Mechanisms and Applications of Dioxirane Chemistry

by

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Abstract

Dimethyldioxirane oxidises nitrogen containing substrates. The sites of oxidation are generally the sp^3 nitrogen atoms in the molecules although other reactive groups can be oxidised if present. An indication of the reactivity of different dioxiranes was obtained qualitatively from the polarographic peak reduction potentials and quantitatively by reaction with the model substrate 4-nitro-*N,N*-dimethylaniline. The polarographic peak potentials were shown to be of a similar order to those of typical acyclic peroxides. The rank order in terms of reactivity was shown to be methyl(trifluoromethyl)dioxirane > dimethyldioxirane > ethylmethyldioxirane. The rate of the reaction was not influenced by pH or ionic strength but was accelerated greatly by the presence of water. An explanation for this observation was proposed through consideration of dielectric constant and hydrogen bonding effects.

In order to measure the rate of reaction of dioxiranes with other substrates a novel constant potential amperometric technique was developed. The disappearance of the dioxirane current arising from application of the polarographic reduction potential on a hanging mercury drop electrode was used to follow the course of oxidations occurring in a timescale of seconds. Measurements of the second order rate constants from reactions involving a series of *para*-substituted *N,N*-dimethylanilines were used to construct a Hammett plot. The excellent correlation obtained for the Hammett relationship, with a ρ value of - 0.89, supported evidence that the DMD oxidation of *N,N*-dimethylanilines is a concerted electrophilic process in terms of the dioxirane. The amperometric technique was further used to show that the reactions with other oxidising species, such as, hydroperoxides, dialkyl peroxides and hydrogen peroxide were not pseudo first order but followed more complex kinetics.

Dimethyldioxirane was shown to be a useful derivatising agent in analytical chemistry. SB202026, a tertiary amine with poor spectroscopic properties, was converted quantitatively into the corresponding *N*-oxide and determined polarographically. The electrochemical properties of the *N*-oxide were such that reduction only occurred readily at the mercury electrode precluding the use of chromatographic detectors based on glassy carbon technology. Other weakly absorbing amines, such as, phenylpropanolamine, ephedrine and phenylephrine were converted into UV active derivatives and determined by HPLC.

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Symbols and Abbreviations

BDS	base deactivated silica
CBZ	carbamazepine
CH_2Cl_2	dichloromethane
CI	chemical ionisation
DABSCl	dimethylaminophenylazobenzenesulphonyl chloride
DC	direct current
DMA	dimethylaniline
DMD	dimethyldioxirane
DME	dropping mercury electrode
DMF	dimethylformamide
Dns-Cl	dansyl chloride
DPP	differential pulse polarography
$E_{1/2}$	half wave potential
Ep	peak potential
ECD	electrochemical detection
EDTA	ethylenediaminetetraacetic acid
EMD	ethylmethyldioxirane
EtOAc	ethylacetate
EtOH	ethanol
FAB	fast atom bombardment
FDNB	1-fluoro-2,4-dinitrobenzene
GC	gas chromatography
GC-MS	gas chromatography with mass spectrometry detection
HFBA	heptafluorobutyric anhydride
HPLC	high performance liquid chromatography
I	current
I_d	limiting diffusion current

KNO_3	potassium nitrate
KOH	potassium hydroxide
KO^tBu	potassium tertiary butoxide
LC	liquid chromatography
LFER	linear free energy relationship
MCPBA	*m*-chloroperbenzoic acid
MeCN	acetonitrile
MeI	methyl iodide
$MgSO_4$	magnesium sulphate
MRI	magnetic resonance imaging
MS	mass spectrometry
MTFMD	methyl(trifluoromethyl)dioxirane
N_2	nitrogen
NaH_2PO_4	sodium dihydrogen orthophosphate
Na_2SO_4	sodium sulphate
p-NBBr	*p*-nitrobenzyl bromide
NBD-Cl	4-chloro-7-nitro-benzo-2,1,3-oxadiazole
NH_4Cl	ammonium chloride
NH_4OH	ammonium hydroxide
NITC	naphthyl isothiocyanate
NM	nanometers
NMR	nuclear magnetic resonance
PITC	phenyl isothiocyanate
R	resistance
R_f	relative fraction
RSD	relative standard deviation
s	standard deviation
SCE	saturated calomel electrode
TLC	thin layer chromatography
TNBS	2,4,6-trinitrobenzenesulphonic acid

UV	ultraviolet
V	voltage
VIS	visible
λ_{em}	emission wavelength
λ_{ex}	excitation wavelength
λ_{max}	wavelength maxima

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Mechanisms and Applications of Dioxirane Chemistry

General Introduction

Dioxiranes - Chemistry and Reactivity

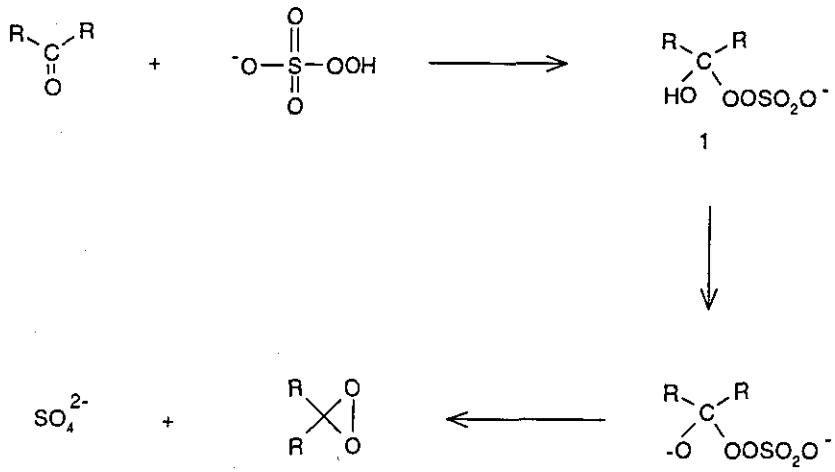
Comprehensive reviews on dioxirane chemistry have been written relatively recently and cover all aspects of dioxirane chemistry.[1-5] It is not intended, therefore, that this introduction be an exhaustive description of dioxirane chemistry but rather a synopsis of pertinent areas of dioxirane chemistry relevant to this thesis.

Dioxiranes are the most strained of cyclic organic peroxides, encompassing one carbon and two oxygen atoms in a three membered ring. They are neutral, thermally unstable compounds and have the longest oxygen-oxygen bond at 1.52Å.[6,7] As a result, the oxygen-oxygen bond is very weak and accounts for both the thermal instability of these compounds and their utility as oxygen atom transfer reagents.

As early as 1899 dioxiranes were postulated as intermediates in the Baeyer-Villiger oxidation of menthone into its lactone by monoperoxysulphuric acid but it was not until 1972 that the first isolation and characterisation of a perfluorodimethyldioxirane was recorded in a United States patent.[8] In this instance the dioxiranes were prepared by fluorinating a compound having 2 oxygen atoms bonded to carbon to effect ring closure to form the peroxide-containing ring.

The events leading to the successful isolation of dioxiranes began with an observation by Montgomery[9] in 1974 that certain ketones enhance the rate of

decomposition of monoperoxysulphuric acid. Furthermore, he discovered that a number of oxidation reactions of caroate, the sulphate equivalent, are catalysed by the presence of ketones. These results led Montgomery to postulate that the monoperoxysulphate anion was adding to the ketone to give an adduct (**1**). The latter is in fact the 'Criegee' intermediate proposed for the Baeyer-Villiger reaction. Since a variety of ketones could enhance the caroate decomposition, Montgomery suggested that intermediate (**1**) reacted further to give a dioxirane. (Scheme 1)



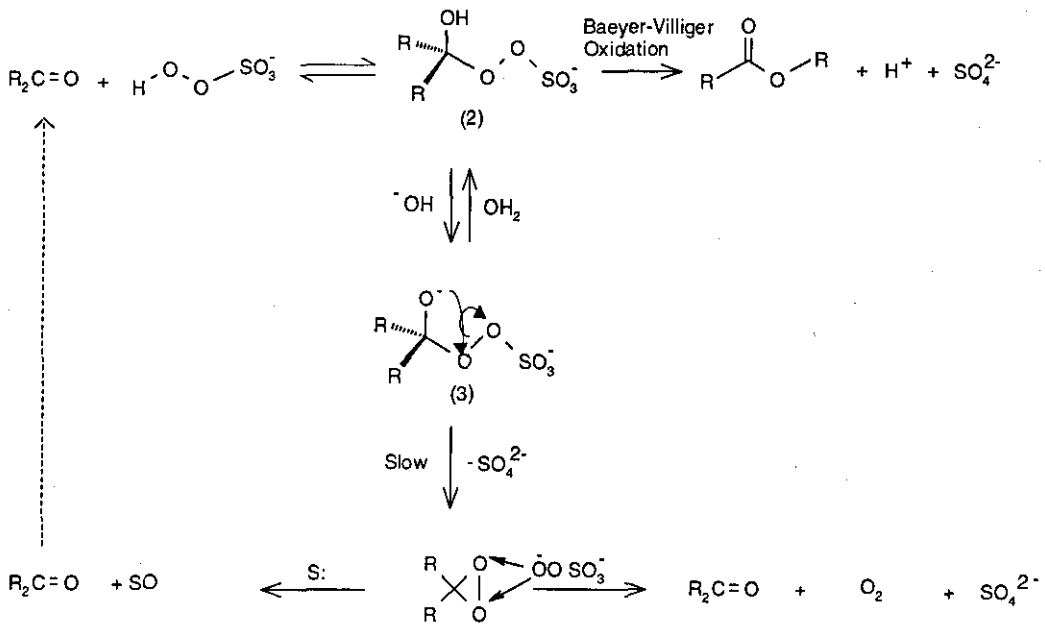
Scheme 1. Generation of dioxiranes

While convinced that dioxiranes were involved as intermediates in the catalysed decompositions, Montgomery was not prepared to say that these dioxiranes were in fact, acting as oxidants. Thus, while he was working in slightly alkaline as opposed to the usual acidic conditions of the Baeyer-Villiger reaction, Montgomery had essentially revived the idea that dioxiranes could be involved in these reactions.

A more detailed study[1] based on extensive kinetic and labelling experiments has since provided the commonly accepted mechanism for dioxirane formation

(Scheme 2).[10] The peracid reacts with the ketone to yield intermediate (2), which, after loss of the hydroxide ion and ring closure with the expulsion of the sulphate ion forms dioxirane.

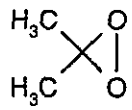
The pH at which dioxirane is formed is critical. At high pH (>8), oxygen transfer by the dioxirane to the substrate (S:) is competed for by the peroxy anion $^{-}OOSO_3^{-}$ leading to destruction of the dioxirane with concomitant formation of molecular oxygen. At low pH (<7) the crucial deprotonation **2** -> **3** is suppressed and dioxirane formation is less probable. Hence the reason why, when *in situ* generated dioxirane is employed it is crucial to control the pH at 7.0-7.5 by means of phosphate or bicarbonate buffer.



Scheme 2. Mechanism for dioxirane formation

Dioxiranes are most commonly prepared today by the oxidation of ketones by peracids, usually, monoperoxysulphuric acid. The most commonly used

dioxirane, dimethyldioxirane (**4**) was first prepared by this method and isolated by Murray and Jeyaraman in 1985.[11]



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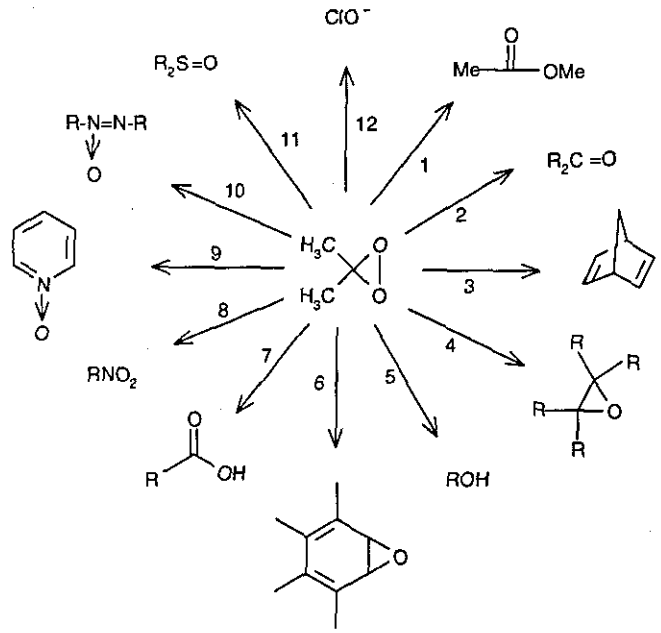
The chemistry reported to date demonstrates that dioxiranes are very powerful oxidants which can be used either in a solution of the parent ketone or generated *in situ*.[11,12]

Solutions of dimethyldioxirane (DMD) in the parent ketone (acetone) have been used to carry out a variety of oxygen atom transfer reactions. They provide a useful synthetic procedure which give products in high yields and, in the case of epoxides, in a regio- and stereo-selective manner.[3,10,12-18] The many transformations of dioxirane reactions are shown in Scheme 3.



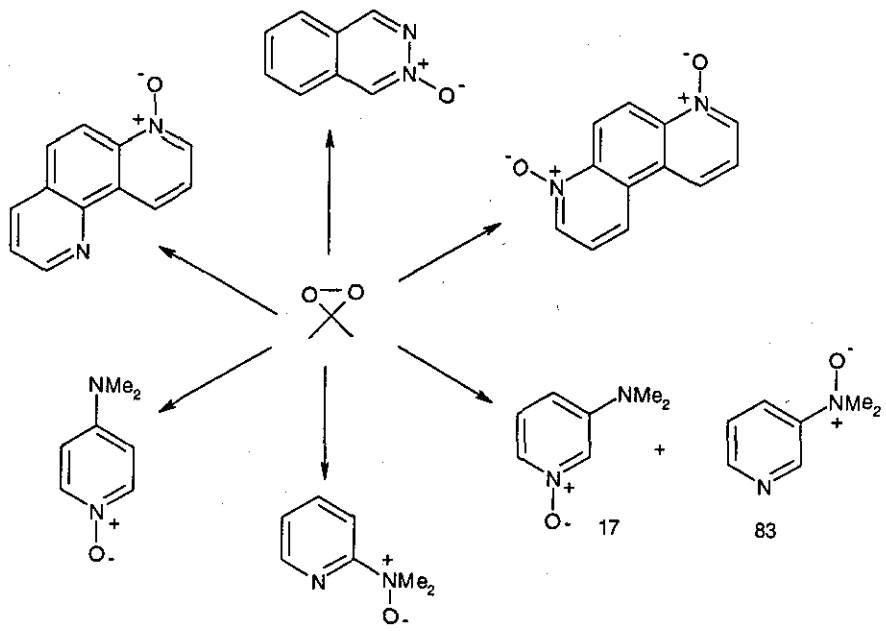
Scheme 3. Dioxirane transformations

The rearrangement of dioxirane into methyl acetate[11] (transformation 1), the oxidation of secondary alcohols into ketones[19] (transformation 2) and the ability of dimethyldioxirane to act as a catalyst in the quadricyclane to norbornadiene valence isomerisation[20,21] (transformation 3) do not formally involve oxygen transfer. However, Marples *et al* have shown that in the oxidation of secondary alcohols into ketones the oxygen is partially derived from DMD as the formation of the ketone results from the intermediate formation of the *gem*-diol.[22]

Effective epoxidations[10,12,13,14] (transformations 4 and 6), as well as, insertions into C-H bonds of alkanes[19] and aldehydes,[11] (transformations 5 and 7), are well known. Indeed contributions to these areas have been made at Loughborough University.[22]

The efficient conversion of primary amines into nitro compounds,[23] (transformation 8), oxidation of tertiary amines, pyridine, and azo compounds[11,24] gives the corresponding *N*-oxides (transformations 9 and 10) and certain secondary amines yield nitroxides upon reaction with dimethyldioxirane which proceed *via* prior oxygen atom insertion into the N-H bond.[25] Organic sulphides[11, 26] and chloride ions[10,12,13,14] are quantitatively oxidised to yield sulphoxides and hypochlorite ion respectively, (transformations 11 and 12).

Dimethyldioxirane has not only been shown to be selective in the case of epoxidation reactions but, also, in the oxidation of nitrogen heteroarenes as shown in Scheme 4.



Scheme 4. Selective oxidations of bidentate nitrogen heteroarenes by DMD

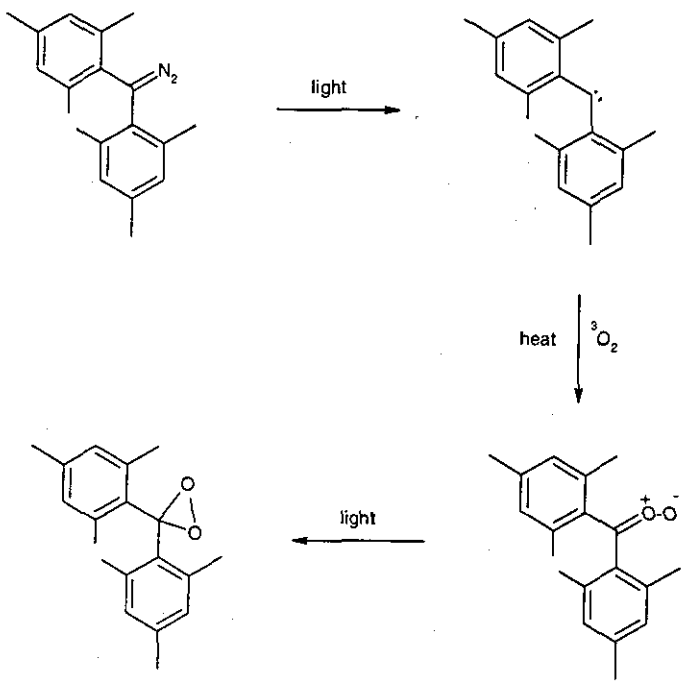
Dimethyldioxirane has, also, been shown to oxidise polycyclic aromatic hydrocarbons[27] to form arene oxides which are of importance in environmentally related mutagenesis and carcinogenesis.

In 1985 Murray and Jeyaraman showed that a few other low molecular weight dialkyldioxiranes could, also, be isolated from buffered aqueous solutions containing caroate and the parent ketone by low temperature distillation.[11] These included ethylmethyldioxirane, methyl-*n*-propyldioxirane, methyl-*n*-butyldioxirane and diethyldioxirane. In 1992 they identified several new non-volatile dioxiranes, for example, menthone dioxirane.[28] These preparations involved the use of a heavy salting-out procedure to obtain solutions of the dioxirane in the parent ketone.

Mello *et al* reported the isolation and full spectroscopic characterisation of methyl(trifluoromethyl)dioxirane in solution in 1988.[16] The latter has been

shown to be much more reactive than dimethyldioxirane. In the case of the oxyfunctionalisation of saturated hydrocarbons methyl(trifluoromethyl)dioxirane is 500 times more reactive than dimethyldioxirane.[29]

In 1993 Russo and DesMarteau reported the synthesis of difluorodioxirane from FCOOF and CsF/ClF which is stable at room temperature for several days.[30] In 1994 Kirschfeld *et al* reported the synthesis of dimesityldioxirane, the first stable, crystalline dioxirane. This was prepared by the photochemical oxidation of a diazo compound in which dimesitylcarbene was photochemically generated followed by a thermal reaction with triplet oxygen to form a carbonyl *O*-oxide which upon irradiation rearranged into dimesityldioxirane.[31] (Scheme 5)

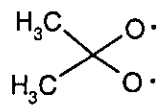


Scheme 5

Schulz *et al* recently published a new method of generating dioxiranes *in situ* using arenesulphonic peracids.[32] This method provides access to the *in situ* generation of dioxiranes in homogeneous organic solution.

An interesting area of research recently has been in the preparation of resin bound dioxirane which would provide a recyclable source of oxidising reagent. The first publication of this type of system was by Shiney *et al*[33] who used a polystyrene-bound dioxirane to oxidise alkenes to epoxides, pyridines to *N*-oxides and amines to nitro compounds in near quantitative yields. Related research has been carried out simultaneously by Marples *et al* at the University of Loughborough.[34]

There is still continuing debate with regards to the mechanism of dimethyldioxirane oxidations with particular attention to the mechanisms of epoxidation and insertion of oxygen into CH bonds. The majority of reactions have been cited as involving a concerted electrophilic mechanism,[35] however, publications by Kazakov *et al*[36] and Minisci *et al*[37] implicate a radical mechanism involving the bis(oxyl)diradical (**5**).



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Miaskiewicz *et al*[38] recently performed ab initio studies of the initial step in the dimethyldioxirane oxidation of primary amines. They demonstrated that dimethyldioxirane displays characteristics of a typical electrophile in the reactions with the primary amines studied.

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1.0 The Oxidation of Nitrogen by Dimethyldioxirane.

1.1 Summary

This chapter represents early investigative work into the reactivity of dimethyldioxirane. Qualitative results only were studied and tentative identification of the products postulated. Similar experiments were, also, conducted in parallel at Loughborough University and are complementary to these studies.

Dimethyldioxirane oxidises a variety of compounds containing nitrogen atoms. The sites of oxidation are generally the sp^3 nitrogen atoms in the molecules. Granisetron and SB202026, both containing tertiary amine groups, oxidised simply to form the corresponding *N*-oxides. It is postulated that quinidine similarly reacts but the quinoline ring nitrogen, also, oxidises to yield a "di-*N*-oxide" as the major product. Ropinirole and SB207266 containing oxygen substitution at the 2-position of the indole ring, were both thought to be oxidised at the 3-position in addition to oxidation of terminal sp^3 nitrogen atoms in the side chains.

1.2 Introduction

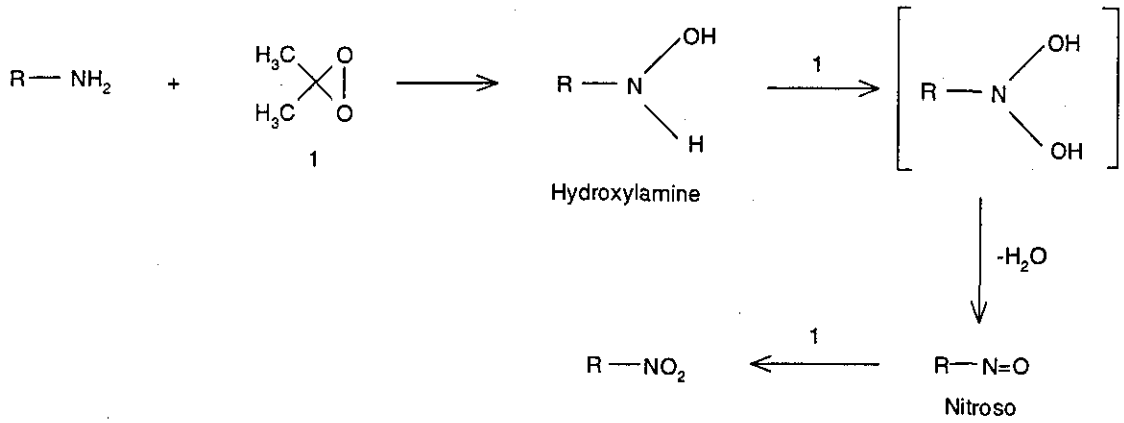
Many drug molecules, *in vitro*, degrade by oxidation. This is particularly true for those containing nitrogen atoms which present a relatively easy target for potential oxidants. Under normal conditions of use the agent responsible for oxidation is molecular oxygen. Since this molecule can react in a number of ways through the formation of surrogate species, such as peroxides it is not an

easy matter to accelerate the process to gain a rapid insight into the potential of any drug for oxidative degradation. It was thought that DMD could be used to mimic such oxidative species and hence help to identify possible products of oxidative degradation.

A review of the existing knowledge of the DMD oxidation of amines is detailed in the remaining section of this introduction.

1.2.1 Oxidation of Primary Amines

A survey of some general methods for the preparation of nitro compounds[1] suggests that dimethyldioxirane (**1**) is the reagent of choice. The conditions are exceedingly mild and the conversion from amine to nitro compound takes place in high yield (Scheme 1).

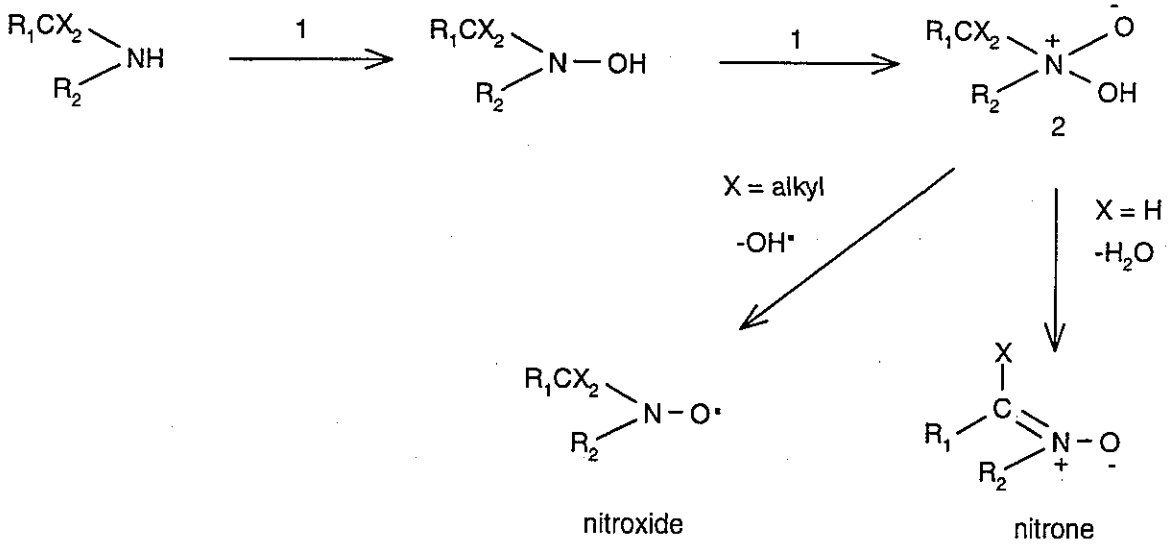


Scheme 1. Oxidation of a primary amine using DMD

1.2.2 Oxidation of Secondary Amines

In general, the oxidation of secondary amines with dimethyldioxirane gives hydroxylamines in a simple, one step and high yielding process. The product obtained is very much a function of the reaction conditions and the structure of

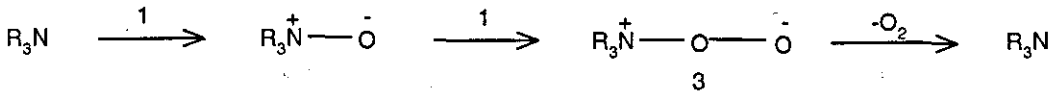
the amine. When equimolar quantities of amine and dioxirane are reacted, the product is the hydroxylamine. When a second mole of dioxirane is available further reaction occurs to give an intermediate (**2**), which if α hydrogens are present, loses water to give a nitrone. If no α hydrogens are present then the product is the nitroxide (Scheme 2).



Scheme 2. Oxidation of secondary amines using DMD

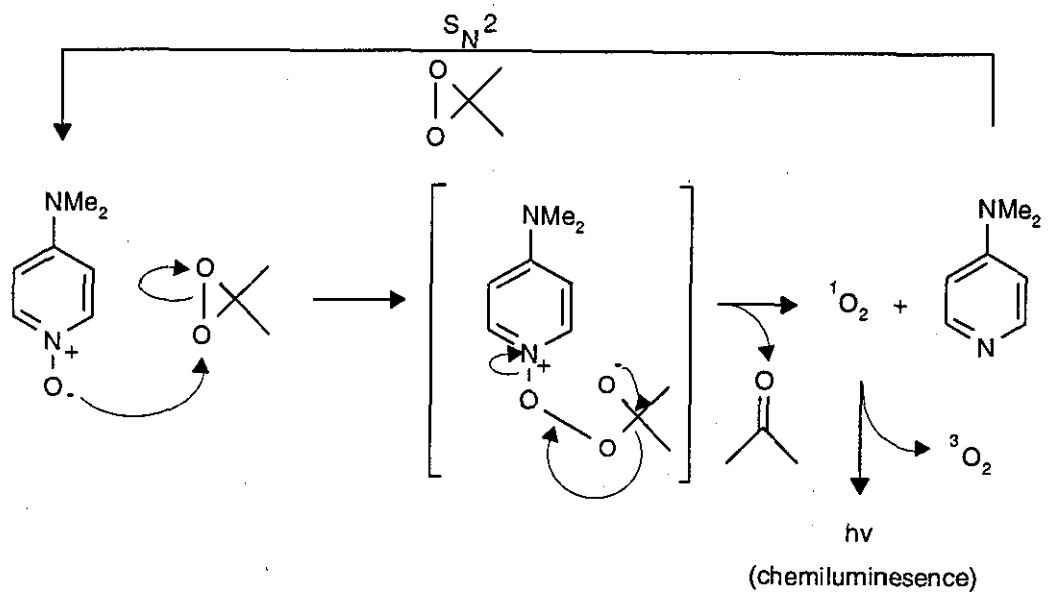
1.2.3 Oxidation of Tertiary Amines

The search for mild, safe and selective reagents for *N*-oxidation of tertiary amines has been and still is the subject of active investigation. Dimethyldioxirane oxidises tertiary amines to the amine oxides in very high yield, an example of this is the oxidation of pyridine to pyridine-*N*-oxide. Murray[2] reports that the reaction conditions are critical if the desired product is the *N*-oxide and postulates that excess dioxirane can lead to an intermediate, presumed to be **3**, which loses oxygen to regenerate the amine (Scheme 3). Further examples are demonstrated in this thesis and were recently published by Ferrer *et al.*[3]



Scheme 3. Oxidation of tertiary amines using DMD

Adam and Smerz[4] observed that certain *N*-heterocyclic substrates could not be converted in high yield to the corresponding *N*-oxides, even when a large excess of DMD was used. They showed that 2,3,5-triacetyladenosine was transformed to the corresponding 1-*N*-oxide in only 62% yield with a fivefold excess of DMD, although the DMD was consumed within a few minutes. The dioxirane was shown to decompose under the reaction conditions to give dioxygen generated in its singlet-excited state. They postulated that the dipolar intermediate fragments into the amine and singlet oxygen as is shown for 4-dimethylaminopyridine-*N*-oxide (Scheme 4). The liberated amine is then reoxidised to the *N*-oxide to close the cycle and to establish a substrate-specific equilibrium mixture of amine and its *N*-oxide.

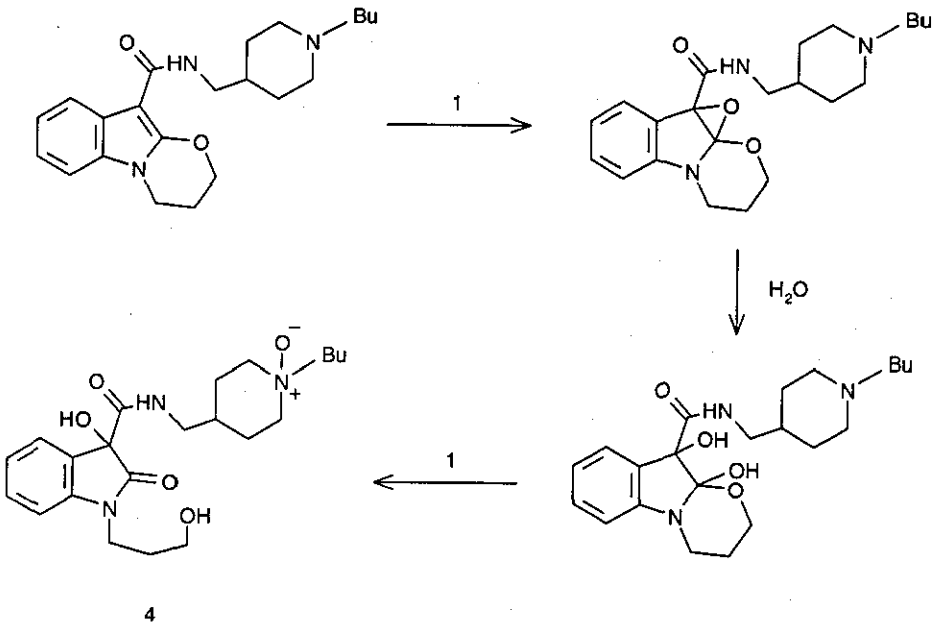


Scheme 4. S_N2 mechanism of the *N*-oxide induced decomposition of DMD

1.3 Results and Discussion

As a preliminary insight into the reactivity of DMD with compounds containing nitrogen, the following reactions were performed. Qualitative experiments only were studied and tentative identification of the products were postulated mainly using mass-spectrometry data.

1.3.1 SB207266

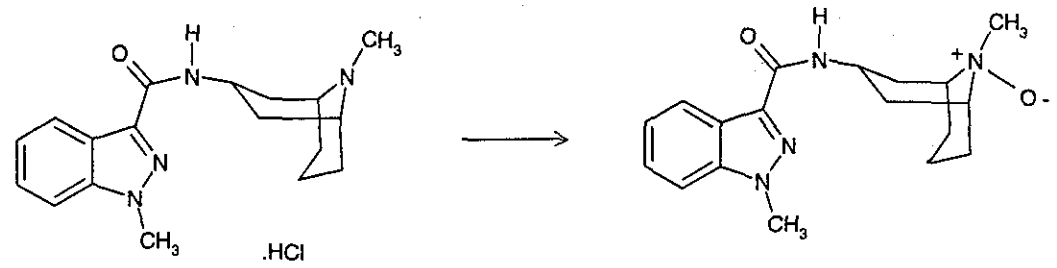


4

Scheme 5

From mass-spectrometry data, MS(FAB); m/z 420 [$(M+H)^+$, 60%], it was postulated that the likely structure of the product of the reaction between SB207266 and DMD was the *N*-oxide (**4**). TLC showed the presence of 4 spots possibly due to the 4 isomers of **4**, 2 axial and 2 equatorial.

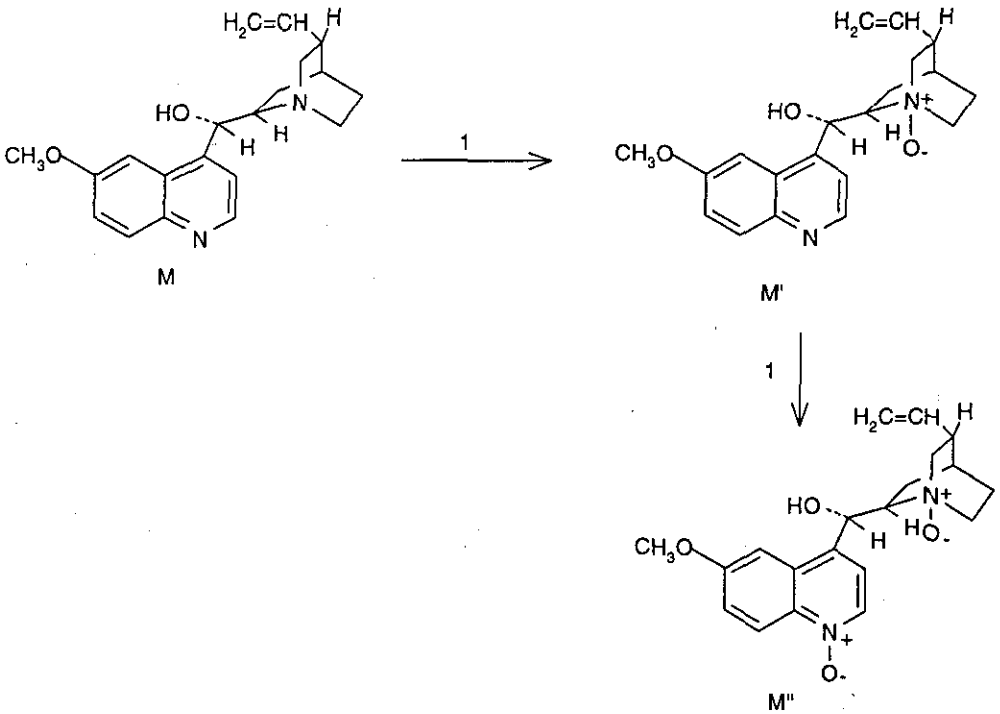
1.3.2 Granisetron hydrochloride



Scheme 6

Granisetron hydrochloride was reacted with DMD and the formation of the axial and equatorial *N*-oxides monitored by HPLC. Both *N*-oxides were formed although not in equal proportions. Positive identification of the products was possible due to the availability of standards of the *N*-oxides.

