Removal of tecnazene from water using UV light and hydrogen peroxide

Saeko Sawata, B.Sc

Thesis presented for
the degree of
Master of Science
(Research course)
December 1998

Agricultural, Food and Environmental Chemistry,
Facility of Science,
University of Glasgow.

Acknowledgements

First of all I am most grateful to Dr H. J. Duncan for his advice and encouragement throughout the period of the research work

The advice and expertise of Dr T. H. Flowers on the working of the Dionex, and of Dr W. J. Coles on the analysis by GC-MS were greatly appreciated

I would also like to thank other members of staff, in particular to Mr M. Beglan and Ms M. Feasby, and the research students in this section, in particular to Mrs F. Karbasi, for their help and encouragement.

Finally, my sincere thanks go to my family especially my husband for constant support and encouragement.

Summary

This thesis describes the efficiency of UV irradiation treatment for removal of tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene) from water in the presence and absence of hydrogen peroxide. It also includes the role of hydrogen peroxide in the treatment, and the identification of decomposition products of tecnazene.

Tecnazene is a fungicide used on potatoes for the purpose of sprout suppression and dry rot control. It is used mainly in the UK, and applied at the time of store loading at a rate of about 0.1g a.i./kg potatoes. In general, toxicity, persistence in the environment and fate are of great concern when using agrochemicals. In the case of tecnazene these aspects are not well understood in spite of the fact the chemical has been used for a long time. However, questions about tecnazene have been raised from the safety point of view since tecnazene has been found to be highly toxic to several aquatic species including fish (Whale *et al.*,1988). In addition to that tecnazene and its metabolites have been identified in sediments and in fish down stream of a potato washing plant (Whale *et al.*, 1988). In this respect, proper treatment and disposal of tecnazene has been of interest. Therefore, the removal of tecnazene from water was studied in this thesis. The method employed for the treatment was UV irradiation treatment combined with hydrogen peroxide (advanced oxidation method) since it is one of the strongest oxidation methods. Hydrogen peroxide was studied as a photooxidant.

UV irradiation treatment of tecnazene in pure water leads to a rapid decomposition, and almost complete decomposition of tecnazene was observed within half an hour. Hydrogen peroxide enhanced the rate of photolysis although hydrogen peroxide alone hardly oxidised any tecnazene. This is because of the formation of hydroxyl radicals, which are known as strong oxidants, as a consequence of photolysis of hydrogen peroxide. The decomposition rate increased with the increase in the concentration of hydrogen peroxide.

Investigation of transformation pathways is an important part of a study for the removal of pesticides, since there is a possibility that more toxic products might be produced as by-products. Therefore, it is necessary to identify as many decomposition products as possible, and investigate the transformation pathways. However, identification of pesticide decomposition products is difficult due to their low concentration versus the concentration of their parent compounds. In this respect, a cosolvent is usually employed for the study of decomposition products due to its capability of dissolving hydrophobic organic chemicals which covers the majority of pesticides.

Although acetonitrile was recommended as a cosolvent by researchers (Leifer, 1988; Bunce, 1978), it was found to be decomposed by UV light and affected the decomposition rate of tecnazene in the case where hydrogen peroxide was present, while the affect of the presence of acetonitrile on the formation of decomposition products was obscure on this occasion.

In terms of identification of decomposition products, trichloronitrobenzene and tetrachlorophenol were identified by GC-MS with the sample irradiated by UV light for 80min in 10% (v/v) acetonitrile-water mixtures. The identification of anionic products was also carried out by ion exchange chromatography, and chloride, nitrite and nitrate were identified as inorganic anions, and lactate, formate, acetate, succinate and oxalate were identified as organic anions. These results may indicate several pathways of tecnazene decomposition, such as reductive dechlorination pathway, replacement of the ring nitro group by hydroxyl and ring opening reaction of tecnazene.

In terms of the role of hydrogen peroxide on the formation of decomposition products, faster oxidation reactions were observed in the case of UV irradiation in the presence of hydrogen peroxide. For instance, the nitrite was oxidised quickly to nitrate permitting control over the form of N. Moreover, organic anions detected as decomposition products were oxidised quicker than in the absence of hydrogen

peroxide. These are advantages of UV irradiation in the presence of a hydrogen peroxide treatment

These supporting results do suggest that UV irradiation treatment of tecnazene brought about reductive dechlorination and the ring opening reaction following the formation of organic anions, and in the case of UV irradiation in the presence of hydrogen peroxide one further advantage was observed which was a faster oxidation reaction toward tecnazene and its decomposition products. These should not cause undue concern. However, further work, such as the study of the influence of the presence of suspended sediment and other substances (humate etc.), temperature and pH on the photodecomposition rate may be required before such an approach could be recommended with confidence for commercial situations, unless it was confined to a final polishing treatment as for drinking water.

Content

CHAPTER 1. Introduction and thesis objectives

1.1 Use of tecnazene (Background information of tecnazene)

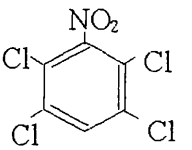
Tecnazene was initially introduced as a fungicide in 1947 for use against dry rot in seed potatoes, and then its sprout suppressant properties were first noted in the following year. Tecnazene and another chemical, chlorpropham (isopropyl N-(3-chlorophenyl) carbamate), in particular are used now as post harvest treatments on stored potato tubers to minimize sprouting. Chlorpropham is employed worldwide and is applied to the potatoes at regular intervals throughout the storage season. It is not unusual to apply up to 0.1 g a.i.*)/kg potatoes. This would amount to some 100 kg of chemical in a 1000-2000 tone store. Tecnazene is used for the purpose of sprout suppression and dry rot control mainly in the UK. It is applied at time of store loading again in about the same amounts as used for chlorpropham (0.1g a.i./kg potatoes). Unlike other commercially available post harvest sprout suppressants (i.e. chlorpropham), tecnazene does not inhibit wound healing and can be used with confidence at time of store filling. It is also generally accepted that tecnazene maintains sprout control and does not 'kill' sprouts. Because it is applied at store loading a more even application of chemical can be made thereby satisfying legislative requirement.

*) a.i.; active ingredient

1.2 The physical and chemical properties of tecnazene

The physical and chemical properties of tecnazene are given in Table 1.1.

Table 1.1 Physical and chemical properties of tecnazene (Hartley *et al.*, 1983).

Structure :	
Chemical name :	1,2,4,5-tetrachloro-3-nitrobenzene (IUPAC, CA)
Common name :	tecnazene (ISO, BSI)
Molecular weight :	260.96
Melting point :	99°C
Boiling point :	304°C (with decomposition)
Physical form :	Colourless crystals
Volatility :	Volatile at room temperature
Solubility :	Practically insoluble in water. Readily soluble in most organic solvents.

As mentioned in the table above, aquatic solubility of tecnazene is low. Leonard (1988) has reported that solubility of tecnazene in water was 0.9mg/L at 20°C.

Determination of tecnazene

Dalziel (1978) reviewed the analytical methods for tecnazene, and described several colorimetric, polarographic and gas chromatographic methods that were reported in the literature. He then employed a gas chromatographic method for residue analysis. This method is most commonly used for residue analysis of tecnazene because of its high specificity and sensitivity. Tecnazene can be detected at extremely low concentrations, i.e. in the ppb range rather than ppm range.

1.3 Tecnazene in aquatic environment

Over the last few years the quality of crop products as presented to the consumer has become paramount. With regard to crop storage, post harvest chemical treatments are of particular concern, i.e. pesticides that are added to the produce either at time of store loading or once the produce is in place in the store. In the case of potatoes, in order to maintain the quality, sprout suppressant chemicals, such as chlorpropham and tecnazene, are applied post harvest as mentioned before. As the percentage of potatoes destined for the processing sector is very much on the increase as is the pre-pack markets, more and more chemicals are being applied to the stored potato crop in this manner.

In terms of supermarket presentation, and the manufacture of crop products, in the case of potatoes, for instance, many new central processing and washing plants have been constructed. These plants release large quantities of wastes in their effluent stream thereby making an impact in the river downstream of these plants, and resulting in more obvious point sources of pollutants. As most of the chemical is either loosely attached to the surface of the potato or absorbed in the skin, the amount released on washing and also peeling can be substantial. Most of this will ultimately reach the water course. Indeed, tecnazene and its metabolites have been identified in sediments and in fish down stream of a potato washing plant (Whale *et al.*, 1988). In the case of potato washing plants the main pollutants are suspended solids and pesticides, while in the case of most potato processing plants much organic matter (potato peelings) is also included.

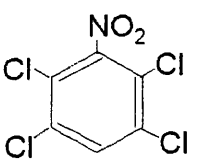
There is also evidence for the possible presence of tecnazene in the environment. Although a large amount of tecnazene is applied for sprout control of potatoes (approximately 0.1g a.i./kg potatoes), the MRL (Maximum Residue Limit) of tecnazene is considered to be 5 mg/kg potatoes for most European countries. MRL is based on lightly washed potatoes. This difference between the applied rate and the MRL indicates that approximately 95% of tecnazene applied to potatoes is supposed to be removed before consumption. In fact, the highest values of tecnazene residues in the washed whole potato tubers detected were just below 3mg/kg in commercial

stores (the rate of tecnazene applied was the maximum of 135mg a.i./kg) (Buckley *et al.*, 1994). Since they detected most of the tecnazene in adhering soil rather than in peel and flesh, tecnazene is likely to be removed with soil when the potato is washed and enter the aquatic environment with waste water unless treated properly.

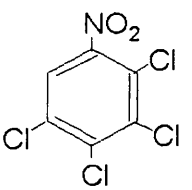
After entering the aquatic environment most likely tecnazene is removed by volatilization, hydrolysis and photolysis. However, it is also likely some will persist in the aquatic environment. Since tecnazene is an example of an organochlorine derivative of nitrobenzene, in general it has a relatively high potential for environmental persistence.

There could be the secondary source of tecnazene as a by-product during metabolism of some chlorinated nitrobenzenes. Pentachloronitrobenzene (PCNB) is an agricultural fungicide and an example of a chlorinated nitorobenzene. The structure is shown below (Figure 1.1). Because of its structural similarity, tecnazene is likely to be obtained easily as a consequence of dechlorination of PCNB. Indeed, in the study of photodecomposition of PCNB, tecnazene and its isomer (2,3,4,5-tetrachloronitrobenzene) have been reported as photodecomposition products of PCNB using UV light (Peterson *et al.*, 1990).

Pentachloronitrobenzene (PCNB)



Tecnazene



2,3,4,5-tetrachloronitrobenzene

Figure 1.1 Molecular structure.

In spite of the fact that there is a possibility of the presence of tecnazene in the aquatic environment, generally speaking, tecnazene has not been considered a hazard to the aquatic environment because of its volatile nature and insolubility in water. However, questions have been raised about its aquatic ecotoxicity and biological accumulation

Toxicity of tecnazene has been studied for several animals, such as mice and rat. Dalziel (1978) reviewed the toxicity of tecnazene for animals from literatures in his work. He summarized that tecnazene was readily metabolized in animals, and could be relatively non-toxic but not absolutely safe. Although tecnazene was found to be less toxic to animals, toxicity to aquatic species was not well understood for a long time. Recently tecnazene has been found highly toxic to several aquatic species including fish (Whale *et al.*,1988). Moreover, Oliver & Niimi (1985) pointed out that tecnazene was unlikely to be readily metabolized by fish.

Biological accumulation is another question from a safety point of view. Tecnazene is an organochlorine which has a moderately high octanol/water partition coefficient (K_{ow}) (Oliver & Niimi, 1985), which may indicate a high tendency of biological accumulation. In fact, Whale *et al.* (1988) have found that tecnazene is bioconcentrated significantly in rainbow trout. Lower chlorinated nitrobenzenes have also been detected in fish but not on a regular basis (Yurawecz & Puma, 1983).

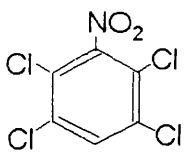
1.4 Breakdown and metabolites in the environment

Generally, volatilisation, hydrolysis and photolysis are the major likely chemical transformation pathways of a pesticide in the environment. In the case of tecnazene volatilization can be one of the major transformation pathways due to its volatile nature, while photolysis by sun light is unlikely to be expected to bring about a major transformation, since tecnazene does not absorb visual light.

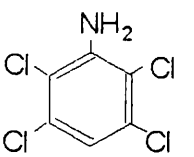
In the case of biological transformation, significant dechlorinating activity for chlorinated benzenes by anaerobic microorganism has been observed (Beurskens *et al.*, 1994). Tecnazene is an example of chlorinated benzene so the decomposition of tecnazene via biological pathways is also possible.

In the case of metabolism of tecnazene in animals, aquatic species and plants, there are a number of publications about them. According to these publications two compounds, namely 2,3,5,6-tetrachloroaniline (2,3,5,6-TCA) and 2,3,5,6-

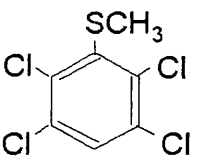
tetrachlorothioanisole (2,3,5,6-TCTA) are likely to be common metabolites. The structures of these compounds are given in Figure 1.2. For instance, these compounds have been reported as metabolites by fish (Allchin and Low, 1988), and have been found in potatoes treated with tecnazene in commercial stores as metabolites of tecnazene (Buckley *et al.*, 1994). Lappin *et al.* (1996) have studied the metabolism of tecnazene in rat, and have found several metabolites including also the aniline derivative.



Tecnazene



Tetrachloroaniline(TCA)



Tetrachlorothioanisole (TCTA)

Figure 1.2 Molecular structure of tecnazene and some of its metabolites.

However, the ultimate fate of these metabolites is as yet not determined. In other words, the fate of tecnazene, parent compound of these metabolites, has not been fully investigated, although it has long been a subject of interest. Some concern has been registered in this connection. However, investigating the fate of pesticides in the environment is difficult due to its complex reaction with several inorganic and organic species in the environment, and various conditions in the environment.

1.5 Removal of pesticide from the aquatic environment

Widespread pollution in the aquatic environment by pesticides has been recognized in recent years, and in general the pollution has increased sharply due to an extensive use in agricultural cultivation, weed control, industrial waste, domestic usage, pest control, etc.

Proper treatment and disposal of pesticide-contaminated wastewater has been a concern for soil and water contamination. Therefore, there has been growing interest

in the removal of pesticides from water. Numerous studies in pesticide removal methods for drinking water, surface and ground water, etc. at laboratory and pilot plant levels have been carried out. Several methods have been studied. For instance, chlorination, and oxidation by ozone, hydrogen peroxide, etc. have been studied for pesticide removal from aqueous solution. These are often used as a treatment method for removal of pathogenic bacteria and viruses from drinking water. Ozone has been found to oxidize several pesticides effectively but to be selective in its oxidation reactions, and has no effect on some pesticides (Roche and Prados, 1995).

UV irradiation is also a conventional method for treatment of drinking water in order to remove pathogenic bacteria and viruses, and in recent years UV irradiation has become an active field of study in order to remove pesticides from water.

As well as removal of pesticides by UV irradiation, photodecomposition of pesticides has been studied by many researchers using several kinds of light source in order to investigate the photolytic fate of pesticides in the aquatic environment from an environmental pollution point of view. It is important that experiments should be conducted with natural light for investigating the fate of pesticides in the environment, while in the case of removal of pesticides UV light can be the most powerful since the energy (E) of the light is related to the frequency (ν) (or the wavelength (λ)) of radiation by the equation:

$$E = h \times \nu$$
$$= h \times c/\lambda$$

Where h is Planck's constant
and c is the speed of light

It is also known that high-energy UV radiation leads electrons to move from one energy level to another with energies that are often able to break chemical bonds.

UV irradiation is often combined with hydrogen peroxide or ozone for the removal of pesticides from water, and is especially known as an advanced oxidation method.

Oxidation methods involving reactions with hydroxyl radicals are termed advanced oxidation methods (Glaze *et al.*, 1987), and UV with hydrogen peroxide, UV with ozone and ozone with hydrogen peroxide are the most known among these methods. In recent years these methods have received attention for water treatment due to these powerful oxidation reactions toward organic pollutants, and the number of publications on this study has increased.

As well as studying efficiency of methods, it is also important to study the decomposition pathways. The details of this issue are discussed later in Chapter 3 which is about the study of tecnazene decomposition products.

1.6 Thesis objectives

As mentioned before, questions of tecnazene contamination in water have been raised from a safety point of view in aquatic ecosystems. Therefore, what now follows is attempts to look at removal of tecnazene from water. The method employed is UV irradiation treatment combined with hydrogen peroxide (advanced oxidation method) since it is one of the strongest oxidation methods. There are two aims in this work;

The first aim is to investigate the efficiency of removal tecnazene from water using UV light with and without hydrogen peroxide which acts as a photooxidant.

The second aim is to identify and quantify tecnazene decomposition products during UV irradiation treatment with and without hydrogen peroxide so that a full picture of tecnazene decomposition pathways can be obtained under both conditions. In terms of toxicity, studying pesticide decomposition pathways is critical since there is a possibility of derivation of undesirable decomposition products.

CHAPTER 2. Decomposition of tecnazene

2.1 Introduction

The combination of UV light and hydrogen peroxide is one of the advanced oxidation methods which involves a radical type of reaction toward substances. With this method hydroxyl radicals are produced as a consequence of photolysis of hydrogen peroxide (Eqn. 1) (Zwiener *et al.*, 1995).

$$H_2O_2 + h\nu = 2 \cdot OH \qquad (1)$$

Hydroxyl radicals are known as the strongest oxidants in aqueous media apart from fluorine (Scheuer *et al.*, 1995). Therefore, in the case of UV irradiation in the presence of hydrogen peroxide there are two decomposition processes, namely direct photolysis by UV light, and indirect photolysis (oxidation) by hydroxyl radicals, while in the case of UV irradiation without hydrogen peroxide there is only a direct photolysis process by UV light as it was found that there was no radical contribution in the case of direct photolysis of pesticide by this method (Benitez *et al.*, 1995; Beltran *et al.*, 1996).

Two types of photochemical processes in aquatic solutions are known among a number of different processes, namely direct and indirect photolysis as mentioned above. Direct photolysis occurs when substances in water absorb light directly, and then leads to chemical reactions (Zepp and Cline, 1977). In the case of indirect photolysis, chemical reaction towards substances occurs through the radicals (e.g. hydroxyl radicals) produced as a consequence of irradiation of additives (e.g. hydrogen peroxide). When there is an additive in the solution, direct photolysis of substances may decrease due to less intensity of absorption. Therefore, the rate of photolysis in the presence of additives could be further complicated. In photochemistry well-known compounds as sources of radicals are hydrogen peroxide and ozone.

As well as the study of the efficiency of the removal method the study of decomposition products is also important because dissipation of parent compound does not mean absolute detoxification. In other words, there is a possibility that decomposition products can be harmful to the environment as well.

However, the analysis of decomposition products is often difficult to perform due to lower concentrations versus the concentration of the parent compound, as a result of low aquatic solubility of the parent compound. Like other organochlorines tecnazene is practically insoluble in water. Therefore, in this study acetonitrile was employed on occasion as a cosolvent to increase the concentration of tecnazene in order to obtain a greater amount of the parent compound. Acetonitrile was recommended for use as a cosolvent in photochemistry studies due to its similarity of refractive index to water (Leifer, 1988), and also was found to have a minimum affect in studies of photodecomposition of pesticides compared to other cosolvents, such as methanol (Bunce, 1978). Therefore, UV irradiation of tecnazene in water-acetonitrile mixtures was carried out as well as in water in order to identify the decomposition products.

Hence, decomposition of tecnazene was carried out in two types of solutions, water and water-acetonitrile mixtures. First of all it was decided to look at the decomposition rate of tecnazene in both solutions in absence and presence of hydrogen peroxide in this Chapter, and then the identification of decomposition products is further discussed later in Chapter 3.

A gas chromatographic method was employed for determination of tecnazene through out the study because of its high specificity and sensitivity.

2.2 Experimental

Common experimental procedures for each set of conditions are described in this section, and then more details of individual experiments are described in individual sections.

2.2.1 Materials

Tecnazene was recrystallised before use. Reagent-grade 30% hydrogen peroxide (BDH Ltd.) was used exclusively. All the organic solvents (Fisher Scientific) used in this study were of HPLC grade. Analytical grade sodium chloride (Fisher Scientific), sodium sulphate anhydrous (BDH Ltd.) and sodium meta-bisulphite (BDH Ltd.) were used as received. 2,3,4,5-tetrachloronitrobenzene was supplied by Aldrich Chemical Co. and was >99% pure. The experiments were carried out in deionized and ultrafiltered water supplied using Purite Selecte (Purite Ltd.).

2.2.2 Preparation of saturated tecnazene solution

In the case of the study of tecnazene decomposition in water saturated aqueous tecnazene solution was used in all experiments.

Procedure

0.01g tecnazene was added into 2.5L of water in a 3 L beaker. The mixture was stirred using a magnetic stirrer hot plate, heated to about 40°C and stirred for 4 hours. This solution was then left overnight in a constant temperature room at 22±3°C. This solution was filtered through Whatmans No1. Filter paper. It was kept in a constant temperature room at 22±3°C in the dark.

2.2.3 UV apparatus

A diagram of UV apparatus is shown in Figure 2.1. 1L reactor vessel wrapped in aluminium foil was used throughout and the contents were stirred using a magnetic stirrer. Irradiation of solution was carried out using an Engelhared Hanovia medium pressure mercury vapour photochemical reactor lamp jacketed with a water cooled quartz sleeve. Specification of the lamp is shown in Table 2.1. Emission spectrum of a typical medium pressure mercury arc lamp (Cox and Kemp, 1971) is also shown Figure 2.2 for reference. The characteristics of the light source are of major concern in photochemical research since the intensity and spectral distribution of the light

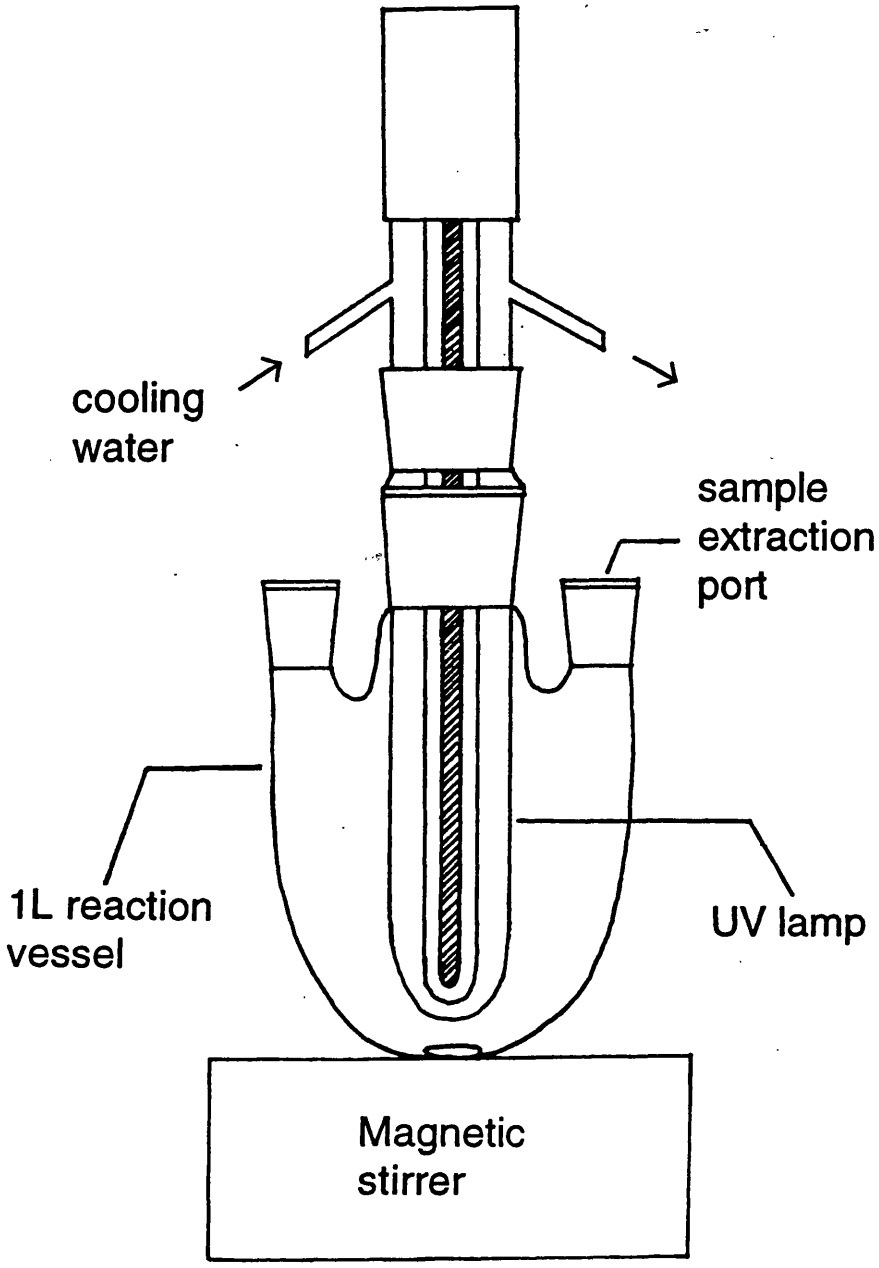


Figure 2.1 Diagram of UV apparatus

Table 2.1 Specification of Hanovia medium pressure mercury lamp

Wavelength (nm)	UV output (mw/cm^2)
254	2.9
265	4.1
297	3.2
303	4.4
313	7.6
366	10.5
405	5.7
436	9.2
546	9.1

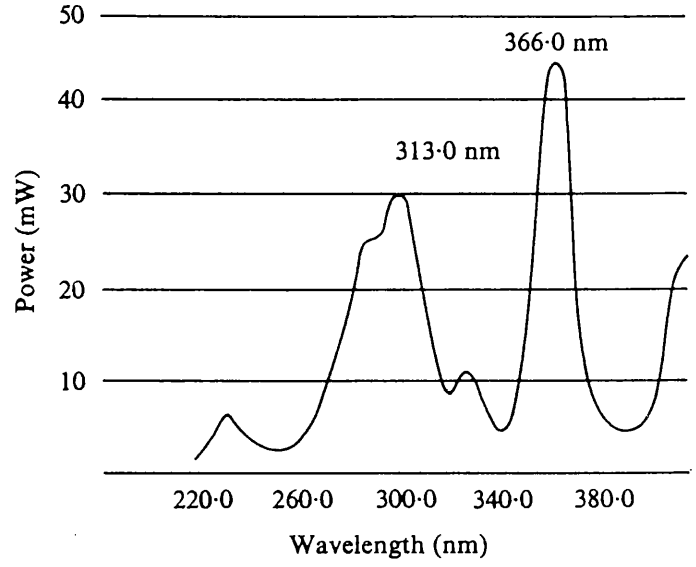


Figure 2.2 Emission spectrum of a typical medium pressure mercury arc lamp (Cox and Kemp, 1971)

source strongly influences photolysis rate. As can been seen in Figure 2.2 medium pressure mercury arc lamp emits polychromatic light rather than monochromatic light.

