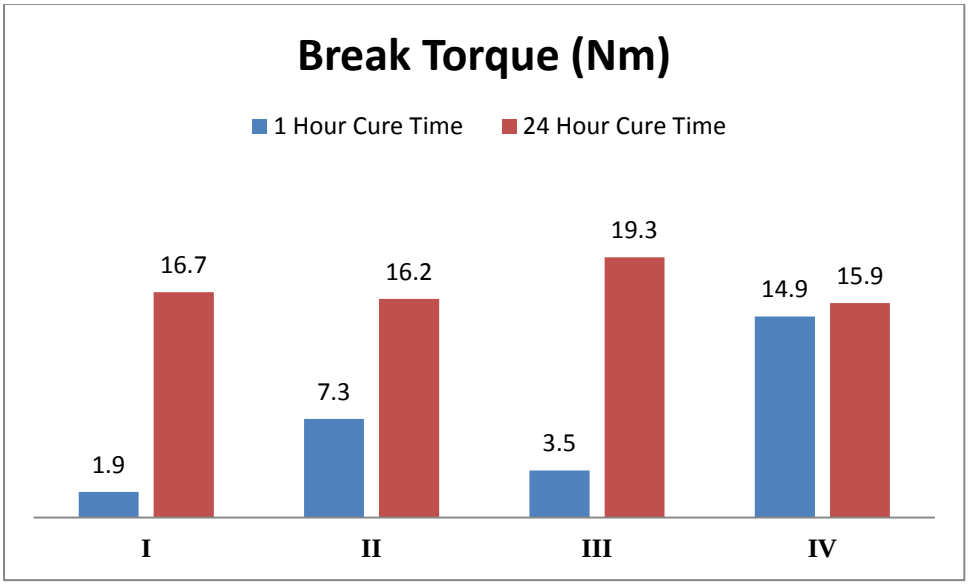
Graph 5.9(a): Mild Steel Pins and Collar - Compressive Strength



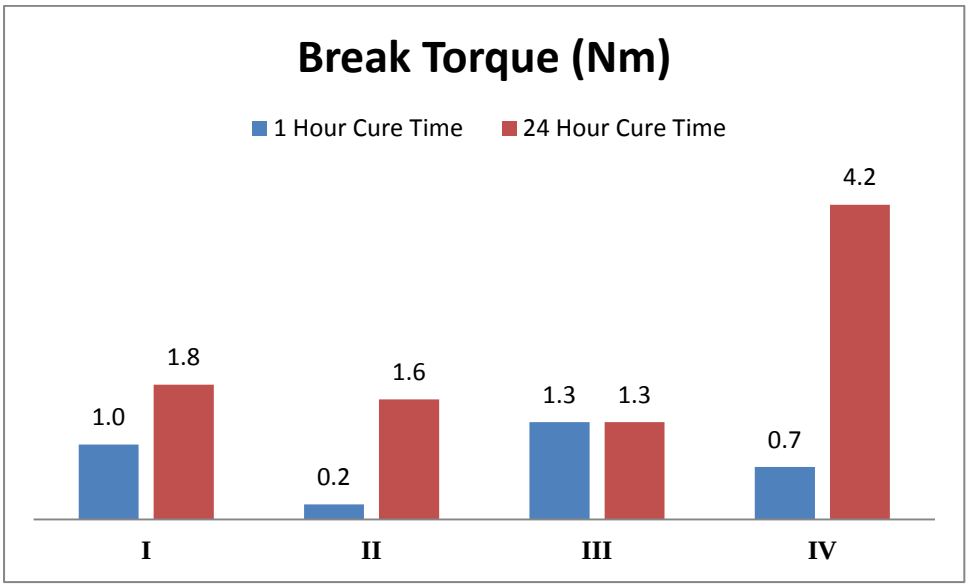
5.3.2 Model Formulations

Component	Amount contained in each formulation (g)			
	No Saccharin with BMIM Saccharinate (I)	Extra Saccharin (II)	No Saccharin with APH (III)	BMIM Saccharinate with Saccharin (IV)
PEGMA	5	5	5	5
Oil Cutting Rigid Resin	4.5	4.5	4.5	4.5
PM17	0.2	0.2	0.2	0.2
Paramenthane Hydroperoxide	0.1	0.1	0.1	0.1
PM40 (Menadione)	0.04	0.04	0.04	0.04
Saccharin	-	0.195	-	0.1
Accelerator	0.095	-	0.095	0.095

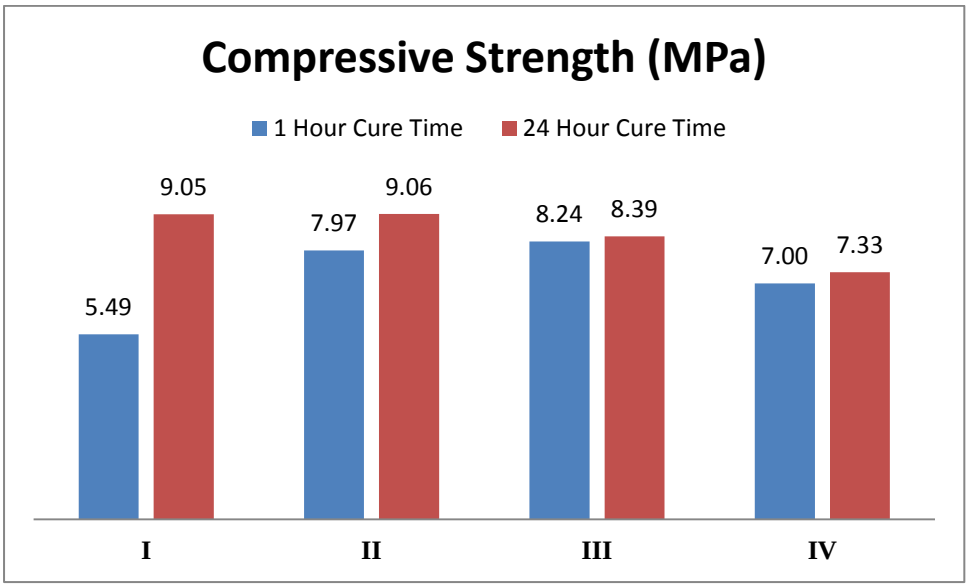
Graph 5.10(a): Black Oxide Bolts with Mild Steel Nuts - Break Torque



Graph 5.11(a): Stainless Steel Nuts and Bolts - Break Torque



Graph 5.12(a): Mild Steel Pins and Collar - Compressive Strength



These results illustrate that the increased cure activity is due to the BMIM saccharinate compound and is not as a result of simply having extra saccharin species in the formulation (Graphs 5.7-11). When we compare the formulation containing the elevated level of saccharin (**II**), to the formulation prepared as normal (**IV**), there is a large difference in cure strength after one hour. For example, we can see the break strength in Graph 5.10(a) of 7.3 Nm for **II** is less than half of that observed for **IV** (14.9 Nm) using the model formulation applied to black oxide bolts and mild steel nuts. This trend is broadly seen across both formulations tested but the performance is somewhat closer in both pins and collars evaluations (Graphs 5.9(a) and 5.12(a)).

The ability of BMIM saccharinate to fulfil the role of both the neutral saccharin and cure accelerator was also investigated. This was carried out by preparing test formulations which contained no saccharin whatsoever using BMIM saccharinate as the accelerator (**I**) as well as another which used APH to act as a bench mark to compare how a representative adhesive formulation would perform when no saccharin was present (**III**). Whilst the results show a marginal increase in activity for the BMIM saccharinate over APH in the PEGMA formulations (Graphs 5.7(a)-9(a)), this difference is not significant and this trend is reversed for the model formulation adhesives (Graphs 5.10(a)-12(a)). Crucially, when they are further compared to the standard formulation (**IV**) in all examinations one can be seen that saccharin still plays a key role in the curing mechanism by its presence in the neutral form and despite the presence of the saccharinate anion.

5.4 The Development of Ionic Liquids as Cure Accelerators.

The results of the full accelerator evaluation of the three BMIM based compounds (**80**, **83**, **100**) confirm the viability of alkyl imidazoliums as cure catalysts. Despite the acetate analogue (**83**) performing very poorly (Graph 5.1(a)), the octyl sulfate (**80**) and particularly the saccharinate (**100**) derivatives displayed activity to warrant further investigation of their properties.

Considering no prior evaluation of the saccharinate compound had been carried out prior to this complete evaluation, the extent of its activity was somewhat unexpected. Given the wide range of different activities displayed by the three BMIM compounds (**80**, **83**, **100**), the initial hypothesis that the activity was primarily as a result of the

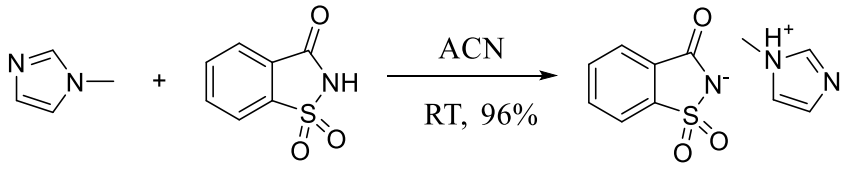
nitrogen containing cation was revisited. The anion is clearly able to alter the cure process significantly, and given the extent to which the saccharinate ion was able to do so, added emphasis was placed on thisimportance in the development of imidazolium ionic liquids as cure accelerators.

In terms of studying the activity of the imidazolium cation, the first position chosen to begin with was the length of the alkyl chain substitution. By altering the chain length an insight into whether this structural characteristic was of significance or not could be gained given the wide range of lipophilicities which could potentially be applied, as well as an opportunity to look more closely at the possible issue of molecule size versus catalytic activity as discussed in the previous chapter. By reducing the cation size, the activity of the BMIM derivatives could possibly be improved upon even further if this turned out to be the case. Thus

1-Methyl- (**102**) and 1-ethyl-3-methylimidazozlium iodide (**103**), and 1-octyl-3-methylimidazolium chloride (**104**) were provisionally selected as compounds of interest along with 1-butyl-2,3-dimethyl imidazolium tetrafluoroborate (**105**). These compounds were preliminarily examined as before by first evaluating their solubility in PEGMA at 1% w/w. Compounds **102** and **103** proved to be soluble and thus were suitable for further analysis using the fixture time test however **104** and **105** proved to not be.

As described previously in Section 1.3, there is the possibility that neutral accelerators are not the active catalytic species in the adhesive formulation and instead form salts with other components such as saccharin which in turn carry out the role of catalyst. It would be advantageous if saccharin salts of neutral accelerators could be prepared for analysis with their performance then compared to the respective "neutral" analogue. This could conceivably allow for the determination of this mechanism characteristic i.e. if the two sets of formulations possessed the same activity it could be said that salt formation leads to the active cure accelerator. In the previous chapter a large number of imidazoles were evaluated for their cure activity and to this end the saccharin salt of 1-methylimidazole was prepared in 96% yield by stirring saccharin and 1.1 equivalents of 1-methyl imidazole in acetonitrile at room temperature (Scheme 5.2). The formation of the salt compound (**106**) was confirmed by NMR spectroscopy and

attempts were made to synthesise the saccharin salts of further imidazoles such as 1-butyl- and 1-benzylimidazole but salt formation did not occur in these cases.



106

Scheme 5.2: Synthesis of 1-Methylimidazolium Saccharinate (106)

The solubility of 1-methylimidazolium saccharinate (**106**) was tested in PEGMA at formulation level and surprisingly was found to be insoluble. This was unexpected given that both neutral compounds are soluble (as well as several similar ionic liquids) and lead to quite good cure acceleration. This may possibly indicate that neutral compounds do not rely on salt formation to become cure active however no definitive statement can be made on this aspect given only one case was investigated. However, the postulation was made that protic imidazolium ionic liquids generated from a simple acid base reaction have poor solubility in PEGMA at 1% w/w, most likely due to low lipophilicity.

The imidazolium halides (**102**, **103**) which proved soluble in the formulation were therefore the only compounds analysed using the fixture time test. Both 1,3-dimethylimidazolium (MMIM) iodide (**102**) and 1-ethyl-3-methylimidazoloium (EMIM) iodide (**103**) were used to prepare separate formulations as per the PEGMA specification outlined in Table 5.1. These formulations were then used to carry out the fixture time test.

Both of the ionic liquids performed very poorly in this examination with no cure observed after three hours. The BMIM compounds (**80**, **83**) analysed previously had displayed cure times of 0-5 minutes in this test (Chapter 4, Table 4.7) and, from investigation of the cation structure, considering that these compounds only differ in shorter alkyl chain length (butyl vs. methyl or ethyl), such a decrease in activity is surprising. Both compounds **80** and **83** contain organic anions and so the halide counter ion was thought to possibly be the cause for the dramatic decrease in

performance. To examine this hypothesis, derivatives of the imidazolium compounds paired with organic anions were preapred.

Of the three organic anions tested to this point (saccharinate, octyl sulfate, and acetate), the saccharinate yielded the best results and so anion metathesis of **102**-**104** was carried out using a modified version of the method used to prepare the BMIM derivative (**100**) with the sodium dihydrate salt of saccharin.[1] Two equivalents of the sodium saccharinate were stirred with the respective imidazolium compound at room temperature in acetone overnight to form MMIM saccharinate (**107**) and EMIM saccharinate (**108**). In order to purify, the excess staring material and inorganic salt by-product were first removed by filtration. The solvent was then evaporated to dryness to yield the crude product (MMIM saccharinate – white crystalline solid, EMIM saccharinate – hygroscopic white solid, and OMIM saccharinate (**109**) – transparent pale yellow oil[4]). The remainder of the inorganic salt was removed by dissolving the product in chloroform and separating the insoluble solid by filtration.

This method worked quite efficiently for OMIM chloride (**104**) but did not result in the complete removal of all of the sodium iodide formed in the case of the shorter chained imidazoliums (**102**, **103**). This was an issue due to the slight solubility of NaI in the same range of solvents as the products meaning NaI could not completely be removed by precipitation and filtration. This also proved to be an issue in other purification methods such as column chromatography as the ionic liquid products required a polar mobile phase.

In order to overcome this problem the decision was made to alter the starting material used. If this were to contain a cation which upon reaction would form an iodide salt insoluble in the majority of polar organic solvents, then the current efficient purification method could be retained. Silver iodide is one such salt and thus silver saccharinate was prepared by stirring equimolar amounts of sodium saccharinate dihydrate with silver nitrate in water at room temperature. This led to the precipitation of the product which was easily isolated and dried.[5]

The silver saccharinate was used in the place of the sodium salt for the successful formation of MMIM (**107**), 92% yield, and EMIM saccharinate **(108**), 86% yield), using the method described above (Scheme 5.2) with the amendment of protecting the reaction mixture from light to avoid degradation of the silver salt.

Both the OMIM (**109**) and EMIM (**108**) saccharinates proved to be soluble in PEGMA at 1% and so were able to be evaluated for their accelerator activity but unfortunately the MMIM derivative (**107**) was insoluble at the 1% w/w loading examined.

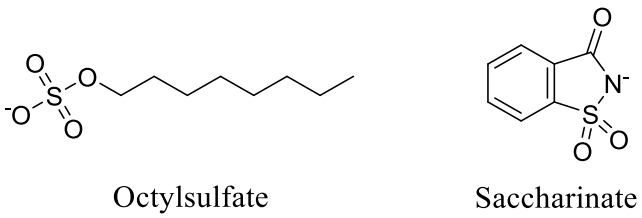


Figure 5.2: Structures of Octylsulfate and Saccharinate Anions

In addition to varying the cation structure, variation of the anion used was also examined for the effect on activity. If the structures of the octyl sulfate and saccharinate anions are compared one can be seen they both contain sulphur moieties, namely sulfate and sulfonyl groups respectively, which may be an indication as to the source of their activity. In addition to this, the saccharinate sulfonyl group exists as part of a sulphonamide functionality (Figure 5.2). It is believed that the activity of cure accelerators is as a result of the amine and so the increased activity of the saccharinate could be due to a combination of these groups.
When proposing possible anion structures, those which contained these characteristics or which were analogous to saccharin were considered. Phthalimide and acesulfame both fulfil some of these requirements (Figure 5.3).

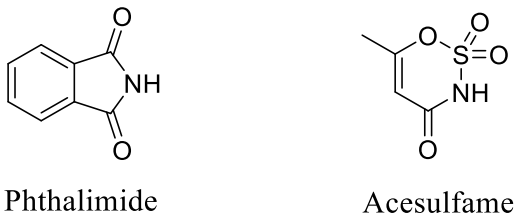


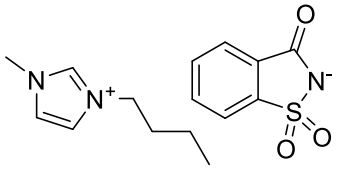
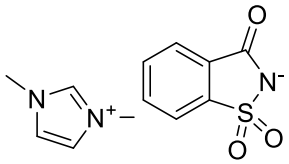
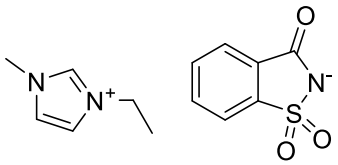
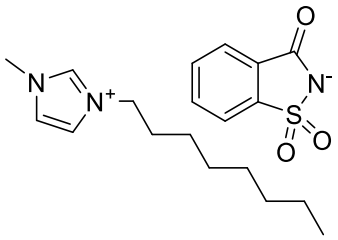
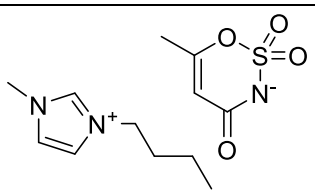
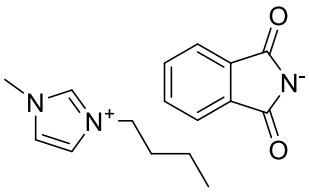
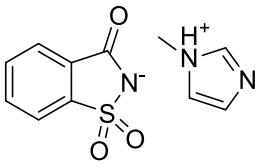
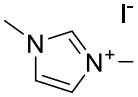
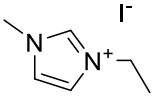
Figure 5.3: Structures of Phthalimide and Acesulfame

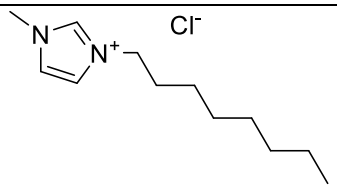
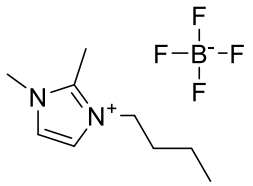
Phthalimide is structurally identical to saccharin but for the substitution of the sulfonyl group for a carbonyl functionality while acesulfame on the other hand contains the possibly key sulphonamide moiety. In order to evaluate their cure

activity both were paired with the BMIM cation. Since several other anions had already been tested as BMIM salts, this would offer a way of quantifying any activity shown in the context of the other accelerators developed. BMIM phthalimate (**111**) was prepared through anion metathesis of BMIM chloride and the potassium salt of phthalimide (86% yield)[6] while BMIM acesulfamate (**110**) was prepared through the same process but using the potassium salt of acesulfame (90% yield).[5] Both products (**110**) and (**111**) were soluble in the monomer and so were eligible to be evaluated as cure accelerators along with the other imidazolium saccharinates (**108** and **109**).

Given the presence of the sulphonamide functional group, acesulfame (**112**) was also evaluayed as a possible alternative to saccharin in the adhesive formulation. This could help elucidate the importance of such a functional group with regards to cure activity.

Table 5.3: Table of Compound Solubility in PEGMA at 1% w/w

Compound	Structure	Solubility
BMIM saccharinate (**100**)		✓
MMIM saccharinate (**107**)		×
EMIM saccharinate (**108**)		✓
OMIM saccharinate (**109**)		✓
BMIM acesulfamate (**110**)		✓
BMIM phthalimate (**111**)		✓
Saccharin-1-methylimidazole (**106**)		×
MMIM iodide (**101**)		✓
EMIM iodide (**102**)		✓

OMIM chloride (**103**)		×
1-Butyl-2,3-dimethyl imidazolium tetrafluoroborate (**104**)		×

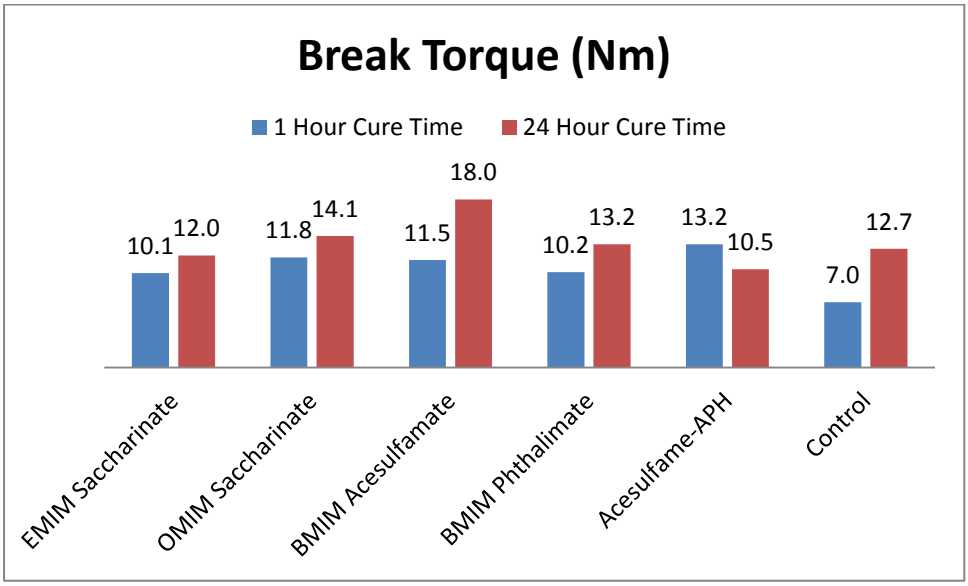
5.4.1 Cure Accelerator Activity Evaluation

The two alkyl-imidazolium saccharinates (**108**, **109**), along with BMIM acesulfamate (**110**), BMIM phthalimate (**111**), and acesulfame (**112**) as a saccharin alternative were tested as cure accelerators. Given the likelihood of positive cure activity, decision was made to evaluate them using the more extensive adhesion tests as opposed to the primary fixture time method. Also, given the greater similarity to commercial products and thus better indication of practical applicability, the accelerators were tested as part of the model formulation only and not the PEGMA formulation as was included previously. The formulations were prepared as per Table 5.2 with the neutral acesulfame paired with APH as an accelerator. As before, APH was used in the control formulation.

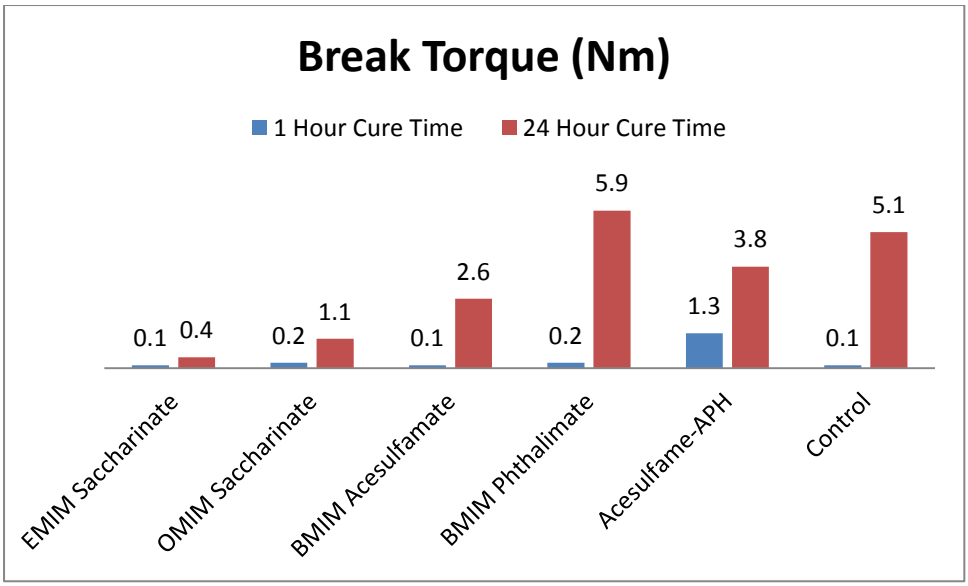
The break torque strength for each compound in both of the nuts and bolts tests is presented below (Graphs 5.13(a) and 5.14(a)). The complete set of results including the prevail torque (Graphs 5.13(b) and 5.14(b)) and maximum (Graphs 5.13(c) and 5.14(c)) torque can be found in Appendix C.

Likewise the compressive strength (Graph 5.15(a)) for each compound tested as part of the pins and collars evaluation is presented below with their maximum load (Graph 5.15(b)) found in Appendix C.

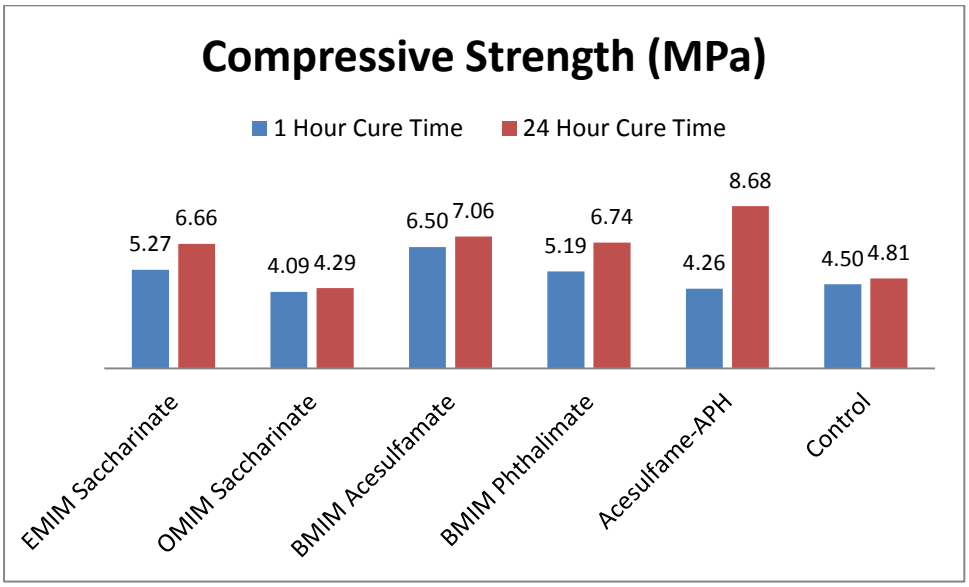
Graph 5.13(a): Black Oxide Bolts with Mild Steel Nuts - Break Torque



Graph 5.14(a): Stainless Steel Nuts and Bolts - Break Torque



Graph 5.15(a): Mild Steel Pins and Collars - Compressive Strength



As can be seen from the results (Graphs 5.13(a)–15(a)), all compounds tested performed at and above the level of APH in the formulation with the exception of the stainless steel substrate for reason discussed in section 5.1.3. Both EMIM (**108**) and OMIM saccharinate (**109**) performed similar to each other as well as to the level exhibited by BMIM saccharinate in the previous analysis (**100**) (Black oxide beak torques of 10.1, 11.8, and 14.9 Nm respectively – Graph 5.13(a) and 5.4(a)). Whilst the BMIM analogue performs the best of the three compounds, the difference in activity observed is not significant and when the activity of **108** and **109** is compared to that of APH across all the tests we can see that they are effective cure accelerators in their own right. This would suggest that the length of alkyl chain substitution on the imidazolium ring has no discernable effect on catalyst activity and that this is possibly due to the properties of the charged heterocyclic ring.

The acesulfamate (**110**) and phthalimate (**111**) derivatives also performed to a high level in the formulation analysis (Graph 5.15(a)). Across all tests both compounds performed to a specification which would suggest their suitability as possible commercial cure accelerators. In particular **111** displayed notable 24 hour cure activity in the stainless steel nuts and bolts test (Graph 5.14 (a-c)) illustrating the excellent level of catalytic activity. The activity of neither acesulfamates nor

phthalimates as anaerobic cure accelerators has been documented before and so the significance of these results should not be understated.

In the case of the acesulfamate, this is potentially further evidence as to the role of the sulphonamide group in polymerisation catalysis. This is further backed up by the performance exhibited by neutral acesulfame (**112**) when used as a replacement for saccharin. When paired with APH in an adhesive system, this resulted in better activity than the control formulation, which was prepared using saccharin, after one hour cure time in both nuts and bolts tests (Graphs 5.13(a)-14(a)). While there is no current requirement to replace saccharin in anaerobic adhesives, it is nevertheless significant to discover an alternative compound capable of performing at and above the same level of efficiency. One should note that the reason neutral phthalimide was not tested in this capacity also was because this compound had previously been found to display poor activity (D. Birkett, personal communication).

5.5 Further Development of Ionic LiquidCure Accelerators

The results described above display that imidazolium based ionic liquids can act as extremely effective cure accelerators regardless of the length of alkyl chain substitution. However, this does not demonstrate whether any unique structural characteristic of the imidazolium cation which is responsible for this activity, only reinforce the weight of evidence for the application of the compound family as cure catalysts.

For example, this activity could be as a result of features such as the aromaticity, the presence of more than one nitrogen centre, or the presence of the lone pair of electrons on the nitrogen atom and/or their confirmation. If the importance of features such as these could be determined it would be invaluable in the design of new cure accelerators.

To study properties such as these a list of cations was drawn up which if tested could possibly lead to more information about the structure activity relationship. (Figure 5.4).

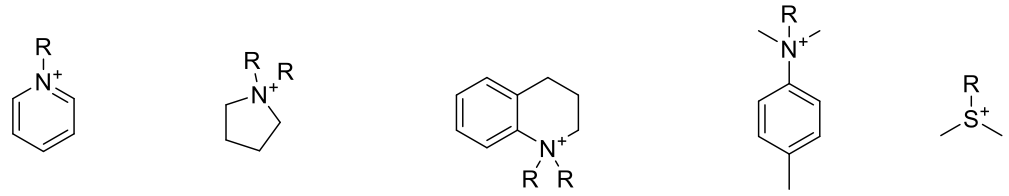


Figure 5.4: Cations of Interest

Testing of a pyridinium cation could determine whether the presence of the lone pair of electrons on the nitrogen group is essential to activity or not. Given that anaerobic accelerators are all nitrogen containing compounds, and given that all in general use largely have no charge, the lone pair is intact in all and could play a key role in the curing mechanism. This would be of particular significance if the active compound relied on the formation of species such as charge transfer complexes.

Quarternarisation of pyrrolidine would also allow for the evaluation of the necessity of the nitrogen lone pair. In addition to this, its evaluation could also lead to information about the importance of aromaticity to the cure process. While the first cure accelerators were compounds such as tributylamine, they were not able to affect the cure time nearly as efficiently as aromatic compounds such as DM*p*T and THQ.

By preparing sulphonium based ionic compounds, the necessity of nitrogen based compounds as a whole to the cure process could be evaluated. The previous tests where the anion has been varied have shown the capability of altering the cure time significantly (Graphs 5.13-15) but it is not known whether this is completely irrespective of the cation present or not. The elucidation of such information would definitively outline the role of each constituent and determine whether one of the cation or anion can be structurally altered to prepare catalysts with custom properties such as; low toxicity, varying solubility/selectivity, environmentally benign without comprising the cure activity attributed to the other.

In addition to compounds selected to directly study the structure-activity relationship, other compounds were prepared to assess their possible cure activity. Compounds such as THQ and DM*p*T have been used to great effect as accelerators in commercial products and display better activity than other neutral compounds such as imidazole. When salts based on imidazole were prepared a substantial increase in activity over the neutral analogues was observed (**80**, **100**, **108-111**).

Therefore, if salts based on compounds which already possessed exemplary activity were to be prepared, and if this had the analogous effect on activity, then compounds capable of extremely efficient cure catalysis could be yielded and even lead to a reduction in the catalyst loading required resulting in an overall more efficient and sustainable product. To this end, quarternarised derivatives of THQ and DM*p*T were therefore targeted for synthesis and evaluation.

N-methylpyridinium iodide (**113**) was prepared through stirring 1.1 equivalents of methyl iodide with pyridine at reflux in methanol. Cooling to room temperature resulted in precipitation of the product which was isolated and washed with ethyl acetate to give 91% yield.[7] *N*,*N*,*N*-Trimethyl-*p*-toluidinium iodide (TM*p*T) (**114**) was prepared from DM*p*T via the same method in 90% yield.

N,*N*-dimethyltetrahydroquinolinium iodide[8] (**115**) and *N*,*N*-dimethylpyrrolidinium iodide[9] (**116**) were prepared using five equivalents of the alkyl halide as well as one equivalent of sodium carbonate by refluxing in 1:1 methanol:H_2O (87% and 75% yields respectively).

Since the imidazolium iodide compounds evaluated in the fixture time test previously performed very poorly (**102**, **103**), anion metathesis was performed using these compounds without any assessment of their applicability as cure accelerators. The acesulfamate (**110**), phthalimate (**111**), and saccharinate (**100**) anions had all performed very well in the previous testing and so by pairing each with the cations prepared, a large library of ionic liquids could be constructed which differed in the structural make up and could help form a basis for the structure-activity relationship.

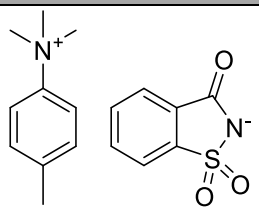
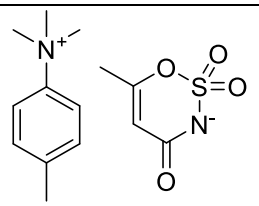
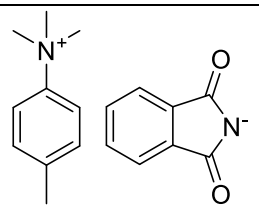
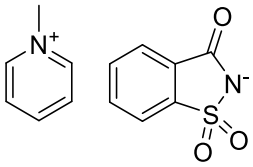
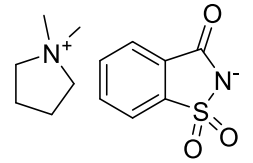
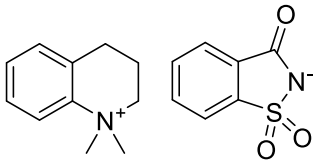
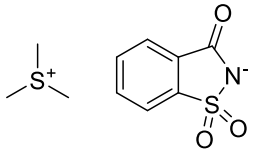
The saccharinate (**117**), acesulfamate (**118**), and phthalimate (**119**) salts of TM*p*T were thus prepared (66%, 89%, and 62% yields respectively) through metathesis of TM*p*T iodide using a single equivalent of the respective silver salt. Once dried and purified, their solubility in PEGMA at 1% w/w was determined. However, all three compounds proved to be insoluble and so could not be evaluated as cure catalysts.

Since all three derivatives of TM*p*T were insoluble, only the saccharinate salt of methylpyridinium (95% yield) (**120**), dimethylpyrrolidinium (84% yield) (**121**), and dimethyltetrahydroquinolinium (77% yield) (**122**) were prepared in order to determine the potential solubility of each series in the formulation instead of preparing the three salts of each. This caution was well founded as these compounds were also found to be insoluble in the monomer and so no further salts of these

cations were prepared as they too most likely would prove to be unsuitable for evaluation.

Trimethylsulfonium iodide[10] (**123**) was prepared by stirring 1.25 equivalents of methyl iodide with dimethyl sulfide neat at room temperature. The white solid was then taken up in acetonitrile before being evaporated to dryness to remove the excess starting material. Silver saccharinate was added and the mixture allowed stir; the saccharinate salt (**124**) was isolated as a white solid but it too proved to be insoluble (87% yield).

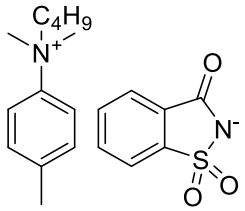
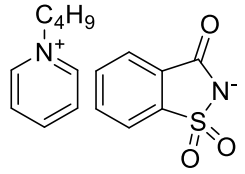
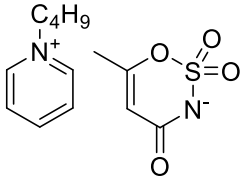
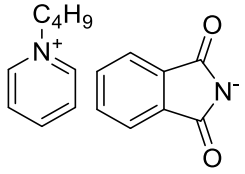
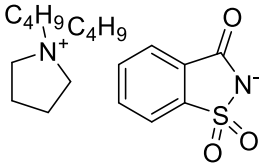
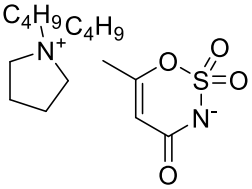
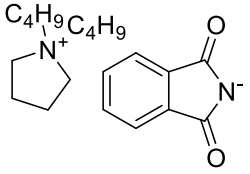
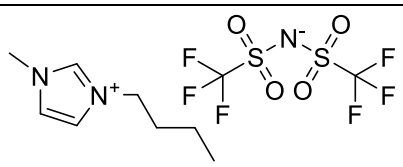
Table 5.4: Solubility of Methyl Derivatised Ionic Liquids in PEGMA at 1% w/w

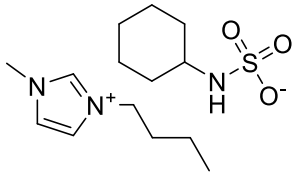
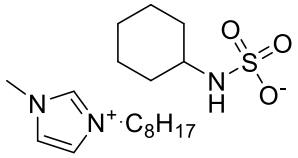
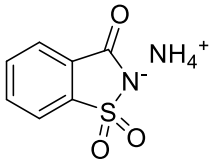
Compound	Structure	Solubility
Trimethyl-*p*-toluidinium saccharinate (**117**)		×
Trimethyl-*p*-toluidinium acesulfamate (**118**)		×
Trimethyl-*p*-toluidinium phthalimate (**119**)		×
N-Methylpyridinium saccharinate (**120**)		×
N,*N*-Dimethylpyrrolidinium saccharinate (**121**)		×
N,*N*-Dimethyltetrahydroquinolinium saccharinate (**122**)		×
Trimethylsulphonium saccharinate (**123**)		×

Quarternarisation using methyl groups did not result in salts which were soluble in PEGMA at 1% w/w (**117**-**123**). This was thought to be as a result of the short alkyl chain substitution as when these compounds are compared to the imidazolium saccharinates synthesised (**100**, **107**-**109**), we also see that the MMIM (**107**) analogue is insoluble in PEGMA but that increasing the chain length resolves this issue (**100**, **108**-**109**). Thus, the decision was made to synthesise more lipophilic derivatives and a chain length of four was chosen as this offered the best balance between potential toxicity and environmental effects whilst still altering the solubility profile of the compounds.

The halide salt of each butyl-derivatised cation was prepared using the same synthetic method described for compounds **117**-**121** with 1-bromobutane and 1-iodobutane acting as the alkyl halides. Evaluation of the accelerator activity was not carried out until anion metathesis was completed and, after doing so, resulted in compounds **127**-**133** (Table 5.5)

Table 5.5: Solubility of Butyl Derivatised Ionic Liquids

Compound	Structure	Solubility	Yield %
N-Butyl-*N*,*N*-dimethyl-p-toluidinium saccharinate (**127**)		×	97
N-Butylpyridinium saccharinate (**128**)		✓	92
N-Butylpyridinium acesulfamate (**129**)		✓	87
N-Butylpyridinium phthalimate (**130**)		×	71
N,*N*-Dibutylpyrrolidinium saccharinate (**131**)		✓	93
N,*N*-Dibutylpyrrolidinium acesulfamate (**132**)		✓	93
N,*N*-Dibutylpyrrolidinium phthalimate (**133**)		×	77
BMIM bistriflimide (**134**)		✓	91

BMIM cyclamate (**135**)		×	89
OMIM cyclamate (**136**)		×	84
Ammonium saccharinate (**137**)		×	93

N-Butyl-*N*,*N*-dimethyl-*p*-toluidinium saccharinate (**127**) proved to be insoluble at 1% w/w in PEGMA and so the acesulfamate and phthalimate derivatives were not prepared. Fortunately, both *N*-Butylpyridinium (NBP) saccharinate (**128**) and *N*,*N*-dibutylpyrrolidinium (BDP) saccharinate (**129**) proved to be so and so the preparation of their acesulfamate and phthalimate salts was proceeded with and their solubility evaluated.