1.3.3 Quinidine



Scheme 7

Treatment of Quinidine (M) with DMD gave a MS (FAB):

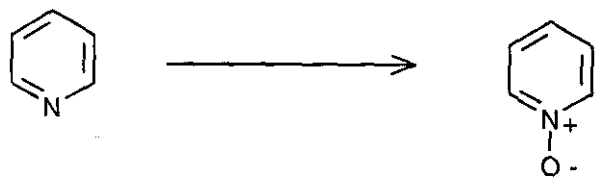
m/z 325 [$(M+H)^+$, 45%] (parent compound)

m/z 341 [$(M'+H)^+$, 65%] (*N*-oxide)

m/z 357 [$(M''+H)^+$, 45%] (di-*N*-oxide)

The structures for the molecular ions are postulated as shown in Scheme 7. As this experiment was an initial investigation into the uses of DMD, further investigation into the exact nature of the products obtained was not thought to be necessary.

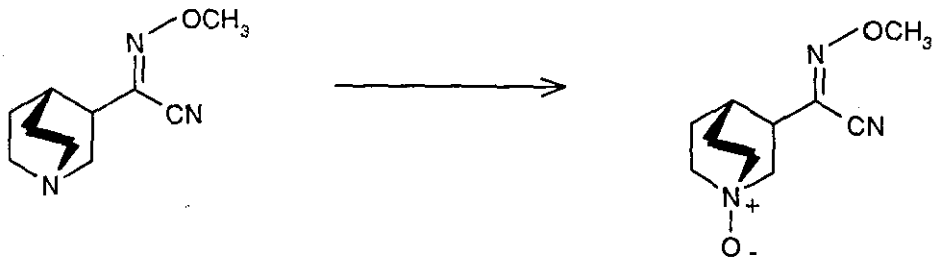
1.3.4 Pyridine



Scheme 8

Treatment of pyridine with DMD gave pyridine-*N*-oxide as evidenced by mass-spectrometry. MS(FAB); *m/z* 96 [$(M+H)^+$, 65%]. The latter is a well known example of the DMD oxidation of a tertiary amine.[2]

1.3.5 SB202026



Scheme 9

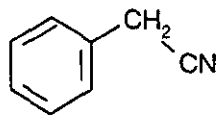
SB202026 is a compound currently under development in the laboratories at SmithKline Beecham Pharmaceuticals. During initial preformulation experiments the drug substance was stressed under acidic and basic conditions in order to determine the possible routes of degradation. The only degradation products identified by these procedures were the geometric (E)-isomer, amide and acid of the drug. The latter two formed from nitrile degradation.

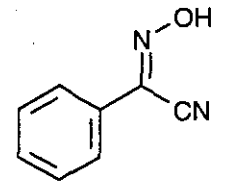
Further into the development programme, during routine stability testing of SB202026 tablets, an unidentified peak was observed in the chromatograms. LC-MS experiments identified the unknown as SB202026-*N*-oxide.

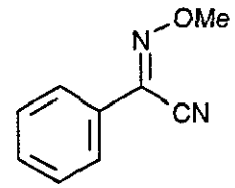
Reaction of DMD with SB202026 gives the *N*-oxide in near quantitative yield. The *N*-oxide was identified using reference standard material. A possible use of DMD within development is, therefore, to provide a better means of predicting what will happen to drug products on store so that unexpected degradation products are not observed during routine stability testing programmes.

In principle, the formation of an *N*-oxide of SB202026 could arise from oxidation of any of the three nitrogen atoms present in the molecule. In order to establish which *N*-oxide was formed a series of related compounds was prepared and subjected to oxidation by DMD.

1.3.6 Benzyl cyanide, 2-Hydroxyimino-2-phenylacetonitrile & 2-Methoxyimino-2-phenylacetonitrile.



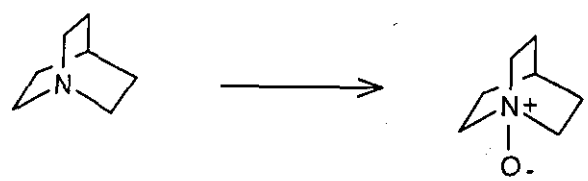




HPLC methods were developed for detecting the above compounds. The preparation of which is detailed in the experimental section. Treatment of benzyl cyanide, 2-hydroxyimino-2-phenylacetonitrile and 2-methoxyimino-2-phenylacetonitrile with DMD showed no reaction.

Due to the failure of DMD to react with those related compounds containing both nitrile and oximino nitrogens it was concluded that the single oxidative product was the *N*-oxide of the bridge nitrogen.

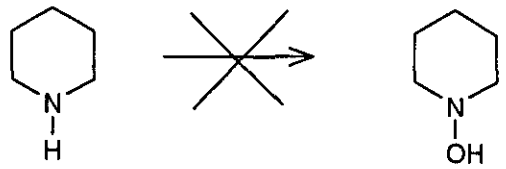
1.3.7 Quinuclidine



Scheme 10

It was postulated from mass-spectrometry data, MS(FAB); m/z 128 [$(M+H)^+$, 100%], that treatment of quinuclidine with DMD gave quinuclidine-*N*-oxide.

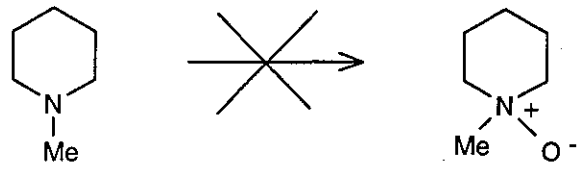
1.3.8 Piperidine



Scheme 11

Mass-spectrometry data on the reaction between piperidine and DMD was inconclusive. A molecular ion was not detected and it was postulated that the hydroxylamine was not formed under the reaction conditions.

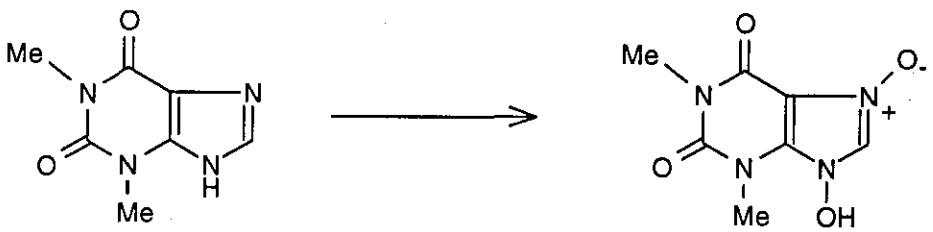
1.3.9 Methyl Piperidine



Scheme 12

Mass-spectrometry data on the reaction between methyl piperidine and DMD was, also, inconclusive. A molecular ion was not detected and it was postulated that the *N*-oxide was not formed under the reaction conditions used.

1.3.10 Theophylline

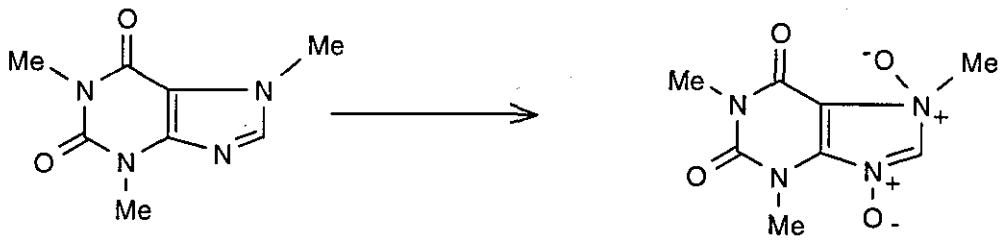


5

Scheme 13

Treatment of Theophylline with DMD gave a MS (CI); m/z 212 [($M^{\cdot+}$), 15%]. This has been postulated as **5**. As this experiment was an initial investigation into the uses of DMD, further investigation into the exact nature of the product obtained was not thought to be necessary.

1.3.11 Caffeine



6

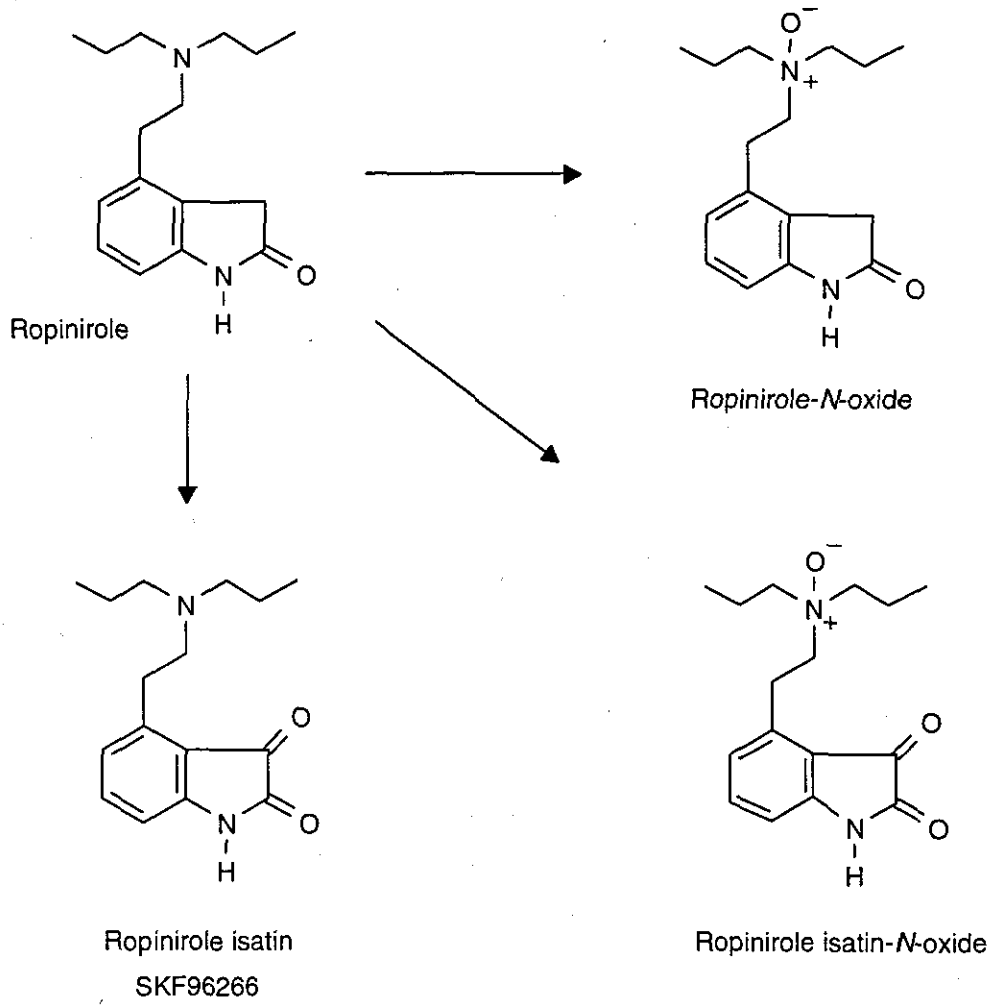
Scheme 14

Treatment of Caffeine with DMD gave a MS (CI); m/z 226 [($M^{\cdot+}$), 20%]. This has been postulated as **6**. As this experiment was an initial investigation into the uses of DMD, further investigation into the exact nature of the product obtained was not thought to be necessary.

1.3.12 Ropinirole

Ropinirole is an indole compound currently in development at SmithKline Beecham Pharmaceuticals for the treatment of Parkinson's disease. A major oxidative degradation product of Ropinirole in tablet formulations under conditions of elevated temperature and humidity is the isatin analogue SKF96266. Other oxidation products arising from dealkylation of the side chain nitrogen atom are, also, known to be formed.

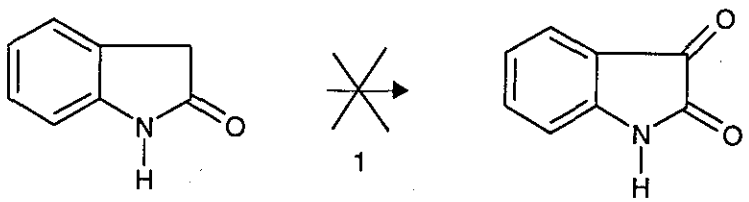
DMD was reacted with Ropinirole and three products observed by HPLC. Using reference material of SKF96266 and UV data on each peak in the chromatogram the structure of the products were postulated as shown in Scheme 15.



Scheme 15

UV scans of the individual peaks in the HPLC chromatogram showed the UV of SKF96266 and Ropinirole to be identical to their proposed *N*-oxides. This would be expected if the structures of the peaks are as postulated.

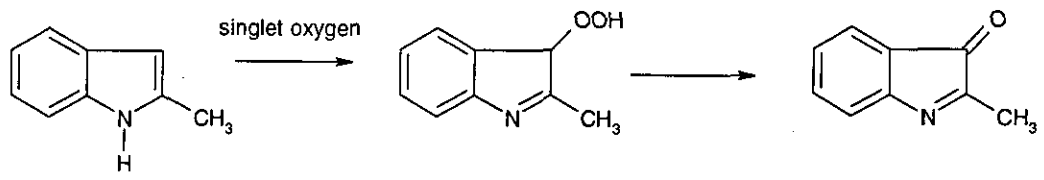
Due to the ease with which Ropinirole was oxidised to the isatin derivative it was decided to try and oxidise oxindole, a close analogue of Ropinirole, with DMD. Interestingly, however, analysis of the reaction mixture showed that in the presence of equimolar or excess of DMD no isatin was formed (Scheme 16).



oxindole isatin

Scheme 16

An investigation into why Ropinirole oxidises easily to its isatin derivative and oxindole does not was carried out. An article by Adam *et al*[4] described how *N*-oxides induce DMD decomposition in which singlet oxygen is produced (Scheme 4, Section 1.2.3). This initiated a study into whether Ropinirole initially forms the *N*-oxide that can generate singlet oxygen in the presence of excess DMD. The singlet oxygen could then add onto the indoline ring in an identical manner to the addition of singlet oxygen onto the C3 position of the indole ring of 2-methylindole (Scheme 17). In this case the ketone is formed *via* a hydroperoxide intermediate [5]



Scheme 17

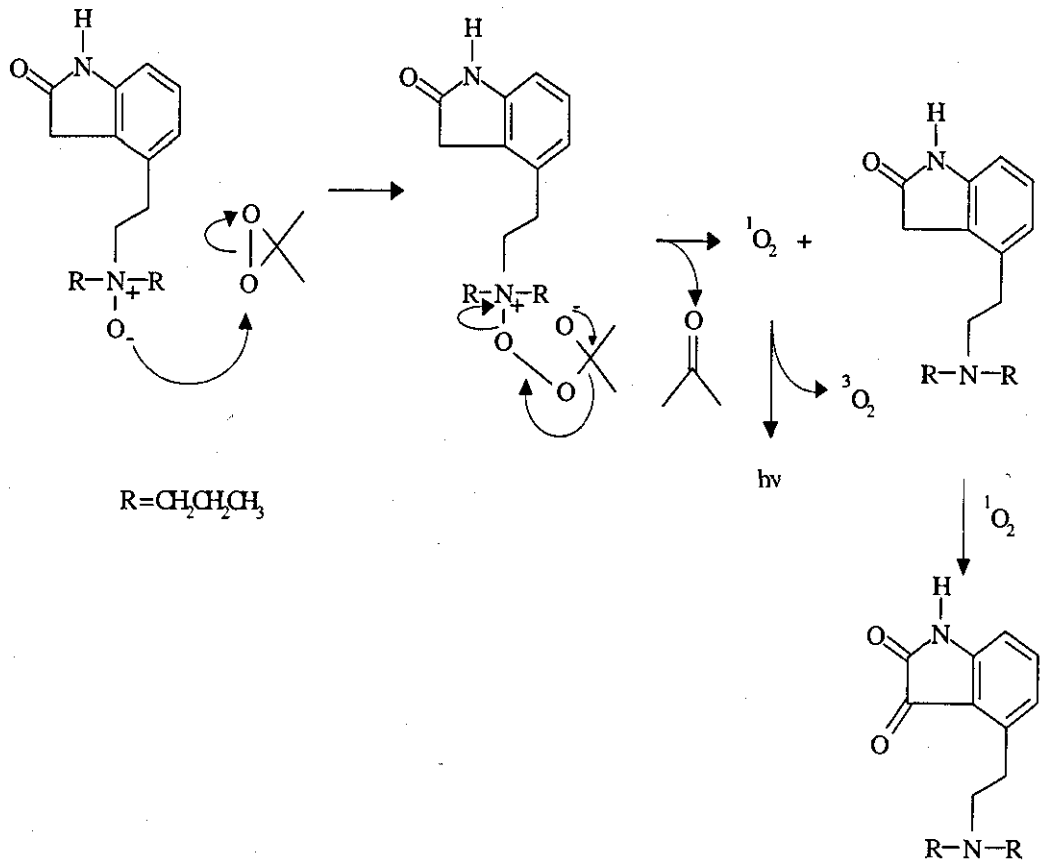
Attempts were made to react oxindole with singlet state oxygen by irradiating aqueous solutions of oxindole, purged with oxygen, with a photochemical lamp in

the presence of Rose Bengal. However, no isatin was observed in the reaction mixture and it was not known if this was due to no reaction between oxindole and singlet oxygen or whether no singlet oxygen had actually been generated.

A pH stability study of Ropinirole in a range of pH solutions indicated that oxidation at the C3 position of the ring occurred most readily at low pH (pH 2 - 5). This was thought to be substantiated by the fact that Ropinirole is present in tablets in the form of the hydrochloride salt which would maintain an acidic environment. It was, therefore, decided to examine the effect of low pH on the DMD oxidation of oxindole.

When the reaction between DMD and oxindole was carried out at low pH (pH 4), again no isatin was formed. However, when the reaction was undertaken at low pH in the presence of excess DMD and triethylamine hydrochloride, a surrogate *N*-oxide source, isatin was observed, albeit, in low yield.

It appears that in order for the oxidation of the indole ring in oxindole to occur an *N*-oxide in the presence of an excess of DMD, at low pH, is required. The conversion of oxindole to isatin was only successful in the presence of triethylamine hydrochloride (which is converted to the *N*-oxide *in situ*) and excess DMD. It could be postulated that the mechanism proceeds in an identical manner to that described in Scheme 4 of the introduction to this chapter. This would involve reaction of Ropinirole with DMD to give the *N*-oxide of the side chain which on reaction with excess DMD generates singlet oxygen. This could possibly add onto the C3 position of the indole, if the enol form was dominant, as described for oxindole earlier in this section (Scheme 18).



Scheme 18

The possibility of direct oxidation of the C3 position in Ropinirole by the *in situ* formed *N*-oxide was investigated by treatment of oxindole with an excess of trimethylamine-*N*-oxide. Oxidation of the C3 position of oxindole in this way proved unsuccessful. However, this is not conclusive evidence that the oxidation of Ropinirole does not occur *via* oxidation by its intramolecular *N*-oxide.

The exact mechanism of the oxidation of Ropinirole with DMD is unclear. A couple of mechanisms have been postulated here and the mechanism described in Scheme 18 seems more favourable. Further investigation into the exact mechanism was thought to be beyond the scope of this work.

1.4 Conclusions

Tentative postulations have been made with regards to the products obtained on reaction of DMD with a variety of nitrogen containing compounds including primary, secondary and tertiary amines. From the initial experiments DMD appears to be a highly reactive and versatile oxidising agent. It appears to react smoothly with drug molecules containing sp^3 nitrogen atoms converting Granisetron, SB202026 and related compounds to the corresponding *N*-oxides. Drug molecules containing the indole ring system substituted at the 2 position with oxygen additionally were oxidised at the adjacent 3 position. Ropinirole yielded the corresponding isatin derivative and the formation of Ropinirole-*N*-oxide and isatin-*N*-oxide was postulated. The presence of a tertiary amine suitably adjacent to the reaction site would appear to be essential for the reaction mechanism to proceed. SB207266 was postulated as being epoxidised at the 2,3 positions and to undergo ring opening to give a 3-hydroxy-oxindole derivative as the major product.

It has, also, been shown that in the case of SB202026 and Ropinirole the oxidative degradation product obtained is that which is formed during stability testing procedures on exposure to atmospheric oxygen. Although in these cases DMD aids in the prediction of oxidative stability, its general utility lies in the fact that in the presence of most tertiary amines it will yield *N*-oxides. This has been confirmed by work carried out at Loughborough University where products have been fully characterised. This is a valuable aid to degradation product identification in the early stages of drug development when chromatographic reference standards are not in existence.

1.5 Experimental

1.5.1 Experimental for Section 1.3

Preparation of dimethyldioxirane:

A round bottom flask was equipped with an efficient mechanical stirrer and a solids addition funnel, connected by a U-tube to a receiving flask and cooled to -78°C by means of a dry ice/acetone bath. The reaction flask was charged with EDTA solution (1.6 g/l), acetone (210 ml) and sodium bicarbonate (160 g). While vigorously stirring, solid caroate (320 g) was added slowly over a period of approximately one hour. The dimethyldioxirane/acetone distillate (≈60 ml, 0.08-0.1 mol l^{-1}) was collected in the cooled (-78°C) receiving flask. A typical apparatus for the preparation of dimethyldioxirane is shown in Figure 1. The use of an ice/water bath and a vacuum were not found to be essential.

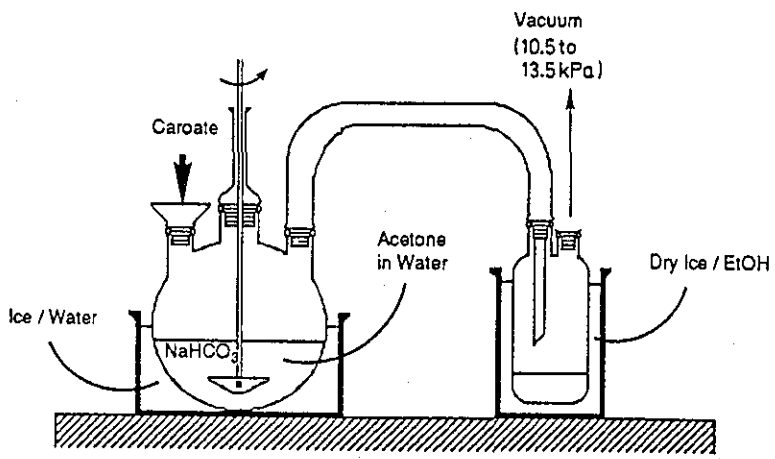


Figure 1. A typical apparatus for dimethyldioxirane preparation

The molarity of the dioxirane solution was determined by titration:
An acetic acid/acetone solution (3:2; 3 ml) and KI solution (10 % w/v; 5 ml) were charged to a conical flask, followed by the dioxirane solution (0.2 ml). The solution was titrated against a sodium thiosulphate solution (0.01 M).

Calculation:

$$\text{Molarity (dioxirane)} = \frac{\text{Vol. } Na_2S_2O_3 \text{ x Mol. } Na_2S_2O_3}{\text{Vol. (dioxirane) x 2}}$$

For all of the reactions in this section the reagents were dissolved in acetone, cooled to 5°C and two equivalents of dioxirane added. When solubility in acetone was limited the compounds were initially dissolved in a small volume of water prior to addition of the acetone. The solution was stirred vigorously for 10 minutes and the product either isolated by vacuum distillation or if volatile identified by mass spectrometry in the solution of acetone. Unless stated otherwise, all chemicals were obtained from Aldrich Chemical Company. Other methods of detection, such as, HPLC were, also, used. For further details see *individual experimental section.*

HPLC conditions:

High performance liquid chromatography with UV detection was performed on the following equipment:
Hewlett Packard 1050 autosampler
Severn Analytical SA 6410B pump
Severn Analytical SA 6504 UV detector

1.5.2 Experimental for Section 1.3.1

The products of the reaction between SB207266 and DMD were investigated using mass-spectrometry data and TLC. The TLC conditions for analysis of the products obtained were as follows:

60 : CH_2Cl_2; 40 : EtOH; 2 : NH_4OH; 5 : H_2O

Alumina plates were used and the R_f values of the postulated isomers were 0.10; 0.15; 0.27; 0.40.

1.5.3 Experimental for Section 1.3.2

Preparation of Granisetron-*N*-oxides as HPLC markers:

The *N*-oxides of Granisetron were prepared by the synthetic chemistry laboratories at SmithKline Beecham. The general procedure used is detailed below:

A solution of Granisetron hydrochloride was basified with dilute sodium hydroxide and the free base extracted into dichloromethane. An excess of MCPBA was added and the reaction monitored by liquid chromatography to completion. The dichloromethane layer was then washed with dilute acid to destroy any excess peroxide, washed out with sodium bicarbonate and dried with anhydrous magnesium sulphate. The solvent was removed by vacuum distillation.

The chromatography conditions for the analysis of Granisetron and the *N*-oxides were as follows:

Column: Spherisorb S5 CN 25 cm x 4.6 mm

Eluent: pH 4.5 0.1 M $NaH_2PO_4.2H_2O$ buffer:MeCN 90:10

Flow rate: 2 ml/min

UV detection at 300 nm

Retention times of the *N*-oxides were approximately 3.8 and 4.2 minutes.

1.5.4 Experimental for Section 1.3.5

A HPLC marker of SB202026-*N*-oxide was obtained from Chemical Development at SmithKline Beecham. SB202026 was dissolved in acetone and reacted with two equivalents of DMD under ambient conditions. The solutions were allowed to stand for at least 10 minutes and analysed by HPLC.

The chromatography conditions for analysis of SB202026 and its *N*-oxide by HPLC with UV detection were as follows:

Column: Spherisorb S5 C1 15 cm x 4.6 mm

Eluent: 0.025 M $NaH_2PO_4.2H_2O$ pH 3.5 buffer solution & 0.1 % triethylamine

Flow rate: 2 ml/min

UV detection at 300 nm

Retention times were approximately 9 and 15 minutes for the drug and its *N*-oxide respectively.

1.5.5 Experimental for Section 1.3.6

The reaction of benzyl cyanide in acetone with two equivalents of DMD under ambient conditions was monitored by HPLC under the following conditions:

Column: Hichrom S5 C1 15 cm x 4.6 mm

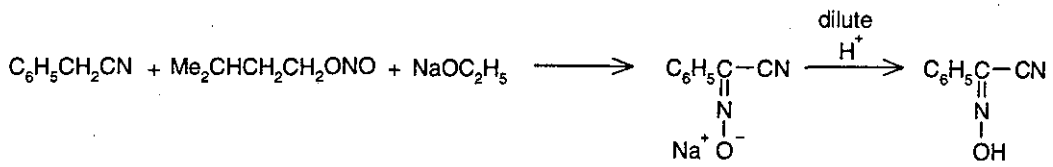
Eluent: pH 4.0 0.1 M acetate buffer:MeCN 80:20

Flow rate: 1 ml/min

UV detection at 210 nm

Retention time was 3.2 minutes

2-Hydroxyimino-2-phenylacetonitrile was prepared by the reaction of benzyl cyanide with isoamyl nitrite (Scheme 19). Fieser & Fieser[6] details the preparation of an identical compound but uses methyl nitrite instead of isoamyl nitrite.

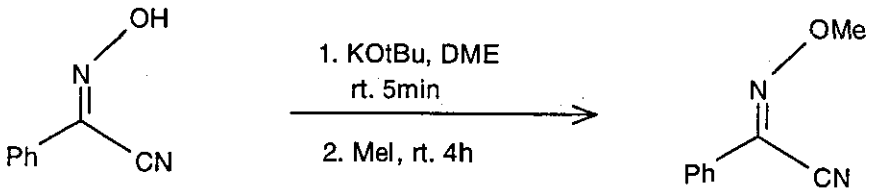


Scheme 19

To a cooled (0°C) mixture of sodium ethoxide (6.8 g, 0.1 mol) in ethanol (100 ml) was added a cooled (0°C) mixture of benzyl cyanide (11.7 g, 0.1 mol) and isoamyl nitrite (14.2 g, 0.12 mol). The yellow precipitate of the sodium salt was dissolved in dilute HCl (0.5 M, 50 ml) and the oxime isolated (5.5 g, 40%) as a white solid, mp 128-130°C; MS(CI) 146 (M^+, 20%), 116 (80), 89 (100); ^1H NMR (400MHz, Acetone-d^6) 12.7 (s, 1H), 7.82 (m, 2H), 7.55 (m, 3H).

The reaction of 2-hydroxyimino-2-phenylacetonitrile in acetone with two equivalents of DMD under ambient conditions was monitored by HPLC under the following conditions:
Column: Spherisorb ODS2 15 cm x 4.6 mm
Eluent: pH 4.0 0.1 M acetate buffer:MeCN 50:50
Flow rate: 1 ml/min
UV detection at 220 nm
Retention time was 3.8 minutes

2-Methoxyimino-2-phenylacetonitrile was prepared by treatment of the oxime with potassium *t*-butoxide followed by methylation with methyl iodide (Scheme 20).



Scheme 20

Oxime (0.5 g, 3.42 mmol) in DME (15 ml) was treated with KO^{t}Bu (0.46 g, 4.10 mmol) under a N_2 atmosphere at room temperature for 5 minutes. MeI (0.23 ml, 3.69 mmol) was then added at room temperature and the suspension stirred for 4 hours. The reaction mixture was washed with NH_4Cl and the organics extracted with EtOAc (3 x 20 ml). The combined organic layers were dried ($MgSO_4$) and the solvent removed by evaporation. Workup afforded the crude product (0.5 g, 91.4%) as a red/brown oil, MS (CI) 160 (M^+, 60%), 178 (MNH_4^+, 100), 195 ($MH + 2NH_4^+$, 60); ^1H NMR (270MHz, $CDCl_3$) 7.8 (m, 2H), 7.48 (m, 3H), 4.2 (s, 3H).

The reaction of 2-methoxyimino-2-phenylacetonitrile in acetone with two equivalents of DMD under ambient conditions was monitored by HPLC under the following conditions:
Column: Spherisorb ODS2 15 cm x 4.6 mm
Eluent: pH 4.0 0.1 M acetate buffer:MeCN 50:50
Flow rate: 2 ml/min
UV detection at 285 nm
Retention time was 5.5 minutes

1.5.6 Experimental for Section 1.3.12

Ropinirole and its degradation products were supplied by the Chemical Development laboratories at SmithKline Beecham Pharmaceuticals. Oxindole and isatin were supplied by Aldrich.

Preparation of Ropinirole, SKF96266, oxindole and isatin solutions

Ropinirole hydrochloride (10 mg) was placed in a volumetric flask (100 ml) dissolved and made up to volume with water. The same quantities and volumes were used for SKF96266, oxindole and isatin. The Ropinirole and isatin solutions were reacted with DMD in acetone (0.08 M) in the following molar ratios: 1:1, 1:2 and 1:5.

Reaction of trimethylamine-*N*-oxide with oxindole

A solution of oxindole and trimethylamine-*N*-oxide (0.1 mg/ml) (1:2 ratio) was prepared and the solution was mixed with equivalent (1:1 ratio) and excess (1:4 ratio) of DMD. This was then analysed using HPLC. The same method was used for reacting oxindole and triethylamine hydrochloride.

pH stability study for oxindole and Ropinirole

A mixture of boric acid, acetic acid and *ortho*-phosphoric acid (0.04 M in each) was made up in a volumetric flask (2 litres) to give the stock Britton-Robinson buffer. The stock solution was divided into 6 different volumetric flasks and an appropriate amount of sodium hydroxide (0.2 M) and potassium chloride added to give the desired pH and the ionic strength.

Ropinirole (10 mg) was weighed into a volumetric flask (10 ml) and dissolved in a solution of the appropriate pH. The solutions of pH 2, 4, 6, 8, and 10 and with

an ionic strength of 0.1 M were analysed initially and stored in a 80°C oven for four weeks. The solutions were analysed after 1 week, 2 weeks and 4 weeks.

Photochemical reaction

Ropinirole (320 mg) and Rose Bengal (10 mg) in water and acetonitrile 50:50 (150 ml) was placed in a conical flask and was photooxygenated with a 500W infrared lamp. Air was pumped throughout the experiment (for 1 hour). The Ropinirole solution (1 ml) was transferred by pipette into a volumetric flask (20 ml) and made upto volume with water. The solution was analysed by HPLC.

The HPLC method for Ropinirole and its degradation products was as follows:
Column: Kromasil C8 5 µm 25 cm x 4.6 mm
Eluent: pH 2.5 0.1 M ammonium acetate buffer:MeCN:MeOH 84:11:5
Flow rate: 1 ml/min
UV detection at 250 nm
Retention times were 18, 20, 28 and 30 minutes for SKF96266, Ropinirole, SKF96266-*N*-oxide and Ropinirole-*N*-oxide respectively

The HPLC method for isatin and oxindole was as follows:
Column: Kromasil C8 5 µm 25 cm x 4.6 mm
Eluent: water:MeOH 65:35
Flow rate: 1 ml/min
UV detection at 250 nm
Retention times were 10 and 16 minutes for isatin and oxindole respectively

1.6 References

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2. Murray, R.W., *Chem. Rev.*, **1989**, *89*, 1187.
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5. Wasserman, H.H.; Murray, R.W., in *Singlet Oxygen*, Academic Press Inc., N.Y., **1979**, pp 468.
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2.0 Reactivity and Kinetics of Dimethyldioxirane Oxidations

2.1 Summary

The reactivity of dioxiranes was investigated by examination of polarographic peak potentials and by measurement of the reaction rates of the oxidation of 4-nitro-*N,N*-dimethylaniline as a model substrate. The polarographic peak potentials were shown to be of a similar order to those of typical acyclic peroxides. Although differences in reactivity between dioxiranes and peroxides were not demonstrated in the peak potential data a trend was observed in the peak potentials within a series of dioxiranes. It was noted that the peak potential of the highly reactive methyl(trifluoromethyl)dioxirane was outside the mercury electrode limit and it is, therefore, probable that higher reactivity can be associated with more positive peak potentials.

Comparison of the rates of oxidation using dimethyldioxirane, methyl(trifluoromethyl)dioxirane and ethylmethyldioxirane in acetonitrile demonstrated the order of reactivity to be MTFMD > DMD > EMD. MTFMD was shown to be 16000 times more reactive than DMD in the oxidation of 4-nitro-*N,N*-dimethylaniline in acetonitrile.

Direct rate measurements of the oxidation of 4-nitro-*N,N*-dimethylaniline were determined spectrophotometrically and showed the reaction to be pseudo first order under conditions of excess dioxirane. The reaction rates were shown to be strongly affected by the solvent and the rank order in terms of decreasing rate was water > acetone > acetonitrile > 1,2-dichloroethane > ethyl acetate.

A novel method of determining reaction rates directly was developed using a constant potential amperometric technique. This involved monitoring the loss of current due to the polarographic response of DMD on addition of a series of anilines (4-nitro, 4-methoxy, 4-chloro-*N,N*-dimethylaniline and *N,N*-dimethylaniline). Both pseudo first order and second order rate constants were determined. The second order rate data showed excellent correlation between different aniline molarities. The Hammett relationship was applied to the data and gave a ρ value of -0.889.

The amperometric technique was, also, used to investigate the mechanisms of oxidation of 4-nitro-*N,N*-dimethylaniline by other oxidants. It was observed that although peroxides are similar to dioxiranes in that they possess an O-O bond the mechanisms of oxidation are different to the electrophilic substitution observed when using DMD.

2.2 Introduction

The determination of the reaction kinetics and mechanisms of dioxirane reactions have to date generally involved the use of UV spectrophotometry to monitor reactions directly or external sampling techniques, such as, HPLC or GC. A brief review of the literature, with regards to, the reaction kinetics and mechanisms of DMD oxidations follows.

In 1988 Baumstark and Vasquez studied the reaction kinetics of dimethyldioxirane with a series of di- and monosubstituted alkenes.[1] The epoxidations were determined to be first order with respect to both the alkene and the dioxirane in dried acetone. They, also, studied the reaction kinetics of a series of substituted styrenes with DMD and observed good LFER with a ρ^+ value of

-0.90, indicating an electrophilic process. The reactions were followed using UV spectrophotometry by monitoring the loss of DMD with time at 332 nm.

Murray and Shiang later studied the relative rates of epoxidation by DMD of a series of 4-substituted ethyl cinnamates.[2] The rate data were subjected to Hammett LFER and gave a ρ value of -1.53, again, indicating electrophilic O-atom transfer. Second order rate coefficients were determined using GC methods of analysis.

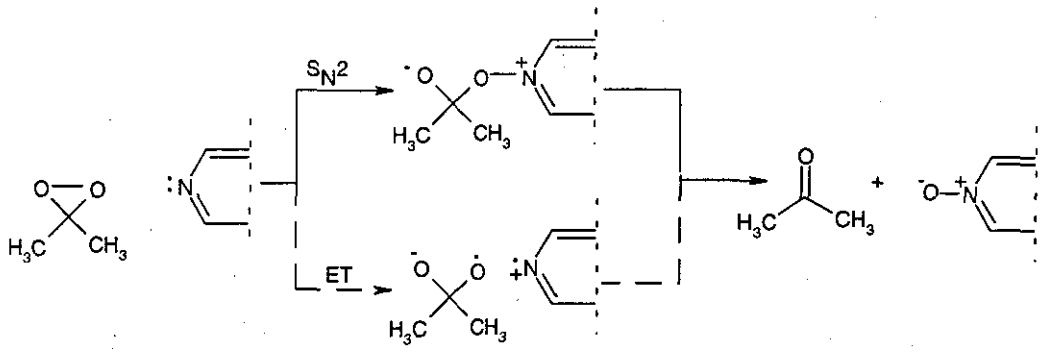
The second order rate coefficients for the epoxidation of ethyl cinnamates recorded by Murray and Shiang were lower than those observed by Baumstark and Vasquez for the epoxidation of substituted styrenes. The lower reactivity of the cinnamate toward DMD is thought to be consistent with the electrophilic nature of DMD.