2.2.4 Gas chromatography

Determination of tecnazene was carried out on a Varian Model 3700 Gas chromatograph instrument equipped with a flame ionization detector (FID). The estimated low detection limit (LOD) with this analysis was approximately 0.5mg/L. GC conditions are as follows;

GC conditions

Column: Length 1m, Diameter 4mm.

Column packing: Packed with 3% OV17 (medium polarity) on a solid support.

Gas settings:

	Flow rate
N_2 (column carrier gas)	30 ml/sec
H_2 (to flame ionisation detector)	30 ml/sec
Air (to flame ionisation detector)	180 ml/sec

Temperatures:

Injector	220°C
Column oven	174°C (isothermal analysis)
Detector	250°C

Others:

Injection volume	5μl

Preparation of standard tecnazene solution for tecnazene GC analysis

1000mg/L stock solution of tecnazene dissolved in hexane was prepared. The stock solution was stored in the dark. 50, 25, 15, 5mg/L standard solutions were prepared from the stock solution. The multiple calibration line with these standards was made before each series of sample analysis since the sensitivity of GC tends to be variable on deferent day. Some example of calibration lines which made on different day for sample analysis were shown in Figure 2.3. As can been seen the slopes are variable

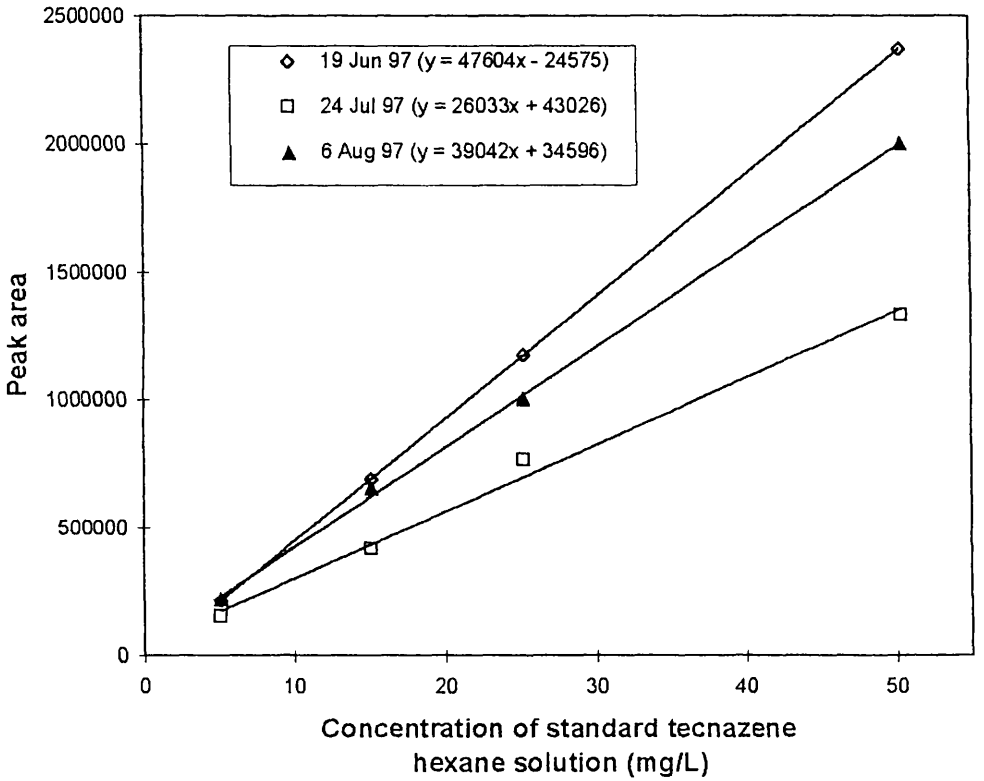


Figure 2.3 Variation of calibration line for tecnazene GC analysis on different date.

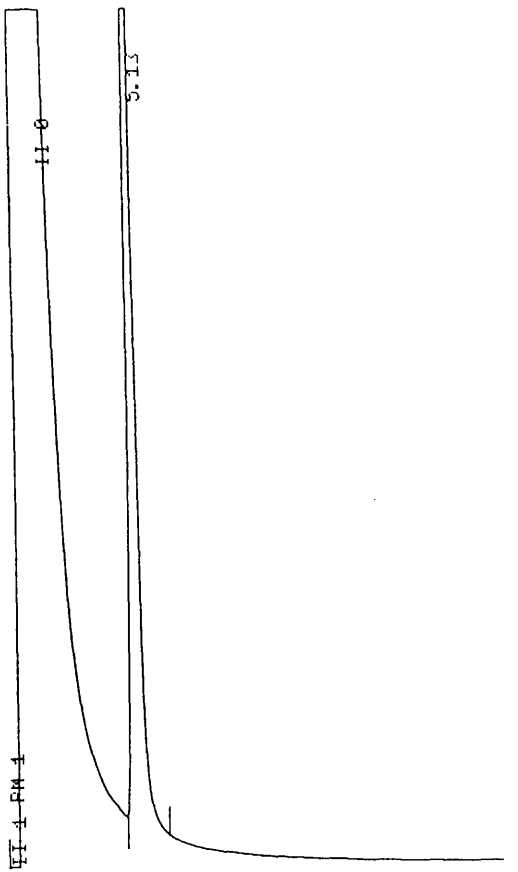


Figure 2.4 Typical Gas chromatogram of 0.25μg of tecnazene standard.

between dates although it is linear all the time. Therefore, it is necessary to make a calibration line before each series of sample analysis.

Data collection

Data collection, calibration and integration was achieved using a SP4400 Integrator (Spectra-Physics). Using the conditions described above a typical chromatogram of standard is shown in Figure 2.4.

2.3 Reproducibility of analysis of tecnazene, and preparation of saturated aqueous tecnazene solution

2.3.1 Reproducibility of analysis of tecnazene

Procedure

In order to investigate the reproducibility of analysis of tecnazene, 6 replicates of 100ml sample were withdrawn from a single batch of saturated tecnazene solution. The samples were quantitatively transferred into a 250ml separating funnel. A little sodium chloride was added in order to reduce the tendency to emulsify. 10ml hexane*) was then added and mixed well. After allowing the two layers to separate, the hexane layer was removed, and the aqueous phase was then re-extracted with a further 10ml of hexane. The hexane extracts were then bulked, and any water was removed from the hexane by adding anhydrous sodium sulphate. This was then filtered into a 100ml round bottomed flask and evaporated to dryness on a rotary evaporator with the water bath at less than 40°C to prevent tecnazene loss. The residue was redissolved in hexane and made up to a final volume of 2ml for GC analysis. GC conditions are the same as described before in Section2.2.4.

*) The distribution coefficient (k) of tecnazene between water and hexane is very high (There is no available literature data but it is possibly more than 1000). Therefore, tecnazene can be effectively extracted with even small amount of hexane. In addition, it was necessary to reduce the volume of hexane extracts for GC analysis. Hence, two 10ml portions of hexane were used for extraction of tecnazene.

Results and Discussion

The results are shown in Table 2.2. High reproducibility was obtained with 6 replicates mean value of 1.13mg/L, standard deviation of 0.045 and R.S.D. of 4.0%. Jardine (1994) also worked on reproducibility of tecnazene analysis. He extracted 25ml tecnazene saturated solution using a separating funnel with 25ml saturated sodium chloride solution and 50ml hexane, and made up to the mark in a 100ml volumetric. The hexane extract was then analysed with a GC equipped with an electron capture detector (ECD). With 4 replicates a mean value of 1.26mg/L, standard deviation of 0.035 and R.S.D. of 2.7% were obtained. Although the amount of sample and hexane were different in the extraction method, high reproducibility was also obtained by extraction with hexane followed by GC analysis. He also compared several extraction techniques, such as using a C-18 extraction column with hexane and extraction using a separating funnel with hexane. He concluded that reproducibility within the extraction techniques was greatest with the shaking method. In addition, the inclusion of sodium chloride in shaking had a slight advantage compared to without it.

Table 2.2 Reproducibility of analysis of tecnazene with 6 replicates from a single batch of saturated aqueous tecnazene solution.

Concentration of tecnazene solution (mg/L)	Mean value (mg/L)	Standard deviation	R.S.D (%) ((Stdev/mean)×100)
1.08 1.20 1.10 1.10 1.16 1.14	1.13	0.045	4.0

2.3.2 Reproducibility of preparation of saturated aqueous tecnazene solution

Procedure

Reproducibility of preparation of saturated tecnazene aqueous solution was calculated with 10 independent batches. The extraction method and GC analysis were same as described previously in Section 2.3.1.

Results and Discussion

The results are shown in Table 2.3. The concentration of saturated tecnazene solution was very variable in the 10 different batches. With 10 independent batches mean value of 0.85mg/L (maximum and minimum value were 1.40mg/L and 0.45mg/L), standard deviation of 0.30 and R.S.D. of 35.0% were obtained. In the previous section high reproducibility of analysis of tecnazene was obtained. Therefore, the variation in the concentration of saturated tecnazene solution is likely to be caused mostly by its preparation. Tecnazene powder is very hydrophobic so it is difficult to mix with water even with agitation. This could be one reason for the low reproducibility in preparation.

As mentioned before tecnazene is practically insoluble in water. Leonard (1988) reported that solubility of tecnazene in water was 0.9mg/L at 20°C, and Jardine (1994) obtained an average concentration for the solubility of tecnazene in water of 1.19mg/L.

Table 2.3 Reproducibility of preparation of 10 independent batches of saturated aqueous tecnazene solution.

Concentration of tecnazene solution (mg/L)	Mean value (mg/L)	Standard deviation	R.S.D (%) ((Stdev/mean)×100)
1.41			
0.96			
0.80			
0.69			
0.56	0.85	0.30	35.0
0.92			
1.00			
0.56			
0.45			
1.13			

2.4 The absorption spectrum of tecnazene solution

The absorption spectrum of a saturated aqueous solution of tecnazene was measured by ultraviolet spectroscopy. Absorption character is important for photolysis since direct photolysis occurs due to direct absorption of UV light by substances (Zepp and

Cline, 1977). In the case of tecnazene, as can be seen in Figure 2.5 it absorbs UV light well, and the peak absorption was at 202.6nm. Therefore, it can be observed that tecnazene is likely to be removed by UV light.

2.5 Decomposition of tecnazene with hydrogen peroxide alone

Dark controls were carried out with hydrogen peroxide alone in order to monitor if there is chemical oxidation and hydrolysis of tecnazene in water. The change in the concentration of hydrogen peroxide was also determined at the same time.

2.5.1 Experimental methods

Procedure

1L saturated aqueous tecnazene solution was transferred into an 1L conical flask wrapped in aluminium foil to prevent any photolysis by sunlight. It was placed in a constant temperature room (22±3°C) and then mixed continuously using a magnetic stirrer. 1ml and 5ml of 30% hydrogen peroxide solution were added to separate flasks to give a starting concentrations of about 10mM and 50mM respectively. 100ml samples were withdrawn using a 100ml bulb pipette at intervals for the determination of tecnazene. The samples treated with 10mM hydrogen peroxide were then transferred to a 100ml conical flask containing 1ml of 1M sodium meta-bisulphite solution. The samples treated with 50mM hydrogen peroxide were transferred to a 100ml conical flask containing 5ml of 1M sodium meta-bisulphite solution which reduces residual hydrogen peroxide immediately. The sample for hydrogen peroxide analysis was withdrawn at the same time. 100ml for the sample treated with 10mM, and 20ml for the sample treated with 50mM were withdrawn at intervals.

Extraction and GC analysis method

The withdrawn sample was extracted with hexane and concentrated into 2ml for GC analysis. The extraction procedure is the same as described in Section 2.3.1. GC condition was described previously in Section 2.2.4.

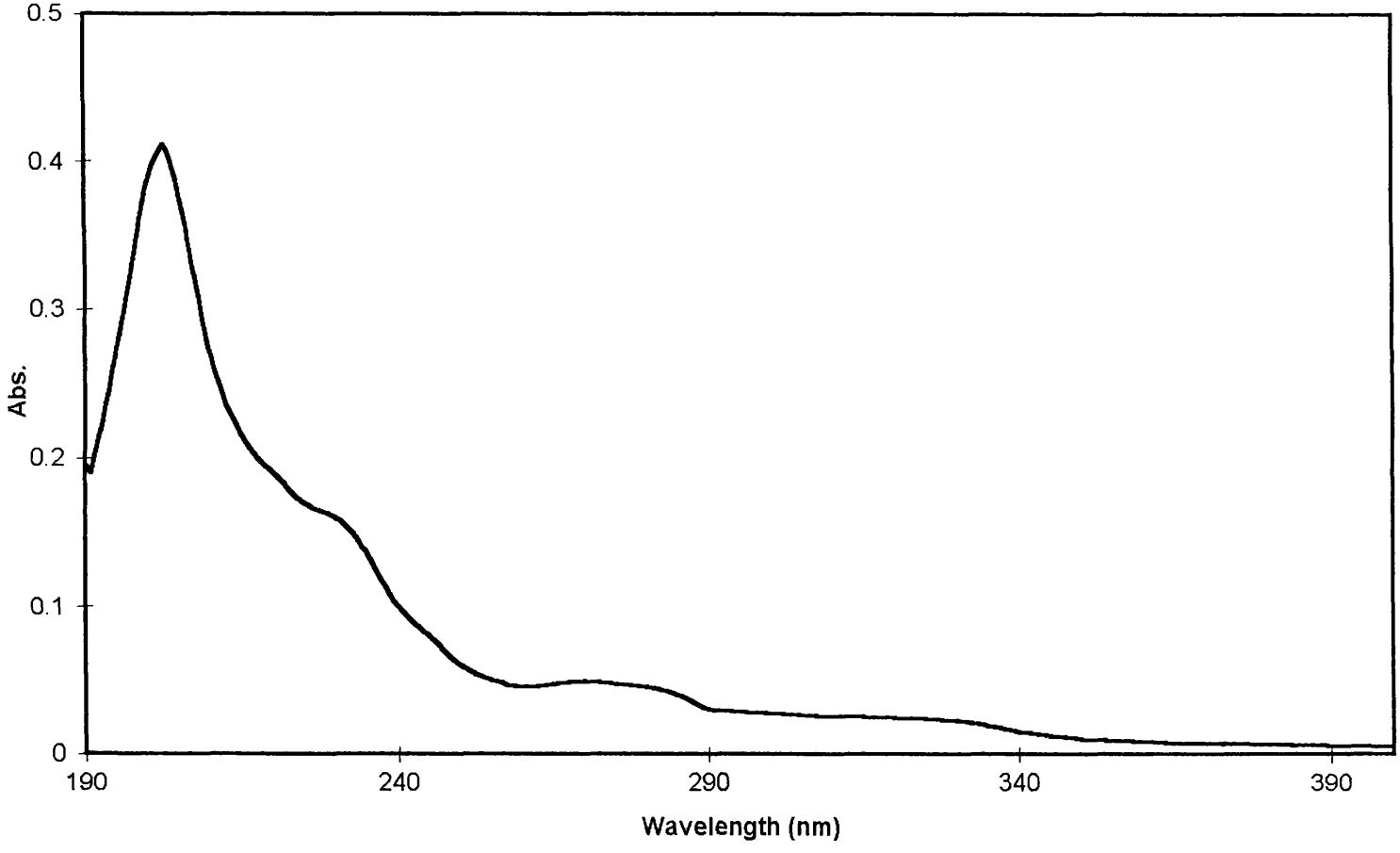


Figure 2.5 The absorption spectrum of tecnazene in aqueous solution

Analysis of hydrogen peroxide

Permanganate titration was employed for the determination of hydrogen peroxide. A 100ml of solution with an initial hydrogen peroxide concentration of 10mM or 20ml of solution with 50mM hydrogen peroxide was transferred into a conical flask. 20ml dilute sulphuric acid (1:5) was then added, and titrated with standard 50mM potassium permanganate to the first permanent, faint pink, colour.

2.5.2 Results and Discussion

The change of the hydrogen peroxide concentration and the remaining percentage of tecnazene during the treatment with hydrogen peroxide alone is shown in Figures 2.6 and 2.7, which compares two experiments with different initial concentrations of hydrogen peroxide. There were negligible changes in concentration of tecnazene and hydrogen peroxide in both conditions. Therefore, it can be observed there was no reaction, such as oxidation and hydrolysis, to decompose tecnazene under these conditions.

2.6 Decomposition of tecnazene in water using UV with and without hydrogen peroxide

2.6.1 Experimental methods

Irradiation procedure

Irradiation of 1L of saturated tecnazene solution was carried out using the UV apparatus described previously. The temperature was maintained at 22±3°C by circulation of cold water through the cooling jackets of the apparatus. 100ml samples were collected at intervals. In the case of UV irradiation in the presence of hydrogen peroxide, withdrawn samples were transferred to glass bottles containing sodium meta-bisulphite solution which reduces residual hydrogen peroxide immediately. Two different concentrations of hydrogen peroxide, 0.1mM and 1mM, were employed. The final concentrations of sodium meta-bisulphite were 0.1mM and 1mM respectively.

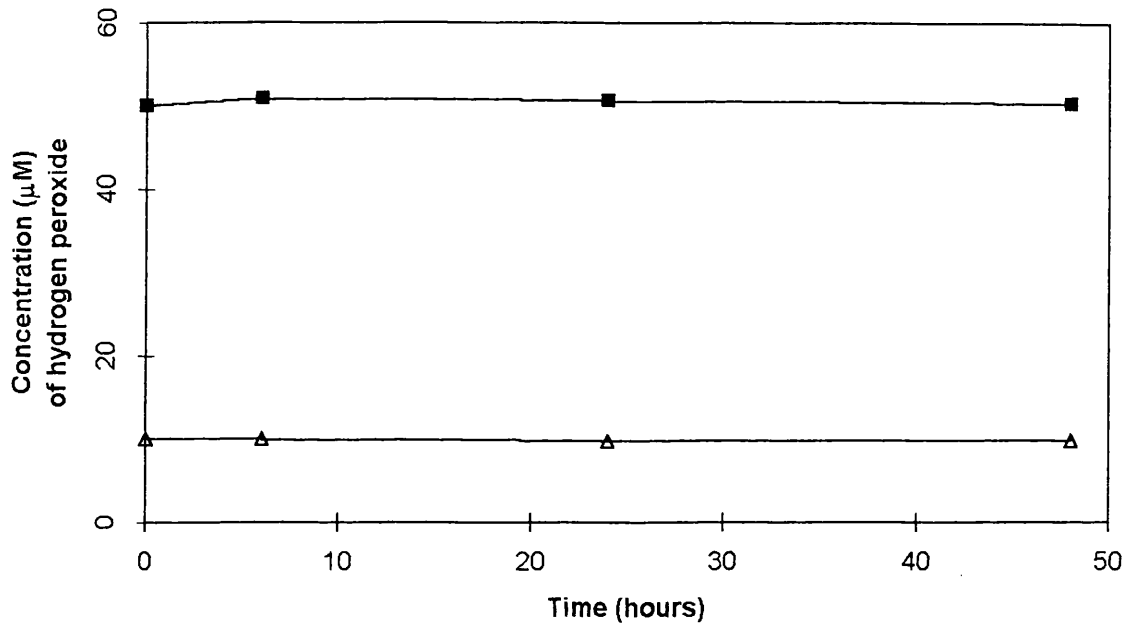


Figure 2.6 Change in the concentration of hydrogen peroxide

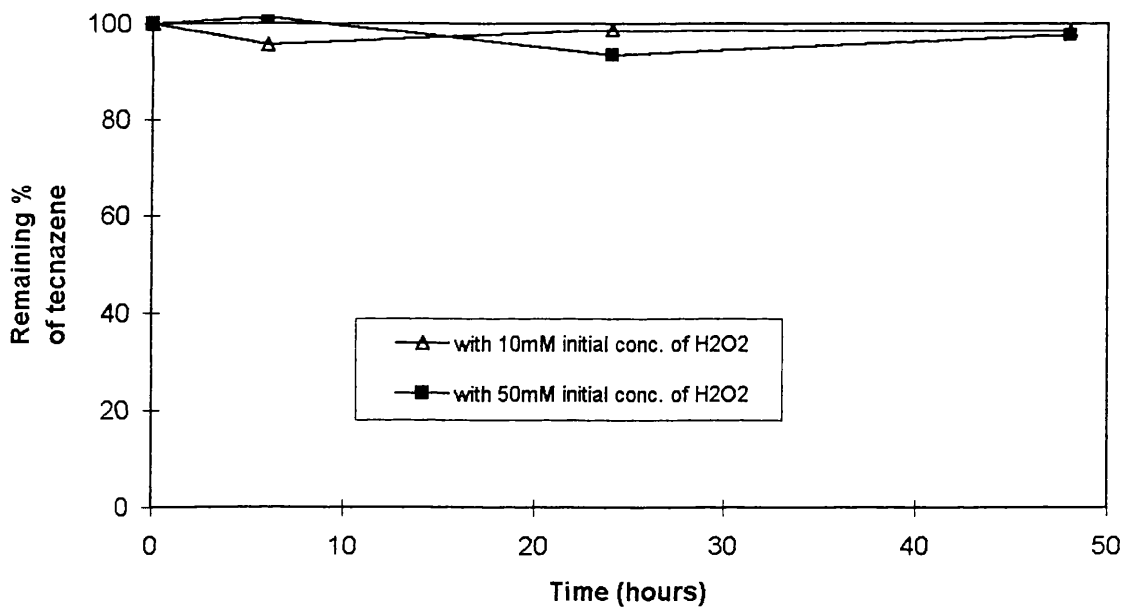


Figure 2.7 Dark control with hydrogen peroxide alone for decomposition of tecnazene

Extraction and GC analysis method

The withdrawn sample was extracted with hexane and concentrated into 2ml for GC analysis. The extraction procedure is the same as described in Section 2.3.1. GC condition was described previously in Section 2.2.4.

2.6.2 Results and Discussion

The GC chromatograms for different irradiation times in the photodecomposition by UV alone and UV with 1mM hydrogen peroxide are given in Figures 2.7 and 2.8.

As mentioned before in Section 2.3.2 the starting tecnazene concentration were variable because of the poor reproducibility in the preparation of the saturated aqueous tecnazene solution. Therefore, the remaining percentage of tecnazene is plotted as (Ct/Co)×100, where Ct and Co are the concentration of tecnazene at time t and zero, rather than concentration against irradiation time in order to make the starting point the same. The result of photodecomposition with UV only is shown in Figures 2.10, and photodecomposition in the presence of hydrogen peroxide was shown in 2.11 with some replicates. These replicates show good agreement. According to these results UV irradiation treatment of tecnazene in pure water leads to a rapid decomposition in either the presence or absence of hydrogen peroxide. The lines drawn through the mean values of replicates at each time points are given in Figure 2.12. for comparison between treatments. As can be seen the sample treated with UV light in the presence of hydrogen peroxide was photodecomposed relatively faster than UV light alone. The decomposition rate increased with the increase in the concentration of hydrogen peroxide. Almost complete decomposition was achieved by 10 minutes irradiation with an initial hydrogen peroxide concentration of 1mM, by 20 minutes with 0.1mM hydrogen peroxide. The levels of tecnazene were below LOD by 15 minutes of UV irradiation with 1mM hydrogen peroxide.