In addition to these cations, other compounds of interest were synthesised. Ammonium saccharinate (**137**) was prepared through dissolution and stirring of saccharin in ethanolic ammonia but it too could not be evaluated as an accelerator due to insolubility.

Likewise, cyclamate based imidazolium salts (**135-136**) were prepared using silver cyclamate. The cyclamate anion contains a sulphonic acid group bonded to an amine which is similar to the sulphonamide moiety found in both saccharin and acesulfame and therefore could potentially possess interesting cure activity. The BMIM derivative[11] was first prepared (**135**) as it could be readily compared to the other BMIM based accelerators (**80**, **83**, **100**, **110-111**) but was found to be insoluble in PEGMA at the required level. In an effort evaluate the activity of the cyclamate group anyway, the OMIM derivative (**136**) was prepared as it was expected the longer alkyl chain could lead to better solvation but no change of the solubility in PEGMA was observed. One additional compound which was able to be evaluated

was BMIM bistriflimide (NTf_2) (**134**). This was prepared through anion metathesis using lithium bistriflimide[12] and also contains the key sulphonamide moiety.

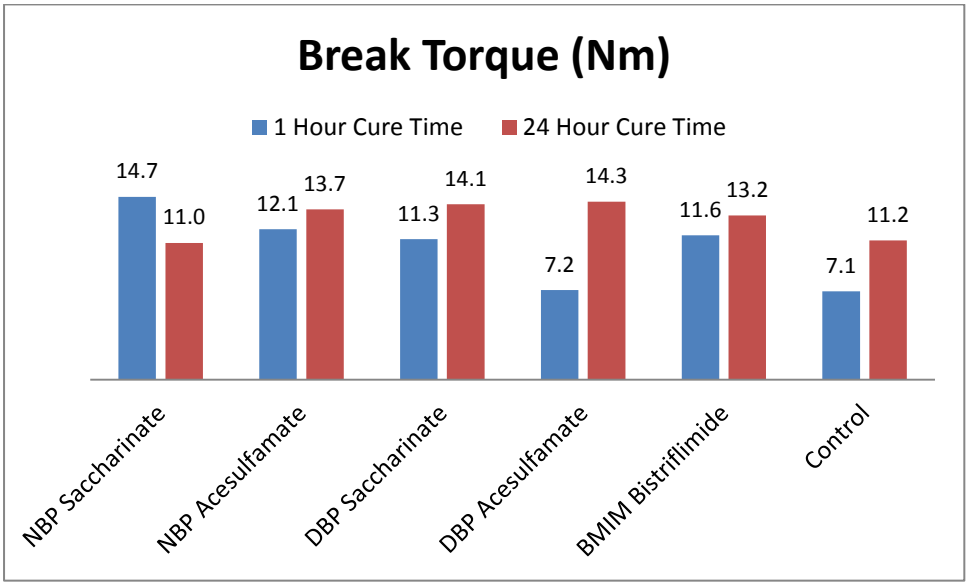
5.5.1 Cure Accelerator Activity Evaluation

All compounds which proved to be soluble in PEGMA were analysed in the lead cure accelerator tests (**128**-**129**, **131**-**132**, **134**).

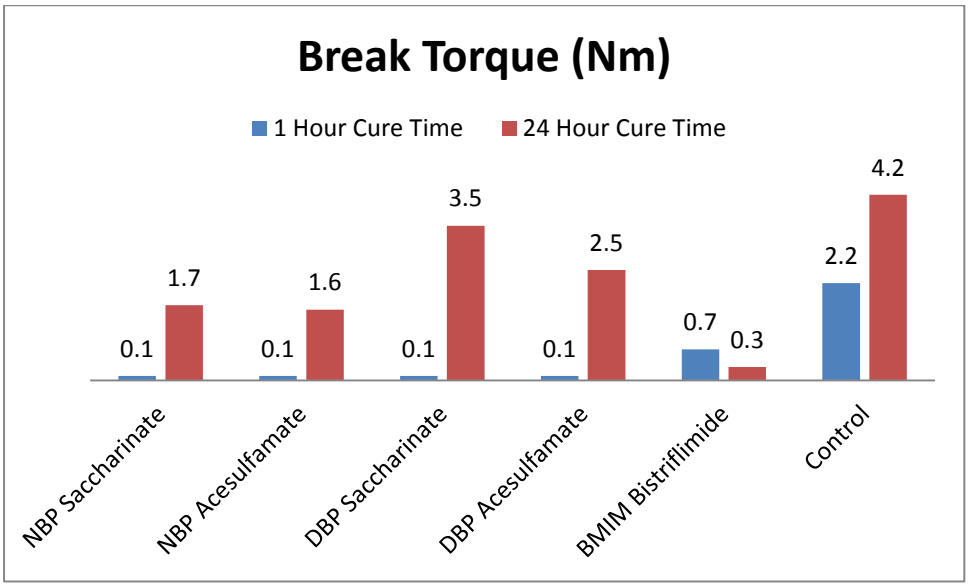
The break torque strength for each compound in both of the nuts and bolts tests is presented below (Graphs 5.16(a) and 5.17(a)). The complete set of results including the prevail torque (Graphs 5.16(b) and 5.17(b)) and maximum torque (Graphs 5.16(c) and 5.17(c)) can be found in Appendix C.

Likewise the compressive strength for each compound tested as part of the pins and collars evaluation is presented below (Graph 5.18(a)) with their maximum load (Graph 5.18(b)) found in Appendix C.

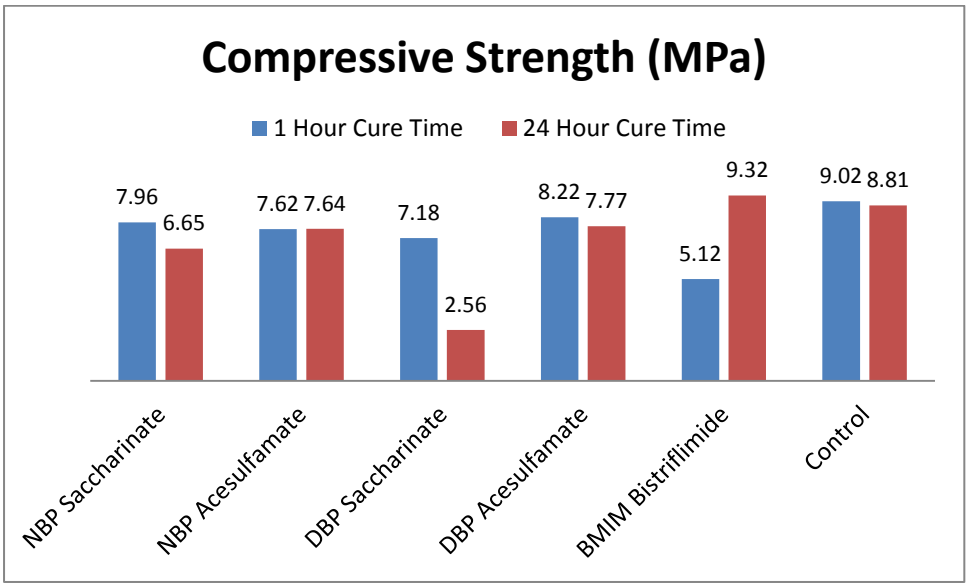
Graph 5.16(a): Black Oxide Bolts with Mild Steel Nuts - Break Torque



Graph 5.17(a): Stainless Steel Nuts and Bolts - Break Torque



Graph 5.18(a): Mild Steel Pins and Collars - Compressive Strength



All compounds displayed cure strength at and above the level of the control formulation (which contained APH as the accelerator) in all tests apart from the stainless steel nuts and bolts examination (Graphs 5.16(a)-18(a)) and is further evidence to illustrate the high cure activity possessed by the saccharinate and acesulfamate anions.

By testing the pyridinium (**128**, **129**) and pyrrolidinium (**131**, **132**) ionic liquids, it had been postulated that this may lead to the importance of properties such as aromaticity and the nitrogen lone pair of electrons to efficient polymerisation catalysis being established. Both cations however performed at comparable levels to each other despite the difference in aromaticity, as well as to the imidazolium saccharinates and acesulfamate (**100**, **108**-**110**) examined in Section 5.3 where the lone pair of electrons is present. This can be seen from their black oxide break torques measured of 14.7 (**128**), 12.1 (**129**), 11.3 (**131**), and 7.2 Nm (**132**) (Graph 5.16(a)) which may point to either of these properties being not as essential to activity as was postulated. Alternatively, this could suggest that the anion is indeed the major source of any cure activity and that the cation may be used to custom design the molecule's properties outside of polymerisation time.

BMIM NTf_2 (**134**) also displayed good catalytic activity and illustrated the advantages of molecules containing a sulphonamide moiety (Graphs 5.16(a)-18(a)). This was not to the same level as BMIM saccharinate (**100**) however (Graphs 5.10(a)-12(a)) and if the anion component is indeed the major source of cure activity, then this difference may be explained by properties such as being non-aromatic in comparison. Significantly, curing of the adhesive bond was observed after one hour when applied to stainless steel (Graph 5.17(a)).

Likewise, the 24 hour cure strengths observed for the dibutylpyrrolidinium based ionic liquids (**131**, **132**) on stainless steel means that they are possibly the most active catalysts of the series (**128**, **129**, **131**, **132**, **134**). The inactivity of the stainless steel surface towards anaerobic adhesives has been discussed previously and so any level of curing achieved can be very significant. Both DBP compounds are able to form 24 hour cure strengths similar to that of APH. Despite the exemplary activity of the previous ionic compounds tested as part of the model formulation, this level of activity had only been seen in BMIM phthalimate (**111**) and is representative of accelerators which are able to greatly affect the curing process. Further to this, in the other tests performed (Graphs 5.16(a), 5.18(a)) both resulted in high cure strengths

when applied as threadlockers in the nuts and bolts tests as well as retainers in the pins and collars examination.

In conclusion, the molecules which were of sufficient solubility in the adhesive formulation (**128-129**, **131-132**, **134**) were evaluated as catalysts using nuts and bolts threadlocking and pins and collars shear tests. The imidazolium saccharinates (**100**, **108-109**), acesulfamate (**110**), and phthalimate (**111**), along with the pyridinium (**128-129**) and pyrrolidinium (**131-132**) compounds were proven in these tests to possess an excellent ability to affect the cure time of the adhesive and suggested that the source of activity was as a result of the anion selected.

5.6 Anti-Microbial Test Results

The set of lead accelerator compounds were submitted for anti-microbial screening by Dr Marcel Spulak at Charles University, Prague to gain a better insight into their possible toxicity profile.

The compounds were submitted for analysis before cure the accelerator studies described in section 5.4.1 were conducted and so compounds within that set were not considered for analysis without knowing their applicability as cure catalysts. The compounds submitted were **80**, **83**, **100**, **107-111**, **122**.

5.6.1 Anti-Bacterial Test Results

For each of the eight strains tested against, where the MIC recorded is >2000 µM no activity was observed within the parameters of the test.

Note: Due to dissolution problems in the media, BMIM phthalimate (**111**) could only be tested to a max concentration of 500 µM and OMIM saccharinate (**109**) to 1000 µM.

Table 5.6: Anti-Bacterial Activity of Compounds 80, 83, 100, 110, and 111

Bacterial Strain	Time (h)	Compound - MIC/IC_{95} (µM)				
		80	**83**	**100**	**110**	**111**
SA	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
MRSA	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
SE	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
EF	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
EC	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
KP	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
KP-E	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
PA	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500

Table 5.7: Anti-Bacterial Activity of Compounds 107-109, and 122

Bacterial Strain	Time (h)	Compound - MIC/IC_{95} (µM)			
		107	**108**	**109**	**122**
SA	24	>2000	>2000	**500**	**62.5**
	48	>2000	>2000	**500**	**62.5**
MRSA	24	>2000	>2000	**100**	**1000**
	48	>2000	>2000	**1000**	**1000**
SE	24	>2000	>2000	**1000**	**125**
	48	>2000	>2000	**1000**	**125**
EF	24	>2000	>2000	**1000**	**2000**
	48	>2000	>2000	**1000**	**2000**
EC	24	>2000	>2000	**500**	**1000**
	48	>2000	>2000	**500**	**1000**
KP	24	>2000	>2000	**1000**	**1000**
	48	>2000	>2000	**1000**	**1000**
KP-E	24	>2000	>2000	>1000	**2000**
	48	>2000	>2000	>1000	**2000**
PA	24	>2000	>2000	**1000**	**1000**
	48	>2000	>2000	**1000**	**1000**

5.6.2 Anti-Fungal Test Results

For each of the twelve strains tested against, where the MIC recorded is >2000 µM no activity was observed within the parameters of the test.

Note: Due to dissolution problems in the media, BMIM phthalimate (**111**) could only be tested to a max concentration of 500 µM and OMIM saccharinate (**109**) to 1000 µM.

Table 5.8: Anti-Fungal Activity of Compounds 80, 83, 100, 110, and 111

Fungal Strain	Time (h)	Fungal Response – MIC IC_{80}/IC_{50}[a] (µM)				
		80	**83**	**100**	**110**	**111**
CA1	24	>2000	**2000**	>2000	>2000	>500
	48	>2000	**2000**	>2000	>2000	>500
CA2	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
CP	24	>2000	**2000**	>2000	>2000	>500
	48	>2000	**2000**	>2000	>2000	>500
CK1	24	>2000	**2000**	>2000	>2000	>500
	48	>2000	**2000**	>2000	>2000	>500
CK2	24	>2000	**2000**	>2000	>2000	>500
	48	>2000	**2000**	>2000	>2000	>500
CT	24	**2000**	>2000	>2000	>2000	>500
	48	**2000**	>2000	>2000	>2000	>500
CG	24	>2000	2000	>2000	>2000	>500
	48	>2000	2000	>2000	>2000	>500
CL	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
TA	24	>2000	**2000**	>2000	>2000	>500
	48	>2000	**2000**	>2000	>2000	>500
AF	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
AC	24	>2000	>2000	>2000	>2000	>500
	48	>2000	>2000	>2000	>2000	>500
TM	24	**2000**	**2000**	>2000	>2000	>500
	48	**2000**	**2000**	>2000	>2000	>500

[a] IC_{50} values were assessed for AF, AC, and TM. For all other fungi strains IC_{80} values were obtained

Table 5.9: Anti-Fungal Activity of Compounds 107-109, and 122

Fungal Strain	Time (h)	Fungal Response – MIC IC_{80}/IC_{50}[a] (µM)			
		107	**108**	**109**	**122**
CA1	24	>2000	>2000	**1000**	**250**
	48	>2000	>2000	**1000**	**250**
CA2	24	>2000	>2000	**1000**	**500**
	48	>2000	>2000	**1000**	**500**
CP	24	>2000	>2000	**500**	**500**
	48	>2000	>2000	**500**	**500**
CK1	24	>2000	>2000	**125**	**250**
	48	>2000	>2000	**125**	**250**
CK2	24	>2000	>2000	**125**	**250**
	48	>2000	>2000	**125**	**250**
CT	24	>2000	>2000	**125**	**250**
	48	>2000	>2000	**125**	**250**
CG	24	>2000	>2000	**500**	**1000**
	48	>2000	>2000	**500**	**1000**
CL	24	>2000	>2000	**1000**	**1000**
	48	>2000	>2000	**1000**	**1000**
TA	24	>2000	>2000	**1000**	**>2000**
	48	>2000	>2000	**1000**	**>2000**
AF	24	>2000	>2000	**1000**	**500**
	48	>2000	>2000	**1000**	**500**
AC	24	>2000	>2000	**1000**	**500**
	48	>2000	>2000	**1000**	**500**
TM	24	>2000	>2000	**1000**	**1000**
	48	>2000	>2000	**1000**	**1000**

[a] IC_{50} values were assessed for AF, AC, and TM. For all other fungi strains IC_{80} values were obtained

As can be seen from the results, all compounds screened against the eight bacterial strains and twelve fungal strains were largely inactive.

The only compounds to display activity in the anti-bacterial screening were OMIM saccharinate (**109**) and *N*,*N*-dimethyltetrahydroquinolinium saccharinate (**122**). This is due to the increased lipophilicity of the eight carbon chain in the case of OMIM, particularly when compared to the inactivity of the other short chain imidazoliums screened (**80**, **83**, **100**, **108**-**111**). This increase in lipophilicity allows for greater interaction between the molecule and the cell membrane which leads to the inhibitory activity observed.

Anti-fungal activity, as explained in Chapter 2, can be a better representation of possible mammalian toxicity due to similarities in their cellular makeup. The same trends that were noted in the anti-bacterial screening were observed for compounds **109** and **122** here also. In addition to this, some activity in certain fungal strains was observed at the upper limit of the test specifications for BMIM octyl sulfate (**80**) and BMIM acetate (**83**). Again this is likely due to similar reasons, particularly for the octyl sulfate derivative where it too will have an increased lipophilicity and in turn cell membrane interaction due to long alkyl chain substitution.

This set of anti-microbial data as a whole is a positive sign with respect to designing safer anaerobic cure accelerators. One can see that several of the compounds which resulted in the highest performance in the cure catalyst studies (**100**, **108**, **110**, **111**) display no inhibition to any of the bacterial or fungal strains whatsoever. Others then such as **80** and **83** either did not exhibit as good cure activity or none at all when analysed as catalysts and so their inhibitory activity, even though only to a low degree, is not of as large a consequence.

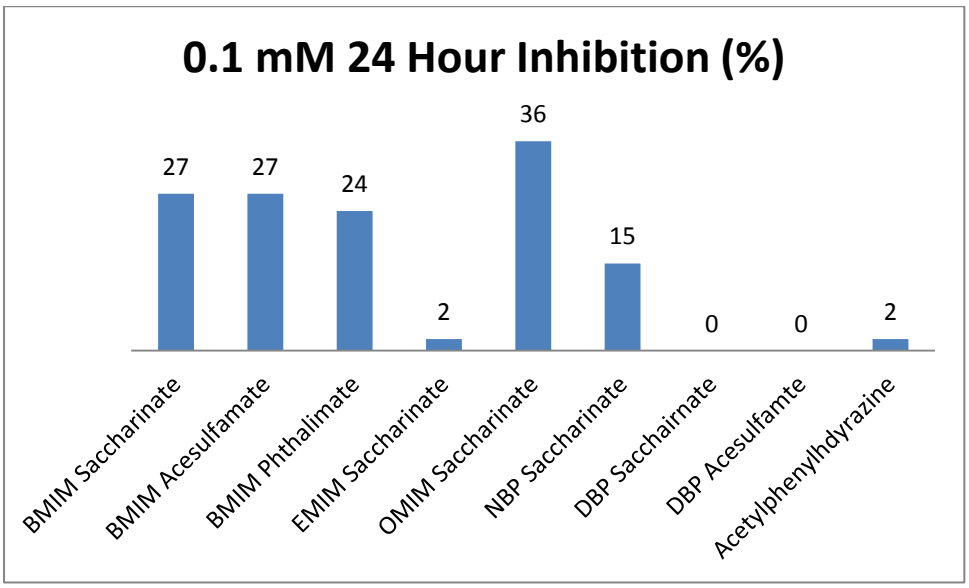
5.7 Cytotoxicity Screening and Direct Comparison with Anti-Microbial Activity

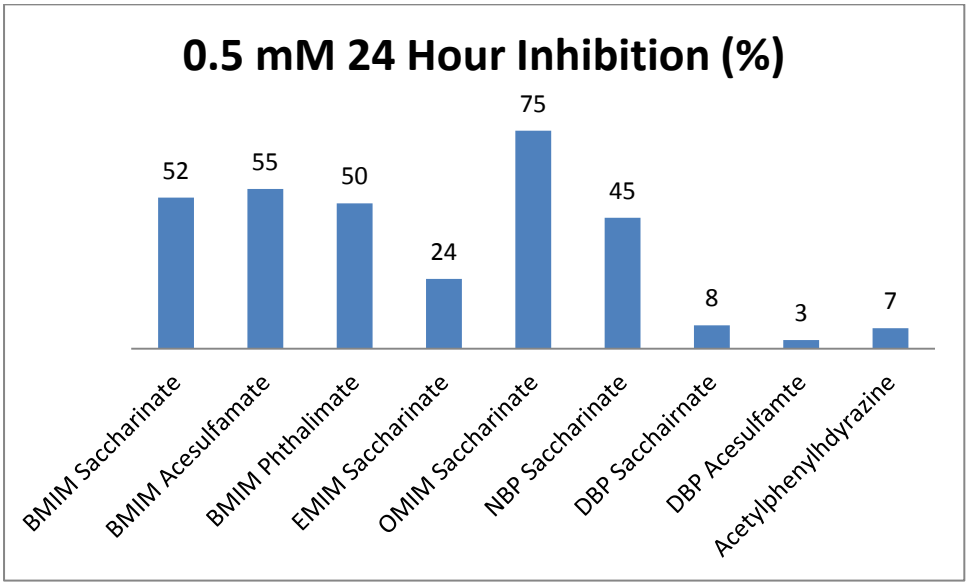
As part of the toxicity evaluation of the lead cure accelerators identified, eight of the compounds which displayed a combination of good catalytic activity and structural variation were selected for analysis along with APH (**100**, **108**-**111**, **128**, **131**, **132**). The work was carried out by Dr. Anne Kahru at the National Institute of Chemical Physics and Biophysics, Tallinn.

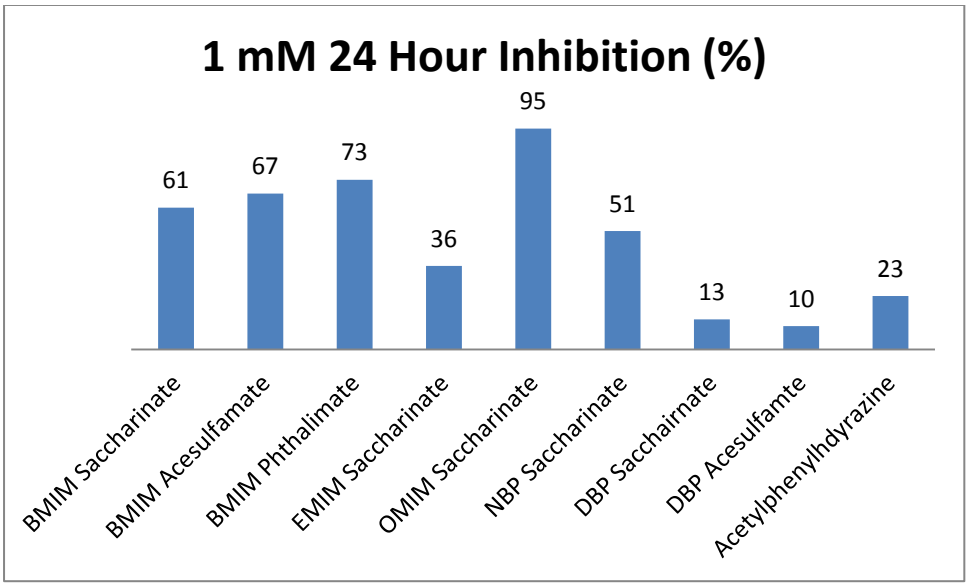
The toxicity of these compounds was first evaluated against two cell lines, A549 cells from carcinomatous tissue of human lungs (ATCC CCL-185) and Caco-2 cells from colorectal adenocarcinomatous tissue of human colon (ATCC HTB-37). This was done by incubating the cells with each compound at 0.1 mM, 0.5 mM, and 1.0 mM concentrations for 24 hours and their inhibition measured using the neutral red uptake assay.

In order to determine any possible correlation between cytotoxicity and anti-microbial activity, the same stock solutions of the eight compounds were evaluated for inhibition activity against the bacteria, *Vibrio fischeri*, in a bioluminescence "flash" assay. This was carried out at 0.1, 0.5, 1.0, 1.5 mM concentrations with the change in luminescence of the bacteria measured after 5 seconds, 15, and 30 minutes.

Graph 5.19: Caco-2 Cell Inhibition:
(a) 0.1 mM, (b) 0.5 mM, and (c) 1 mM

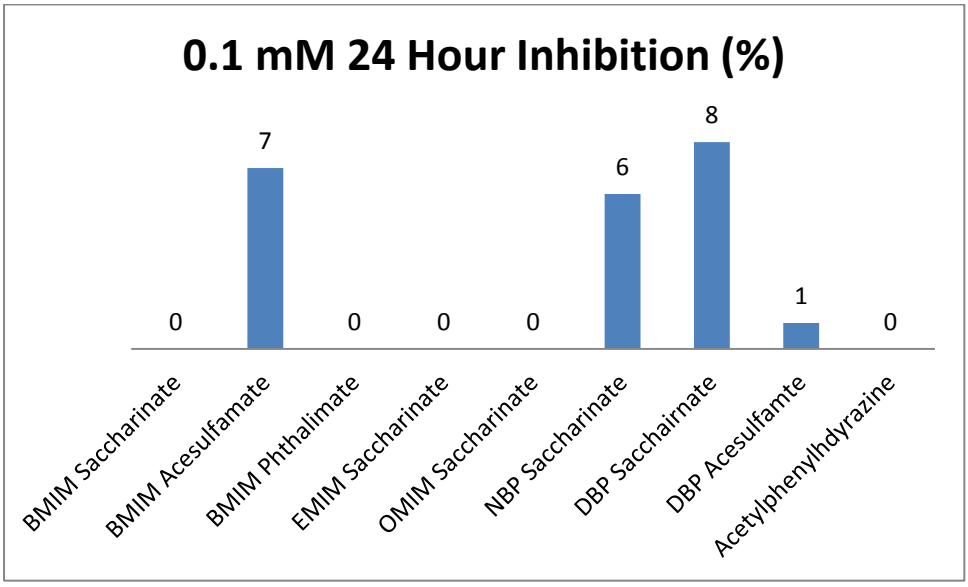


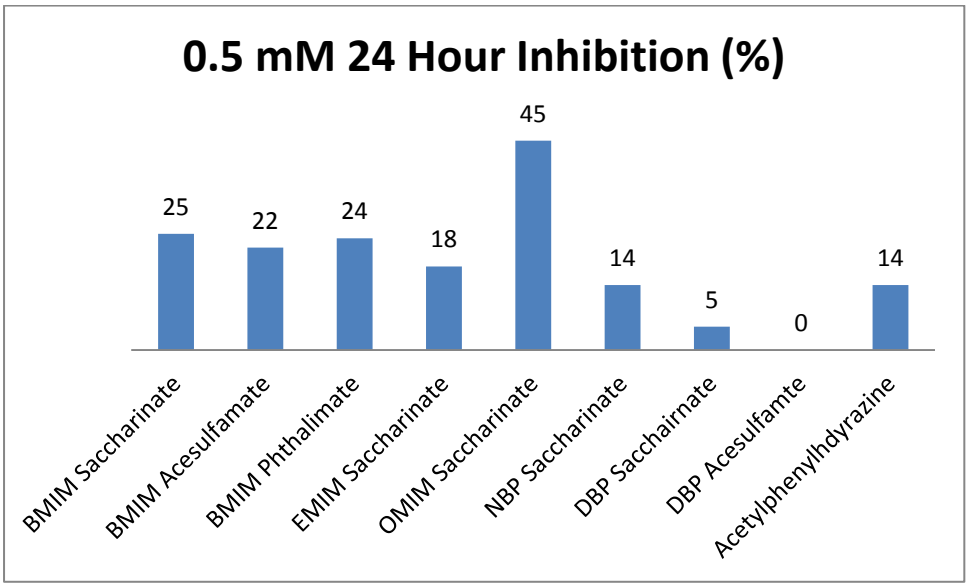


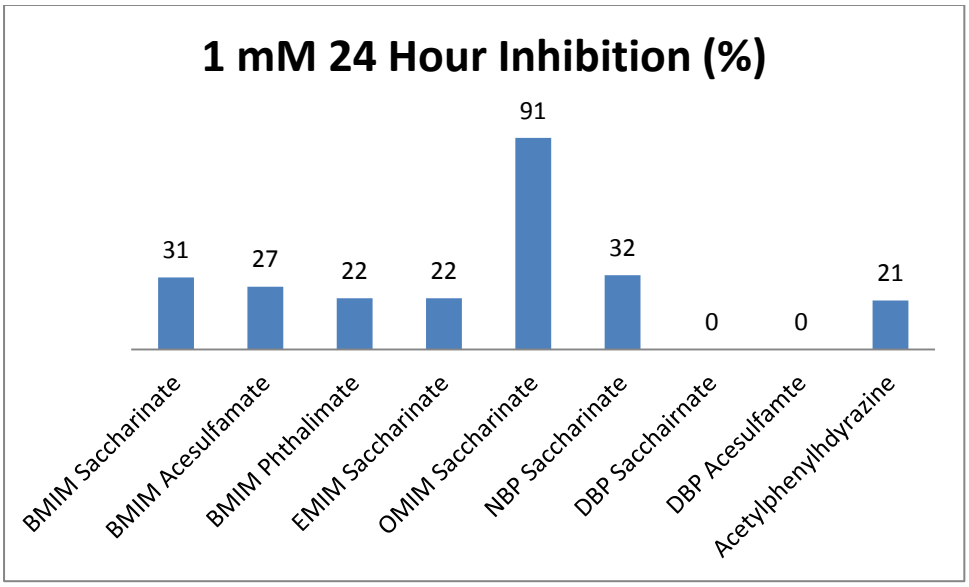

1 mM 24 Hour Inhibition (%)
61
67
73
36
95
51
13
10
23
BMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
EMIM Saccharinate
OMIM Saccharinate
NBP Saccharinate
DBP Sacchairnate
DBP Acesulfamte
Acetylphenylhdyrazine

Graph 5.20: A549 Cell Inhibition

(a) 0.1 mM, (b) 0.5 mM, and (c) 1 mM

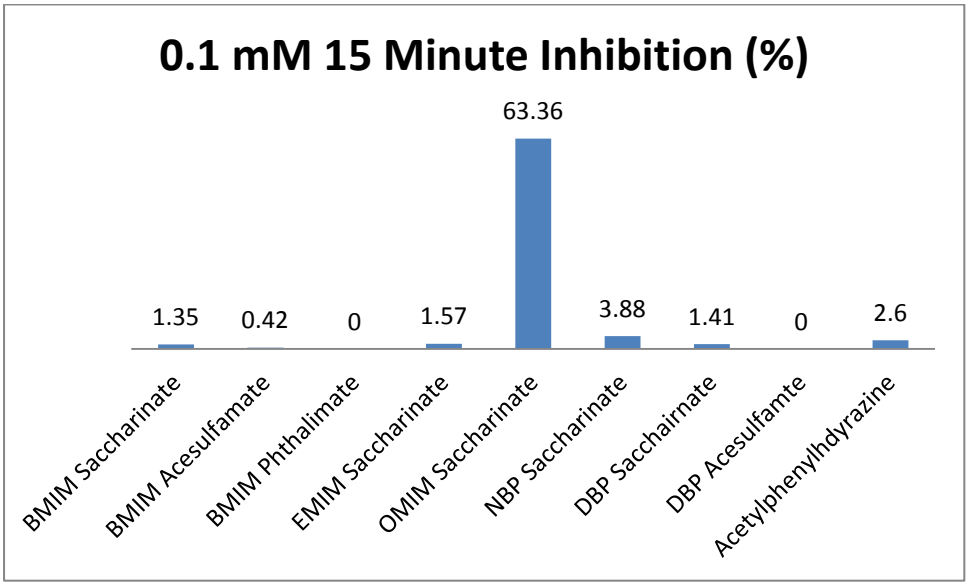


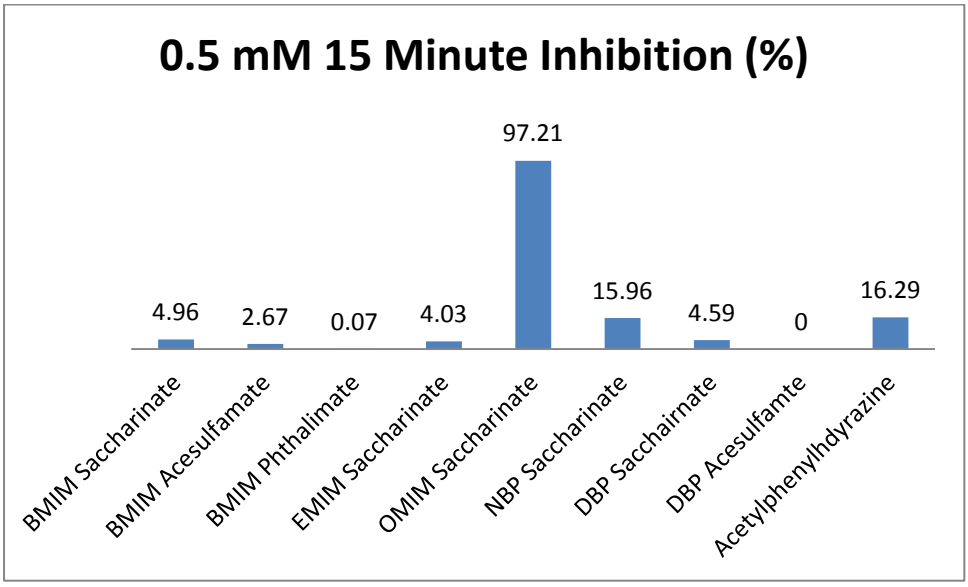


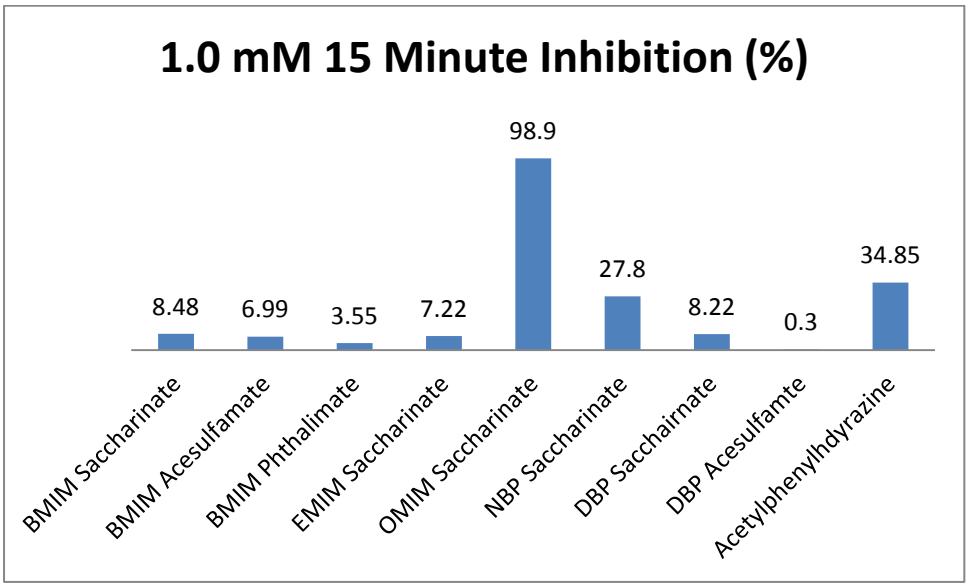

1 mM 24 Hour Inhibition (%)
31
27
22
22
91
32
0
0
21
BMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
EMIM Saccharinate
OMIM Saccharinate
NBP Saccharinate
DBP Sacchairnate
DBP Acesulfamte
Acetylphenylhdyrazine

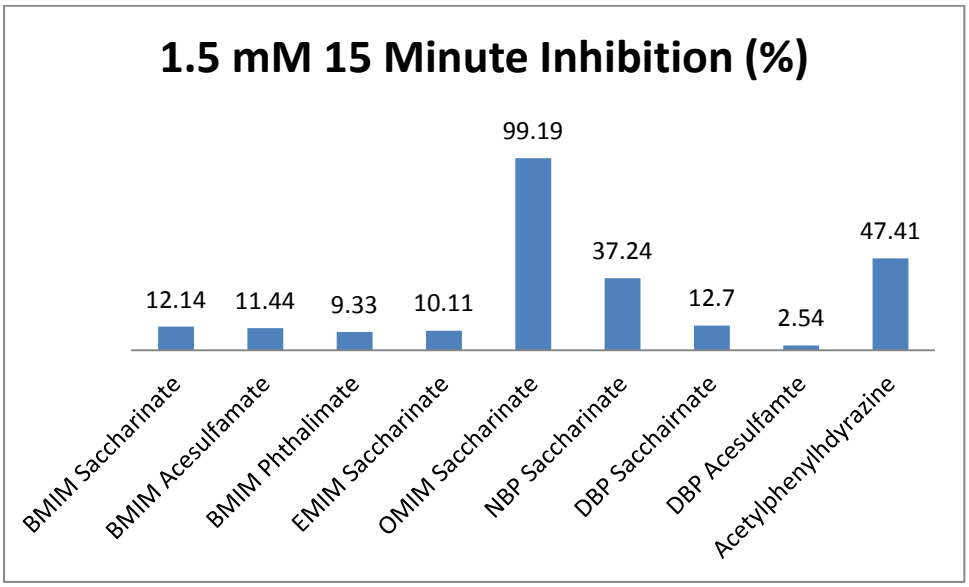
Graph 5.21: *Vibrio fischeri* 15 Minute Luminescence Inhibition:

(a) 0.1 mM, (b) 0.5 mM, (c) 1.0 mM, and (d) 1.5 Mm



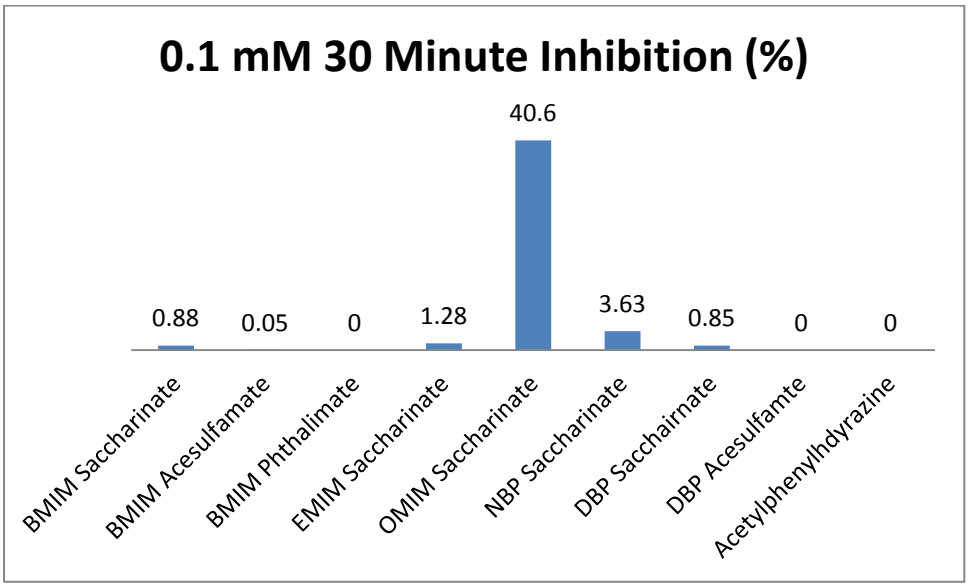


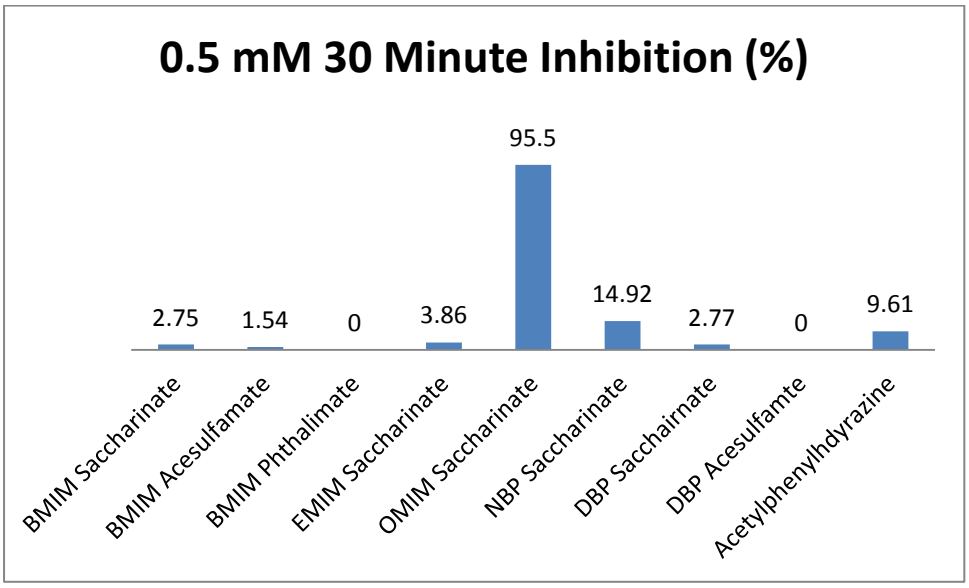

1.0 mM 15 Minute Inhibition (%)
8.48
6.99
3.55
7.22
98.9
27.8
8.22
0.3
34.85
BMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
EMIM Saccharinate
OMIM Saccharinate
NBP Saccharinate
DBP Sacchairnate
DBP Acesulfamte
Acetylphenylhdyrazine

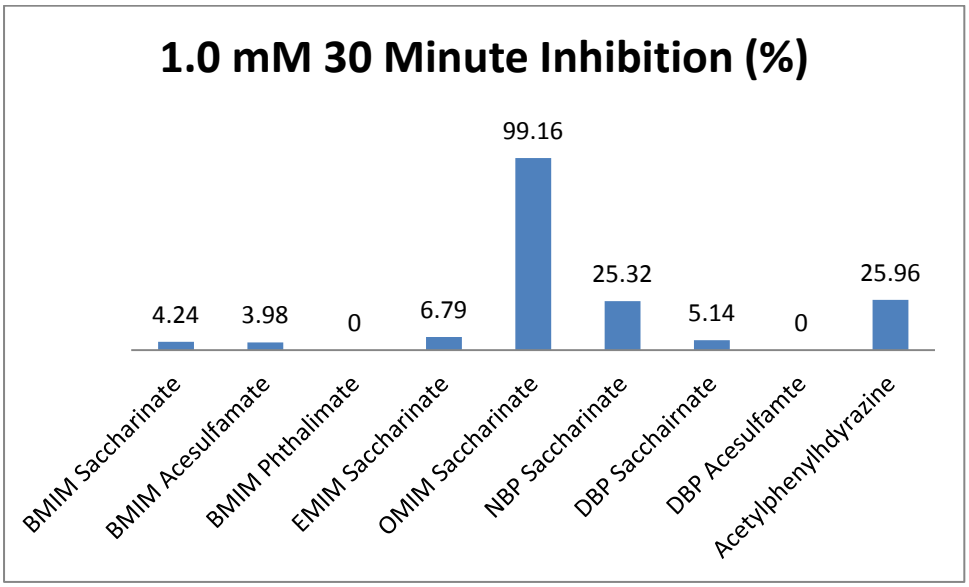

1.5 mM 15 Minute Inhibition (%)
12.14
11.44
9.33
10.11
99.19
37.24
12.7
2.54
47.41
BMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
EMIM Saccharinate
OMIM Saccharinate
NBP Saccharinate
DBP Sacchairnate
DBP Acesulfamte
Acetylphenylhdyrazine

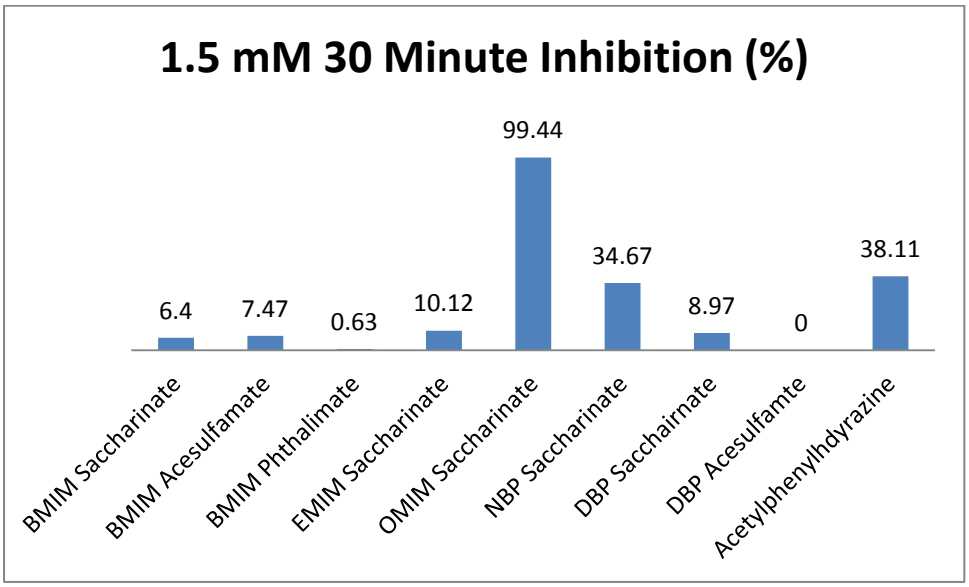
Graph 5.22: *Vibrio fischeri* 30 Minute Luminescence Inhibition:

(a) 0.1 mM, (b) 0.5 mM, (c) 1.0 mM, and (d) 1.5 Mm






1.0 mM 30 Minute Inhibition (%)
99.16
25.32
25.96
4.24
3.98
0
6.79
5.14
0
BMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
EMIM Saccharinate
OMIM Saccharinate
NBP Saccharinate
DBP Sacchairnate
DBP Acesulfamte
Acetylphenylhdyrazine


1.5 mM 30 Minute Inhibition (%)
99.44
34.67
38.11
6.4
7.47
0.63
10.12
8.97
0
BMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
EMIM Saccharinate
OMIM Saccharinate
NBP Saccharinate
DBP Sacchairnate
DBP Acesulfamte
Acetylphenylhdyrazine

As part of the cell line assay (Graphs 5.19-20), a level of cell inhibition of 50% or higher a compound can be considered toxic towards the cells in question.