Baumstark and McCloskey showed that (Z)-alkenes are more reactive than their (E)-counterparts.[3] They proposed a spiro transition state for epoxidation which could explain differences in the reaction rates described above. Such a transition state would permit a less hindered approach of DMD to the (Z)-alkenes.

Murray and Gu determined second order rate coefficients for the epoxidation of *trans*-ethyl cinnamate in a number of binary solvents and observed that solvents with hydrogen bond donor capacity increase the rate of the reactions while solvents with hydrogen bond acceptor capacity decrease the rate.[4,5] In 1995 they studied the reaction kinetics of a series of *p*-substituted cumenes with DMD to give the corresponding cumyl alcohols.[6] Treatment of the rate data with the Hammett substituent constants revealed that the insertion reaction is an electrophilic process with a ρ value of -2.76.

Murray and Gu, also, carried out absolute rate studies on a series of C-H insertion reactions of DMD.[7] The substrates were chosen so that the distance between a single tertiary C-H bond and an OH group could be varied. The measured rate constants indicated that a rate acceleration occurs when the distance between the reacting C-H bond and the OH group permits intramolecular H-bonding stabilisation of the transition state. A related study of the epoxidation reaction of DMD, also, found an increased rate when chain length permitted intramolecular H-bonding by an OH group.

Adam and Golsch investigated the mechanism of the oxygen transfer by DMD to nitrogen heteroarenes.[8] While most oxygen atom transfer reactions appear to take place by direct substitution (S_N2) on the dioxirane peroxide bond by the nucleophile, more complex electron-transfer (ET) activity has been implicated (Scheme 1) particularly for substrates with low oxidation potentials.[9-11]



Scheme 1

They compared the relative rates and regioselectivities of DMD oxygen transfer with those of CH_3I methylation and found the nitrogen lone pair oxidation by DMD to be a clear-cut S_N2 rather than ET mechanism.

More recently Adam *et al*[12] were able to show with the help of radical-clock and isomerisation experiments that the mechanisms of epoxidation and insertion of oxygen into C-H bonds do not involve a radical pathway, as implicated by Kazakov *et al*[13] and Minisci *et al.*[14]

2.3 Results and Discussion

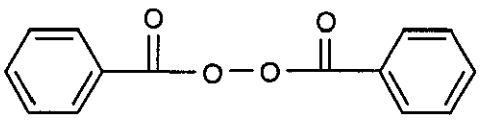
Chapter 1 showed that DMD oxidises a variety of nitrogen containing compounds to give simple products which in the case of SB202026 and Ropinirole were identical to those obtained on exposure to atmospheric oxygen. The oxidations using DMD, however, were much more rapid taking only a matter of minutes. It would be extremely useful if a parameter could be found which would provide a means of predicting reactivity towards oxidation.

Oxidation reactions, which involve electron transfer, can easily be monitored by electrochemical techniques, such as, polarography. The polarographic detection of peroxides has been documented in the literature[15] and it was decided to determine whether dioxiranes could be detected using polarography and if so whether the peak potentials could be used to predict reactivity. The correlation between peak potentials and reactivity has been documented in the literature, with regard to the influence of molecular structure on reduction potentials. In this instance molecular structure was correlated with reported inhibitory activity against various cancer cells.[16]

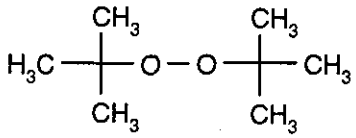
2.3.1 Electrochemistry of Peroxides

Before attempting to characterise DMD and other dioxiranes a number of peroxides, shown overleaf, were analysed by polarography and their peak potentials recorded. This initial data would provide an indication of the region of the O-O polarographic reduction.

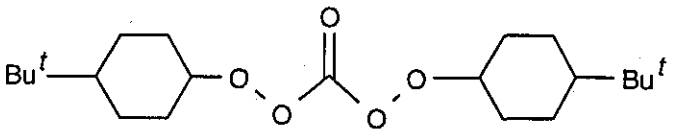
For an account of the principles and applications of polarography the reader is referred to the appendix at the end of this chapter.



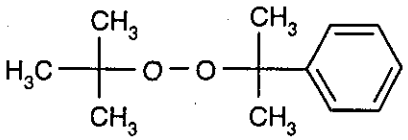
benzoyl peroxide



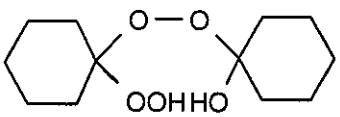
di-*tert*-butyl peroxide



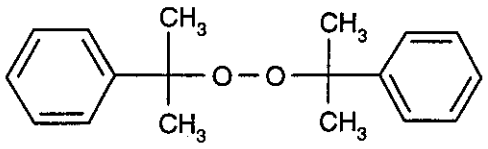
di-(4-*tert*-butyl cyclohexyl) peroxy dicarbonate



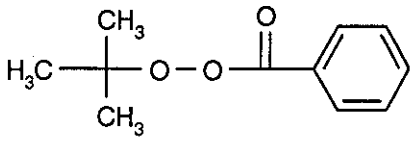
tert-butyl cumyl peroxide



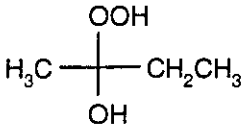
cyclohexanone peroxide



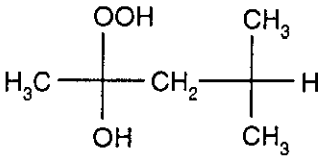
dicumyl peroxide



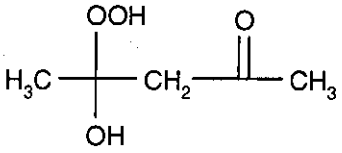
tert-butylperoxy benzoate



methyl ethyl ketone peroxide

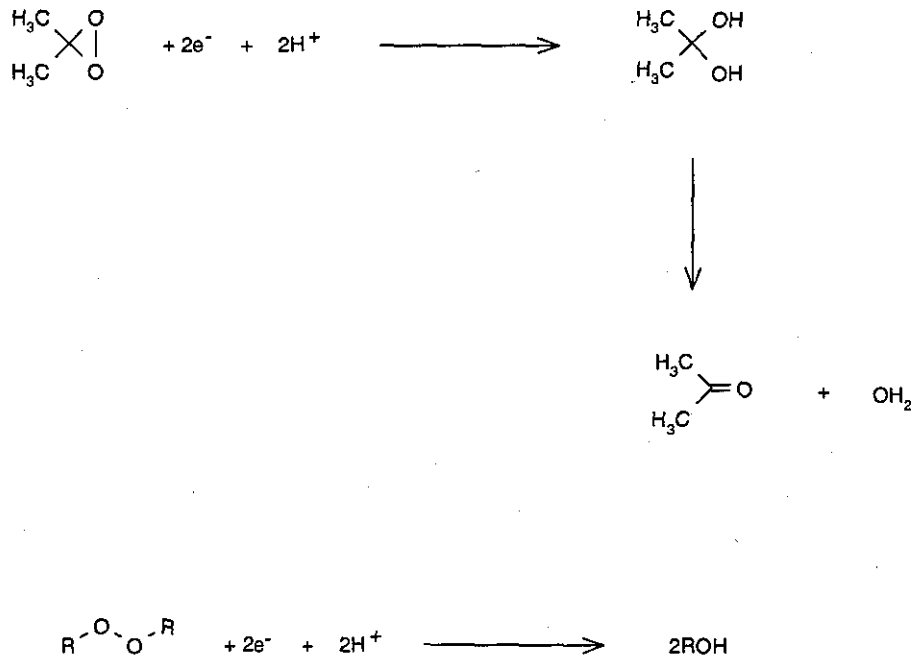


methyl isobutyl ketone peroxide



acetyl acetone peroxide

It may be postulated that the polarographic reduction of dimethyldioxirane and peroxides proceeds *via* a $2e^-$ process (Scheme 2).



Scheme 2. Predicted polarographic reduction of dioxiranes and peroxides

The peak potentials recorded are tabulated (Table 1). An unbuffered supporting electrolyte system of 50:50 0.1M KNO_3:MeCN was used to allow the maximum scope for detection at the positive range of the mercury electrode and good solubility of the peroxides.

The mercury electrode was adopted as the only material on which reduction could be demonstrated. No responses were observed when glassy carbon or gold were used, both being electrodes for which a higher positive electrode limit would be expected. The reasons for differences in behaviour between these electrodes is unclear but could relate to the greater ease of electron transfer in the case of the mercury electrode. One exception to this behaviour was benzoyl peroxide which displayed a peak reduction potential of + 0.2 V on the glassy carbon electrode.

In the case of di-(4-*tert*-butyl cyclohexyl) peroxy dicarbonate two peak potentials were observed. It is probable that in this instance the sample of peroxide was not chemically pure.

Table 1. Peak potentials of various peroxides

Peroxide	**Peak Potential *vs* AgCl/Ag (V)**
Benzoyl peroxide	0.23
Di-*tert*-butyl peroxide	0.25
Di-(4-*tert*-butyl cyclohexyl) peroxy dicarbonate	0.18 (-0.05)
tert-Butylcumyl peroxide	0.20
Cyclohexanone peroxide	0.00
Dicumyl peroxide	0.02
Methylethylketone peroxide	-0.07
Methylisobutylketone peroxide	-0.05
Acetylacetone peroxide	0.03
tert-Butylperoxybenzoate	-0.08

The lack of response with the carbon electrode on the polarograph was confirmed by HPLC-EC & UV detection. The peroxides were monitored by UV spectrophotometry, however, no electrochemical response was observed with the exception of benzoyl peroxide.

Having successfully detected the peroxides using polarography it was decided to record peak potentials for various cyclic peroxides i.e. dioxiranes.

2.3.2 Electrochemistry of Dioxiranes

In order to investigate the relationship between peak potential and chemical structure/reactivity several dioxiranes were synthesised and their peak potentials recorded (Table 2). DMD, MTFMD and EMD were isolated prior to measurement but all others were prepared *in situ* and diluted with supporting electrolyte.

For dimethyldioxirane a peak potential of + 0.14 V *vs* AgCl/Ag was measured (Figure 1). This result fitted in very well with the peak potentials recorded for the series of peroxides in Table 1. An attempt was made to measure the peak potential of dimethyldioxirane in a buffered medium in order to obtain a pH profile of the dioxirane. However, the satisfactory polarography obtained with the potassium nitrate and acetonitrile system was not reproduced using Britton Robinson or phosphate buffer solutions.

Figure 1. A differential pulse polarogram of dimethyldioxirane

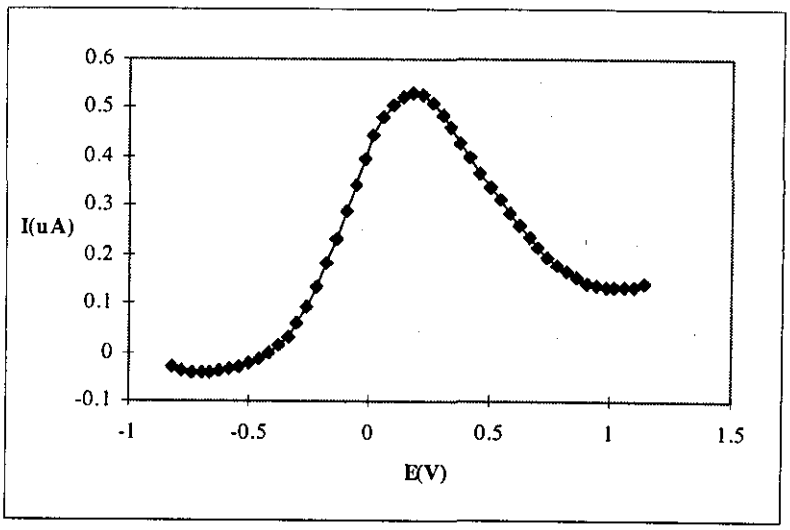


Table 2. Peak potentials and currents of dioxiranes

Dioxirane	Peak Potential (V)	Current (nA)	Dilution (ml)
Methyl(trifluoromethyl)	> 0.2	ND*	NA**
Dimethyl	0.140	1045.6	1/100 x 4/100
Ethylmethyl	0.012	1145.4	1/100
Methyl-*n*-propyl	0.020	744.7	1/50
Methyl-*n*-butyl	0.100	20.0	1/25
Diethyl	0.032	267.5	1/25
2-Methylcyclohexanone	0.020	752.5	1/100
3-Methylcyclohexanone	0.104	14.4	1/200
4-Methylcyclohexanone	0.140	20.0	1/100
Cyclohexanone	-0.004	640.4	1/50 x 5/50
3-Methyl-2-butanone	0.020	239.0	1/50 x 5/50
2,2-Dimethyl-3-butanone	0.088	81.9	1/100
Methylphenyl	0.114	19.0	1/50
2-Methoxyphenylmethyl	0.060	144.8	1/50
2-Nitrophenylmethyl	0.144	45	1/50

*ND Not detected

**NA Not applicable

The polarographic data obtained did not show a significant difference between the peak potentials of the peroxides and the dioxiranes. Due to the much greater reactivity of dioxiranes over peroxides it may have been postulated that the peak potentials would be more positive for the dioxiranes. In order to assess the relationship between peak potential and reactivity further it was decided to try and measure dioxirane reactivity directly.

2.3.3 Determination of Dioxirane Reactivity using a Spectrophotometric Technique

To assess the reactivity of dioxiranes directly it was decided to select nitrogen containing compounds which were readily amenable to oxidation by dioxiranes. The compounds chosen for the investigation were a series of *N,N*-dimethylanilines including the parent compound itself, 4-methoxy-*N,N*-dimethylaniline and 4-nitro-*N,N*-dimethylaniline.

During initial investigations into the possible methods of analysis of these compounds it was discovered that *N,N*-dimethylaniline had strong fluorescence properties (λ_{ex} = 244 nm, λ_{em} = 362 nm). It was initially thought, therefore, that fluorescence detection could provide a novel basis for measuring reaction rates of dioxiranes as the *N*-oxide oxidation product is non fluorescent. Hence, the rates could be followed directly by monitoring the loss of fluorescence as the reaction proceeds. It was discovered, however, that a severe limitation of this proposal was the fact that the acetone present in the dioxirane solution quenched the strong fluorescence of *N,N*-dimethylaniline.

Fluorescence activity results from a compound absorbing a quantum of ultraviolet or visible light and in doing so the molecule undergoes a transition from its ground state into an electronically excited state. This excited state is normally very short lived, typically 10^{-8} seconds and if the molecule then returns to its ground state by emission of light the emission band that results is known as the fluorescence emission band. The excited states of molecules can, also, be degraded by transfer of large quanta of energy to other species in the system. This results in the molecule itself, returning to its ground state and the light energy being lost. The acetone in the dioxirane solution was found to quench the fluorescence of *N,N*-dimethylaniline in this manner.

Neither of the other substrates proved amenable to fluorescence analysis. 4-Nitro-*N,N*-dimethylaniline showed no fluorescence activity and 4-methoxy-*N,N*-dimethylaniline (λ_{ex} = 242 nm, λ_{em} = 383 nm) was quenched by the acetone in the same manner as *N,N*-dimethylaniline. An alternative means of measuring the rates of reactions was, therefore, required to monitor the loss of concentration of the anilines.

UV spectroscopic properties had been used by Edwards and Gallopo in 1980 to follow the loss of pyridine in its reaction with DMD[17] and by Baumstark and Vasquez in 1988 to monitor the loss of DMD on reaction with various alkenes.[1] Both *N,N*-dimethylaniline and 4-methoxy-*N,N*-dimethylaniline have $\lambda_{max} \approx 240$ nm which renders them unusable for this method due to the fact that the acetone in the dioxirane solution completely overlaps the UV absorption in this region. 4-Nitro-*N,N*-dimethylaniline, however, has a λ_{max} = 425 nm which is clear of any interference from the addition of acetone.

In order to determine the suitability of this method for measuring the rates of reaction a linearity of 4-nitro-*N,N*-dimethylaniline was carried out over the concentration range 0.4 - 21.1 μg/ml (Table 3).

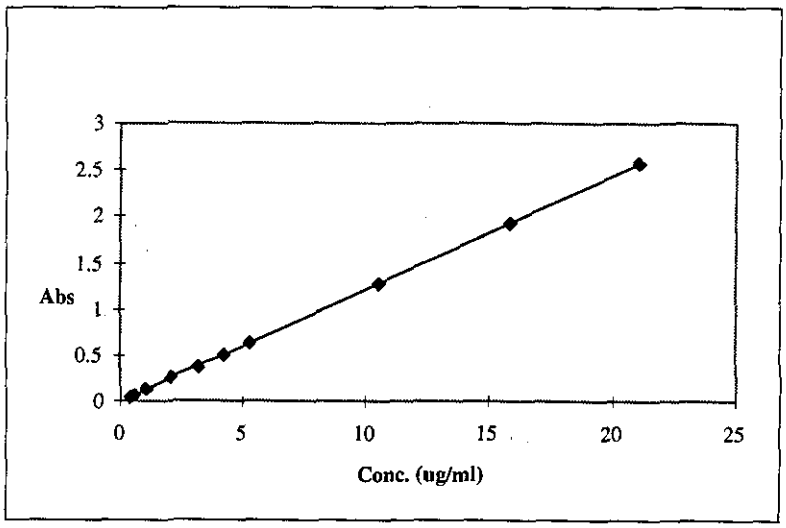
The following data and graph show that the absorbance of 4-nitro-*N,N*-dimethylaniline is linear over the entire concentration range shown and the intercept is not significant (Figure 2). Since the absorbance of the *N*-oxide at 425 nm is zero it was possible to use the absorbance measurements directly to represent the concentration. From a practical point of view measurements were not taken during the initial mixing period of the reagents.

Using this method it has been possible to investigate the reactivity of dioxiranes directly by following the reaction between the dioxiranes and 4-nitro-*N,N*-dimethylaniline.

Table 3. Linearity over concentration range 0.4 - 21.1 µg/ml

Concentration (µg/ml)	Absorbance
0.42	0.0508
0.53	0.063
1.06	0.1259
2.11	0.2502
3.17	0.3766
4.22	0.5005
5.28	0.6291
10.56	1.2745
15.83	1.9184
21.11	2.5707
Correlation coefficient	**0.9999**
Slope	**0.1218**
Intercept	**-0.0069**

Figure 2. Plot of linearity (0.4 - 21.11 µg/ml)



2.3.3.1 Oxidation Rates of 4-Nitro-*N,N*-Dimethylaniline by Dimethyl, Methyl(Trifluoromethyl) and EthylMethylDioxirane

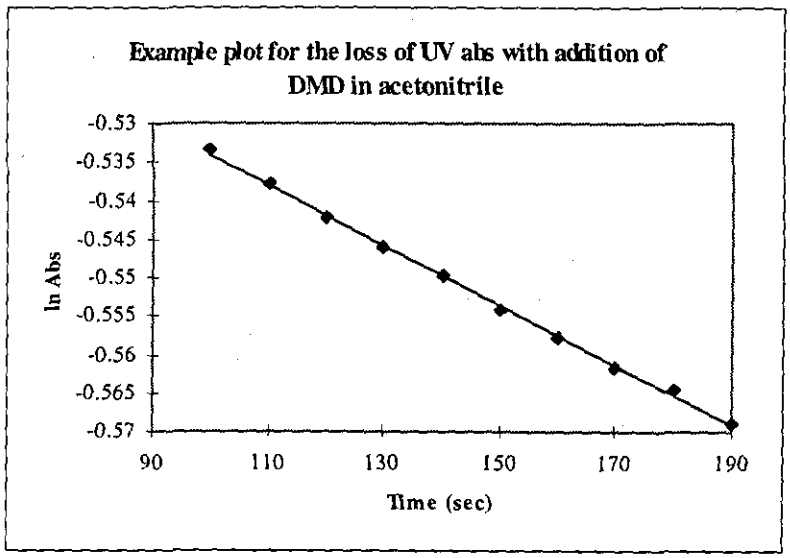
4-Nitro-*N,N*-dimethylaniline was reacted with dimethyl, methyl(trifluoromethyl) and ethylmethyldioxirane and the pseudo first order rate constants determined in triplicate. The results are reported in Tables 4 to 6 and illustrated in Figures 3 to 5.

a) Reaction between DMD and 4-nitro-*N,N*-dimethylaniline in acetonitrile (Table 4, Figure 3).

Table 4

	Rate constant (s^{-1})	**Correlation Coefficient**
1	0.00042	0.996
2	0.00039	0.999
3	0.00043	0.999
Mean	**0.00041**	

Figure 3

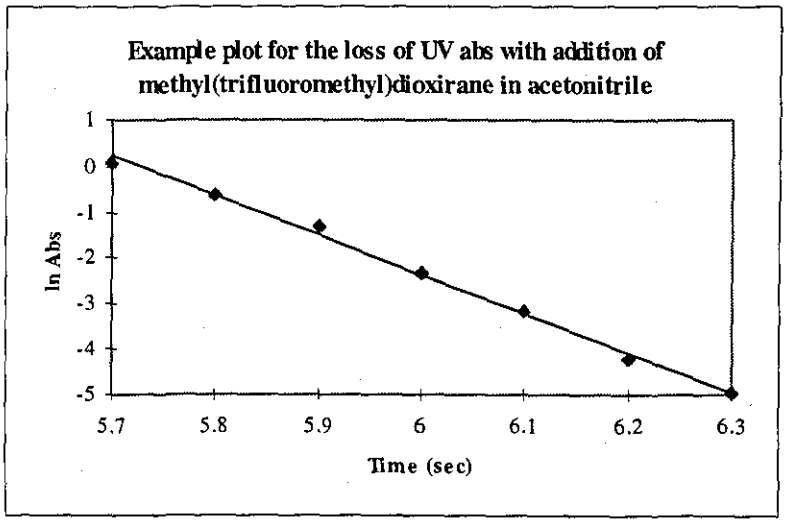


b) Reaction between methyl(trifluoromethyl)dioxirane and 4-nitro-*N,N*-dimethylaniline in acetonitrile (Table 5, Figure 4).

Table 5

	Rate constant (s^{-1})	**Correlation Coefficient**
1	8.6552	0.998
2	6.2380	0.999
3	5.7561	0.994
Mean	**6.8831**	

Figure 4

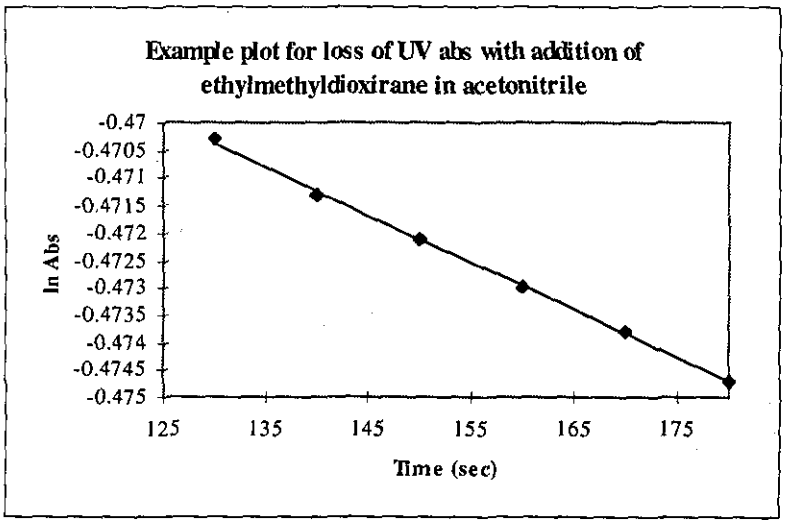


c) Reaction between ethylmethyldioxirane and 4-nitro-*N,N*-dimethylaniline in acetonitrile (Table 6, Figure 5).

Table 6

	Rate constant (s^{-1})	**Correlation Coefficient**
1	0.00009	0.999
2	0.00006	0.997
3	0.00011	0.996
Mean	**0.00009**	

Figure 5



The extremely high reactivity of methyl(trifluoromethyl)dioxirane compared to dimethyldioxirane has been well documented in the literature.[18-22] The above data show that for the oxidation of 4-nitro-*N,N*-dimethylaniline in acetonitrile methyl(trifluoromethyl)dioxirane is around sixteen thousand times more reactive than dimethyldioxirane. Acetonitrile was used as the solvent because the reactivity of methyl(trifluoromethyl)dioxirane in water was too fast to obtain

sufficient data points to establish linearity, even though the spectrophotometer could sample at intervals as low as 0.05 seconds.

This data would suggest that if dioxirane reactivity is indeed linked with the peak potential of the dioxirane then the peak reduction potential for methyl(trifluoromethyl)dioxirane would be much more positive than DMD. The fact that a peak potential was not observed (see Table 2, Section 2.3.2) suggests that it may be higher than + 0.2 V, which would be beyond the electrode limit and be hidden by the mercury response at high potentials.

It was observed that the rate of the reaction of ethylmethyldioxirane with 4-nitro-*N,N*-dimethylaniline was much slower than the rate when using DMD or MTFMD. This is consistent with the peak potential data in that ethylmethyldioxirane has a peak potential less positive than DMD (Table 2, Section 2.3.2). The trend in peak potentials appears, therefore, to represent the trend in reactivity of dioxiranes towards the oxidation of 4-nitro-*N,N*-dimethylaniline.

It was not possible to measure the rates of other dioxiranes, for example, methylphenyl, 2-methoxyphenylmethyl or 2-nitrophenylmethyldioxirane since these dioxiranes could not be isolated and the presence of Oxone® would interfere with the results. However, the peak potential data of the above series indicates that 2-nitrophenylmethyldioxirane would be the most reactive and 2-methoxyphenylmethyldioxirane would be the least reactive. This is the trend which would be expected due to the electron-withdrawing and electron-donating properties of the groups respectively.

Although there is little difference between the peak potentials of the peroxides and the dioxiranes it would appear that the peak potential data is predicting the reactivities within the series of dioxiranes in good agreement with actual

reactivities. Using this technique, therefore, the reactivity of novel dioxiranes could be determined relatively quickly and easily.

The peak potentials and reactivities of the three dioxiranes towards 4-nitro-*N,N*-dimethylaniline in acetonitrile are summarised in Table 7.

Table 7

Dioxirane	**Peak Potential (V)**	**Reaction Rate (s^{-1}) Mean (n = 3)**
DMD	0.140	0.00041
MTFMD	> 0.2	6.88
EMD	0.012	0.00009

2.3.3.2 Effect of Solvent on the Rate of Reaction of DMD and 4-Nitro-*N,N*-Dimethylaniline

Due to the success of the spectrophotometric technique in measuring the reactivity of dioxiranes it was decided to investigate the effect of solvent changes on the rate of reaction of DMD with 4-nitro-*N,N*-dimethylaniline (Tables 8 to 13 and Figures 6 to 11).

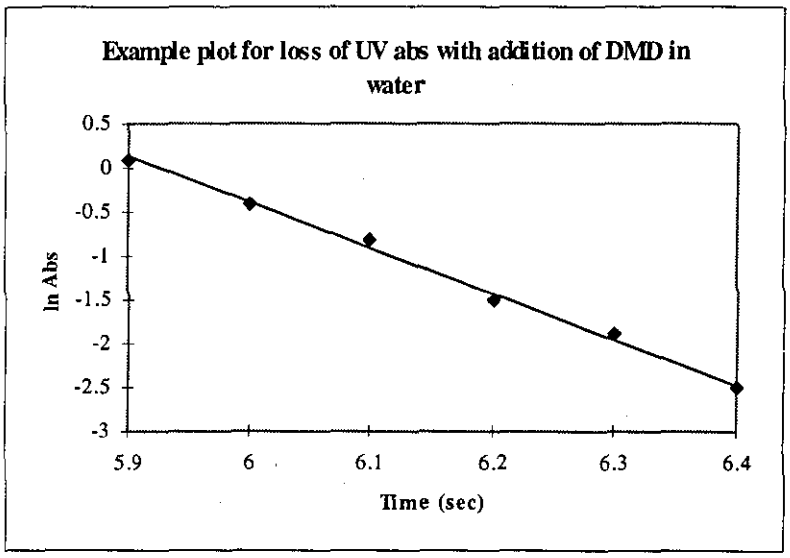
The rate constant measurements were determined in triplicate and the mean taken. All reactions were pseudo first order in the presence of excess dioxirane.

a) Water

Table 8

	Rate constant (s^{-1})	Correlation Coefficient
1	5.19649	0.998
2	6.36342	0.993
3	6.0059	0.996
Mean	**5.85527**	

Figure 6

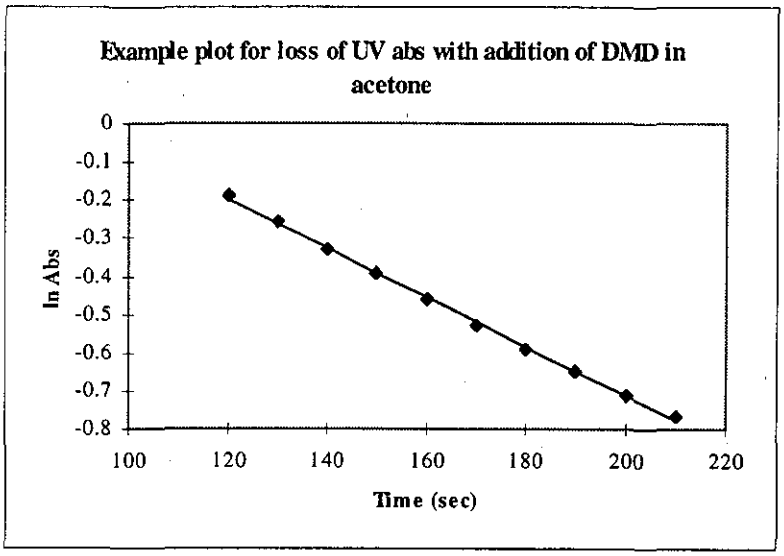


b) Acetone

Table 9

	Rate constant (s^{-1})	Correlation Coefficient
1	0.00643	0.999
2	0.00634	0.999
3	0.00612	0.999
Mean	**0.00630**	

Figure 7

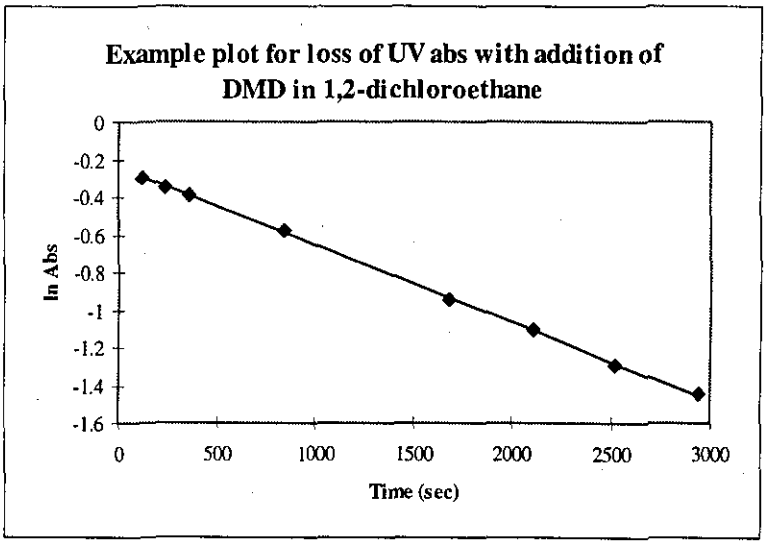


c) 1,2-Dichloroethane

Table 10

	Rate constant (s^{-1})	Correlation Coefficient
1	0.00042	0.998
2	0.00041	0.999
3	0.00039	0.999
Mean	**0.00041**	

Figure 8

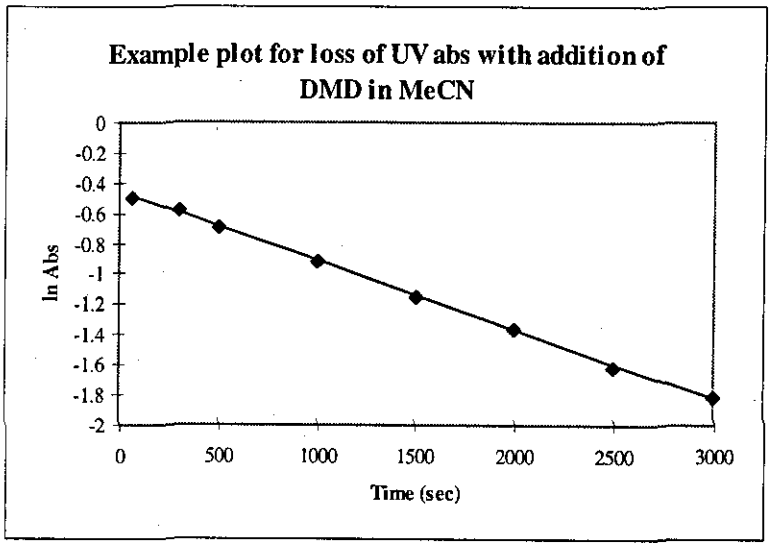


d) Acetonitrile

Table 11

	Rate constant (s^{-1})	Correlation Coefficient
1	0.00042	0.997
2	0.00045	0.999
3	0.00047	0.998
Mean	**0.00045**	

Figure 9

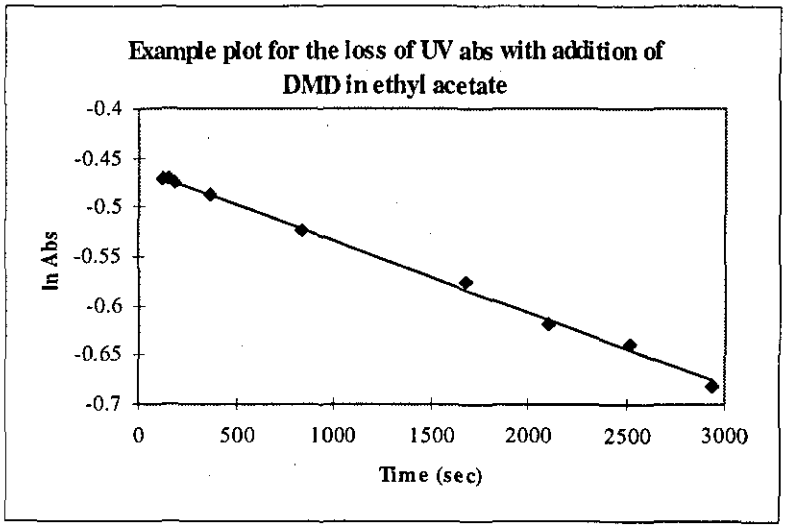


e) Ethyl acetate

Table 12

	Rate constant (s^{-1})	Correlation Coefficient
1	0.00009	0.999
2	0.00007	0.997
3	0.00008	0.998
Mean	**0.00008**	

Figure 10

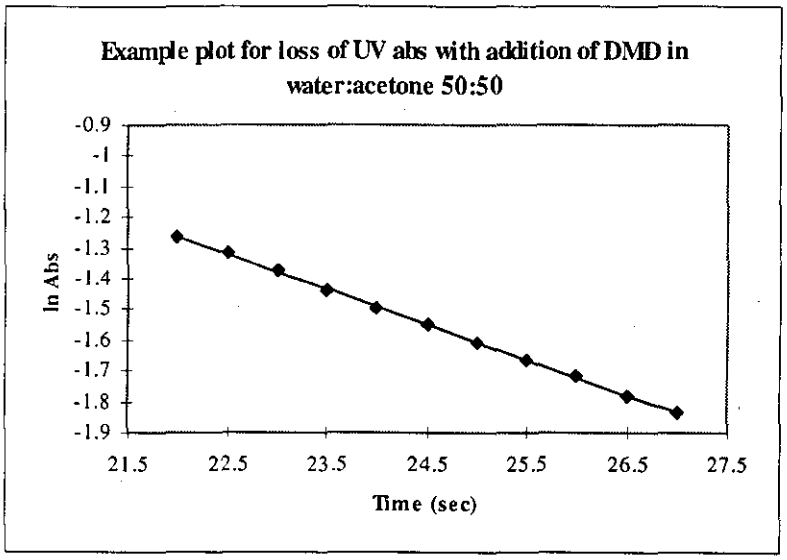


f) Water:acetone 50:50

Table 13

	Rate constant (s^{-1})	Correlation Coefficient
1	0.18322	0.999
2	0.11496	0.999
3	0.16711	0.999
Mean	**0.15510**	

Figure 11



The effect of solvent on the rate of the DMD oxidation of 4-nitro-*N,N*-dimethylaniline is summarised in Table 14.