As can be seen in Figures 2.8 and 2.12, in the case of photodecomposition with UV light alone, the remaining percentage was high (more than 90%) at the very first stage of photodecomposition (after a few minutes). The possible reason for this is that UV

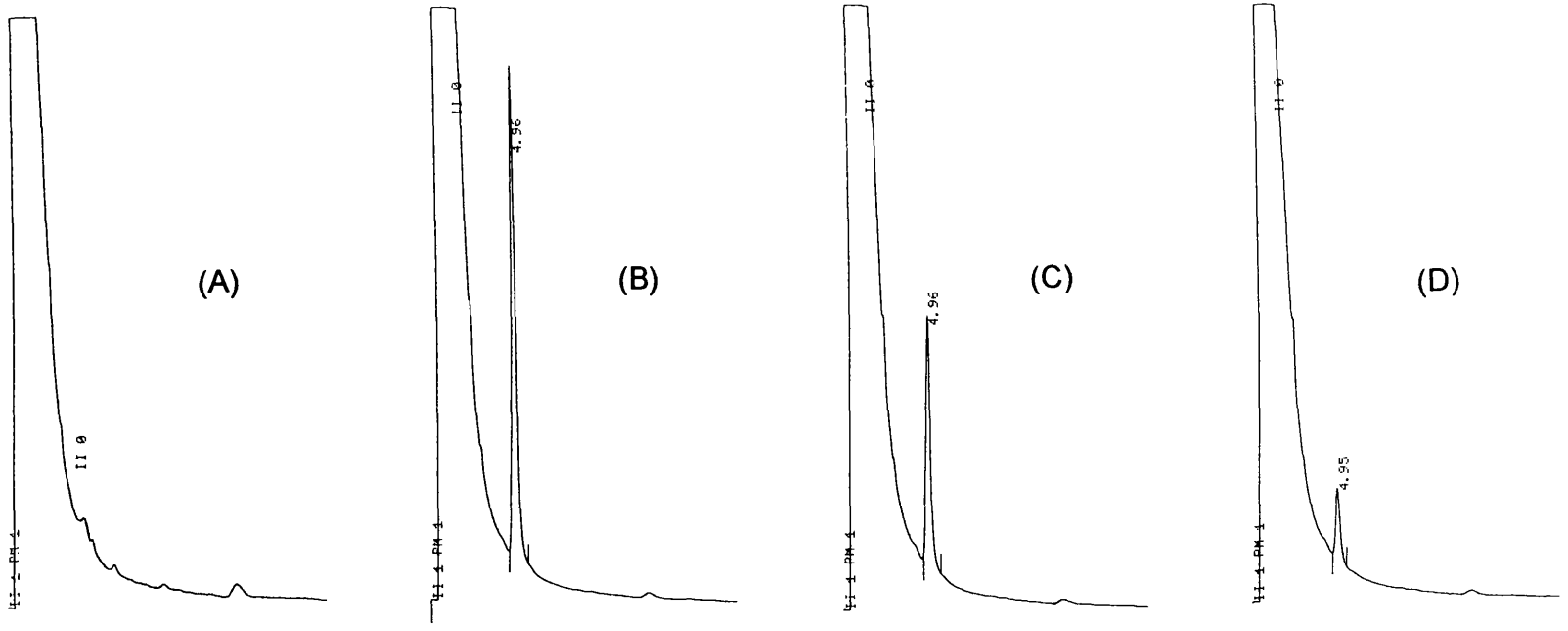


Figure 2.8 GC chromatograms from tecnazene irradiated by UV light in water for (B) 0min, (C) 5min and (D) 10min. (A) is control.

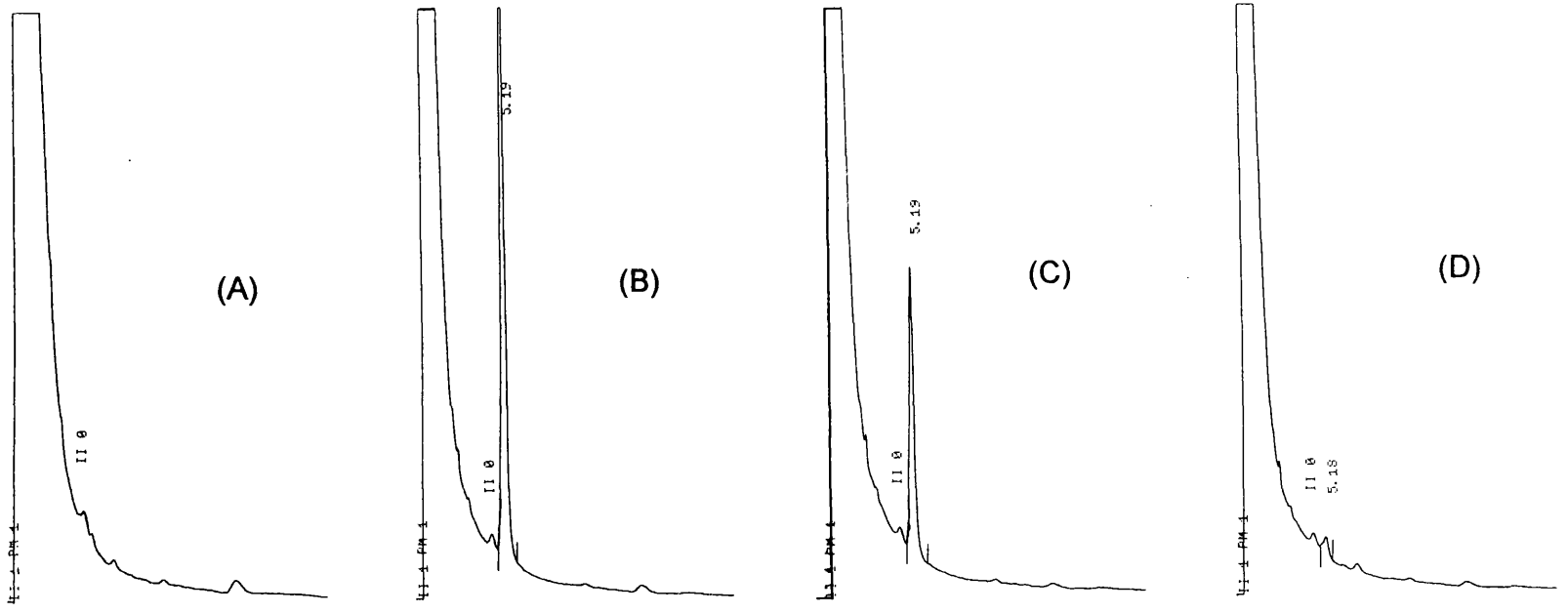


Figure 2.9 GC chromatograms from tecnazene irradiated by UV light with 1mM H_2O_2 in water for (B) 0min, (C) 5min and (D) 10min. (A) is control.

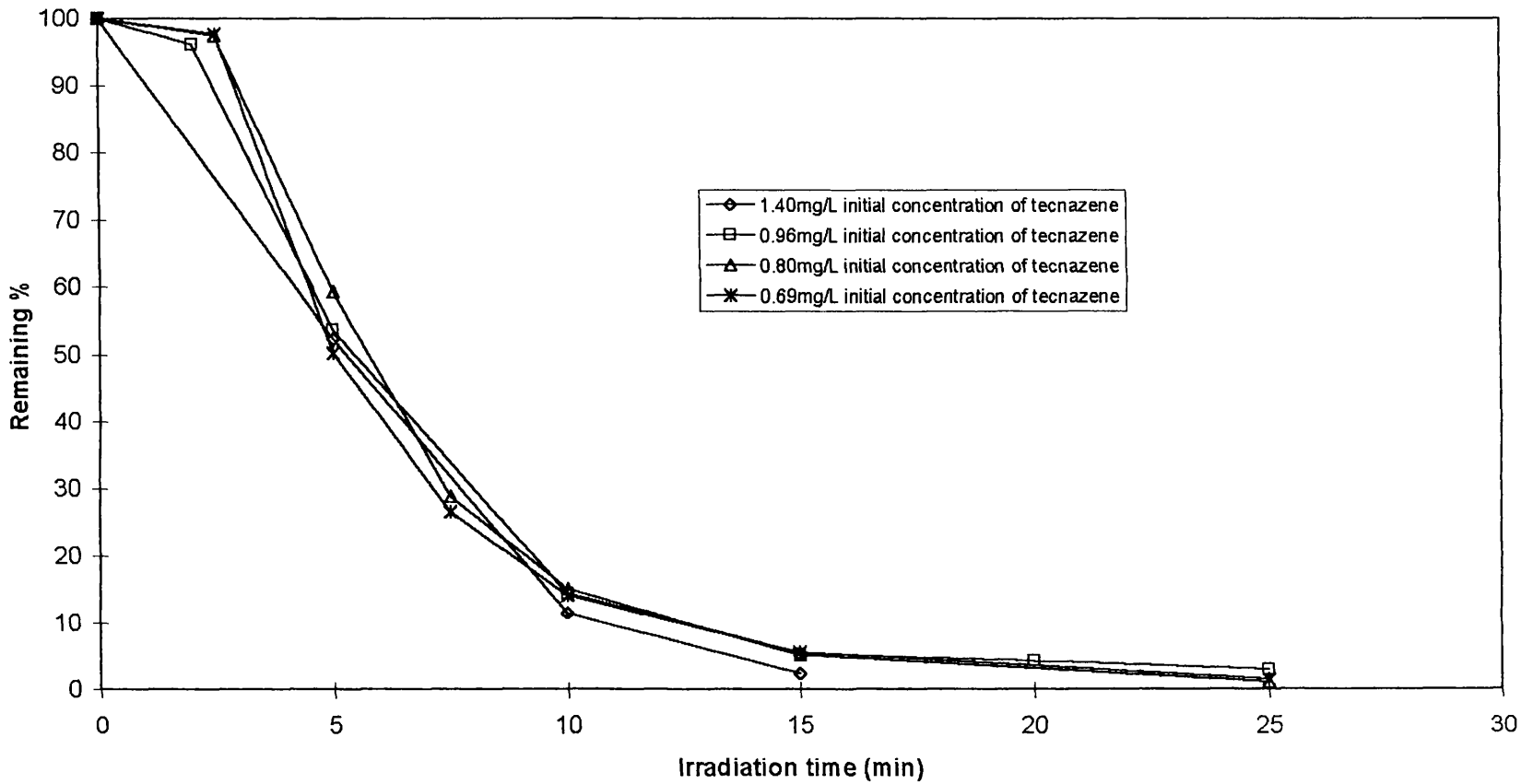


Figure 2.10 Decomposition of tecnazene with UV alone in water (4 individual runs using different batches of saturated tecnazene solution) - normalised data.

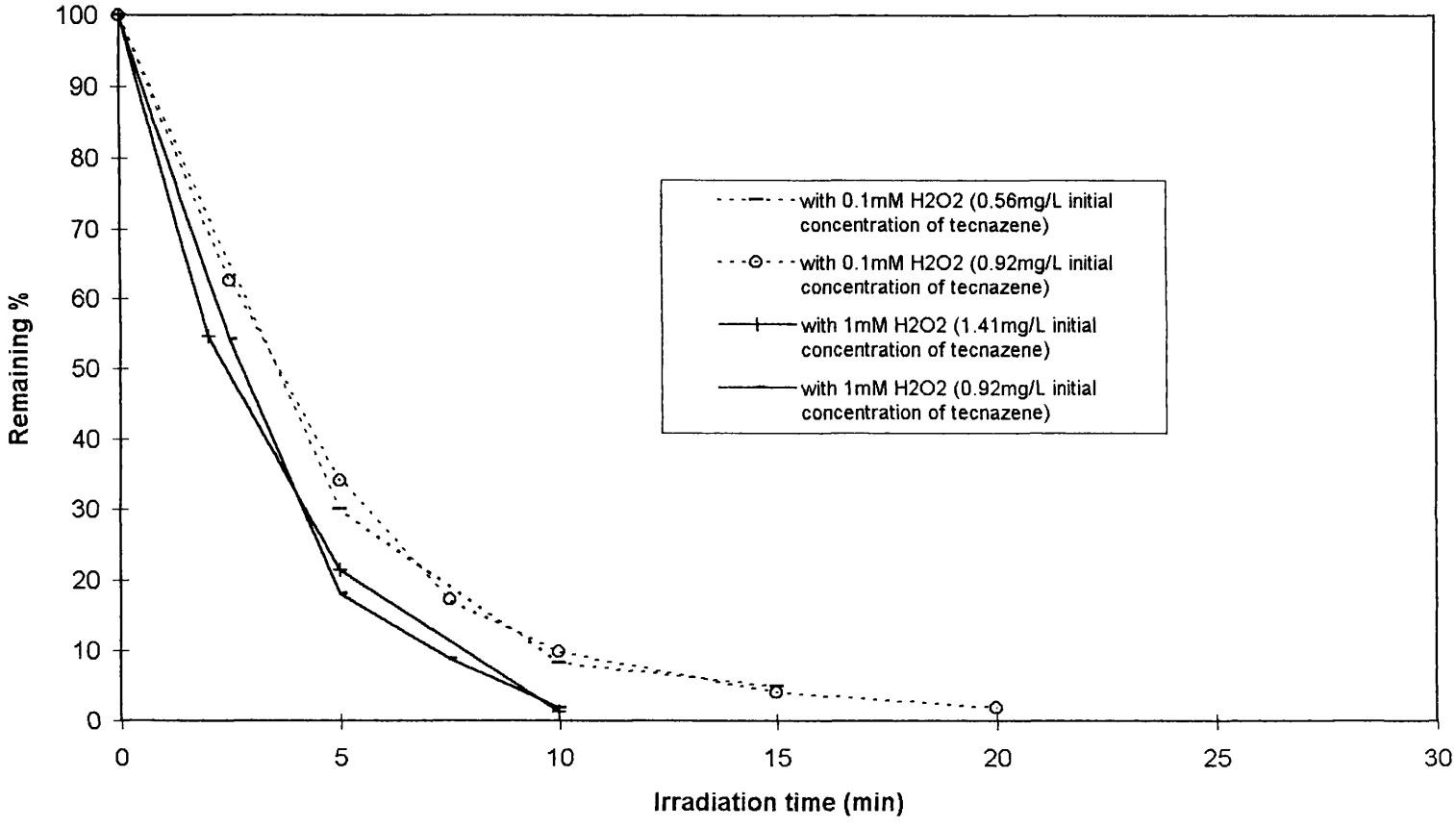


Figure 2.11 Decomposition of tecnazene with UV in the presence of hydrogen peroxide (2 each individual runs using different batches of saturated tecnazene solution) - normalised data.

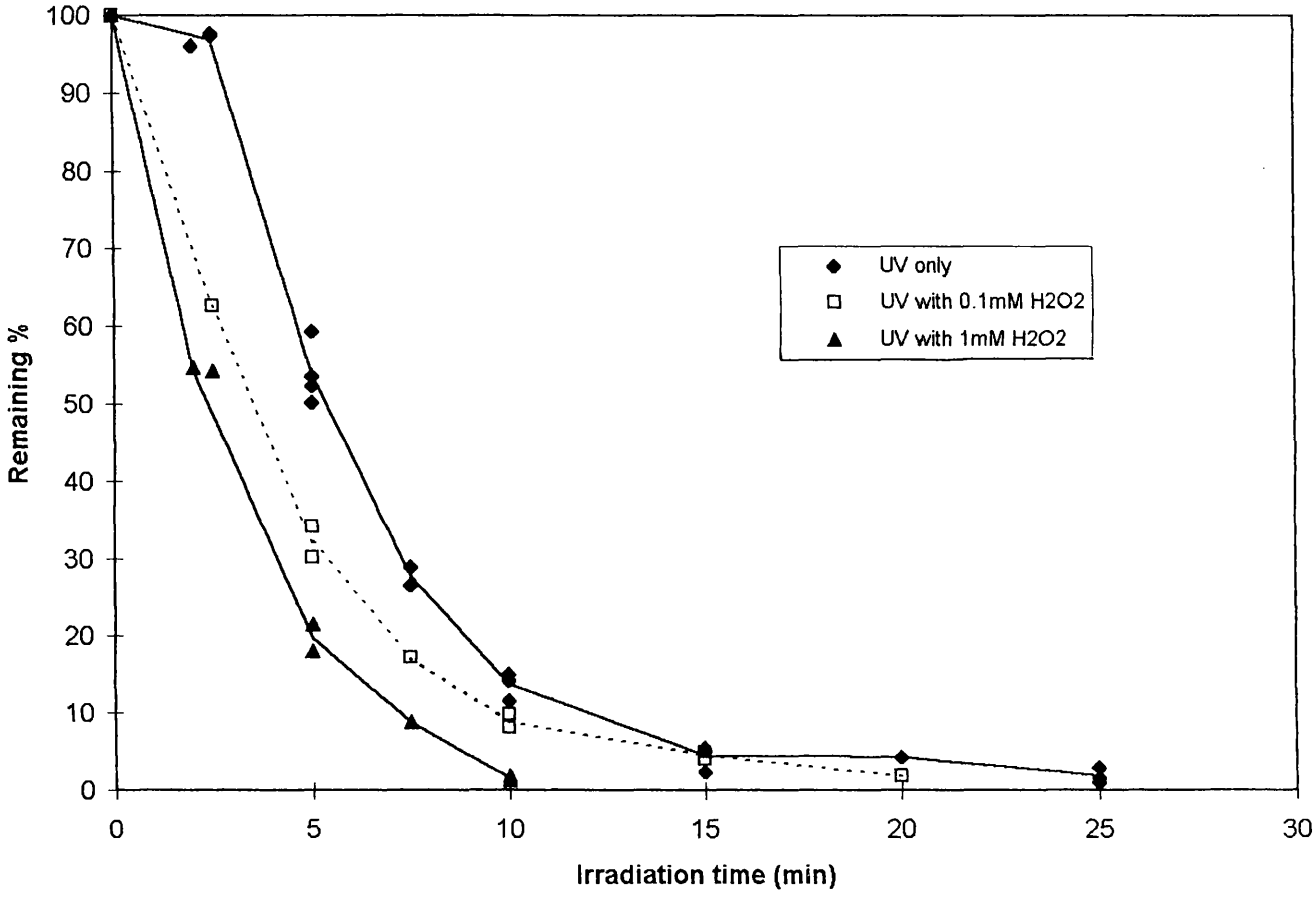


Figure 2.12 Effect of treatment on the photodecomposition of tecnazene. Replicates at each time points are independent runs with the line drawn through the mean value.

lamp could have been warming up at the first stage of the irradiation time because UV lamp was switched on at irradiation time zero. The UV lamp took some time to reach maximum output power. However, in the presence of hydrogen peroxide this phenomenon did not appear in both concentrations although the lamp was switched on in the same way. In other words in the presence of hydrogen peroxide, tecnazene is likely to be quickly photodecomposed even with low energy output of the UV lamp.

The reason for different decomposition rates on UV irradiation between samples with and without hydrogen peroxide is that hydroxyl radicals are produced as a consequence of photolysis of hydrogen peroxide as described in Section 2.1. Therefore, it can be concluded that in the case of UV irradiation in the presence of hydrogen peroxide tecnazene was photodecomposed by UV light (direct photolysis) and hydroxyl radical oxidation reaction (indirect photolysis), while tecnazene was photodecomposed by UV light (direct photolysis) only in the case of UV irradiation without hydrogen peroxide.

Beltran *et al.* (1996) pointed out that there was an optimum concentration of hydrogen peroxide in their study of photolysis of deethylatrazine and deisopropylatrazine as first decomposition products of atrazine using UV irradiation in the presence of hydrogen peroxide. It was reported that a greater amount of hydrogen peroxide led to quenching of the hydroxyl radicals and produced hydroperoxyl radicals (Eqn. 2) (Buxton *et al.*, 1988) which were much less reactive and scarcely oxidised organic substances (Legrini *et al.*, 1993).

$$\cdot OH + H_2O_2 \rightarrow H_2O + HO_2\cdot \leftrightarrow O_2\cdot^- + H^+ \qquad (2)$$

$$k = 2.7\times10^7\ M^{-1}\ s^{-1}$$

Furthermore, absorption of UV light by substances is less intense due to high concentrations of hydrogen peroxide, and this reduces the rate of direct photolysis.

Competition with hydroxyl radical scavengers, such as additives and hydrogen peroxide itself is one of main factors which limit the oxidation rate of organic

pollutants with hydroxyl radicals. The rate constants for reaction of hydroxyl radicals with organic pollutants is usually used for predicting the oxidation rate. Although the hydroxyl radical reaction rate constant with tecnazene is not available in the available literature, hydroxyl radicals react with polychlorinated aromatic compounds (trichlorobenzenes and dichlorobenzenes) relatively faster (10^9 M^{-1} s^{-1}) (Haag and Yao, 1992) which is faster than the rate constant of hydroxyl radicals with hydrogen peroxide. Therefore, because tecnazene is an example of polychlorinated aromatic compounds, hydroxyl radical reaction rate constant with tecnazene is possibly high which could also be faster than rate constant with hydrogen peroxide itself. This could be the reason that the decrease in decomposition rate of tecnazene due to a higher concentration of hydrogen peroxide as described above was not observed on this occasion. Because of the possible high rate constant of hydroxyl radicals with tecnazene UV irradiation treatment of tecnazene in the presence of hydrogen peroxide seems to be effective, and photodecomposition rate increased with the increase in the concentration of hydrogen peroxide.

Half-life

Generally the photodecomposition of pesticide is expected to follow the first-order kinetics (Eqn. 3):

$$\ln(Ct/Co) = kt \qquad (3)$$

Where Ct and Co are the concentration of pesticide at time t and zero,
k is the photolysis rate constant and t is time.

Therefore, the half-life ($t_{1/2}$) for the photodecomposition of the pesticides can be determined by (Eqn. 4):

$$t_{1/2} = \ln(1/2)/k = -0.693/k \qquad (4)$$

The rate constant (k) can be obtained as the slope of a straight line plotted ln(Ct/Co) against t. In Figure 2.13 ln(Ct/Co) of tecnazene is plotted against the irradiation time in the case of UV only. In order to reduce the experimental error resulted from warming up time of the UV lamp, the regression line was calculated without the value at irradiation time zero. In addition, in the range of low remaining percentage, small

differences within replicates are magnified as a results of calculation of ln(Ct/Co). Therefore, it was decided to reject the values where Ct was less than 5% (ln(Ct/Co) ≅ -3). As can be seen in Figure 2.13, in these conditions first-order kinetics is followed. In the case of photodecomposition of tecnazene in the presence of hydrogen peroxide, the kinetic can be complicated because the radical type of reaction is involved. However, as can be seen in Figure 2.14 the first-order seems to be followed. The points at irradiation time zero was included at this time because regression lines seem to be pass through that point. ln(Ct/Co) value of less than -3 were excluded as well in order not to magnify small differences. The half-life of tecnazene in each conditions of treatments was calculated using equation (4), and is given in Table 2.4 with the rate constant values calculated as slopes. It seems there is no significant difference in half-life between the treatment with UV only and in the presence of 0.1mM hydrogen peroxide although the photodecomposition rate was faster in the presence of hydrogen peroxide in Figure 2.12. As mentioned before there is experimental error because of warming up time of UV lamp. Therefore, half life can not be simply compared between treatment. In the case of UV irradiation with 1mM hydrogen peroxide half life seems to be high compared with other two treatments. However, more replicates are needed for statistical comparison.