The postulation was made that cytotoxicity screening could act as a model and offer an accurate representation of the mammalian toxicity of established accelerators, such as APH, of which it is the aim to design safer alternatives to. However, it can be seen from the cell viabilities measured for each cell line and concentration that APH is of low toxicity towards them. This does not mean that APH can be considered a safe, low toxicity accelerator however as it is already known to be acutely toxic towards mammals.[13] Instead this means that the toxicity and risk of APH does not come from anability to target and inhibit cells but from another mode of action.

Comparing the data generated by both cell lines, one can see that the Caco-2 cell line (Graph 5.19) is more sensitive to these types of compounds than the A549 cells (Graph 5.20) and so it is recommended that the Caco-2 cells could be used as part of a primary indicator of cell toxicity.

When the structures of the cure accelerators screened are compared, there is a definite trend between alkyl chain length and cell inhibition. The imidazolium compounds illustrate this with EMIM saccharinate (**108**) displaying quite low inhibition across all concentrations and both cell lines but as the chain length increases as in the three BMIM analogues (**100**, **110**, **111**), the inhibition % gets larger and rises above the 50% threshold at 0.5 mM for the Caco-2 cells (Graph 5.19 (b)). In the case of the eight carbon chain of OMIM saccharinate (**109**) it possesses cell inhibition of >90% for both cell lines at 1 mM. This interaction is most likely due to the enhanced lipophilicity of long alkyl chains resulting in interaction with the cell membrane.

One can also see from the BMIM derivatives analysed (**100**, **110**, **111**) that they all largely possess similar cell activity at the different concentrations. This may possibly mean that saccharinate, acesulfamate, and phthalimate anions are of low toxicity towards cells with the inhibition mainly being caused by the cation. Thus when *N*-butylpyridinium saccharinate is considered, (**128**) which also displayed some inhibitory activity, particularly at higher concentrations, it may be reduced through substitution of the pyridinium cation for a more benign alternative.

In fact, this can be readily seen in the results of *N*,*N*-dibutylpyrrolidinium saccharinate (**131**) and acesulfamate (**132**). Both display very low inhibition of the

two cell lines, even at the higher concentration, and lower than that of APH. This suggests that DBP is a cation which does not readily interact with cells and when paired with saccharinate or acesulfamate results in a compound which is benign in terms of cellular toxicity.

The activity of compounds **100**, **109**, and **110** towards Caco-2 cells has been previously investigated with the same outcomes observed.[14] OMIM saccharinate (**109**) displays higher toxicity than the shorter chained BMIM derivatives (**100, 110**) which are similar in their inhibitory effect. The study also included pyrrolidinium and pyridinium derivatives which, while different structurally, also displayed the same trends observed in graphs 5.19-20 with pyrrolidinium being the least active.

The bioluminescence assay using *Vibrio fischeri* largely showed the same trends (Graphs 5.21-22). *N*-Butylpyridinium saccharinate (**128**) showed similar levels of activity to APH. The bacteria were not as sensitive to the change in alkyl chain length as was observed for the two mammalian cell lines however. BMIM saccharinate (**100**) and **110-111** unexpectedly display similarly low levels of inhibition to EMIM saccharinate (**108**). This may be due to the relative low toxicity of the saccharinate anion present as studies of the alkyl chain length of imidazolium compounds involving other anions have shown a clear increase in inhibition as the chain grows.[15–17] OMIM saccharinate on the other hand possesses remarkable inhibition of the metabolic activity of the bacteria owing to the C8 chain (Graph 5.21(a)). This level of almost 100% inhibition at concentrations of 0.5 mM and higher (Graphs 5.21 (b-d)) is to a level that merited further investigation at 0.05 mM. At this concentration the compound still retained sizeable inhibitive activity of 41% after 15 minutes and 23% after 30 minutes. In fact after 5 seconds, the compound showed sizeable inhibitory activity with an EC_{50} of 0.37 mM.

While the flash assay was not as sensitive to structural modifications such as alkyl chain length as the Caco-2 or A549 mammalian cells, it was able to identify the higher cytotoxicity of the pyridinium based compound (**128**) and APH over the pyrrolidiniums (**130**-**131**) evaluated. Further to this OMIM saccharinate (**62**) was identified as the most inhibitive compound and illustrates that anti-microbial analysis can be used as a primary indicator of the cytotoxicity of a compound.

5.8 Environmental Fate Study of Adhesive Formulations and Possible Biodegradation of Lead Cure Accelerators

5.8.1 Environmental Fate of Anaerobic Adhesives

The environmental fate and persistence of cure accelerators has been a key property in this project to design novel polymerisation catalysts. However, when we consider that they often only constitute 1% w/w of an anaerobic adhesive product, then the biodegradability of the rest of the formulation components cannot be ignored if a truly environmentally benign product is to be designed.

In order to investigate this, two complete anaerobic adhesive products Loctite 2400 (**138**) and 2700 (**139**), were analysed. Since the monomer(s) is the largest formulation component by some extent, four monomers frequently used in anaerobic products were submitted in addition for evaluation. These monomers were PEGMA (**1**), flex III resin (**140**), oil cutting rigid resin (**141**), and ethoxylated bisphenol A dimethacrylate (**142**). Compounds **140**-**142** are custom monomers developed by Henkel for use in their adhesive product line.

The five compounds were submitted for biodegradation analysis using the CO_2 headspace test (OECD Test No. 310) and eco-toxicity evaluation using the microtox assay and *daphnia magna*. This work was carried out by Dr. Teresa Garcia, IQAC, Barcelona. Samples **1** and **138** were analysed separately to the other three and so their biodegradation results are reported along with a second SDS standard control reference.

Table 5.10: Microtox Activity of Formulation Components

Compound	MICROTOX (30 min)		EC_{50} concentration range (mg/L)
	EC_{50} mg/L	IC95%	
PEGMA (1)	17	16-18	10 to 100
Loctite 2400 (138)	34	27-41	
Loctite 2700 (139)	30	21-43	
Flex III Resin* (140)	-	-	
Oil Cutting Rigid Resin* (141)	-	-	
Ethoxylated Bisphenol A Dimethacrylate* (142)	-	-	

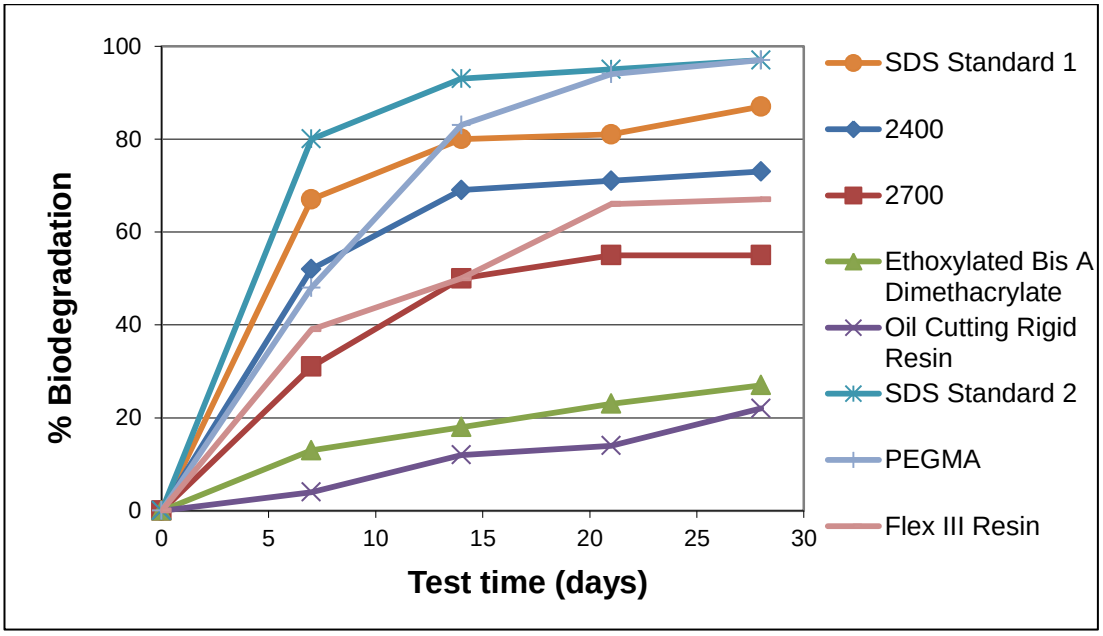
*Due to compound insolubility in the test medium no toxicity could be determined

Table 5.11: *Daphnia Magna* Activity of Formulation Components

Compound	***Daphnia magna* (48 h)**		**EC_{50} concentration range (mg/L)**	**Hazard Classes (OECD, 2001)**
	EC_{50} mg/L	**IC95%**		
Oil Cutting Rigid Resin (141)	55	30-80	10 to 100	Acute toxicity III (harmful to aquatic life)
PEGMA (1)	>100	-	>100	-
Loctite 2400 (138)	>100	-		
Loctite 2700 (139)	>100	-		
Flex III Resin (140)	>100	-		
Ethoxylated Bisphenol A Dimethacrylate (142)	>100	-		

Table 5.12: Biodegradability of Formulation Components

Compound	Biodegradation (%)				Inhibition (%)
	6 Days	13 Days	21 Days	28 Days	
SDS Standard 1	67	80	81	87±4.6	
Loctite 2400 (138)	52	69	71	73±4.3	0
Loctite 2700 (139)	31	50	55	55±1.7	0
Oil Cutting Rigid Resin* (141)	13	18	23	27±2.9	0
Ethoxylated Bisphenol A Dimethacrylate* (142)	4	12	14	22±7.4	0
SDS Standard 2	80	93	95	97±3.6	
PEGMA (1)	48	83	94	97±6.0	0
Flex III Resin (140)	39	50	66	67±4.6	0



In terms of their threat to the aquatic environment, the samples evaluated largely pose little risk. Under OECD classification for the *daphnia magna* evaluation, only one of the monomers required to be assigned a hazard label and this was at the lowest level, acute toxicity III (**141**). The remaining compounds (**1**, **138**-**140**, **142**) have an EC_{50} of >100 mg/L and therefore outside of the hazard limits. In the microtox bioluminescence assay, differing activity was observed with both adhesive formulations (**138**-**139**) and PEGMA (**1**) displaying lower EC_{50} values of 34, 30, and 17 mg/L respectively.

Of the samples, one of the complete formulations, **138**, and two of the monomers, **1** and **140**, were found to reach the 60% degradation threshold to be considered readily biodegradable. PEGMA will readily undergo degradative processes such as hydrolysis and so over 28 days nearly mineralises completely. Due to this extremely high level of degradation, any adhesive product primarily made up of PEGMA will also pass biodegradation evaluations and this is evident in the case of **138**.
Historically anaerobic adhesives have been based on PEGMA but in order to customise the bond properties such as strength or elasticity, there is often additional monomers added to the formulation. Since these are also included in large amounts, they have the ability to significantly affect the environmental fate of the product. Monomer **140** is made up of 1,6-hexanediol, toluene diisocyanate (TDI), and adipic acid groups bonded and end capped with PEGMA. Whilst this monomer still offers several sites for cleavage to occur, the degradation has dropped to 67% and in the case of **141** and **142** this level has dropped even further below the threshold. There is conflicting evidence as to the biodegradability of bisphenol A with this chemcial being classified as readily biodegradable under the OECD 301F manometric test but not readily biodegradable in the OECD closed bottle test, OECD modified sturm test, and MITI test for biodegradability.[18] There is no previously published biodegradation information on ethoxylated bisphenol A (**142**) but it is conceivable that, if the bisphenol A fragment is indeed persistent, the ethoxy portion may degrade to some extent leading to the low level of degradation observed but not enough to class the compound as readily degradable.

By investigating complete adhesive products and their major components, a greater perspective of the environmental fate of the complete formulation as opposed to simply a single component is gained. This also serves to highlight the areas where

improvements can be made if further readily degradable adhesives are to be designed and how monomers can be altered to achieve this.

5.8.2 Possible Environmental Fate of Lead Accelerator Compounds

The lead cure accelerators have been identified based on their catalyst activity and their potential risk has been investigated using anti-microbial and cytotoxicity analysis, however the environmental fate of the compounds remains unknown.

Of the ionic liquid cure accelerators investigated, BMIM saccharinate (**100**) is the only compound to have published biodegradation data. Using the CO_2 headspace test, **100** was found to degrade by 41% over 28 days and as such is deemed not readily biodegradable.[1] The persistence of short chain alkyl imidazolium ionic liquids is well studied[19] and so this does not come as a surprise. For these reasons, similarly low biodegradation levels would be expected if the other short chain imidazolium cure accelerators were to be analysed (**108**, **110**-**111**). An investigation into the biodegradability of ionic liquids which incorporate an acesulfamate anion has been carried out where it is paired with 1-alkoxymethyl-3-hydroxypyridinium based cations.[20] As part of this study the biodegradation of acesulfamate, saccharinate, and chloride anions of several pyridinium derivatives under the OECD closed bottle test was compared. None of the acesulfamate compounds were found to be readily degradable and possessed degradation levels similar to that of the chloride analogues. However, this does not necessarily mean the acesulfamate anion encourages persistence in the environment as only one of the saccharinate compounds, an anion which we believe to be readily degradable, was found to pass the test. This may instead point to the persistence of the cation itself with partial degradation of the C11 chain found in the readily degradable compound along with degradation of the saccharinate anion owing to the positive result obtained.

If we consider the possible fate of OMIM saccharinate, there are several reported biodegradation studies on OMIM halide ionic liquids which indicate that it degrades, at least partially, unlike the shorter chain imidazoliums. Docherty et al. determined OMIM bromide to not be fully degradable with a level of 41%. However this level is significant when we compare it to the lack of degradation of BMIM halides which degrade only by 0-1%.[21] Further to this, Stolte carried out a primary biodegradation

study of OMIM chloride coupled with metabolite analysis which indicated that the cation is degraded to a 1-carboxymethyl-3-methylimidazolium ion.[22]

If we compare the low degradability of BMIM halides (0-1%[21]) to the level of BMIM saccharinate (**100**) (41%[1]) it could be inferred that the saccharinate anion is almost fully degradable. Likewise, if we then consider the moderate degradation of OMIM bromide (41%[23]), substitution of the counter ion to form OMIM saccharinate (**109**) may result in a readily biodegradable compound (>60%).

The *N*,*N*-dibutylpyrrolidinium (**131**-**132**) and *N*-butylpyridinium (**128**-**129**) cure accelerators may not be determined to be readily biodegradable however if already published data is followed.[24] *N*-alkylpyridinium halides have been found to be persistent[22] but if ester functionality can be incorporated into the alkyl chain then their fate is markedly improved and readily degradable compounds may be prepared.[25] Likewise, alteration of the alkyl chain substitution of the pyrrolidinium cation may be required to prepare readily degradable compounds. While no degradation studies of the *N*,*N*-dibutyl- derivative have been published to date, *N*-methyl-*N*-butylpyrrolidinium compounds amongst others have been shown to be non-degradable[26].

5.9 Conclusion

Through analysis of imidazolium based ionic liquids as cure accelerators using the fixture time test, the lead compound BMIM saccharinate (**100**) was identified displaying activity equivalent to existing commercial accelerators. In an effort to identify the source of this efficient cure activity, further alkyl imidazolium ionic liquids as well as those with pyridinium and pyrrolidinium based cations amongst others were prepared. These compounds were paired with saccharinate, acesulfamate, and phthalimate counter ions before analysis.

The molecules which were of sufficient solubility in the adhesive formulation were evaluated as catalysts using nuts and bolts threadlocking and pins and collars shear tests. The imidazolium saccharinates (**100**, **108**-**109**), acesulfamate (**110**), and phthalimate (**111**), along with the pyridinium (**128**-**129**) and pyrrolidinium (**131**-**132**) compounds were proven in these tests to possess an excellent ability to affect the

cure time of the adhesive and suggested that the source of activity was as a result of the anion selected.

In order to evaluate the potential hazard or risk posed by these compounds, and in turn their suitability to replace existing harmful cure accelerators, their activity against several strains of bacteria, fungi, as well as two mammalian cell lines was determined. Through these tests it was discovered that increasing alkyl chain length leads a growth in toxicity whilst use of the saccharinate, acesulfamate, or phthalimate anions appears to have no effect as observed from the imidazoliums examined (**100**, **108**-**111**). Although EMIM saccharinate (**108**) displayed low inhibition of the mammalian cells and bacterial strains, the *N*,*N*-dibutylpyrrolidinium compounds (**131**-**132**) prepared resulted in the lowest activity of all compounds examined in the cytotoxicity assay, even lower than that of APH.

From these studies on cure activity and cytotoxicity, the compounds which offer the best combination of polymerisation time efficiency with low risk are the pyrrolidiniums (**131**-**132**) examined. They possess cure times and strengths analogous to that of commercial accelerators (Graphs 5.16-18) whilst also performing the best in the series examined as part the cytotoxicity/anti-microbial investigation exhibiting no discernable cell inhibition.

5.10 References

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Chapter 6:

Conclusion and Future Work

6.1 Conclusion

Existing commercial cure accelerators used are done so with the caveat that they pose health risks to the user and are labelled accordingly as acutely toxic or hazardous. For these reasons it would be desirable if alternative compounds could be developed which were capable of efficient catalytic performance but did not possess any of these drawbacks and, furthermore, were environmentally benign.

There are many mechanisms proposed which may account for cure acceleration activity and at the start of this project the suitability of each mechanistic pathway for the different compound classes was unknown. A similar problem regarding accelerator toxicity also was also present and, due to limited toxicity data, SAR studies would not generate reliable identification of trends to predict low toxicity examples. Additional data was required and this was achieved through first the identification of a primary "first generation" set of compounds which were of varying accelerator activity but of which their level of toxicity was unknown. These compounds were then screened for properties such as their anti-microbial toxicity, cytotoxicity, biodegradability, eco-toxicity, and accelerator activity in order to begin to elucidate the structure-activity relationship. Further to this, given that the set contained several compounds which are currently applied as commercial accelerators, they were to act as a bench mark for these properties against which the performance of novel accelerators designed could be compared.

Anti-microbial toxicity evaluation was carried out in order to investigate whether a correlation between the mammalian toxicity and anti-microbial activity of these compounds could be constructed. This could then be used to act as an efficient primary indicator of the possible mammalian toxicity of any compounds designed. Whilst a complete relationship between the two properties could not be constructed for this first generation of accelerator compounds, there was a clear link between the anti-fungal activity and mammalian toxicity of some compounds. This was evident in the case of APH (**16**) displaying the application of anti-microbial screening as a primary means of evaluating the mammalian toxicity of compounds.

Biodegradation of the same set of compounds illustrated how persistent this family of nitrogen heterocycles can be with only three of the fifteen compounds proving to be readily biodegradable (**16**, **30**, **33**). This persistence is due to structural features such as fused multi-cyclic frames, heterocyclic fragments, and tertiary amines but

methods of alleviating their effect were illustrated with the presence of oxygen containing functional groups and hydrolysable bonds in the compounds which were readily degradable. Likewise, the aquatic toxicity study displayed the design rules by which low toxicity compounds can be made. By increasing the water solubility of any compound designed, their ability to interact with and cross the membrane of any organism is reduced along with their potential to cause harm.

As a whole, this first generation of compounds provided a base set of data from which design traits could be used to synthesise new cure accelerators. For example the data showed APH to be of low aquatic toxicity and to be readily biodegradable, two qualities which should be retained, but since APH is also acutely toxic then further development is required.

These design traits were put into practice through synthesising and testing benzomorpholine derivatives. It was postulated that the introduction of an oxygen atom into the fused heterocyclic framework may result in increased biodegradability and lower the aquatic toxicity whilst still possessing cure activity comparable to the THQ or indoline analogues. However this was not the case with the compounds synthesised proving to be just as persistent in the environment.

This research was carried out with green chemistry principles in mind and with that; the possibility of designing cure catalysts from natural sources was explored. Given their structural similarity to existing accelerators such as indoline, the application of tryptophan and several purines was explored. The synthesis of derivatives of these compounds highlighted a significant challenge when attempting to design compounds which combine several properties, such as cure activity, low toxicity, and biodegradability, in that structural changes to enhance them can be made on paper, but once synthesised there is no guarantee that the compound prepared will be soluble in the adhesive formulation and be suitable for testing. For these reasons significant time was spent developing these compounds simply to be able to primarily evaluate the presence of any activity as opposed to improve upon and develop this property.

Emphasising the development of compounds with enhanced biodegradation and lower toxicity profiles meant less priority was given to aspects such as the cure activity of the molecules. For this reason, heterocyclic compounds which were capable of variation and derivitisation without the loss of solubility were explored.

Imidazoles met this criterion with a preliminary study of a broad variety of derivatives displaying their wide ranging solubility in PEGMA at 1% w/w. With their suitability assessed, and after altering the adhesive formulation based on mole amounts to make the results more comparable, the catalyst applicability of a range of imidazoles was assessed with several outcomes.

The study illustrated the effect of catalyst loading on cure activity displaying that an increased amount of accelerator may not result in an increase in cure time observed, along with a potential relationship between molecule size and cure activity.

The investigation of imidazoles as cure accelerators also allowed for the exploration of the application of charged compounds through the synthesis of imidazolium derivatives. Primary evaluation of BMIM octyl sulfate (**80**) and BMIM acetate (**83**) in the fixture time test resulted in activity on a par with established commercial accelerators. This level of activity merited further investigation and the compounds were tested in a further range of adhesive tests as lead cure accelerator candidates.

Along with these two compounds, a further BMIM based molecule was also tested (**100**). This compound incorporated the saccharinate anion, the charged analogue of saccharin which is already known to be cure active and widely included in anaerobic adhesives. Testing of these lead compounds showed that **100** possessed a high level of activity and the possibility for commercial application.

In order to explore the source of this activity, a number of ionic liquid compounds with varying cations and anions were synthesised along with further alkyl imidazoliums. They were tested for their capability to act as threadlockers and their shear strength as part of the nuts & bolts and pins & collars tests. This investigated structural properties such as aromaticity, and the nitrogen lone pair of electrons through pyridinium and pyrrolidinium derivatives, and paired them with the acesulfamate, phthalimate, and saccharinate anions.

All of these compounds tested displayed efficient activity comparable to that of APH and illustrated the primary role which the anion plays in the cure accelerator ability of the molecule. The phthalimate, acesulfamate, and saccharinate anions all exhibited their ability to act as effective anaerobic cure accelerators.

In order to assess the potential level of risk they may pose, a range of these lead cure accelerators were submitted for evaluation of their cytotoxicity against two mammalian cell lines and anti-microbial toxicity against a range of fungal and

bacterial strains. Study of imidazolium saccharinates **100**, **108**, and **109** showed that by increasing the alkyl chain length, the toxicity increased due to enhanced interaction with the cell membrane. Substitution of the anion (**100**, **110**, **111**) resulted in no discernable change in toxicity and suggested that the acesulfamate, phthalimate, and saccharinate may all be of a low toxicity and given this, if paired with a cation also of low toxicity, may result in an effective cure accelerator which poses low risk to the user.

In fact, this was readily achieved with the results of the two pyrrolidinium compounds designed. Both *N*,*N*-dibutylpyrrolidinium saccharinate (**131**) and acesulfamate (**132**) possessed remarkable cure accelerator ability whilst also proving to be of lower microbial toxicity and cytotoxicity than APH – a key goal of this research. The two compounds resulted in strong adhesive bonds after short cure times, whilst also displaying low cell inhibition against the cell lines and strains tested.

In terms of their environmental impact, it is likely that both **131** and **132** may not be readily degradable but alteration of the *N*-alkyl substation to contain alcohol and ether functional groups has proven in previous studies to result in readily biodegradable pyrrolidinium compounds. Such derivitisations would also likely increase the water solubility of the compounds and in turn lower the potential aquatic toxicity.

6.2 Future Work

From these studies, the source of cure activity within ionic liquid compounds may possibly be solely the result of the anion constituent. This would allow freedom to develop the cation with the promotion of other properties such as toxicity, biodegradability, bond strength in mind.

This could potentially lead to custom designed cure accelerators which may be altered depending on the application of the adhesive and durability required. Before any such development can take place however, the mechanism through which polymerisation occurs and the role of the cure accelerator within it should be further elucidated.

This may be done through studying the potential interaction of each of the components of the formulation with the cure accelerator in isolation. For instance, ionic liquids may form metal based compounds in the presence of a suitable metal ion and likewise may interact with peroxides through hydrogen bonding. Occurrences such as these could have a significant effect on the polymerisation observed and thus their likelihood to arise may be evaluated through reaction of the cure accelerator with the respective analyte at concentrations representative of formulation conditions.

Examination of the properties of the polymer and bond formed may also lead to information regarding the curing mechanism of anaerobic adhesives. Specifically this would be with the aim of determining the source of bond properties such as the strength. In this thesis, measurements of the force required to break the adhesive bond formed after specific cure times were used as an indicator of the level of polymerisation which had occurred. However, the chance remains that the level of force required to cause bond failure may have been as a result of polymer properties such as cross-linking as opposed to solely the result of the accelerator increasing the rate of polymerisation. Experiments should therefore be carried out in order to study the effect of the cure accelerator on the level of cross-linking which occurs within the polymer system.

With more information about the role of the accelerator within the adhesive curing mechanism established, development of other properties of the compounds may be carried out. As alluded to earlier, if the anion is the major source of cure activity then this may allow the cation to be designed with respect to affecting other properties of the overall molecule such as toxicity, biodegradability, and adhesive bond properties. Ionic liquids containing a *N*-methyl-*N*-hydroxypropyl pyrrolidinium cation have been shown to be readily biodegradable and if similar transformations were made to the *N*,*N*-dibutylpyrrolidinium compounds prepared as part of this research then this may result in a highly active, biodegradable cure accelerator which is also of low anti-microbial and cytotoxicity.

If we are to consider long-term adhesive applications where high strength and durability are matters of priority, then the cation component may be designed to maximise bond life and strength. Initially this would entail evaluation of cations such

as tetrahydroquinoliniums and alkyl-toluidiniums which are based on neutral compounds already of high cure activity.

In addition, adhesive properties other than the overall strength may also be incorporated into the design of ionic liquid cure accelerators. Maleimides can be used to enhance the thermal resistance of adhesive bonds and so if the cation were to incorporate this functionality the analogous effect may be achieved. This is an example whereby ionic liquid cure accelerators could potentially offer multi-functionality with respect to the adhesive curing process and remove the need for additives to be included in product formulations.

As a result of this research the capability of ionic liquids to act as anaerobic cure accelerators has been established. This has not only lead to the discovery of several new accelerator molecules, but perhaps more importantly has opened a whole new avenue for their development and expansion.

Chapter 7:

Experimental

7.1 Biodegradation Screening – IQAC, Barcelona

In order to evaluate the biodegradability of anaerobic cure accelerators and accelerator-like compounds, samples of each compound were submitted to a collaborating research group at the "Department of Chemical and Surfactants Technology, Institute of Advanced Chemistry of Catalonia". The samples were evaluated using the "CO_2 Headspace" test which is a standardised method of analysing the aerobic degradation of a test compound.

A bottle containing a mineral salts medium was inoculated with activated sludge containing micro-organisms, which was collected from an aeration tank at a sewage treatment plant located in Manresa, Barcelona. The test samples were prepared in either water or, for poorly water soluble samples; analytical grade hexane or ethyl acetate. A sample was then incubated in the test bottle at a carbon concentration of 20 mg/L., leaving a "headspace" of air in the bottle.

Since the test sample is the only source of carbon within the bottle, any CO_2 produced is as a direct result of its biodegradation and thus the extent to which the sample degrades can be calculated by measuring this level of CO_2. Each sample was tested in quadruplicate with the same number of "blank" bottles used and sodium n-dodecyl sulfate was used as a control. The level of inorganic carbon was measured at seven day intervals, and by subtracting the mean amount of inorganic carbon in the blank bottles from that of the test samples, the level of total inorganic carbon (TIC) produced as a result of sample degradation was determined. This level was then expressed as a percentage of the theoretical maximum amount of inorganic produced by the sample (ThIC) and the confidence levels calculated.

In order for a compound to be considered "readily biodegradable", degradation by a minimum level of 60% over 28 days is required.

7.2 Aquatic Toxicity Screening – IQAC, Barcelona

In order to evaluate the aquatic toxicity of anaerobic cure accelerators and accelerator-like compounds, samples of each compound were submitted to a collaborating research group at the "Department of Chemical and Surfactants Technology, Institute of Advanced Chemistry of Catalonia". Each sample was evaluated using MICROTOX and *Daphnia magna* tests.

Microtox uses bioluminescent bacteria, *Vibrio fischeri*, which when exposed to toxic substances, exhibit a decrease in light output as a result of their respiratory process being disrupted. By measuring this change in luminescence, the toxicity of a test sample may be evaluated.

Evaluations carried out using *Daphnia magna* involve adding a defined number of organisms to a test medium containing the substance to be evaluated. At various time intervals the number of offspring produced as well as the number of deceased organisms are counted and the toxicity of the substance determined.

7.3 Anti-Bacterial Screening – Charles University, Prague

In vitro anti-bacterial evaluation of anaerobic cure accelerators and accelerator-like compounds were carried out by a collaborating research group at the "Department of Biological and Medical Sciences, Charles University".

The compounds were evaluated on a panel of three ATCC strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and five clinical isolates (*Staphylococcus aureus* MRSA H 5996/08, *Staphylococcus epidermidis* H 6966/08, *Enterococcus sp.* H 14365/08, *Klebsiella pneumoniae* D 11750/08, *Klebsiella pneumoniae* ESBL H 14368/08) from the collection of bacterial strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. The abovementioned ATCC strains also served as the quality control strains. All the isolates were maintained on Mueller-Hinton dextrose agar prior to being tested.

Dimethyl sulfoxide (100 %) served as a diluent for all compounds; the final concentration did not exceed 2 %. Mueller-Hinton agar (MH, HiMedia, adersky-Envitek, Czech Republic) buffered to pH 7.4 (±0.2) was used as the test medium. The wells of the microdilution tray contained 200 μL of the Mueller-Hinton medium with 2-fold serial dilutions of the compounds (2000 or 1000 to 0.488 μmol/l) and 10 μL of inoculum suspension. Inoculum in MH medium was prepared to give a final concentration of 0.5 McFarland scale (1.5×10^8 cfu.mL-1). The trays were incubated at 36°C and MICs were read visually after 24 h and 48 h. The MICs were defined as 95 % inhibition of the growth of control. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

7.4 Anti-Fungal Screening – Charles University, Prague

In vitro anti-fungal evaluation of anaerobic cure accelerators and accelerator-like compounds were carried out by a collaborating research group at the "Department of Biological and Medical Sciences, Charles University".

In vitro antifungal activities of the compounds were evaluated on a panel of four ATCC strains (*Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258) and eight clinical isolates of yeasts (*Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon asahii* 1188) and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three of the above ATCC strains (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258) also served as the quality control strains. All the isolates were maintained on Sabouraud dextrose agar prior to being tested. Minimum inhibitory concentrations (MICs) were determined by the microdilution format of the NCCLS M27-A guidelines. Dimethyl sulfoxide (100 %) served as a diluent for all compounds; the final concentration did not exceed 2 %. RPMI 1640 (Sevapharma, Prague) medium supplemented with L-glutamine and buffered with 0.165 M morpholinepropanesulfonic acid (Serva) to pH 7.0 by 10 N NaOH was used as the

test medium. The wells of the microdilution tray contained 100 μL of the RPMI 1640 medium with 2-fold serial dilutions of the compounds (2000 or 1000 to 0.48 μmol/L) and 100 μL of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2$ cfu.mL-1. The trays were incubated at 35°C and MICs were read visually for filamentous fungi and photometrically for yeasts as an absorbance at 540 nm after 24 h and 48 h. The MIC/IC50 values for the dermatophytic strain (*T. mentagrophytes*) were determined after 72 h and 120 h and for *A. fumigatus*, *A. corymbifera* after 24 and 48 h. For all other strains MIC/IC80 values were evaluated. The MICs were defined as 50 % or 80 % inhibition of the growth of control. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

7.5 Mammalian Cell Culture Screening – NICPB, Tallinn

Autoclaved MilliQ water (18 MΩ, Millipore) was used throughout the study. Media components for cell culture were from the following companies: Minimum Essential Medium (MEM) with GlutaMAX, sodium pyruvate, non-essential amino acids (NEAA), streptomycin-penicillin (10 000 U/mL) all from Gibco, Life Technologies; fetal bovine serum (FBS) obtained from Biological Industries. Phosphate buffered saline (PBS) from Lonza, neutral red (NR) dye was from AppliChem GmbH. Acetic acid was from Sigma-Aldrich along with sodium dodecyl sulphate (SDS) and dimethyl sulfoxide (DMSO).

All samples except sample OMIM saccharinate were dissolved in water by sonicating for 1 minute (40W, Branson probe sonicator, USA). OMIM Saccharinate was dissolved in 5% DMSO in water with all other stock solutions prepared as 3 mM in MilliQ water. APH and BMIM phthalimate were prepared fresh before use in any experiments. The stock solutions were sterilized by filtration through 0.2 μm filter and stored at +4oC in the dark. All the chemicals were tested at their initial pH values except *N*-butyl pyridinium saccharinate which had an initial pH of 2.4 but due to the buffering capacity of the culture medium even at the highest tested concentration (1 mM) the pH was neutral: pH of the 1mM *N*-butyl pyridinium saccharinate in both test media was 8.1.

Cell lines used for the toxicity testing were obtained from American Type Culture Collection (ATCC): A549 cells originate from carcinomatous tissue of human lungs (ATCC CCL-185) and Caco 2 cells from colorectal adenocarcinomatous tissue of human colon (ATCC HTB-37). The cell cultures were maintained according to ATCC guidance. A549 cells were cultured in MEM with GlutaMAX and 10%. FBS. Caco 2 cells were cultured in MEM with 15% FBS, 1% NEAA, 1% sodium pyruvate. All cell culture media were supplemented with 100 μg/mL and 100 U/mL of streptomycin-penicillin, respectively.

The neutral red uptake (NRU) assay in which neutral red dye is taken up only by viable cells and is accumulated in lysosomes was carried out essentially according to the protocol by Sigma Aldrich. Briefly, the cells were seeded on 96-well plates (Cellstar, Greiner) at various initial densities: A549 cells at $5x10^3$ cells per well and Caco 2 cells at $1x10^4$ cells per well. The cells were incubated in respective media at 37°C (95% humidity and 5% CO_2).