Table 14. Effect of solvent on oxidation rates of 4-nitro-*N,N*-dimethylaniline by DMD

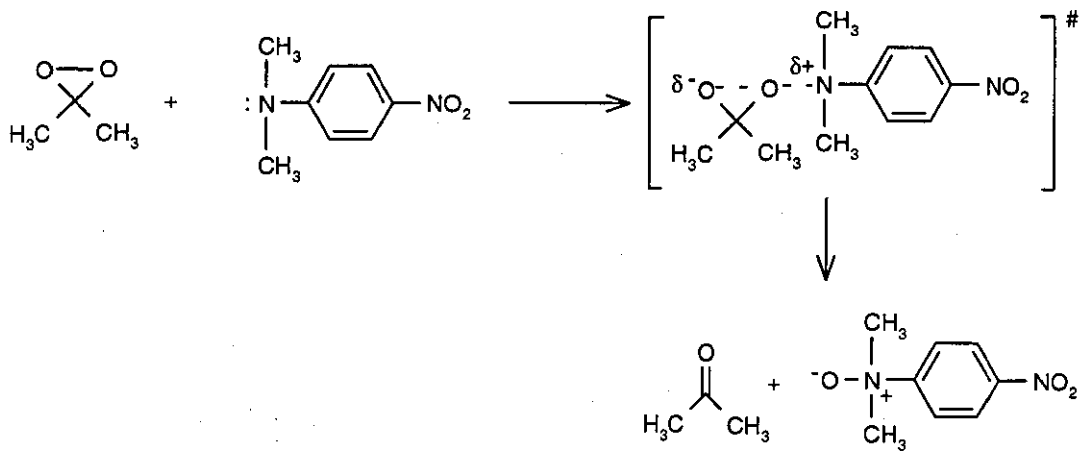
Solvent	Rate (s^{-1})	Dielectric constant
Water	5.85527	78.5
Acetone	0.00630	20.7
1,2-Dichloroethane	0.00039	10.4
Acetonitrile	0.00041	37.5
Ethylacetate	0.00008	6.02
Water:acetone 50:50	0.15510	

The rate constants observed in water are comparable to those obtained for the oxidation of 4-nitro-*N,N*-dimethylaniline using methyl(trifluoromethyl)dioxirane in a relatively unreactive solvent such as acetonitrile. It is speculated that the presence of water promotes faster reaction times and yields **without recourse to the use of fluorinated dioxiranes which are much more expensive to make and are experimentally more difficult to handle.**

The oxygen transfer mechanism for the heteroatom oxidation of tertiary amines with dioxiranes has been proposed as following a strictly S_N2 pathway.[23] It is known that if the transition state in such a reaction mechanism is capable of greater solvation than are the reagents, then the reaction rate will be increased by a change to a more solvating solvent since the activation energy will be lowered.

Perhaps the most important contribution to solvation is polar interactions. For the heteroatom oxidation of 4-nitro-*N,N*-dimethylaniline the activation step is accompanied by an increase in electrical charge on the reactants (Scheme 3) and

hence a change to a more polar solvent will cause a large increase in rate, as is observed.



Scheme 3. Oxidation of 4-nitro-*N,N*-dimethylaniline

There is no easy indication of solvation power and it is rare that a solvation scale is a measure purely of one type of intermolecular interaction. In many cases the response appears to be a blend of polar (non-specific), hydrogen bonding and donor acceptor (specific) solvation capabilities. Table 14, also, shows that with the exception of acetonitrile there is a rank order correlation with the dielectric constant of the medium.

There are obviously many factors influencing the effect of solvent on the rate of the DMD oxidation. However, there is evidence to suggest that the large increase in rate in the presence of water probably arises from the polarity and strong hydrogen bonding nature of the solvent. The role of hydrogen bonded solvents in accelerating oxidation rates has previously been recognised by Murray and Gu in studies of the dimethyldioxirane epoxidation of *trans*-ethyl cinnamate[4] and the oxidation of C-H bonds.[5] They observed an acceleration in the absolute second order rate constants for DMD C-H insertion reactions when the distance between

the reacting C-H bond and the OH group permits intramolecular H-bonding stabilisation of the transition state.[7]

Miaskiewicz *et al*[24a] very recently performed ab initio studies and showed that the reaction barrier for the reaction between DMD and primary amines is substantially decreased in the presence of polar solvents. The effects were particularly dramatic when solvent molecules are hydrogen bonded to the peroxy oxygen.

Stabilisation of the transition state may, also, occur in water due to the formation of stable hydrates of the *N*-oxide reaction products. Direct evidence of *N*-oxide hydrate formation was observed by other workers at Loughborough University[24b] in the ^{1}H NMR spectrum of 4-nitro-*N,N*-dimethylaniline *N*-oxide. It was observed that when water or deuterium oxide was added to solutions of the *N*-oxide in deuterochloroform additional resonances were observed consistent with the reversible formation of a strongly bound hydrate.

2.3.3.3 Effect of pH and Ionic Strength on the Rate of Reaction of DMD and 4-Nitro-*N,N*-Dimethylaniline

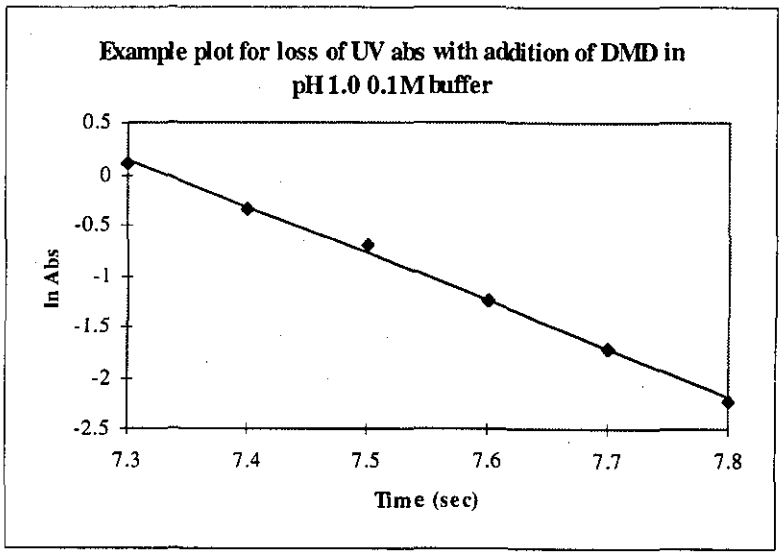
In a further study the effect upon rate of the pH and ionic strength was explored on the reaction of DMD with 4-nitro-*N,N*-dimethylaniline. The findings are reported in Tables 15 to 18 and Figures 12 to 15.

a) pH 1.0, 0.1M Britton Robinson buffer

Table 15

	Rate constant (s^{-1})	Correlation Coefficient
1	5.64145	0.998
2	4.65682	0.998
3	4.86223	0.997
Mean	**5.0535**	

Figure 12

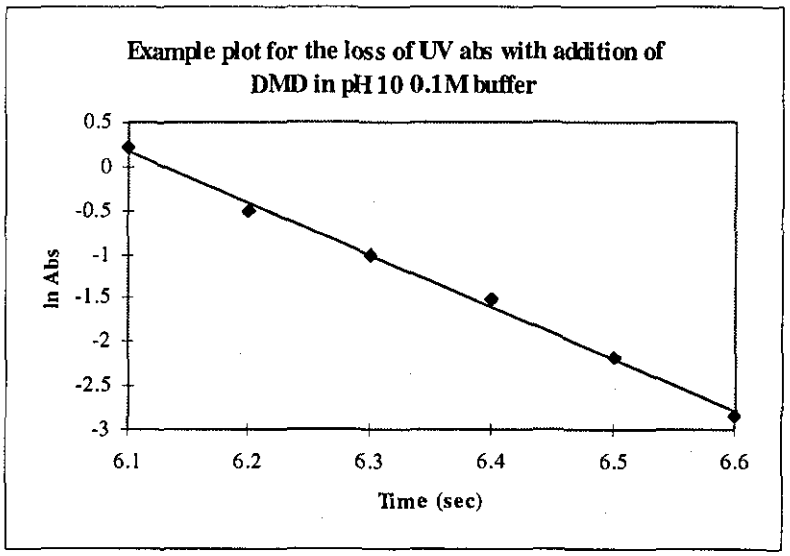


b) pH 10, 0.1M Britton Robinson buffer

Table 16

	Rate constant (s^{-1})	Correlation Coefficient
1	7.14647	0.997
2	5.94349	0.998
3	6.48939	0.996
Mean	**6.52645**	

Figure 13

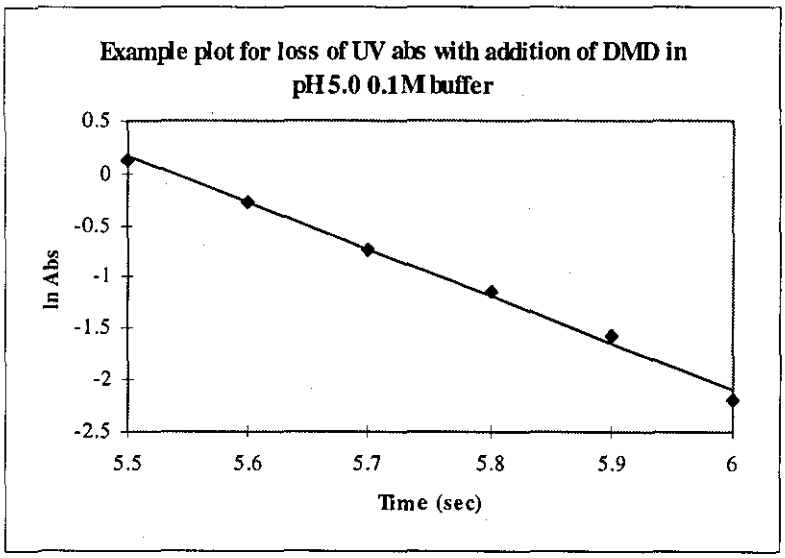


c) pH 5.0, 0.1M Britton Robinson buffer

Table 17

	Rate constant (s^{-1})	Correlation Coefficient
1	6.02514	0.996
2	4.53799	0.998
3	5.00421	0.997
Mean	**5.1891**	

Figure 14

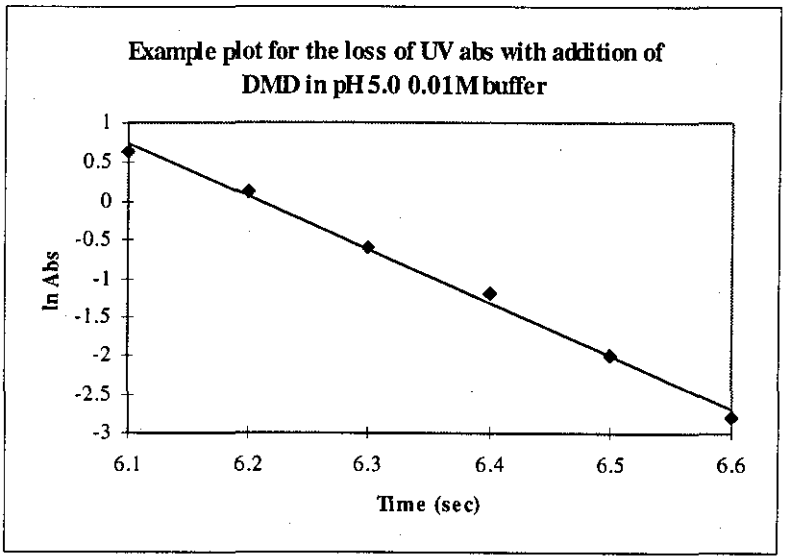


d) pH 5.0, 0.01M Britton Robinson buffer

Table 18

	Rate constant (s^{-1})	Correlation Coefficient
1	5.43364	0.996
2	6.86333	0.997
3	6.73784	0.996
Mean	**6.3449**	

Figure 15



The rate data on the reaction of DMD with 4-nitro-*N*,*N*-dimethylaniline show that there is little difference between the rates at the pH and ionic strengths investigated. The rates of S_N2 reactions are only affected by addition of ionic solutes, insofar as, this effectively increases the polarity of the medium. The reaction rates recorded, however, are all extremely fast and it would be difficult to state that ionic strength has a measurable effect on the reaction.

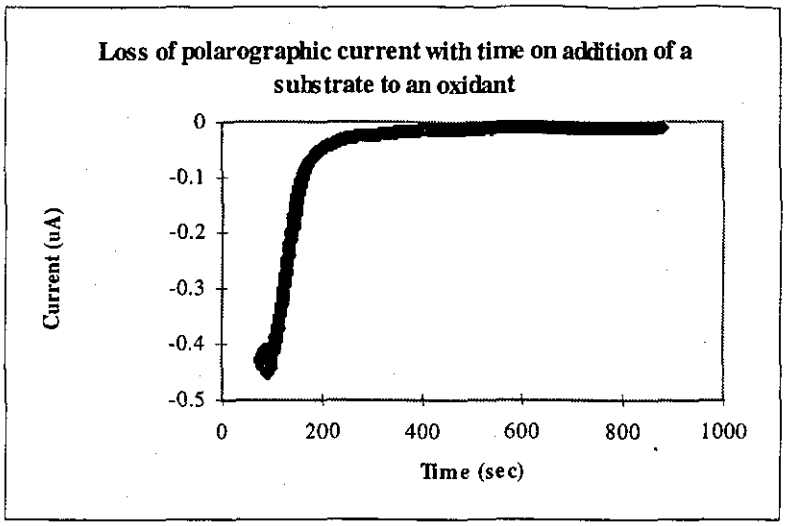
It would, also, be expected that the rates would be faster the higher the pH (more basic) since under acidic conditions the nitrogen would be protonated and hence less readily available for oxidation. However, the rates are not significantly different between pH 1, 5 and 10. At pH 1 there will be a significant proportion of protonated species ($pK_a = 2.36$; equation $5.06 - 3.46\Sigma\sigma$ where $\sigma = 0.78$ for *para* nitro)[25] and it may be inferred that the acid/base equilibrium proceeds at a faster rate than the oxidation reaction itself.

2.3.4 Determination of Reaction Rates using a Constant Potential Amperometric Technique

In order to assess the reactivity of other substrates which are not amenable to UV analysis it was desirable to develop a technique capable of specific quantitation of the oxidising agent (DMD). Using an electrochemical technique it has been possible to determine reaction rates by monitoring the loss of the polarographic current of oxidising agents, at a constant potential, on addition of a given substrate. The technique is based on hydrodynamic voltammetry but rather than keeping the solution still and rotating the electrode, the electrode is held still and the solution stirred. In this way mass transport of the oxidising agent is occurring by both convection, in the bulk solution and diffusion, at the electrode surface.

In hydrodynamic voltammetry the concentration gradient only extends across the diffusion layer, so the oxidant is brought to the electrode (and the reduced species is removed) at a fast rate. As a result of this the limiting current is a plateau which on addition of the substrate to the stirred solution of the oxidant decreases as the reaction between the oxidant and substrate proceeds (Figure 16).

Figure 16



The amperometric response is directly proportional to the concentration of oxidant and by measurement of its loss with time gives information pertaining to the rate of oxidation.

Whereas in Section 2.3.3, using the UV method of monitoring reaction rates, it was only possible to determine the rate of reaction between DMD and 4-nitro-*N,N*-dimethylaniline, using this novel method the reaction rates between DMD and any number of substrates could be determined. For the purpose of this work the effect of substitution on the benzene ring on the oxidation of a series of *N,N*-dimethylanilines was investigated. The anilines used were 4-nitro, 4-methoxy, 4-chloro-*N,N*-dimethylaniline and *N,N*-dimethylaniline itself. Full identification of the products obtained on reaction of DMD with the anilines was conducted by colleagues at Loughborough University.

In order to determine the analytical acceptability of the technique the linearity of DMD in 0.1M KNO_3:MeCN 50:50 was determined using the hydrodynamic technique (Table 19) and by polarography (Table 20). This was done by recording the current over the working concentration range and good linearity was achieved.

Table 19. Hydrodynamic linearity over concentration range 11 - 59 μg/ml

Concentration (μg/ml)	Current (nA)
59.20	1010
23.68	388
17.76	248
11.84	154
Correlation coefficient	**0.999**
Slope	**18.1**
Intercept	**59.2**

Table 20. Polarographic linearity over concentration range 10 - 48 μg/ml

Concentration (μg/ml)	Current (nA)
47.57	477
23.79	259
19.03	215
14.27	168
9.51	112
Correlation coefficient	**0.999**
Slope	**9.4**
Intercept	**30.4**

The 95% confidence intervals intersected the origin in both cases.

2.3.4.1 Determination of the Second Order Rate Constant for the Reaction of DMD with 4-Nitro-*N,N*-Dimethylaniline

4-Nitro-*N,N*-dimethylaniline was reacted with DMD and the loss in current at a potential of 80 mV monitored with time. A potential slightly less positive than the peak potential recorded for DMD (140 mV) was used to ensure complete reduction at the electrode surface.

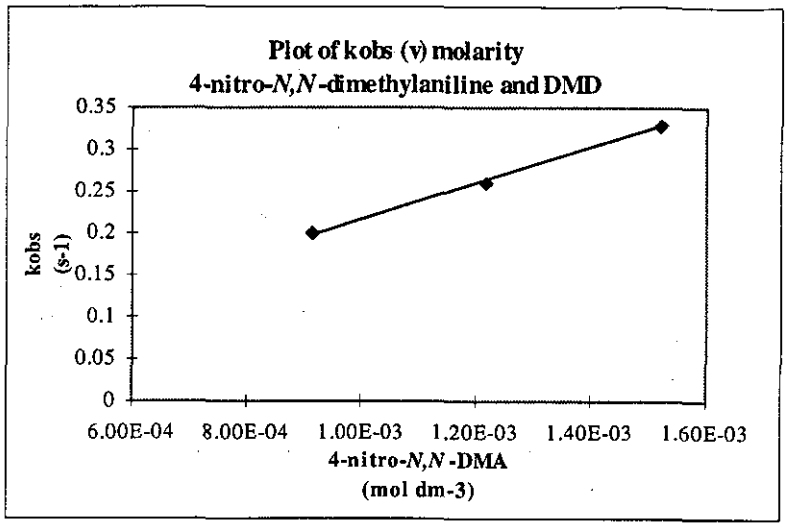
The pseudo first order rate constants (k_{obs}) were obtained from the slope of ln current against time and are shown in Table 21. From this data and the molarity of the aniline the second order rate constant was determined.

Table 21. Second order rate data for 4-nitro-*N,N*-dimethylaniline and DMD

Molarity of Aniline (mol dm^{-3})	**Pseudo first order rate constant (k_{obs}) s^{-1}**	**Correlation coefficient**
9.13×10^{-4}	0.1955 *mean* = 0.1998	0.996
	0.2041	0.999
1.22×10^{-3}	0.2662 *mean* = 0.2604	0.999
	0.2546	0.997
1.52×10^{-3}	0.3223 *mean* = 0.3289	0.996
	0.3355	0.998

A plot of the pseudo first order rate constants against molarity of 4-nitro-*N,N*-dimethylaniline is shown in Figure 17.

Figure 17



The plot is linear and the origin is within the 95% confidence intervals. The second order rate constant was determined from the slope to be 212.6 mol^{-1} dm^3 s^{-1} @ 21°C with a correlation coefficient of 0.999.

2.3.4.2 Determination of the Second Order Rate Constant for the Reaction of DMD with *N,N*-Dimethylaniline

Initial attempts to determine the second order rate constant of *N,N*-dimethylaniline on reaction with DMD using the same concentrations and volumes as used for 4-nitro-*N,N*-dimethylaniline proved to be unsuccessful and the results obtained were variable. This was thought to be due to the much faster reaction rate between *N,N*-dimethylaniline and DMD in that the mixing rate of the larger volumes of the aniline was inefficient and meant that there was not always an excess of aniline relative to DMD at the electrode surface.

To overcome this problem the volume and hence concentration of the aniline was decreased three fold and the experiment repeated. In this case good correlation was achieved between each concentration.

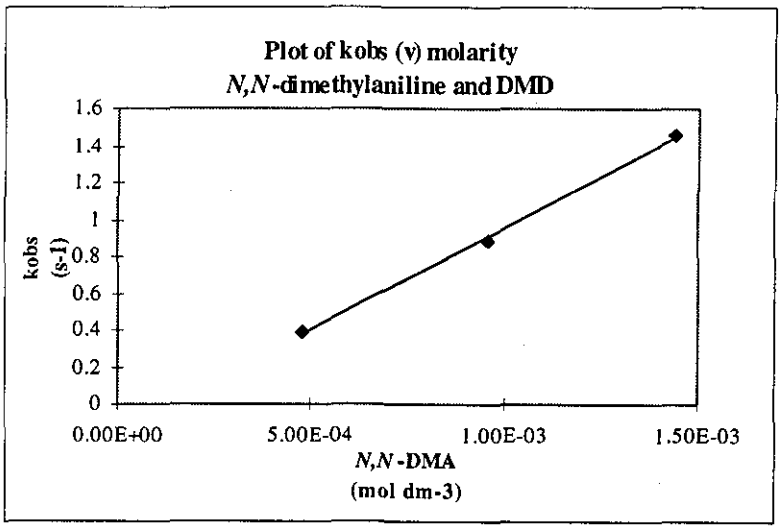
The pseudo first order rate constants were determined in the same way as in section 2.3.4.1 and the results are shown in Table 22.

Table 22. Second order rate data for *N,N*-dimethylaniline and DMD

Molarity of Aniline (mol dm^{-3})	**Pseudo first order rate constant (k_{obs}) s^{-1}**	**Correlation coefficient**
0.48 x 10^{-3}	0.3831 *mean* = 0.3936	0.997
	0.4040	0.996
0.96 x 10^{-3}	0.8962 *mean* = 0.8831	0.995
	0.8700	0.996
1.44 x 10^{-3}	1.5205 *mean* = 1.4584	0.993
	1.3963	0.995

A plot of the pseudo first order rate constants against molarity of *N,N*-dimethylaniline is shown in Figure 18.

Figure 18



The plot is linear and the origin within the 95% confidence intervals. The second order rate constant was determined from the slope to be 1109.2 mol^{-1} dm^3 s^{-1} @ 21°C with a correlation coefficient of 0.998.

2.3.4.3 Determination of the Second Order Rate Constant for the Reaction of DMD with 4-Methoxy-*N,N*-Dimethylaniline

In the reaction of 4-methoxy-*N,N*-dimethylaniline with DMD it again proved necessary to reduce the concentration and volume of the aniline added as in the *N,N*-dimethylaniline case.

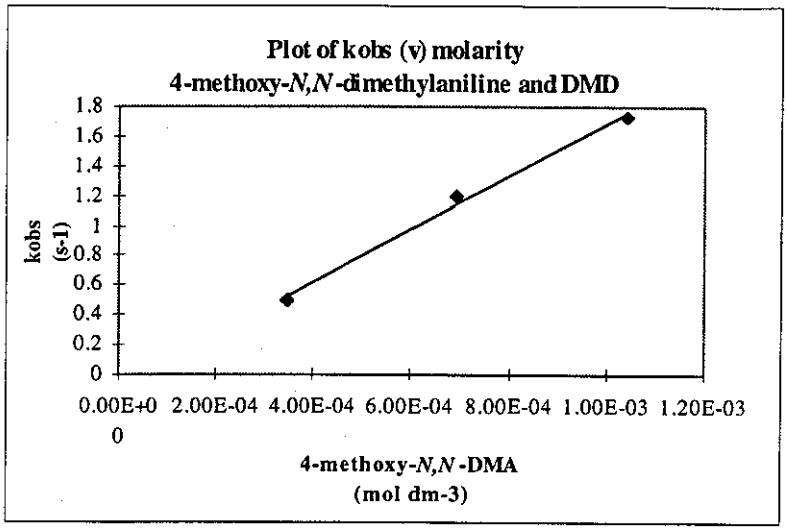
The pseudo first order rate constants were determined in the same way as in section 2.3.4.1 and the results are shown in Table 23.

Table 23. Second order rate data for 4-methoxy-*N,N*-dimethylaniline and DMD

Molarity of Aniline (mol dm^{-3})	Pseudo first order rate constant (k_{obs}) s^{-1}	Correlation coefficient
0.35×10^{-3}	0.4831 *mean* = 0.4936	0.997
	0.5040	0.996
0.69×10^{-3}	1.2615 *mean* = 1.1955	0.994
	1.1295	0.996
1.04×10^{-3}	1.9231 *mean* = 1.7267	0.995
	1.5303	0.993

A plot of the pseudo first order rate constants against molarity of 4-methoxy-*N,N*-dimethylaniline is shown in Figure 19.

Figure 19



The plot is linear and the origin within the 95% confidence intervals. The second order rate constant was determined from the slope to be 1776.8 mol^{-1} dm^3 s^{-1} @ 21°C with a correlation coefficient of 0.997.

2.3.4.4 Determination of the Second Order Rate Constant for the Reaction of DMD with 4-Chloro-*N,N*-Dimethylaniline

In analogy to work with *N,N*-dimethylaniline, it again proved necessary to reduce the concentration and volume of the aniline added.

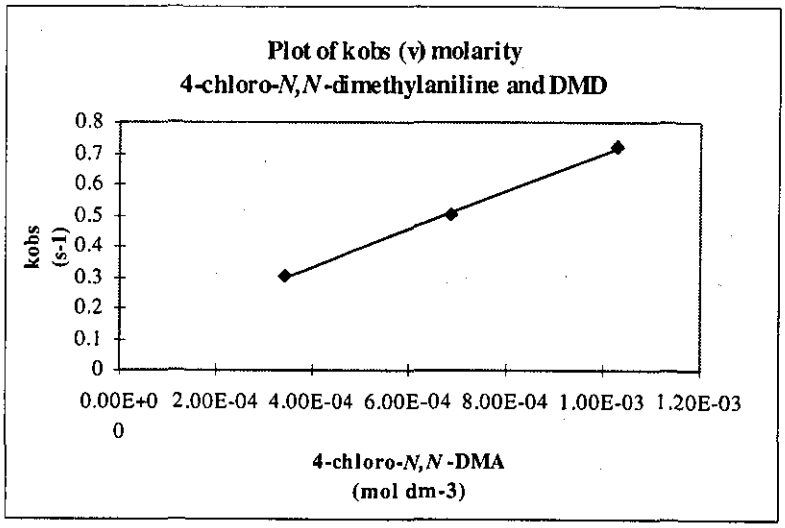
The pseudo first order rate constants were determined in the same way as in section 2.3.4.1 and the results are shown in Table 24.

Table 24. Second order rate data for 4-chloro-*N,N*-dimethylaniline and DMD

Molarity of Aniline (mol dm^{-3})	Pseudo first order rate constant (k_{obs}) s^{-1}	Correlation coefficient
3.43×10^{-4}	0.3010 *mean* = 0.3003	0.997
	0.2996	0.998
6.87×10^{-4}	0.5127 *mean* = 0.5012	0.995
	0.4897	0.994
1.03×10^{-3}	0.7258 *mean* = 0.7201	0.996
	0.7144	0.995

A plot of the pseudo first order rate constants against molarity of 4-chloro-*N,N*-dimethylaniline is shown in Figure 20.

Figure 20



The plot is linear and the origin within the 95% confidence intervals. The second order rate constant was determined from the slope to be 611.3 mol^{-1} dm^3 s^{-1} @ 21°C with a correlation coefficient of 0.999.

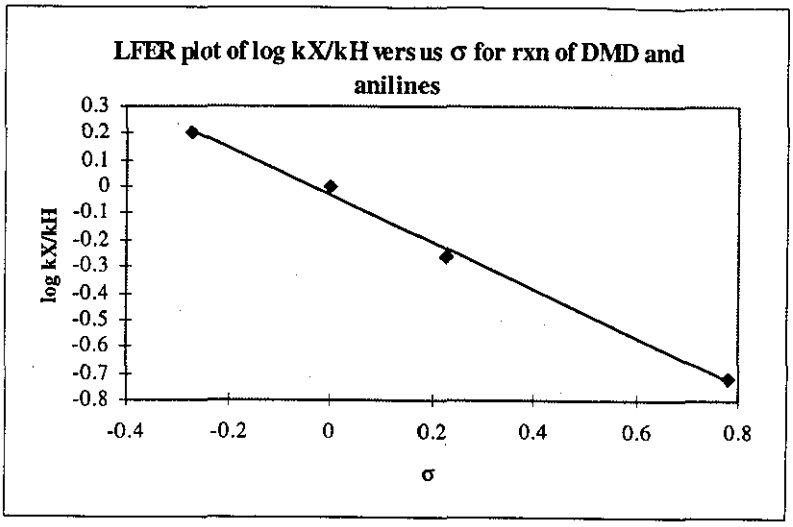
The data shows that there is a dramatic effect on the rate of oxidation when the substituent on the 4-position of the benzene ring is changed. As would be expected due to the electron-withdrawing and electron-donating properties of the nitro and methoxy groups respectively the reactivity is of the order MeO > H > Cl > NO_2. Substitution of the 4-position with a methoxy group results in an 8 fold increase in the rate of oxidation compared to nitro substitution.

The Hammett[26] relationship was applied to the dimethyldioxirane results (Table 25, Figure 21) and gave a ρ value of -0.889.

Table 25

Substituent	**k**	**k_{rel}**	**log k_{rel}**	**σ**
Methoxy	1776.8	1.60	0.20	-0.27
Unsubstituted	1109.2	1.00	0.00	0.00
Chloro	611.3	0.55	-0.26	0.23
Nitro	212.6	0.19	-0.72	0.78
r_H			**0.998**	
ρ			**-0.889**	

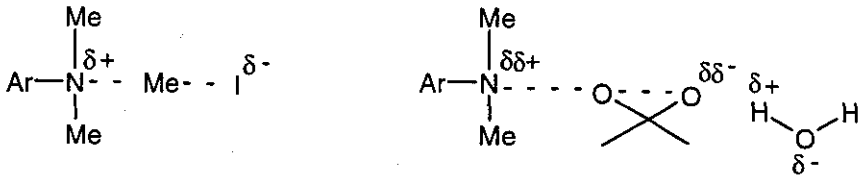
Figure 21



The highest negative ρ value reported for a dimethyldioxirane reaction is -2.76 by Murray and Gu[6] for the C-H insertion reaction into *para*-substituted cumenes. Lower values (-0.77 and -0.76) have been reported by Murray[27] for the electrophilic dimethyldioxirane oxidation of *para*-substituted aryl methyl sulphides and sulphoxides respectively.

The reaction between DMD and the *N,N*-dimethylanilines is similar to the reaction of *N,N*-dimethylanilines with methyl iodide (Menschutkin reaction) which has a reported[28] ρ = -3.30 at 35°C in 90 % aqueous acetone. Whereas, in these reactions the transition state is thought to have developed almost a full positive charge, the reactions with DMD clearly have much less charge development (Figure 22) and may be a function, in part, of steric crowding in the transition state and hydrogen bonding.

Figure 22



It has been demonstrated that the constant potential amperometric technique can be used to determine both the pseudo first order and second order rate constants for the reaction between DMD and several substituted anilines. The second order rate data indicates that the precision of the technique is extremely good, particularly with 4-nitro-*N,N*-dimethylaniline where the correlation between different molarities of aniline is 0.999.

The data does show, however, that as the rates of the reactions being studied increase it becomes more difficult to achieve such high precision between duplicate determinations and between the differing molarities. The reaction between 4-methoxy-*N,N*-dimethylaniline and DMD is much faster than the reaction with the other two anilines and this increase in reaction rate is reflected in the lower correlation coefficient obtained during the second order rate determination.

Not only has this constant potential amperometric technique shown to be an extremely good way of determining the reaction kinetics between dioxiranes and a series of substituted anilines it has the potential to study the reaction kinetics of other oxidations. To this end it was decided to investigate the mechanisms of oxidation of other oxidising agents using 4-nitro-*N,N*-dimethylaniline as a model substrate.

2.3.4.5 Determination of the Pseudo First Order Rate Constant for the Reaction of (1S)-(+)-(10-Camphorsulphonyl) Oxaziridine with 4-Nitro-*N,N*-Dimethylaniline

4-Nitro-*N,N*-dimethylaniline was reacted with (1S)-(+)-(10-camphorsulphonyl) oxaziridine and the loss in current monitored with time at a constant potential. A polarographic peak potential was recorded for (1S)-(+)-(10-camphorsulphonyl) oxaziridine in 0.1M KNO_3:MeCN 50:50 at + 160 mV *vs* Ag/Ag^+. This enabled the determination of the appropriate potential for the experiment.

The pseudo first order rate constants (k_{obs}) were obtained from the slope of ln current against time (Figure 23) and are shown in Table 26.

Figure 23

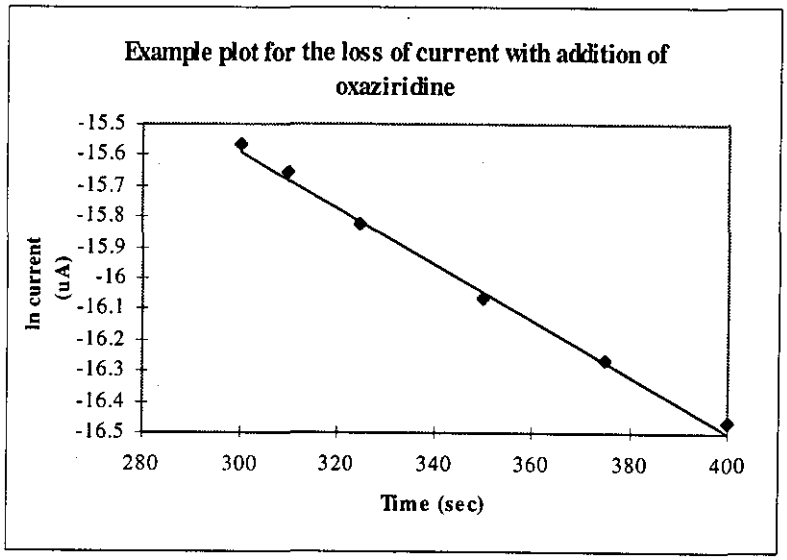


Table 26. Pseudo first order rate data for 4-nitro-*N,N*-dimethylaniline and (1S)-(+)-(10-camphorsulphonyl) oxaziridine

	Rate constant (s^{-1})	Correlation Coefficient
1	0.01181	0.998
2	0.01178	0.999
Mean	**0.01180**	

The rate of reaction between (1S)-(+)-(10-camphorsulphonyl) oxaziridine and 4-nitro-*N,N*-dimethylaniline is 10 times slower than the reaction between DMD and 4-nitro-*N,N*-dimethylaniline. The fact that a straight line plot is achieved indicates that the same mechanism of oxidation is occurring for the reaction with (1S)-(+)-(10-camphorsulphonyl) oxaziridine as for DMD. Literature references, also, observe the oxidation of tertiary amines to *N*-oxides by oxaziridines.[29]

2.3.4.6 Determination of the Pseudo First Order Rate Constant for the Reaction of Oxone® with 4-Nitro-*N,N*-Dimethylaniline

4-Nitro-*N,N*-dimethylaniline was reacted with Oxone® and the loss in current monitored with time at a constant potential. A polarographic peak potential was recorded for Oxone® in 0.1M KNO_3:MeCN 50:50 at + 90 mV *vs* Ag/Ag^+.

The pseudo first order rate constants were determined in the same way as in section 2.3.4.5. The results are represented in Figure 24 and Table 27.

Figure 24

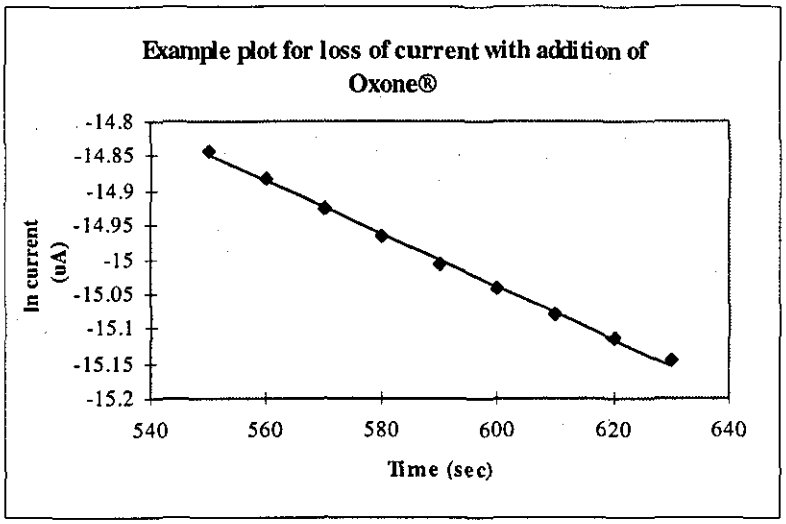


Table 27. *Pseudo first order rate data for 4-nitro-N,N-dimethylaniline and Oxone®*

	Rate constant (s^{-1})	Correlation Coefficient
1	0.00271	0.999
2	0.00382	0.999
Mean	**0.00327**	

The rate of reaction between Oxone® and 4-nitro-*N,N*-dimethylaniline is 8 times slower than the reaction between DMD and 4-nitro-*N,N*-dimethylaniline. The fact that a straight line plot is achieved indicates that the same mechanism of oxidation is occurring for the reaction with Oxone® as for DMD. Literature references, also, observe the oxidation of tertiary amines to *N*-oxides by Oxone®.[30]

The pseudo first order rate constants of Oxone® and (1S)-(+)-(10-camphorsulphonyl) oxaziridine are compared to that for the reaction with DMD

in Table 28. The absolute rate data indicate that DMD is a much faster oxidant of 4-nitro-*N,N*-dimethylaniline than either Oxone® or (1S)-(+)-(10-camphorsulphonyl) oxaziridine.