Table 2.4 Half-life of tecnazene in water

Treatment method	UV only	UV with H_2O_2	
		with 0.1mM	with 1mM
Rate constant(k) (Slope)	-0.303	-0.251	-0.309
	-0.229	-0.238	-0.335
	-0.241		
	-0.230		
Mean	-0.251	-0.245	-0.322
Standard deviation	0.035		
Half-life (min)	2.76	2.83	2.15

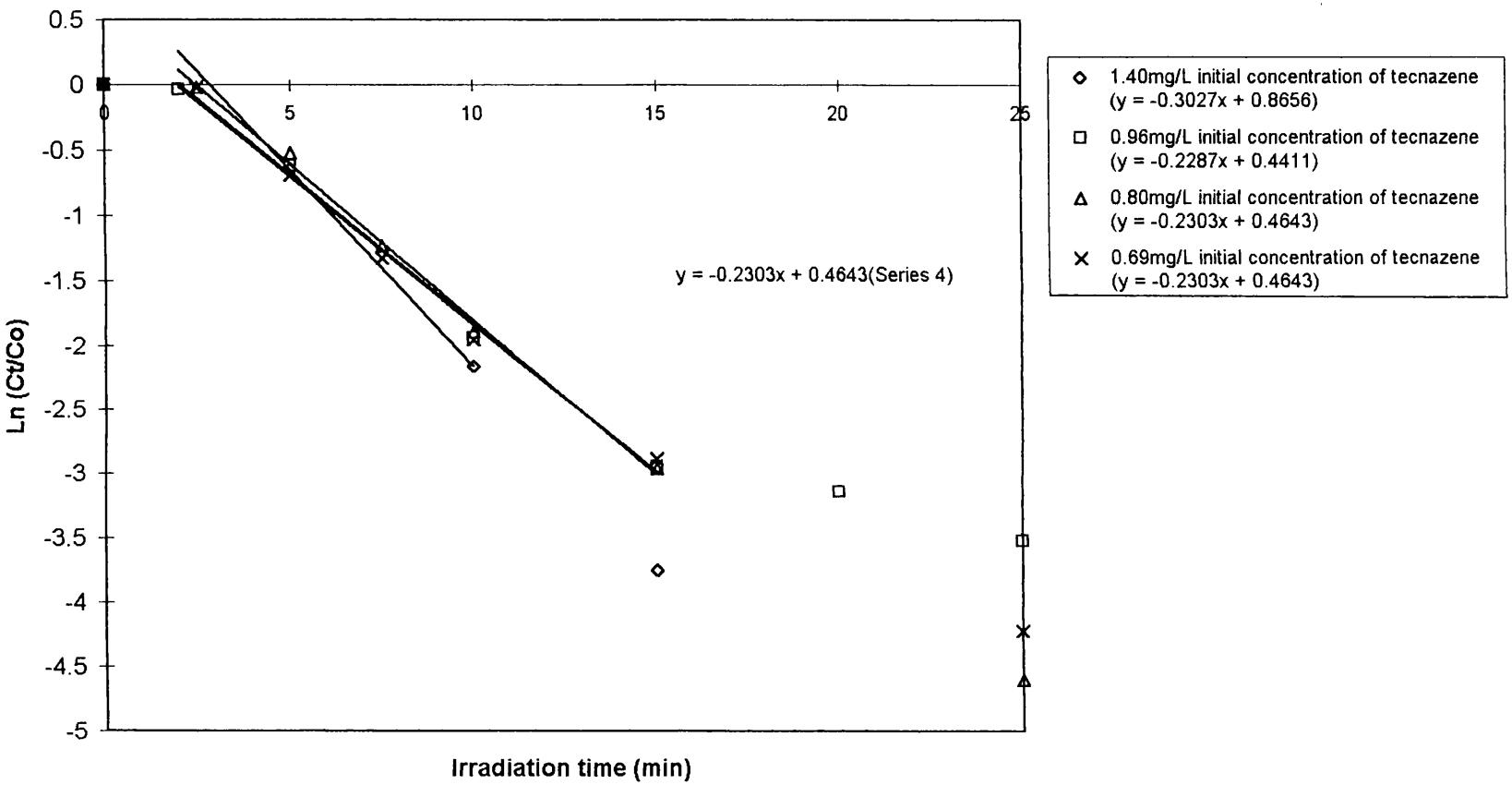


Figure 2.13 First-order treatment of the photodecomposition (by UV only) data for tecnazene in water (4 individual runs using different batches of saturated tecnazene solution). The data of ln(Ct/Co) less than -3 were excluded for first order treatment.

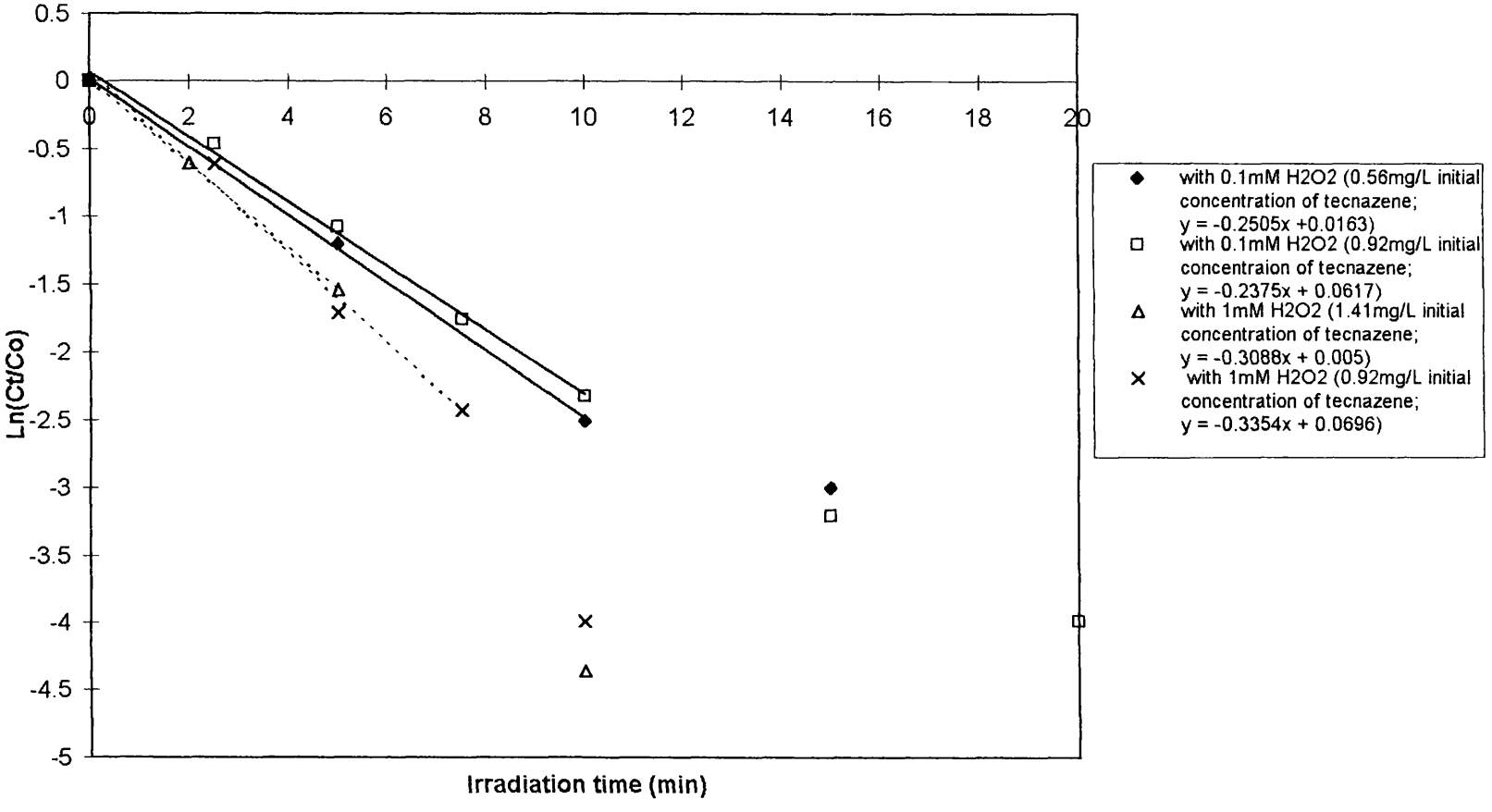


Figure 2.14 First-order treatment of the photodecomposition (by UV in the presence of hydrogen peroxide) data for tecnazene in water (2 each individual runs using different batches of saturated tecnazene solution).
The data of ln(Ct/Co) less than -3 were excluded for first order treatment.

2.7 Decomposition of tecnazene in water-acetonitrile mixtures using UV with and without hydrogen peroxide

2.7.1 Experimental methods

UV irradiation of tecnazene in 10% (v/v) acetonitrile-water mixtures was carried out using the UV apparatus described previously. The temperature was maintained at 22±3°C by circulation of cold water through the cooling jackets of the apparatus. 100ml samples were withdrawn at intervals. The samples were then extracted with hexane and concentrated into 2ml for GC analysis. The extraction method was the same as described in section 2.3.1. GC condition was described previously in Section 2.2.4. The each point of data was obtained from the mean of duplicate. As control 10%(v/v) acetonitrile-water mixture was irradiated without tecnazene. Irradiation procedure, extraction method and GC analysis for control were same as described above.

In the case of decomposition using UV light only, 10mg/L of tecnazene concentration was used in the first set up. However, the photolysis rate was slower than in water (Figure 2.18). The initial concentration of tecnazene in water averaged 0.85mg/L, while in 10% (v/v) acetonitrile-water mixtures it was 10.0mg/L that was almost 10 times higher than in water. Therefore, different initial concentrations were set up in order to investigate the affect of initial concentration against the decomposition rate. 10% (v/v) acetonitrile-water mixtures was used for both experiments to make the conditions the same. UV irradiation of 5.0mg/L and 1.0mg/L of tecnazene in 10% (v/v) acetonitrile-water mixtures were carried out.

In the case of UV irradiation with hydrogen peroxide in water-acetonitrile mixtures two different initial concentrations of tecnazene, 10.0 and 1.0mg/L, were set up, and a 1mM concentration of hydrogen peroxide was used for both conditions. The withdrawn samples were transferred to glass bottles containing sodium meta-bisulphite solution which reduces residual hydrogen peroxide immediately. The final concentration of sodium meta-bisulphite was 1mM.

2.7.2 Results and Discussion

Decomposition of tecnazene in water-acetonitrile mixtures using UV only

GC chromatograms at different irradiation time points of control and a sample are given in Figures 2.15 and 2.16. The decomposition rate was found to decrease with the decrease of initial concentration of tecnazene, and the decomposition rate was almost the same as in water when 1.0mg/L tecnazene was irradiated (Figure2.18). Therefore, the major reason for the difference in the photolysis rate appears to be the initial concentration of tecnazene, and acetonitrile itself seems to have no effect on the photolysis rate. This result led to the conclusion that the initial concentration of tecnazene is one important factor governing the rate of decomposition. This aspect that the photolysis rate depends on the initial concentration of compounds was also reported by Benitez *et al.* (1994) in experiments of decomposition of an organic pollutant using UV light.

Decomposition of tecnazene in water-acetonitrile mixtures using UV irradiation in the presence of hydrogen peroxide

GC chromatograms at different irradiation time points are given in Figures 2.17 (also see Figure 2.15 as control). The graph plotted remaining percentage against irradiation time is shown in Figure 2.19. When starting the decomposition of tecnazene in 10% (v/v) acetonitrile using UV irradiation in the presence of hydrogen peroxide, a different behaviour was observed compared with water. In the case of water hydrogen peroxide accelerated the decomposition rate during UV irradiation, and the decomposition rate increased with the increase in the concentration of hydrogen peroxide. However, in the case of 10% (v/v) acetonitrile-water mixtures, hydrogen peroxide had no effect on the decomposition rate for 10.0mg/L initial concentration of tecnazene, while hydrogen peroxide slowed down the decomposition rate in the case of 1.0mg/L initial concentration of tecnazene.

Acetonitrile itself was found to be decomposed by UV light and produced inorganic and organic ions (see Chapter 3). Although it was not investigated, it could be possible to produce substances that cause reduction of decomposition rate using UV light in the presence of hydrogen peroxide.

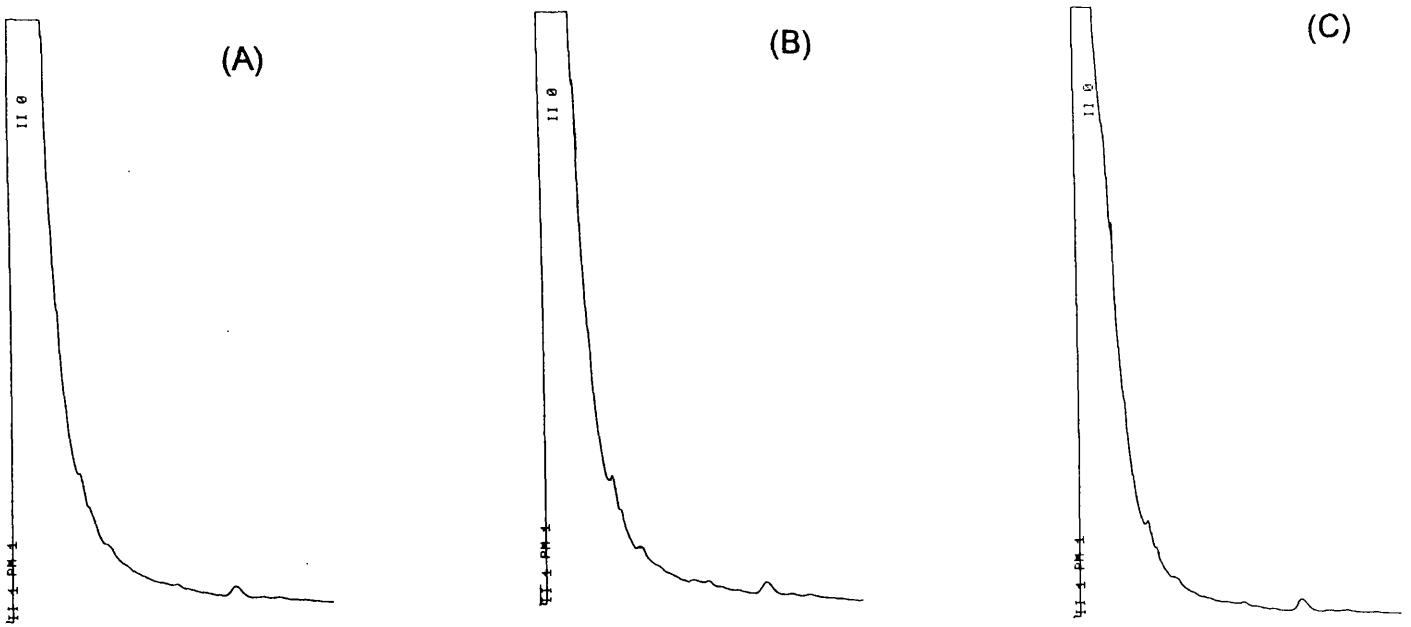


Figure 2.15 GC chromatograms of control. 10%(v/v) acetonitrile-water mixtures was irradiated by UV light without tecnazene for (A) 0min, (B) 40min and (C) 80min.

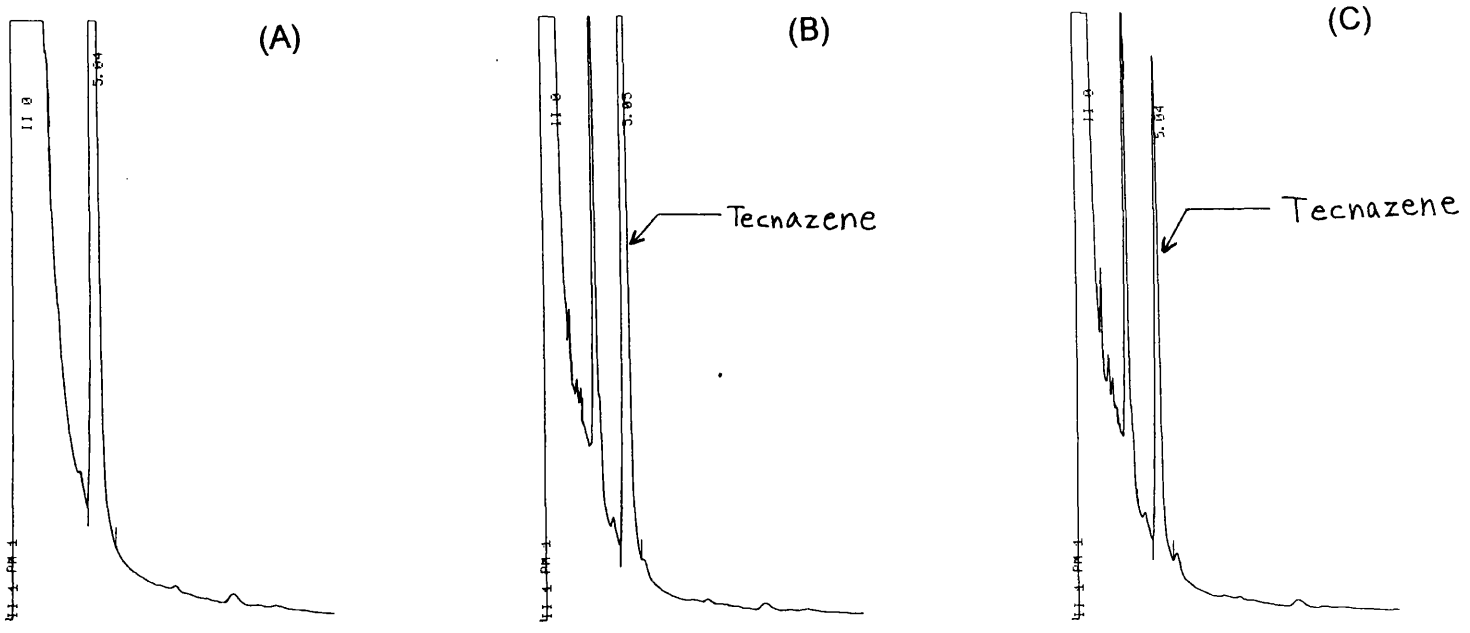


Figure 2.16 GC chromatograms from tecnazene irradiated by UV light in 10%(v/v) acetonitrile-water mixtures for (A) 0min, (B) 40min and (C) 80min. Initial concentration of tecnazene; 10mg/L.

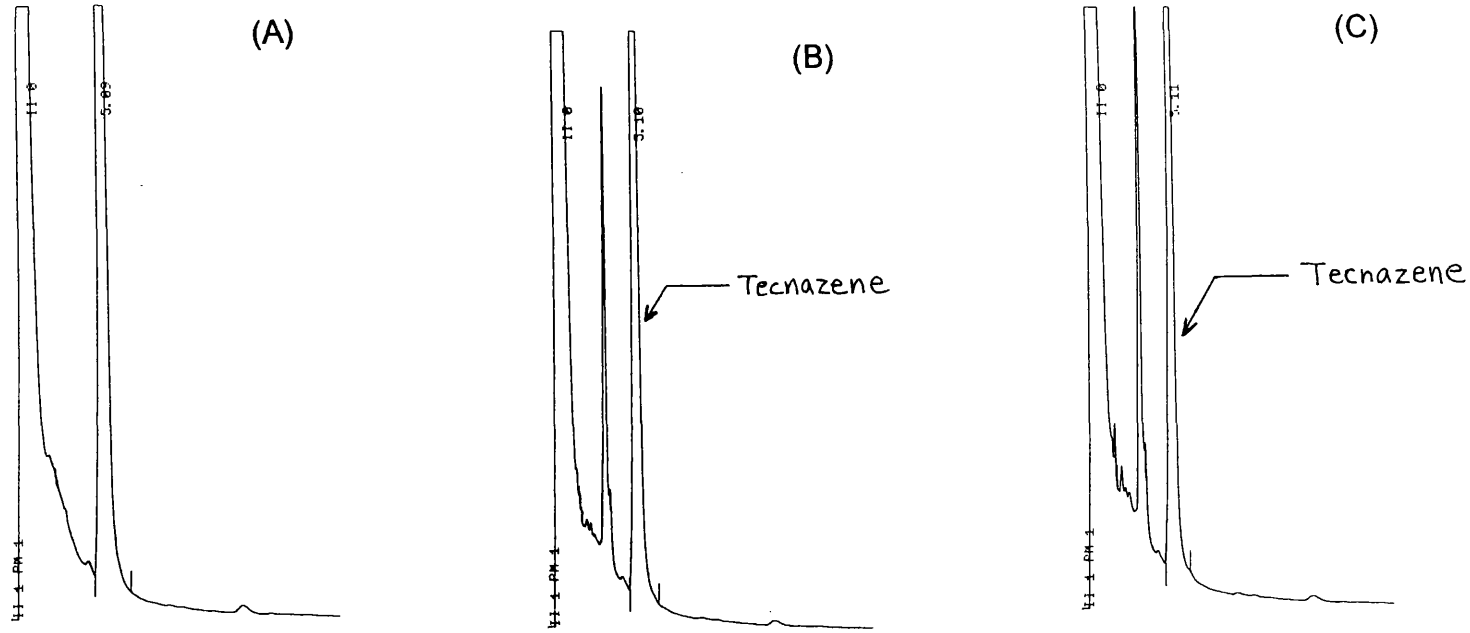


Figure 2.17 GC chromatograms from tecnazene irradiated by UV light with 1mM H_2O_2 in 10%(v/v) acetonitrile-water mixtures for (A) 0min, (B) 40min and (C) 80min. Initial concentration of tecnazene; 10mg/L.

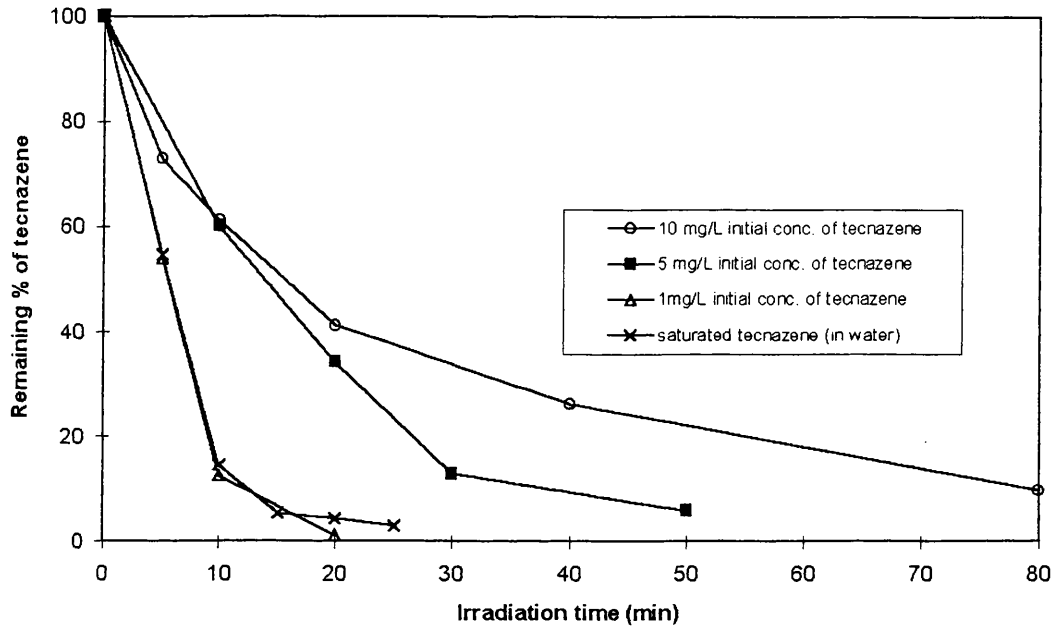


Figure 2.18 Decomposition of tecnazene in 10%(v/v) acetonitrile-water mixtures (influence of initial concentration of tecnazene)

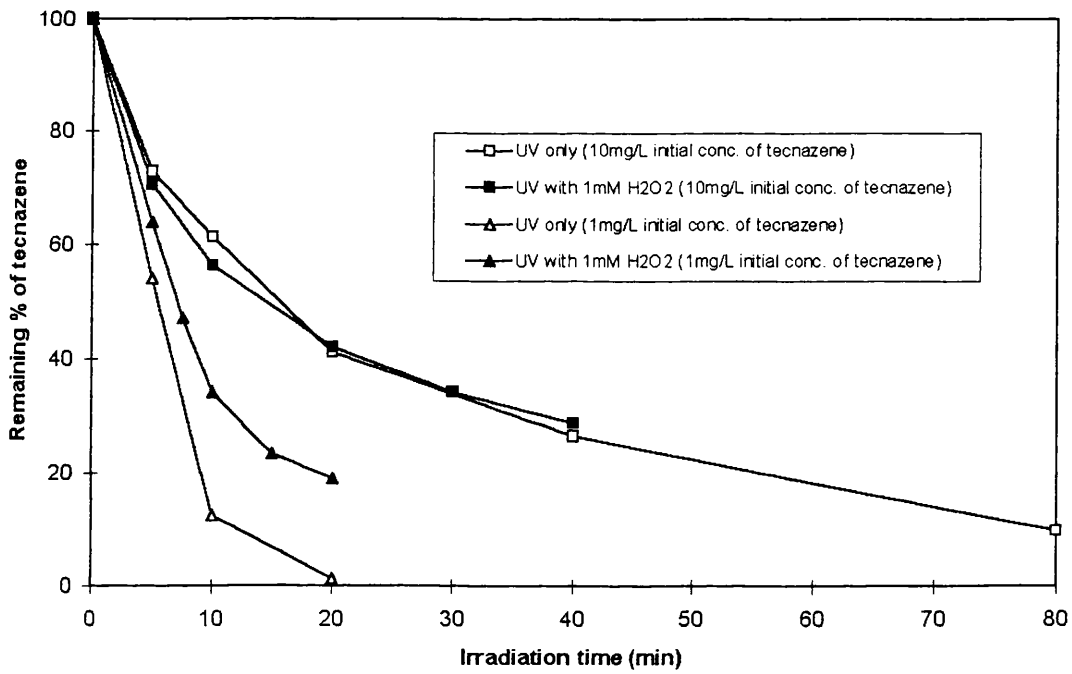


Figure 2.19 Decomposition of tecnazene in 10% (v/v) acetonitrile-water mixtures using UV with and without hydrogen peroxide

Soil mineral and organic constituents, such as fulvic acid, humic acid, kaolinite and so on, were found to tend to reduce the photolysis rate of organic pollutants by quenching the excited states of organic molecules or by reducing their absorption intensity (Mathew and Khan, 1996; Sanlavilley *et al.*, 1996). However, it is unlikely that these types of compounds were produced as a consequence of photolysis of acetonitrile because as discussed in the previous section acetonitrile has been found to have no effect on the photolysis rate using UV alone in the case of 10%(v/v) acetonitrile water mixtures.