The stocks of chemicals were prepared at 3 mM as described. Further dilutions were prepared in cell culture media. Cells were exposed to 100 μl of chemicals or solvent controls at concentrations 0.1 mM, 0.5 mM, and 1 mM. 6.25 - 200 μg/mL sodium dodecyl sulphate (SDS) was used as a positive control. The exposed cells were incubated at 37°C (95% humidity and 5% CO_2) for 24 h. After that, test chemicals or cell culture medium were removed, the adhered cells washed with PBS, and incubated for 3 h in a medium supplemented with neutral red (50 μg/ml). After 3 h the neutral red reagent was removed, cells were washed with PBS and 100 μL of NR solubilisation solution (1% acetic acid in 50% ethanol) was added. The plates were gently shaken for 1 h at room temperature and the absorbance was measured at 540 nm using a microplate reader (Multiskan, Thermo Scientific). Cell viability was determined by the formula: cell viability (%) = $(OD_{test} - OD_{blank}) / (OD_{control} - OD_{blank}) \times 100$.

Where the data is expressed as the loss of viability (INH%), the INH% value was calculated as 100% - viability%. All exposures were performed in at least three technical replicates in three independent experiments (0.1 mM concentration) or in one independent experiment (0.5 mM and 1.0 mM concentration). The positive control SDS was used in all runs.

7.6 Anti-Bacterial Screening – NICPB, Tallinn

All samples except OMIM saccharinate were dissolved in water by sonicating for 1 minute (40W, Branson probe sonicator, USA). OMIM saccharinate was dissolved in 5% DMSO in water at 3 mM with all other stock prepared at a concentration of 3 mM in MilliQ water. For the analysis, the stocks prepared for the mammalian cell culture testing (sterilized by filtration through 0.2 µm filter and stored at +4^{o}C in the dark) were used in order to compare the data of bacterial assays and mammalian cell assays *in vitro*. Most of the stock solutions were prepared on June 2 or June 9, 2015 with stocks for APH and BMIM phthalimate prepared June 22, 2015.

For the testing with *Vibrio fischer*i, NaCl was added to the stocks of the chemicals (as an average, 60 mg of NaCl was added to the 3 ml of the stock solution), to adjust the salinity of the sample to 2% NaCl, i.e. physiologically optimal salinity for this marine bacterium.

As a positive control 3,5-dichlorophenol (3,5-DCP) was used (suggested by ISO 21338:2010). All samples except *N*-butyl pyridinium saccharinate were tested at their initial pH values. Stock of *N*-butyl pyridinium saccharinate had an initial pH 2.4 and as 2% NaCl has no buffering capacity the pH of this chemical was adjusted with NaOH to pH 6.3 prior to toxicity testing**.**

The kinetic *Vibrio fischeri* bioluminescence inhibition assay (flash assay) is an ISO standardized acute toxicity test. It is a very rapid and sensitive method to evaluate/screen toxic properties of different chemical substances (including synthetic NPs and solid/coloured environmental samples, e.g. sediments, soil suspensions, wastewater, sludge extracts, etc.) by measuring the reduction of light production of test bacteria (naturally luminescent marine bacteria *V. fischeri*) due to interactions between bacteria and toxic compounds. The decrease in bacterial luminescence occurs already after brief contact of bacteria with toxicants (in the scale of seconds to minutes, depending on the compounds). The decrease in bioluminescence reflects the inhibition of bacterial metabolic activity and is proportional to the toxicity of test sample.

Toxicity tests were conducted with *Vibrio fischeri* strain NRRL B-11177. The bacterial suspension used for the toxicity measurements was reconstituted from freeze-dried *V. fischeri* reagent (Aboatox, Turku, Finland). The *V. fischeri* bioluminescence inhibition assay (an acute test, exposure times of 5 seconds, 15 min

and 30 min) was performed at room-temperature (~ 20° C) in 96-well polypropylene white microplates (Greiner Bio-One, Frickenhausen, Germany) following the modified Flash Assay protocol (ISO, 2010). Briefly, 100 µl of test solution in 2% NaCl was pipetted into each well, which was supplemented with 100 µl of bacterial suspension by automatic dispensing in the Microplate Luminometer Orion II (Berthold Detection Systems, Pforzheim, Germany) testing chamber. The changes in the bioluminescence of the test bacteria upon exposure to different concentrations (0.1-1.5 mM) of different test chemicals were recorded during the first 5 seconds after the dispensing of the bacterial suspension in each well, after 15 and 30 minute incubation, the light output was recorded again.

All chemicals and their dilutions were supplemented by NaCl to contain 2% NaCl. Each test was performed in at least 4 technical replicates in two independent experiments. Controls, both negative (2% NaCl) and positive (3,5-dichlorophenol, 3,5-DCP), were included in each measurement series.

The inhibition of bacterial bioluminescence (INH%) by different concentrations of the tested compounds at 15 and 30 min was calculated as a percentage of the unaffected control (2% NaCl):

$$INH\,\% = 100 - \left(\frac{IT_t * 100}{IC_t} \right)$$

where

IT_t – luminescence of bacteria exposed to certain concentration of chemicals after certain time of exposure (t=15 min, 30 min);

IC_t – luminescence of bacteria in the control solution (2% NaCl) after certain time of incubation (t=15 min, 30 min).

EC_{50} values are usually applied as a quantitative measure of the toxicity of a chemical. In the current study 15 minute and 30 minute EC_{50} is the concentration of the chemicals (mM) that causes the inhibition of bacterial luminescence by 50% after 15 or 30 minute exposure to the chemical, respectively. The EC_{50} value was calculated from the concentration-effect data. Due to the low toxicity of most of the chemicals in the concentration range tested (up to 1.5 mM), only the EC_{50} for the most toxic chemical, OMIM saccharinate, was able to be calculated. The coefficient of variation of INH% values obtained in different days did not exceed 5%.

7.7 Cure Accelerator Evaluation

In order to evaluate cure accelerator activity, adhesive formulations were prepared as per Tables 7.1 or 7.2 as stated.

Table 7.1: PEGMA Formulation Specification

Component	% Weight
Polyethylene glycol dimethacrylate	95.5
Saccharin	0.5
Trigonox TMBH-L	2
PM 16	0.5
PM 17	0.5
Accelerator	1

Table 7.2: Model Formulation Specification

Component	% Weight
Polyethylene glycol dimethacrylate	50
RROC	45.05
PM17	2
Saccharin	1
Paramenthane Hydroperoxide	1
PM40	0.4
Accelerator	0.95

These formulations were then applied as part of several tests:

Fixture Time Test

The fixture time test was used as primary method of evaluation of possible cure accelerators. The mild steel substrate surface is pre-treated prior to use by grit-blasting. A series of bonds are prepared by bonding two pieces of the mild steel such that they have a half inch overlap. At 30 minute cure time intervals, the strength of the bond is tested by attach a 3 kg weight by means of a spring to one of the pieces

of steel. If the bond is capable of holding the weight for five minutes it is deemed to have passed the test. Through preparing further series' of bond specimens, the cure time is determined to within five minutes.

Determination of Torques Strength – Nuts and Bolts (ISO 10964)

The torque strength of the adhesive bond formed was measured as per ISO 10964. The test was carried out suing two types of specimens; stainless steel nuts & bolts and black oxide bolts with mild steel nuts. The adhesive was applied to bolts and the threaded joint fastened. These bonds were allowed cure for either 1 hour or 24 hours cure time after which the torque strength was measured in terms of the break torque, prevail torque at 180°, and maximum torque.

Determination of Shear Strength – Pins and Collars (ISO 10123)

The shear strength of the anaerobic adhesive bonds formed were measured as per ISO 10123.

7.8 Chemicals

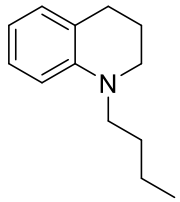
All chemicals were purchased from Sigma Aldrich, TCI Chemicals, or Acros Organics. Silica gel from Sigma Aldrich was used for column chromatography. THF, methanol, ethanol, DCM, and diethyl ether were all dried over molecular sieves and distilled prior to use. THF was also dried over sodium/benzophenone.

7.9 NMR Analysis

All NMR analysis was performed using a Bruker 400 MHz spectrometer, operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, or, a Bruker 600 MHz spectrometer, operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. Samples were dissolved in chloroform-*d*, DMSO-d_6, deuterium oxide, or 35% deuterium chloride in D_2O prior to analysis. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are measured in Hertz (Hz). Splitting patterns of sample peaks are reported using the following abbreviations: s – singlet, d – doublet, t – triplet, q – quartet, dd – doublet of doublets, ddd – doublet of doublet of doublets, dt – doublet of triplets, tt – triplet of triplets, m – multiplet.

7.10 Synthetic Procedures

N-Butyl Tetrahydroquinoline (29)[1]



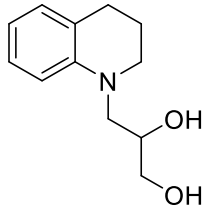
The compound was prepared by addition of tetrahydroquinoline (2.50 g, 2.36 mL, 19.0 mmol), diisopropylethylamine (3.64 g, 4.90 mL, 28.0 mmol), and DMF (20 mL) to a round bottomed flask. 1-Bromobutane (3.86 g, 3.62 mL, 28.0 mmol) was added gradually and the solution was stirred at reflux for 2 hours. The reaction solution was allowed cool to RT upon which the DIPEA bromide salt precipitated and was removed by filtration. The reaction mixture was diluted with deionised water (40 mL) and diethyl ether (20 mL) and washed with a further 20 mL of Et_2O. The organic layers were combined and washed with deionised water (3x20 mL) and dried over $MgSO_4$. The organic layer was filtered, then evaporated to dryness to yield a brown oily liquid (86%, 3.2 g, 17 mmol).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 – 7.07 (m, 1H, -Ar*H*), 7.05 – 6.98 (m, 1H, -Ar*H*), 6.68 – 6.58 (m, 2H, -Ar*H*), 3.39 – 3.25 (m, 4H, -ArNC*H*$_2$CH$_2$CH$_2$Ar, -NC*H*$_2$CH$_2$CH$_2$CH$_3$), 2.83 (t, *J* = 6.4 Hz, 2H, -ArC*H*$_2$CH$_2$CH$_2$N), 2.07 – 1.96 (m, 2H, -ArCH$_2$C*H*$_2$CH$_2$), 1.72 – 1.59 (m, 2H, -C*H*$_2$CH$_2$CH$_3$), 1.52 – 1.38 (m, 2H, -C*H*$_2$CH$_3$), 1.04 (t, *J* = 7.3 Hz, 3H, -C*H*$_3$).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 145.38 (-Ar*C*), 129.18 (-Ar*C*H), 127.10 (-Ar*C*H), 122.16 (-Ar*C*), 115.20 (-Ar*C*H), 110.47 (-Ar*C*H), 51.28 (-O*C*H$_2$CH$_2$N), 49.52 (-*C*H$_2$CH$_2$CH$_2$CH$_3$), 28.44 (-ArCH$_2$CH$_2$CH$_2$N), 28.30 (-*C*H$_2$CH$_2$CH$_3$), 22.32 (-ArCH$_2$*C*H$_2$CH$_2$), 20.55 (-*C*H$_2$CH$_3$), 14.16 (-*C*H$_3$).

^{1}H Spectrum in agreement with reported literature spectrum[1].

Tetrahydroquinoline "Diol" (35)[1]



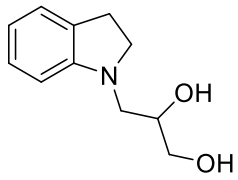
The compound was prepared as per a slight modified method of that outlined[1]. To a 250 mL round bottomed flask, tetrahydroquinoline (7.00 g, 6.60 mL, 54.0 mmol) and glycidol (4.00 g, 3.60 mL, 54.0 mmol) were added under nitrogen. The mixture was heated to 60°C for one hour before a paste formed upon which 20 mL of methanol was added in order to dissolve and allow the reaction mixture to be stirred. The mixture was heated to 60°C and allowed stir for 18 hours after which deionised water (100 mL) was added before the mixture was stirred at 60°C for a further 9 hours. The precipitate was isolated via vacuum filtration and recrystallized from IPA/deionised water to give the title compound as a white solid in 59% yield (5.48g, 32.0 mmol).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.11 – 7.01 (m, 1H, Ar*H*), 6.98 (d, 1H, Ar*H*), 6.74 – 6.60 (m, 2H, Ar*H*), 4.11 – 4.01 (m, 1H, -CH_2C*H*$(OH)CH_2OH$), 3.81 (dd, *J* = 11.3, 3.3 Hz, 1H, -$CH(OH)C$*H*$_2OH$), 3.60 (dd, *J* = 11.3, 5.4 Hz, 1H, -$CH(OH)C$*H*$_2OH$), 3.45 – 3.23 (m, 4H, -$ArCH_2CH_2C$*H*$_2N$, -NC*H*$_2CH(OH)CH_2OH$), 2.83 – 2.75 (m, 2H, -ArC*H*$_2CH_2CH_2N$), 2.51 (s, 1H, -O*H*), 2.10 (s, 1H, -O*H*), 2.02 – 1.89 (m, 2H, -$ArCH_2C$*H*$_2CH_2N$).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 145.71 (-Ar*C*), 129.53 (-Ar*C*H), 127.24 (-Ar*C*H), 123.19 (-Ar*C*), 117.06 (-Ar*C*H), 111.86 (-Ar*C*H), 69.54 (-CH_2*C*$H(OH)CH_2OH$), 64.49 (-$CH(OH)$*C*H_2OH), 54.99 (-$ArCH_2CH_2$*C*H_2N), 50.90 (-$NCH_2CH(OH)CH_2OH$), 28.03 (-Ar*C*$H_2CH_2CH_2N$), 22.12 (-$ArCH_2$*C*H_2CH_2N).

^{1}H and ^{13}C spectra in agreement with literature[2].

Indoline "Diol" (36)[1]

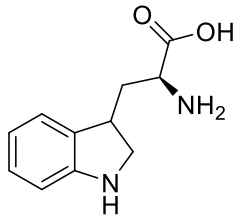


Indoline (2.50 g, 2.35 mL, 21.0 mmol), glycidol (1.55 g, 21.0 mmol, 1.40 mL) and deionised water (5 mL) were added to a round bottomed flask and the solution stirred at 0°C for 2 hours upon which a white solid formed. This precipitate was isolated by vacuum filtration and recrystallized from acetone to give the title compound (**15**) in 62% yield (2.54 g, 13.0 mmol).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.17 – 7.05 (m, 2H, -Ar*H*), 6.78 – 6.69 (m, 1H, -Ar*H*), 6.57 (d, *J* = 7.9 Hz, 1H. -Ar*H*), 4.04 – 3.94 (m, 1H, -C*H*(OH)CH_2OH), 3.83 (dd, *J* = 11.4, 3.4 Hz, 1H, -CH(OH)C*H*$_2$OH), 3.62 (dd, *J* = 11.4, 5.3 Hz, 1H, -CH(OH)C*H*$_2$OH), 3.59 – 3.48 (m, 1H, -ArCH_2C*H*$_2$N), 3.38 – 3.20 (m, 2H, -ArCH_2C*H*$_2$N, -NC*H*$_2$CH(OH)CH_2), 3.09 – 2.91 (m, 3H, -NC*H*$_2$CH(OH)CH_2, -ArC*H*$_2$$CH_2$), 2.84 (s, 1H, -O*H*), 2.35 (s, 1H -O*H*).

^{13}C NMR (100 MHz, Deuterium Oxide) δ 152.73 (-Ar*C*), 130.08 (-Ar*C*), 127.52 (-Ar*C*H), 124.74 (-Ar*C*H), 118.82 (-Ar*C*H), 107.72 (-Ar*C*H), 69.61 (-*C*H(OH)CH_2OH), 64.56 (-CH(OH)*C*H_2OH), 54.94 (-ArCH_2*C*H_2N), 53.61 (-N*C*H_2CH(OH)CH_2), 28.90 (-ArC*H*$_2$$CH_2$).

Dihydrotryptophan (42)[3]

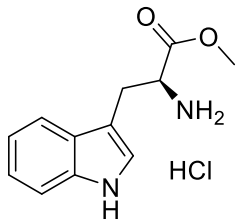


L-Tryptophan (1.50 g, 7.30 mmol) was dissolved in 1 M hydrochloric acid (30 mL) in a round bottomed flask along with 10% Pd/C (300 mg). A balloon of hydrogen was attached and the mixture allowed stir at RT for 24 hours before the flask was flushed with nitrogen gas and the catalyst removed by filtration. The reaction solution made alkaline using 1 M ammonium hydroxide (40 mL). The reaction solution was evaporated to dryness and the product recrystallised from deionised water resulting in a mixture of stereoisomers (55%, 0.82 g, 4.0 mmol).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.27 (dd, *J* = 18.2, 7.2 Hz, 1H, -Ar*H*), 7.17 (t, *J* = 7.6 Hz, 1H, -Ar*H*), 6.95 – 6.84 (m, 2H, -Ar*H*), 3.81 (m, 1H, -C*H*$_2$NH), 3.74 – 3.63 (m, 1H, -C*H*$_2$NH), 3.48 (m, 1H, -C*H*NH$_2$), 3.24 – 3.17 (m, 1H, -C*H*CH$_2$NH), 2.36 – 2.17 (m, 1H, -C*H*$_2$CH(NH$_2$), 2.16 – 1.97 (m, 1H, -C*H*$_2$CH(NH$_2$).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 174.71 (-*C*(O)O), 174.44 (-*C*(O)O), 150.14 (-Ar*C*), 132.93 (-Ar*C*), 132.74 (-Ar*C*), 128.23 (-Ar*C*), 128.19 (-Ar*C*), 124.27 (-Ar*C*), 124.07 (-Ar*C*), 120.62 (-Ar*C*), 120.55 (-Ar*C*), 111.93 (-Ar*C*), 111.92 (-Ar*C*), 53.47 (-*C*HNH$_2$), 53.41 (-*C*HNH$_2$), 52.01 (-*C*H$_2$NH), 51.97 (-*C*H$_2$NH), 38.32 (-*C*HCH$_2$NH), 37.97 (-*C*HCH$_2$NH), 35.84(-*C*H$_2$CH(NH$_2$)), 35.34 (-*C*H$_2$CH(NH$_2$)).

L-Tryptophan Methyl Ester Hydrochloride Salt (43)[4]



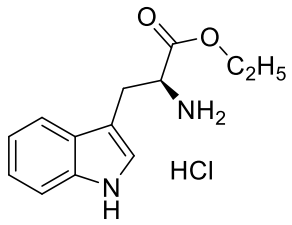
L-Tryptophan (2.01 g, 9.8 mmol) was added to MeOH (30 mL) and the mixture cooled to 0°C. $SOCl_2$ (2.0 mL, 29.0 mmol) was added drop wise before the solution was allowed warm to RT and stir overnight. Et_2O was added causing the product to precipitate. The title compound was isolated and washed with Et_2O to yield the hydrochloride salt (82%, 2.06 g, 8.1 mmol).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.56 (d, *J* = 7.9 Hz, 1H, -Ar*H*), 7.49 (d, *J* = 8.2 Hz, 1H, -Ar*H*), 7.29 – 7.18 (m, 2H, -Ar*H*, -Indole*H*), 7.19 – 7.10 (m, 1H, -Ar*H*), 4.45 – 4.37 (m, 1H, -C*H*(NH_2)C(O)O), 3.76 (s, 2H, -CH_2CH(NH_2)), 3.51 – 3.35 (m, 3H, -CH_3).

^{13}C NMR (100 MHz, Deuterium Oxide) δ 170.35 (-*C*(O)OCH_3), 136.18 (-Ar*C*), 126.26 (-Ar*C*), 125.29 (-Ar*C*), 122.15 (-Ar*C*), 119.52 (-Ar*C*), 117.94 (-Ar*C*), 111.97 (Ind*C*), 105.87 (Ind*C*), 53.46 (-*C*H_3), 53.21 (-*C*H(NH_2)C(O)O), 25.57 (-*C*H_2CH(NH_2)).

^{1}H and ^{13}C spectra in agreement with literature[5].

L-Tryptophan Ethyl Ester Hydrochloride Salt (44)[4]

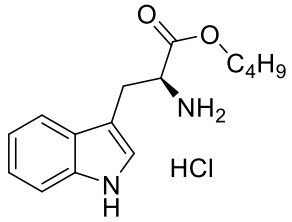


The product was prepared as per the method described for L-tryptophan methyl ester HCl using L-tryptophan (0.50 g, 2.4 mmol), EtOH (8 mL), and $SOCl_2$ (0.40 mL, 5.5 mmol). The title compound was isolated a white solid (85%, 0.55 g, 2.0 mmol).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.59 (d, *J* = 7.9 Hz, 1H, -Ar*H*), 7.49 (d, *J* = 8.2 Hz, 1H, -Ar*H*), 7.29 – 7.18 (m, 2H, -Ar*H*, -Indole*H*), 7.15 (td, *J* = 7.6, 1.0 Hz, 1H, -Ar*H*), 4.38 (t, *J* = 6.4 Hz, 1H, -C*H*(NH_2)C(O)O), 4.18 (q, *J* = 7.1 Hz, 2H, -C$H_2$$CH_3$), 3.54 – 3.36 (m, 2H, -CH_2CH(NH_2)), 1.16 (t, *J* = 7.2 Hz, 3H, -CH_3).

^{13}C NMR (100 MHz, Deuterium Oxide) δ 169.91 (-*C*(O)OC_2H_5), 136.18 (-Ar*C*), 126.30 (-Ar*C*), 125.25 (-Ar*C*H), 122.14 (-Ar*C*H), 119.48 (-Ar*C*H), 117.96 (-Ar*C*H), 111.95 (Ind*C*), 105.96 (Ind*C*), 63.50 (-*C*$H_2$$CH_3$) , 53.29 (-*C*H($NH_2$)C(O)O), 25.65 (-*C*$H_2$CH($NH_2$)), 12.91 (-*C*$H_3$).

L-Tryptophan Butyl Ester Hydrochloride Salt (45)[4]

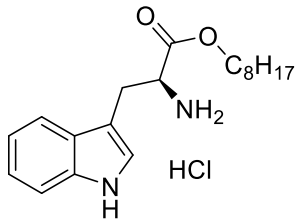


The product was prepared as per a modified method of that described for L-tryptophan methyl ester using L-tryptophan (1.0 g, 4.9 mmol), BuOH (16 mL), and $SOCl_2$ (1.06 mL, 14.7 mmol). This mixture was heated at 100°C overnight and upon cooling to RT, the precipitate was isolated from solution and washed with cold pet-ether to yield the title compound (70%, 1.01 g, 3.4 mmol).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.58 (d, *J* = 8.0 Hz, 1H, -Ar*H*), 7.49 (d, *J* = 8.2 Hz, 1H, -Ar*H*), 7.29 – 7.20 (m, 2H, -Ar*H*, -Indole*H*), 7.15 (t, *J* = 7.3 Hz, 1H, Ar*H*), 4.40 (t, *J* = 6.4 Hz, 1H, -C*H*(NH_2)C(O)O), 4.12 (t, *J* = 6.5 Hz, 2H, -C*H*$_2$$CH_2CH_2CH_3$), 3.45 (d, *J* = 6.4 Hz, 2H, -C*H*$_2$CH(NH_2)C(O)O), 1.53 – 1.41 (m, 2H, -C*H*$_2$$CH_2CH_3$), 1.15 (tq, *J* = 7.4, 7.4 Hz, 2H, -C*H*$_2$$CH_3$), 0.79 (t, *J* = 7.4 Hz, 3H, -C*H*$_3$).

^{13}C NMR (100 MHz, Deuterium Oxide) δ 167.41 (-*C*(O)OC_4H_9), 133.50 (-Ar*C*), 123.60 (-Ar*C*), 122.44 (-Ar*C*H), 119.43 (-Ar*C*H), 116.76 (-Ar*C*H), 115.23 (-Ar*C*H), 109.26 (Ind*C*), 103.27 (Ind*C*), 64.42 (-*C*$H_2CH_2CH_2CH_3$, 50.61 (-*C*H(NH_2)C(O)O), 26.74 (-*C*$H_2CH_2CH_3$), 23.09 (-*C*H_2CH(NH_2)), 15.49 (-*C*H_2CH_3), 10.02 (-*C*H_3).

L-Tryptophan Octyl Ester Hydrochloride Salt (46)[4]

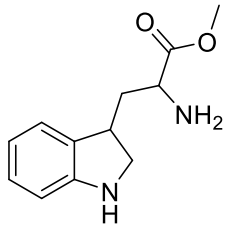


The product was prepared as per a modified method of that described for L-tryptophan methyl ester using L-tryptophan (1.0 g, 4.9 mmol), Octanol (40 mL), and $SOCl_2$ (1.0 mL, 14.7 mmol). This mixture was stirred at 100°C overnight and cooling to RT, the precipitate was isolated from solution and washed with DCM to yield the title compound (52%, 0.9 g, 2.55 mmol).

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.22 (s, 1H, -N*H*) 8.81 (br. s, 3H, -N*H*$_3$), 7.52 (d, *J* = 7.9 Hz, 1H, -Ar*H*), 7.37 (d, *J* = 8.1 Hz, 1H, -Ar*H*), 7.26 (d, *J* = 2.2 Hz, 1H, -Indole*H*), 7.06 (t, *J* = 7.4 Hz, 1H, -Ar*H*), 6.98 (t, *J* = 7.4 Hz, 1H, -Ar*H*), 4.19 – 4.11 (m, 1H, -C*H*(NH_2)C(O)O), 3.95 (m, 2H, -C*H*$_2$$C_7H_{15}$), 3.39 (m, 1H, -C*H*$_2$CH($NH_2$)C(O)O), 3.28 (dd, *J* = 14.6, 7.5 Hz, 1H, - C*H*$_2$CH(NH_2)C(O)O), 1.45 – 1.32 (m, 2H, -CH_2C*H*$_2$$C_5H_{10}CH_3$), 1.30 – 1.01 (m, 10H,- $CH_2CH_2C_5$*H*$_{10}$$CH_3$), 0.85 (t, *J* = 7.0 Hz, 3H, -C*H*$_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ 169.44 (-*C*(O)OC_8H_{17}), 136.27 (-Ar*C*), 126.99 (-Ar*C*), 124.87 (Ind*C*), 121.05 (-Ar*C*H), 118.47 (-Ar*C*H), 118.01 (-Ar*C*H), 111.60 (-Ar*C*H), 106.52 (Ind*C*), 65.51 (-O*C*$H_2C_7H_{15}$), 60.76 (-*C*H(NH_2), 52.79 (-OCH_2*C*$H_2C_6H_{13}$), 31.31 (-*C*H_2CH(NH_2)), 28.67 (-OCH_2CH_2*C*$H_2C_5H_{11}$), 28.62 (-*C*$H_2C_4H_9$), 27.80(-*C*$H_2C_3H_7$), 25.16 (-*C*$H_2C_2H_5$), 22.18 (-*C*H_2CH_3), 14.02 (-*C*H_3).

Dihydrotryptophan Methyl Ester (47)[3]



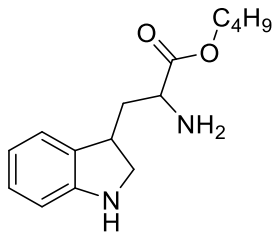
Dihydrotryptophan methyl ester was prepared using a modified method of that used to prepare dihydrotryptophan. L-tryptophan methyl ester hydrochloride (1.01 g, 3.4 mmol) was added to a flask along with deionised water (30 mL) and 10% Pd/C (20% w/w). Conc. HCl (0.2 mL) was added before a balloon of hydrogen gas was attached and the mixture allowed stir overnight. Upon work-up, the reaction was not complete and so fresh catalyst was added and the mixture allowed stir at RT for 24 hours. This process was repeated once more before the catalyst was removed and the solution neutralised using 1M ammonium hydroxide. The solvent was removed by rotary evaporation and the crude product recrystallised from water to yield the title compound as a mixture of stereoisomers. (52%, 0.39 g, 1.8 mmol).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.18 (t, *J* = 6.7 Hz, 1H, -Ar*H*), 7.12 (t, *J* = 7.6 Hz, 1H, -Ar*H*), 6.86 (t, *J* = 7.4 Hz, 1H, -Ar*H*), 6.81 (d, *J* = 7.8 Hz, 1H, -Ar*H*), 3.74 – 3.68 (m, 3H, -OCH_3), 3.68 – 3.58 (m, 1H, -C*H*(NH_2)), 3.58 – 3.48 (m, 1H, m, 1H, -CH_2NH), 3.45 – 3.28 (m, 1H, -C*H*CH_2CH(NH_2)), 3.14 – 3.05 (m, 1H, -CH_2NH), 2.21-1.97 (m, 1H, -CH_2CH(NH_2)), 1.97-1.76 (m, 1H, -CH_2CH(NH_2)).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 176.38 (-*C*(O)O), 176.27 (-*C*(O)O), 150.33 (-Ar*C*), 150.25 (-Ar*C*), 133.24 (-Ar*C*), 133.21 (-Ar*C*), 128.05 (-Ar*C*), 128.02 (-Ar*C*), 124.19 (-Ar*C*), 120.42 (-Ar*C*), 120.38 (-Ar*C*), 111.81 (-Ar*C*), 52.73 (-

*C*H(NH_2)), 52.69 (-*C*H(NH_2)), 52.43 (-O*C*H_3), 52.08 (-*C*H_2NH), 51.96 (-*C*H_2NH), 38.26 (-*C*HCH_2CH(NH_2), 38.17 (-*C*HCH_2CH(NH_2), 37.64 (--*C*H_2CH(NH_2), 37.61 (-*C*H_2CH(NH_2)).

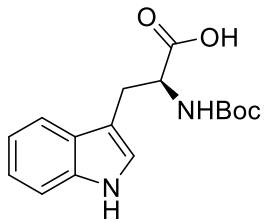
Dihydrotryptophan Butyl Ester (48)[3]



The product was prepared as per the procedure for dihydrotryptophan methyl ester using L-tryptophan butyl ester hydrochloride (0.25 g, 0.9 mmol). This title compound was isolated in 39% yield (100 mg, 0.38 mmol).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.43 – 7.24 (m, 2H, -Ar*H*), 7.18 – 7.08 (m, 2H, -Ar*H*), 4.30 – 4.16 (m, 3H, -C*H*$_2$C$_3$H$_7$, -C*H*$_2$NH), 3.88 – 3.78 (m, 1H, -C*H*$_2$NH), 3.72 – 3.64 (m, 1H, -C3*H*), 3.42 – 3.34 (m, 1H, -C*H*(NH_2)C(O)O), 2.54 – 2.13 (m, 2H, -C*H*$_2$CH(NH_2)), 1.72 – 1.62 (m, 2H, -C*H*$_2$CH$_2$CH$_3$), 1.44 – 1.32 (m, 2H, -C*H*$_2$CH$_3$), 0.94 – 0.86 (m, 3H, -C*H*$_3$).

***N*-Boc-L-Tryptophan (49)**[6]

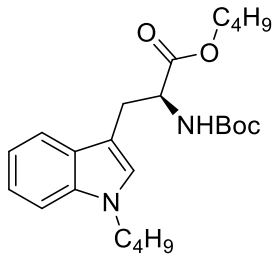


Boc protected tryptophan was prepared as per the published method. L-Tryptophan (2.05 g, 10.0 mmol) was dissolved in 1:1 H_2O:dioxane (40 mL) before 1M NaOH (10 mL) and di-tert-butyl carbonate (2.21 g, 10.0 mmol) were added. The mixture was stirred at RT for 24 hours before the pH was adjusted to 2 using 1 M HCl. The product was extracted using EtOAc (3 x 80 mL) and the organic layers pooled and dried over $MgSO_4$. After filtration, the solvent was removed by rotary evaporation to yield the title compound (77% yield, 2.33 g, 7.66 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (s, 1H –O*H*), 10.87 (s, 1H, -IndoleN*H*), 7.57 (d, *J* = 7.8 Hz, 1H, -Ar*H*), 7.37 (d, *J* = 8.0 Hz, 1H, -Ar*H*), 7.19 (s, 1H, -Indole*H*), 7.10 (t, *J* = 7.1 Hz, 1H, -Ar*H*), 7.05 – 6.97 (m, 2H, -Ar*H*, -N*H*(COO)), 4.22 (m, 1H, -C*H*(NH)C(O)O) 3.19 (dd, *J* = 14.5, 4.6 Hz, 1H, -CH_2CH(NH)C(O)O), 3.03 (dd, *J* = 14.6, 9.4 Hz, 1H, -CH_2CH(NH)C(O)O), 1.35 (s, 9*H*, -C(CH_3)$_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ 174.22 (-CH$_2$*C*(O)O), 155.61 (-NH*C*(O)O), 136.26 (-Ar*C*), 127.35 (-Ar*C*), 124.01 (IndC), 121.09 (-Ar*C*H), 118.54 (-Ar*C*H), 118.33 (-Ar*C*H), 111.58 (-Ar*C*H), 110.33 (Ind*C*), 78.21 (-*C*(CH$_3$)$_3$), 66.51 (-*C*H(NH)C(O)O), 54.72 (-*C*H$_2$CH(NH)C(O)O), 28.33 (-C(*C*H$_3$)$_3$).

***N*-Boc-1-Butyl-L-Tryptophan Butyl Ester (50)**[6]



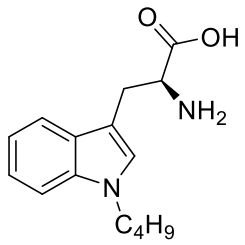
N-Boc-L-Tryptophan (1.51 g, 5.0 mmol) was dissolved in DMSO (15 mL) before NaOH (10 mmol) was added. This solution was heated to 40°C before bromobutane (1.62 mL, 15.0 mmol) was added and stirred for 4 hours. The product solution was diluted with H_2O (100 mL) and extracted using EtOAc (3 x 30 mL). The organic layers were dried using Na_2SO_4, filtered, and evaporated to dryness to yield the crude product. The title compound was purified by column chromatography (DCM:MeOH, 80:20) to yield a white solid (72%, 3.6 mmol, 1.32 g).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 (t, *J* = 8.2 Hz, 1H, -Ar*H*), 7.32 (m, 1H, -Ar*H*), 7.24 –7.16 (m, 1H, -Ar*H*), 7.15-7.06 (m, 1H, -Ar*H*), 6.90 (s, 1H, -Indole*H*), 5.08 (m, 1H, -CH_2CH(NH_2)), 4.67 – 4.57 (m, 1H, -CH_2CH(NH_2)), 4.05 (m, 4H, -OCH_2, -NCH_2), 3.27 (m, 1H, -C*H*(NH)), 1.83-1.74 (m, 2H, -$OCH_2CH_2C_2H_5$), 1.62 – 1.47 (m, 2H, -$NCH_2CH_2C_2H_5$), 1.44 (s, 9H, C(CH_3)$_3$), 1.41 – 1.21 (m, 4H, -$OC_2H_4CH_2CH_3$, -$NC_2H_4CH_2CH_3$), 0.91 (m, 6H, -$OC_3H_6CH_3$, -$NC_3H_6CH_3$).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 172.56 (-CH*C*(O)O), 155.36 (-NH*C*(O)O), 136.24 (-Ar*C*N(C4H9)), 128.40 –Ar*C*), 126.56 (-Indole*C*N(C_4H_9)), 121.61 (-Ar*C*H), 119.05 (-Ar*C*H), 109.49 (-Ar*C*H), 108.67 (-Ar*C*H), 79.79 (-*C*(CH_3)$_3$), 65.27 (-O*C*H_2), 54.45 (-*C*HNH), 46.07 (-N*C*H_2), 32.39 (-OCH_2CH_2), 30.58 (-NCH_2CH_2), 28.45 (-C(*C*H_3)$_3$), 20.27 (-$OC_2H_4CH_2CH_3$), 19.12 (-$NC_2H_4CH_2CH_3$), 13.83 (-$OC_3H_6CH_3$), 13.80 (-$NC_3H_6CH_3$).

^{1}H and ^{13}C spectra in agreement with literature[5].

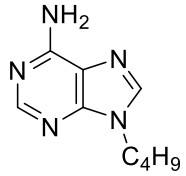
1-Butyl Tryptophan (51)[6]



N-Boc-1-Butyl-L-Tryptophan Butyl Ester (1.48 g, 3.6 mmol) was dissolved in DMSO (20 mL) and 1M NaOH (12 mL) and stirred at RT for 30 minutes whilst monitoring by TLC (DCM:MeOH, 9:1). H_2O (100 mL) was added to the solution and the pH adjusted to 2 using 1M HCl before extraction using EtOAc (6 x 30 mL). The solvent was removed by rotary evaporation and the crude product dissolved in TFA:DCM 1:4 (20 mL) and stirred for 2 hours. The solvent was removed and the crude product taken up in H_2O (10 mL) and adjusting to neutral pH precipitated the product in 40% yield (1.4 mmol, 0.36 g).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.57 (d, *J* = 7.8 Hz, 1H, -Ar*H*), 7.42 (d, *J* = 8.2 Hz, 1H, -Ar*H*), 7.21 (s, 1H, -Indole*H*), 7.15 – 7.08 (m, 1H, -Ar*H*), 7.05 – 6.96 (m, 1H, -Ar*H*), 4.08 (t, *J* = 7.1 Hz, 2H, -C*H*$_2$CH$_2$CH$_2$CH$_3$), 3.61-3.58 (m, 1H, -C*H*CH$_2$(NH$_2$)) 3.29 (dd, *J* = 15.1, 4.3 Hz, 1H, -C*H*$_2$(NH$_2$)C(O)O), 3.01 (dd, *J* = 15.1, 8.4 Hz, 1H, -C*H*$_2$(NH$_2$)C(O)O), 1.70 (m, 2H, -C*H*$_2$CH$_2$CH$_3$), 1.40 – 1.21 (m, 2H, -C*H*$_2$CH$_3$), 0.89 (t, *J* = 7.4 Hz, 3H, -C*H*$_3$).

9-Butyl Adenine (54)[7]

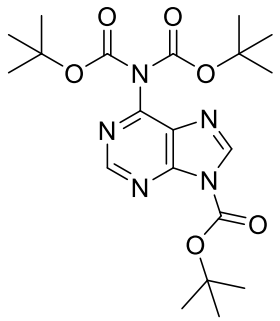


The compound was prepared using adenine (0.27 g, 2.0 mmol) dissolved in DMF (8 mL) along with K_2CO_3 (400 mg) before 1-bromobutane (0.26 mL, 2.40 mmol) was added. The mixture was stirred at RT under nitrogen for 96 hours before the solvent was removed by rotary evaporation and the title compound purified by column chromatography to yield a white solid (8:1:1, $CHCL_3$:MeOH:cC_6H_{12}) (0.35 g, 1.8 mmol, 90%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, *J* = 2.7 Hz, 2H, *H*2, *H*8), 7.21 (s, 2H, -NH_2), 4.12 (t, *J* = 7.1 Hz, 2H, -CH_2CH_2CH_2), 1.81 – 1.70 (m, 2H, -CH_2CH_2CH_3), 1.28 – 1.18 (m, 2H, -CH_2CH_3), 0.87 (t, *J* = 7.4 Hz, 3H, -CH_3).
^{13}C NMR (100 MHz, DMSO) δ 155.98, 152.39 (*C*2), 149.57, 140.89 (*C*8), 118.75, 42.62 (-*C*H_2CH_2CH_2CH_3), 31.49 (-*C*H_2CH_2CH_3), 19.30 (-*C*H_2CH_3), 13.44 (-*C*H_3).