Table 28

Oxidant	**Pseudo First Order Rate (s^{-1})**
DMD	0.1998
Oxone®	0.0033
(1S)-(+)-(10-camphorsulphonyl) oxaziridine	0.0118

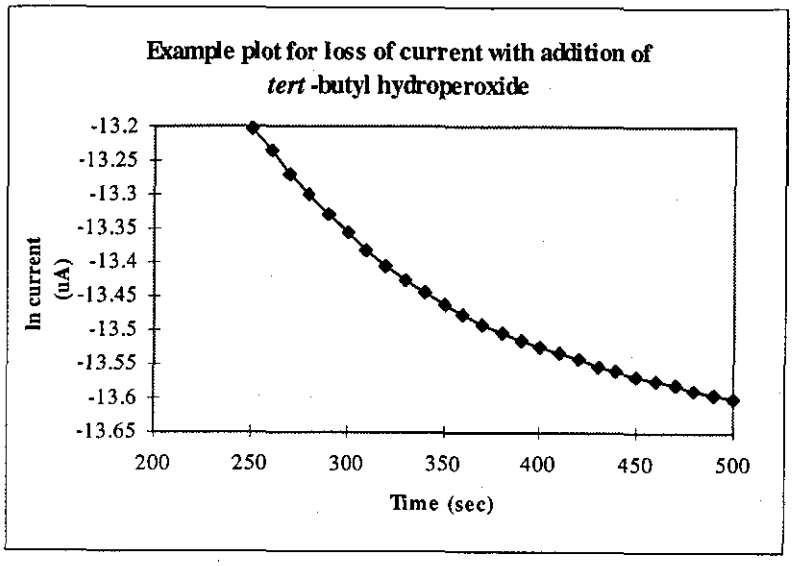
2.3.4.7 Determination of the Pseudo First Order Rate Constant for the Reaction of *tert*-Butyl Hydroperoxide with 4-Nitro-*N,N*-Dimethylaniline

Due to the success of monitoring the reaction rates of 4-nitro-*N,N*-dimethylaniline with DMD, Oxone® and (1S)-(+)-(10-camphorsulphonyl) oxaziridine it was decided to try and study the rates of other oxidants, for example, peroxides.

4-Nitro-*N,N*-dimethylaniline was, therefore, reacted with *tert*-butyl hydroperoxide and the loss in current monitored with time at a constant potential. A polarographic peak potential was recorded for *tert*-butyl hydroperoxide in 0.1M KNO_3:MeCN 50:50 at - 284 mV *vs* Ag/Ag^+.

Pseudo first order kinetics were applied to the reaction, however, a straight line plot was not observed for the oxidation using this peroxide, as shown in Figure 25.

Figure 25



The fact that the reaction between *tert*-butyl hydroperoxide and 4-nitro-*N,N*-dimethylaniline does not give a straight line plot when pseudo first order kinetics are applied would indicate that the reaction does not proceed *via* an identical S_N2 mechanism to that of the other oxidants investigated.

Subsequent literature precedent revealed that tertiary amines on reaction with *tert*-butyl hydroperoxide give dealkylation products (Scheme 4).[31,32] The usual product obtained from hydroperoxides (amine oxides) can be obtained by including the presence of catalytic amounts of V_2O_5 with *tert*-butyl hydroperoxide, in which case no dealkylation is observed.[33]

$$(RCH_2)_3N + t\text{-BuOOH} \quad \text{-----}> \quad (RCH_2)_2NH + RCHO + t\text{-BuOH}$$

Scheme 4

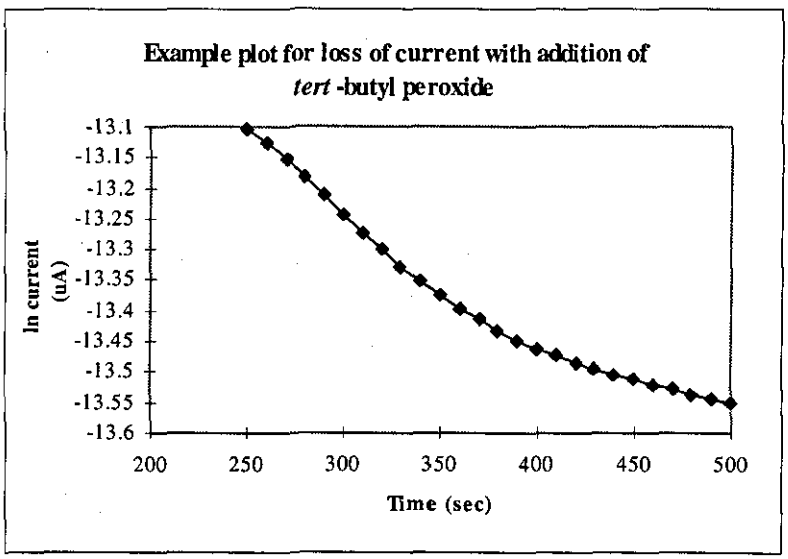
Due to the commercial availability of monomethylaniline (the dealkylation product of *N,N*-dimethylaniline) the above example was investigated using *N,N*-

dimethylaniline instead of 4-nitro-*N,N*-dimethylaniline. Chromatography was established to identify *N,N*-dimethylaniline and monomethylaniline as HPLC markers. It was observed that when *tert*-butyl hydroperoxide was reacted with *N,N*-dimethylaniline the major product of the reaction was monomethylaniline. This confirmed the reaction mechanism would not be expected to be the same as for DMD, Oxone® and oxaziridine and, therefore, explains the deviation from linearity.

2.3.4.8 Determination of the Pseudo First Order Rate Constant for the Reaction of *tert*-Butyl Peroxide with 4-Nitro-*N,N*-Dimethylaniline

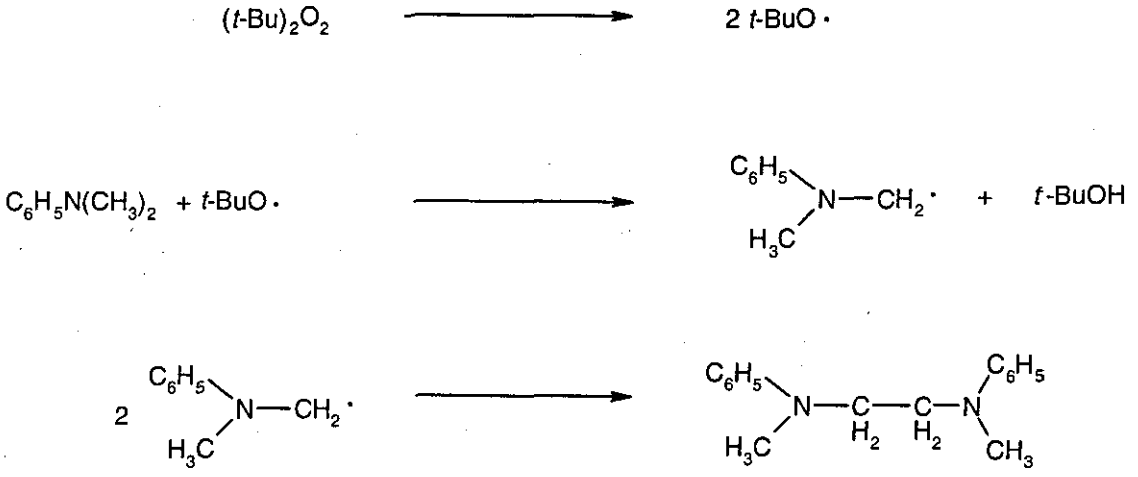
In analogy to the work with *tert*-butyl hydroperoxide, 4-nitro-*N,N*-dimethylaniline was reacted with *tert*-butyl peroxide and the loss in current monitored with time at a constant potential. A polarographic peak potential was recorded for *tert*-butyl peroxide in 0.1M KNO_3:MeCN 50:50 at - 284 mV *vs* Ag/Ag^+. Pseudo first order kinetics were applied to the reaction, however, a straight line plot was not observed for the oxidation using this peroxide, as shown in Figure 26.

Figure 26



The fact that the reaction between *tert*-butyl peroxide and 4-nitro-*N,N*-dimethylaniline does not give a straight line plot when pseudo first order kinetics are applied would indicate that the reaction does not proceed *via* an identical S_N2 mechanism to that of the other oxidants investigated.

Initial thoughts lead to an investigation of whether the product of the reaction between *tert*-butyl peroxide and 4-nitro-*N,N*-dimethylaniline was, also, the product of dealkylation, as for *tert*-butyl hydroperoxide. This was again studied using *N,N*-dimethylaniline. However, no monomethylaniline was observed. Subsequently literature references were found which documented the reaction product between *N,N*-dimethylaniline and *tert*-butyl peroxide to be the dimer, *N,N'*-dimethyl-*N,N'*-diphenylethylenediamine (Scheme 5).[34]



Scheme 5

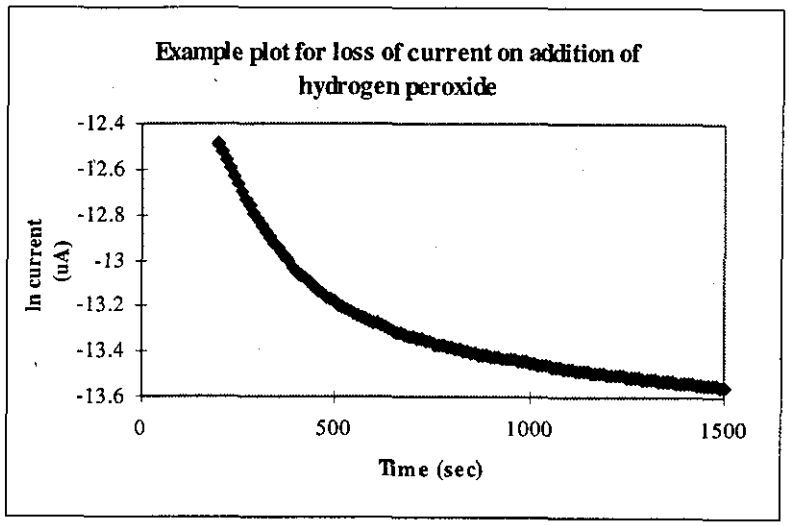
2.3.4.9 Determination of the Pseudo First Order Rate Constant for the Reaction of Hydrogen Peroxide with 4-Nitro-*N,N*-Dimethylaniline

Another reaction which was investigated was that between 4-nitro-*N,N*-dimethylaniline and hydrogen peroxide. A polarographic peak potential was

recorded for hydrogen peroxide in 0.1M KNO_3:MeCN 50:50 at - 320 mV *vs* Ag/Ag^+ and the loss in current monitored with time at a constant potential.

Pseudo first order kinetics were applied to the reaction, however, a straight line plot was only observed for the initial stages of the oxidation (Figure 27).

Figure 27



Literature references document that the product of the reaction between tertiary amines and hydrogen peroxide is the amine oxide.[35] However, it is stated that hydrogen peroxide is very ineffective at this transformation due to the poor leaving characteristics of the hydroxide anions. It may be that under the reaction conditions employed here other products are being formed which would result in a more complex mechanism. Without identification of the products it is difficult to conclude the reasons for non-linearity.

2.3.4.10 Determination of the Pseudo First Order Rate Constant for the Oxidation of SB202026 by DMD

In Chapter 1 the reaction between DMD and SB202026 was studied and the single oxidation product determined to be the corresponding *N*-oxide. Due to the success of the hydrodynamic technique in measuring the rates of reactions of various substrates it was decided to attempt to measure the rate of the reaction.

The pseudo first order rate constant (k_{obs}) for the reaction between SB202026 and DMD was obtained from the slope of ln current against time (Figure 28). The resultant pseudo first order data is shown in Table 29.

Figure 28

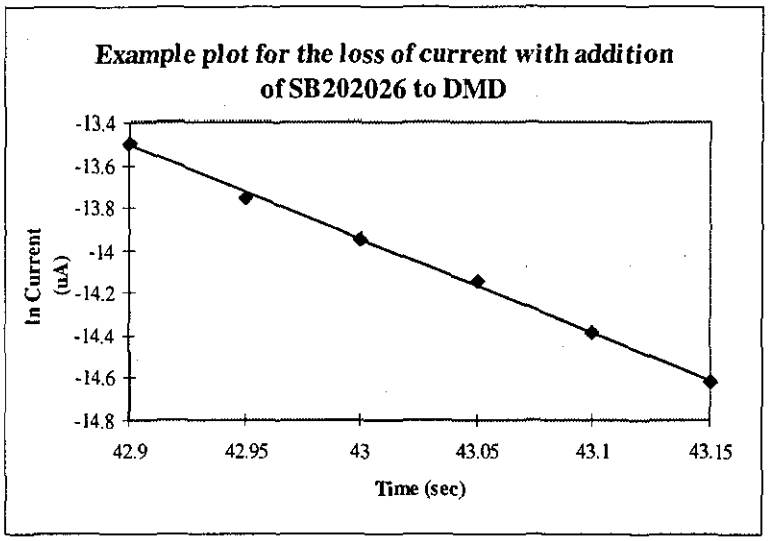


Table 29

	Rate constant (s^{-1})	Correlation Coefficient
1	4.42	0.997
2	3.48	0.996
Mean	**3.95**	

The pseudo first order rate constant for the reaction of SB202026 with DMD is similar to the rates of reaction between DMD and the series of anilines investigated in Sections 2.3.4.1 to 2.3.4.4. This is a good indication that a similar reaction mechanism is taking place in the two reactions. It, also, indicates that this technique could be used to study the kinetics of the reaction of dioxiranes with many types of substrates including drug molecules.

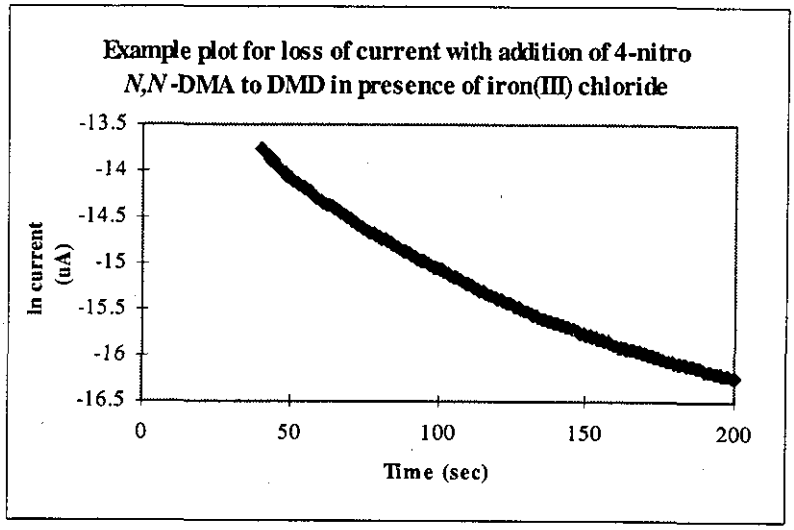
2.3.4.11 Determination of the Pseudo First Order Rate Constant for the Oxidation of 4-Nitro-*N,N*-Dimethylaniline by DMD in the Presence of Iron(III) Chloride

Oxygen transfer reactions from acyl and alkyl hydroperoxides to metal (III) porphyrins have been documented in the literature by Thomas C. Bruice.[36] In these reactions it is shown that transfer of an "oxene equivalent" molecule from the peroxide to metalloporphyrins results in the formation of hypervalent metal-oxo porphyrins. Epoxidation and oxygen insertion reactions occurring in this way have been reported. It was decided to investigate if the presence of iron would have an effect on the mechanism of the DMD oxidation of 4-nitro-*N,N*-dimethylaniline.

The reaction was carried out with an equimolar amount of DMD and iron(III) chloride. As can be seen from Figure 29 the reaction does not follow first order reaction kinetics even though the reaction was first order in the absence of iron. It may be concluded, therefore, that a different reaction mechanism is taking place, possibly where the oxygen is first transferred to the iron to form a metallo-oxo species which then transfers oxygen to the aniline. It is the reaction between the metallo-oxo species and the aniline which has a different reaction mechanism to that between DMD and the aniline.

This experiment was carried out to see if different reaction mechanisms could be detected using the electrochemical technique. Determination of the exact nature of the products and mechanism in the presence of iron(III) chloride was thought to be beyond the scope of this work.

Figure 29



The amperometric technique has been shown, therefore, to be capable of studying not only the rates and mechanisms of DMD reactions but, also, the rates and mechanisms of other oxidation processes.

2.4 Conclusions

Dioxiranes have been characterised electrochemically by polarography. The peak reduction potentials observed were similar to those for acyclic peroxides. It was shown that although there is little difference between the peak potentials of the series of peroxides and the dioxiranes investigated there does appear to be a trend between the reactivity and peak potentials within the series of dioxiranes themselves.

The reactivity of dioxiranes was investigated using a spectrophotometric technique by monitoring the loss of the UV absorption of 4-nitro-*N,N*-dimethylaniline on addition of a dioxirane. The reaction rates were determined to be pseudo first order in the presence of excess dioxirane consistent with an electrophilic S_N2 mechanism and significantly faster in more polar solvents such as water. Good correlation coefficients were achieved for the reactions studied, all were greater than 0.996.

A novel electrochemical technique was used to monitor the reaction kinetics between DMD and a series of substituted anilines. This technique used constant potential amperometry to monitor the loss in the polarographic current due to DMD on addition of the aniline. The technique displays significant advantage over previous literature methods in that it avoids the large solvent background interferences. The amperometric response was found to be directly proportional to the concentration of dioxirane and by measurement of its loss with time, gave information pertaining to the rate of oxidation. The reaction rates were determined to be second order in the presence of excess aniline.

All the data is consistent with the overall conclusion that the DMD oxidation of *N,N*-dimethylanilines is purely an electrophilic S_N2 process and does not involve

such species as the bis(oxyl)diradical implicated by Minisci *et al* in other oxidations.[14] This was observed to be in contrast to the mechanisms of oxidation of 4-nitro-*N,N*-dimethylaniline by other oxidants.

It was observed that although peroxides are similar to dioxiranes in that they have an O-O bond, the mechanisms of oxidation are different to the electrophilic substitution observed when using DMD and, also, that in the presence of metals, such as, iron(III) chloride the reaction mechanism appears to change.

2.5 Experimental

2.5.1 Experimental for Section 2.3.1

Samples of peroxides were supplied by Peroxid-Chemie GmbH.

Polarographic equipment:

Polarographic analysis was performed on a Princeton Applied Research (PAR) 174A polarographic analyser in conjunction with a PAR 303 polarographic detector and an advance X-Y recorder. For some experiments the mercury drop electrode was replaced with a glassy carbon electrode. Latter experiments were carried out on a PAR 394 polarographic analyser in conjunction with a PAR 303 polarographic detector.

Peroxide sample preparation:

Approximately 40 mg or 1 ml of peroxide was dissolved in 200 ml of 50:50 0.1 M KNO_3 (supporting electrolyte):MeCN and then diluted 4 ml into 100 ml 50:50 0.1 M KNO_3:MeCN.[15]

Polarographic conditions:

Initial potential: + 0.3 V (mercury electrode) ; + 1.0 V (carbon electrode)
Final potential: - 1.0 V
Scan rate: 5 mV/sec
Drop time: 0.5 sec
Modulation amplitude: 20 mV
Purge time: 360 sec

HPLC conditions:

High performance liquid chromatography with electrochemical and UV detection was performed on the following equipment:

Hewlett Packard 1050 autosampler

Severn Analytical SA 6410B pump

Severn Analytical SA 6504 UV detector

Coulochem II electrochemical detector

The peroxides were analysed under the following conditions:

Column: Spherisorb ODS2 S5 15 cm x 4.6 mm

Eluent: pH 5.0 0.1 M $NaH_2PO_4.2H_2O$ buffer:MeCN 50:50

Flow rate: 1 ml/min

UV detection at 240 nm

Electrochemical detection at -0.7 V

2.5.2 Experimental for Section 2.3.2

Polarographic equipment and conditions as in section 2.5.1. If necessary the molarity of the dioxiranes was determined according to the titration method described in Chapter 1, Section 1.5.1.

Preparation of dioxiranes:

Preparation of dimethyl, methyl(trifluoromethyl) and ethylmethyldioxirane:

A round bottom flask was equipped with an efficient mechanical stirrer and a solids addition funnel, connected by means of a U-tube to a receiving flask and cooled to -78°C by means of a dry ice/acetone bath. The reaction flask was charged with EDTA solution (1.6 g/l), ketone (210 ml) and sodium bicarbonate (160 g). While vigorously stirring solid Oxone® (320 g) was added slowly over

a period of approximately one hour. The dioxirane/ketone distillate was collected in the cooled (-78°C) receiving flask.

Preparation of methyl-*n*-propyl, methyl-*n*-butyl and diethyldioxirane:

The above dioxiranes were prepared according to a literature procedure.[37] Oxone® (40 g, 0.065 mol) was added in small portions to a well-stirred mixture of 18-crown-6 (0.20 g), ketone (25 ml), water (50 ml), and sodium bicarbonate (30 g). The organic layer was separated from the two-layer mixture obtained and dried ($MgSO_4$). In the preparation of methyl-*n*-propyldioxirane 2-pentanone was used as the ketone, in the preparation of methyl-*n*-butyldioxirane 2-hexanone was used as the ketone and in the preparation of diethyldioxirane 3-pentanone was used as the ketone.

Preparation of 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, cyclohexanone, 3-methyl-2-butanone, 2,2-dimethyl-3-butanone, methylphenyl, 2-methoxyphenylmethyl and 2-nitrophenylmethyldioxirane:

The above dioxiranes were prepared according to a literature procedure.[38] A mixture of ketone (45 ml), phosphate buffer pH 7.4 (50 ml) and ice (10-15 g) was stirred at 0°C (ice-salt bath). Cooled Oxone® (90 g) was added as a slurry in water (200 ml) over 5 minutes. A KOH solution (10-15 %) cooled to 0-5°C, was added simultaneously to maintain the pH at 7-8.5. A yellow colour is formed immediately upon combining the reagents. The mixture was stirred vigorously for 2-3 minutes and then poured into a beaker containing a cooled mixture of anhydrous Na_2SO_4, $NaH_2PO_4.H_2O$, $Na_2HPO_4.7H_2O$ (4:2:1) and the combined mixture was stirred vigorously in an ice-salt bath. The liquid phase was transferred rapidly to a cooled separating funnel and the aqueous phase separated out. The dark yellow organic phase was dried with cold anhydrous Na_2SO_4 and

the polarographic response recorded on dilution in a 50:50 mix of 0.1 M KNO_3: MeCN.

The ketones used for the above dioxiranes were 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, cyclohexanone, 3-methyl-2-butanone, 2,2-dimethyl-3-butanone, acetophenone, 2-methoxyacetophenone and 2-nitroacetophenone respectively.

2.5.3 Experimental for Section 2.3.3

Linearity of 4-nitro-*N,N*-dimethylaniline

4-Nitro-*N,N*-dimethylaniline (10 mg) supplied by Lancaster Synthesis Ltd was accurately weighed into a volumetric flask (100 ml) and dissolved in acetonitrile (25 ml). The volume was made up with distilled water. The following volumes of the stock solution were then transferred by pipette 20, 15, 10, 5, 4, 3, 2, & 1 ml into a volumetric flask (100 ml) and 1 ml transferred into a 200 and 250 ml volumetric flask. The volumes of each of the flasks were again made up using distilled water.

The UV absorbance of each of the solutions was recorded at 425 nm (λ_{max} in water) using a Beckman DU 640*i* spectrophotometer. Linear regression statistics were used to calculate the slope, intercept and correlation coefficient of the line.

2.5.4 Experimental for Section 2.3.3.1

Dimethyl, ethylmethyl and methyltrifluoro(methyl)dioxirane were prepared according to the method in section 2.5.2.

The λ_{max} of 4-nitro-*N,N*-dimethylaniline in acetonitrile was first determined by running a scan over the range 200 to 600 nm using a Beckman DU 640*i* spectrophotometer. A λ_{max} of 393 nm in acetonitrile was recorded.

The kinetic runs were performed on a Beckman DU 640*i* spectrophotometer using a 1 cm UV cell with stirring thermostated to 25°C by a Thermomix 1460 unit. The 4-nitro-*N,N*-dimethylaniline solution was prepared by weighing 10 mg into a volumetric flask (100 ml) and making up to volume using acetonitrile. The stock solution (5 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume made up with acetonitrile. The acetonitrile used was A.C.S grade and supplied by Fisher Scientific.

The instrument parameters were the minimum possible and were as follows:
Interval time = 0.1 sec
Read average time = 0.05 sec

Dioxirane (60 µl, 0.07 M) was added using a microsyringe (100 µl) and the aniline solution (2.5 ml) aspirated into the cell using a syringe (5 ml). The loss in UV absorbance with time was then recorded using the kinetics programme on the instrument. The rate data obtained was calculated by plotting the natural log of UV absorbance against time. The final absorbance reading was recorded by taking a reading of the solution after addition and if the absorbance had not returned to zero the value was subtracted from each UV reading prior to taking the natural log. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line.

2.5.5 Experimental for Section 2.3.3.2 to 2.3.3.3

For each of the solvents used the λ_{max} of the aniline was first determined by running a scan over the range 200 to 600 nm. All scans and kinetic runs were recorded using a Beckman DU 640*i* spectrophotometer. The λ_{max} readings were 425, 400, 394, 393 & 383 nm in water, acetone, 1,2-dichloroethane, acetonitrile and ethyl acetate respectively. Britton Robinson buffer was prepared according to the method in Chapter 1, Section 1.5.6 and the λ_{max} the same as in water.

The kinetic runs were performed using a 1 cm UV cell with stirring thermostated to 25°C by a Thermomix 1460 unit. The 4-nitro-*N,N*-dimethylaniline solution was prepared by weighing 10 mg into a volumetric flask (100 ml) and making up to volume using acetonitrile. The stock solution (5 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume made up with the appropriate solvent. The solvents used were A.C.S grade and supplied by Fisher Scientific. The aqueous solutions at pH 1, 5 and 10 were Britton Robinson buffer.

The instrument parameters were the minimum possible and were as follows:
Interval time = 0.1 sec
Read average time = 0.05 sec

Dioxirane (60 µl, 0.07 M) was added using a microsyringe (100 µl) and the aniline solution (2.5 ml) aspirated into the cell using a syringe (5 ml). The loss in UV absorbance with time was then recorded using the kinetics programme on the instrument. The rate data obtained was calculated by plotting the natural log of UV absorbance against time. The final absorbance reading was recorded by taking a reading of the solution after addition and if the absorbance had not returned to zero the value was subtracted from each UV reading prior to taking

the natural log. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line.

2.5.6 Experimental for Section 2.3.4

Linearity of dimethyldioxirane

Dimethyldioxirane (5 ml, 0.067 M), prepared according to section 1.5.1, was accurately transferred using a pipette into a volumetric flask (100 ml) and the volume made up with potassium nitrate solution (0.1 M):MeCN 50:50. The following volumes of the stock solution were then transferred by pipette 10, 5, 4, 3, 2, & 1 ml into a volumetric flask (50 ml) and the volume made up with potassium nitrate solution (0.1 M):MeCN 50:50.

The polarographic current of each of the solutions was recorded under the following conditions using a Bioanalytical Systems CV50W voltammetric analyser with a controlled growth mercury electrode detector:
Mode: differential pulse polarography
Initial potential: + 0.15 V
Final potential: - 0.15 V
Purge time: 100 sec (nitrogen)
Pulse amplitude: 20 mV
Scan rate: 5 mV/sec
Scan increment: 4 mV
Step drop time: 0.8 sec
Drop size: 8

Linear regression statistics were used to calculate the slope, intercept and correlation coefficient of the line.

The linearity experiment was repeated over a similar concentration range by recording the current of the solutions using the hydrodynamic technique. For this the polarographic conditions were as follows:

Bioanalytical Systems CV50W voltammetric analyser with a controlled growth mercury electrode detector.
Mode: Timebase function
Initial potential: 80 mV
Sampling time: 50 msec
Run time: 100 sec
Sensitivity: 1 μA
Drop size: 8
Stirrer speed: 600 rpm

2.5.7 Experimental for Section 2.3.4.1 to 2.3.4.4

Kinetic experiments were performed using a Bioanalytical Systems CV50W voltammetric analyser with a controlled growth mercury electrode detector under the following conditions:
Mode: Timebase function
Initial potential: 80 mV
Sampling time: 50 msec
Run time: 100 sec
Sensitivity: 1 μA
Drop size: 8
Stirrer speed: 600 rpm
There was no need to purge the system due to the fact that a potential of 80 mV is not sufficiently negative to reduce any oxygen in the system.

Dioxirane solution (1 ml) was transferred by pipette into a volumetric flask (100 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock solution (20 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. The working solution (10 ml) was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established a solution of the aniline in acetonitrile at a concentration of at least 5 times the concentration of the dioxirane (50 - 250 μl) was added to the cell using a microsyringe.

Pseudo first order rates (k_{obs}) were calculated by plotting the natural log of initial current minus the final current against time. The final current reading was often zero and so no subtraction was necessary. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line.

Second order rate constants were calculated by plotting the pseudo first order rate constants against the molarities of the aniline. The different molarities of the aniline were achieved by pipetting different volumes of the aniline in the range 50 - 250 μl. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line. All readings were recorded at room temperature which was 21°C.

4-Chloro- and 4-methoxy-*N,N*-dimethylaniline were supplied by Loughborough University. 4-Nitro-*N,N*-dimethylaniline and *N,N*-dimethylaniline were supplied by Lancaster Synthesis Ltd.

2.5.8 Experimental for Section 2.3.4.5 to 2.3.4.6

Polarographic experiments were performed using a Bioanalytical Systems CV50W voltammetric analyser with a controlled growth mercury electrode detector. All experiments were carried out at 21°C.

The polarographic peak potentials for (1S)-(+)-(10-camphorsulphonyl) oxaziridine and Oxone® were recorded under the following conditions:
Mode: differential pulse polarography
Initial potential: + 0.25 V
Final potential: - 0.15 V
Purge time: 100 sec (helium)
Pulse amplitude: 50 mV
Pulse width: 50 msec
Drop time: 1000 msec
Sensitivity: 1 μA
Drop size: 8

Kinetic experiments were performed using the timebase function with the following parameters:
Initial potential: 80 mV (oxaziridine); 60 mV (Oxone®)
Sampling time: 1 sec
Run time: 200 sec
Sensitivity: 1 μA
Drop size: 8
Stirrer speed: 600 rpm

There was no need to purge the system due to the fact that potentials of 60 and 80 mV are not sufficiently negative to reduce any oxygen in the system.

(1S)-(+)-(10-Camphorsulphonyl) oxaziridine/Oxone® (1 mg) was accurately weighed into a volumetric flask (100 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock solution (20 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. The working solution (10 ml) was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established a solution of 4-nitro-*N,N*-dimethylaniline in acetonitrile at a concentration of 5 times the concentration of oxaziridine/Oxone® (100 - 200 µl) was added to the cell using a microsyringe.

Pseudo first order rates (k_{obs}) were calculated by plotting the natural log of initial current minus the final current against time. The final current reading was often zero and so no subtraction was necessary. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line.

Oxone® and (1S)-(+)-(10-Camphorsulphonyl) oxaziridine were supplied by Aldrich Chemical Company.

2.5.9 Experimental for Section 2.3.4.7 to 2.3.4.8

The polarographic peak potentials for *tert*-butyl hydroperoxide and *tert*-butyl peroxide were recorded under the same conditions as in section 2.5.8.

Kinetic experiments were performed using the timebase function with the following parameters:
Initial potential: - 400 mV
Sampling time: 1 sec

Run time: 500 sec

Sensitivity: 1 μA

Drop size: 8

Stirrer speed: 600 rpm

Purge Time: 5 mins (helium)

Tert-butyl hydroperoxide/*tert*-butyl peroxide (30 & 35 mg respectively) was accurately weighed into a volumetric flask (100 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock solution (10 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. The working solution (10 ml) was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established a solution of 4-nitro-*N,N*-dimethylaniline in acetonitrile at a concentration of 5 times the concentration of *tert*-butyl hydroperoxide/*tert*-butyl peroxide (100 - 200 μl) was added to the cell using a microsyringe.

The chromatographic parameters for the detection of *N,N*-dimethylaniline and monomethylaniline were as follows:

Column: Hypersil BDS C18 15 cm x 4.6 mm

Eluent: pH 4.0 acetate buffer:MeCN 80:20

Detection @ 240 nm

Flow rate: 1 ml/min

Retention times: monomethylaniline: 7.5 mins, *N,N*-dimethylaniline: 14 mins

The peroxides were supplied by Peroxid-Chemie GmbH.

2.5.10 Experimental for Section 2.3.4.9

The polarographic peak potential for hydrogen peroxide and the kinetic experiments were carried out using the same conditions as in section 2.5.8. The hydrogen peroxide was supplied by Aldrich Chemical Company.

Hydrogen peroxide (20 volume; 1 ml) was transferred by pipette into a volumetric flask (100 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock solution (1 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. The working solution (10 ml) was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established a solution of 4-nitro-*N,N*-dimethylaniline in acetonitrile at a concentration of 5 times the concentration of hydrogen peroxide (150 µl) was added to the cell using a microsyringe.

2.5.11 Experimental for Section 2.3.4.10

Kinetic experiments for the reaction between DMD and SB202026 were performed using the timebase function with the following parameters:
Initial potential: 80 mV
Sampling time: 50 msec
Run time: 100 sec
Sensitivity: 1 µA
Drop size: 8
Stirrer speed: 600 rpm

DMD (0.08 M; 1 ml) was transferred by pipette into a volumetric flask (100 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock

solution (15 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. The working solution (10 ml) was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established a solution of SB202026 in acetonitrile at a concentration of 5 times the concentration of DMD (100 µl) was added to the cell using a microsyringe.

Pseudo first order rates (k_{obs}) were calculated by plotting the natural log of initial current minus the final current against time. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line.

SB202026 was supplied by the chemical development department at SmithKline Beecham Pharmaceuticals.

2.5.12 Experimental for Section 2.3.4.11

Kinetic experiments for the reaction between DMD and 4-nitro-*N,N*-dimethylaniline in the presence of iron(III) chloride were performed using the timebase function with the following parameters:
Initial potential: 80 mV
Sampling time: 50 msec
Run time: 100 sec
Sensitivity: 1 µA
Drop size: 8
Stirrer speed: 600 rpm

DMD (0.08 M; 2 ml) was transferred by pipette into a volumetric flask (100 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock

solution (15 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. Iron(III) chloride (20 mg) was accurately weighed into a volumetric flask (50 ml) and the volume made up with potassium nitrate (0.1 M):MeCN 50:50. The stock solution (10 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with potassium nitrate (0.1 M):MeCN 50:50. The working solution was prepared by mixing the dioxirane solution (5 ml) with the iron(III) chloride solution (5 ml). This solution was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established a solution of 4-nitro-*N,N*-dimethylaniline in acetonitrile at a concentration of 5 times the concentration of DMD (100 µl) was added to the cell using a microsyringe.

Pseudo first order rates (k_{obs}) were calculated by plotting the natural log of initial current minus the final current against time. Linear regression statistics were used to calculate the slope (rate), intercept and correlation coefficient of the line.

Iron(III) chloride was supplied by Aldrich Chemical Company.

2.6 Appendix

The Principles and Applications of Polarography

Polarography is a special type of voltammetry in which the working electrode is a dropping mercury electrode. It is an analytical technique where electrical currents are measured in solution when a changing potential (voltage) is applied to that solution. The simplest relationship between current (I) and potential is in the case of an electrical resistance (R) where current (I) is proportional to the applied potential (V). This is a statement of Ohm's law (V=IR). Liquids such as water conduct electricity very poorly and the currents obtained are relatively small even when large potentials are applied. If, however, that solution contains a substance that may be chemically oxidised or reduced, large currents sometimes appear in the form of 'waves' which occur at well defined potentials.