It could be possible that acetonitrile produces substances that quench possible radical type of reactions toward tecnazene as a consequence of UV irradiation. This could be a possible reason that hydrogen peroxide did not accelerate the photodecomposition rate in acetonitrile-water mixtures. Many substances that have a property as a radical scavenger have been reported. As high rate constants of hydroxyl radicals with several inorganic and organic substances have been reported (Buxton *et al.*, 1988; Haag and Yao, 1992), hydroxyl radical are relatively non-selective although aliphatic polyhaloganated have been reported to be less reactive (Haag and Yao, 1992). Therefore, there may be many compounds that are capable of acting as hydroxyl radical scavengers. Hoigen and Bader (1983) used propanol and *t*-butanol in order to scavenge hydroxyl radicals, and carbon monoxide was studied as a scavenger for hydroxyl radicals produced using UV light by Buxton and Wilmarth (1963). Bicarbonate is also well-known as a strong hydrogen radical inhibitor (Staehelin and Hoigne, 1982). In the case of bicarbonate, Beltran *et al.* (1996) found that the presence of bicarbonate had no effect on photolysis of deethylatrazine and deisopropylatrazine as first decomposition products of atrazine, while hydrogen peroxide accelerated the photolysis rates of these compounds. However, they also found when hydrogen peroxide is present together with bicarbonate the decomposition rate decreases significantly even slower than the rate of photolysis without any additives.

The likely reason why hydrogen peroxide did not accelerate the photolysis rate in acetonitrile-water mixtures even though it accelerates in water is that acetonitrile may produce some types of substances like these mentioned above or acetonitrile itself act like them. Furthermore, the possible reason for the difference in behaviour that hydrogen peroxide has no effect on the photolysis rate for 10.0mg/L initial concentration of tecnazene, while it slowed down the photolysis rate in the case of 1.0mg/L initial concentration is that in the case of higher concentrations of tecnazene the concentration may dominate its photolysis rate, while in the case of lower concentrations the photolysis rate may depend more on the nature of the additives.

Half-life

Half-lives under each condition in 10%(v/v) acetonitrile-water mixtures were calculated using the methods described in Section 2.6.2, but this time slopes (constant rates) were calculated from the mean of duplicate runs. The results are given in Table 2.5. In the case of UV irradiation only, the half-life is increasing with the increase of the initial concentration of tecnazene. The half life in the case of 1mg/L initial concentration of tecnazene is almost the same as in the case of saturated aqueous tecnazene solution(0.85mg/L average concentration). In the case of UV irradiation in the presence of hydrogen peroxide, it seems there is no significant difference between the treatment with UV irradiation alone in the case of 10mg/L initial concentration of tecnazene, while half life is longer compared with UV irradiation alone in the case of 1mg/L initial concentration of tecnazene. All these results reflect what was discussed previously.

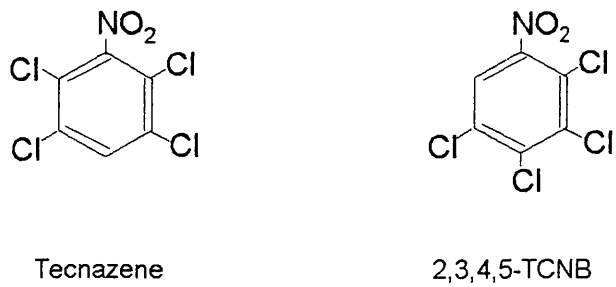
Table 2.5 Half-life of tecnazene in 10%(v/v) acetonitrile-water mixtures

Initial concentration of tecnazene (mg/L)	Treatment method	Half-life (min)
10.0	UV light only	24.8
	UV light with 1mM H_2O_2	23.1
5.0	UV light only	11.7
1.0	UV light only	3.3
	UV light with 1mM H_2O_2	8.1

2.8 Decomposition of 2,3,4,5-tetrachloronitrobenzene, an isomer of tecnazene

2.8.1 Introduction

2,3,4,5-tetrachloronitrobenzene (2,3,4,5-TCNB) is an isomer of tecnazene and the structures are given in Figure 2.20.



Tecnazene 2,3,4,5-TCNB

Figure 2.20 Chemical structure of tecnazene and its isomer, 2,3,4,5-TCNB

The objectives of the study in this section was to determine how a difference in the structure of tetrachloronitrobenzene affect their decomposition rates in water.

2,3,4,5-TCNB is expected as one of the decomposition products of tecnazene due to an isomerisation pathway of tecnazene (see Chapter 3). Therefore, the study of the decomposition of 2,3,4,5-TCNB is also important in order to study the further decomposition of one of the decomposition products as well as the study of the influence of difference in the structure of tetrachloronitrobenzene on their decomposition rates in water.

Two conditions were conducted for this study, namely UV irradiation in the presence and absence of hydrogen peroxide in water.

2.8.2 Experimental methods

Preparation of saturated aqueous 2,3,4,5-TCNB solution

Like tecnazene the solubility of 2,3,4,5-TCNB in water is very low. Therefore, saturated aqueous solution was used for all experiment. The procedure was the same as for tecnazene (see Section 2.2.2).

Reproducibility of analysis of 2,3,4,5-TCNB, and preparation of saturated aqueous 2,3,4,5-TCNB solution

The procedures are the same as for tecnazene (see Section 2.3). The reproducibility of analysis of 2,3,4,5-TDNB was obtained with 10 replicates from a single batch, while the reproducibility of preparation of saturated aqueous 2,3,4,5-TCNB solution was obtained from 6 independent batches.

The absorption spectrum of 2,3,4,5-TCNB

The absorption spectrum of a saturated aqueous solution of 2,3,4,5-TCNB was measured by ultraviolet spectroscopy.

Gas chromatography

Determination of 2,3,4,5-TCNB was carried out on a Varian Model 3700 Gas chromatograph instrument equipped with a flame ionization detector (FID). The estimated low detection limit (LOD) with this analysis was approximately 0.5mg/L. GC conditions are the same as for tecnazene (see Section 2.2.4). 1000mg/L stock solution of 2,3,4,5-TCNB dissolved in hexane was prepared. The stock solution was stored in the dark. 50, 25, 15, 5mg/L standard solutions were prepared from the stock solution. The multiple calibration line with these standards was freshly made before each series of sample analysis since the sensitivity of GC tends to be variable with the time. Some example of calibration lines which made on different day for sample analysis were shown in Figure 2.21. Using the conditions described above typical chromatograms of standard 2,3,4,5-TCNB, a hexane extract of 2,3,4,5-TCNB from saturated solution and its control of hexane extract are shown in Figure 2.22.

Decomposition of 2,3,4,5-TCNB with hydrogen peroxide alone

Dark controls were carried out with hydrogen peroxide alone in order to monitor if there is chemical oxidation and hydrolysis of 2,3,4,5-TCNB in water. The change in the concentration of hydrogen peroxide was also determined at the same time. The procedure was the same as for tecnazene (see Section 2.5).

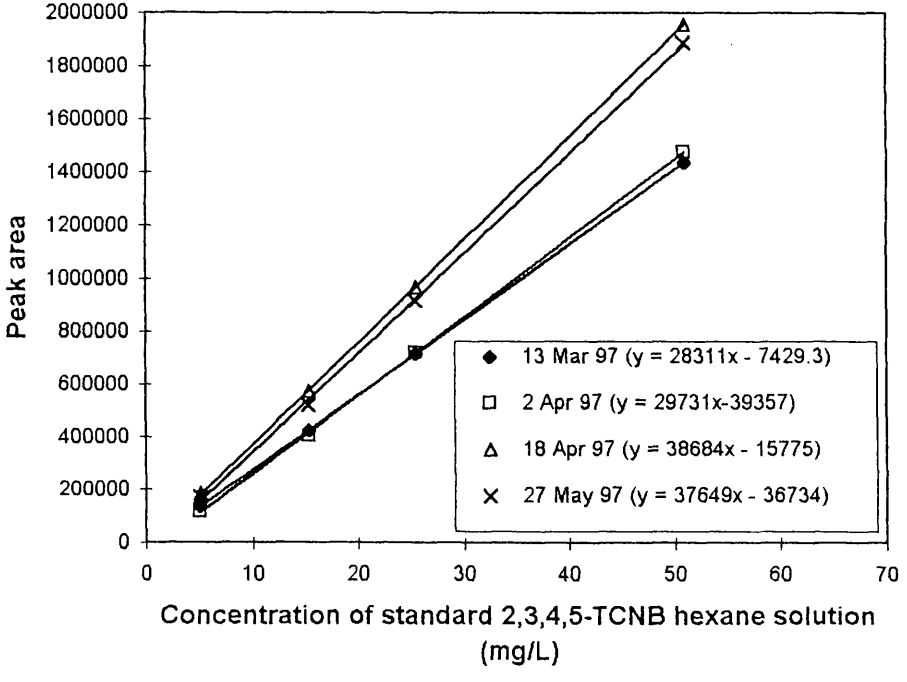


Figure 2.21 Variation of calibration line for 2345-TCNB GC analysis on different date.

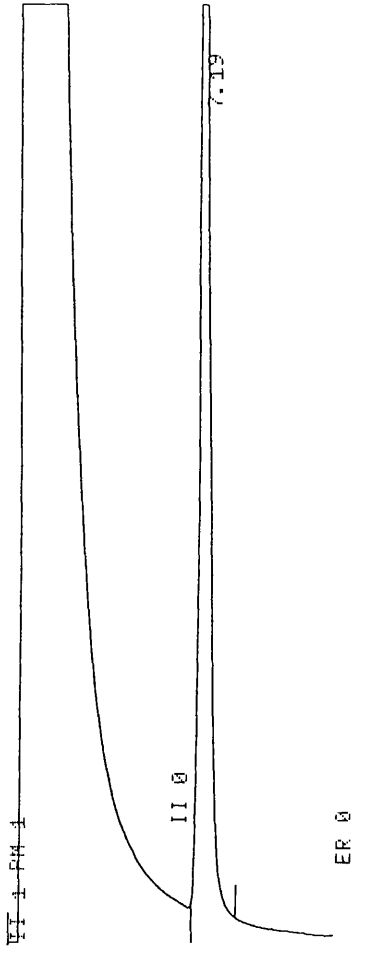


Figure 2.22 Typical Gas chromatogram of 0.25μg of 2,3,4,5-TCNB standard.

Decomposition of 2,3,4,5-TCNB in water using UV light with and without hydrogen peroxide

The all experimental procedure was the same as for tecnazene (see Section 2.6).

2.8.3 Results and Discussion

Reproducibility of analysis of 2,3,4,5-TCNB, and preparation of saturated aqueous 2,3,4,5-TCNB solutions

Reproducibility of analysis of 2,3,4,5-TCNB was determined with 10 replicates from a single batch. The results are given in Table 2.6. Like tecnazene high reproducibility was obtained with mean value of 1.48mg/L, standard deviation of 0.054 and R.S.D. of 3.6% from 10 replicates.

Table 2.6 Reproducibility of analysis of 2,3,4,5-TCNB with 10 replicates from a single batch of saturated aqueous 2,3,4,5-TCNB solution.

Concentration of 2,3,4,5-TCNB solution (mg/L)	Mean value (mg/L)	Standard deviation	R.S.D (%) ((Stdev/mean)×100)
1.44			
1.45			
1.44			
1.41			
1.54	1.48	0.054	3.6
1.57			
1.47			
1.51			
1.53			
1.43			

Reproducibility of preparing saturated aqueous 2,3,4,5-TCNB solution was calculated with 6 independent batches. The extraction method and GC analysis are the same as for tecnazene. The results are given in Table 2.7. The concentration of saturated aqueous 2,3,4,5-TCNB solution was very variable in 6 different batches. With 6 independent batches, mean value of 1.53mg/L (maximum and minimum value were 2.12mg/L and 1.07mg/L), standard deviation of 0.41 and R.S.D. of 26.7% were obtained. Because a high reproducibility of analysis of 2,3,4,5-TCNB was obtained, variation in the concentration of saturated solution is likely to be caused mostly by its

preparation. Like tecnazene 2,3,4,5-TCNB powder is hydrophobic so it is difficult to mix with water even with agitation with. This could be one reason for the low reproducibility in preparation. The solubility of 2,3,4,5-TCNB (1.53mg/L) was slightly higher than tecnazene (0.85mg/L on average). However, there is no solubility data of 2,3,4,5-TCNB available in the literature.

Table 2.7 Reproducibility of preparation of 6 independent batches of saturated aqueous 2,3,4,5-TCNB solution.

Concentration of 2,3,4,5-TCNB solutions (mg/L)	Mean value (mg/L)	Standard deviation	R.S.D (%) ((Stdev/mean)×100)
1.47 2.12 1.87 1.07 1.52 1.14	1.53	0.41	26.7

The absorption spectrum of 2,3,4,5-TCNB

As can be seen in Figure 2.23 it absorbs UV light well, and the peak absorption was at 206.0nm. Therefore, it can be observed that 2,3,4,5-TCNB is likely to be removed by UV light.

Decomposition of 2,3,4,5-TCNB with hydrogen peroxide alone

The change of concentration of hydrogen peroxide and the remaining percentage of 2,3,4,5-TCNB during treatment with hydrogen peroxide alone are shown in Figures 2.24 and 2.25, which compares two experiments with different initial concentrations of hydrogen peroxide. Like tecnazene there were negligible changes in the concentration of 2,3,4,5-TCNB and hydrogen peroxide in both conditions. Therefore, it can be observed there was no reaction, such as oxidation and hydrolysis, to decompose 2,3,4,5-TCNB in these conditions.

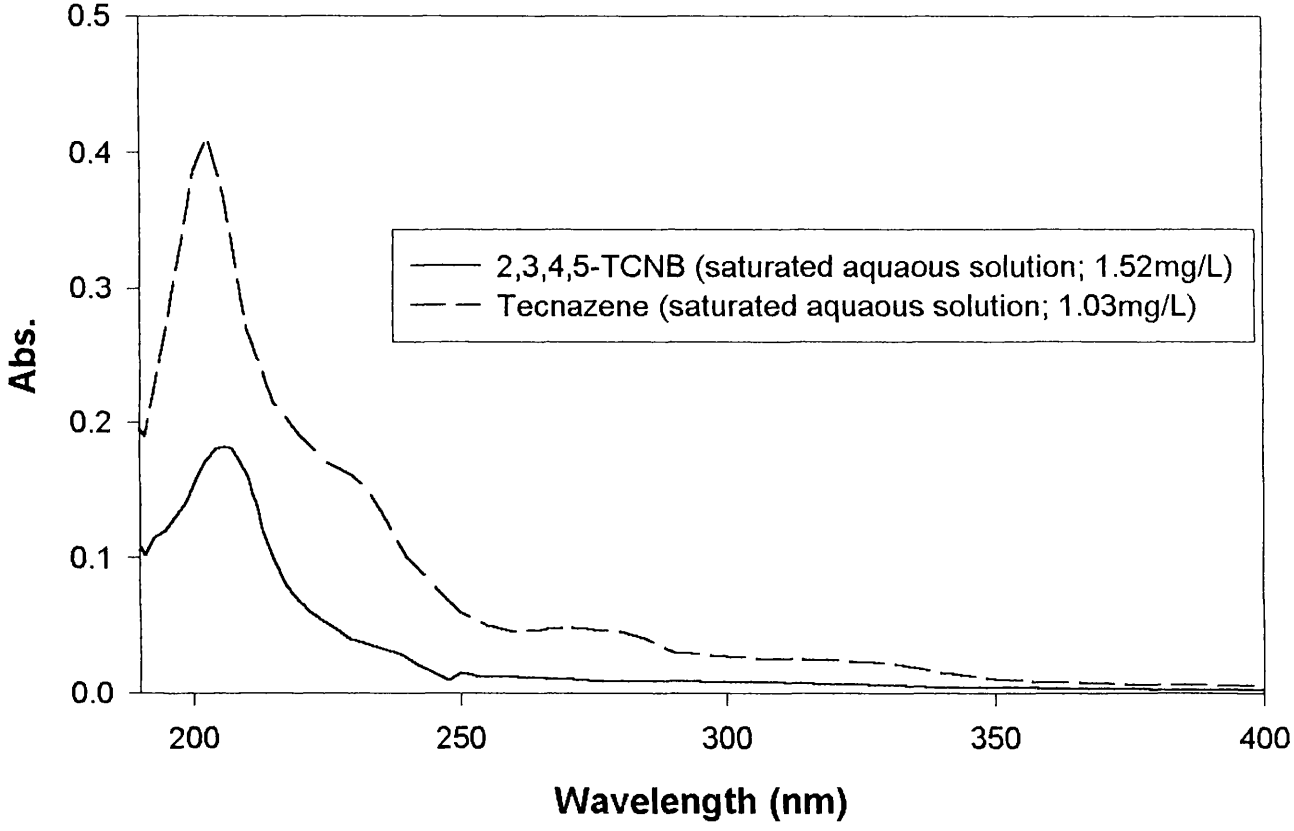


Figure 2.23 The absorption spectra of 2,3,4,5-TCNB and tecnazene in aquaous solution

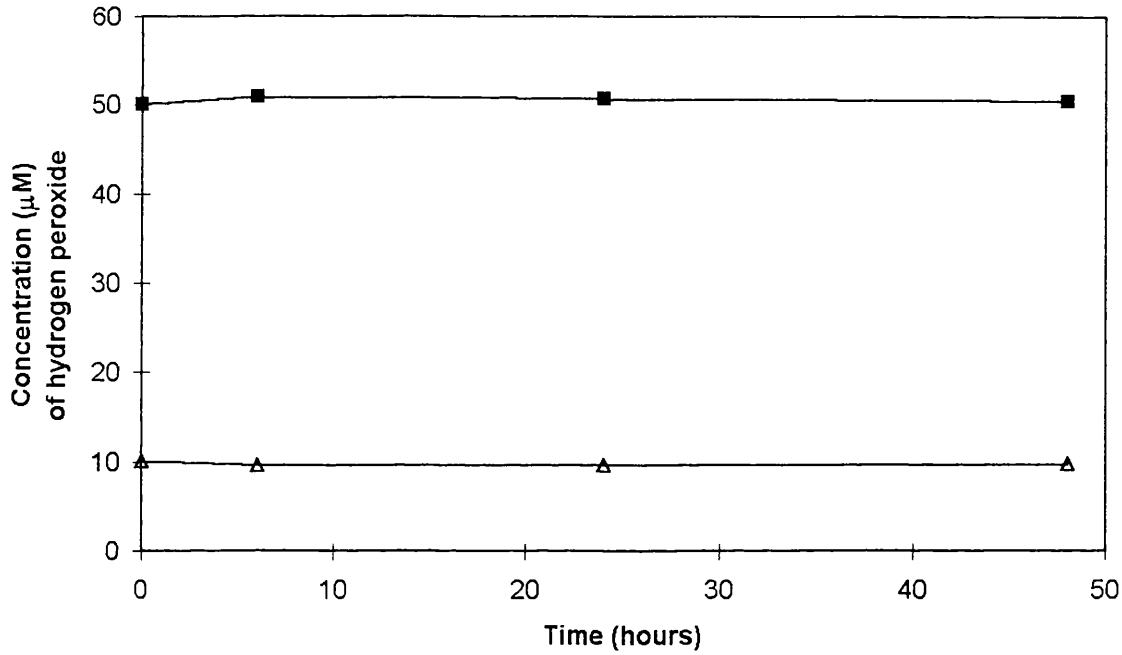


Figure 2.24 Change in the concentration of hydrogen peroxide

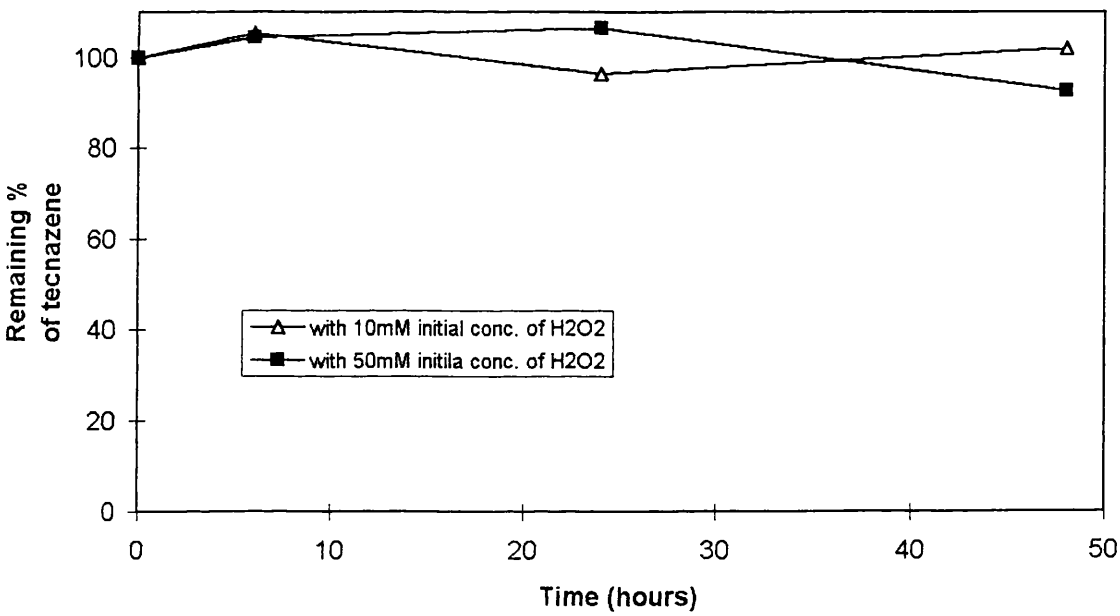


Figure 2.25 Dark control with hydrogen peroxide alone for decomposition of 2,3,4,5-TCNB

Decomposition of 2,3,4,5-TCNB in water using UV light with and without hydrogen peroxide

The GC chromatograms at different irradiation time points are given in Figures 2.26 and 2.27.

The remaining percentage of 2,3,4,5-TCNB are plotted against irradiation time (Figures 2.28 and 2.29). UV irradiation treatment of 2,3,4,5-TCNB in pure water leads to a rapid decomposition in either presence or absence of hydrogen peroxide. The lines drawn through the mean values of replicates at each time points are given in Figure 2.30 with the data of tecnazene (see also Section 2.5). The sample treated with UV in the presence of hydrogen peroxide was decomposed relatively faster than with UV alone. The decomposition rate increased with the increase in the concentration of hydrogen peroxide. Almost complete decomposition was achieved by 20 minutes irradiation with an initial hydrogen peroxide concentration of 1mM, by 30 minutes irradiation with 0.1mM hydrogen peroxide and by 40min irradiation with UV only. All these phenomena are quite similar to the case of tecnazene. However, the decomposition rates in all conditions are relatively slower than tecnazene except for the early stage of photodecomposition in the case of tecnazene with UV alone. Because the early stage of photodecomposition values in the case of 2,3,4,5-TCNB with UV only were not obtained, it can not be compared but the result will probably show the same phenomena. Apart from the structural difference there are a few possible reasons for the different photodecomposition rate between 2,3,4,5-TCNB and tecnazene. Firstly the higher aquatic solubility of 2,3,4,5-TCNB than tecnazene could be one of the reasons for the different photodecomposition rate between 2,3,4,5-TCNB and tecnazene because the initial concentration of the compound has a great influence on the decomposition rate as discussed in Chapter 2. The characteristics of the absorption of UV light may be another reason. As can be seen in Figure 2.23 the intensity of UV light absorption of 2,3,4,5-TCNB is less than tecnazene even if the concentration is higher than tecnazene. Absorption character is important for its photolysis since direct photolysis occurs due to direct absorption by substances (Zepp and Cline, 1977). However, these reasons are unlikely to be the main reasons because in terms of the difference in saturated concentration between

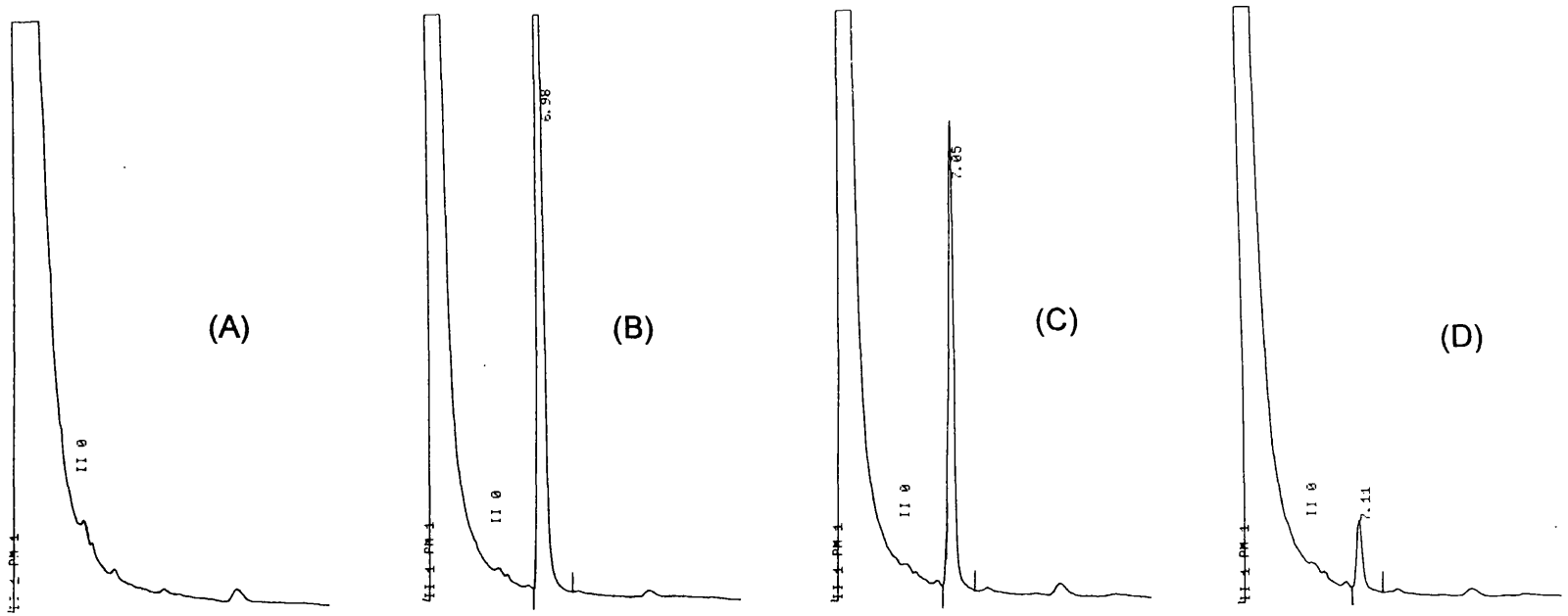


Figure 2.26 GC chromatograms from 2,3,4,5-TCNB irradiated by UV light in water for (B) 0min, (C) 10min and (D) 20min. (A) is control.