^{1}H and ^{13}C spectra in agreement with literature[5].
ESI-HRMS *m/z* calcd. for $C_9H_{13}N_5[M+H]^+$: 192.1244 Found: 192.1236

Tris-Boc Adenine (55)[8]

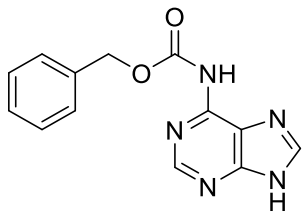


Adenine (1.35 g, 10.0 mmol), DMAP (0.12 g, 1.0 mmol), and Boc_2O (9.04 g, 40 mmol) were added to a flask along with dried THF (50 mL). The mixture was allowed stir at RT under nitrogen for 36 hours whilst being monitored by TLC (7:3, Hexane:EtOAc). The solvent was evaporated to dryness and the product purified by column chromatography (7:3, Hexane:EtOAc) to yield 85% (3.7 g, 8.5 mmol).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.00 (s, 1H, *H*2), 8.50 (s, 1H, *H*8), 1.70 (s, 9H, -N(C(O)OC(CH_3)$_3$, 1.41 (s, 18H, -N(C(O)OC(CH_3)$_3$)$_2$.
^{13}C NMR (100 MHz, Chloroform-*d*) δ 154.18 (*C*2), 152.54, 151.28, 150.11, 145.70, 143.30 (*C*8), 129.67, 87.61 (-*C*(CH_3)$_3$), 84.08 (-(*C*(CH_3)$_3$)$_2$), 28.02 (-N(C(O)OC(*C*H_3)$_3$), 27.83 (-N(C(O)OC(*C*H_3)$_3$)$_2$).

^{1}H and ^{13}C spectra in agreement with literature[8].

***N*^{6}-Cbz-Adenine (56)**[9]



0.66 g of NaH (60% dispersion in oil, 17.0 mmol) was washed with dry DMF before being added to a flask containing DMF (25 mL) along with adenine (0.29 g, 2.0 mmol). The mixture was cooled on ice before ClCOOBn (0.43 mL, 3.0 mmol) was added. The reaction mixture was warmed to RT and stirred for 36 hours. Ice-cold

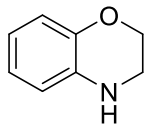
deionised water (50 mL) was added before the pH was adjusted to 7 using 1 M HCl. A white solid precipitate was formed which was collected by filtration and the title compound was recrystallised from DCM:MeOH (0.21 g, 0.78 mmol, 39%).

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.30 (s, 1H, -N^{9}*H*), 11.00 (s, 1H, -N^{6}*H*), 8.59 (s, 1H, *H*2), 8.44 (s, 1H, *H*8), 7.49 – 7.33 (m, 5H, -C6*H*5), 5.28 (s, 2H, -NHCH_2C$_6$H$_5$).

^{13}C NMR (150 MHz, DMSO) δ 161.81 (*C*4), 153.63 (-*C*O), 151.20 (*C*2), 145.97(*C*6), 144.47 (*C*8), 135.92 (-Ar*C*), 128.49 (-Ar*C*), 128.20 (-Ar*C*), 128.00 (-Ar*C*), 112.84 (*C*5), 66.88 (-*C*H$_2$).

^{1}H and ^{13}C spectra in agreement with literature[9].

Benzomorpholine (57)[10]



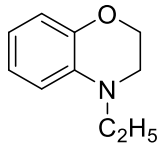
Benzomorpholine was prepared by dissolving 2H-1,4-benzoxazin-3(4H)-one (5.01 g, 33.5 mmol) in dry THF (60 mL). This solution was added drop wise to a flask containing 2.4 M $LiAlH_4$ in THF (83 mmol) whilst cooling on ice. The mixture stirred at reflux for 24 hours before being quenched using 6 mL of deionised water and 3 mL of 15% w/w NaOH. It was diluted with a further 100 mL if deionised water and filtered to remove the salts formed which were washed with THF and added to the reaction solution. The product was then extracted using EtOAc (3 x 100 mL) and the layers combined and dried over Na_2SO_4. The solvent was removed by rotary evaporation to yield a brown oil (94%, 4.27 g, 31.6 mmol).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.91 – 6.79 (m, 2H, -Ar*H*), 6.73 (td, *J* = 7.6, 1.6 Hz, 1H, -Ar*H*), 6.63 (dd, *J* = 7.7, 1.6 Hz, 1H, -Ar*H*), 4.31 – 4.24 (m, 2H, -COCH_2CH$_2$), 3.77 (s, 1H, -N*H*), 3.43 – 3.36 (m, 2H, -CNHCH_2CH$_2$).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 143.97 (-Ar*C*), 133.68 (-Ar*C*), 121.22 (-Ar*C*H), 118.62 (-Ar*C*H), 116.58 (-Ar*C*H), 115.56 (-Ar*C*H), 65.09 (-O*C*H2CH2N), 40.84 (-N*C*H2CH2O).

ESI-HRMS *m/z* calcd. for $C_8H_9NO[M+H]^+$: 136.0757 Found: 136.0754

***N*-Ethyl Benzomorpholine (58)**[11]



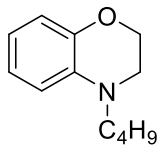
The product was prepared as per the described method. Benzomorpholine (7.7 mmol, 1.05 g) was added to a flask along with acetic acid (25 mL) and cooled on ice before $NaBH_4$ (3.03 g, 80 mmol) was added in portions. The solution was stirred at RT for 3 hours before being neutralised with 15% w/w NaOH and the product extracted with EtOAc (2 x 100 mL). The organic layers were combined and evaporated to dryness and the product purified by column chromatography (9:1, Hexane:EtOAc) to yield the title compound as brown oil (47%, 0.60 g, 3.6 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.80 – 6.71 (m, 1H, -Ar*H*), 6.68 (ddd, *J* = 7.8, 4.1, 1.5 Hz, 2H, -Ar*H*), 6.55 – 6.45 (m, 1H, -Ar*H*), 4.19 – 4.12 (m, 2H, -COCH_2CH$_2$), 3.29 (q, *J* = 7.0 Hz, 2H, -CH_2CH$_3$), 3.25 – 3.20 (m, 2H, -CNCH_2CH$_2$), 1.04 (t, *J* = 7.0 Hz, 3H, -CH_3).
^{13}C NMR (100 MHz, DMSO-d_6) δ 143.90 (-Ar*C*), 134.86 (-Ar*C*) , 121.32 (-Ar*C*H), 116.73 (-Ar*C*H) , 115.83 (-Ar*C*H), 112.16 (-Ar*C*H), 64.14 (-O*C*H$_2$CH$_2$N) , 45.29 (-*C*H$_2$CH$_3$), 44.04 (-N*C*H$_2$CH$_2$O), 10.00 (-*C*H$_3$).

^{1}H and ^{13}C spectra in agreement with literature[11].

ESI-HRMS *m/z* calcd. for $C_{10}H_{13}NO[M+H]^+$: 164.1070 Found: 164.1066

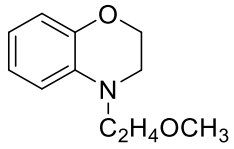
***N*-Butyl Benzomorpholine (59)**[11]



The product was prepared as per the method described for *N*-ethyl benzomorpholine using benzomorpholine (1.1 g, 8.4 mmol), butyric acid (40 mL), and $NaBH_4$ (3.3 g, 85 mmol). The title compound was purified by column chromatography (DCM) to yield the title compound as a brown oil (61%, 5.1 mmol, 0.98 g).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.01 – 6.89 (m, 2H, -Ar*H*), 6.83 – 6.76 (m, 1H, -Ar*H*), 6.77 – 6.68 (m, 1H, -Ar*H*), 4.37 – 4.29 (m, 2H, -COCH_2CH$_2$), 3.46 – 3.31 (m, 4H, -CNCH_2CH$_2$, -CH_2CH$_2$CH$_2$), 1.77 – 1.70 (m, 2H, -CH_2CH$_2$CH$_3$), 1.62 – 1.41 (m, 2H, -CH_2CH$_3$), 1.11 (t, *J* = 7.4 Hz, 3H, -CH$_2$CH_3).
^{13}C NMR (100 MHz, Chloroform-*d*) δ 143.90 (-Ar*C*), 135.28 (-Ar*C*), 121.47 (-Ar*C*H), 116.91 (-Ar*C*H), 116.16 (-Ar*C*H), 111.89 (-Ar*C*H), 64.39 (-O*C*H$_2$CH$_2$N), 50.64 (-*C*H$_2$CH$_2$CH$_2$CH$_3$), 46.87 (-N*C*H$_2$CH$_2$O), 28.10 (*C*H$_2$CH$_2$CH$_3$), 20.36 (-*C*H$_2$CH$_3$), 13.97 (-*C*H$_3$).

***N*-Methoxyethyl Benzomorpholine (60)**[11]



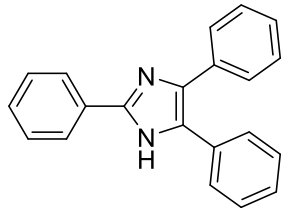
The product was prepared as per the method described for *N*-ethyl benzomorpholine using benzomorpholine (0.92 g, 6.8 mmol), methoxyacetic acid (30 mL), and $NaBH_4$ (2.7 g, 70 mmol). The title compound was purified by column chromatography (DCM) to yield an orange/brown oil (65%, 4.4 mmol, 0.85 g).

^{1}H NMR (400 MHz DMSO-d_6) δ 6.81 – 6.71 (m, 1H, -Ar*H*), 6.70 (ddd, *J* = 8.1, 2.8, 1.6 Hz, 2H, -Ar*H*), 6.57 – 6.48 (m, 1H, -Ar*H*), 4.16 – 4.08 (m, 2H, -COCH_2CH$_2$),

3.55 – 3.48 (m, 2H, -C*H*$_2$OCH$_3$), 3.45 – 3.38 (m, 2H, -NC*H*$_2$CH$_2$OCH$_3$), 3.38 – 3.33 (m, 2H, -CNC*H*$_2$CH$_2$), 3.26 (s, 3H, -OC*H*$_3$).

^{13}C NMR (100 MHz, DMSO-d_6) δ 143.59 (-Ar*C*), 135.00 (-Ar*C*), 121.31 (-Ar*C*H), 116.60 (-Ar*C*H), 115.88 (-Ar*C*H), 111.94 (-Ar*C*H), 69.21 (-NCH2*C*H2OCH3), 63.98 (-O*C*H2CH2N), 58.19 (-O*C*H3), 49.75 (-N*C*H2CH2OCH3), 47.37 (-N*C*H2CH2O).

2,4,5-Triphenylimidazole (78)[12]



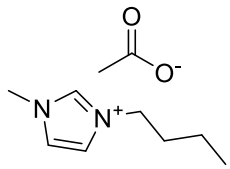
The compound was prepared using a modified method of that reported in the literature[12]. Benzoin (0.23 g, 1.0 mmol), ammonium acetate (5.0 mmol), sodium bisulphite (0.02 g, 0.2 mmol), and benzaldehyde (0.15 mL, 1.5 mmol) were added to a flask along with 1:1 ethanol:H_2O (10 mL). The solution was heated to 90°C and stirred for five hours. Upon cooling, the precipitate was collected by filtration and the title compound recrystallised from ethanol in 72% yield (0.21 g, 0.7 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H, -N*H*), 8.11 – 8.04 (m, 2H, -C2Ar*H*), 7.57 – 7.18 (m, 13H, -Ar*H*).
^{13}C NMR (151 MHz, DMSO) δ 145.81 (*C*2), 137.36, 135.25, 131.16, 130.42, 128.97, 128.93, 128.62, 128.60, 128.50, 128.46, 128.06, 127.36, 126.86, 125.45

^{1}H and ^{13}C spectra in agreement with literature[13].

ESI-HRMS *m/z* calcd. for $C_{21}H_{16}N_2[M+H]^+$: 297.1386 Found: 297.1391

1-Butyl-3-methylimidazolium Acetate (83)[14]

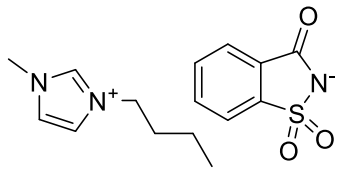


Equimolar amounts of BMIM chloride (0.18 g, 1 mmol) and silver acetate (0.17 g, 1 mmol) were added to H_2O and the flask wrapped in aluminium foil. The solution was stirred at RT for 3 hours following which the AgCl was removed by filtration. Charcoal was added to the solution and stirred for 12 hours to remove coloured impurities before being filtered off. The solvent was removed by rotary evaporation and to yield the title compound as a colourless oil (0.19 g, 0.96 mmol, 96%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H, -C2*H*), 7.97 (t, *J* = 1.7 Hz, 1H, -C4/5*H*), 7.89 (t, *J* = 1.6 Hz, 1H, -C4/5*H*), 4.20 (t, *J* = 7.2 Hz, 2H, -C*H*$_2$C$_3$H$_7$), 3.88 (s, 3H, -C*H*$_3$), 1.78 – 1.67 (m, 2H, -CH$_2$C*H*$_2$C$_2$H$_5$), 1.58 (s, 3H, -C*H*$_3$COO), 1.20 (h, *J* = 7.4 Hz, 2H, -C$_2$H$_4$C*H*$_2$CH$_3$), 0.84 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$C*H*$_3$).
^{3}C NMR (150 MHz, DMSO-d_6) δ 175.02 (-*C*OO), 136.92 (*C*2), 123.82 (*C*4/5), 122.47 (*C*4/5), 48.79 (-*C*H$_2$C$_3$H$_7$), 35.95 (-*C*H$_3$), 31.64 (-CH$_2$*C*H$_2$C$_2$H$_5$), 25.52 (-*C*H$_3$COO), 19.04 (C$_2$H$_4$*C*H$_2$CH$_3$), 13.54 (-C$_3$H$_6$*C*H$_3$).

^{1}H and ^{13}C spectra in agreement with literature[15].

1-Butyl-3-methylimidazolium Saccharinate (100)[16]



BMIM chloride (1.9 g, 11 mmol) was added to acetone (70 mL) before sodium saccharinate.2H_2O (4.8 g, 20 mmol) was added. The mixture was stirred at RT for 24 hours before the excess starting material and NaCl were removed by filtration

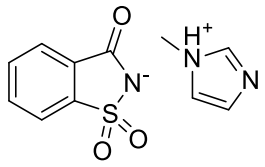
through celite. The solvent was removed by rotary evaporation to yield the title compound (3.2 g, 10 mmol, 87%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.80 (s, 1H, -C2*H*), 7.79 – 7.70 (m, 2H, -Ar*H*), 7.59 – 7.51 (m, 2H, -Ar*H*), 7.40 (t, *J* = 1.7 Hz, 1H, -C4/5*H*), 7.31 (t, *J* = 1.8 Hz, 1H, --C4/5*H*), 4.24 (t, *J* = 7.4 Hz, 2H, -C$H_2$$C_3H_7$), 4.03 (s, 3H, -C$H_3$), 1.84 – 1.76 (m, 2H, -$CH_2CH_2$$C_2H_5$), 1.33 – 1.26 (m, 2H, -$C_2H_4CH_2$$CH_3$), 0.87 (t, *J* = 7.4 Hz, 3H, -C_3H_6CH_3).
^{13}C NMR (150 MHz, Chloroform-*d*) δ 170.42 (-*C*O), 144.65 (-Ar*C*), 137.68 (*C*2), 134.78 (-Ar*C*), 132.09 (-Ar*C*H), 131.47 (-Ar*C*H), 123.55 (-Ar*C*H), 123.24 (-Ar*C*H), 121.92 (*C*4/5), 119.71 (*C*4/5), 49.83 (-*C*$H_2$$C_3H_7$), 36.59 (-*C*$H_3$), 32.12 (-$CH_2$*C*$H_2$$C_2H_5$), 19.45 ($C_2H_4$*C*$H_2$$CH_3$), 13.43 (-$C_3H_6$*C*$H_3$).

^{1}H and ^{13}C spectra in agreement with literature[16].

ESI-HRMS *m/z* calcd. for $[C_8H_{15}N_2]^+$: 139.1230 Found: 139.1228
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9913

Saccharin-1-methylimidazole (106)[17]



The title compound was synthesised through addition of saccharin (0.2 g, 1 mmol) to 1-methylimidazole (0.9 mL, 1.1 mmol) in acetonitrile (20 mL). The solution was stirred at RT for 18 hours before the reaction mixture was concentrated under reduced pressure. The solid was washed with hexane to remove residual impurities (10 mL) and the title compound dried (0.25 g, 0.96 mmol, 96%).

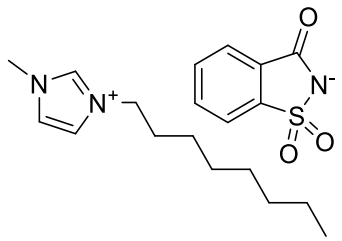
^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (s, 1H, -C2*H*), 7.74 – 7.65 (m, 2H, -C4/5*H*), 7.65 – 7.57 (m, 4H, -Ar*H*), 3.86 (s, 3H, -CH_3).

^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 167.73 (-*C*O), 144.99 (-Ar*C*), 135.92 (*C*2), 134.40 (-Ar*C*), 131.78 (-Ar*C*H), 131.29 (-Ar*C*H), 123.03 (*C*4/5), 122.63 (-Ar*C*H), 120.18 (*C*4/5), 119.26 (-Ar*C*H), 35.30 (-*C*H_3).

^{1}H and ^{13}C spectra in agreement with literature[18].

ESI-HRMS *m/z* calcd. for $[C_4H_7N_2]^+$: 83.0609 Found: 83.0607
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9914

1-Octyl-3-methylimidazolium Saccharinate (109)[16]



The title compound was prepared through a modified version of the published method. OMIM chloride (0.23g, 1 mmol) was dissolved in acetone (20 mL) before sodium saccharinate.2H_2O (0.48 g, 2 mmol) was added. The mixture was allowed stir at RT for 24 hours before the excess starting material and NaCl were removed by filtration through celite. The title compound was isolated as a pale yellow oil after removal of the solvent by rotary evaporation (0.31 g, 0.82 mmol, 82%).

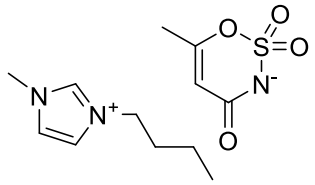
^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.30 (s, 1H, -C2*H*), 7.74 – 7.64 (m, 2H, -Ar*H*), 7.55 – 7.47 (m, 2H, -Ar*H*), 7.36 (t, *J* = 1.8 Hz, 1H, -C4/5*H*), 7.24 (t, *J* = 1.8 Hz, 1H, -C4/5*H*), 4.13 – 4.06 (m, 2H, -C*H*$_2$C$_7$H$_{15}$), 3.93 (s, 3H, -C*H*$_3$), 1.73 – 1.63 (m, 2H, -CH$_2$C*H*$_2$C$_6$H$_{13}$), 1.21 – 1.07 (m, 10H, -C$_2$H$_4$C$_5$*H*$_{10}$CH$_3$), 0.78 (t, *J* = 7.2 Hz, 3H, -C*H*$_3$).

^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.28 (-*C*O), 144.36 (-Ar*C*), 136.93 (*C*2), 134.34 (-Ar*C*), 132.23 (-Ar*C*H), 131.62 (-Ar*C*H), 123.72 (*C*4/5), 123.33 (-Ar*C*H), 121.92 (*C*4/5), 119.72 (-Ar*C*H), 49.91 (-*C*H$_2$C$_7$H$_{15}$), 36.47 (-*C*H$_3$), 31.67 (-*C*$_6$H$_{12}$CH$_3$), 30.11 (-*C*$_6$H$_{12}$CH$_3$), 28.99 (-*C*$_6$H$_{12}$CH$_3$), 28.89 (-*C*$_6$H$_{12}$CH$_3$), 26.15 (-*C*$_6$H$_{12}$CH$_3$), 22.57 (-*C*$_6$H$_{12}$CH$_3$), 14.07 (-C$_7$H$_{14}$*C*H$_3$).

^{1}H and ^{13}C spectra in agreement with literature[19].

ESI-HRMS *m/z* calcd. for $[C_{12}H_{23}N_2]^+$: 195.1856 Found: 195.1847
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9919

1-Butyl-3-methylimidazolium Acesulfamate (110)[20]



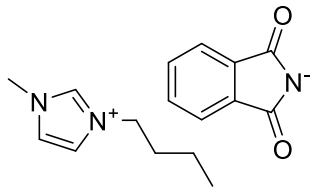
BMIM choride (0.71 g, 4 mmol) was added to a flask along with potassium acesulfamate (1.61 g, 8 mmol) and acetone (30 mL). The mixture was allowed stir at RT for 24 hours before the excess starting material and KCl were removed by filtration. The acetone was removed by rotary evaporation and the crude product resuspended in $CHCl_3$ and filtered once more to remove solid impurities. The title compound was dried to yield a colourless oil (1.1 g, 3.5 mmol, 90%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.54 (s, 1H, -C2*H*), 7.37 (t, *J* = 1.8 Hz, 1H, -C4/5*H*), 7.31 (t, *J* = 1.8 Hz, 1H, -C4/5*H*), 5.42 (s, 1H, -C*H*), 4.20 (t, *J* = 7.4 Hz, 2H, -CH_2C$_3$H$_7$), 3.96 (s, 3H, -CH_3), 1.98 (s, 3H, -C(O)CH_3), 1.86 – 1.73 (m, 2H, -CH$_2$CH_2C$_2$H$_5$), 1.36 – 1.25 (m, 2H, , -C$_2$H$_4$CH_2CH$_3$), 0.89 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$CH_3).
^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.12 (-*C*O), 161.25 (-*C*O(CH$_3$)), 137.40 (*C*2), 123.57 (*C*4/5), 122.00 (*C*4/5), 102.20 (-*C*H), 49.76 (-*C*H$_2$C$_3$H$_7$), 36.45 (-*C*H$_3$), 32.11 (-CH$_2$*C*H$_2$C$_2$H$_5$), 20.00 (C$_2$H$_4$*C*H$_2$CH$_3$), 19.43 (-C(O)*C*H$_3$), 13.45 (-C$_3$H$_6$*C*H$_3$).

^{1}H and ^{13}C spectra in agreement with literature[21].

ESI-HRMS *m/z* calcd. for $[C_8H_{15}N_2]^+$: 139.1230 Found: 139.1232
m/z calcd. for $[C_4H_4NO_4S]^-$: 161.9867 Found: 161.9867

1-Butyl-3-methylimidazolium Phthalimate (111)

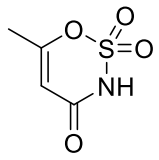


The title compound was prepared through the addition of BMIM chloride (0.72 g, 4 mmol) to a flask along with potassium phthalimate (1.48 g, 8 mmol) and acetone (40 mL). The mixture was allowed stir at RT for 24 hours before the excess starting material and KCl were removed by filtration. The acetone was removed by rotary evaporation and the crude product resuspended in $CHCl_3$ and filtered once more to remove solid impurities. The product was dried to yield the title compound as a colourless oil (0.98 g, 3.4 mmol, 86%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.51 – 7.36 (m, 4H), 7.35 (d, *J* = 1.8 Hz, 1H), 7.30 (d, *J* = 1.7 Hz, 1H), 4.06 (t, *J* = 7.2 Hz, 2H, -C*H*$_2$C$_3$H$_7$), 3.76 (s, 3H), 1.72 (p, *J* = 7.3 Hz, 2H), 1.20 (h, *J* = 7.4 Hz, 2H), 0.82 (t, *J* = 7.4 Hz, 3H).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 178.47 (-*C*O), 177.50 (-*C*O), 140.44 (-Ar*C*), 135.81 (-Ar*C*), 133.00 (-Ar*C*H), 131.56 (-Ar*C*H), 130.16 (-Ar*C*H), 129.65 (-Ar*C*H), 125.79 (*C*4/5), 124.53 (*C*4/5), 51.61 (-*C*H$_2$C$_3$H$_7$), 37.96 (-*C*H$_3$), 33.66 (-CH$_2$*C*H$_2$C$_2$H$_5$), 21.17 (C$_2$H$_4$*C*H$_2$CH$_3$), 15.07 (-C$_3$H$_6$*C*H$_3$).

ESI-HRMS *m/z* calcd. for $[C_8H_{15}N_2]^+$: 139.1230 Found: 139.1232
m/z calcd. for $[C_8H_4NO_2]^-$: 146.0248 Found: 146.0247

Acesulfame (112)[22]



Potassium acesulfamate (1.41 g, 7 mmol) was dissolved in H_2O (10 mL) before the pH was adjusted to 2 using conc. HCl (2 mL). The solution was allowed stir for 12 hours before the product was extracted using EtOAc (3 x 20 mL). The organic layers were combined and dried over $MgSO_4$ and filtered before being evaporated to dryness. Residual starting material was removed by re-suspending the white solid in DCM and filtering off the solid impurity.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.98 (br. s, 1H, -N*H*), 6.03 (s, 1H, -C*H*), 2.18 (d, *J* = 1.0 Hz, 3H, -CH_3).
^{13}C NMR (150 MHz, DMSO-d_6) δ 170.15 (-*C*O), 168.12 (-*C*O(CH$_3$)), 96.90 (-*C*H), 19.83 (-*C*H$_3$).

General Method A: Preparation of Silver salts of Saccharin, Acesulfame, Phthalimide, and Cyclamate[20]

Equimolar amounts of the sodium or potassium salt of the required anion were combined with $AgNO_3$ in H_2O. The solution was protected from light and allowed stir at RT for 18 hours. The silver salt of the product precipitated from solution and was isolated by filtration before being washed with ethanol and H_2O and dried.

General Method B: Preparation of *N*-Alkylpyridinium and *N*,*N*,*N*-Trimethyl-*p*-toluidinium Halides[23]

N-methyl pyridinium and *N*,*N*,*N*-trimethyl-*p*-toluidinium halides were prepared through reaction of pyridine or DM*p*T with 1.1 equivalents of the respective alkyl halide. The reagents were refluxed in methanol for 18 hours before being cooled to RT and EtOAc added causing a precipitate to occur. The precipitate was isolated and washed with EtOAc (10 mL) to yield the title compound.

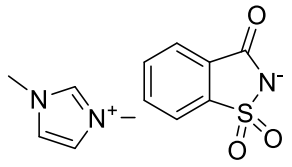
General Method C: Preparation of N,N-Dialkyl-Pyrrolidinium Halides[24]**.**

To a solution of pyrrolidine in 1:1 MeOH:H_2O was added one equivalent of Na_2CO_3 and four equivalents of the respective alkyl halide. The solution was stirred at reflux for 18 hours before being cooled to RT and the solvent removed by rot. evap. The crude product was suspended in DCM and the insoluble impurities removed to yield the title compound.

General Method D: Preparation of Saccharinate, Acesulfamate, Phthalimate, and Cyclamate Salts of Nitrogen Heterocycles

The halide salt of the nitrogen heterocycle was dissolved in 1:1 Acetone:H_2O (50 mL) before an equimolar amount of the silver salt of the respective anion was added. The mixture was protected from light and allowed stir at RT for 18 hours. The silver halide salt was removed by filtration before the solvent was evaporated to dryness. The product was then dissolved in DCM or ACN and the insoluble impurities removed before being dried once more to yield the purified title compound.

1,3-Dimethylimidazolium Saccharinate (107)

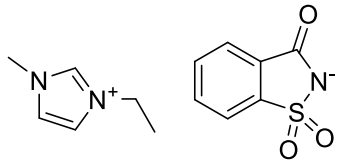


The title compound was prepared as per general method D using 1,3-dimethylimidazolium iodide (0.68 g, 3.0 mmol) and silver saccharinate 0.87 g, 3.0 mmol) to yield the title compound as a white solid (0.77 g, 2.8 mmol, 92%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.56 (s, 1H, -C2*H*), 7.75 – 7.66 (m, 2H, -Ar*H*), 7.56 – 7.49 (m, 2H, -Ar*H*), 7.31 (d, *J* = 1.5 Hz, 2H, -C4/5*H*), 3.94 (s, 6H, -(CH_3)$_2$).
^{13}C NMR (150 MHz, Chloroform-*d*) δ 170.34 (-*C*O), 144.59 (-Ar*C*), 138.07 (*C*2), 134.73 (-Ar*C*), 132.18 (-Ar*C*H), 131.55 (-Ar*C*H), 123.42 (-Ar*C*H), 123.22 (*C*4/5), 119.68 (-Ar*C*H), 36.45 (-(*C*H_3)$_2$).

ESI-HRMS *m/z* calcd. for $[C_5H_9N_2]^+$: 97.0760 Found: 97.0757
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9914

1-Ethyl-3-methylimidzolium Saccharinate (108)



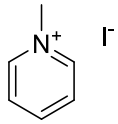
The title compound was prepared as per general method D using 1-ethyl-3-methylimidazolium iodide (0.71 g, 3.0 mmol) and silver saccharinate (0.85 g, 3 mmol) to yield the title compound (0.76 g, 2.6 mmol, 86%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 – 7.67 (m, 1H, -Ar*H*), 7.72 – 7.63 (m, 3H, -Ar*H*), 7.32 (d, *J* = 2.0 Hz, 1H, -C4/5*H*), 7.25 (d, *J* = 2.0 Hz, 1H, -C4/5*H*), 4.08 (q, *J* = 7.4 Hz, 2H, -CH_2CH$_3$), 3.75 (s, 3H, -CH_3), 1.36 (t, *J* = 7.4 Hz, 3H, -CH$_2$CH_3).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.83 (-*C*O), 144.24 (-Ar*C*), 136.25 (-Ar*C*H), 135.74 (-Ar*C*H), 134.74 (-Ar*C*), 126.01 (-Ar*C*H), 125.71 (*C*4/5), 124.13 (*C*4/5), 122.67 (-Ar*C*H), 47.09 (-*C*$H_2$$CH_3$), 37.89 (-*C*$H_3$), 16.78 (-$CH_2$*C*$H_3$).

ESI-HRMS *m/z* calcd. for $[C_6H_{11}N_2]^+$: 111.0917 Found: 111.0921
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9908

N-Methylpyridinium Iodide (113)[23]

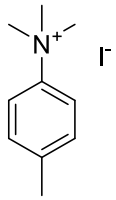


The title compound was prepared as per general method B using pyridine (6.11 mL, 76 mmol) and methyl iodide (5.23 mL, 84 mmol) to yield the title compound as a white solid (15.23 g, 69 mmol, 91%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.81 (d, *J* = 5.9 Hz, 2H, -Ar*H*), 8.54 (t, *J* = 7.9 Hz, 1H, -Ar*H*), 8.07 (t, *J* = 7.0 Hz, 2H, -Ar*H*), 4.41 (s, 3H, -C*H*$_3$).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 145.31 (-Ar*C*H), 145.10 (-Ar*C*H), 128.00 (-Ar*C*H), 48.44 (-*C*H_3).

^{1}H and ^{13}C spectra in agreement with literature[25].

***N*,*N*,*N*-Trimethyl-*p*-toluidinium Iodide (114)**

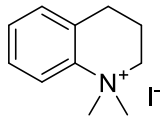


The title compound was prepared as per general method B using DM*p*T (6.32 mL, 44 mmol) and methyl iodide (3.42 mL, 55 mmol) to yield the title compound as a white solid (11.16 g, 39 mmol, 90%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.64 (d, *J* = 9.0 Hz, 2H, -Ar*H*), 7.39 (d, *J* = 8.8 Hz, 2H, -Ar*H*), 3.57 (s, 9H, -(CH_3)$_3$), 2.32 (s, 3H, -CH_3).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 143.99 (-Ar*C*N), 141.04 (-Ar*C*), 130.65 (-Ar*C*H), 119.25 (-Ar*C*H), 56.91 (-(*C*H$_3$)$_3$), 19.90 (-*C*H$_3$).

^{1}H spectrum in agreement with literature[26]

***N*,*N*-Dimethyltetrahydroquinolinium Iodide (115)**[27]

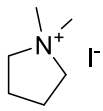


To a flask was added THQ (0.13 mL, 1 mmol) along with Na_2CO_3 (0.11 g, 1 mmol), methyl iodide (0.31 mL, 10 mmol) and 50 mL of 1:1 MeOH:H_2O. The solution was heated to reflux and stirred for 24 hours before being cooled to RT and the solvent removed. The crude product was dissolved in H2O and extracted using $CHCl_3$ (3 x 20 mL). The organic layers were pooled and dried over $MgSO_4$ being evaporated to dryness to yield the pure product sample (0.23 g, 0.87 mmol, 87%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 – 8.09 (m, 1H, -Ar*H*), 7.46 – 7.31 (m, 2H, -Ar*H*), 7.28 – 7.20 (m, 1H, -Ar*H*), 4.31 – 4.23 (m, 2H, -NCH_2), 3.93 (s, 6H, -(CH_3)$_2$), 3.04 (t, *J* = 6.6 Hz, 2H-CH_2), 2.31 (dt, *J* = 12.0, 6.5 Hz, 2H, -CH_2).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 142.81 (-Ar*C*), 131.42 (-Ar*C*), 130.29 (-Ar*C*H), 129.75 (-Ar*C*H), 129.09 (-Ar*C*H), 121.87 (-Ar*C*H), 65.05 (-N*C*H$_2$), 58.22 (-(*C*H$_3$)$_2$), 25.90 (-*C*H$_2$), 17.88 (-*C*H$_2$).

N,*N*-Dimethylpyrrolidinium Iodide (116)[24]

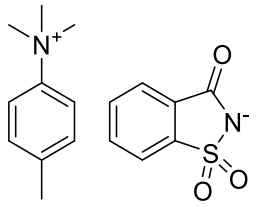


The title compound was prepared as per general method C using pyrrolidine (0.16 mL, 2 mmol) and methyl iodide (0.50 mL, 8 mmol) to yield the title compound (0.34 g, 1.5 mmol, 75%).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 3.56 – 3.47 (m, 4H, -N(C*H*$_2$)$_2$), 3.14 (s, 6H, -(C*H*$_3$)$_2$), 2.27 – 2.18 (m, 4H, -(C*H*$_2$)$_2$).
^{13}C NMR (150 MHz, Deuterium Oxide) δ 65.84 (-(N*C*H$_2$)$_2$), 51.74 (-*C*H$_3$), 21.65 (-(*C*H$_2$)$_2$).

^{1}H and ^{13}C spectra in agreement with literature[24].

***N*,*N*,*N*-Trimethyl-*p*-toluidinium Saccharinate (117)**

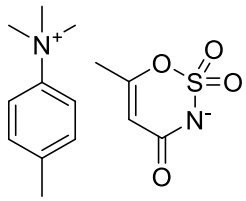


The title compound was prepared as per general method D using TM*p*T iodide (1.25 g, 4.5 mmol) and silver saccharinate (1.30 g, 4.5 mmol) to yield the title compound as a white salt (0.98 g, 3.0 mmol, 66%).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.78 – 7.65 (m, 4H, -Ar*H*), 7.54 (d, *J* = 8.2 Hz, 2H, -Ar*H*), 7.27 (d, *J* = 8.1 Hz, 2H, -Ar*H*), 3.53 (s, 9H, -(CH_3)$_3$), 2.26 (s, 3H, -CH_3).
^{13}C NMR (150 MHz, Deuterium Oxide) δ 172.36 (-*C*O), 144.12 (-Ar*C*N), 142.00 (-Ar*C*), 141.14 (-Ar*C*), 133.84 (-Ar*C*H), 133.31 (-Ar*C*H), 132.49 (-Ar*C*), 130.70 (-Ar*C*H), 123.66 (-Ar*C*H), 120.25 (-Ar*C*H), 119.16 (-Ar*C*H), 56.96 (-(*C*H_3)$_3$), 19.93 (-*C*H_3).

ESI-HRMS *m/z* calcd. for $[C_{10}H_{16}N]^+$: 150.1277 Found: 150.1281
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9918

***N*,*N*,*N*-Trimethyl-*p*-toluidinium Acesulfamate (118)**



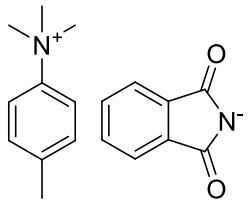
The title compound was prepared as per general method D using TM*p*T iodide (1.32 g, 4.8 mmol) and silver acesulfamate (1.31 g, 4.8 mmol) to yield the title compound (1.33 g, 4.3 mmol, 89%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.63 – 7.54 (m, 2H, -Ar*H*), 7.36 – 7.28 (m, 2H, -Ar*H*), 5.49 (s, 1H, -C*H*), 3.52 (s, 9H, -(CH_3)$_3$), 2.27 (s, 3H, -ArCH_3), 1.93 (s, 3H, -CH_3).

^{13}C NMR (100 MHz, Deuterium Oxide) δ 171.76 (-*C*O), 163.68 (-*C*O(CH$_3$)), 144.00 (-Ar*C*N), 140.98 (-Ar*C*), 130.65 (-Ar*C*H), 119.11 (-Ar*C*H), 100.86 (-*C*H), 56.76 (-(*C*H$_3$)$_3$), 19.86 (-*C*H$_3$), 18.84 (-*C*H$_3$).

ESI-HRMS m/z calcd. for [$C_{10}H_{16}N$]$^+$: 150.1277 Found: 150.1282
m/z calcd. for [$C_4H_4NO_4S$]$^-$: 161.9867 Found: 161.9872

***N*,*N*,*N*-Trimethyl-*p*-toluidinium Phthalimate (119)**



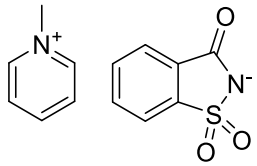
The title compound was prepared as per general method D using TM*p*T iodide (1.41 g, 5.1 mmol) and silver phthalimate (1.31 g, 5.1 mmol) to yield the title compound (0.94 g, 3.2 mmol, 62 %).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.64 (d, *J* = 8.8 Hz, 2H, -Ar*H*), 7.54 – 7.37 (m, 6H, -Ar*H*), 3.55 (s, 9H, -(CH_3)$_3$), 2.35 (s, 3H, -CH_3).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 175.12 (-*C*O), 174.16 (-*C*O), 143.33 (-Ar*C*N), 140.35 (-Ar*C*), 137.33 (-Ar*C*), 132.49 (-Ar*C*), 129.90 (-Ar*C*H), 129.74 (-Ar*C*H), 128.22 (-Ar*C*H), 126.90 (-Ar*C*H), 126.43 (-Ar*C*H), 118.39 (-Ar*C*H), 56.07 (-(*C*H$_3$)$_3$), 19.07 (-*C*H$_3$).

ESI-HRMS *m/z* calcd. for [$C_{10}H_{16}N$]$^+$: 150.1277 Found: 150.1284

***N*-Methylpyridinium saccharinate (120)**

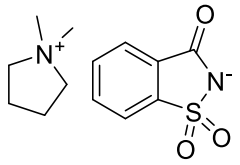


The title compound was prepared as per general method D using *N*-methylpyridinium iodide (1.2 g, 5.4 mmol) and silver saccharinate (1.57 g, 5.4 mmol) to yield the title compound (1.42 g, 5.1 mmol, 95%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.60 (d, *J* = 5.8 Hz, 2H, -Ar*H*), 8.32 (t, *J* = 7.9 Hz, 1H, -Ar*H*), 7.84 (t, *J* = 7.1 Hz, 2H, -Ar*H*), 7.71 – 7.53 (m, 4H, -Ar*H*), 4.25 (s, 3H, -CH_3).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 172.02 (-*C*O), 144.97 (-Ar*C*), 144.75 (-Ar*C*), 141.65 (-Ar*C*H), 133.66 (-Ar*C*H), 133.18 (-SaccAr*C*H), 132.10 (-SaccAr*C*H), 127.70 (-Ar*C*H), 123.41 (-SaccAr*C*H), 120.09 (-SaccAr*C*H), 47.93 (-*C*H_3).