Potential is applied to the solution by means of electrodes. A supporting electrolyte containing inert ions must be used in approximately a 100 fold excess of the substance under measurement. This ensures that reducible compounds arrive at the electrode by diffusion processes and fixes the linear relationship between concentration (c) and the limiting diffusion current (i_d). From the point of view of obtaining a response we could merely immerse two copper wires in the solution, but to obtain consistent results which can be assessed scientifically a polarograph uses a reference electrode (saturated calomel - SCE) and a dropping mercury electrode (DME) as working electrode. As the voltage is varied there comes a point when the solute begins to reduce at the mercury electrode, the material at the surface of the electrode, once reduced, is rapidly replaced by fresh material further from the electrode. This movement of solute through the solution controls the amount of material reduced and hence the current. Material cannot be reduced at the electrode faster than it arrives from other parts of the

solution. This is why there is a limiting diffusion current and a wave results rather than the current increasing indefinitely.

A dropping mercury electrode is used for two reasons. It has a relatively large operating range (+ 0.2 V to - 2.0 V) and since drops are continually being formed the electrode surface is always fresh and uncontaminated by reduction products. The range can, also, be extended beyond - 2.0 V in non aqueous media. In the case of organic compounds the reduction process usually involves hydrogen ions, for example:

$$PhNO_2 + 4e^- + 4H^+ \rightarrow PhNHOH + H_2O$$
$$PhNHOH + 2e^- + 2H^+ \rightarrow PhNH_2 + H_2O$$

Scheme 6

In such cases a buffer solution is necessary to control the pH and, also, act as the supporting electrolyte.

The main disadvantages of DC polarography are that the wave heights are difficult to measure reproducibly and the technique is not very sensitive. Differential pulse polarography is more commonly used where pulses are applied and the difference in current during the duration of the pulse is plotted against a variable potential. In this case peaks are obtained instead of waves and are much easier to measure.

Many nitrogen containing compounds are pharmacologically active and are used widely in the pharmaceutical industry. Analysis of such compounds can prove difficult if strong UV absorbing chromophores are not present in the molecular structure, particularly when determination of degradation products and metabolites is required. When drug metabolism and pharmacokinetic

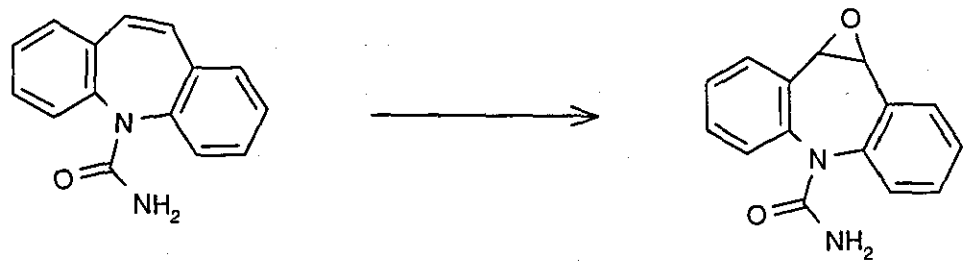
experiments are carried out drug solutions may, also, be present in complex biological matrices which makes detection difficult. In these circumstances polarographic techniques can be used to advantage.

Advanced polarographic and voltammetric techniques are among the most sensitive instrumental techniques. In these methods, an analyte is oxidised or reduced at an appropriate electrode and the amount of current involved is related to the amount of analyte. The fraction of analyte electrolysed may be very small, in fact negligible, in the current-voltage techniques of voltammetry or polarography. The potential at which a given analyte will be oxidised or reduced is dependent on a particular substance and selectivity can be achieved by appropriate choice of electrode potential.

Renewal of interest occurred in the area of polarography in the 1970's when reliable polarographs offering pulse mode with sensitivity down to 10^{-7} and 10^{-8} M and higher resolving power became available. A better understanding of the electrode processes of compounds of pharmaceutical interest (active agents, excipients, additives, antioxidants etc.) and the application of oxidation waves, making the technique available to a wide range of pharmaceuticals, also, improved interest. A practical advantage of polarography and voltammetric methods in the determination of active agents or impurities in pharmaceutical forms is that undissolved excipients or coloured solutions do not interfere to the extent that they do in other methods.

Frequently electroactive compounds are, also, strongly therapeutically active or toxic. Since many metabolic mechanisms and polarographic activity both involve changes of oxidation state or redox character, the polarographic analysis can reflect clinical changes. Thus polarographic methods are often suitable for metabolic studies.

There are many examples of drugs which are detectable using voltammetry.[39] For example, Carbamazepine (**1**), the active ingredient of Tegretol, a Ciba-Geigy drug widely used for treatment of epilepsy and trigeminal neuralgia, is metabolised *via* oxidation to the 10,11-epoxide compound (**2**) (Scheme 7).[40, 41]



1 2

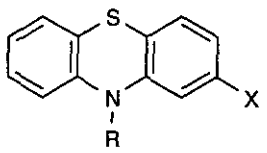
Scheme 7. Oxidation of Carbamazepine

In Carbamazepine (CBZ) biotransformation, the epoxide pathway is quantitatively the most important one. The first product of the pathway is 10,11-epoxy-CBZ, which is chemically stable under physiological conditions. Since the epoxide has shown to possess anticonvulsant activity of the same order of magnitude as the parent drug it is necessary to quantitate both in order to evaluate the relationship between plasma levels of the drug and its effects. Several gas chromatographic (GC), GC-MS, and HPLC methods have been developed for the analysis of **1** and **2**.[42]

Epoxide (**2**) gives in tetrabutylammonium perchlorate/DMF in the presence of $MgSO_4$ a well formed differential pulse polarograph reduction wave with a peak potential (*Ep*) of -2430 $\pm$ 20mV (*vs* SCE).

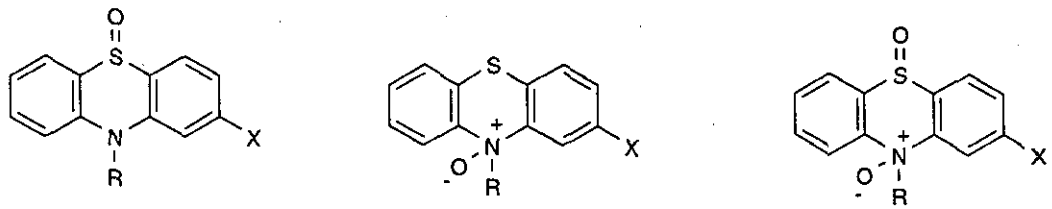
Another good example is the phenothiazine range of drugs, such as, chlorpromazine (Largactil) and promethazine (Phenergan) (Scheme 8). The

parent drug itself is not electroactive at the DME but its metabolites the *S*-oxides and *N*-oxides are electroactive, by reduction, and can be selectively determined by voltammetry at a gold or graphite solid electrode.



Chlorpromazine R = $-(CH_2)_3N(CH_3)_2$, X = Cl

Promethazine R = $-CH_2CHCH_3N(CH_3)_2$, X = H



S-oxide *N*-oxide *S*-oxide-*N*-oxide

Metabolites

Scheme 8. Metabolites of Chlorpromazine & Promethazine

Dc polarographic methods have a sensitivity quite suitable for the determination of many drugs in tablets, creams and other pharmaceutical formulations. Frequently it is possible to simply crush and grind the tablet or tablets in water or some suitable solvent such as methanol and then dilute with the chosen supporting electrolyte for direct polarographic analysis. Most excipients used to carry the drug, such as lactose or starch are not electroactive. Fragments of undissolved excipient will not interfere with polarographic analysis. These undissolved fragments would interfere badly with a UV spectroscopy method.

Metabolic studies and clinical analyses of drugs in body fluids such as blood plasma, urine, cerebrospinal fluid require much higher sensitivity of normal or differential pulse polarography.

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3.0 Dimethyldioxirane as a Derivatising Agent in Analytical Chemistry

3.1 Summary

This chapter demonstrates the use of dimethyldioxirane as an efficient and effective derivatising agent of amines with particular reference to ephedrine and a quinuclidine based compound. It illustrates how DMD can be employed as an analytical tool utilising a polarographic method and high performance liquid chromatography method as a means of identification and detection of the oxidation products obtained. Other methods of derivatisation are reviewed and the advantages and disadvantages of DMD are discussed.

3.2 Introduction

Analytical derivatisation finds application in a wide range of disciplines. In addition to the traditional areas of chemistry and biochemistry, derivatisation methods are an integral part of much of the chemical analysis practised in the medical, forensic and environmental sciences to name but a few. Derivatisation has enabled the use of analytical methods, such as, gas chromatography (GC) and mass spectrometry (MS) on samples not otherwise amenable to these techniques and has thus extended the application of these powerful tools to new fields.

Although high performance liquid chromatography has undergone explosive growth, a limitation in the use of this technique has been the sensitivity of available detection methods. Ultraviolet detection has been most widely applied, but many compounds have only a weak UV absorption. Increasing use is being

made of chromogenic derivatisation to impart or increase UV detection sensitivity.

Amines exist in many biologically important compounds such as amino acids and polyamines. There are many pharmaceutical amines, for example, ephedrine, amphetamine and phenmetrazine. Direct HPLC analysis with UV-VIS detection of these amines is limited by the weak or non-existent UV-VIS absorption of these compounds. Only by converting amines to strongly absorbing derivatives can they be determined by HPLC with high sensitivity when present in biological and clinical samples at low concentrations.

Primary and secondary amines are usually derivatised as aromatic derivatives by nucleophilic substitution reactions. Tertiary amines are more difficult to detect and their determination involves quaternisation. The derivatising reagents of primary, secondary and tertiary amines and the derivatising procedures are summarised in Table 1.

This chapter will show that compared to the derivatising procedures detailed in this introduction the use of dimethyldioxirane as a derivatising reagent of amines has significant advantages. The reactions between dimethyldioxirane and amines are extremely rapid, give products in high yield and the residual acetone remaining can be removed simply by evaporation.

Further details of each procedure with appropriate examples are presented in Sections 3.2.1 to 3.2.4.

Table 1

Amine	Derivatising Agent	Product	Derivatising Procedure
Primary amines Secondary amines	Acyl chlorides	Amides	50°C for 3 hours 12 hour reflux
Polyfunctional amines	Only *m*-toluoyl chloride gives a reaction free of by-products	Amides	in pyridine at room temperature for 5 minutes
Primary amines Secondary amines	Arylsulphonyl chlorides	Sulphonamides	70°C for 1 hour
Primary amines Secondary amines	Nitrobenzenes	Nitrobenzene derivative	with FDNB* 85°C for 15 minutes in pH 9.0 aqueous solution
Primary amines Secondary amines	Isocyanates	*N,N*-disubstituted ureas	in *N,N*-dimethyl-formamide for few minutes
Primary amines Secondary amines	NBD-Cl**	NBD-Cl fluorescent derivative	in aqueous solution (pH 8) at 55°C for 1 hour
Tertiary aliphatic amines	*p*-nitrobenzyl bromide (*p*-NBBr)	*p*-NBBr derivative	in acetonitrile at 40°C for 24 hours

*FDNB - 1-Fluoro-2,4-dinitrobenzene
**NBD-Cl - 4-Chloro-7-nitro-benzo-2,1,3-oxadiazole

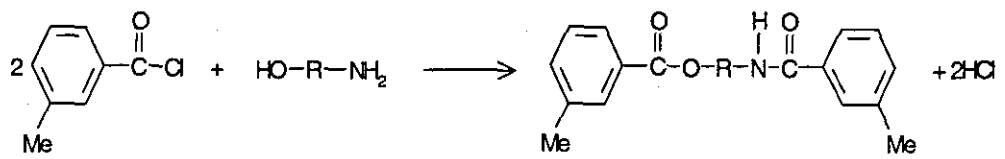
3.2.1 Acyl Chlorides

Acyl chlorides react readily with amines, in alkaline medium, to form amides (Scheme 1). The use of amide derivatives for the HPLC analysis of amines has been extensively studied. Several acyl chlorides such as *p*-nitrobenzoyl, *p*-methoxybenzoyl, *p*-methylbenzoyl and *p*-chlorobenzoyl chlorides have been used.[1] The reaction with *p*-methoxybenzoyl chloride, for example, involves dissolving the acyl chloride and amine in THF followed by reaction in potassium

carbonate at pH 8. For primary amines the reaction is carried out at 50°C for 3 hours and for secondary amines the reaction mixture is refluxed for 12 hours. The amides formed are then extracted with chloroform and analysed using reverse phase HPLC. The detection limit for amphetamine has been determined as 3 ng and the recovery 90.8 +/- 4.4 %.

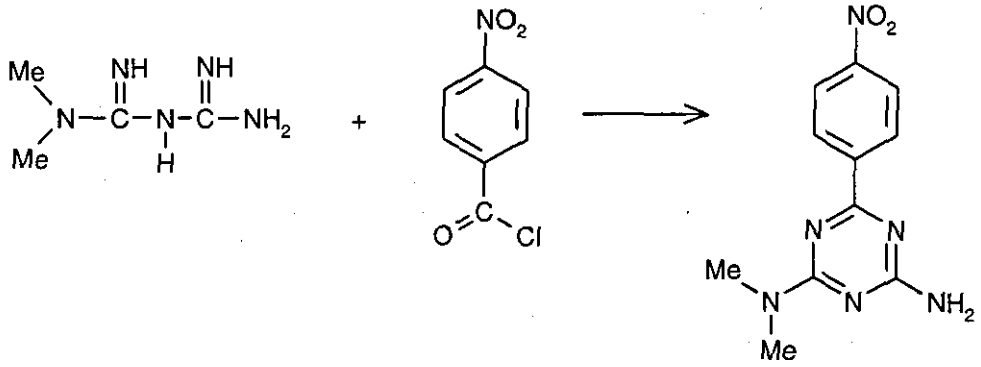
Scheme 1. The formation of amide derivatives by reaction of primary or secondary amines with benzoyl chloride

Polyfunctional amines can, also, be analysed by HPLC as amides.[2] Acyl chlorides, such as, benzoyl, *m*-nitrobenzoyl, *m*-toluoyl, *p*-*t*-butylbenzoyl, phenoxyacetyl and *p*-anisoyl chloride have been investigated for derivatisation and measurement of piperazine. Of these reagents only *m*-toluoyl chloride gives a reaction which is free of by-products and derivatives which are soluble in water-immiscible solvents and hence easily separated (Scheme 2). The reaction is carried out in pyridine at room temperature for 5 minutes and the amides extracted with dichloromethane prior to reverse phase HPLC analysis. The detection limit for the reaction has been determined to be approximately 5 ng and the recovery exceeds 94 %.



Scheme 2. The reaction of *m*-toluoyl chloride with polyfunctional amines

Metformin is a biguanide compound with high polarity and poor chromatographic properties. Reaction with *p*-nitrobenzoyl chloride (Scheme 3) results in the triazine derivative which has lower polarity and is suitable for HPLC separation and detection at 280 nm.[3] The reaction conditions involve dissolving Metformin in acetonitrile and 20 % sodium hydroxide followed by treatment with *p*-nitrobenzoyl chloride. The reaction takes 6 hours at room temperature with a yield of 94 %. The derivative of Metformin in acetonitrile can be analysed directly by reverse phase HPLC.



Scheme 3. The reaction of metformin with *p*-nitrobenzoyl chloride

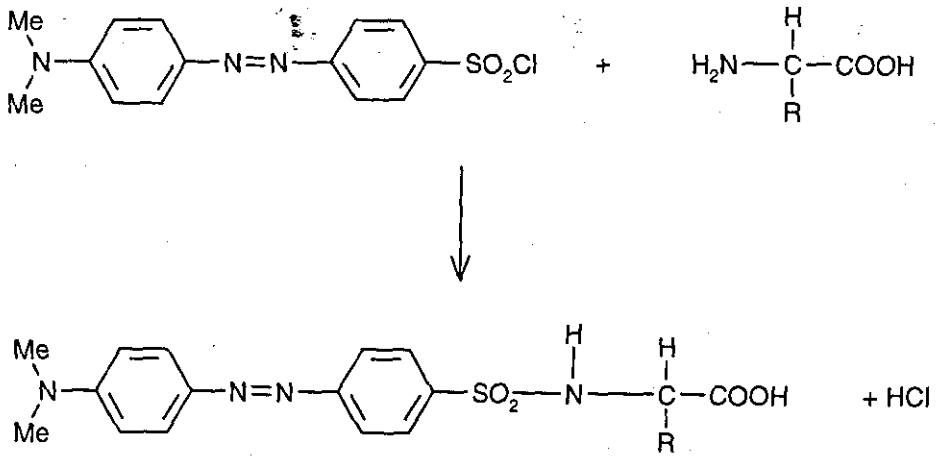
Benzoyl chloride is the preferred derivatising agent for polyamines, as it reacts with most of the naturally occurring diamines and polyamines. The assay procedure has been optimised and evaluated.[4] The procedure involves incubation of samples under basic conditions for 30 minutes at room temperature and the derivatives extracted with chloroform prior to analysis by reverse phase HPLC. The linear range of the method has been determined to be up to 100 nmol of polyamine and the limit of detection 1 pmol.

3.2.2 Arylsulphonyl Chlorides

In analogy to acyl chlorides, arylsulphonyl chlorides react with primary and secondary amines.[1] The determination of polyamines based on the formation of

tosylated derivatives has been reported by Sugiura *et al.*[5] The tosylation results not only in an increase in the absorptivity but, also, in the improvement of HPLC separation. The reaction is carried out at 70°C for 1 hour and the mixture washed with *n*-hexane to remove the toluenesulphonyl chloride which interferes with the peaks of some of the products. After extraction with chloroform the derivatives are separated by reverse phase HPLC.

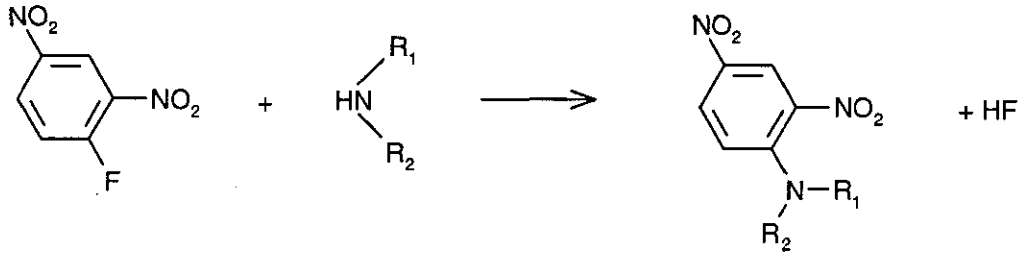
Gentamicin, a polyfunctional amino compound, has been determined by using reverse phase HPLC after labelling with benzenesulphonyl chloride.[6] The derivatisation reaction is complete in 10 minutes at 75°C.
Dimethylaminophenylazobenzenesulphonyl chloride (DABSCl) is one of the derivatising agents for amino acids.[7] The resulting sulphonamides show an absorbance maximum at 420-450 nm, which allows detection at long wavelength and excludes interference from endogenous substances in clinical samples to a large extent. The derivatisation is performed at 50°C and gives the product in quantitative yield (Scheme 4).



Scheme 4. The reaction of dimethylaminoazobenzenesulphonyl chloride with amines

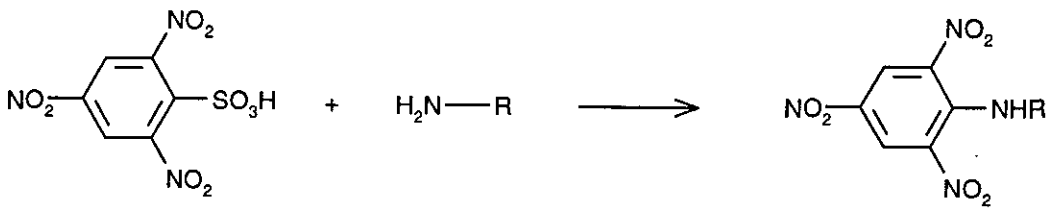
3.2.3 Nitrobenzenes

Nitrobenzenes are most commonly used for the derivatisation of amino compounds. The 4-nitrobenzoyl group has been shown to impart high UV absorptivity to amines. 1-Fluoro-2,4-dinitrobenzene (FDNB) has been used as a label for aminoglycosides such as neomycin,[8] fortimicin A[9] and amikacin (Scheme 5).[10] The derivatisation of fortimicin A with FDNB is carried out in 1 % potassium phosphate buffer, pH 9.0 and is complete in 15 minutes at 85°C.



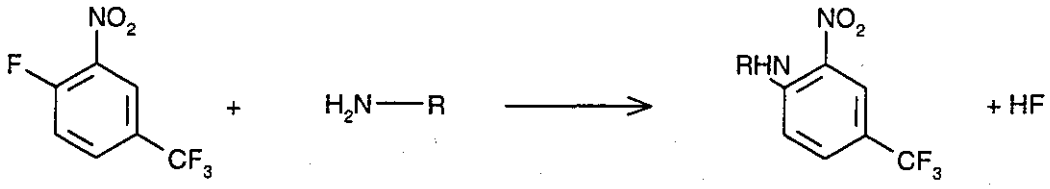
Scheme 5. The reaction of 1-fluoro-2,4-dinitrobenzene with amines

2,4,6-Trinitrobenzenesulphonic acid (TNBS) has been used for the derivatisation of aminoglycosides in biological fluids, for example, amikacin[11] and tobramycin[12] in serum (Scheme 6). In order to obtain a single *N*-trinitrophenyl derivative in quantitative yield a large excess of TNBS dissolved in acetonitrile, a temperature above 70°C at pH 9.5 - 10.0 and a reaction time of not less than 30 minutes are required. An advantage of TNBS is its selectivity because it reacts with amines preferentially over hydroxyl groups.



Scheme 6. The formation of *N*-trinitrophenyl derivatives by reaction of trinitrobenzenesulphonic acid with amines

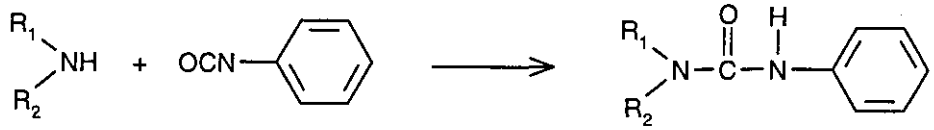
4-Fluoro-3-nitrobenzotrifluoride (FNBT)[13] has been used for labelling diamines and polyamines such as putrescine, spermidine and spermine to give the nitrotrifluoromethylphenyl derivatives (Scheme 7). FNBT does not, however, react with secondary amines or molecules containing polar groups such as amino acids.



Scheme 7. The reaction of 4-fluoro-3-nitrobenzotrifluoride with amines

3.2.4 Isocyanates and Isothiocyanates

Phenyl isocyanate reacts with aliphatic and aromatic primary and secondary amines to yield *N,N*-disubstituted ureas (Scheme 8).[14] The reaction is quantitative and completed in a few minutes. The derivatisation is carried out in *N,N*-dimethylformamide and an aliphatic alcohol added to destroy the excess of reagent. The detection limit by reverse phase HPLC has been determined to be 1-10 ng.



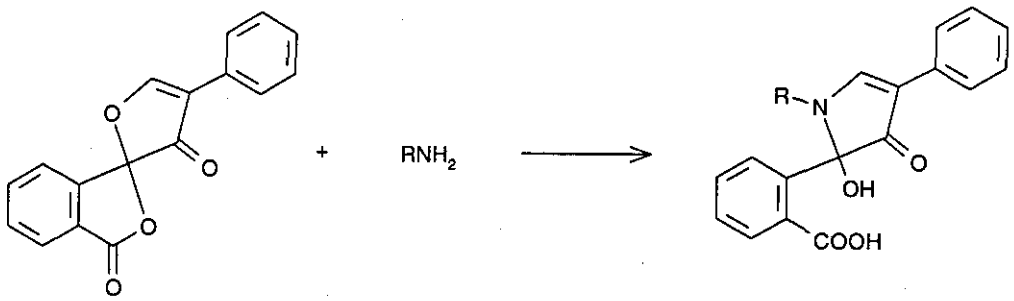
Scheme 8. The reaction of phenyl isocyanate with amines

Isothiocyanates are widely used for derivatising primary amines. They possess higher selectivity and are less reactive towards water, hydroxyl and carboxylic groups compared with isocyanates.[15-17] Labelling with phenyl isothiocyanate (PITC) or naphthyl isothiocyanate (NITC) has been used for the determination of C3-C10 alkylamines in ethanol.[18]

The derivatisation of 6-amino-2-methyl-2-heptanol (heptaminol) with 4-*N,N*-dimethylaminoazobenzene-4-isothiocyanate has been used for the HPLC determination of this amino compound in pharmaceutical preparations.[19,20] Labelling with *p*-phenylbenzoyl isothiocyanate[21] is, also, a good technique for the HPLC determination of amines.

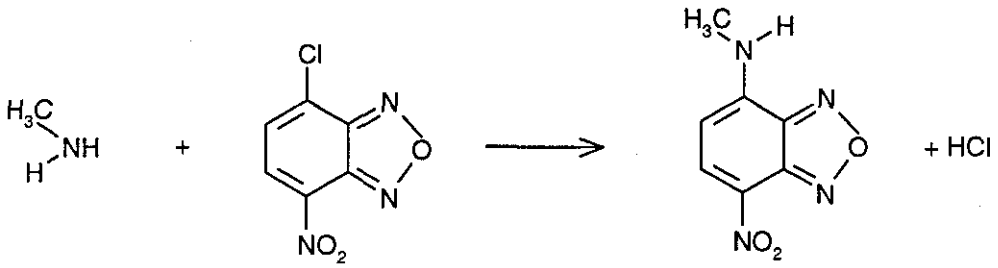
Other chromogenic derivatising agents of amines include the use of dansyl chloride (Dns-Cl) for derivatising amino acids. These derivatives are usually detected fluorimetrically, however, the fluorescence is quenched by phosphate ions and the derivatives have been detected using UV detection at 250 nm.[22] The reaction between *o*-phthaldehyde and primary amines has been employed to detect amino acid derivatives by UV absorption at 340 nm[23] and ninhydrin has been used as a post-column derivatising reagent in the automatic analysis of amino acids.[24]

With increasing availability of suitable fluorometric detectors, fluorogenic derivatisation is, also, becoming increasingly applied. Fluram or fluorescamine is a non-fluorescent compound which reacts with primary amino acid groups to form highly fluorescent pyrrolidone derivatives (Scheme 9). The reaction occurs virtually instantaneously with excess reagent hydrolysed to a non-fluorescent water soluble product.[25-29]



Scheme 9. Reaction of Fluram with a primary amine to form a pyrrolidone

4-Chloro-7-nitro-benzo-2,1,3-oxadiazole (NBD-Cl) reacts with primary and secondary aliphatic amines to produce intensely fluorescent derivatives (Scheme 10).[30-31] The reagent is selective since although reaction occurs with anilines and phenols, the derivatives are only weakly fluorescent.



Scheme 10. NBD-Cl reacts with primary and secondary aliphatic amines to produce intensely fluorescent derivatives

Tertiary aliphatic amines are, as aforementioned, rather difficult to detect, but they can be detected by UV detection at 254 nm after quaternisation with *p*-nitrobenzyl bromide (*p*-NBBr).[32] Pilocarpine and isopilocarpine have been derivatised in this way. Tertiary amines have, also, been analysed by post-column derivatisation using 1 % citric acid in acetic anhydride at 120°C.[33] There are far fewer methods available for the derivatisation of tertiary amines, however, than there are for primary and secondary amines. A reagent which would derivatise these compounds rapidly and in high yield would, therefore, be highly advantageous.

3.3 Results and Discussion

3.3.1 SB202026

As mentioned in chapter 1, SB202026 is a compound currently under development at SmithKline Beecham for the treatment of Alzheimer's disease. It has poor UV absorption and is present in tablets at levels below 20 µg. The main part of the molecule is a quinuclidine ring which, being a tertiary amine, is difficult to detect at low levels and to derivatise, as detailed in the introduction to this chapter. In an attempt to improve the detection limits currently achieved with this compound using conventional HPLC procedures with UV detection, an investigation into its electrochemical behaviour was carried out.

The variation of peak potential and peak height with pH was recorded on a polarograph using a glassy carbon electrode to determine the optimum conditions for the oxidative electrochemical analysis of SB202026 (Table 2).

Table 2

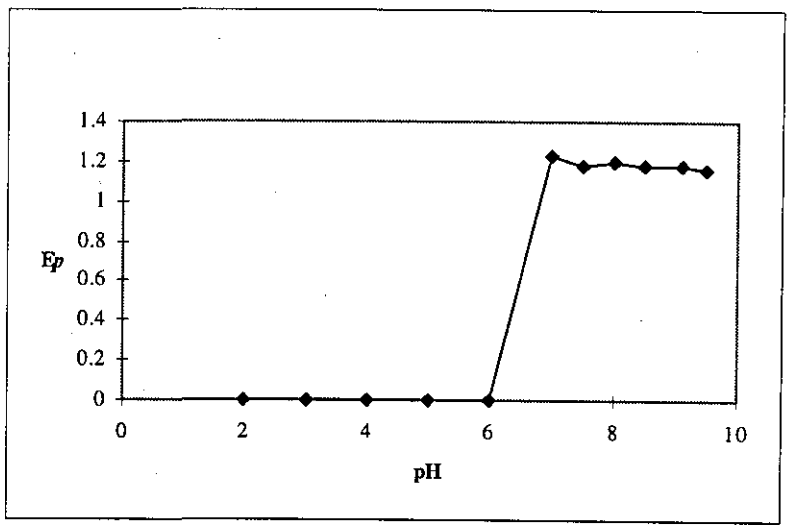
pH	Peak Potential (V)	Peak Height (mm)
2	Not Detected (ND)	Not Detected (ND)
3	ND	ND
4	ND	ND
5	ND	ND
6	ND	ND
7	1.23	62
7.5	1.18	62
8.0	1.20	136
8.5	1.18	140
9.1	1.18	160
9.5	1.16	170

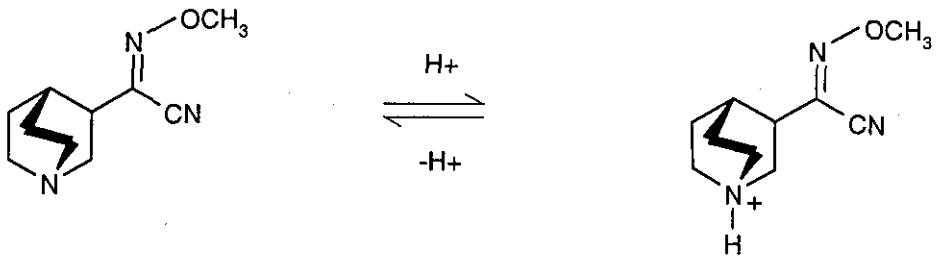
It was noted that the optimum pH for electrochemical detection of SB202026 would be pH 9.5. The peak shape achieved for the analysis even at pH 9.5, however, was not ideal and it would have been preferable to carry out HPLC with electrochemical detection. Unfortunately, chromatography is very difficult above pH 7.0 due to destruction of column packing and eluents of this pH could not be used routinely. Columns capable of withstanding higher pH were examined but were unsuccessful.

A pH of 7.0 is, therefore, the highest routine pH that could be used for HPLC with oxidative electrochemical detection. However, a relatively weak response was observed at this pH and 1.2 V is at the limit of the glassy carbon electrode under basic conditions.

What was interesting to note was that a plot of E*p versus* pH (Figure 1) gave a clear indication of the pKa of SB202026 and showed the electroactive species to be solely the unprotonated form, as would be expected (Scheme 11). The actual pKa of SB202026 has been determined to be 8.0 using a titration method.

Figure 1. E*p vs* pH for SB202026





unprotonated species
electroactive

protonated species
electroinactive

Scheme 11

Due to the very high peak potential of SB202026 and the difficulties involved with oxidative voltammetry it was decided to try and use DMD as a derivatising agent to form the *N*-oxide of SB202026 which may be easier to detect using reductive voltammetry. Direct UV detection of the *N*-oxide was inappropriate due to the fact that the *N*-oxide was determined to be less UV active than the drug itself. A relative response factor of 0.65 was recorded.

Attempts to detect the *N*-oxide of SB202026 by voltammetry on a carbon electrode or with a gold electrode were, however, unsuccessful. The literature suggests that a mercury amalgam with a gold electrode may need to be used in order to detect *N*-oxides using electrochemistry.[34] The necessity for the presence of mercury was confirmed when no response was observed with a glassy carbon electrode but the reduction of the *N*-oxide was readily observed using the dropping mercury electrode. Although attempts to detect the *N*-oxide on a gold electrode amalgamated with mercury were successful using the polarograph, detection by HPLC using a corresponding electrode proved unsuccessful in our laboratories. Although there may be effects from the moving eluent it was likely that other instrumental factors were involved and the detection by HPLC was not pursued further.

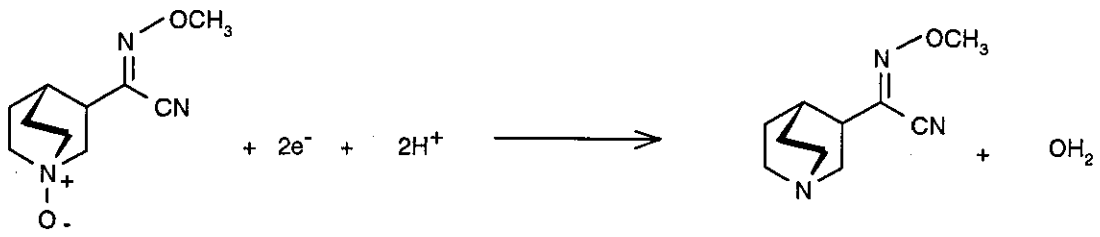
Although a voltammetric HPLC method was unsuccessful the ability to detect the *N*-oxide using polarography still provided a method of detecting the *N*-oxide at lower levels than was possible with the parent compound. The yield of the derivatisation of SB202026 to the *N*-oxide using DMD was determined to be in excess of 95 %.

In order to determine the optimum conditions for reductive polarographic analysis the variation of electrode potential and peak height with pH of SB202026-*N*-oxide was measured (Table 3).

Table 3. Peak potential and peak height *vs* pH for reductive polarographic detection of SB202026-*N*-oxide

pH	Peak Potential (V)	Peak Height (mm)
2	-0.85	87
3	-0.97	98
4	-1.36	87
5	-1.34	37
6	-1.31	30
7	-1.34	51
8	-1.34	57
9	-1.34	57

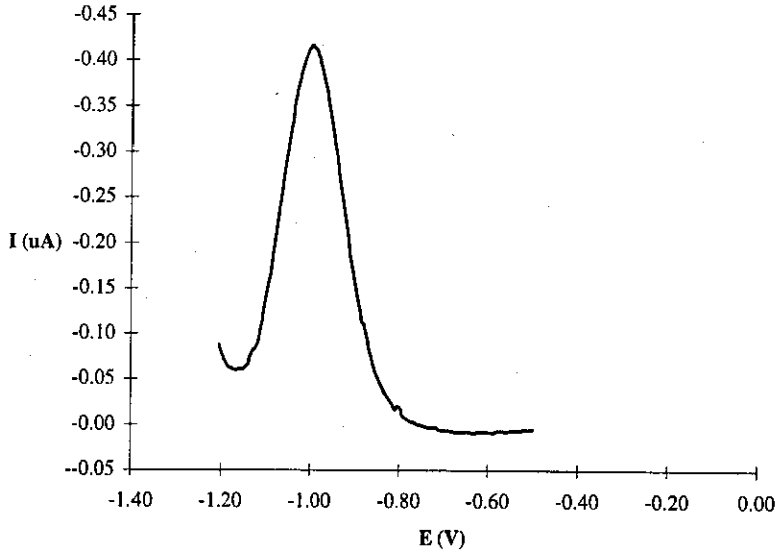
The polarographic reduction of SB202026-*N*-oxide probably proceeds *via* a 2 electron process as shown in Scheme 12.



Scheme 12. Polarographic reduction of SB202026-*N*-oxide

The optimum pH, in terms of maximum response, for polarographic analysis of SB202026-*N*-oxide was pH 3.0. Accordingly SB202026 was derivatised with DMD and the *N*-oxide analysed by polarography (Figure 2). The *N*-oxide was detectable at concentrations of 1 µg/ml although by increasing the pulse amplitude from 20 mV to 100 mV a 5.0 ng/ml solution could be detected.

Figure 2. Differential Pulse Polarogram of SB202026-*N*-oxide (10 µg/ml @ 20 mV pulse height



The polarographic method was validated and the following data obtained. The derivatisation was, also, successful by simply crushing a tablet, extracting the

drug and adding DMD. A limit of detection of 1.2 μg/ml was calculated from the linearity plot by conventional statistical means.