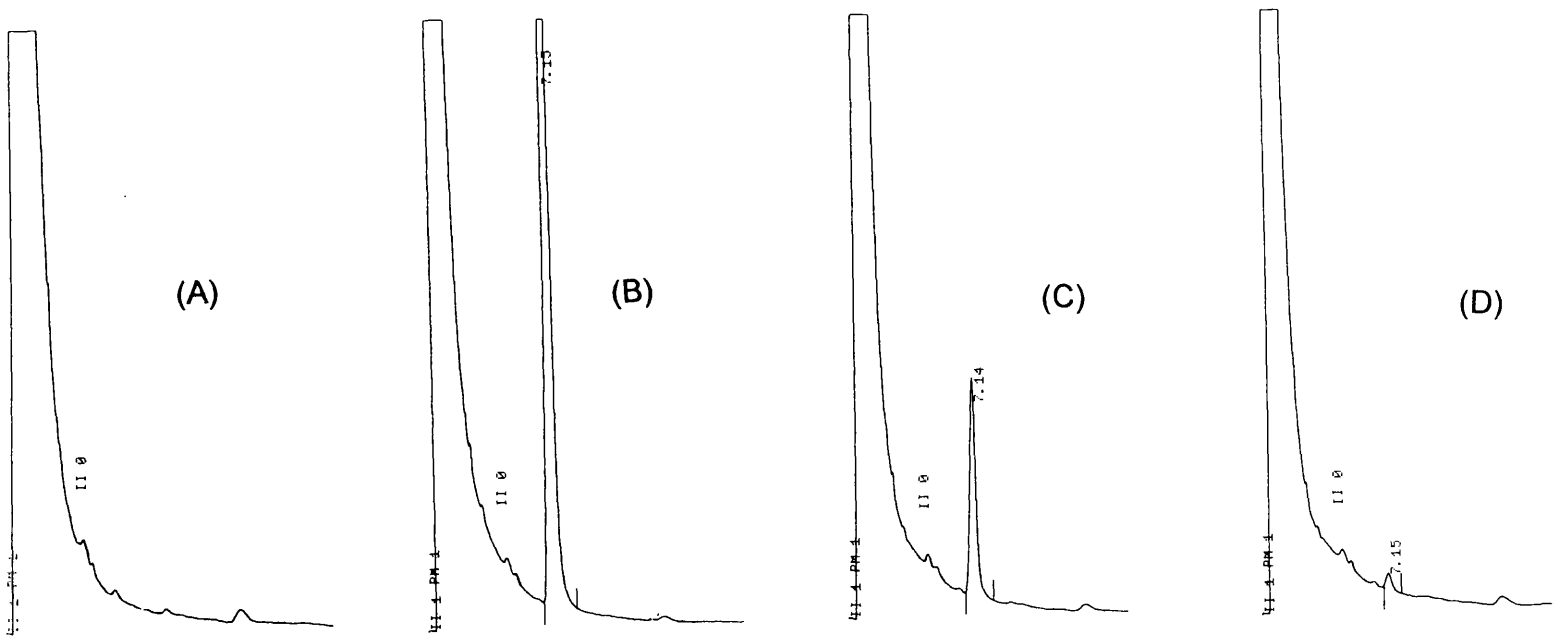


Figure 2.27 GC chromatograms from 2,3,4,5-TCNB irradiated by UV light with 1mM H_2O_2 in water for (B) 0min, (C) 10min and (D) 20min. (A) is control.

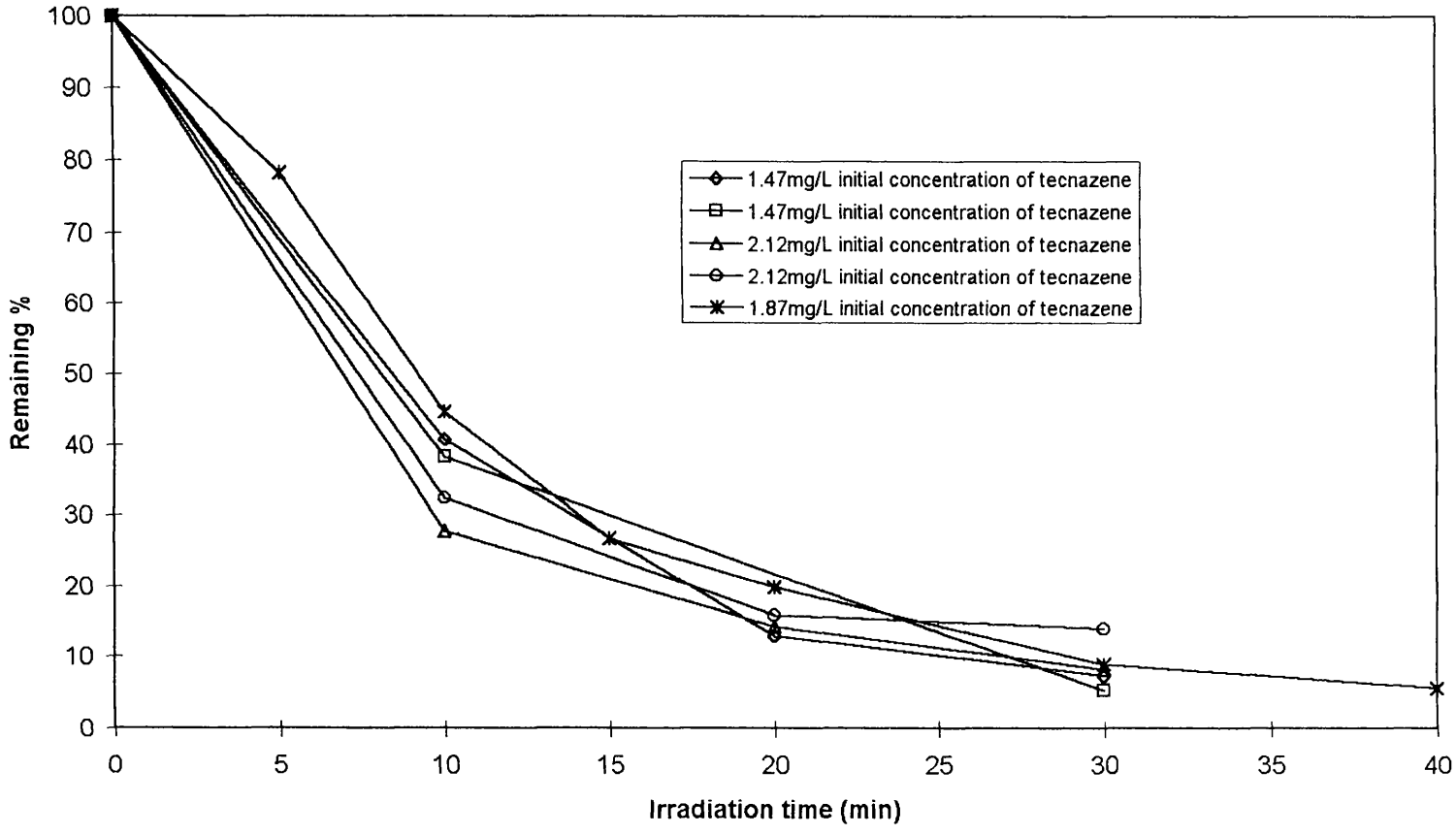


Figure 2.28 Decomposition of 2,3,4,5-TCNB with UV alone in water (4 individual runs using different batches of saturated 2,3,4,5-TCNB solution) - normalised data.

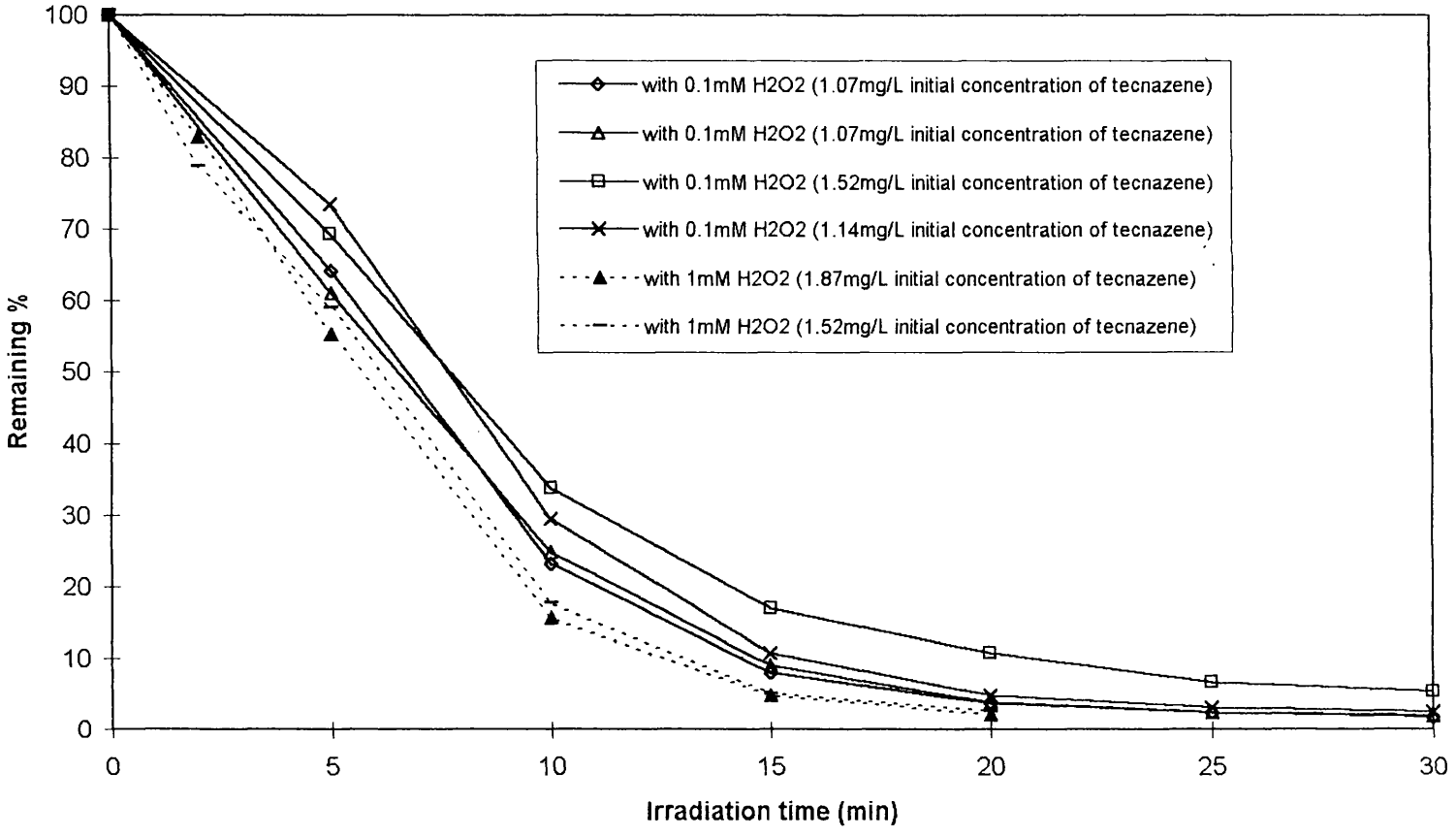


Figure 2.29 Decomposition of 2,3,4,5-TCNB with UV in the presence of hydrogen peroxide (2 each individual runs using different batches of saturated 2,3,4,5-TCNB solution) - normalised data.

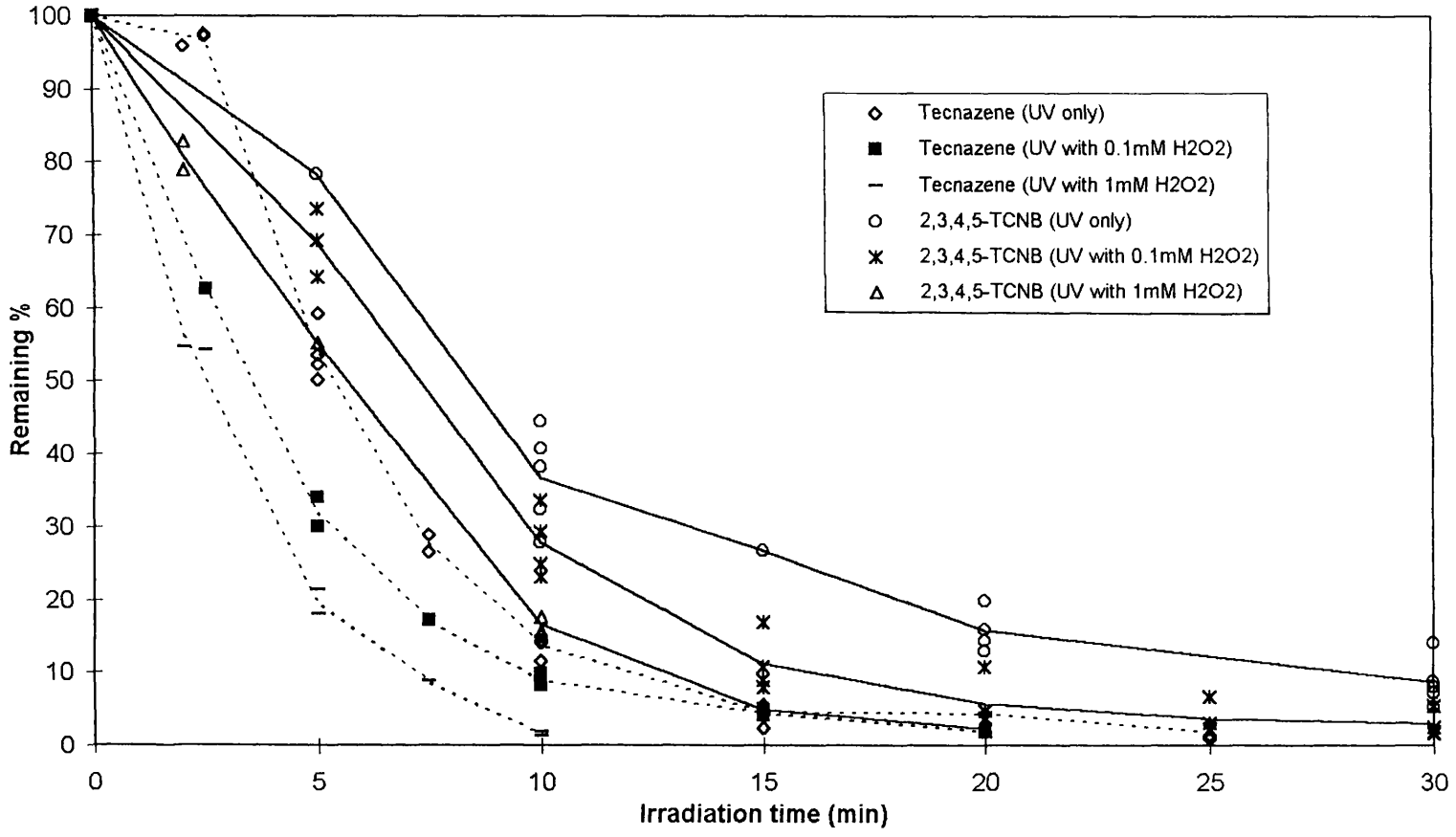


Figure 2.30 Effect of treatment on the photodecomposition of 2,3,4,5-TCNB and tecnazene. Replicates at each time points are independent runs with the line drawn through the mean value.

2,3,4,5-TCNB and tecnazene, within the differences in the starting concentration of replicates, the photodecomposition rates seem not to depend on the starting concentration(see Figures 2.10, 2.11, 2.28 and 2.29). The difference in the concentration of saturated solution between 2,3,4,5-TCNB and tecnazene is probably in this range of differences. In terms of the deference in absorption character, the range of wavelength of UV lamp is mainly >254nm. In this range the difference in absorption is relatively small. With these reasons the differences in solubility and absorption character are unlikely to be main reasons for differences in photodecomposition rate. Therefore, the structural difference is likely to be the main reason. Simmons and Zepp (1986) studied the influence of variations in the structure of nitroaromatic compounds, such as 2-nitrobenzene, its isomers, 2-nitro-1,3-xylene and its isomers, on the photolysis rate. Their results indicate that photolysis rate is strongly dependent on molecular structure.

As mentioned before 2,3,4,5-TCNB is expected as one of the decomposition products of tecnazene due to an isomerisation pathway of tecnazene. Since UV irradiation treatment in pure water in either presence or absence of hydrogen peroxide led to a rapid decomposition of 2,3,4,5-TCNB, the further decomposition of the compound which could be one of the decomposition products of tecnazene was obtained.

Half-life

Values of ln(Ct/Co) was plotted against irradiation time in Figure 2.31 and 2.32. ln(Ct/Co) values of less than -3 were excluded for calculation of the regression line because of the same reason in the case of tecnazene. The values at irradiation time zero were included for all calculations of the regression line. The half-life in each treatments was calculated using equation (4) described in 2.6.2, and is given in Table 2 with the rate constant values calculated as slopes. The half life value of tecnazene is included for reference. According to t-test ($P<0.01$), in the case of 2,3,4,5-TCNB, there is a significant difference between UV irradiation treatment in the absence and presence of 0.1mM hydrogen peroxide. This is a big difference compared with the case of tecnazene. In other words the presence of hydrogen peroxide is more effective for photodecomposition of 2,3,4,5-TCNB. In the case of 1mM hydrogen peroxide

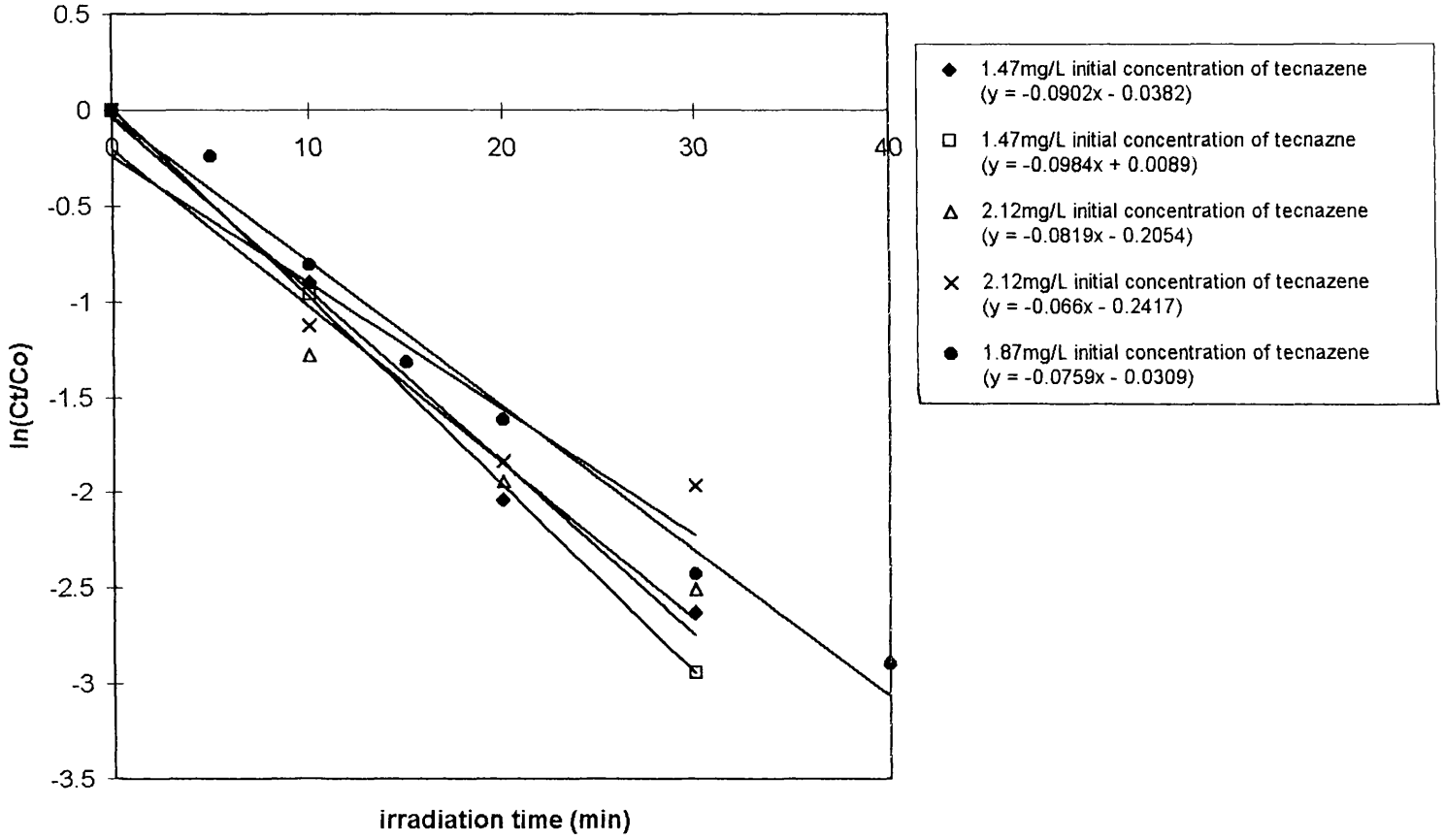


Figure 2.31 First-order treatment of the photodecomposition (by UV only) data for 2,3,4,5-TCNB in water (4 individual runs using different batches of saturated solution).
The data of ln(Ct/Co) less than -3 were excluded for first order treatment.