ESI-HRMS *m/z* calcd. for $[C_6H_8N]^+$: 94.0651 Found: 94.0648
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9921

***N*,*N*-Dimethylpyrrolidinium saccharinate (121)**

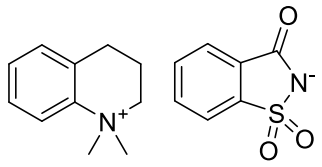


The title compound was prepared as per general method D using *N*,*N*-dimethylpyrrolidinium iodide (0.34 g, 1.5 mmol) and silver saccharinate (0.43 g, 1.5 mmol) to yield the title compound (0.36 g, 1.3 mmol, 84%).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.77 – 7.73 (m, 1H, -Ar*H*), 7.70 – 7.64 (m, 3H, -Ar*H*), 3.40 – 3.34 (m, 4H, -N(CH_2)$_2$), 3.00 (s, 6H, -(CH_3)$_2$), 2.11 – 2.06 (m, 4H, -(CH_2)$_2$).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 172.45 (-*C*O), 141.78 (-Ar*C*), 133.86 (-Ar*C*), 133.34 (-Ar*C*H), 132.33 (-Ar*C*H), 123.66 (-Ar*C*H), 120.27 (-Ar*C*H), 65.72 (-(N*C*H$_2$)$_2$), 51.56 (-(*C*H$_2$)$_2$), 21.56 (-(*C*H$_3$)$_2$).

***N*,*N*-Dimethyltetrahydroquinolinium saccharinate (122)**

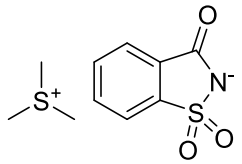


The title compound was prepared as per general method D using N,N-tetrahydroquinolinium iodide (0.15 g, 0.55 mmol) and silver saccharinate (0.16 g, 0.55 mmol) to yield the title compound (0.14 g, 0.42 mmol, 77%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 – 7.94 (m, 1H, -Ar*H*), 7.74 – 7.56 (m, 4H, -Ar*H*), 7.48 – 7.32 (m, 3H, -Ar*H*), 3.93 – 3.85 (m, 2H, -NC*H*$_2$), 3.56 (s, 6H, -(C*H*$_3$)$_2$), 2.91 (t, *J* = 6.5 Hz, 2H, -C*H*$_2$), 2.25 – 2.16 (m, 2H, -C*H*$_2$).
^{13}C NMR (100 MHz, DMSO-d_6) δ 167.77(-*C*O), 145.02 , 142.92 , 134.50 , 131.88 , 131.37 , 131.15 , 130.81 , 129.67 , 128.14 , 122.73 , 121.45 , 119.35 , 63.79 (-*C*H$_2$), 56.79 (-(*C*H$_3$)$_2$), 25.51 (-*C*H$_2$), 16.91 (-*C*H$_2$).

ESI-HRMS *m/z* calcd. for $[C_{11}H_{16}N]^+$ 162.1277 Found: 162.1276
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9925

Trimethylsulphonium Saccharinate (123)

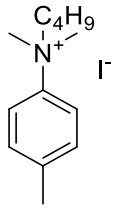


Dimethylsulfide (0.15 mL, 2 mmol) was added to a small vial along with methyl iodide (0.16 mL, 2.5 mmol) and the solution was allowed stir neat at RT for 3 hours to yield a white solid. The reaction mixture was taken up in ACN (10 mL) and transferred to a flask where the solvent was removed to yield a white solid.
The white solid was dissolved in 1:1 acetone:H_2O without any further purification before silver saccharinate was added (0.16 g, 0.5 mmol). The mixture was protected from light and allowed stir at RT for 18 hours. The AgI was removed by filtration and the solvent by rotary evaporation.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 – 7.57 (m, 4H, -Ar*H*), 2.86 (s, 9H, -(CH_3)$_3$).
^{13}C NMR (100 MHz, DMSO-d_6) δ 167.83 (-*C*O), 144.98 (-Ar*C*), 134.47 (-Ar*C*), 131.93 (-Ar*C*H), 131.42 (-Ar*C*H), 122.74 (-Ar*C*H), 119.37 (-Ar*C*H), 26.21 (-(*C*H$_3$)$_3$).

ESI-HRMS *m/z* calcd. for $[C_3H_9S]^+$: 77.0419 Found: 77.0418
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9912

***N*-Butyl-*N*,*N*-dimethyl-p-toluidinium Iodide (124)**



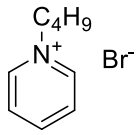
To a flask was added DM*p*T (0.90 mL, 6.3 mmol) along with 1-iodobutane (0.93 mL, 6.9 mmol) and MeOH (30 mL). The solution was heated to reflux and stirred for 24 hours. Upon cooling to RT the solvent was removed and EtOAc (50 mL) added to the flask. The product was extracted using H_2O (3 x 20 mL) and, after pooling, the

aqueous layer was washed with EtOAc. The solvent was removed by rotary evaporation to yield the title compound as an orange/red oil (1.44 g, 4.5 mmol, 71%)

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.60 (d, *J* = 8.9 Hz, 2H, -Ar*H*), 7.42 (d, *J* = 8.8 Hz, 2H, -Ar*H*), 3.83 – 3.77 (m, 2H, -C*H*$_2$C$_3$H$_7$), 3.54 (s, 6H, -(C*H*$_3$)$_2$), 2.35 (s, 3H, -C*H*$_3$), 1.41 – 1.33 (m, 2H, -CH$_2$C*H*$_2$C$_2$H$_5$), 1.24 (h, *J* = 7.4 Hz, 2H, -C*H*$_2$CH$_3$), 0.79 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$C*H*$_3$).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 141.44 (-Ar*C*N), 141.03 (-Ar*C*), 130.77 (-Ar*C*H), 120.23 (-Ar*C*H), 69.24 (-*C*H$_2$C$_3$H$_7$), 54.21 (-(*C*H$_3$)$_2$), 24.80 (-CH$_2$*C*H$_2$C$_2$H$_5$), 19.94 (-*C*H$_3$), 18.80 (-*C*H$_2$CH$_3$), 12.74 (-C$_3$H$_6$*C*H$_3$).

***N*-Butylpyridinium Bromide (125)**



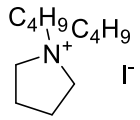
The title compound was prepared as per general method B using pyridine (1.12 mL, 13.9 mmol) and 1-bromobutane (1.62 mL, 13.9 mmol) to yield the title compound (2.48 g, 11.5 mmol, 83%)

^{1}H NMR (600 MHz, Deuterium Oxide) δ 8.83 (d, *J* = 5.7 Hz, 2H, -Ar*H*), 8.52 (t, *J* = 7.8 Hz, 1H, -Ar*H*), 8.04 (t, *J* = 6.9 Hz, 2H, -Ar*H*), 4.60 (t, *J* = 7.4 Hz, 2H, -C*H*$_2$C$_3$H$_7$), 1.98 (p, *J* = 7.5 Hz, 2H, -CH$_2$C*H*$_2$C$_2$H$_5$), 1.34 (h, *J* = 7.4 Hz, 2H, -C*H*$_2$CH$_3$), 0.92 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$C*H*$_3$).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 145.43 (-Ar*C*H), 144.16 (-Ar*C*H), 128.15 (-Ar*C*H), 61.71 (-*C*H$_2$C$_3$H$_7$), 32.54 (-CH$_2$*C*H$_2$C$_2$H$_5$), 18.69 (-*C*H$_2$CH$_3$), 12.63 (-*C*H$_3$).

^{1}H and ^{13}C spectra in agreement with literature[28].

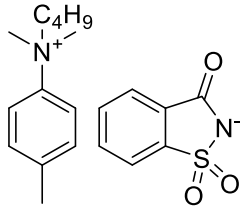
***N*,*N*-Dibutylpyrrolidinium Iodide (126)**



The title compound was prepared as per general method C using pyrrolidine (1.31 mL, 16 mmol) and 1-iodobutane (4.71 mL, 35 mmol) to yield the title compound (3.43 g, 11.0 mmol, 69%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 3.44 (s, 4H, -N(C*H*$_2$)$_2$), 3.23 – 3.14 (m, 4H, -(C*H*$_2$C$_3$H$_7$)$_2$), 2.13 – 2.07 (m, 4H, -(C*H*$_2$)$_2$), 1.71 – 1.58 (m, 4H, -(CH$_2$C*H*$_2$C$_2$H$_5$)$_2$), 1.31 (q, *J* = 7.4 Hz, 4H, -(C*H*$_2$CH$_3$)$_2$), 0.89 (t, *J* = 7.4 Hz, 6H, -(C*H*$_3$)$_2$).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 62.67 (-(N*C*H$_2$)$_2$), 59.32 (-(*C*H$_2$C$_3$H$_7$)$_2$), 24.52 (-(CH$_2$*C*H$_2$C$_2$H$_5$)$_2$), 21.36 (-(*C*H$_2$)$_2$), 19.12 (-(*C*H$_2$CH$_3$)$_2$) 12.74 (-(*C*H$_3$)$_2$).

***N*-Butyl-*N*,*N*-dimethyl-p-toluidinium Saccharinate (127)**



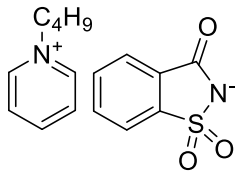
The title compound was prepared as per general method D using *N*-butyl-*N*,*N*-dimethyl-p-toluidinium iodide (0.47 g, 1.45 mmol) and silver saccharinate (0.42 g, 1.45 mmol) to yield the title compound (0.53 g, 1.41 mmol, 97%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.79 – 7.64 (m, 4H, -Ar*H*), 7.46 (d, *J* = 8.9 Hz, 2H, -TolAr*H*), 7.28 (d, *J* = 8.6 Hz, 2H, -TolAr*H*), 3.73 – 3.64 (m, 2H, -C*H*$_2$C$_3$H$_7$), 3.44 (s, 6H, -(C*H*$_3$)$_2$), 2.26 (s, 3H, -C*H*$_3$), 1.33 – 1.20 (m, 2H, -CH$_2$C*H*$_2$C$_2$H$_5$), 1.14 (h, *J* = 7.2 Hz, 2H, -C*H*$_2$CH$_3$), 0.70 (t, *J* = 7.3 Hz, 3H, -C$_3$H$_6$C*H*$_3$).

^{13}C NMR (100 MHz, Deuterium Oxide) δ 172.28 (-*C*O), 141.71 (-Ar*C*N), 141.24 (-Ar*C*), 140.85 (-TolAr*C*), 133.72 (-Ar*C*H), 133.19 (-Ar*C*H), 132.25 (-Ar*C*), 130.56 (-TolAr*C*H), 123.51 (-Ar*C*H), 120.12 (-Ar*C*H), 119.95 (-TolAr*C*H), 69.06 (-*C*$H_2C_3H_7$), 53.90 (-(*C*H_3)$_2$), 24.60 (-*C*H_3), 19.75 (-CH_2*C*$H_2C_2H_5$), 18.63 (-*C*H_2CH_3), 12.53 (-C_3H_6*C*H_3).

ESI-HRMS *m/z* calcd. for $[C_{13}H_{22}N]^+$: 192.1747 Found: 192.1744
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9912

***N*-Butylpyridinium Saccharinate (128)**

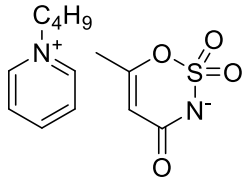


The title compound was prepared as per general method D using *N*-butylpyridinium bromide (0.34 g, 1.57 mmol) and silver saccharinate (0.45 g, 1.57 mmol) to yield the product compound (0.46 g, 1.44 mmol, 92%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.64 (d, *J* = 5.4 Hz, 2H, -PyAr*H*), 8.37 – 8.31 (m, 1H, -PyAr*H*), 7.87 (t, *J* = 7.1 Hz, 2H, -PyAr*H*), 7.71 – 7.54 (m, 4H, -Ar*H*), 4.41 (t, *J* = 7.4 Hz, 2H, -C*H*$_2C_3H_7$), 1.78 (p, *J* = 7.5 Hz, 2H, -CH_2C*H*$_2C_2H_5$), 1.17 (dq, *J* = 14.8, 7.4 Hz, 2H, -C*H*$_2CH_3$), 0.77 (t, *J* = 7.4 Hz, 3H, -C_3H_6C*H*$_3$).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 171.58 (-*C*O), 145.17 (-PyAr*C*H), 143.89 (-PyAr*C*H), 141.59 (-Ar*C*), 133.71 (-Ar*C*H), 133.27 (-Ar*C*H), 131.98 (-Ar*C*), 127.93 -PyAr*C*H), 123.48 (-Ar*C*H), 120.13 (-Ar*C*H), 61.52 (-*C*$H_2C_3H_7$), 32.43 (-CH_2*C*$H_2C_2H_5$), 18.57 (-*C*H_2CH_3), 12.50 (-*C*H_3).

ESI-HRMS *m/z* calcd. for $C_9H_{14}N^+$: 136.1121 Found: 136.1125
m/z calcd. for $[C_7H_4NO_3S]^-$: 181.9917 Found: 181.9915

***N*-Butylpyridinium Acesulfamate (129)**

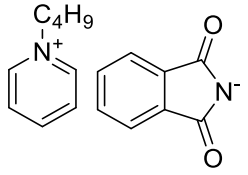


The title compound was prepared as per general method D using *N*-butylpyridinium bromide (0.73 g, 3.4 mmol) and silver acesulfamate (0.92 g, 3.4 mmol) to yield the title compound (0.86 g, 2.9 mmol, 87%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.78 (d, *J* = 5.6 Hz, 2H, -Ar*H*), 8.47 (t, *J* = 7.9 Hz, 1H, -Ar*H*), 7.99 (t, *J* = 6.9 Hz, 2H, -Ar*H*), 5.63 – 5.57 (m, 1H, -C*H*), 4.54 (t, *J* = 7.4 Hz, 2H, -C*H*$_2$C$_3$H$_7$), 2.04 (s, 3H), 1.92 (p, *J* = 7.5 Hz, 2H, -CH$_2$C*H*$_2$C$_2$H$_5$), 1.29 (h, *J* = 7.4 Hz, 2H, -C*H*$_2$CH$_3$), 0.87 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$C*H*$_3$).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 172.11 (-*C*O), 164.14 (-*C*O(CH$_3$)), 145.29 (-Ar*C*H), 144.07 (-Ar*C*H), 128.02 (-Ar*C*H), 100.70 (-*C*H), 61.62 (-*C*H$_2$C$_3$H$_7$), 32.47 (-*C*H$_3$), 18.85 (-CH$_2$*C*H$_2$C$_2$H$_5$), 18.60 (-*C*H$_2$CH$_3$), 12.51 (-*C*H$_3$).

ESI-HRMS *m/z* calcd. for $C_9H_{14}N^+$: 136.1121 Found: 136.1123
m/z calcd. for $[C_4H_4NO_4S]^-$: 161.9867 Found: 161.9869

***N*-Butylpyridinium Phthalimate (130)**



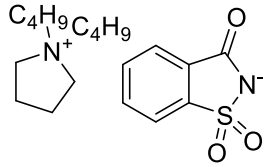
The title compound was prepared as per general method D using *N*-butylpyridinium bromide (0.68 g, 3.2 mmol) and silver phthalimate (0.83 g, 3.2 mmol) to yield the title compound (0.78 g, 2.8 mmol, 71%).

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.12 (d, *J* = 5.5 Hz, 2H, -Ar*H*), 8.64 – 8.55 (m, 1H, -Ar*H*), 8.16 (t, *J* = 7.1 Hz, 2H, -Ar*H*), 7.70 (d, *J* = 7.6 Hz, 1H, -Ar*H*), 7.34 –

7.16 (m, 3H, -Ar*H*), 4.60 (t, *J* = 7.4 Hz, 2H, -C*H*$_2$C$_3$H$_7$), 1.95 – 1.82 (m, 2H, -CH$_2$C*H*$_2$C$_2$H$_5$), 1.27 (dq, *J* = 14.7, 7.4 Hz, 2H, -C*H*$_2$CH$_3$), 0.90 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$C*H*$_3$).

^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 172.68 (-*C*O), 169.37 (-*C*O), 145.42 (-PyAr*C*H), 144.84 (-PyAr*C*H), 143.92 (-Ar*C*), 134.21 (-Ar*C*), 130.78 (-Ar*C*H), 129.66 (-Ar*C*H), 128.87 (-Ar*C*H), 128.03 (-PyAr*C*H), 125.80 (-Ar*C*H), 60.46 (-*C*H$_2$C$_3$H$_7$), 32.70 (-CH$_2$*C*H$_2$C$_2$H$_5$), 18.74 (-*C*H$_2$CH$_3$), 13.32 (-*C*H$_3$).

N,*N*-Dibutylpyrrolidinium Saccharinate (131)



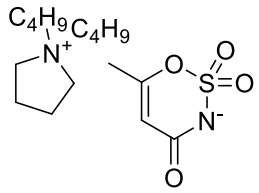
The title compound was prepared as per general method D using *N*,*N*-Dibutylpyrrolidinium iodide (0.52 g, 1.67 mmol) and silver saccharinate (0.49 g, 1.67 mmol) to yield the title compound (0.57 g, 1.55 mmol, 93%).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.86 – 7.81 (m, 1H, -Ar*H*), 7.79 – 7.73 (m, 3H, -Ar*H*), 3.43 – 3.38 (m, 4H, -N(C*H*$_2$)$_2$), 3.18 – 3.12 (m, 4H, -(C*H*$_2$C$_3$H$_7$)$_2$), 2.10 – 2.06 (m, 4H, -(C*H*$_2$)$_2$), 1.64 – 1.58 (m, 4H, -(CH$_2$C*H*$_2$C$_2$H$_5$)$_2$), 1.32 – 1.25 (m, 4H, -(C*H*$_2$CH$_3$)$_2$), 0.87 (t, *J* = 7.3 Hz, 6H, -(C*H*$_3$)$_2$).

^{13}C NMR (150 MHz, Deuterium Oxide) δ 172.52 (-*C*O), 142.01 (-Ar*C*), 133.93 (-Ar*C*), 133.40 (-Ar*C*H), 132.52 (-Ar*C*H), 123.73 (-Ar*C*H), 120.33 (-Ar*C*H), 63.06 (-(N*C*H$_2$)$_2$), 59.49 (-(*C*H$_2$C$_3$H$_7$)$_2$), 24.59 (-(CH$_2$*C*H$_2$C$_2$H$_5$)$_2$), 21.49 (-(*C*H$_2$)$_2$), 19.20 (-(*C*H$_2$CH$_3$)$_2$), 12.80 (-(*C*H$_3$)$_2$).

ESI-HRMS *m/z* calcd. for [C$_{12}$H$_{26}$N]$^+$: 184.2060 Found: 184.2068
m/z calcd. for [C$_7$H$_4$NO$_3$S]$^-$: 161.9917 Found: 181.9915

***N*,*N*-Dibutylpyrrolidinium Acesulfamate (132)**

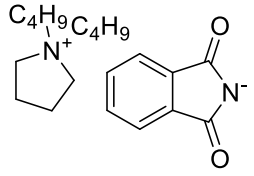


The title compound was prepared as per general method D using *N*,*N*-Dibutylpyrrolidinium iodide (0.90 g, 2.9 mmol) and silver acesulfamate (0.78 g, 2.9 mmol) to yield the title compound (0.94 g, 2.7 mmol, 93%).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 5.61 (s, 1H, -C*H*), 3.45 – 3.40 (m, 4H, -N(CH_2)$_2$), 3.22 – 3.13 (m, 4H, -(CH_2C$_3$H$_7$)$_2$), 2.12 – 2.06 (m, 4H, -(CH_2)$_2$), 2.05 (s, 3H, -CH_3), 1.70 – 1.57 (m, 4H, -(CH$_2$CH_2C$_2$H$_5$)$_2$), 1.30 (h, *J* = 7.4 Hz, 4H, -(CH_2CH$_3$)$_2$), 0.88 (t, *J* = 7.4 Hz, 6H, -(CH_3)$_2$).
^{13}C NMR (100 MHz, Deuterium Oxide) δ 172.14 (-*C*O), 164.04 (-*C*O(CH$_3$)), 100.79 (-*C*H), 62.62 (-(N*C*H$_2$)$_2$), 59.30 (-(*C*H$_2$C$_3$H$_7$)$_2$), 24.49 (-(CH$_2$*C*H$_2$C$_2$H$_5$)$_2$), 21.33 (-(*C*H$_2$)$_2$), 19.09 (-(*C*H$_2$CH$_3$)$_2$), 18.84 (-CH$_3$), 12.70 (-(*C*H$_3$)$_2$).

ESI-HRMS *m/z* calcd. for [C$_{12}$H$_{26}$N]$^+$: 184.2060 Found: 184.2066
m/z calcd. for [C$_4$H$_4$NO$_4$S]$^-$: 161.9867 Found: 161.9864

***N*,*N*-Dibutylpyrrolidinium Phthalimate (133)**



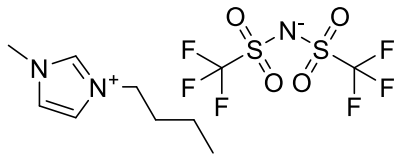
The title compound was prepared as per general method D using *N*,*N*-Dibutylpyrrolidinium iodide (0.93 g, 3.0 mmol) and silver phthalimate (0.76 g 3.0 mmol) to yield the title compound (0.76 g, 2.3 mmol, 77%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.74 – 7.69 (m, 1H, -Ar*H*), 7.33 – 7.28 (m, 2H, -Ar*H*), 7.24 – 7.17 (m, 1H, -Ar*H*), 3.48 – 3.41 (m, 4H, -N(CH_2)$_2$), 3.21 – 3.15 (m,

4H, -CH_2C$_3$H$_7$), 2.06 – 1.99 (m, 4H, -(CH_2)$_2$), 1.63 – 1.54 (m, 4H, -(CH$_2$CH_2C$_2$H$_5$)$_2$), 1.30 (h, *J* = 7.4 Hz, 4H, -(CH_2CH$_3$)$_2$), 0.92 (t, *J* = 7.4 Hz, 6H, -(CH_3)$_2$).

^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 172.43(-*C*O), 169.23 (-*C*O), 130.71, 129.68 , 128.93(-Ar*C*H), 128.08 (-Ar*C*H), 125.86 (-Ar*C*H), 99.55 , 62.10 (-(N*C*H$_2$)$_2$), 58.49 (-(*C*H$_2$C$_3$H$_7$)$_2$), 24.47 (-(CH$_2$*C*H$_2$C$_2$H$_5$)$_2$), 21.40 (-(*C*H$_2$)$_2$), 19.26 (-(*C*H$_2$CH$_3$)$_2$), 13.53 (-(*C*H$_3$)$_2$).

1-Butyl-3-methylimidazolium Bistriflimide (134)[29]



Anion metathesis was carried by stirring BMIM chloride (0.57g, 3 mmol) and LiNTf$_2$ (0.87 g, 3 mmol) in 15 mL oh H$_2$O. The solution was stirred at RT for 18 hours forming a biphasic mixture which was then further diluted with H$_2$O (10 mL) before the product was extracted using chloroform (3 x 20 mL). The organic layers were pooled, washed with H$_2$O, and dried over MgSO$_4$ before the solvent was evaporated to dryness to yield the product as a7 colourless liquid (1.13 g, 2.7 mmol, 91%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.72 (s, 1H, -C2*H*), 7.32 – 7.28 (m, 2H, -C4/5*H*), 4.14 (dt, *J* = 14.5, 7.4 Hz, 2H, -CH_2C$_3$H$_7$), 3.92 (s, 3H, -CH_3), 1.90 – 1.79 (m, 2H, -CH$_2$CH_2C$_2$H$_5$), 1.41 – 1.27 (m, 2H, -CH_2CH$_3$), 0.95 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$CH_3).

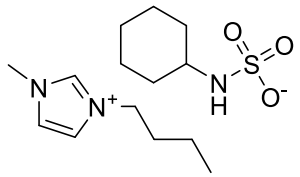
^{13}C NMR (100 MHz, Chloroform-*d*) δ 136.13 (*C*2), 123.77 (*C*4/5), 122.35 (*C*4/5), 119.34 (q, *J* = 320.4 Hz), 50.04 (-*C*H$_2$C$_3$H$_7$), 36.42 (-*C*H$_3$), 32.04 (-CH$_2$*C*H$_2$C$_2$H$_5$), 19.44 (-*C*H$_2$CH$_3$), 13.33 (-*C*H$_3$).

^{19}F NMR (376 MHz, Chloroform-*d*) δ -79.13 .

^{1}H and ^{13}C spectra in agreement with literature[30].

ESI-HRMS *m/z* calcd. for [C$_8$H$_{15}$N$_2$]$^+$: 139.1230 Found: 139.1231
m/z calcd. for [C$_2$F$_6$NO$_4$S$_2$]$^-$: 279.9178 Found: 279.918

1-Butyl-3-methylimidazolium Cyclamate (135)

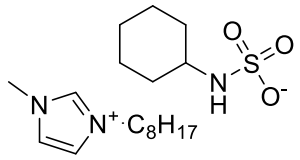


The title compound was prepared as per general method D using BMIM chloride (1.65 g, 9.5 mmol) and silver cyclamate (2.70 g, 9.5 mmol) to yield the title compound (2.66 g, 8.4 mmol, 89%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.84 (s, 1H, -C2*H*), 7.52 (t, *J* = 1.7 Hz, 1H, -C4/5*H*), 7.38 (t, *J* = 1.7 Hz, 1H, -C4/5*H*), 4.22 (t, *J* = 7.4 Hz, 2H, -C*H*$_2$C$_3$H$_7$), 4.00 (s, 3H, -C*H*$_3$), 3.22 – 3.14 (m, 1H, -C*H*), 2.08 – 2.02 (m, 2H), 1.86 – 1.77 (m, 2H), 1.67 – 1.60 (m, 2H), 1.55 – 1.48 (m, 1H), 1.36 – 1.19 (m, 4H), 1.16 – 1.03 (m, 3H), 0.90 (t, *J* = 7.4 Hz, 3H, -C$_3$H$_6$C*H*$_3$).
^{13}C NMR (150 MHz, Chloroform-*d*) δ 138.12 (*C*2), 123.77 (*C*4/5), 122.0177 (*C*4/5) , 52.91 , 49.70 , 36.45 , 34.51 , 32.19 , 25.83 , 25.12 , 19.49 , 13.47 (-*C*H$_3$).

ESI-HRMS *m/z* calcd. for $[C_8H_{15}N_2]^+$: 139.1230 Found: 139.1229
m/z calcd. for $[C_6H_{12}NO_3S]^-$: 178.0543 Found: 178.0543

1-Octyl-3-methylimidazolium Cyclamate (136)



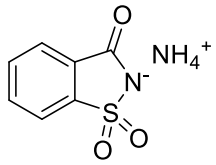
The title compound was prepared as per general method D using OMIM chloride (0.37 g, 1.6 mmol) and silver cyclamate (0.46 g, 1.6 mmol) to yield the title compound (0.49 g, 1.3 mmol, 84%).

^{1}H NMR (600 MHz, Deuterium Oxide) δ 7.43 (d, *J* = 2.0 Hz, 1H, -C4/5*H*), 7.39 (d, *J* = 2.0 Hz, 1H, -C4/5*H*), 4.15 (t, *J* = 7.1 Hz, 2H, -C*H*$_2$C$_7$H$_{15}$), 3.85 (s, 3H, -C*H*$_3$), 3.07 – 3.00 (m, 1H, -C*H*), 1.97 – 1.91 (m, 2H, -cC$_6$*H*$_{11}$, -C$_7$*H*$_{15}$), 1.87 – 1.79 (m, 2H,

-cC$_6$*H*$_{11}$, -C$_7$*H*$_{15}$), 1.72 – 1.65 (m, 2H, -cC$_6$*H*$_{11}$, -C$_7$*H*$_{15}$), 1.58 – 1.51 (m, 1H, -cC$_6$*H*$_{11}$, -C$_7$*H*$_{15}$), 1.33 – 1.06 (m, 15H, -cC$_6$*H*$_{11}$, -C$_7$*H*$_{15}$), 0.83 (t, *J* = 7.0 Hz, 3H, -C$_7$H$_{14}$C*H*$_3$).
^{13}C NMR (150 MHz, Deuterium Oxide) δ 123.37 (*C*4/5), 122.10 (*C*4/5), 53.27, 49.49, 35.51, 33.27, 30.91, 29.08, 28.12, 27.92, 25.21, 25.02, 24.63, 21.92, 13.32 (-*C*H$_3$).

ESI-HRMS *m/z* calcd. for [C$_{12}$H$_{23}$N$_2$]$^+$: 195.1856 Found: 195.1856
m/z calcd. for [C$_6$H$_{12}$NO$_3$S]$^-$: 178.0543 Found: 178.0543

Ammonium Saccharinate (137)



Saccharin (1.99 g, 11 mmol) was added to ethanol (40 mL) along with 1M NH$_3$ (16 mL). The solution was stirred for 18 hours resulting in a white precipitate. The product was isolated by filtration and washed with ethanol (5 mL) to yield 93% (2.04 g, 10.2 mmol).

^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 7.71 – 7.64 (m, 1H, -Ar*H*), 7.64 – 7.56 (m, 3H, -Ar*H*), 7.20 (br. s, 4H, -N*H*$_4$).
^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 168.13 (-*C*O), 145.16 (-Ar*C*), 134.55 (-Ar*C*), 131.78 (-Ar*C*H), 131.27 (-Ar*C*H), 122.62 (-Ar*C*H), 119.27 (-Ar*C*H).

ESI-HRMS *m/z* calcd. for [C$_7$H$_4$NO$_3$S]$^-$: 181.9917 Found: 181.9925

7.11 References

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Appendices

Appendix A: Compounds used in Evaluation of BIOWIN Prediction Software

BIOWIN Prediction Results for THQ's									
Compound	**SMILES**	**BIOWIN Model:**							**Ready Biodegradability Prediction**
		1	**2**	**3**	**4**	**5**	**6**	**7**	
THQ	**c12c(cccc1)CCCN2**	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
n-Bu THQ	**c12c(cccc1)CCCN2CCCC**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
THQ Diol	**c12c(cccc1)CCCN2CC(O)CO**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
Sulfonated THQ	**c12c(cc(S(=O)(=O)O)cc1)CCCN2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
1	**c12c(cccc1)CC(C)(C)CN2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
2	**c12c(cccc1)CC(=O)CN2**	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
3	**c12c(cccc1)CC(N)CN2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
4	**c12c(cccc1)CC(=S)CN2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
5	**c12c(cccc1)NCCO2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
6	**c12c(cccc1)CCN=N2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
7	**O=C1c2c(cccc2)NCC1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
8	**O=C1c2c(cccc2)N=NC1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
9	**c12c(cccc1)CCNN2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
10	**c12c(cccc1)CC(C(=O)O)CN2**	BF	DNBF	W	D	NRD	NRD	DNBF	No
11	**c12c(cccc1)CC(C(=O)OC)CN2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
12	**c12c(cc(O)nn1)cccc2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
13	**c12c(cccc1)CC(=O)N=N2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
14	**c12c(cc(OC)nn1)cccc2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
15	**O=C(O)c1cc2c(cccc2)n**	BF	BF	W	D-	R	R	BF	Yes

	n1				W	D	D		
16	**O=C1C=C2C(C=C1)NCCC2**	BF	BF	W	D-W	RD	NRD	DNBF	Yes
17	**O=C1C2C(CC(=O)CN2)CC(=O)C1**	BF	BF	W	D-W	RD	RD	DNBF	Yes

BIOWIN Prediction Results for Indolines									
Compound	**SMILES**	**BIOWIN Model:**							**Ready Biodegradability Prediction**
		1	**2**	**3**	**4**	**5**	**6**	**7**	
Indoline	**c12c(cccc1)CCN2**	BF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
n-Bu Indoline	**CCCCN1CCC2=C1C=CC=C2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
Indoline Diol	**OCC(O)CN1CCC2=C1C=CC=C2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
Sulfonated-Indoline	**OS(=O)(=O)C1=CC2=C(NCC2)C=C1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
Isatin	**O=C1C(=O)c2c(cccc2)N1**	BF	BF	W	D	NRD	NRD	DNBF	No
1	**C1(C)(C)c2c(cccc2)NC1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
2	**O=C1c2c(cccc2)NC1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
3	**c12c(C(N)CN1)cccc2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
4	**C1(=S)c2c(cccc2)NC1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
5	**c12c(cccc1)NCO2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
6	**c12c(cccc1)CN=N2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
7	**O=C1c2c(cccc2)N=N1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
8	**c12c(cccc1)CNN2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
9	**c12c(C(C(=O)O)CN1)ccccc2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
10	**c12c(C(OC)CN1)cccc2**	BF	BF	W	D	NRD	NRD	DNBF	No
11	**c12c(C(O)N=N1)cccc2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
12	**O=C1c2c(cccc2)N=N1**	BF	DNBF	W	D-W	NR	NR	BF	No

						D	D		
13	**c12c(C(OC)N=N1)cccc2**	BF	BF	W	D-W	NRD	NRD	DNBF	No
14	**c12c(C(C(=O)O)N=N1)cccc2**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No
15D	**O=C1C=C2C(C=C1)NCC2**	BF	DNBF	W	D	NRD	NRD	BF	No
16	**O=C1C2C(C(=O)CC(=O)C2)NC1**	BF	BF	W	D-W	RD	NRD	DNBF	Yes
17	**O=C1CNC2C1CC(=O)CC2=O**	BF	BF	W	D-W	RD	RD	DNBF	Yes

BIOWIN Prediction Results for Benzoxazines									
Compound	**SMILES**	**BIOWIN Model:**							**Readily Biodegradable Prediction**
		1	**2**	**3**	**4**	**5**	**6**	**7**	
1	**C1(C)(C)C=C(O)C=C(N2CCOCC2)C1**	DNBF	DNBF	W-M	W	NRD	NRD	DNBF	No
2	**O=C1C=C(N2CCOCC2)CC(C)(C)C1**	DNBF	DNBF	M	W	NRD	NRD	DNBF	No
3	**C1(C)(C)C=C(N)C=C(N2CCOCC2)C1**	DNBF	DNBF	W-M	W	NRD	NRD	DNBF	No
4	**C1(=S)C=C(N2CCOCC2)CC(C)(C)C1**	DNBF	DNBF	M	W	NRD	NRD	DNBF	No
5	**c32c(cc(C)cc3)CN(c1ccc(OC)cc1)CO2**	BF	BF	M	D-W	NRD	NRD	DNBF	No
6	**c32c(cc(C)cc3)CN(c1ccc(N)cc1)CO2**	DNBF	DNBF	M	W	NRD	NRD	DNBF	No
7	**c32c(cc(C)cc3)CN(c1ccc(O)cc1)CO2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
8	**C(#N)c1ccc(N2Cc3c(ccc(C)c3)OC2)cc1**	BF	BF	M	W	NRD	NRD	DNBF	No
9	**c32c(cc(C)cc3)CN(c1ccc(S)cc1)CO2**	BF	DNBF	M	W	NRD	NRD	DNBF	No
10	**c32c(cc(C)cc3)CN(C1C=CC(=O)C=C1)CO2**	BF	DNBF	W-M	W	NRD	NRD	DNBF	No
11	**O=C1C(c2ccccc2)C(=O)CNC1**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
12	**O=C(O)c1cc(C2C(C(=O)O)CNCC2)ccc1**	BF	BF	W	D	RD	NRD	BF	Yes
13	**O=C(C2CCNCC2)Oc1ccccc1**	BF	BF	W	D	RD	RD	DNBF	Yes
14	**O=C1c2c(cccc2)OC(=O)N1**	BF	BF	W	D-W	NRD	NRD	DNBF	No
15	**c12c(cccc1)NCC(=O)O2**	BF	BF	W	D	RD	RD	DNBF	Yes
16	**c32c(cccc3)Nc1c(cccc1)O2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
17	**c12c(cccc1)NCCO2**	BF	BF	W-M	D-W	NRD	NRD	DNBF	No
18	**O=C1C(=N)Nc2c(cccc2)O1**	BF	BF	W	D-W	NRD	NRD	DNBF	No
19	**O=C(O)c1cc(C2C(=O)CNCC2)ccc1**	BF	BF	W-M	D-W	RD	RD	DNBF	No
20	**c1(O)cc(N2CCOCC2)ccc1**	DNBF	DNBF	W-M	D-W	NRD	NRD	DNBF	No

Legend:
BF - Biodegrades fast
DNBF - Does not biodegrade fast
W-M - Weeks to months
W - Weeks
D-W - Days to weeks
D - Days
RD - Readily degradable
NRD - Not readily degradable

Appendix B: Results of BIODEG Database Survey

Chemical	Aerobic Test						Anaerobic Test			#Refs
	Screen	BTSim	GrSoi	GrWat	Field	Sumry	Soil	Watr	Sum	
Aniline	BF1	BFA1	BS2	BF1	BF3	BF1	BSA3	BF3	BST3	33
p-Dodecylaniline	BF3					BF3				1
3-Methoxyaniline	BF1					BF1				4
4-Methoxyaniline	BF1					BF1				4
Pyridoxal	BF3					BF3				1
Acetanilide	BF1	BFA3		BFA3		BF1				8
Benzylamine	BF1	BFA3		BF1		BF1				5
Cyclohexanamine	BF3					BF3				1
1,2-Cyclohexanediamine	BF3					BF3				1
1,4-Cyclohexanediamine	BF3					BF3				1
Indole	BF3		BF2			BF1		BFA3	BFA3	3
2-Methylpyridine	BF1		BF3			BF1	BSA3		BSA3	4
3-Pyridinecarboxylic Acid	BF2		BF3			BF1	BF3		BF3	3
Pyridine-2,4-Dicarboxylic Acid	BF3		BF3			BF2				2
Pyridine-2,5-Dicarboxylic Acid	BF3					BF3	BSA3		BSA3	1
Pyridine-2,6-Dicarboxylic Acid	BF2					BF2	BF3		BF3	2
2-Hydroxypyridine	BF3		BF3			BF2	BFA3		BFA3	3
4-Hydroxypyridine	BF3		BF3			BF2	BSA3		BSA3	3
Piperidine	BF2				BF2					2
2-Pyrrolidone	BF3					BF3				1
N-Methylpyrrolidone	BF1	BFA1	BF3			BF1				4
Hydroxyproline	BF3					BF3				1
o-Toluidine	BF1	BF3				BF1				9
p-Toluidine	BF1					BF1		BSA3	BSA3	8
1,4-Benzenediamine	BFA3					BFA3				2
2,3-Dimethylaniline	BFA3					BFA3				1
2,4-Dimethylaniline	BFA2					BFA2				3
N,N-Dimethylaniline	BFA1					BFA1				5
M-Hydroxyacetanilide	BFA1					BFA1				4
3-Hydroxypyridine	BFA3		BF1			BF2	BSA3		BSA3	3
3-Methylphthalimide	BFA3					BFA3				1
4-Methylpyridine	BFA3		BFA3			BFA2		BFA3	BFA3	2
N-Methylpyrrole	BFA3					BFA3				1
N-Phenyl-1-naphthylamine	BFA2		BF3	BFA3		BFA1				2
Quinoline	BFA1			BFA1		BFA1				4
m-Toluidine	BFA2					BFA2				4
2,4-Diaminotoluene	BS3	BF3				BFA2				2
o-Anilinesulfonic Acid	BS2					BS2				1

N,N-Diethylaniline	BS1					BS1				3
N-Phenylanthranilic Acid	BS3					BS3				1
Melamine	BS2		BS3			BS2				3
Morpholine	BS1	BST2				BS1				8
p-Toluidine-m-sulfonic Acid	BS2	BSA3				BS1				3
N-Methyl-m-toluidine	BSA3					BSA3				1
1,5-Naphthalenediamine	BSA3					BSA3				1
2,6-Diaminopyridine	BSA3		BSA 3			BSA2				2
3-(3-Pyridyl)-1-propanol	BSA3					BSA3				1
m-Tolyldiethanolamine	BSA3					BSA3				1
2,3,6-Trimethylpyridine	BSA3					BSA3	BSA 3		BSA 3	1
2-Vinylpyridine	BSA3					BSA3				1
3-Aminobenzenesulfonic Acid	BSA1	BSA2				BSA1				7
2,4,6-Trimethylpyridine	BFA3					BSA3	BSA 3		BSA 3	1
1,2-Benzenediamine	BST1					BST1				3
1,3-Benzenediamine	BST2					BST2				3
1-Naphthylamine	BST1		BSA 3			BST1				4
N-Phenyl-2-napthylamine	BST2					BST2				2
Pyridine	BST1	BFA3	BF3			BST1		BFA3	BFA 3	10
4-Pyridinecarboxylic Acid	BST2		BF3			BST1	BFA 3		BFA 3	3
2,4-Dimethylpyridine	BST2		BFA 3			BST2	BSA 3		BSA 3	3
2,6-Dimethylpyridine	BST2		BF3			BST2	BSA 3		BSA 3	3
3-Methylpyridine	BST2		BF3			BST1				3
4-Ethoxyaniline	BST2					BST2				2
2-Methoxyaniline	BST1					BST1				4
N-Methylaniline	BST1	BFA3				BST1				5
p-Phenylazoaniline	BST2					BST2				4
4-Aminobenzenesulfonic Acid	BST1	BST1				BST1				9
Benzidine	BST2	BST2	BST2			BST2				4
Pendimethalin				BSA1	BST 1	BST1		BST1	BST 1	11
2,4- Dimethylaniline	BF1		BF3			BST1				3
2,6- Dimethylaniline	BS3		BST2			BST2				3
2,3-Dihydroxypyridine	BSA2		BFA 3			BST1	BSA 3		BSA 3	3
5,5-Dimethylhydantoin	BSA3	BFA2				BST2				1

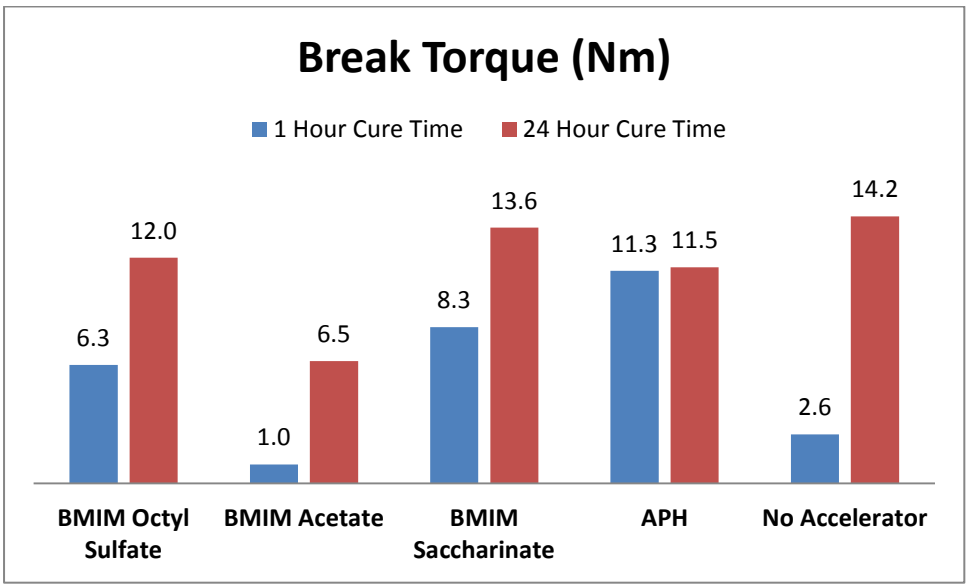
BF – Biodegrades fast
BFA – Biodegrades fast with acclimation
BS – Biodegrades slow
BSA – Biodegrades slow with acclimation
BST – Biodegrades sometimes

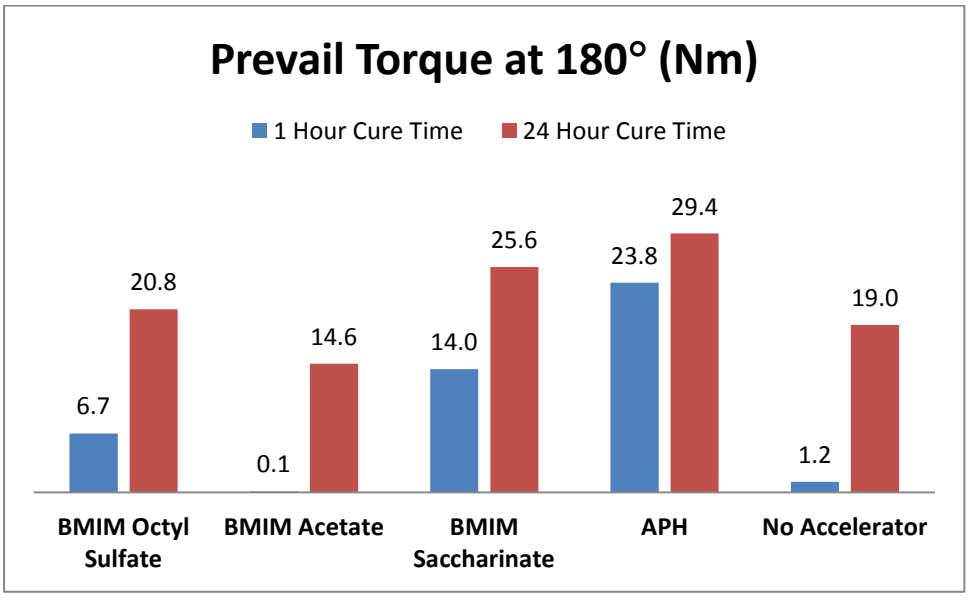
1 - Chemical tested in three or more tests, consistent results.
2 - Chemical tested in two tests, or results in more than two tests are interpretable, but conflicting data.
3 - Only one test or uninterpretable, conflicting data.