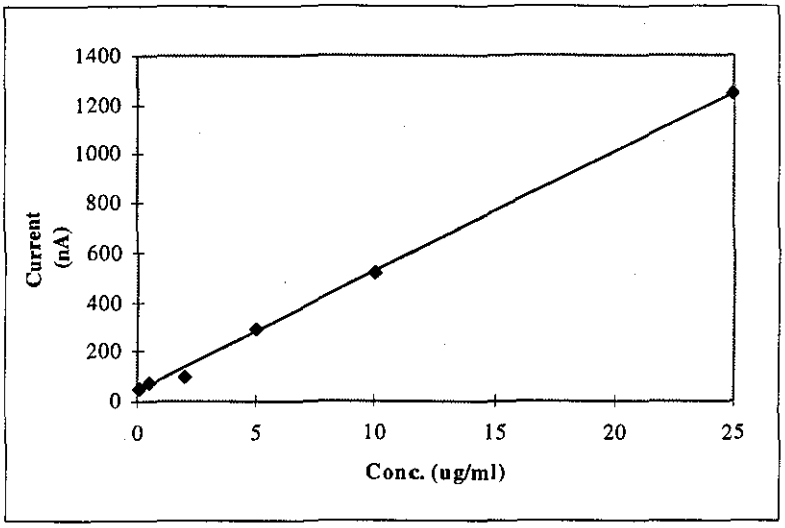
Linearity

In the range from 0.05 to 25 μg/ml a good linearity was observed for the peak current *versus* concentration relationship, a correlation coefficient of 0.9993 was achieved (Table 4, Figure 3).

Table 4. Method performance for SB202026 derivatisation - Linearity

Concentration (μg/ml)	Current (nA)
0.05	50
0.50	75
2.00	100
4.99	290
10.00	520
24.94	1250
Correlation coefficient	**0.9993**
Slope	**48.6**
Intercept	**36.8**

Figure 3. Plot of current *vs* concentration for SB202026 derivatisation with polarographic detection



Derivatisation Efficiency

The derivatisation efficiency of DMD with respect to the oxidation of SB202026 was demonstrated by oxidising 75 - 125 % of the nominal concentration, Table 5.

Table 5. Method performance for the derivatisation of SB202026 - Accuracy

Mass of SB202026 (mg)	Conc. SB202026 added expressed as *N*-oxide (µg/ml)	Conc. SB202026 *N*-oxide determined (µg/ml)	% Recovery	Mean
3.75	8.2	8.2	100	101
	8.2	8.3	101	
5.00	10.9	11.0	101	101
	10.9	11.0	101	
6.25	13.6	13.6	100	101
	13.6	13.8	101	

Precision

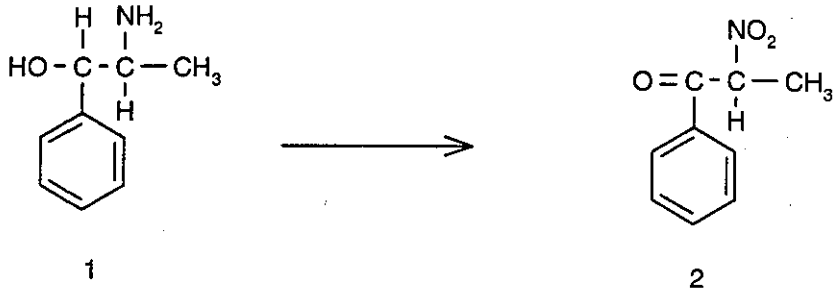
The derivatisation was carried out on six tablets to produce method repeatability data and two standard solutions injected six times to give the system repeatability data which are summarised in Table 6.

Table 6. Method performance for the derivatisation of SB202026 - Precision

	System Repeatability		**Method Repeatability**
Units	Current (nA)		µg/tablet
Concentration of SB202026	0.5 µg/ml	10.0 µg/ml	2.5 µg/ml (nominally 125 µg tablet)
	74	345	123.3
	75	343	122.6
	71	344	122.5
	73	345	121.8
	75	346	123.3
	72	342	123.4
Mean	**73**	**344**	**122.8**
s	**1.6**	**1.47**	**0.63**
RSD (%)	**2.2**	**0.43**	**0.50**

The good RSD data shown in Table 6 demonstrates the use of DMD as an efficient and effective derivatising agent of a tertiary amine with poor UV activity. The derivatisation is achieved quickly and simply and utilises DMD's ability to oxidise tertiary amines to amine oxides. The product obtained has good polarographic activity and can be detected at lower concentrations than the parent compound.

3.3.2 Phenylpropanolamine Hydrochloride

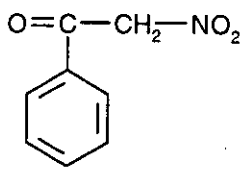


1 2

Scheme 13

Phenylpropanolamine hydrochloride (norephedrine) (**1**) is a compound which is prescribed for the symptomatic relief of nasal congestion. It has no strongly absorbing UV chromophore and detection is difficult at low doses.

Treatment of norephedrine with DMD gave α-benzoylnitroethane (**2**) as evidenced by mass spectrometry MS(FAB); m/z 179 [(M·+) , 30%] (Scheme 13). When the product was analysed using polarography, in pH 7.0 phosphate buffer, two peak potentials were observed at -720 mV and -1232 mV. For further confirmation of the structure of the product, polarographic analysis of benzoylnitromethane (**3**), similar in structure to **2**, was carried out under the same conditions and, also, produced two peak potentials at -648 mV and -1068 mV.



3

Benzoylnitromethane (**3**) can be used to confirm the structure of the product of phenylpropanolamine and DMD because polarographic determination by itself is not generally specific for a particular organic compound. Usually the

determination is, in practice, of a particular electroactive group and hence a class of compounds, for example, nitro or ketone.

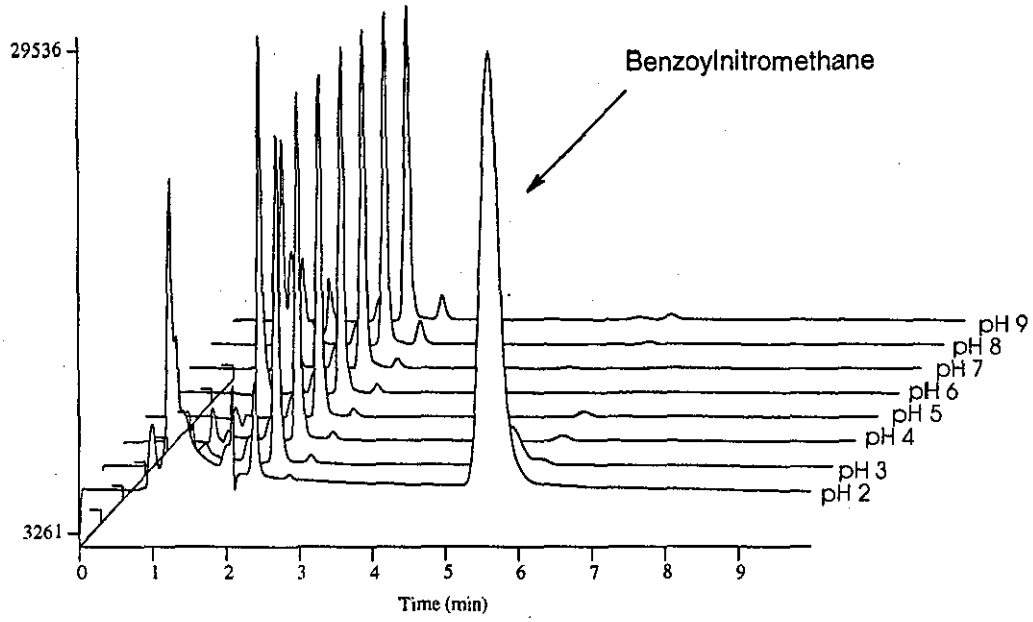
Phenylpropanolamine (**1**) and oxidation product (**2**) with DMD were, also, analysed by HPLC. On reaction with DMD no trace of phenylpropanolamine was found in the sample solutions and the product of the reaction had the same λ_{max} of 250 nm as benzoylnitromethane. A fluorescence examination, also, showed the two compounds as having an identical emission maxima at 353 and 359 nm respectively.

During polarographic experiments it was observed that if the benzoylnitromethane solutions were left for two days and reanalysed a change in the peak profile occurred. This was possibly due to the instability of benzoylnitromethane in solution and was, also, observed for the product of phenylpropanolamine and DMD. It was, therefore, decided to monitor the stability of benzoylnitromethane in solution using chromatography, the stability after 1 month at pH 2 - 9 is illustrated in Figure 4.

Since the loss of benzoylnitromethane (**3**) was so rapid above pH 2 it was decided to carry out a short term (2 days) solution stability study. Literature suggests[35] that α-nitroketones will degrade to the corresponding carboxylic acid, in this case benzoic acid.

$$R^1COCHR^2NO_2 \quad \rightarrow \quad R^1CO_2H + R^2CONHOH$$

Figure 4. Stability of benzoylnitromethane (3) in solution after 1 month



For the duration of the study, therefore, the level of benzoic acid was, also, monitored. The results of which are summarised in Tables 7 & 8.

Table 7. Stability of benzoylnitromethane (3) in solution after 2 days

pH	Benzoic acid (µg/ml)*	Benzoyl nitromethane (BNM) (µg/ml)	Accountance of material	% Initial BNM
2	3.9	97.5	101.4	97
3	23.9	73.0	96.9	73
4	72.4	10.9	83.3	11
5	80.6	0.0	80.6	0
6	60.3	0.0	60.3	0
7	34.9	1.8	36.7	2
8	25.9	3.6	29.5	4
9	23.9	5.2	29.1	5

Table 8. Stability of benzoylnitromethane (3) in solution after 5 days

pH	Benzoic acid (μg/ml)*	Benzoyl nitromethane (BNM) (μg/ml)	Accountance of material	% Initial BNM
2	6.4	95.3	101.7	95
3	43.1	48.0	91.1	48
4	81.5	0.5	82.0	0.5
5	81.0	0.0	81.0	0
6	60.7	0.0	60.7	0
7	35.1	0.0	35.1	0
8	25.3	0.0	25.3	0
9	27.3	0.1	27.4	0.1

* Benzoic acid adjusted for molecular weight difference and expressed as equivalent BNM.

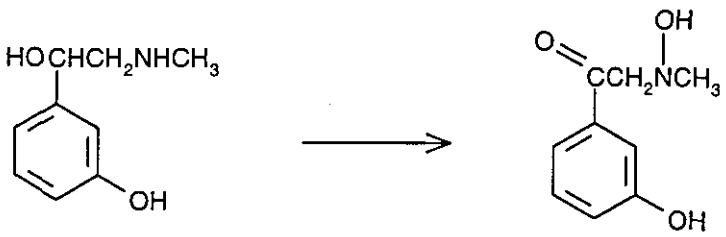
The data indicated the instability of benzoylnitromethane (**3**) in basic solution. It was observed that up to pH 6 the concentration of benzoic acid increased as the concentration of benzoylnitromethane (**3**) decreased, however, above pH 6 a different pathway of degradation seems to be occurring. α - Benzoylnitroethane (**2**), also, showed identical instability but at a much slower rate presumably due to the lower number of α-hydrogen atoms present. It has been shown that the α-hydrogen dissociates readily in nitrocompounds.[36]

Analysis of α-benzoylnitroethane (**2**) by HPLC with UV detection provided a good means of detection due to the products high UV activity. DMD has, therefore, converted an otherwise poor UV absorbing parent molecule into a

strongly absorbing product. Having shown the potential of DMD to derivatise phenylpropanolamine reference material it was decided to attempt to derivatise a tablet containing phenylpropanolamine hydrochloride. Sinutab, sold by Warner Wellcome Consumer Healthcare, contains phenylpropanolamine at a level of 12.5 mg per tablet. However, the tablet, also, contains 500 mg of paracetamol which impeded the derivatisation by as much as 50 %. This may be due to DMD reacting with paracetamol itself, the oxidation of phenols by DMD being well documented.[37]

The derivatisation was tried on other products which contain phenylpropanolamine including Eskornade spansules, Dimotapp tablets and elixir but all proved unsuccessful due to successful competition for DMD from other components in the formulations. During the derivatisation of the Dimotapp products an additional peak was observed in the chromatograms. It was thought that this additional peak could be the product of DMD reacting with phenylephrine which is another active ingredient in Dimotapp products.

3.3.3 Phenylephrine Hydrochloride



Scheme 14

Phenylephrine hydrochloride is a compound which is used as a nasal decongestant in rhinitis and sinusitis. It is used in ophthalmology as a mydriatic and conjunctival decongestant. Reaction of DMD with phenylephrine hydrochloride present in Minims eye drop solution, manufactured by Chauvin Pharmaceuticals

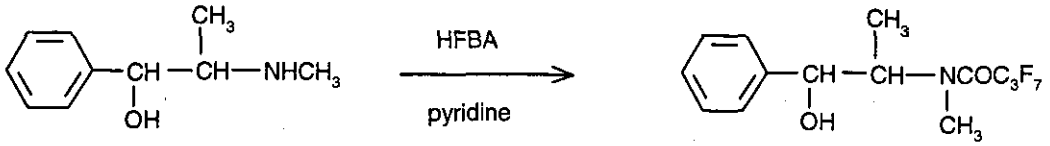
Ltd, gave 96 % conversion to the product shown in Scheme 14, compared with a standard solution when monitored by HPLC, MS(FAB); m/z 182 [(M.+), 25 %].

When the product was analysed by polarography, in pH 7.0 phosphate buffer, two peak potentials were observed at -0.9 V and -1.3 V. For further confirmation of the structure of the product the peak potentials of acetophenone and *N,N*-dimethylhydroxylamine were recorded under the same conditions and produced peaks at -1.4 V and -1.0 V respectively. The evidence is, therefore, conclusive that DMD oxidises both the hydroxy and secondary amine group of phenylephrine during the derivatisation.

Although the derivatisation was successful this molecule already has acceptable UV activity without derivatisation due to the conjugation of the hydroxy group on the ring.

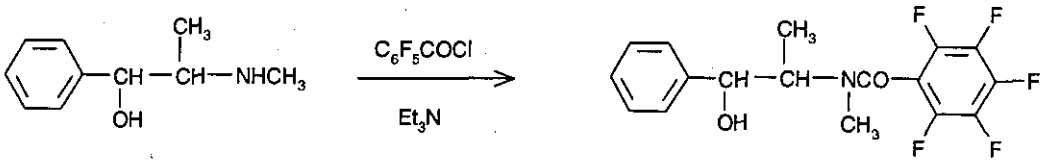
Ephedrine, however, has a similar structure to phenylephrine but does not have the hydroxy group on the ring. Derivatisation of this compound would, therefore, provide a means of achieving lower detection limits.

Ephedrines are particularly difficult compounds to detect at low concentrations. Examples of derivatisations of ephedrines are referred to in the literature using electron capture detection with gas chromatography (ECD-GC). Heptafluorobutyric anhydride (HFBA) in the presence of pyridine has been used to derivatise pseudoephedrine in plasma samples (Scheme 15).[38]



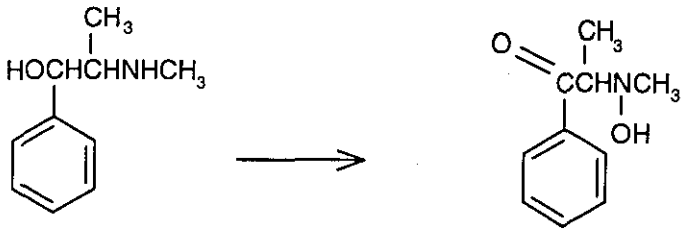
Scheme 15. Derivatisation of ephedrine using HFBA

Another method used to detect ephedrines by ECD-GC is derivatisation with pentafluorobenzoyl chloride in the presence of triethylamine (Scheme 16).[39]



Scheme 16. Derivatisation of ephedrine using pentafluorobenzoyl chloride

3.3.4 Ephedrine Hydrochloride



Scheme 17

Ephedrine hydrochloride is present in CAM syrup at a concentration of 4 mg/5 ml. It is manufactured by Rybar Laboratories and is a children's antispasmodic mixture. Reaction of DMD with ephedrine hydrochloride in CAM syrup gave 98 % conversion to the product shown in Scheme 17 compared with a standard solution when monitored by HPLC, MS(FAB); m/z 179 [(M·+) , 35 %].

When the product was analysed by polarography, in pH 7.0 phosphate buffer, two peak potentials were observed at -0.9 V and -1.4 V. The peak potentials of

acetophenone and *N,N*-dimethylhydroxylamine previously recorded under the same conditions produced peaks at -1.4 V and -1.0 V respectively.

Due to the increased UV activity of the product compared to the starting material it was considered a worthwhile exercise to validate this derivatisation procedure.

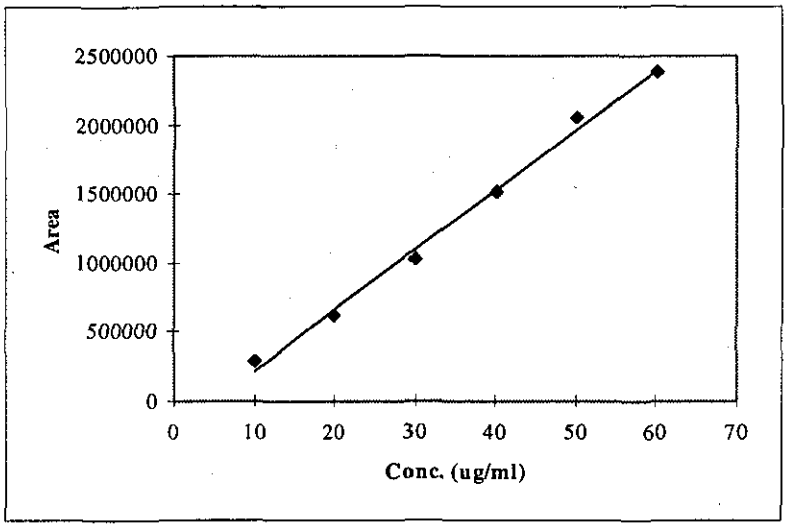
Linearity

In the range from 10.02 to 60.11 µg/ml the peak area *versus* concentration data gave a correlation coefficient of 0.997 as shown in Table 9. A limit of detection of 4.8 µg/ml was calculated from the linearity plot by conventional statistical means (Figure 5).[40]

Table 9. Method performance for the derivatisation of ephedrine - Linearity using 2 ml of DMD solution

Concentration (µg/ml)	Area
10.02	289795
20.04	615229
30.05	1025441
40.07	1510305
50.09	2054388
60.11	2385975
Correlation coefficient	**0.997**
Slope	**43589.3**
Intercept	**-214864.9**

Figure 5. Plot of area *vs* concentration for ephedrine derivatisation by HPLC with UV detection



Derivatisation efficiency

The following data was obtained using a single standard at the 100 % level (Table 10) and was intended to demonstrate the effectiveness of DMD as a derivatising agent of ephedrine.

Table 10. Single standard accuracy

Mass of Ephedrine added (mg)	**Mass of Ephedrine determined (mg)**	**% Recovery**	**Mean**
3.01	2.68	89	89
	2.68	89	
4.02	4.01	100	100
	4.02	100	
5.03	5.30	105	107
	5.40	109	

The results, however, indicated a problem with the linearity when using a single standard at the nominal working concentration of 0.04 mg/ml. Due to the large negative intercept of the linearity plot the amount of ephedrine would be underestimated or overestimated depending on whether the analytical sample concentrations were greater than or less than the analytical standard concentration. The accuracy experiment was, therefore, repeated using three standards one at each of the 75, 100 and 125 % ephedrine concentration level (Table 11).

Table 11. Accuracy repeated with three standards

Mass of Ephedrine added (mg)	**Mass of Ephedrine determined (mg)**	**% Recovery**	**Mean**
3.00	2.87	96	98
	3.01	100	
4.01	3.92	98	98
	3.90	97	
5.01	4.82	96	99
	5.08	101	

The above data show that good accuracy is achieved if a standard is used at the same concentration as the samples which again highlights the non-linearity of the derivatisation.

Investigation into non-linearity

It was initially thought that the non-linearity may be a result of some aspect of the HPLC equipment as experienced by other analysts.[41] For this reason the linearity was repeated using a separate technique, in this case polarography.

However, when the current *versus* concentration data was plotted a large negative intercept was again recorded, indicating that the non-linearity was not due to the HPLC procedure.

When the initial linearity experiment was carried out the same amount of DMD solution (2 ml) was used in each sample over the 10 to 60 μg/ml concentration range. It was thought that perhaps the different DMD to ephedrine ratio was somehow causing a problem with the linearity. It appears that when the DMD to ephedrine ratio is large, less of the product is being detected than when the ratio is smaller. The linearity was, therefore, repeated using the same ratio of DMD to ephedrine over the entire concentration range. The data is tabulated in Table 12 and graphically presented in Figure 6.

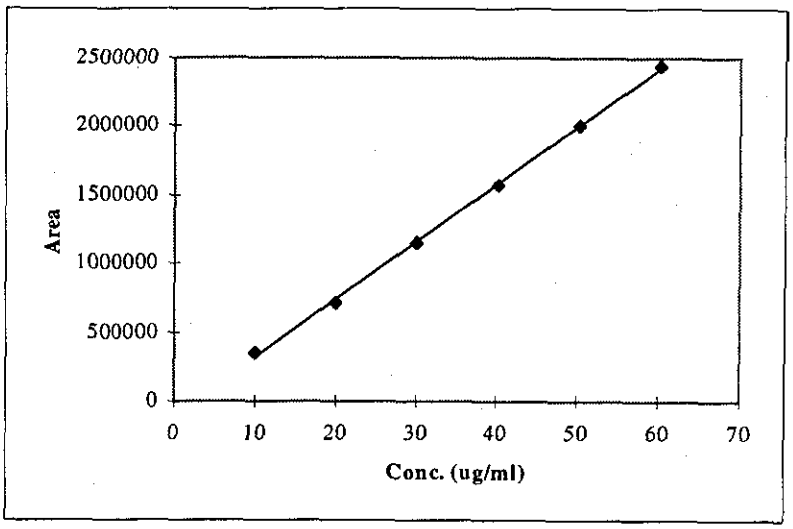
Linearity repeated

In the range from 10.01 to 60.09 μg/ml the peak area *versus* concentration data gave a correlation coefficient of 0.9997. A limit of detection of 1.6 μg/ml was calculated from the linearity plot by conventional statistical means.[40]

Table 12. Linearity with constant DMD:ephedrine ratio

Concentration (μg/ml)	Area
10.01	343630
20.03	717912
30.04	1145129
40.06	1568254
50.07	2009813
60.09	2445629
Correlation coefficient	**0.9997**
Slope	**42245.7**
Intercept	**-108983.6**

Figure 6. Plot of area *vs* concentration for ephedrine derivatisation by HPLC with UV detection (constant ratio of DMD:ephedrine)



It is seen that by keeping the DMD to ephedrine ratio constant the line is much more linear and the negative intercept is halved. It is necessary, therefore, to ensure that when the derivatisation is carried out the ratio of DMD to ephedrine is kept constant between the samples and the standards and that the samples and standards are at the same concentration. Alternatively, a conventional calibration curve can be generated avoiding the use of a single point standard. When this is so, good data will be achieved. It is probable that when a very large excess of dioxirane is used the product is being oxidised further, possibly resulting in the formation of a nitrone (see Scheme 2, Chapter 1).

Precision

The derivatisation was carried out on six 5 ml samples of CAM syrup to produce method repeatability data and a standard solution injected six times to give the system repeatability data which are summarised in Table 13.

Table 13. Method performance for the derivatisation of ephedrine - Precision

	System Repeatability	**Method Repeatability**
Units	Area	mg/5 ml
	1457772	3.90
	1455232	3.96
	1451853	3.91
	1449600	3.93
	1447363	3.94
	1450660	3.92
Mean	**1452080**	**3.93**
s	**3815.8**	**0.02**
RSD %	**0.26**	**0.6**

The use of DMD as an efficient and effective derivatising agent of a secondary amine and an alcohol with poor UV activity has again been demonstrated. This example illustrates DMD's ability to oxidise alcohols to ketones and secondary amines to hydroxylamines quickly and easily. The product obtained has improved UV activity and can be detected at lower concentrations than the parent compound.

DMD is an ideal reagent for chromogenic derivatisation because it is not necessary to remove excess reagent prior to the analysis to avoid "swamping" the detector with reagent absorption, as is the case for other derivatising agents.

3.4 Conclusions

In the case phenylpropanolamine, phenylephrine, ephedrine and SB202026 dimethyldioxirane has been shown to be an efficient and effective derivatising agent of a primary, secondary and tertiary amine. In each example it has produced a product which has an improved absorptivity over the parent compound and can, therefore, be detected at lower concentrations.

The main emphasis of this chapter has been on the derivatisation of amines, however, as the derivatisation of phenylpropanolamine and ephedrine have shown, the application may be extended to other chemical groups, for example, alcohols. Indeed the oxidising properties of DMD are so extensive that many different types of compounds could potentially be derivatised in this way. Also, the derivatisation process does not necessarily fix the method of analysis since the examples reported lend themselves to electrochemical and chromatographic methodology.

A further application could be in the drug metabolism and clinical analysis areas where the levels of compounds of interest are far lower than we have been concerned with here. The particular advantage of dioxirane solutions is that excess reagent and acetone can be easily removed from the samples by evaporation, unlike, many other derivatising agents which require complicated purification steps.

One disadvantage is that there may be competitive side reactions which can complicate the derivatisation process. An example of this was the presence of paracetamol in the Sinutab tablets.

3.5 Experimental

All HPLC equipment as in Chapter 2 (Section 2.5.1). All reagents were analytical grade and unless otherwise stated sourced from the Aldrich Chemical Company Ltd. Dimethyldioxirane solutions were prepared as described in Chapter 2 (Section 2.5.2).

The molarity of the dioxirane solution was determined by titration:
An acetic acid/acetone solution (3:2; 3 ml) and KI solution (10 % w/v; 5 ml) were charged to a conical flask, followed by the dioxirane solution (0.2 ml). The solution was titrated against a sodium thiosulphate solution (0.01 M).

Calculation:

$$\text{Molarity (dioxirane)} = \frac{\text{Vol. } Na_2S_2O_3 \text{ x Mol. } Na_2S_2O_3}{\text{Vol. (dioxirane) x 2}}$$

3.5.1 Experimental for Section 3.3.1

A HPLC marker of SB202026-*N*-oxide was obtained from Chemical Development at SmithKline Beecham. SB202026 was dissolved in acetone and reacted with two equivalents of DMD under ambient conditions. The solutions were allowed to stand for at least 10 minutes and analysed by HPLC.

The chromatography conditions for analysis of SB202026 and its *N*-oxide by HPLC with UV detection were as follows:
Column: Spherisorb S5 C1 15 cm x 4.6 mm
Eluent: 0.025 M $NaH_2PO_4.2H_2O$ pH 3.5 buffer solution & 0.1 % triethylamine
Flow rate: 2 ml/min
UV detection at 300 nm

The chromatography conditions for analysis of SB202026 and its *N*-oxide by HPLC with electrochemical detection were as follows:
Column: Hypersil Cyano 15 cm x 4.6 mm
Eluent: 0.025 M $NaH_2PO_4.2H_2O$ pH 7.0 buffer solution:MeCN 50:50 v/v
Flow rate: 1 ml/min
Electrochemical detection at + 1.2 V
A Shandon Base Deactivated Silica (BDS) column was evaluated to allow analysis at a higher pH but was unsuccessful.
The optimum potential for the electrochemical detection of SB202026 was determined by polarography using a glassy carbon electrode in place of the dropping mercury electrode and scanning over the range + 0.0 V to + 2.0 V.
The electrolyte for determining the optimum pH for polarographic detection was Britton Robinson buffer solutions (pH 2.0 - 9.5), 0.04 M in acetic acid, orthophosphoric acid and boric acid.

The chromatography conditions for analysis of SB202026 and its *N*-oxide by HPLC with UV detection and LC-MS were as follows:
Column: Spherisorb S5 C1 15 cm x 4.6 mm
Eluent: 0.025 M $NaH_2PO_4.2H_2O$ pH 3.5 buffer solution & 0.1% triethylamine
Flow rate: 1 ml/min
UV detection at 230 nm

The electrolyte for determining the optimum pH for polarographic detection was Britton Robinson buffer (pH 2 - 9).

Polarographic conditions were as follows:
The electrolyte used was Britton Robinson buffer solution adjusted to pH 3.0 with 1 M sodium hydroxide. Phosphate buffer solutions were pH 7.0 with a molarity of 0.05 M.

Initial potential: -0.5 V

Final potential: -1.4 V

Scan rate: 5 mV/sec

Large drop size

Scan increment: 4 mV

Step/drop time: 0.8 sec

Pulse height: 20 mV

Derivatisation Efficiency

Duplicate samples of a stock solution of SB202026 at a concentration of 0.252 mg/ml, were transferred by pipette (15, 20 and 25 ml) into 100 ml volumetric flasks to achieve concentrations of 8.2, 10.9 and 13.6 μg/ml. The weight of SB202026 in each flask corresponding to 3.75, 5.00 and 6.25 mg. Acetone (20 ml) and dimethyldioxirane solution (5 ml) were then added to each flask and the volume made up with distilled water. The solutions were allowed to stand for 10 minutes and 20 ml transferred by pipette into a 100 ml volumetric flask and the volume made up with pH 3.0 Britton Robinson buffer. The solutions were analysed by polarography against an *N*-oxide of SB202026 standard prepared at the Chemical Development laboratories of SmithKline Beecham Pharmaceuticals.

Method Performance Conditions

Precision

System repeatability:

Standard solutions of an *N*-oxide of SB202026 in pH 3.0 buffer solution (10.0 μg/ml) were measured six times and the peak current recorded.

Method repeatability:

Five tablets containing nominally 125 µg of SB202026, in a lactose based formulation, were placed in a volumetric flask (50 ml) and dissolved in pH 7.0 phosphate buffer solution (20 ml). Acetone (20 ml) and DMD solution (5 ml) were added and the solution allowed to stand for 10 minutes. The flasks were made up to volume with further phosphate buffer pH 7.0 and the resultant stock solution (5 ml) transferred into a volumetric flask (25 ml) and made up to volume with electrolyte solution pH 3.0. Six replicates were prepared in this way and analysed.

Quantitation

Sample solutions were quantified by reference to an externally prepared standard solution of the *N*-oxide of SB202026 at a concentration of 2.5 µg/ml in electrolyte solution. The content of SB202026 was calculated from the following formula:

$$\text{Content} = \frac{I_S}{I_R} \cdot C_R \cdot \frac{V_1}{V_2} \cdot \frac{V_3}{N} \ \mu g/\text{Tablet}$$

Where:

I_S = Sample peak current

I_R = Reference peak current

C_R = Reference concentration

V_1 = Working sample volume (25)

V_2 = Dilution factor (5)

V_3 = Stock sample volume (50)

N = Number of tablets (5)

3.5.2 Experimental for Section 3.3.2

Phenylpropanolamine hydrochloride (20 mg) was dissolved in a minimal amount of water (≈10 ml) and subsequently diluted in acetone (10 ml). Five equivalents of DMD were added under ambient conditions and the solution left for 15 minutes. The volume was made up using distilled water. The product obtained was analysed by mass spectrometry, polarography and HPLC.

The polarographic conditions were as follows:
Electrolyte: 0.05 M phosphate buffer solution pH 7.0
Initial potential: -0.5 V
Final potential: -1.4 V
Scan rate: 5 mV/sec
Large drop size
Scan increment: 4 mV
Step/drop time: 0.8 sec
Pulse height: 20 mV

HPLC conditions for detection of phenylpropanolamine:
Column: Spherisorb S5 C1 15 cm x 4.6 mm
Eluent: 0.05 M $NaH_2PO_4.2H_2O$ buffer solution pH 7.2:MeCN 85:15
Flow rate: 1 ml/min
UV detection at 210 nm

HPLC conditions for detection of product of phenylpropanolamine and DMD:
Column: Novapak C8 15 cm x 3.9 mm
Eluent: 0.05 M $NaH_2PO_4.2H_2O$ buffer solution pH 7.2:MeCN 80:20
Flow rate: 1 ml/min
UV detection at 250 nm

HPLC conditions for detection of benzoylnitromethane and benzoic acid:
Column: Novapak C8 15 cm x 3.9 mm
Eluent: 0.05 M $NaH_2PO_4.2H_2O$ buffer solution pH 3.0:MeCN 75:25
Flow rate: 1 ml/min
UV detection at 230 nm for first 10 minutes (detection of benzoic acid) followed by wavelength switching to 250 nm (detection of benzoylnitromethane).

Fluorescence examinations were carried out on a Perkin Elmer LS50B fluorescence spectrophotometer. λ_{ex} = 315 nm, λ_{em} = 353 and 359 nm (product of phenylpropanolamine and DMD and benzoylnitromethane respectively) for a 1 μg/ml solution.

Reaction of DMD with phenylpropanolamine in tablets was carried out by initially disintegrating a tablet in water, followed by addition of acetone and finally a ten fold excess of DMD, the increase in the excess of DMD was to allow for the more complicated matrix of the reaction. The spansules required grinding in a pestle and mortar prior to dissolution in water. The elixir was mixed directly with acetone. The solutions were diluted to the appropriate concentration for HPLC analysis using distilled water. A working concentration of 0.01 mg/ml was typically used.

3.5.3 Experimental for Section 3.3.3

Phenylephrine hydrochloride (20 mg) was dissolved in a minimal amount of water (≈10 ml) and subsequently diluted in acetone (10 ml). Five equivalents of DMD were added under ambient conditions and the solution left for 15 minutes. The volume was made up using distilled water. The product obtained was analysed by mass spectrometry, polarography and HPLC. For derivatisation of

the eye drop solution 0.3 ml of the solution was used in place of the drug substance above.

HPLC conditions for detection of product of phenylephrine and DMD:
Column: Novapak C8 15 cm x 3.9 mm
Eluent: 0.05 M $NaH_2PO_4.2H_2O$ buffer solution pH 3.0:MeCN 75:25
Flow rate: 1 ml/min
UV detection at 250 nm

The polarographic conditions were as follows:
Electrolyte: 0.05 M phosphate buffer solution pH 7.0
Initial potential: -0.5 V
Final potential: -1.4 V
Scan rate: 5 mV/sec
Large drop size
Scan increment: 4 mV
Step/drop time: 0.8 sec
Pulse height: 20 mV

3.5.4 Experimental for Section 3.3.4

Ephedrine hydrochloride (20 mg) was dissolved in a minimal amount of water (≈10 ml) and subsequently diluted in acetone (10 ml). Five equivalents of DMD were added under ambient conditions and the solution left for 15 minutes. The volume was made up using distilled water. The product obtained was analysed by mass spectrometry, polarography and HPLC. For derivatisation of the CAM syrup 5 ml of the syrup was taken in place of the drug substance.

HPLC conditions for detection of product of ephedrine and DMD:
Column: Novapak C8 15 cm x 3.9 mm

Eluent: 0.05 M $NaH_2PO_4.2H_2O$ buffer solution pH 3.0:MeCN 75:25

Flow rate: 1 ml/min

UV detection at 250 nm

The polarographic conditions were as follows:

Electrolyte: 0.05 M phosphate buffer solution pH 7.0

Initial potential: -0.5 V

Final potential: -1.4 V

Scan rate: 5 mV/sec

Large drop size

Scan increment: 4 mV

Step/drop time: 0.8 sec

Pulse height: 20 mV

Derivatisation Efficiency

Duplicate samples of a stock solution of ephedrine at a concentration of 0.201 mg/ml, were transferred by pipette (5, 10 and 15 ml) into 100 ml volumetric flasks each containing CAM syrup (2.5 ml, 4.0 mg per 5 ml). The weight of ephedrine in each flask corresponding to 3.00, 4.01 and 5.01 mg. Acetone (10 ml) and dimethyldioxirane solution (2 ml) were then added to each flask and the volume made up with distilled water. The solutions were allowed to stand for 10 minutes and analysed by HPLC against standards of derivatised ephedrine drug substance at the nominal concentration (0.02 mg/ml) and, also, at 3 different concentrations (0.015, 0.02 and 0.025 mg/ml).

Method Performance Conditions

Precision

System repeatability:

Standard solutions of derivatised ephedrine (40 µg/ml) were measured six times and the peak area recorded.

Method repeatability:
CAM syrup (5 ml) containing nominally 4 mg per 5 ml of ephedrine was placed in a volumetric flask (100 ml), acetone (10 ml) and DMD solution (2 ml) were added and the solution allowed to stand for 10 minutes. The flasks were made up to volume with distilled water. Six replicates were prepared in this way and analysed by HPLC.