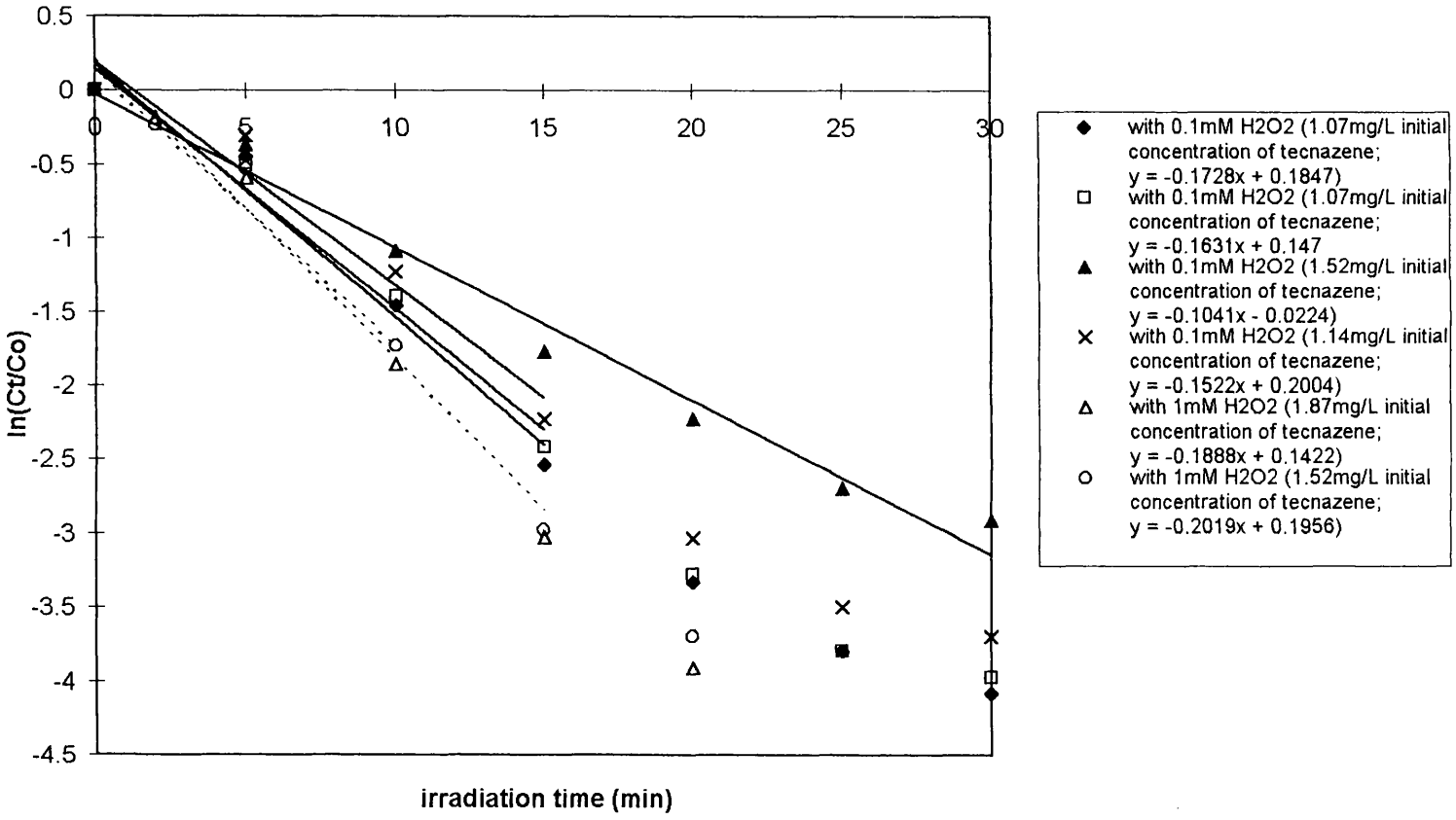


Figure 2.32 First-order treatment of the photodecomposition (by UV in the presence of hydrogen peroxide) data for 2,3,4,5-TCNB in water (2 each individual runs using different batches of saturated solution).
The data of ln(Ct/Co) less than -3 were excluded for first order treatment.

the half-life seems to be shorter than the other tow treatments but more replicates are needed for statistical comparison. In comparison between half-life of 2,3,4,5-TCNB and tecnazene, in the case of UV irradiation only the half-life of 2,3,4,5-TCNB was 3 times longer than tecnazene. This is the same result discussed previously. However, in the case of UV irradiation in the presence of hydrogen peroxide, values of half-life are just 1.5 times longer than tecnazene. This again shows that the presence of hydrogen peroxide is more effective than in the case of tecnazene.

Table 2.8 Comparison of half-life of 2,3,4,5-TCNB and tecnazene in water in different treatments.

Treatment method	UV only	UV with H_2O_2	
		with 0.1mM	with 1mM
Rate constant(k) (Slope)	-0.090 -0.098 -0.082 -0.066 -0.076	-0.173 -0.163 -0.104 -0.152	-0.189 -0.202
Mean values	-0.0825	-0.148	-0.196
Standard deviation	0.013	0.031	
Half-life (min)	8.4	4.7	3.5
Half-life (min) of tecnazene	2.8	2.8	2.2

CHAPTER 3. Study of decomposition products of tecnazene

3.1. Introduction

Investigation of transformation pathways is an important part of a study for the removal of a pesticide, since there is a possibility that more toxic products might be produced as a by-product. However, identification of pesticide decomposition products is difficult due to their low concentration versus the concentration of their parent compounds. Furthermore, the reaction pathway is generally complicated. As a result many kinds of decomposition products might be produced during the treatment. Therefore, it is necessary to identify as many decomposition products as possible, and investigate the transformation pathways.

Use of a cosolvent is helpful for the study of decomposition products due to its capability of dissolving hydrophobic organic chemicals which covers many pesticides. Durand *et al.* (1994) have studied the photodecomposition of fenitrothion, an insecticide in water-methanol mixtures in order to increase the starting concentration of a parent compound so that decomposition products could be identified as many as possible. Acetonitrile is another major solvent used as a cosolvent. Choudhry and his colleagues (1983; 1985a; 1985b) have investigated the photodecomposition of several organic pollutants in water-acetonitrile mixtures, and studied their transformation pathways. As mentioned in Chapter 2 as well, among these solvents used as a cosolvent acetonitrile was recommended for use in photochemistry studies due to its similarity of refractive index to water (Leifer, 1988), and also it was found to have a minimum affect in studies of photodecomposition of pesticides compared to other cosolvents, such as methanol (Bunce, 1978). However, as discussed in Chapter 2 in the case of UV irradiation of tecnazene in water-acetonitrile mixtures in the presence of hydrogen peroxide different behaviour was observed compared with the situation which existed in the case of water. This result may indicate the transformation pathway might be different from what happens in water. However, as Choudhry and Barrie Webster (1985a) emphasised the requirement of the investigation of the mechanism of the decomposition using a cosolvent even though it is different from

what happens in the environment, the determination of decomposition products was still carried out in acetonitrile-water mixtures as well as in water if possible.

Methods for the determination of decomposition products are important. Many procedures have been employed to characterize product structures and quantify them. One of the classical methods which has been used widely to separate the compounds can be thin-layer chromatography (TLC). High performance liquid chromatography (HPLC) can also be a classical method. However, many researchers have employed gas chromatography combined with mass spectrometry (GC-MS) which can be an effective method for identification of pesticide decomposition products. Liquid chromatography combined with mass spectrometry (LC-MS) is another popular and effective method, particularly for polar compounds and the compounds that are susceptible to decomposition during gas chromatographic separation. In the case of the determination of anionic decomposition products ion-exchange chromatography could be efficient and versatile for either inorganic or organic anions. Indeed, a number of environmental applications based on ion exchange separation of anions have been published (Legrand *et al.*, 1993; Chen, 1996).

The purpose of the work in this Chapter is to identify and quantify as many photodecomposition products as possible, and propose a possible pathway for the decomposition process. In order to characterize product structures and quantify them, GC-MS and ion-exchange chromatographic methods were employed. GC-MS was used for the determination of organic decomposition products in the hexane extracts, and ion-exchange chromatography was used for the determination of anionic decomposition products.

3.2. Identification of decomposition products using GC-MS

3.2.1 Materials and methods

Materials

2,4,5-trichloronitrobenzene (97%, Aldrich Chemical Co.) was of the highest available purity and was used as received. 2,3,5,6-tetrachlorophenol (Aldrich Chemical Co.)

was recrystallised once previously. Analytical grade of acetic anhydride was supplied by Hopkin & Williams ltd. Pyridine was supplied by Sigma Chemical Co. and was >99% pure.

Sample preparation

Four experimental conditions, UV irradiation in the presence and absence of hydrogen peroxide in water, and UV irradiation in the presence and absence of hydrogen peroxide in 10% (v/v) acetonitrile-water mixtures, were conducted for the determination of anions. 0.1mM hydrogen peroxide for UV irradiation of tecnazene in water, and 1mM for the 10% (v/v) acetonitrile-water mixtures were set up. The withdrawn sample was extracted with hexane and concentrated into 2ml for GC or GC-MS analysis. The extraction procedure is the same as described in section 2.4.1.

GC-MS

Identification of decomposition products in the hexane extracts were carried out on a Hewlett-Packard 5890 Series 2 gas chromatograph interfaced to a 5971 Series Mass Selective detector using a fused silica capillary column (12m×0.2mm i.d.) coated with 0.3μm HP1 Non-polar Methyl Silicone polymer.

Acetylation method

A 100μl of sample was transferred into a small reaction vial, and evaporated to dryness with blowing the surface of the sample using nitrogen gas. 15μl of acetic anhydride, and 10μl of pyridine as a catalyst were then added. The sample mixture was heated up to 90°C for 30 minutes using a digestion block. If the sample was not yet dried it was evaporated to dryness with blowing surface of the sample using nitrogen gas. The residue was redissolved with 10μl hexane for GC-MS analysis.

3.2.2 Results and discussion

There were no extra peaks on the GC chromatogram after irradiation of tecnazene in water either in the presence or absence of hydrogen peroxide. However, using a higher starting concentration of tecnazene (10mg/L) in 10% (v/v) acetonitrile-water

mixtures saveral unknown peaks were detected on the GC chromatogram either in the presence or the absence of hydrogen peroxide (see GC chromatograms in Figures 2.16 and 17 in Chapter 3). The chromatogram pattern was the same in both conditions.

Therefore, identification of these unknown peaks was carried out on GC-MS with the representative sample which was irradiated by UV light in the absence of hydrogen peroxide for 80 minutes in 10% (v/v) acetonitrile-water mixtures. With the conditions in GC-MS analysis two unknown peaks were obtained. The GC chromatogram is given in Figure 3.1. The first unknown peak on the chromatogram (RT 9.29 in Figure 3.1) was identified as trichloronitrobenzene (the reliability of library match by computer was 87%) by its typical ions at *m/z* values of 179 and 167, corresponding to $[M^+-NO_2]$ and $[M^+-NO-Cl]$ respectively, although one of typical ions at *m/z* values of 195, corresponding to $[M^+-NO]$ is missing in the sample mass spectra. The ion obtained at *m/z* 225 corresponded to the molecular ion. Its mass spectra is shown in Figure 3.2 with comparison of standard, 2,4,5-trichloronitrobenzene, and mass spectral ion fragments of 2,4,5-trichloronitrobenzene is given in Table 3.1. However, actual distribution of chlorine atoms are still unknown because mass spectra gives almost the same spectral patterns between isomers. The structures of the product can be established by comparison of chromatographic properties with its authentic standards. However, as was pointed out in a recent paper about the identification of photodecomposition products of a pesticide (Durand *et al.*, 1994) one of the main problems in this field of study is that most of the products are not commercially available. Indeed, although 2,3,5-trichloronitrobenzene and 2,3,6-trichloronitrobenzene were expected as decomposition products from the tecnazene structure, neither of them is commercially available. 2,4,5-trichloronitrobenzene, used as a standard for mass spectra, does not match the chromatographic properties of the decomposition products. Therefore, the actual structure of trichloronitrobenzene has not been established on this occasion.

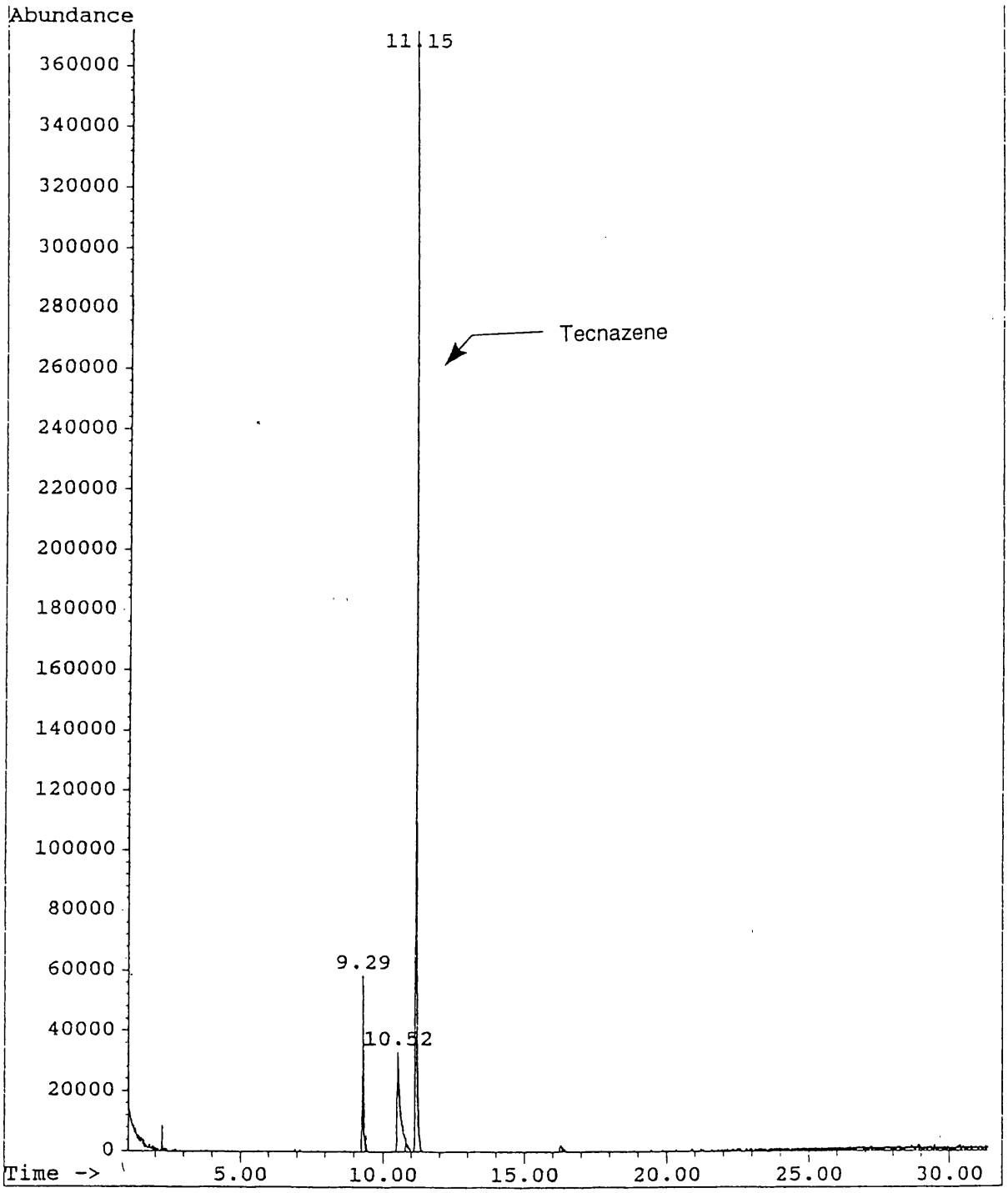


Figure 3.1 Total ion current (TIC) chromatogram by GC-MS from tecnazene irradiated by UV light for 80min in 10%(v/v) acetonitrile-water mixtures.

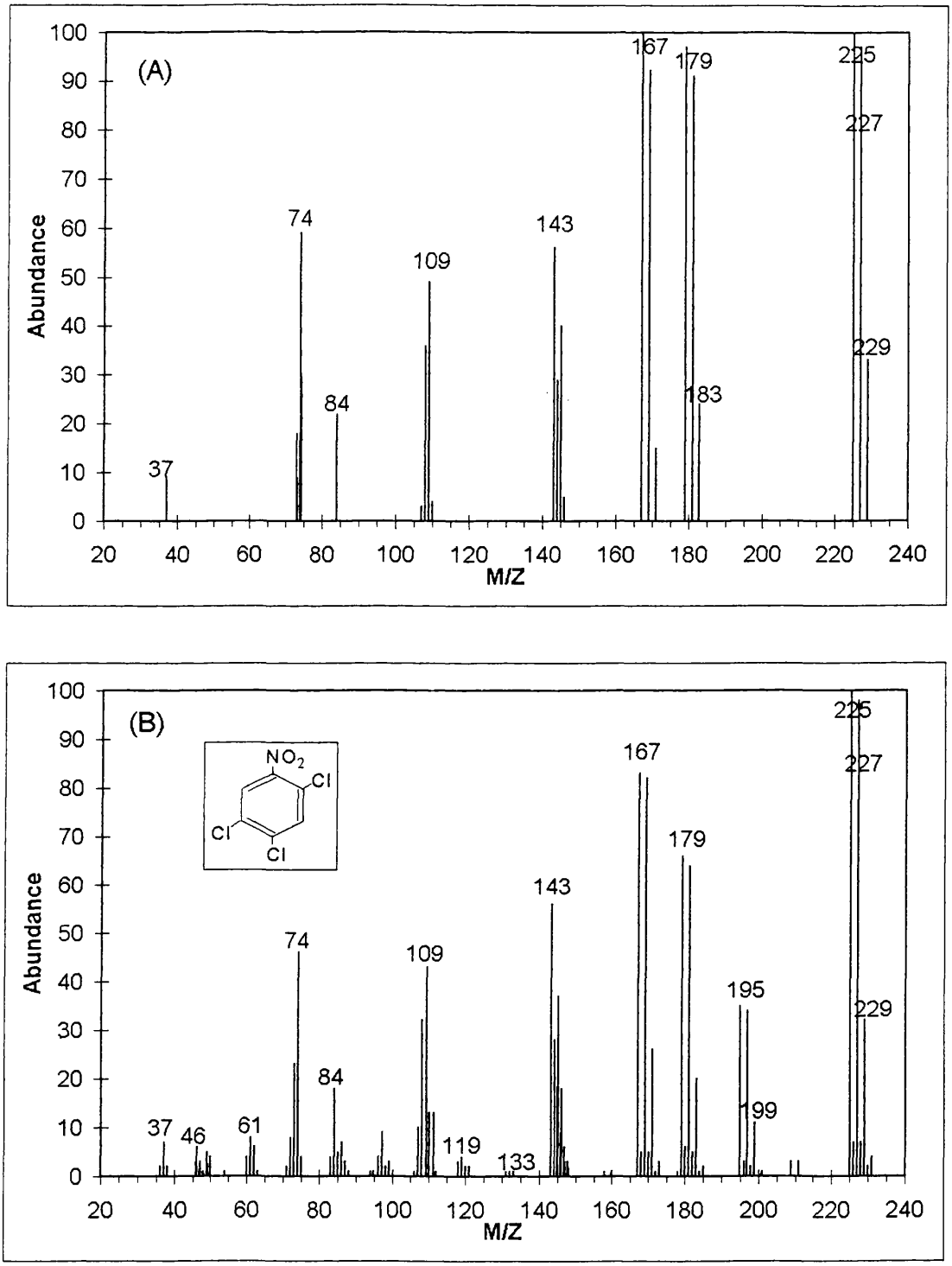


Figure 3.2 Mass spectra of (A) first unknown peak and (B) standard 2,4,5-trichloronitrobenzene

Table 3.1 Mass spectral ion fragments of standard 2,4,5-trichloronitrobenzene.

Mass (m/e)	Relative intensity	Proposed fragment
225	100	M^+
195	35	M^+-NO
179	66	M^+-NO_2
167	83	M^+-NO-Cl
144	28	M^+-NO_2-Cl

Hamadmad (1967) studied photolysis of tecnazene in hexane using UV light at a wavelength of 253.7nm, and found that 1,2,4,5-tetrachlorobenzene and 2,3,5-trichloronitrobenzene were the major products formed, whilst 2,3,6-trichloronitobenzene was a minor product. According to this result, the decomposition product identified as trichloronitrobenzene is likely to be 2,3,5-trichloronitrobenzene, although the UV irradiation of tecnazene in hexane solution conducted by Hamadmad is probably not representative of what happens to the UV irradiation treatment in acetonitrile-water mixtures. Another major decomposition product, 1,2,4,5-tetrachlorobenzene reported was not detected on this occasion.

The formation of trichloronitrobenzene could indicate that UV irradiation of tecnazene in water acetonitrile mixtures brought about the replacement of the chlorine by hydrogen. Reductive dechlorination has been reported as one of the most important transformation pathway for polychlorinated aromatic compounds (Choudhry *et al.*, 1986). However, other dechlorinated tecnazene compounds, such as dichloronitorobenzene and monochloronitrobenzene were not detected on this occasion.

The second unknown (RT 10.52 in Figure 3.1) peak was broad and small. This compound was suspected to be a phenolic compound due to its chromatographic properties (flat peak). Therefore, acetylation with acetic anhydride with pyridine as a catalyst was carried out in order to obtain higher sensitivity for GC-MS with the following reaction (Figure 3.3).

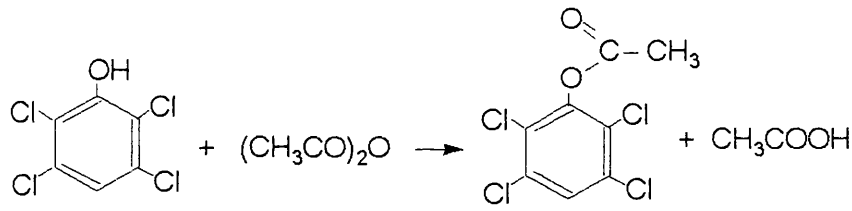
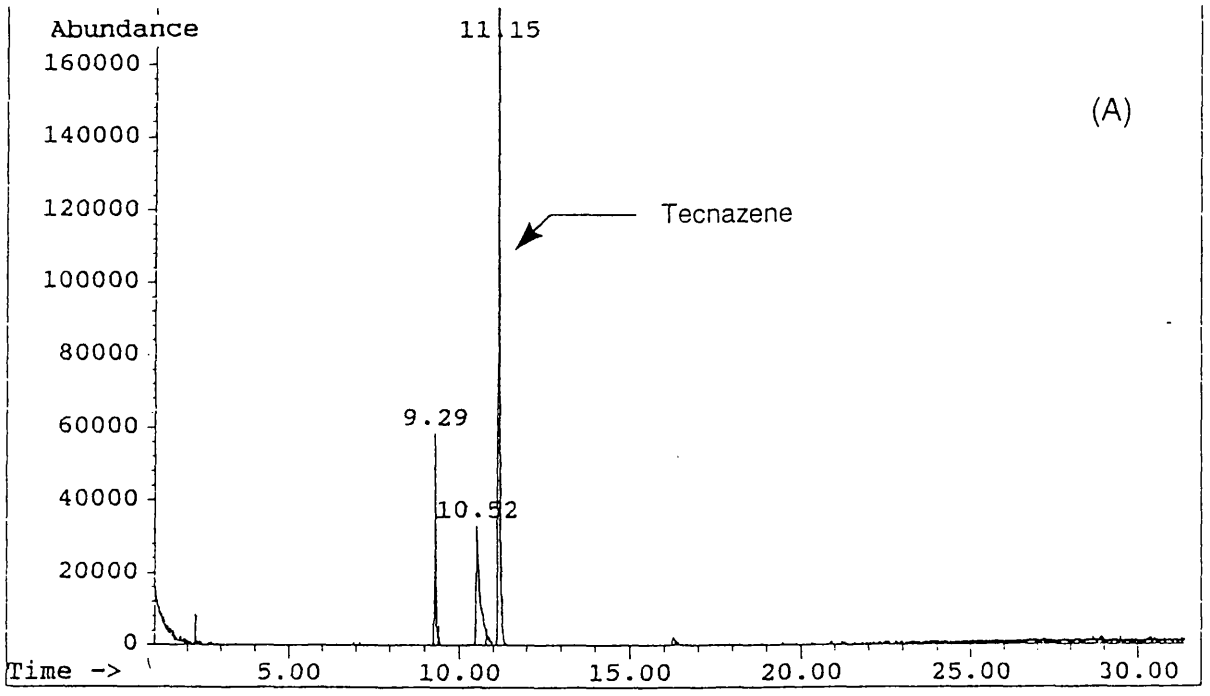


Figure 3.3 The reaction of acetylation

As shown in Figure 3.4, the acetylated compound showed a sharp peak (RT 11.68), and its mass spectra is shown in Figure 3.5 with comparison of the standard, acetylated standard 2,3,5,6-tetrachlorophenol. Because there is not standard reference mass spectra in the computer library, the identification was carried out by comparison with the determined acetylated standard mass spectra. Both spectra contained typical ions of acetylated compound, at *m/z* values of 43 corresponded $[COCH3]^+$. Other important ions of acetylated tetraclorophenol were obtained at 230, corresponding to $[M^+–C=C-CH_3]$. The ion obtained at *m/z* 232 corresponded to the molecular ion. At *m/z* value of 232 another important ion was obtained in both mass spectra. This ion is 1 molecule bigger than the ion corresponded to $[M^+–COCH3]$ at *m/z* 231 that has low abundance in both mass spectra. Therefore, ion obtained at *m/z* 232 seems to be corresponded to hydrided ion of $[M^+–COCH3]$. Changing values from *m/z* 201 to 166, from 166 to 131, from 131 to 96, and from 96 to 61 are because of the loss of chlorines. In accordance with the above comparison there is a high level of confidence that they are the same compound. Hence, it can be observed that one of the decomposition products is tetrachlorophenol. Since mass spectra gives almost the same spectral patterns between isomers, the actual distribution of chlorine atoms was established by comparison of chromatographic properties with authentic standard. The retention time of the acetylated unknown compound was the same as the acetylated standard 2,3,5,6-tetrachlorophenol. On this basis, there is a high level of confidence that they are the same compound.