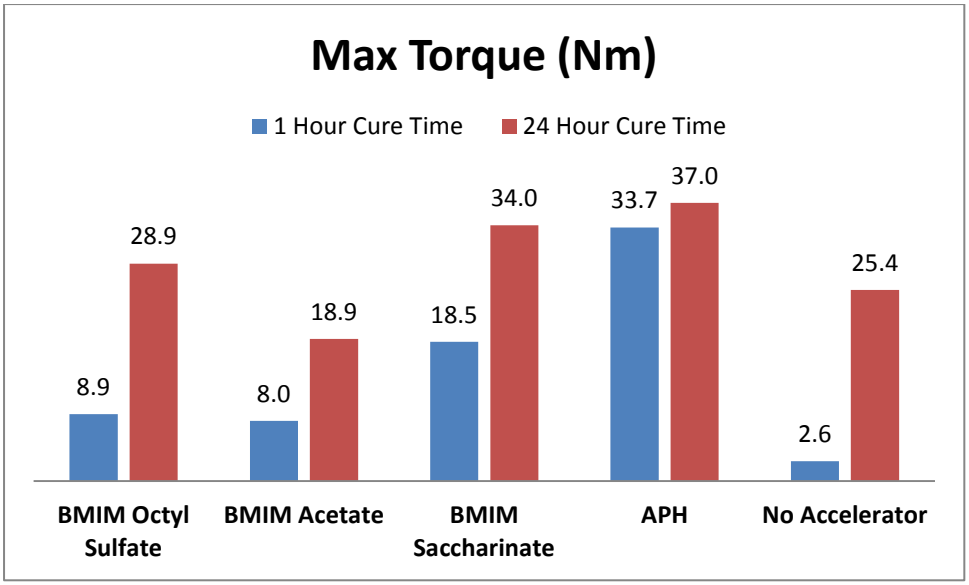
Appendix C: Complete Accelerator Performance Results for All Compounds Evaluated in the Nuts & Bolts and Pins & Collar Tests.

Graph 5.1: Black Oxide Bolts with Mild Steel Nuts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

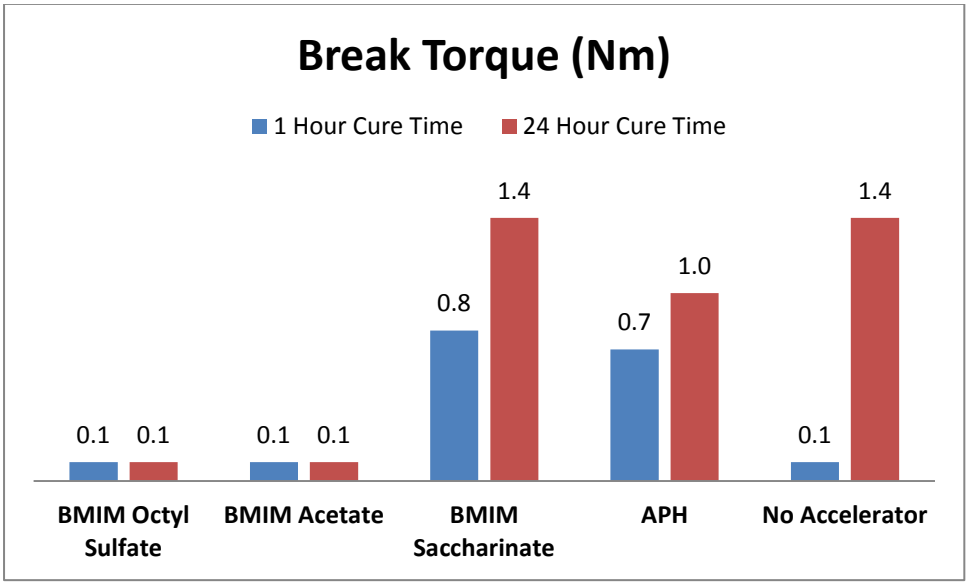


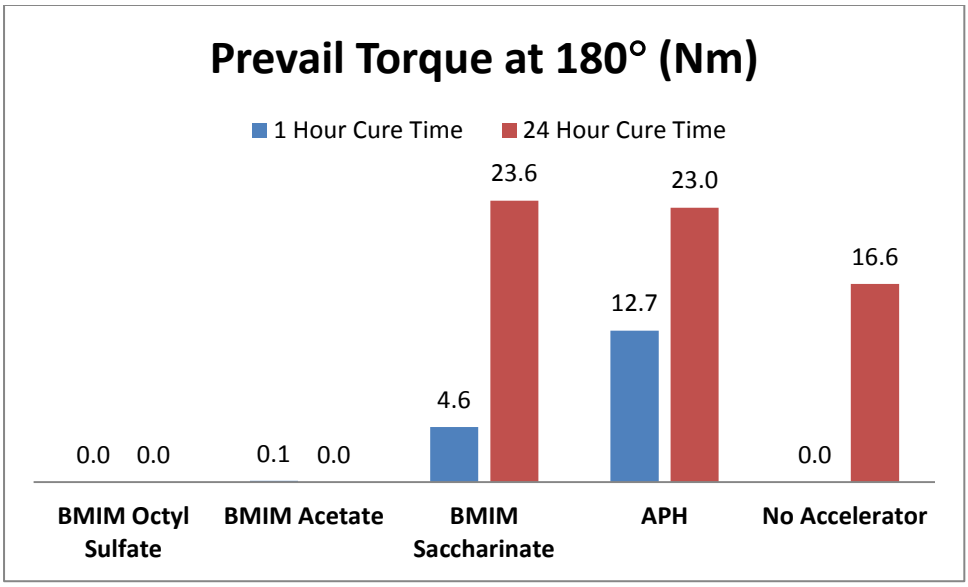


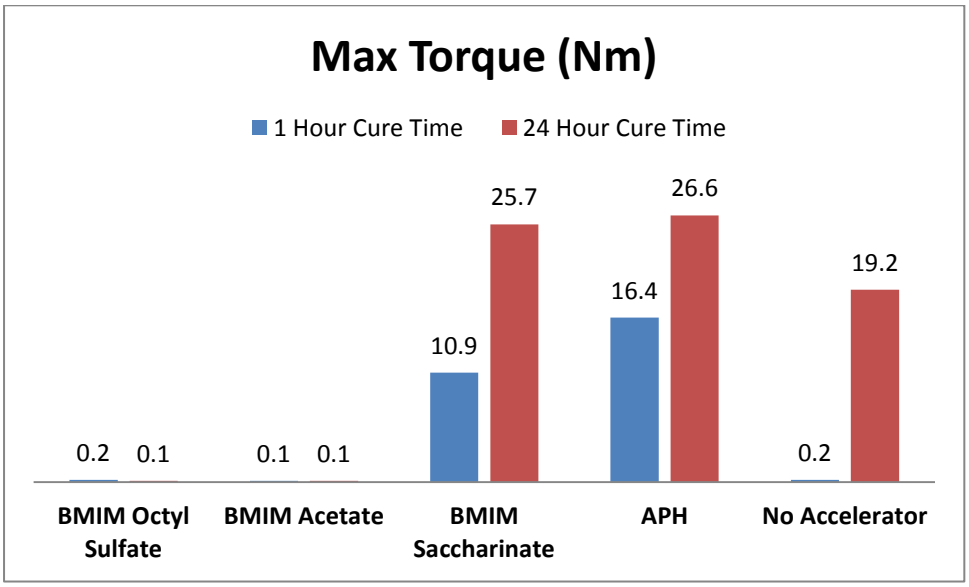

Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
8.9
28.9
8.0
18.9
18.5
34.0
33.7
37.0
2.6
25.4
BMIM Octyl Sulfate
BMIM Acetate
BMIM Saccharinate
APH
No Accelerator

Graph 5.2: Stainless Steel Nuts and Bolts:

(a) Max Torque, (b) Prevail Torque, and (c) Maximum Torque

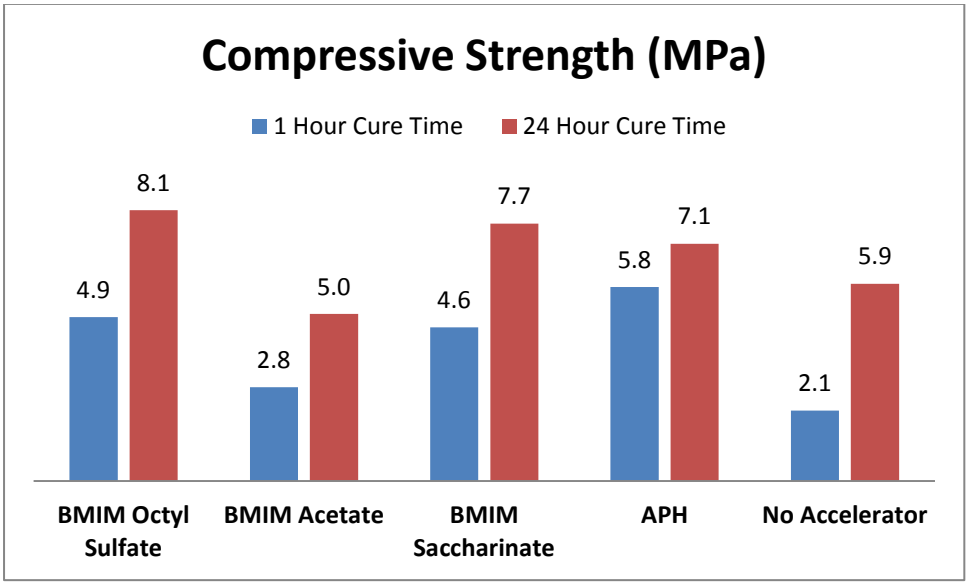


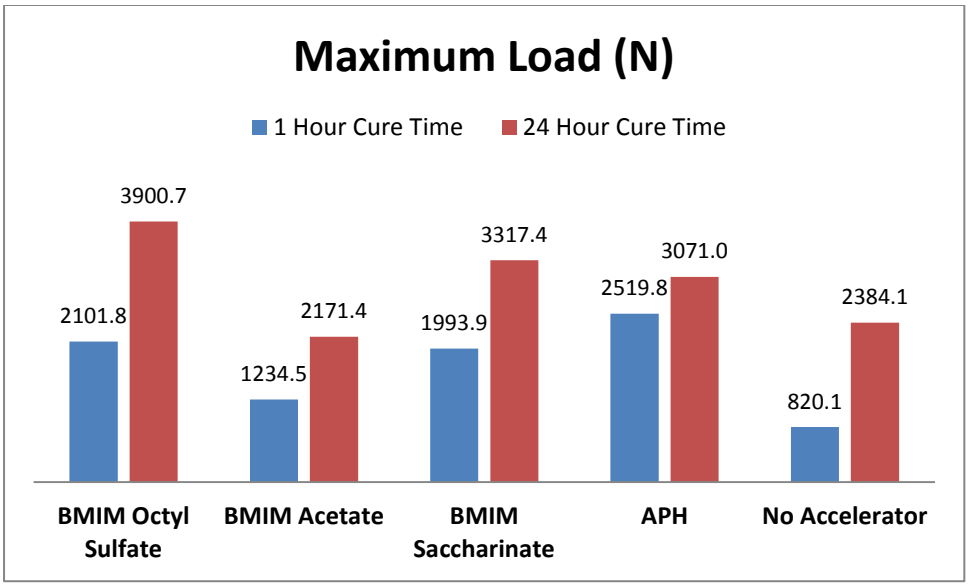



Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
0.2
0.1
0.1
0.1
10.9
25.7
16.4
26.6
0.2
19.2
BMIM Octyl Sulfate
BMIM Acetate
BMIM Saccharinate
APH
No Accelerator

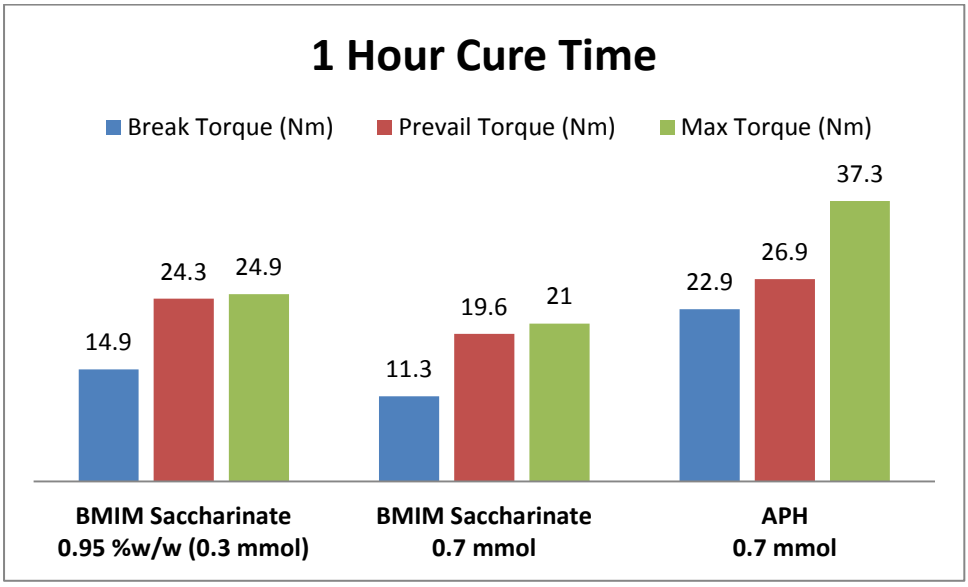
Graph 5.3: Mild Steel Pins and Collars:

(a) Compressive Strength and (b) Maximum Load

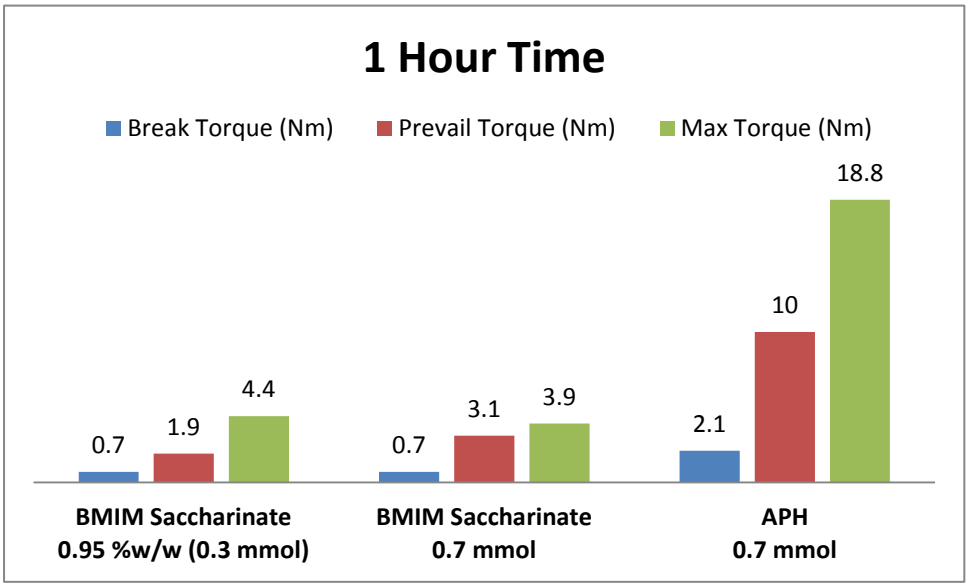




Graph 5.4: Black Oxide Bolts with Mild Steel Nuts

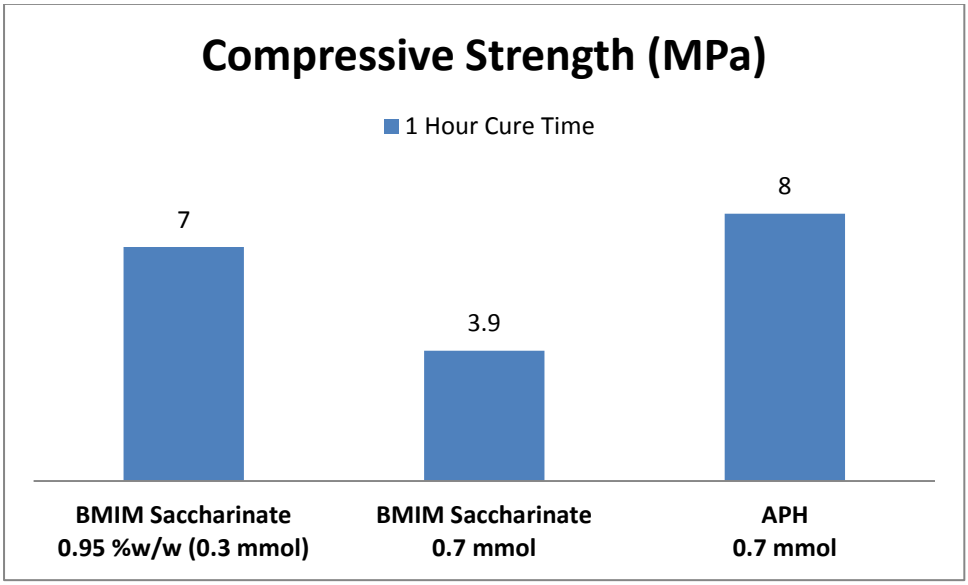


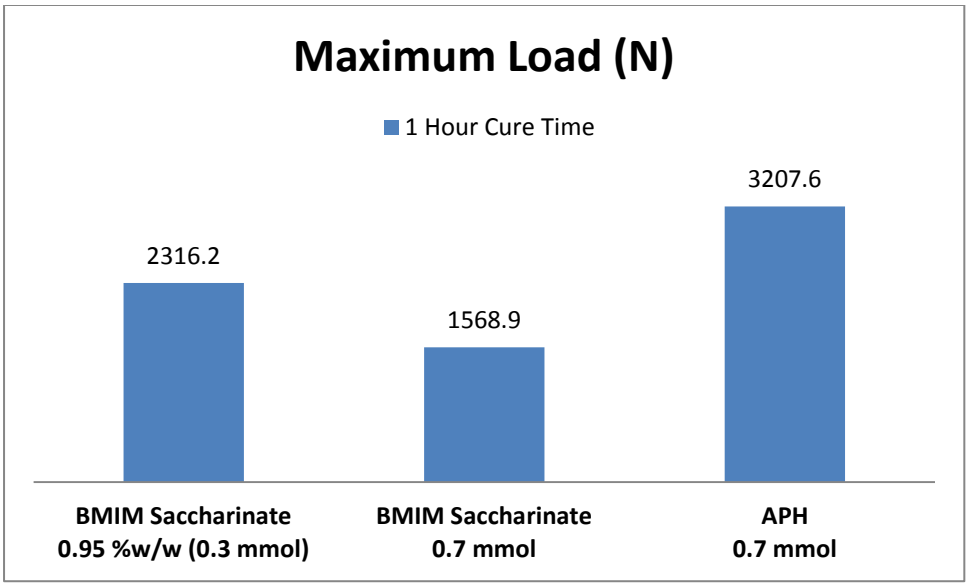
Graph 5.23: Stainless Steel Nuts and Bolts



Graph 5.6: Mild Steel Pins and Collars:

(a) Compressive Strength and (b) Maximum Load

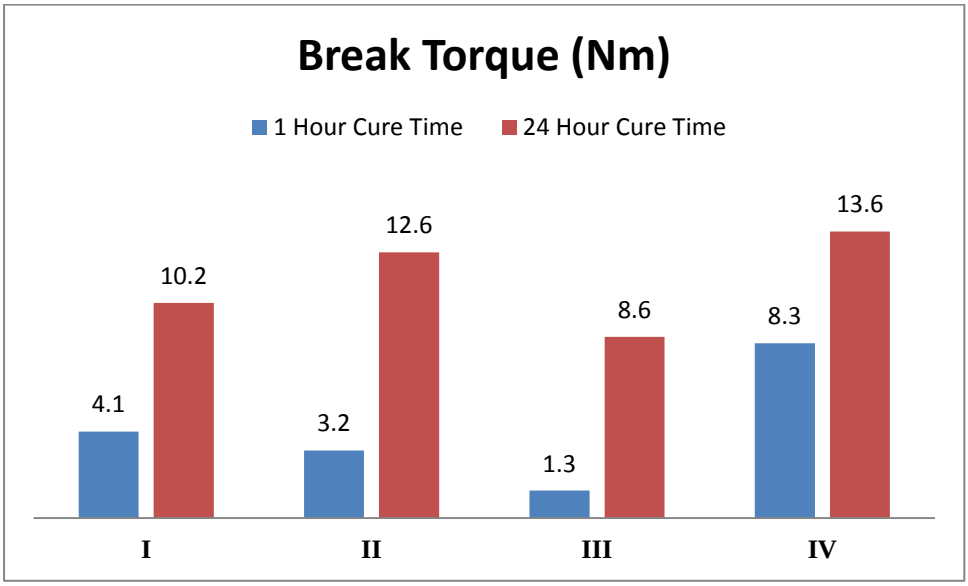


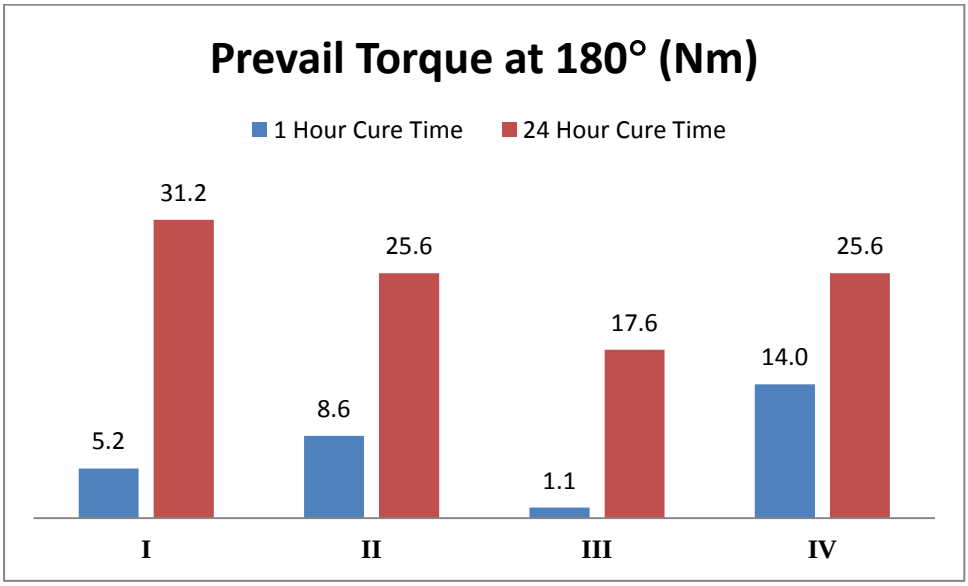


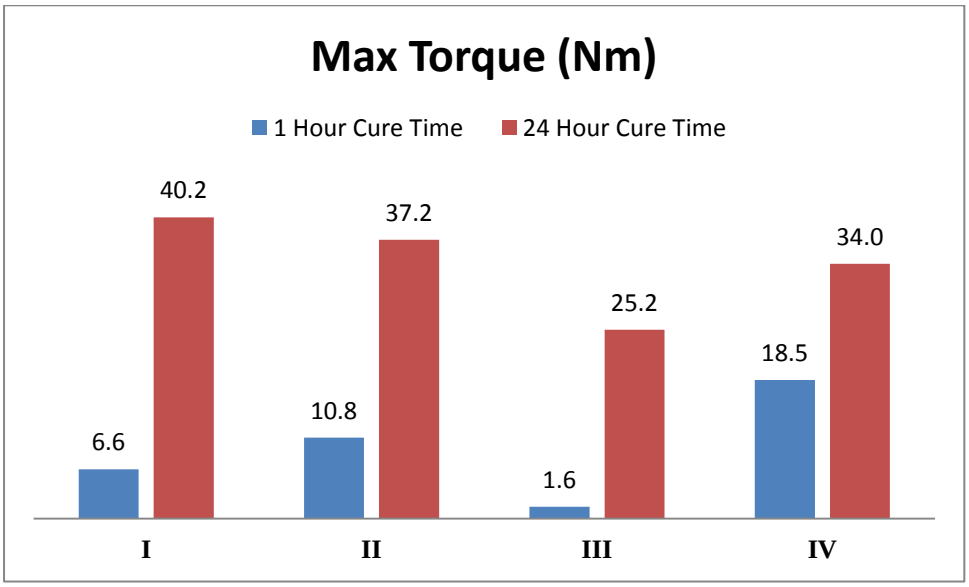
Graph 5.7: Black Oxide Bolts with Mild Steel Nuts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

The composition of each formulation (I-IV) may be found in Section 5.3.1.



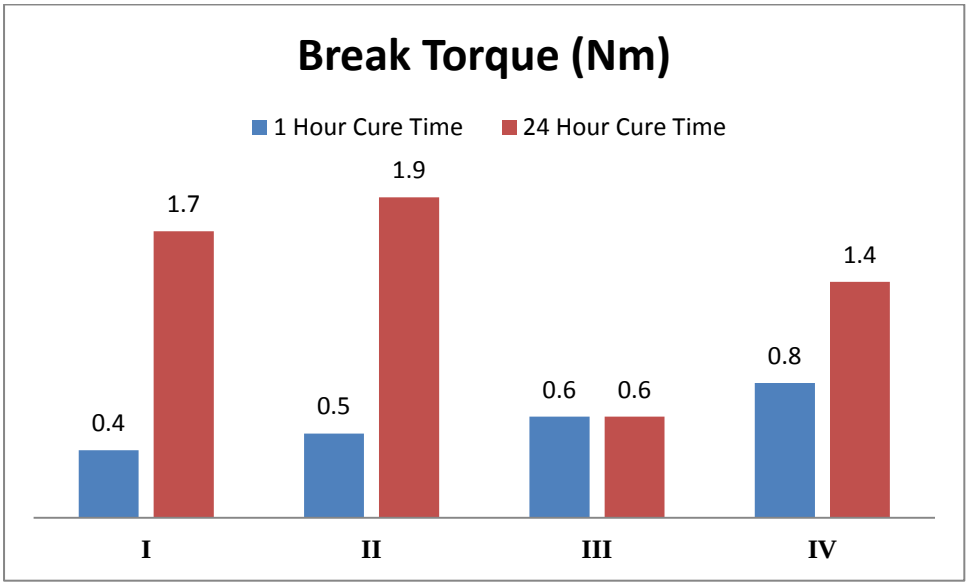


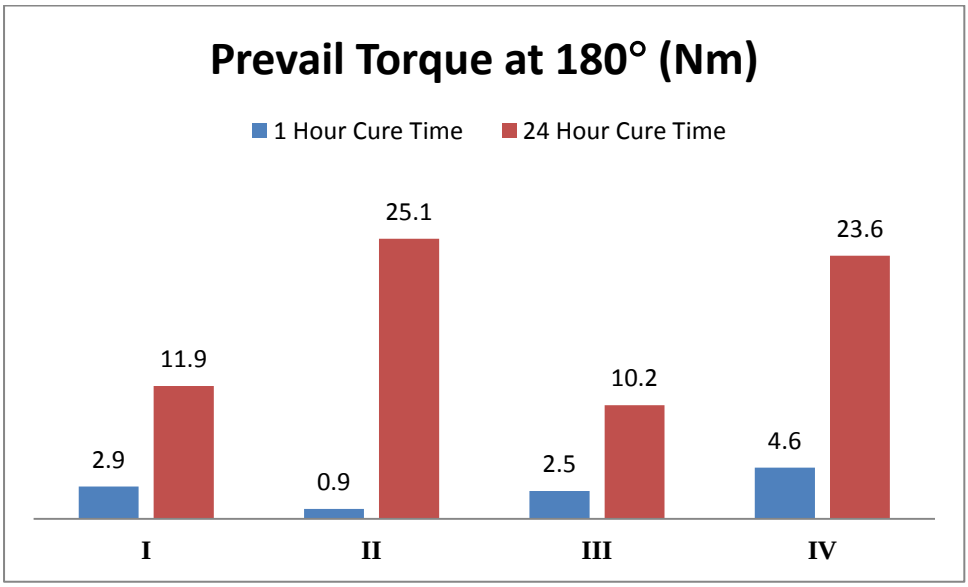

Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
40.2
37.2
34.0
25.2
18.5
10.8
6.6
1.6
I
II
III
IV

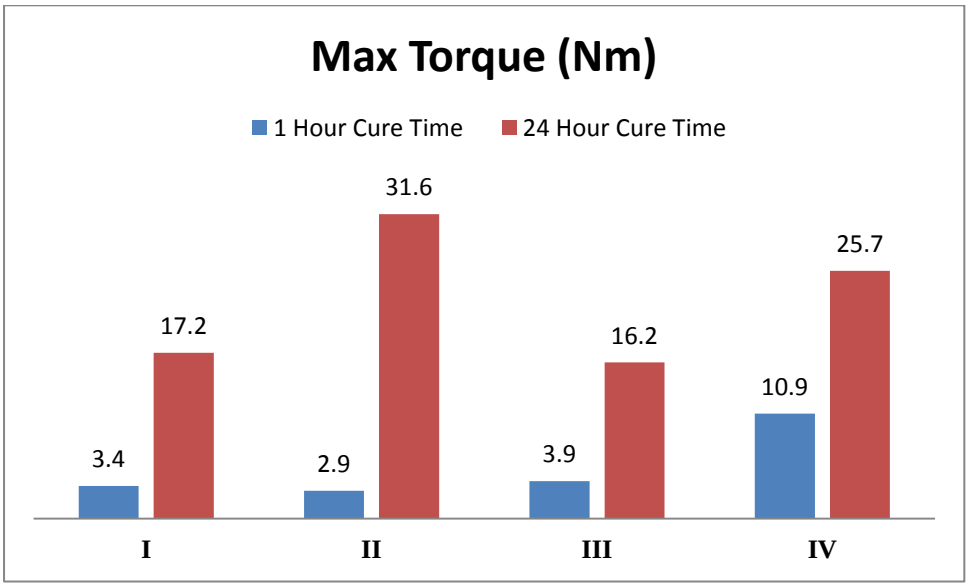
Graph 5.8: Stainless Steel Nuts and Bolts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

The composition of each formulation (I-IV) may be found in Section 5.3.2.