Quantitation

Sample solutions were quantified by reference to an externally prepared standard solution of derivatised ephedrine at a concentration of 40 µg/ml. The content of ephedrine was calculated from the following formula:

$$\text{Content} = \frac{I_S . C_R . V_1}{I_R} \quad \text{mg/5 ml}$$

Where:

I_S = Sample peak current
I_R = Reference peak current
C_R = Reference concentration
V_1 = Working sample volume (100)

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ADDENDUM

Differential Pulse Polarographic Determination of [*R*-(*Z*)]-α-(methoxyimino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile Monohydrochloride in Tablets Following Derivatization With Dimethyldioxirane

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Dimethyldioxirane was used as a derivatizing agent to convert [*R*-(*Z*)]-α-(methoxyimino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile monohydrochloride (I) to the corresponding quinuclidine *N*-oxide which was then determined by differential pulse polarography in the presence of common excipients. The polarographic method yielded an LOD of 1.2 μg ml^{-1} calculated from the linearity plot by conventional statistical means. However, by increasing the pulse amplitude to 100 mV, a 5.0 ng ml^{-1} solution could be detected. This constituted an approximate eight-fold improvement over conventional HPLC procedures. The method was suitable for dosage forms containing I at levels as low as 100 ppm. Samples were extracted with phosphate buffer solution of pH 7.0, diluted into Britton Robinson buffer solution of pH 3.0 and the polarographic response recorded at a peak potential of −0.97 V. The method was linear over the sample concentration range of 0.05 to 25 μg ml^{-1} with r = 0.9993. The precision for the determination of I at the 100 ppm level was 0.5%.

New drug candidates are increasingly of high potency and low dose. When combined with poor spectroscopic properties the analysis of formulated products containing such drugs presents a significant challenge. Typical of this class of compound is [*R*-(*Z*)]-α-(methoxyimino)-1-azabicyclo[2.2.2]octane-3-acetonitrile monohydrochloride (I; Fig. 1) which has a modest UV response (A^{1}_{1} = 260, λ = 232 nm) and requires formulated product dosages in the microgram range. Good sensitivity can be attained by HPLC but large injection volumes are required. Furthermore HPLC currently provides the only means of product identification and there is, therefore, a requirement for an independent means of routine qualitative analysis.

It is common practice in analytical chemistry to increase sensitivity or specificity by derivatization and this has been particularly successful for primary and secondary amines. Tertiary amines such as I are not so readily derivatized but it is conceptually possible to oxidize the quinuclidine moiety to the corresponding *N*-oxide and render the molecule susceptible to reductive electrochemical analysis. Such a derivatization was considered desirable following the failure to determine the compound directly by anodic oxidation. In principle the electron pair on the quinuclidine nitrogen atom is available for anodic oxidation using a solid electrode such as glassy carbon and applied potentials in the range 0–1.0 V. In practice the peak oxidation potential of I proved to be close to the electrode limit and severely limited the sensitivity of the technique. Reductive polarography of the *N*-oxide of I would be expected to occur in a potential region well clear of electrode or supporting electrolyte responses.

Dimethyldioxirane (DMD) possesses advantageous properties as a derivatizing agent in that it is volatile and hence easily removed from the sample matrix. It is also highly reactive and has been shown to carry out a wide variety of oxygen transfer reactions frequently in high yield.[1,2]

It was the aim of this work to examine the feasibility of this concept using the highly strained peroxide DMD as the derivatizing agent and a differential pulse polarographic method to analyse the resultant *N*-oxide (Fig. 2). Differential pulse polarography was selected in preference to other forms of polarography due to its high sensitivity and ease of quantitation.

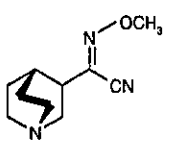

Fig. 1 Chemical Structure of [*R*-(*Z*)]-α-(methoxyimino)-1-azabicyclo-[2.2.2]octane-3-acetonitrile monohydrochloride (I).

Experimental

Reagents and Instrumentation

All reagents were of analytical-reagent grade. The polarographic equipment consisted of a Princeton Applied Research (PAR) polarographic analyser in conjunction with a PAR 303 polarographic detector. The polarographic detector was operated under the following conditions: initial potential, −0.5 V; final potential, −1.4 V; scan rate, 5 mV s^{-1}; large drop size; scan increment, 4 mV; step–drop time, 0.8 s; pulse height, 20 mV. The electrolyte used was Britton Robinson buffer (0.04 mol l^{-1} in acetic acid, orthophosphoric acid and boric acid), adjusted to pH 3.0 with 1 mol l^{-1} sodium hydroxide. Phosphate buffer solutions were pH 7.0 with a molarity of 0.05 mol l^{-1}.

Preparation of Dimethyldioxirane

A round-bottom flask was equipped with an efficient mechanical stirrer and a solids addition funnel, connected by means of

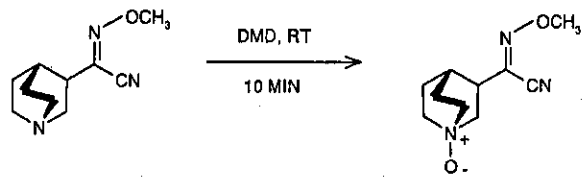

Fig. 2 Derivatization of I with DMD.

a U-tube to a receiving flask which was cooled to −78 °C by means of a dry ice–acetone bath. The reaction flask was charged with an aqueous EDTA solution, 4.3 mmol l^{-1} (300 ml), distilled acetone (210 ml) and sodium hydrogen carbonate (160 g). While vigorously stirring, solid oxone (potassium caroate; 320 g) was added slowly over a period of approximately 1 h. The DMD–acetone distillate (60 ml) was collected in the cooled (−78 °C) receiving flask.

The molarity of the DMD solution was determined by titration. Acetic acid–acetone solution (3+2; 3 ml) and KI solution (10% m/v; 5 ml) were charged to a flask, followed by the DMD solution (0.2 ml). This solution was titrated against a sodium thiosulfate solution (0.01 mol l^{-1}). The DMD molarities obtained were in the range 0.08–0.1 mol l^{-1}.

Derivatization Efficiency

Duplicate samples of a stock solution of **I** at a concentration of 0.252 mg ml^{-1}, were transferred by pipette (15, 20 and 25 ml) into 100 ml calibrated flasks to achieve concentrations of 8.2, 10.9 and 13.6 μg ml^{-1}. The mass of **I** in each flask corresponded to 3.75, 5.00 and 6.25 mg. Acetone (20 ml) and dimethyldioxirane solution (5 ml) were then added to each flask and the volume made up with distilled water. The solutions were allowed to stand for 10 min, 20 ml portions were then transferred by pipette into 100 ml calibrated flasks and diluted to volume with Britton Robinson buffer of pH 3.0. The solutions were analysed by polarography against an *N*-oxide of **I** standard prepared at the Chemical Development Laboratories of SmithKline Beecham Pharmaceuticals.

Method Performance Conditions

System repeatability

Standard solutions of an *N*-oxide of **I** in pH 3.0 buffer solution (10.0 μg ml^{-1}) were measured six times and the peak current recorded.

Method repeatability

Five tablets containing nominally 125 μg of **I**, in a lactose-based formulation, were placed in a calibrated flask (50 ml) and dissolved in phosphate buffer solution of pH 7.0 (20 ml). Acetone (20 ml) and DMD solution (5 ml) were added and the solution was set aside for 15 min. The flasks were made up to volume with further phosphate buffer of pH 7.0 and the resultant stock solution (5 ml) was transferred into a calibrated flask (25 ml) and diluted to volume with electrolyte solution of pH 3.0. Six replicates were prepared in this way and analysed.

Quantification

Sample solutions were quantified by reference to an externally prepared standard solution of the *N*-oxide of **I** at a concentration of 2.5 μg ml^{-1} in electrolyte solution. The content of **I** was calculated from the following formula:

$$\text{Content} = \frac{i_S}{i_R}.C_R.\frac{V_1}{V_2}.\frac{V_3}{N}\ \mu\text{g per tablet}$$

Where: i_S = sample peak current; i_R = reference peak current; C_R = reference concentration; V_1 = working sample volume (25); V_2 = dilution factor (5); V_3 = stock sample volume (50); and N = number of tablets (5).

Results and Discussion

Optimization of Detection Conditions

In order to determine the optimum conditions for reductive polarographic analysis the variation of electrode potential and peak response with pH of the *N*-oxide of **I** was measured (Table 1).

The maximum response and separation from the reduction of the supporting electrolyte was observed at pH 3.0. This pH was, therefore, chosen as the optimum for the analysis and yielded well defined polarograms (Fig. 3).

Derivatization Efficiency

The quantitative nature of the conversion (Fig. 2) was illustrated by derivatizing **I** and analysing the resulting solutions against a reference standard of the *N*-oxide of **I**. The percentage conversions are recorded in Table 2 with an overall mean of 101%.

Table 1 Variation of electrode potential and peak height with pH for the polarographic reduction of **I** *N*-oxide

pH	Peak potential/V	Peak current/nA
2	−0.85	435
3	−0.97	490
4	−1.36	435
5	−1.34	185
6	−1.31	150
7	−1.34	255
8	−1.34	285
9	−1.34	285

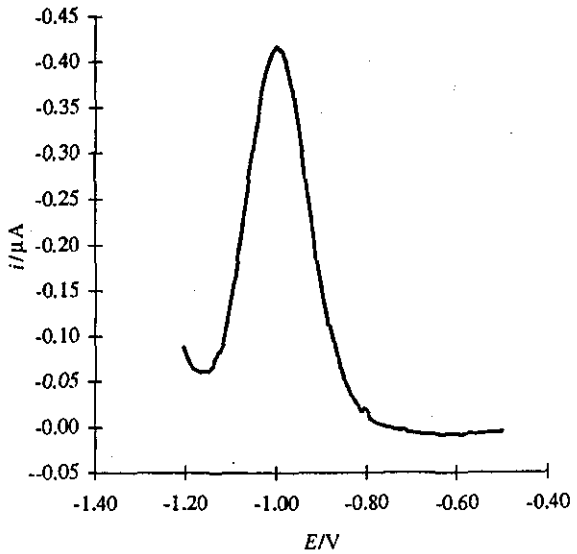

Fig. 3 Differential pulse polarogram of **I** *N*-oxide (10 μg ml^{-1}; 20 mV pulse height).

Table 2 Conversion of **I** to the *N*-oxide using DMD

Mass of **I**/mg	Working concentration of **I** *N*-oxide/ μg ml^{-1}	Concentration of **I** *N*-oxide determined/ μg ml^{-1}	Recovery (%)	Mean recovery (%)
3.75	8.2	8.2	100	101
	8.2	8.3	101	
5.00	10.9	11.0	101	101
	10.9	11.0	101	
6.25	13.6	13.6	100	101
	13.6	13.8	101	

The high conversions indicate that the derivatization may be regarded as quantitative on analytical solutions containing milligram quantities of I.

Method Performance

Linearity and limit of detection

For solutions containing I in the absence of excipients good linearity was observed over the concentration range of 0.05 to 25.0 μg ml^{-1} with r = 0.9993.

The LOD calculated from the linearity plot by conventional statistical means[3] was found to be 1.2 μg ml^{-1} reflecting the uncertainties at the lower end of the calibration plot. By increasing the pulse amplitude to 100 mV, a 5 ng ml^{-1} solution could be detected with a peak current of 13 nA representing an approximately eight-fold improvement over conventional HPLC procedures with respect to the LOD (Fig. 4).

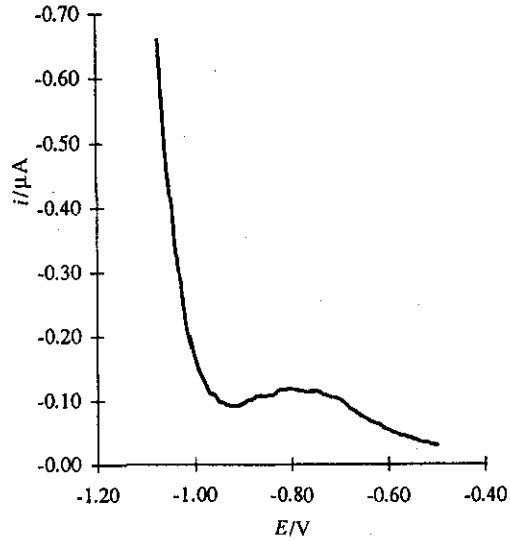

Fig. 4 Differential pulse polarogram of I (*N*-oxide (5 ng ml^{-1}; 100 mV pulse height).

Table 3 Method performance for the dedrivatization of I precision

	System repeatability Current (nA)		Method repeatability μg I tablet^{-1} 2.5 μg ml^{-1} (nominally 125 μg per tablet)
Concentration of I	0.5 μg ml^{-1}	10.0 μg ml^{-1}	
	74	345	123.3
	75	343	122.6
	71	344	122.5
	73	345	121.8
	75	346	123.3
	72	342	123.4
Mean	73	344	122.8
s	1.6	1.47	0.63
RSD (%)	2.2	0.43	0.50

Precision

Precision measurements conducted on solutions with concentrations in the anticipated range were good. The system repeatability improved with increasing concentration as expected and the method repeatability carried out by analysing tablets yielding a nominal assay concentration of 2.5 μg ml^{-1} was 0.5% (Table 3). This compared favourably with HPLC procedures which yielded a method repeatability of 2.4%.

Accuracy

The tablets analysed for method repeatability contained an accurately known quantity of I of 125 μg per tablet. The mean result of 122.8 μg yielded an accuracy of 98.2%.

Scope of Analysis

Analysis of tertiary amines such as I can be achieved at low analytical concentrations. Limiting factors for the analysis of I were mainly matrix effects from excipient material rather than inherent lack of sensitivity. Simple carbohydrates such as lactose appear to be unreactive to DMD but other excipients that contain oxidizable material can limit the scope of the analysis. The tablet formula analysed in this work displayed large backgrounds sufficient to make the quantification impossible if the dose of I was reduced below 100 μg. The LODs reported here are those derived from the drug alone and in practice usable limits will depend more on the nature and level of excipients present in the samples.

In addition to improvements in sensitivity and precision compared to conventional HPLC the generation of a polarogram characteristic of the drug provided an alternative means of identification. This is useful in satisfying regulatory requirements with an experimentally straightforward procedure. Further benefits of this derivatization procedure might also be anticipated in the analysis of biological fluids where sensitivity and specificity are key requirements. For such procedures the ability to remove the volatile derivatizing agent could be a useful advantage.

We would like to thank Professor B. A. Marples at Loughborough University of Technology for his help in the discussions of this project.

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Mechanism of the dimethyldioxirane oxidation of *N*,*N*-dimethylanilines

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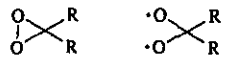
Relative rates of dimethyldioxirane oxidation of a number of *para*-substituted *N*,*N*-dimethylanilines in acetone at 5 °C are compared with those of reactions with methyl iodide and other oxidants. The reactions with dimethyldioxirane followed the Hammett relationship with a ρ value of −1.0. Measurement of the second order rate constants for the dimethyldioxirane reactions in aqueous acetonitrile containing potassium nitrate at 21 °C, showed better correlation with the Hammett relationship (ρ = 0.89) than with the Okamoto–Brown model (ρ^+ = 0.56). The reaction rates are accelerated greatly in the presence of water such that the respective pseudo first order rate constants for the oxidation of *N*,*N*-dimethyl-4-nitroaniline in acetone and water are 6.3×10^{-3} and 5.86 s^{-1}, respectively. All of the data are consistent with a concerted electrophilic mechanism and there is no evidence of free radical or electron transfer reactions.

Introduction

In recent years, dioxiranes **1** have been used to carry out a wide variety of synthetically useful transformations.[1] However, some mechanistic details remain unclear and evidence has been presented suggesting that some dioxirane reactions may occur *via* bis(oxyl) diradicals **2**.



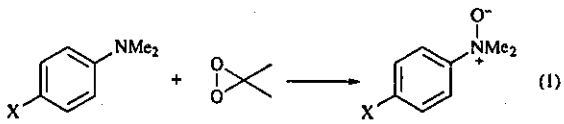
1 **2**

Epoxidation,[2] sulfur oxidation[3] and C–H insertion[4] reactions of dimethyldioxirane have been shown to be electrophilic by means of linear free energy relationship (LFER) correlations and a mechanistic study by Adam and Golsch[5] concluded that the dimethyldioxirane oxidation of nitrogen heteroarenes follows an S_N2 mechanism. Other studies, however, on the oxidation of *para*-substituted benzaldehydes to the corresponding acids and an LFER study on the oxidation of some α-methylbenzyl alcohols by Baumstark and co-workers[6,7] have shown evidence of radical character, as has recent work on alkene epoxidation[8] and alkane oxidation[9] by Minisci and co-workers. Also, studies by Crandell[10] and Curci[11] and their co-workers on the dimethyldioxirane oxidation of phenols and by Adam and Schonberger[12] on the oxidation of hydroquinones propose electron transfer processes.

We report an investigation into the nature of the dimethyldioxirane oxidation of a series of *para*-substituted *N*,*N*-dimethylanilines in an attempt to further probe the mechanism of amine oxidations and explore the possibility of substituent-induced changes of mechanism. The relative rates of oxygen transfer by dimethyldioxirane to the nitrogen of several *N*,*N*-dimethylanilines are described and compared to the relative rates obtained for methyl iodide methylation, benzoyl peroxide oxidation and *tert*-butyl hydroperoxide oxidation. The substrates are sterically identical around the nitrogen so the only factor affecting the rate of reaction is the electron demand of the *para*-substituent. Methyl iodide was used because, as in Adam's study,[5] it is reported to react with tertiary amines *via* a well established S_N2 mechanism. Benzoyl peroxide and *tert*-butyl hydroperoxide, like dimethyldioxirane, are both neutral peroxides. Benzoyl peroxide dealkylates tertiary amines and its rate determining step is thought to be electrophilic.[13] *tert*-Butyl hydroperoxide, also, dealkylates tertiary amines but is thought to react *via* a homolytic process involving *tert*-butoxyl radicals.[14] *tert*-Butyl hydroperoxide in the presence of a vanadium catalyst, which is reported[15] to lead to *N*-oxidation rather than dealkylation, was also used.

Results and discussion

The competition reactions were carried out by mixing one equivalent of the *para*-substituted *N*,*N*-dimethylaniline with one equivalent of the unsubstituted *N*,*N*-dimethylaniline in an acetone solution and adding one equivalent of dimethyldioxirane–acetone solution. The reactions were carried out at 0–5 °C and continued overnight to ensure total consumption of the dimethyldioxirane, enabling 50% conversion to be assumed. Relative reaction rates were determined by monitoring the consumption of starting material by HPLC. In each case, the *N*-oxide was the major product and no evidence was found for dealkylation [reaction (1)]. The reactions of dimethyldioxirane



(1)

with each *N*,*N*-dimethylaniline were carried out to confirm this and the products obtained were compared with *N*-oxides prepared using performic acid.[16]

The competition reactions were repeated, replacing the dimethyldioxirane with (*i*) methyl iodide, (*ii*) benzoyl peroxide, (*iii*) *tert*-butyl hydroperoxide and (*iv*) *tert*-butyl hydroperoxide and vanadyl acetylacetonate. As with dimethyldioxirane, these reactions were run at 0–5 °C and continued to completion. The *tert*-butyl hydroperoxide reaction was also carried out at 70 °C as this is the more usual temperature at which to carry out dealkylations of tertiary amines with this reagent.[14]

The results of the competition reactions are shown in Table 1 and were calculated by determining the ratio of the reactants remaining at the end of the reaction. Here the unsubstituted

Table 1 Summary of the relative data for the reaction of *para*-substituted *N,N*-dimethylanilines in acetone with the reagents shown

	k_{rel}					
X	Dimethyldioxirane 0–5 °C	Methyl iodide 0–5 °C	Benzoyl peroxide 0–5 °C	*tert*-Butyl hydroperoxide 0–5 °C	*tert*-Butyl hydroperoxide 70 °C	*tert*-Butyl hydroperoxide + catalyst
MeO	2.13 ± 0.19	3.65 ± 1.14	27.6 ± 12.9	1.19 ± 0.08	0.89 ± 0.01	1.03 ± 0.10
H	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Cl	0.87 ± 0.01	0.70 ± 0.02	0.06 ± 0.02	0.86 ± 0.01	0.52 ± 0.12	1.13 ± 0.03
NO_2	0.18 ± 0.04	0.65 ± 0.10	0.03 ± 0.02	0.91 ± 0.08	0.52 ± 0.10	0.43 ± 0.06

Table 2 Summary of the relative rate data and substituent constants used in the LFER plot for the reaction of dimethyldioxirane with *para*-substituted *N,N*-dimethylanilines in acetone at 0–5 °C

			Run 1		Run 2	
X	σ[a]	σ^+[b]	k_{rel}	log k_{rel}	k_{rel}	log k_{rel}
MeO	−0.27	−0.78	2.13	0.33	2.91	0.46
H	0.00	0.00	1.00	0.00	1.00	0.00
Cl	0.23	0.11	0.87	−0.06	0.49	−0.31
NO_2	0.78	0.79	0.18	−0.74	0.23	−0.64
R_H[c]				0.987		0.964
ρ[d]				−0.99		−1.01
R_{OB}[e]				0.963		0.979
ρ^+[f]				−0.67		−0.71

[a] Taken from ref. 18. [b] Taken from ref. 19. [c] R_H, Correlation coefficient for Hammett plot. [d] R_{OB}, Correlation coefficient for Okamoto–Brown plot. [e] ρ, Hammett constant. [f] ρ^+, Okamoto–Brown constant.

amine in each series has been given an arbitrary value of 1 and the other rates in the series were calculated relative to this.

The results obtained indicate a similar qualitative trend for dimethyldioxirane oxidation as for the electrophilic methyl iodide and benzoyl peroxide reactions. The reactivity decreased in the order MeO > H > Cl ≥ NO_2.[17] The *tert*-butyl hydroperoxide reactions are less susceptible to changes in substituent, the difference in rates being less marked, as would be expected for a non-electrophilic reaction. These trends suggest that the dimethyldioxirane oxidation of *N,N*-dimethylanilines is electrophilic. All the reactions were run to completion, therefore, the relative rates obtained reflect a minimum difference in reactivity.

The Hammett[18] relationship was applied to the dimethyldioxirane results giving an approximate ρ value of −0.99 (run 1, Table 2). Similar treatment of the data with the Okamoto–Brown[19] relationship gave $\rho^+ = -0.67$. The highest negative ρ value reported for a dimethyldioxirane reaction is −2.76 by Murray and Gu[4] for the C–H insertion reaction into *para*-substituted cumenes. Lower values (−0.77 and −0.76) have been reported by Murray *et al.*[3] for the electrophilic dimethyldioxirane oxidation of *para*-substituted aryl methyl sulfides and sulfoxides, respectively.

The dimethyldioxirane competition reactions were repeated in a second run (Table 2) using a slightly different approach. In this case the relative rates were calculated by comparing the initial concentrations of anilines with the final concentrations making no assumptions about the percentage conversion. The Hammett[18] relationship was applied to data from both runs (Table 2) yielding a mean ρ value of −1.00 which suggests that the conclusions drawn by Murray on the electrophilic nature of the cumene and sulfide oxidations can be equally applied to the dimethylaniline oxidations.

Although the relative rate measurements enable a competitive assessment to be made between substrates they provide no direct measure of the rate of reaction itself. In a third approach, therefore, absolute second order rate constants for reactions of dimethyldioxirane each with a known excess of the dimethylaniline were determined by controlled potential amperometry on the polarographic reduction wave of dimethyldioxirane. This avoids the large background interferences inherent with spectroscopic methods for the detection of dimethyldioxirane. The data obtained are summarised in Table 3.

Table 3 Run 3: absolute second order rate constants for the reactions of dimethyldioxirane with *para*-substituted *N,N*-dimethylanilines in 50:50 acetonitrile–0.1 M KNO_3 solution

X	k_x/dm^3 mol^{-1} s^{-1}	k_{rel}	log k_{rel}
MeO	1777	1.60	0.20
H	1109	1.00	0.00
Cl	611	0.55	−0.26
NO_2	213	0.19	−0.72
R_H			0.998
ρ			−0.89
R_{OB}			0.952
ρ^+			−0.58

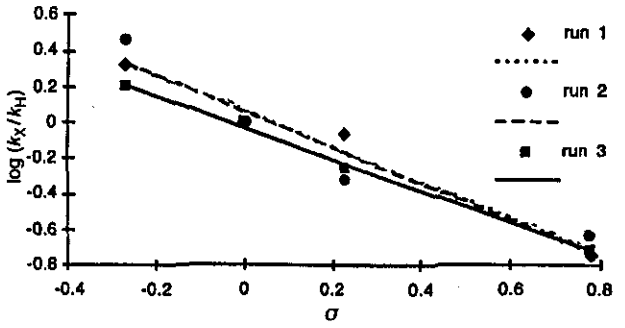


Fig. 1 LFER plots of log (k_X/k_H) *versus* σ for the reaction of dimethyldioxirane with *N,N*-dimethylanilines

The reactions of dimethyldioxirane and *para*-substituted *N,N*-dimethylanilines follow the Hammett relationship with a ρ value of −0.89 which is similar to that obtained from the relative rate data (Fig. 1). The agreement is good considering that the two experimental approaches were conducted at different temperatures and in different solvents. The electrochemical measurements were carried out in aqueous acetonitrile at 21 °C whereas the relative rate data were generated in acetone at 0–5 °C. The excellent correlation observed with the Hammett plot of the absolute rate data suggests that this relationship is more appropriate than that from the Okamoto–Brown[20] plot and lends further support to the hypothesis that the dimethyldioxirane oxidation of substituted dimethylanilines is a concerted electrophilic process.

The dimethyldioxirane oxidations show obvious similarities to reactions of dimethylanilines with methyl iodide (Menschutkin reaction) which has a reported[21] ρ value of −3.30 at 35 °C in 90% aqueous acetone. Whereas, in the latter reactions the transition state is thought to have developed almost a full positive charge, the reactions with dimethyldioxirane clearly have much less charge development (Fig. 2) and may be a result, in

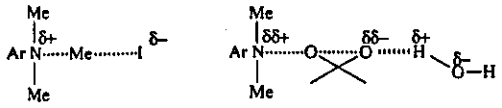


Fig. 2 Transition states for the reactions of dimethylanilines with MeI and dimethyldioxirane

Table 4 Pseudo first order rate constants for the reaction between dimethyldioxirane and *N,N*-dimethyl-4-nitroaniline in various solvents at 25 °C

Solvent	k/s^{-1}	ε
Water	5.86	78.5
Water–acetone	1.55×10^{-1}	—
Acetone	6.30×10^{-3}	20.7
Dichloroethane	4.10×10^{-4}	10.4
Acetonitrile	4.50×10^{-4}	37.5
Acetonitrile[a]	6.88	37.5
Ethyl acetate	8.00×10^{-5}	6.02
pH 1, 0.1 M Buffer soln	5.05	—
pH 10, 0.1 M Buffer soln	6.52	—
pH 5, 0.1 M Buffer soln	5.19	—
pH 5, 0.01 M Buffer soln	6.34	—

[a] (Trifluoromethyl)methyldioxirane.

part, of steric crowding in the transition state. Hydrogen bonding is also thought to be important (see below).

The rapidity of the reactions in aqueous acetonitrile is surprising in view of our earlier observation that *N,N*-dimethyl-4-nitroaniline was effectively unchanged after many hours of reaction with dimethyldioxirane in acetone. Interestingly oxidation of *N,N*-dimethyl-4-nitroaniline at 0 °C with dimethyldioxirane (10 equiv.) in 50% aqueous acetone on a preparative scale showed significant conversion to the *N*-oxide after only one hour. The ^{1}H NMR spectrum of the *N*-oxide in the reaction mixture showed the expected peaks at δ 8.37 ($2H^3$, d, J 9), 8.21 ($2H^2$, d, J 9) and 3.7 (NMe_2, s). The reaction mixture also contained some starting material and a minor impurity that we were unable to isolate.

The possibility of a strong solvent effect, as has been observed[22–24] in other dioxirane oxidations, was investigated kinetically by monitoring the loss of the UV absorbance of *N,N*-dimethyl-4-nitroaniline when reacted with an excess of dimethyldioxirane. The pseudo first order rate constants were calculated in a number of solvents and the results are summarised in Table 4.

With the exception of acetonitrile the pseudo first order rate constant decreases with the relative permittivity of the medium. This is a similar trend to that observed in the Menschutkin reaction in which, as here, factors other than relative permittivity are involved.[21] Significantly there is a large increase in the reaction rate in water and the increased reaction rate remains relatively constant irrespective of the pH or ionic strength. The magnitude of the rate increase in water is almost certainly not due to the effects of relative permittivity alone and probably arises from the strong hydrogen bonding nature of the solvent. Such solvents would be expected to stabilise both the transition state (Fig. 2) and the *N*-oxide reaction products which readily form stable hydrates.[25] Direct evidence of the latter was observed particularly in the ^{1}H NMR spectrum of *N,N*-dimethyl-4-nitroaniline *N*-oxide. D_2O solutions of the *N*-oxide showed resonances at δ 8.19 ($2H^3$, d, J 9), 7.89 ($2H^2$, d, J 9) and 3.5 (NMe_2, s). Evaporation of the D_2O and suspension of the hydrated residue in $CDCl_3$ containing sieves afforded a ^{1}H NMR spectrum identical to that of the *N*-oxide originally isolated (see earlier). The role of hydrogen bonded solvents in accelerating oxidation rates has previously been recognised by Murray and Gu in studies of the dimethyldioxirane epoxidation of ethyl (*E*)-cinnamate[23] and oxidation of C–H bonds.[24] We and others have suggested intramolecular hydrogen bonding could be important in some dimethyldioxirane oxidations[26,27] and very recently *ab initio* model studies on primary amine oxidations with dimethyldioxirane has confirmed the importance of solvent and hydrogen bonding effects.[28]

The rate constants observed in water are comparable to those obtained for the oxidation of *N,N*-dimethyl-4-nitroaniline using (trifluoromethyl)methyldioxirane in a relatively unreactive solvent such as acetonitrile. It is speculated that the presence of water promotes faster reaction times and yields without recourse to the use of fluorinated dioxiranes which are experimentally more difficult to handle.

All the data obtained are consistent with conclusions that the dimethyldioxirane oxidation of dimethylanilines is electrophilic in nature and does not involve the bis(oxyl) diradical **2** or electron transfer. In this respect the mechanism agrees with the conclusions drawn by Curci and co-workers for the epoxidation and oxygen insertion into alkane C–H bonds.[29]

Experimental

Materials

Acetone was distilled from potassium permanganate prior to use. Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) was supplied by Aldrich and used without further treatment. *N,N*-Dimethylaniline and *N,N*-dimethyl-4-nitroaniline were supplied by Lancaster and used as received. Dimethyldioxirane–acetone solutions were synthesised according to literature procedure.[30] Methyl iodide, benzoyl peroxide, *tert*-butyl hydroperoxide and vanadyl acetylacetonate were supplied by Lancaster and used as received.

N,N-Dimethyl-4-methoxyaniline and *N,N*-dimethyl-4-chloroaniline were prepared according to literature procedures using sodium cyanoborohydride and *para*-formaldehyde in glacial acetic acid.[31] The purity was checked using ^{1}H NMR spectroscopy and melting point data.[32] The *N*-oxides of the *N,N*-dimethylanilines were prepared using performic acid also according to literature procedures.[16]

Instrumentation

NMR spectra were recorded using a Bruker AC 250 spectrometer. Amperometric measurements were recorded using a BAS CV50W voltametric analyser with a hanging mercury drop electrode and using the timebase mode for the kinetic measurements. Spectrophotometric measurements were recorded on a Beckmann 640i spectrophotometer using the kinetics accessory. HPLC was performed using: Perkin-Elmer series 3 pump, Perkin-Elmer LC55B spectrophotometric detector and Spectraphysics integrator. A Hewlett Packard 1090 diode array detector was also used. The chromatographic conditions used for the first run were as follows: column, μBondapak C18 10 μm 3.9 × 300 mm; eluent, 6:4 methanol–water (NO_2 and Cl), 1:1 methanol–water (MeO); flow rate, 1 ml min^{-1}; detection, UV@240 nm; injection volume, 20 μl.

The second set of results were obtained using the HPLC method given below: column, Spherisorb S5 C1 15 cm × 4.6 mm; eluent, 0.05 M phosphate buffer, 0.1% triethylamine pH 3.5 (NO_2 and Cl), pH 4.0 (MeO); flow rate, 1 ml min^{-1}; detection, UV@210 nm; injection volume, 20 μl.

Relative rate studies of *N,N*-dimethylanilines

An equimolar stock solution of *N,N*-dimethylaniline and *para*-substituted *N,N*-dimethylaniline in acetone was prepared. An aliquot of this solution was removed, placed in a flask covered with aluminium foil to protect it from light, and cooled to 0–5 °C in an ice bath. One equivalent of dimethyldioxirane solution was then added to the flask and the reaction stirred magnetically overnight. Starch–iodide paper showed no remaining oxidant. A 50 μl sample was removed and analysed by HPLC. Two determinations were made for each reaction and each reaction was carried out in duplicate.

The above procedure was repeated using aliquots of the same stock solution and the following reagents: methyl iodide, benzoyl peroxide and *tert*-butyl hydroperoxide. The *tert*-butyl hydroperoxide reactions were also carried out at 70 °C and in the presence of vanadyl acetylacetonate (0.025 equiv.). All the reactions were run to completion. The relative rates were calculated by determining the ratio of reactants remaining at the end of the reaction. This was achieved by first constructing a calibration graph of actual ratio of unsubstituted aniline to substituted aniline *versus* observed peak area ratio for a series of standard solutions containing varying known ratios of aniline to substituted aniline. Using this graph, observed peak area ratios of aniline to substituted aniline obtained from the chromatogram were converted to actual ratios. The reactions were run to completion, consuming all the dioxirane, therefore, 50% conversion could be assumed and the relative rates calculated by taking the reciprocal of the ratio of aniline to substituted aniline remaining at the end of the reaction. A summary of the relative rate data obtained is given in Table 1.

The dimethyldioxirane competition reactions were repeated and analysed using quantitative HPLC. Again two determinations were made for each reaction and each reaction was done in duplicate. Standards of each *N*,*N*-dimethylaniline were prepared and used to quantify the amounts of each present at the end of the reaction. The relative rates were calculated using eqn. (2),[4] where ΔC = change in concentration of reactants.

$$k_{rel} = \frac{k_{p\text{-substituted aniline}}}{k_{aniline}} = \frac{\Delta C_{p\text{-substituted aniline}}}{\Delta C_{aniline}} \quad (2)$$

Oxidation of the *N*,*N*-dimethylanilines with dimethyldioxirane

Solutions of each of the *N*,*N*-dimethylanilines in acetone were oxidised using 2 equiv. of dimethyldioxirane–acetone solution. Concentration of the product solution *in vacuo* gave the *N*-oxides as the only products by ^{1}H NMR and HPLC.

Constant potential amperometric rate studies

The absolute rates of reaction were determined by monitoring the loss of current due to the polarographic reduction of dimethyldioxirane on addition of a solution of the anilines. A solution of dimethyldioxirane (0.13 mM, 10 ml) prepared by adding an appropriate volume of dimethyldioxirane in acetone to a mixture of potassium nitrate solution (0.1 M) and acetonitrile (50% v/v), was placed in the electrochemical cell. The solution was stirred at 600 rpm while the test aniline solution (50–250 μl, 0.06 M in acetonitrile) was added to the cell. The loss in current was monitored with time under the following instrumental settings: potential = +80 mV, sampling time = 100 s, interval time = 50 ms, sensitivity = 1 μA, reference electrode Ag^+/Ag, temperature 21 °C. The pseudo first order rate constants were determined by plotting the ln *I versus* time (final current = zero). Second order rate constants were determined by plotting the pseudo first order rate constants *versus* molar concentration of aniline. Standard linear regression statistics were used to calculate the slope of these lines.

Spectrophotometric rate studies

The spectophotometric rates were determined by monitoring the loss of UV absorbance of *N*,*N*-dimethyl-4-nitroaniline on addition of dimethyldioxirane. A solution of *N*,*N*-dimethyl-4-nitroaniline (0.03 mM, 2.5 ml) in the appropriate solvent was placed in a 1 cm cuvette and the solution stirred magnetically. Dimethyldioxirane solution in acetone (0.07 M, 60 μl) was aspirated into the cell and the loss of absorbance as a function of time was recorded with the following instrumental settings: λ = 400–425 nm depending on λ_{max} in each solvent, interval time = 0.1 s, read average time = 0.05 s, run time = 30–3000 s, temperature thermostatted at 25 °C. The pseudo first order rate constants were determined by plotting ln absorbance *versus* time (the absorbances of the *N*-oxides at these wavelengths were negligible) using standard linear regression statistics to calculate the slopes and hence rate constants.

Note added in proof. Dimethyldioxirane oxidation of *N*,*N*-dimethylaniline to the *N*-oxide has very recently been reported.[33]

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