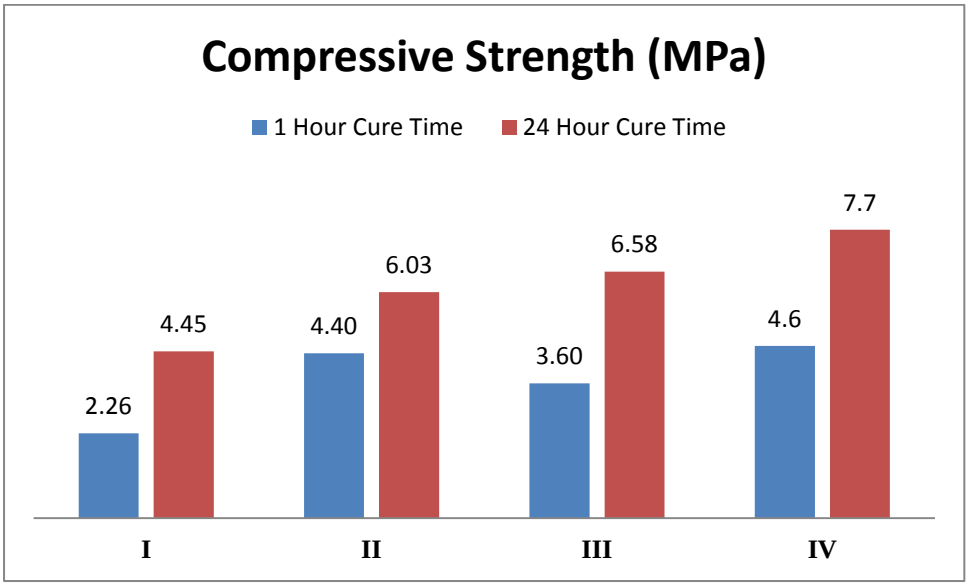


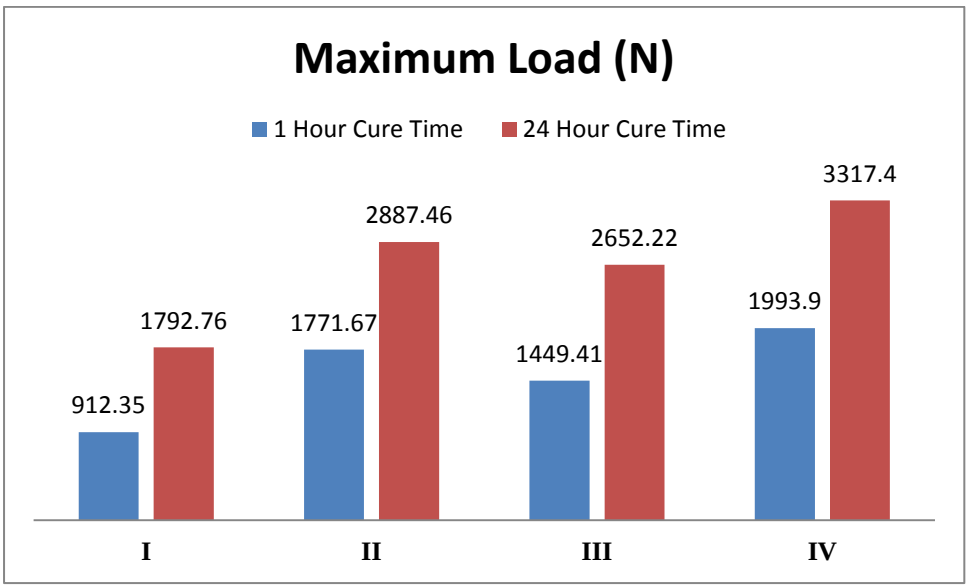



Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
3.4
17.2
2.9
31.6
3.9
16.2
10.9
25.7
I
II
III
IV

Graph 5.9: Mild Steel Pins and Collar:

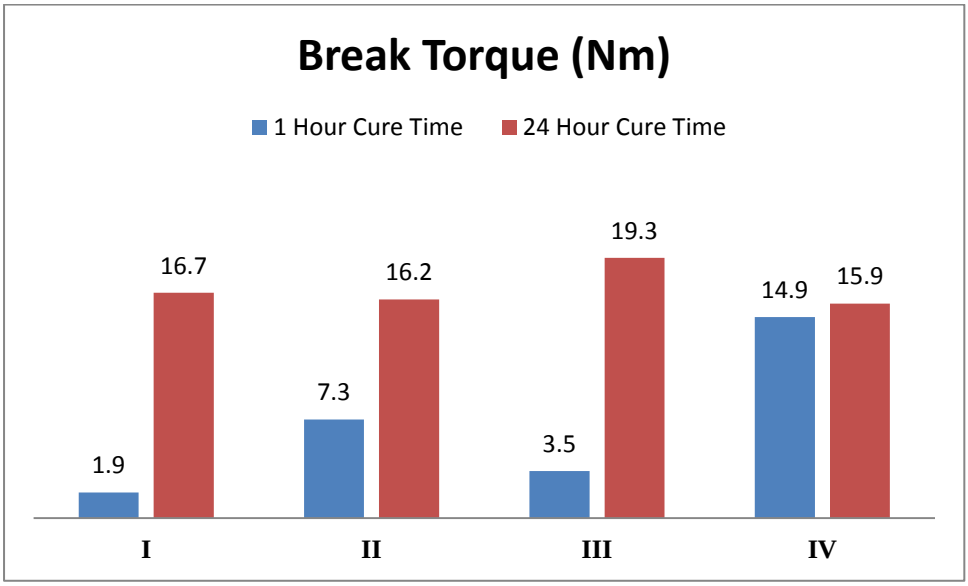
(a) Compressive Strength and (b) Maximum Load

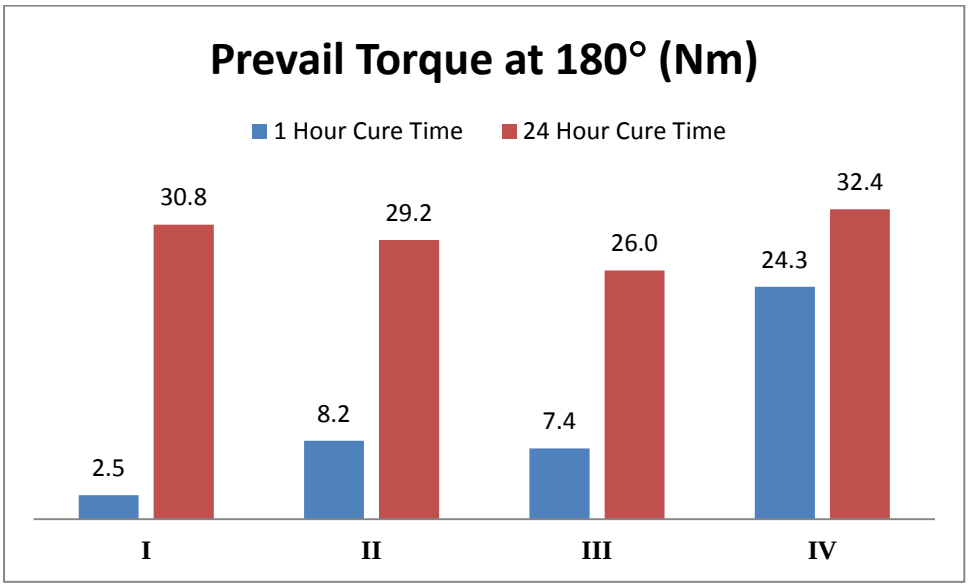


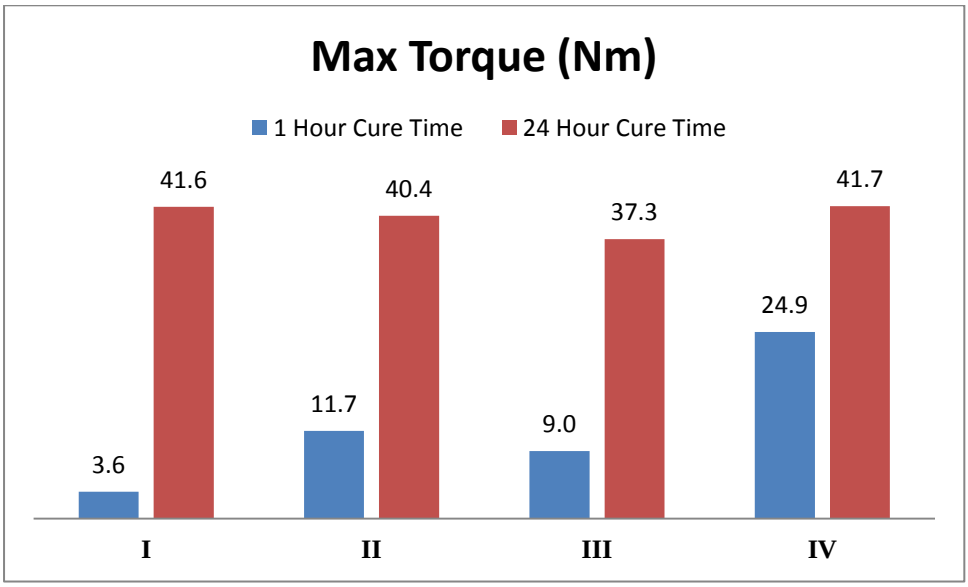


Graph 5.10: Black Oxide Bolts with Mild Steel Nuts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

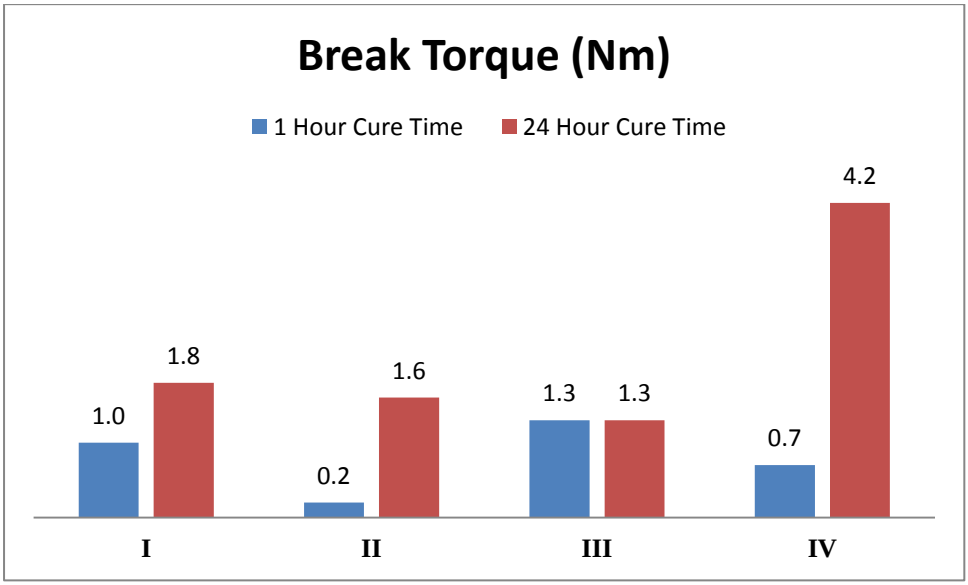


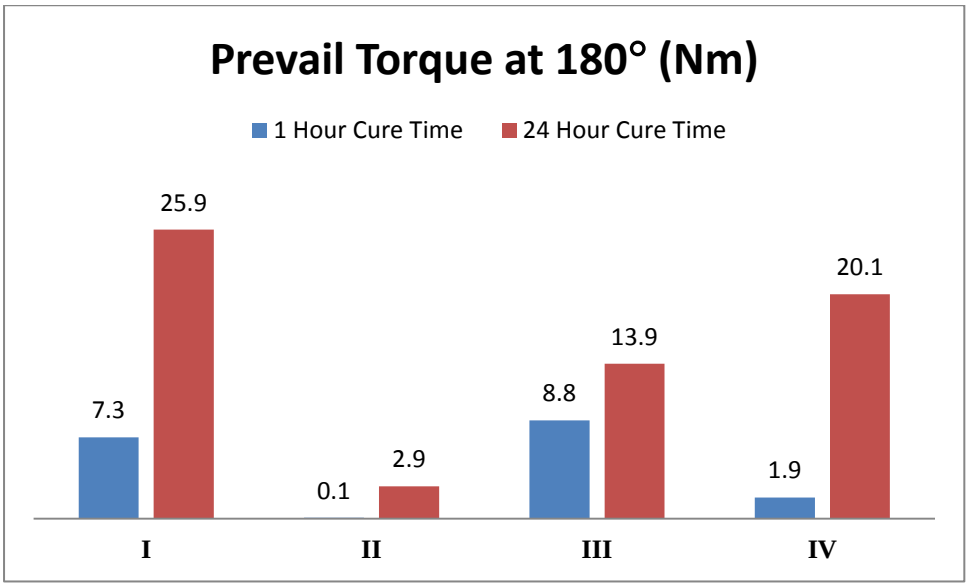


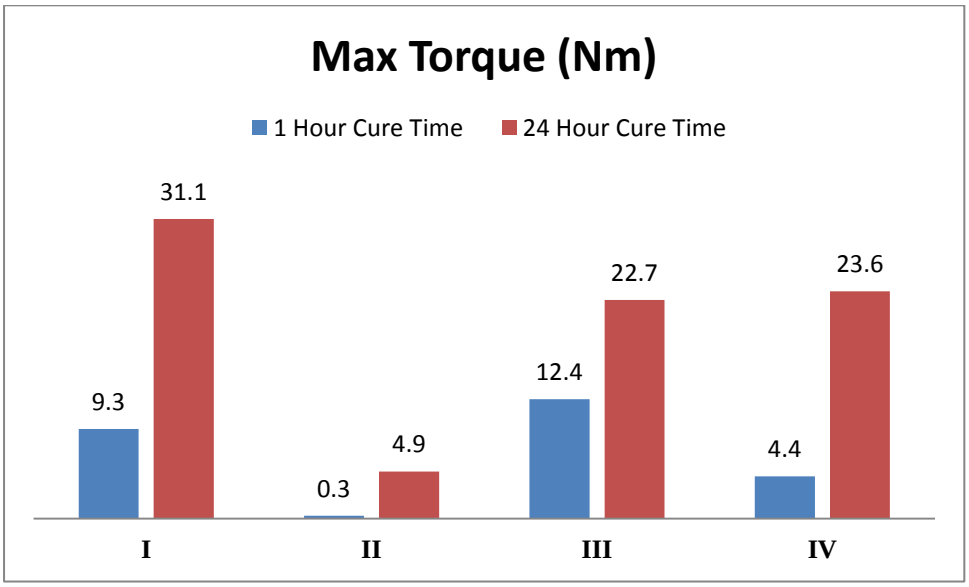

Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
41.6
40.4
37.3
41.7
24.9
11.7
9.0
3.6
I
II
III
IV

Graph 5.11: Stainless Steel Nuts and Bolts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

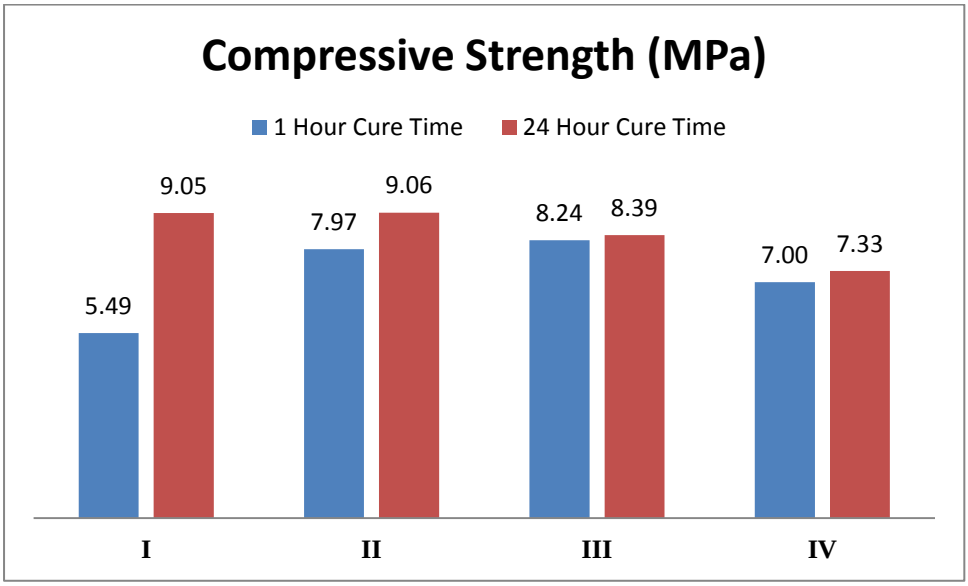


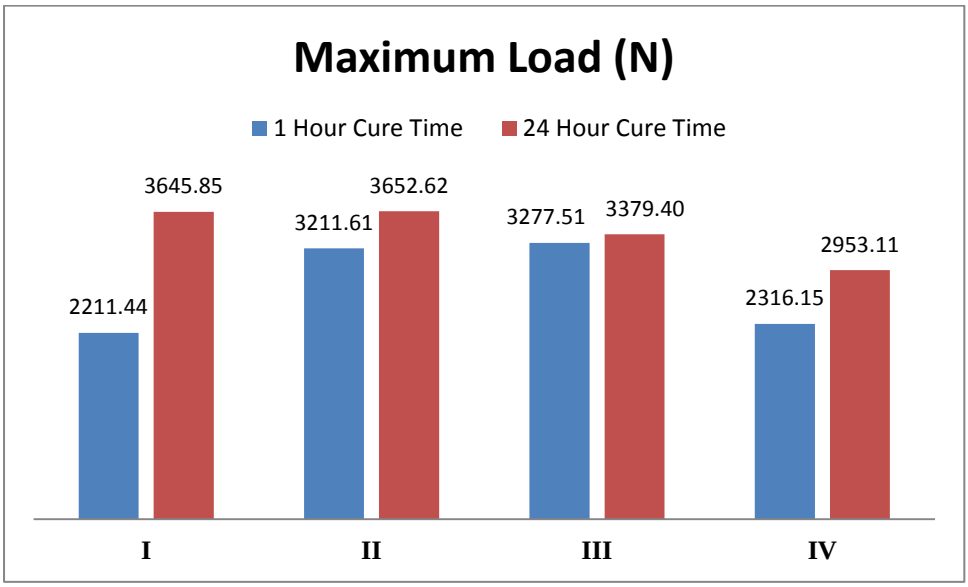



Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
31.1
9.3
0.3
4.9
12.4
22.7
4.4
23.6
I
II
III
IV

Graph 5.12: Mild Steel Pins and Collar:

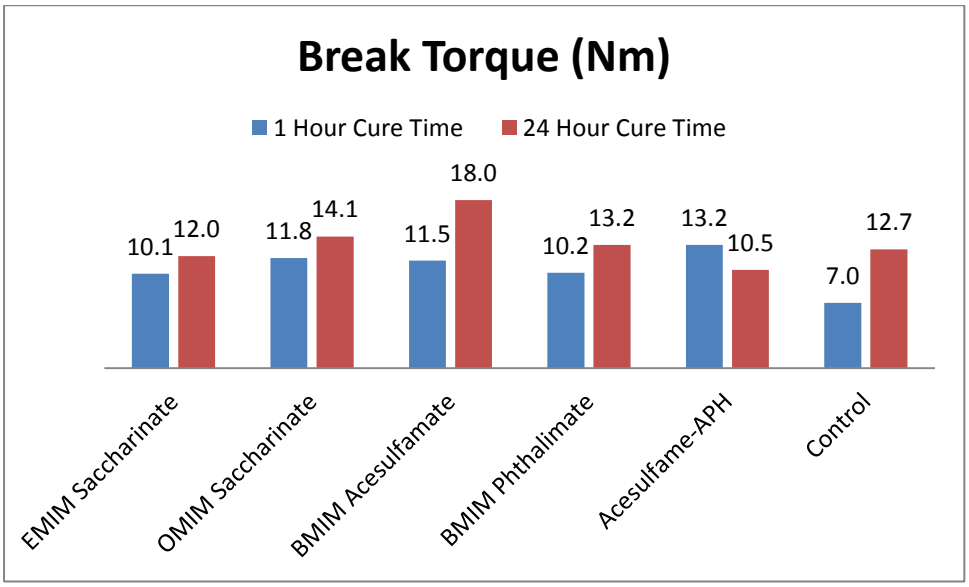
(a) Compressive Strength and (b) Maximum Load

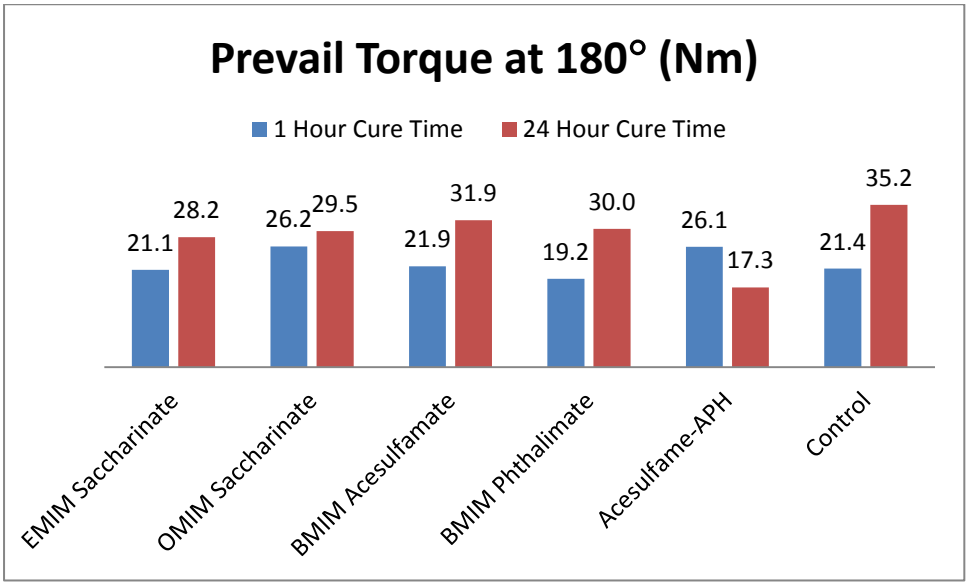


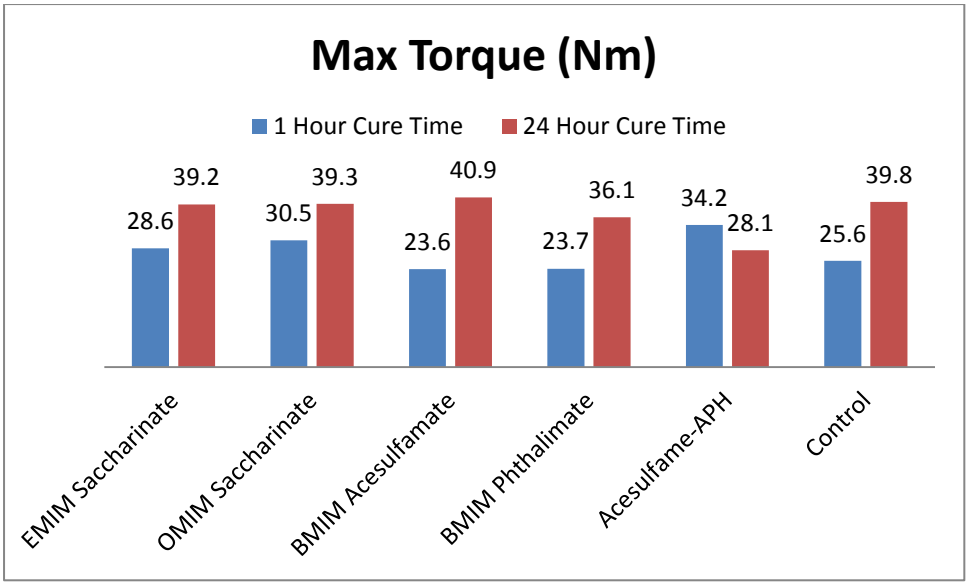


Graph 5.13: Black Oxide Bolts with Mild Steel Nuts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

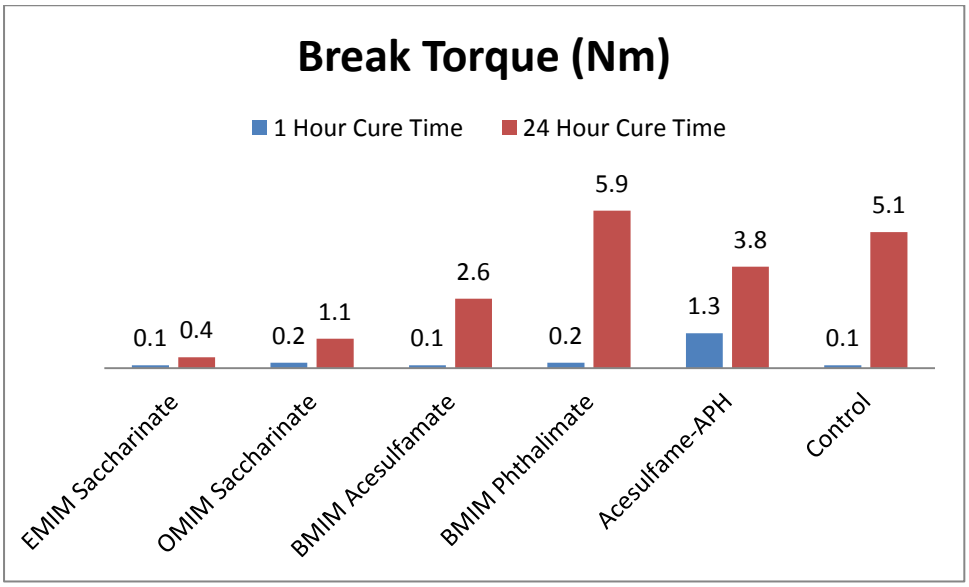


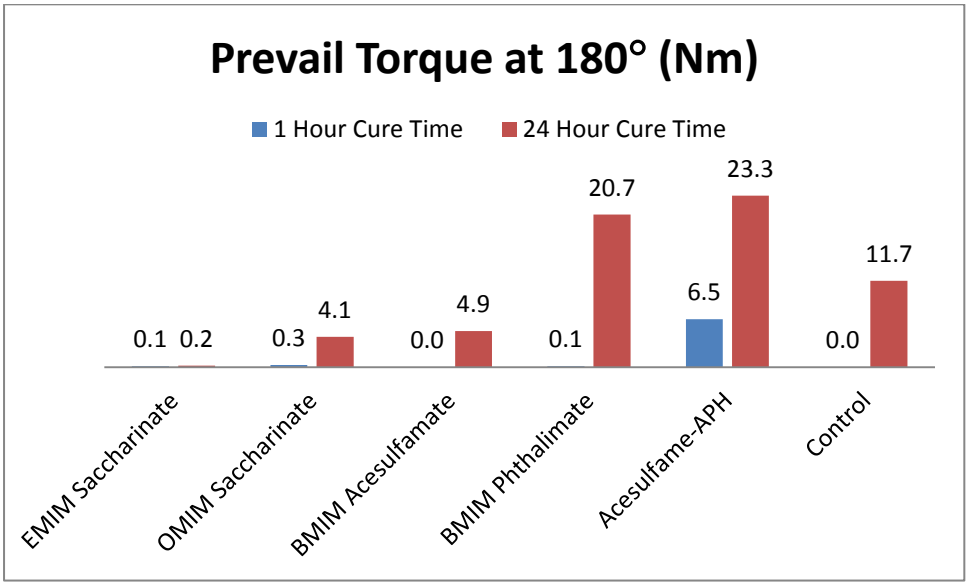


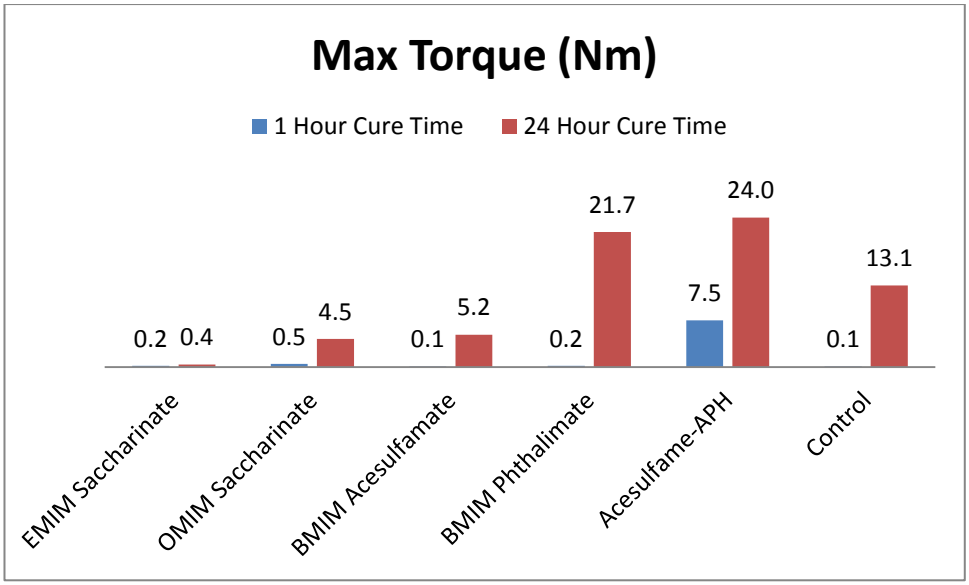

Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
28.6
39.2
30.5
39.3
23.6
40.9
23.7
36.1
34.2
28.1
25.6
39.8
EMIM Saccharinate
OMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
Acesulfame-APH
Control

Graph 5.14: Stainless Steel Nuts and Bolts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

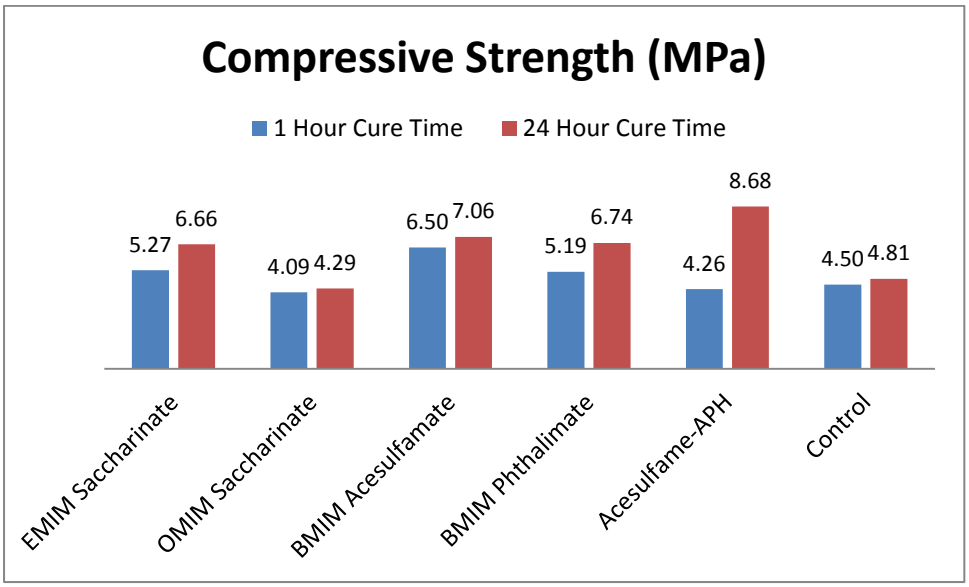


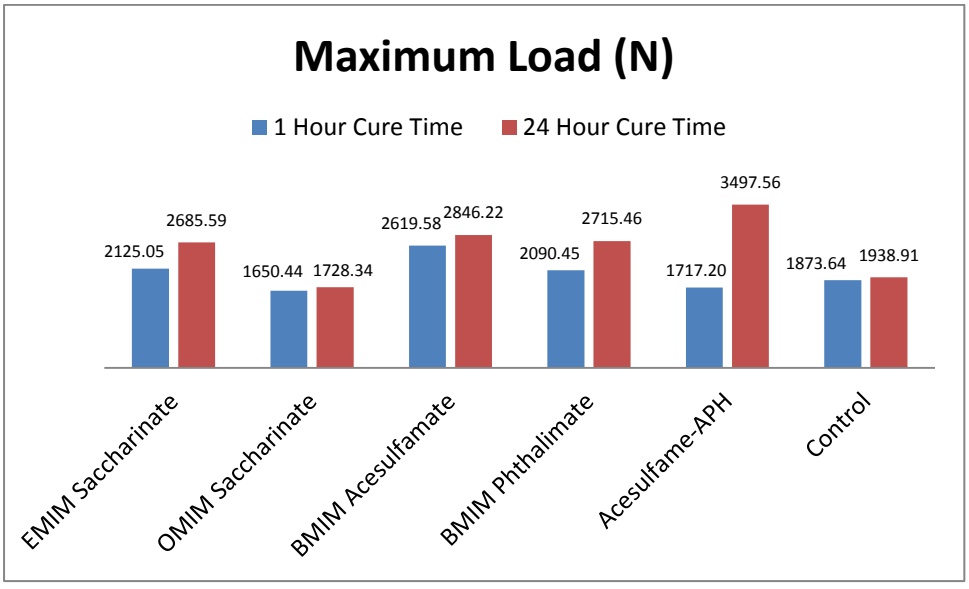



Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
0.2
0.4
0.5
4.5
0.1
5.2
0.2
21.7
7.5
24.0
0.1
13.1
EMIM Saccharinate
OMIM Saccharinate
BMIM Acesulfamate
BMIM Phthalimate
Acesulfame-APH
Control

Graph 5.15: Mild Steel Pins and Collars:

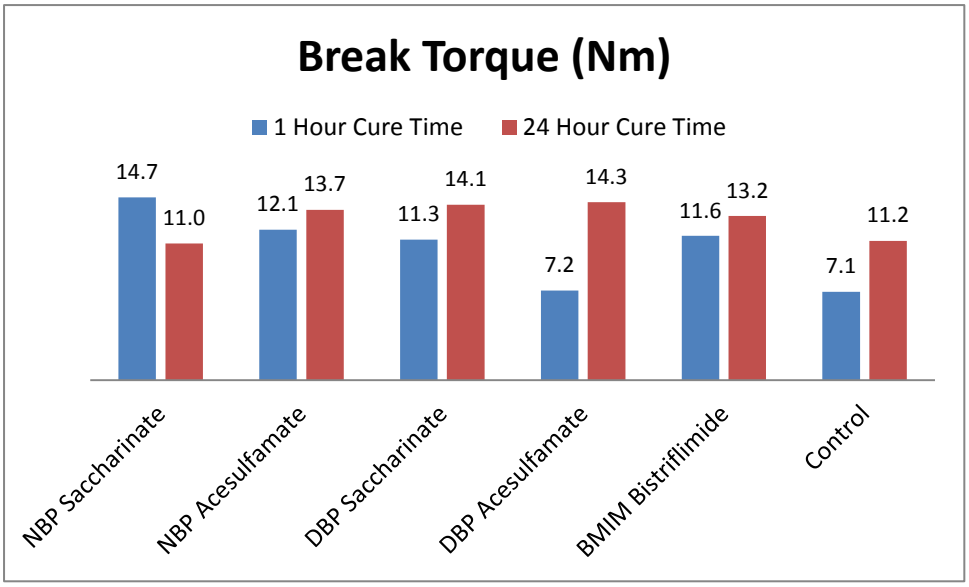
(a) Compressive Strength and (b) Maximum Load

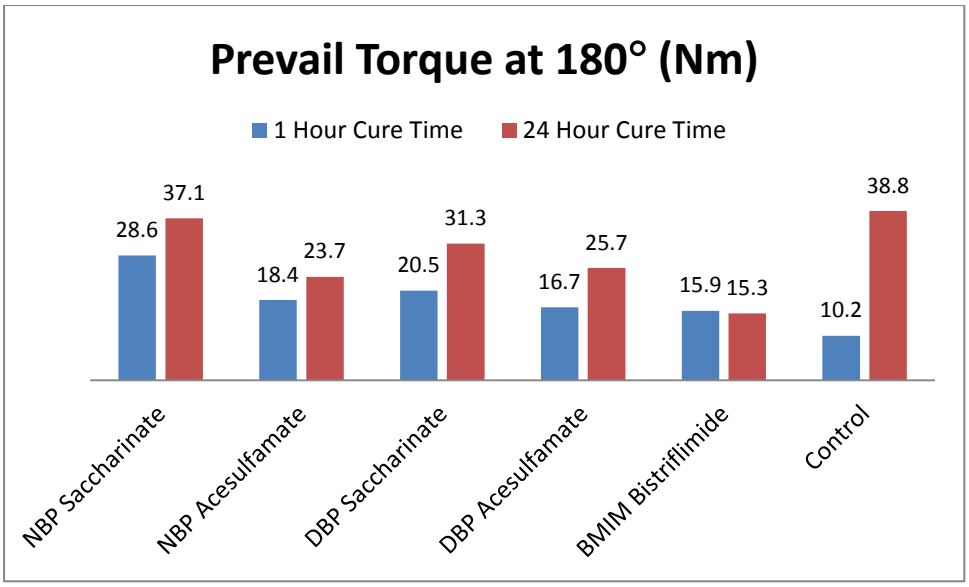


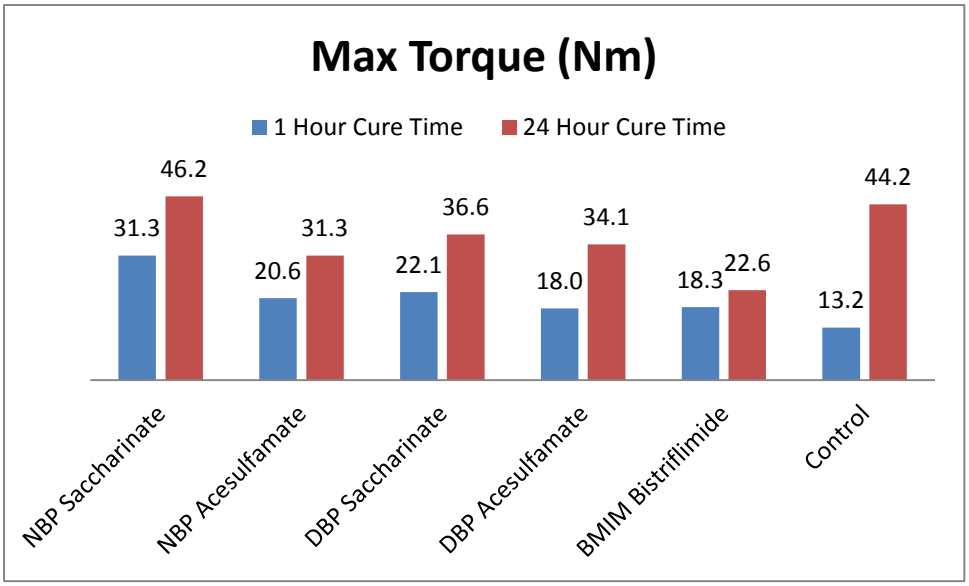


Graph 5.16: Black Oxide Bolts with Mild Steel Nuts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque

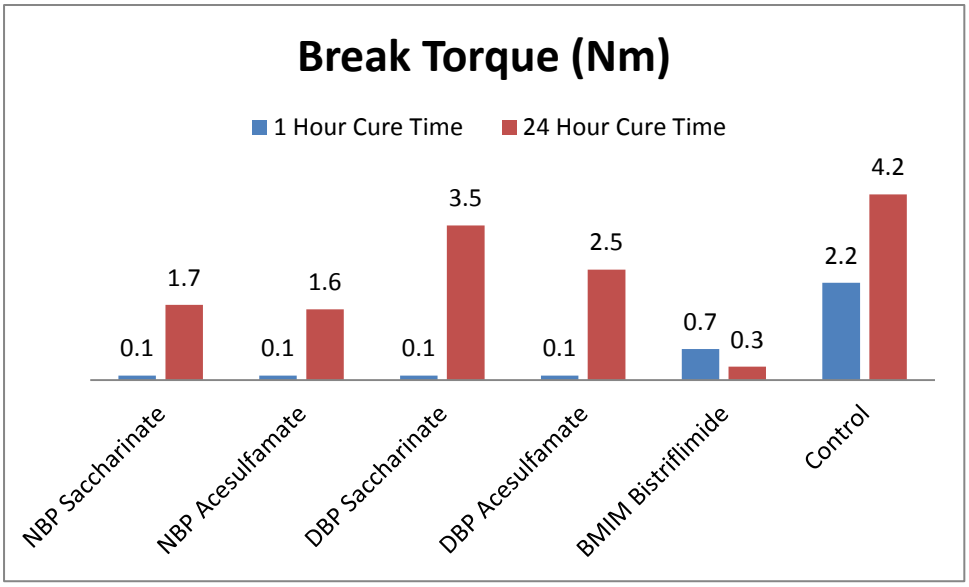


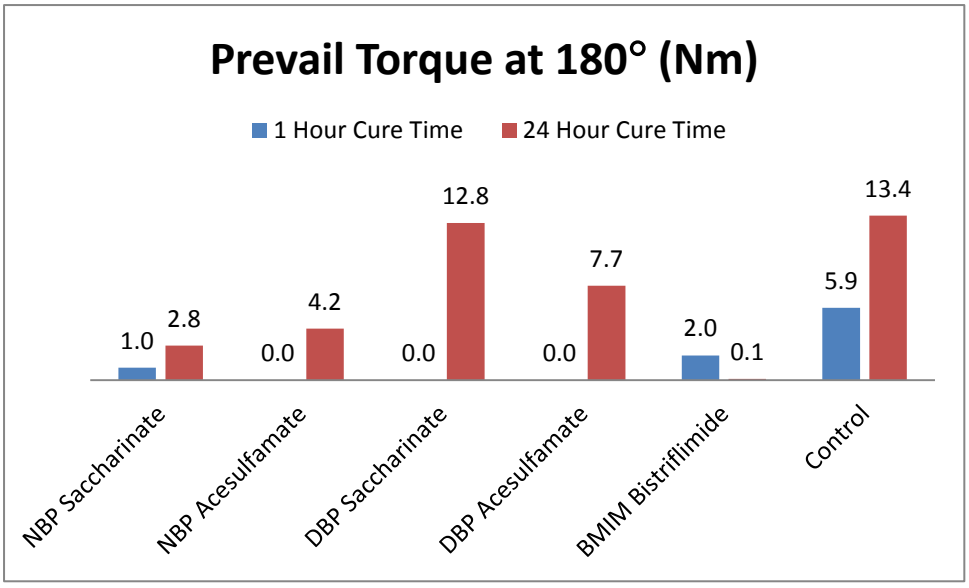


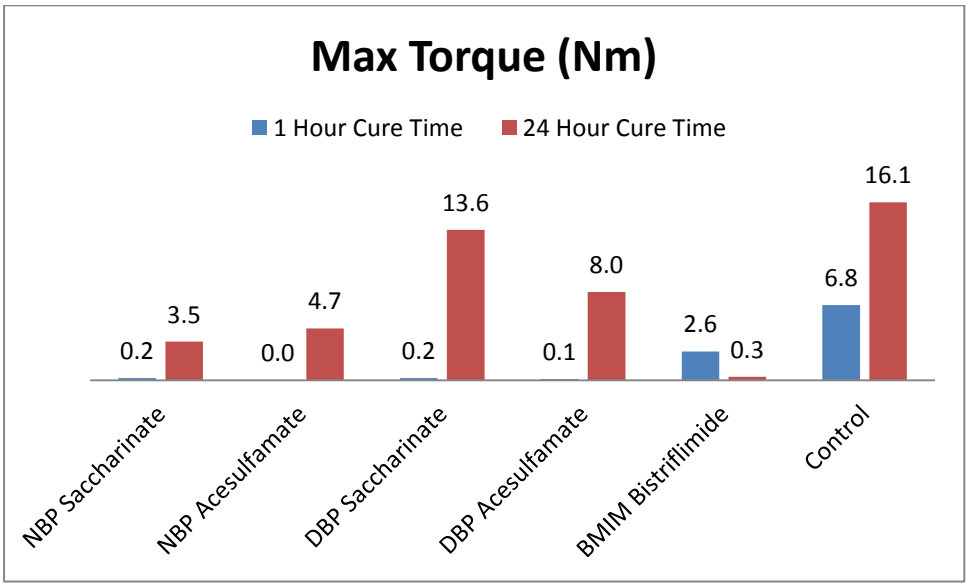

Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
31.3
46.2
20.6
31.3
22.1
36.6
18.0
34.1
18.3
22.6
13.2
44.2
NBP Saccharinate
NBP Acesulfamate
DBP Saccharinate
DBP Acesulfamate
BMIM Bistriflimide
Control

Graph 5.17: Stainless Steel Nuts and Bolts:

(a) Break Torque, (b) Prevail Torque, and (c) Maximum Torque






Max Torque (Nm)
1 Hour Cure Time
24 Hour Cure Time
0.2
3.5
0.0
4.7
0.2
13.6
0.1
8.0
2.6
0.3
6.8
16.1
NBP Saccharinate
NBP Acesulfamate
DBP Saccharinate
DBP Acesulfamate
BMIM Bistriflimide
Control

Graph 5.18: Mild Steel Pins and Collars:

(a) Compressive Strength and (b) Maximum Load