Choudhry *et al.* (1986) reported that further decomposition of 2,3,5,6-tetrachlorophenol in water-acetonitrile mixtures (1:1 v/v) led to the production of several chlorinated phenolic compounds. According to their work, 2,3,5-



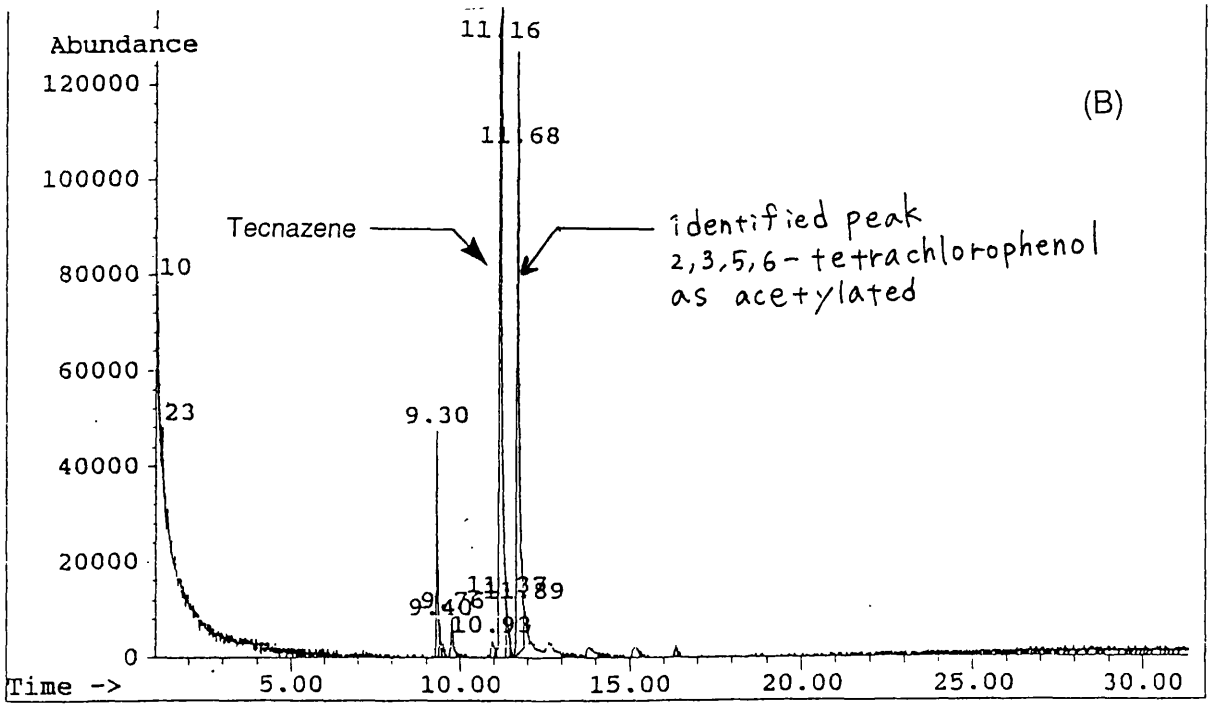


Figure 3.4 Total ion current (TIC) chromatogram by GC-MS from tecnazene irradiated by UV light for 80min in 10%(v/v) acetonitrile-water mixtures. (A) before acetylation and (B) after acetylation.

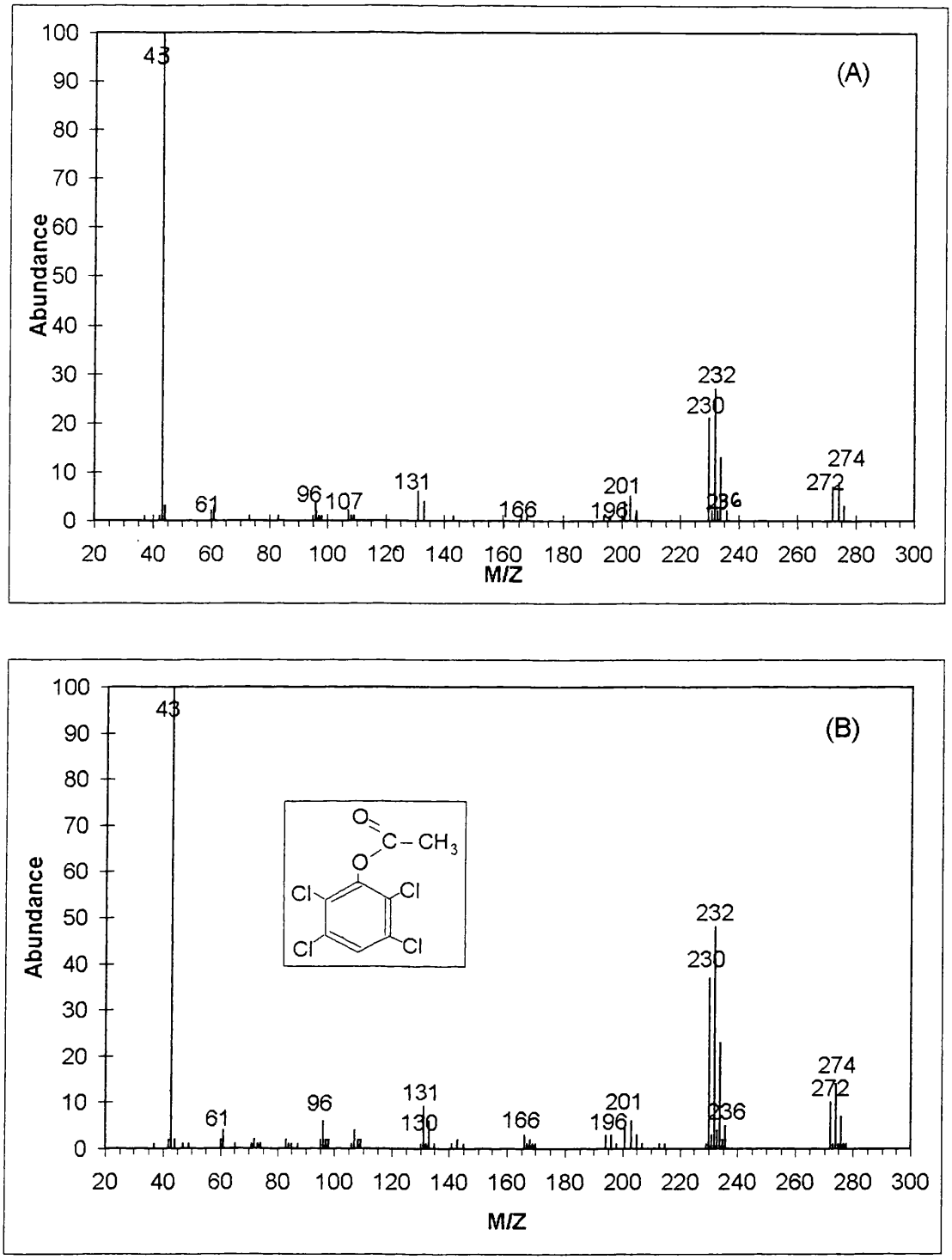


Figure 3.5 Mass spectra of (A) second unknown peak and (B) acetylated standard 2,3,5,6-tetrachlorophenol.

trichlorophenol is one of the major decomposition products of 2,3,5,6-tetrachlorophenol. Therefore, when tecnazene is irradiated further by UV light several more phenolic compounds including 2,3,5-trichlorophenol could be expected as decomposition products of tecnazene as a result of further decomposition of 2,3,5,6-tetrachlorophenol the identified decomposition product of tecnazene.

Although two unknown peaks were identified by GC-MS the quantification of the identified these compounds were not carried out on this occasion so the percentage of tecnazene used for producing the compounds was not established. No difference in the formation of decomposition products was observed by GC between the two conditions, UV irradiation of tecnazene in 10% (v/v) acetonitrile-water mixtures in the absence and presence of hydrogen peroxide because the same chromatogram patterns were obtained.

Finally, it can be said that the study of decomposition products of tecnazene using GC-MS was limited to volatile, thermally stable and relatively non-polar compounds due to the extraction by hexane, a non-polar organic solvent, and characterisation by GC analysis. However, UV irradiation possibly leads to the formation of transformation products much more polar and possibly less volatile than tecnazene. Consequently, such products would not be sufficiently extracted by hexane, and would not be volatile enough for GC analysis.

3.3. Identification and determination of anionic decomposition products using ion exchange chromatography

3.3.1 Introduction

Several anionic decomposition products were expected as a result of UV irradiation of tecnazene. Therefore, ion exchange chromatography was employed for their determination. The determination was divided into mainly three parts, inorganic, phenolic and organic carboxylic anion analysis.

3.3.2 Determination of inorganic anionic decomposition products

3.3.2.1 Introduction

Chloride, nitrate and nitrite were expected as inorganic anionic decomposition products from the structure of tecnazene. In the case of UV irradiation in the presence of hydrogen peroxide chloride oxide, perchlorate, chlorate, chlorite and hypochlorite were also expected to be present as decomposition products as a result of oxidation of chloride due to the presence of hydrogen peroxide. Therefore, determination of these inorganic anions was carried out using ion exchange chromatography.

3.3.2.2 Materials and methods

Materials

Analytical grade of potassium nitrite, sodium nitrate and potassium chloride were supplied by BDH Ltd.. Potassium perchlorate and potassium chlorate (Aldrich Chemical Co.) were >99% pure. Sodium chlorite (Technical grade, 80%, Aldrich Chemical Co.) was of highest available purity and was used as received. Sodium hypochlorite was supplied by BDH Ltd., and in which available chlorine was 12% (M/V).

Preparation of standard solutions

1000mg/L stock solution of inorganic ions were prepared from the salts. Stock solutions were stored at 4°C in order to minimize any micro-organism activity. 1mg/L standard solutions were freshly prepared from the stock solution each time for each analysis.

Sample preparation

Four conditions, UV irradiation in presence and absence of hydrogen peroxide in water, and UV irradiation in presence and absence of hydrogen peroxide in 10% (v/v) acetonitrile-water mixtures, were conducted for the determination of anions. 0.1mM hydrogen peroxide for UV irradiation of tecnazene in water, and 1mM for in 10% (v/v) acetonitrile-water mixtures were set up. 10%(v/v) acetonitrile-water mixture was

irradiated without any samples as control. All samples were filtered through a 0.2 micron filter (Millipore) for Dionex analysis after being withdrawn.

Ion exchange chromatography

Determination of inorganic anions was carried out using a Dionex DX-500 Ion Chromatograph equipped with a conductivity detector and Dionex ASRS-1 suppressor using a sodium hydroxide gradient to measure ions. Separation was performed on a Dionex IonPac AS11 column (250×4 mm I.D.) with Dionex AG11 guard column. The injection volume was 25μl using a sample loop. AS40 auto sampler was used when analysing a series of samples.

Preparation of eluents for Dionex

The eluents were prepared under a helium atmosphere using 50% (w/v) of sodium hydroxide solution (Vickers Laboratories Ltd.). The concentration of sodium hydroxide eluent prepared for inorganic anion analysis was 200mM. Helium atmosphere was constantly applied over eluents.

Method of Dionex analysis

Isocratic separation of chloride, nitrite and nitrate in 4mM sodium hydroxide followed by gradient to 20mM and re-equlibriation at 4mM was used. The flow rate was 2ml/min. Using this method typical chromatograms of standard and samples are shown in Figure 3.6. The quantification of organic anions in samples was carried out using single point standard calibration with 1mg/L of a mixed standard. In the case of determination of oxyanions of chlorine, isocratic separation in 6.2mM sodium hydroxide was used.

3.3.2.3 Results and discussion

Identification of product was established by comparison of chromatographic properties with authentic standards. As a result chloride, nitrite and nitrate were identified as inorganic anionic decomposition products, and quantified.

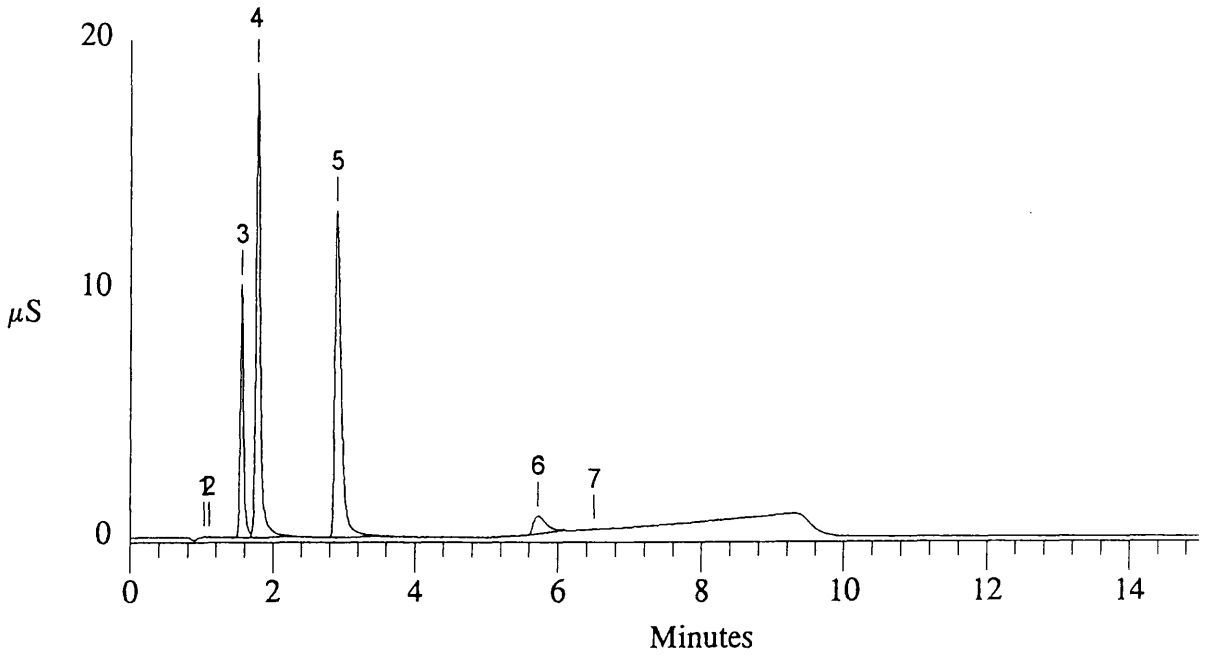


Figure 3.6 Typical ion exchange chromatogram of inorganic anions. Mixed standard.
Peaks 3=chloride, 1mg/L; 4=nitrite, 1mg/L, 5=nitrate, 1mg/L and 6=disolved carbon dioxide.

In the case of UV irradiation in water in the presence and absence of hydrogen peroxide, ion exchange chromatograms at different time points from tecnazene irradiated were given in Figures 3.7 and 3.8, and the results of the determination of chloride, nitrite and nitrate are shown in Figures 3.9 and 3.10. In Figure 3.9 Y axis shows the percentage of nitrogen that became either nitrite or nitrate from the nitrogen of tecnazene, and also shows remaining percentage for tecnazene, while in Figure 3.10 the Y axis shows percentage of chlorine that became chloride ion from the chlorine of tecnazene, and also shows the remaining percentage for tecnazene

In the case of UV irradiation in water, the rate of release of total-nitrogen produced as nitrite and nitrate is relatively faster in the presence of hydrogen peroxide (Figure 3.9). In addition, after 7.5min the release of nitrite decreased, and nitrate was released significantly compared to it in the case of UV only. Therefore, it can be observed that nitrite was oxidized quicker to nitrate in the presence of hydrogen peroxide than in the case of UV only. This could be an advantage of the treatment using hydrogen peroxide for UV irradiation of tecnazene. The rate of release of chlorine as chloride is also relatively faster in the presence of hydrogen peroxide (Figure 3.10). The rate of release of total-nitrogen and chlorine mirrored very closely the rate of loss of the tecnazene in both treatment conditions. Therefore, it can be concluded that almost all nitrogen and chlorine from decomposed tecnazene become nitrite, nitrate and chloride ions, and it seems there is no build up of intermediate decomposition products containing nitrogen and chlorine. Furthermore, in the case of UV irradiation in the presence of hydrogen peroxide, although it was observed that nitrite was quickly oxidised to nitrate, further oxidation of chloride was not observed because the most of chlorine from the decomposition of tecnazene was accounted for as chloride ions. However, as chlorine oxyanions were expected as decomposition products as a consequence of further oxidation of chloride due to the presence of hydrogen peroxide, some limited method development was carried. The chromatogram of each standard of the trial were shown in Figure 3.11. In the case of determination of hypochlorite and chlorite, there was a big extra peak in addition to the standard peak which is possibly chloride ions as a consequence of decomposition of the oxyanions. The retention time of chlorate is close to the nitrate so they might be co-eluted. The

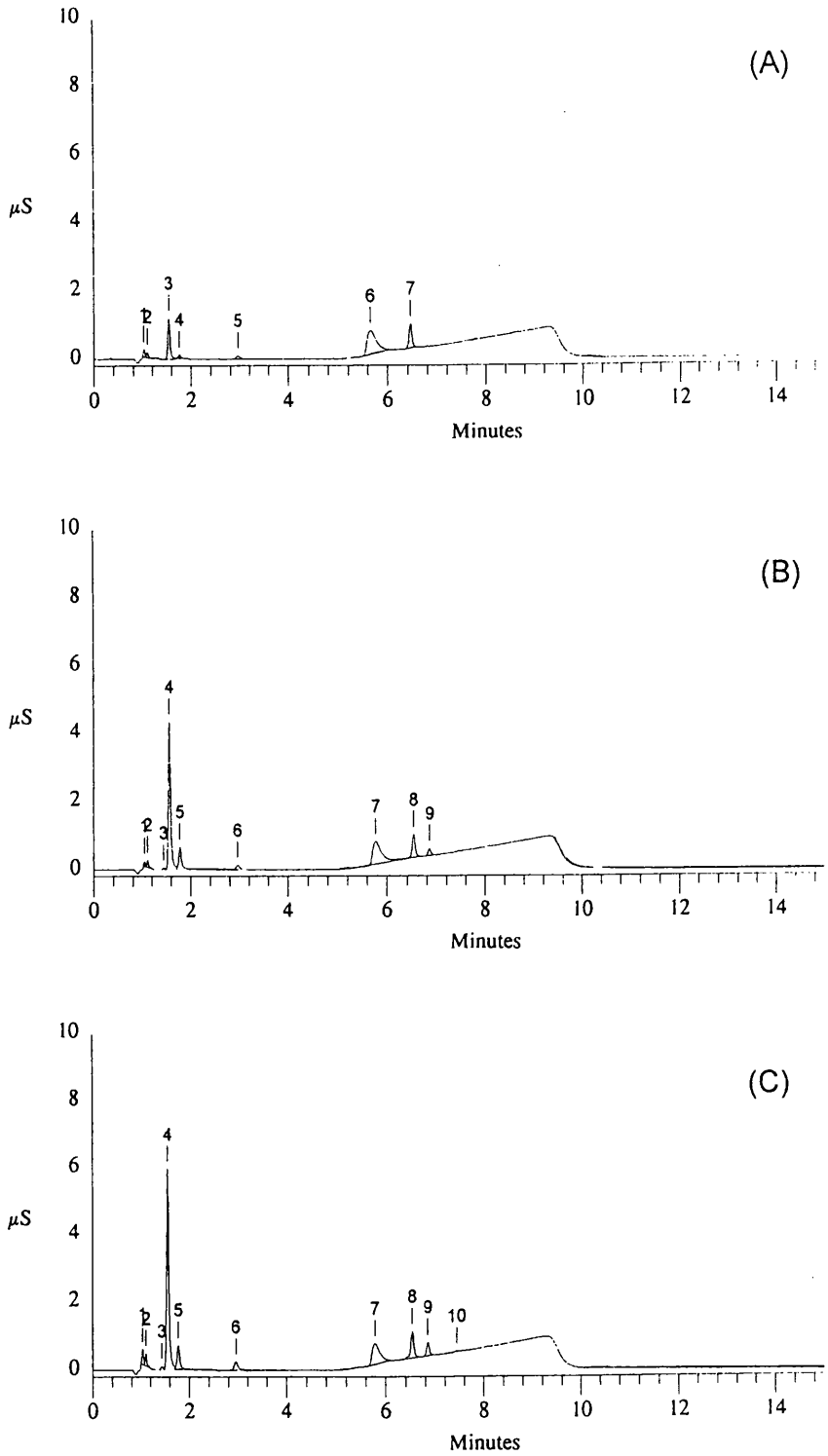


Figure 3.7 Ion exchange chromatograms for inorganic anion analysis from tecnazene irradiated by UV light in water for (A) 0min, (B) 10min and (C) 25min.

Name of anions and peak number

	Unidentified (possibly organic anions)	chloride	nitrite	nitrate	dissolved CO_2	sulphate	Unidentified (possibly organic anions)
(A) 0min	1,2,	3	4	5	6	7	-
(B) 10min	1,2,3	4	5	6	7	8	9
(C) 25min	1,2,3	4	5	6	7	8	9,10

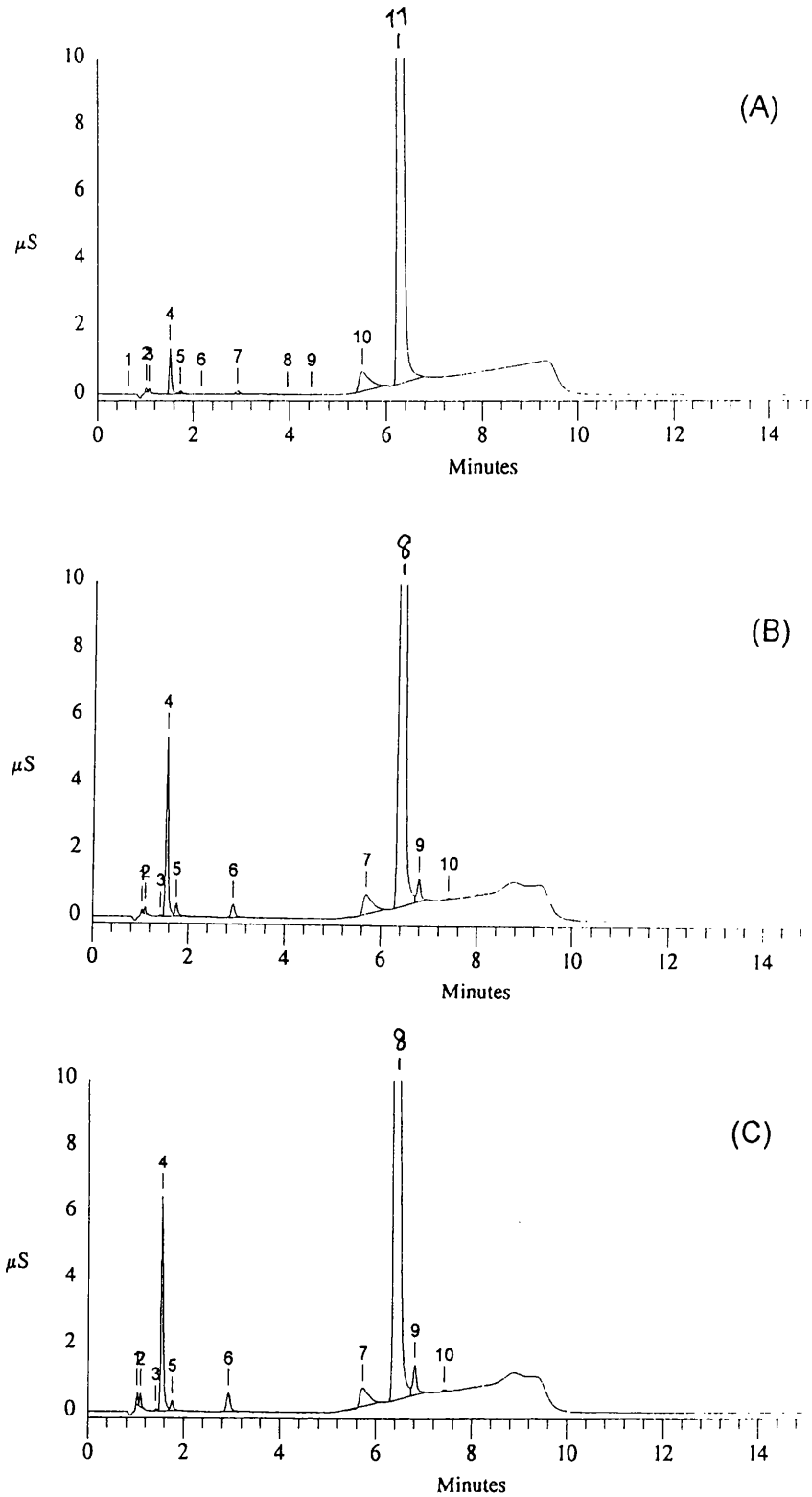


Figure 3.8 Ion exchange chromatograms for inorganic anion analysis from tecnazene irradiated by UV light with 0.1mM H_2O_2 in water for (A) 0min, (B) 10min and (C)20min.

Name of anions and peak number

	Unidentified (possibly organic anions)	chloride	nitrite	nitrate	dissolved CO_2	sulphate*)	Unidentified (possibly organic anions)
(A) 0min	2,3	4	5	7	10	11	-
(B) 10min	1,2,3	4	5	6	7	8	9,10
(C) 20min	1,2,3	4	5	6	7	8	9,10

*) from oxidised disulphite that added in order to reduce residual H_2O_2 after collecting samples

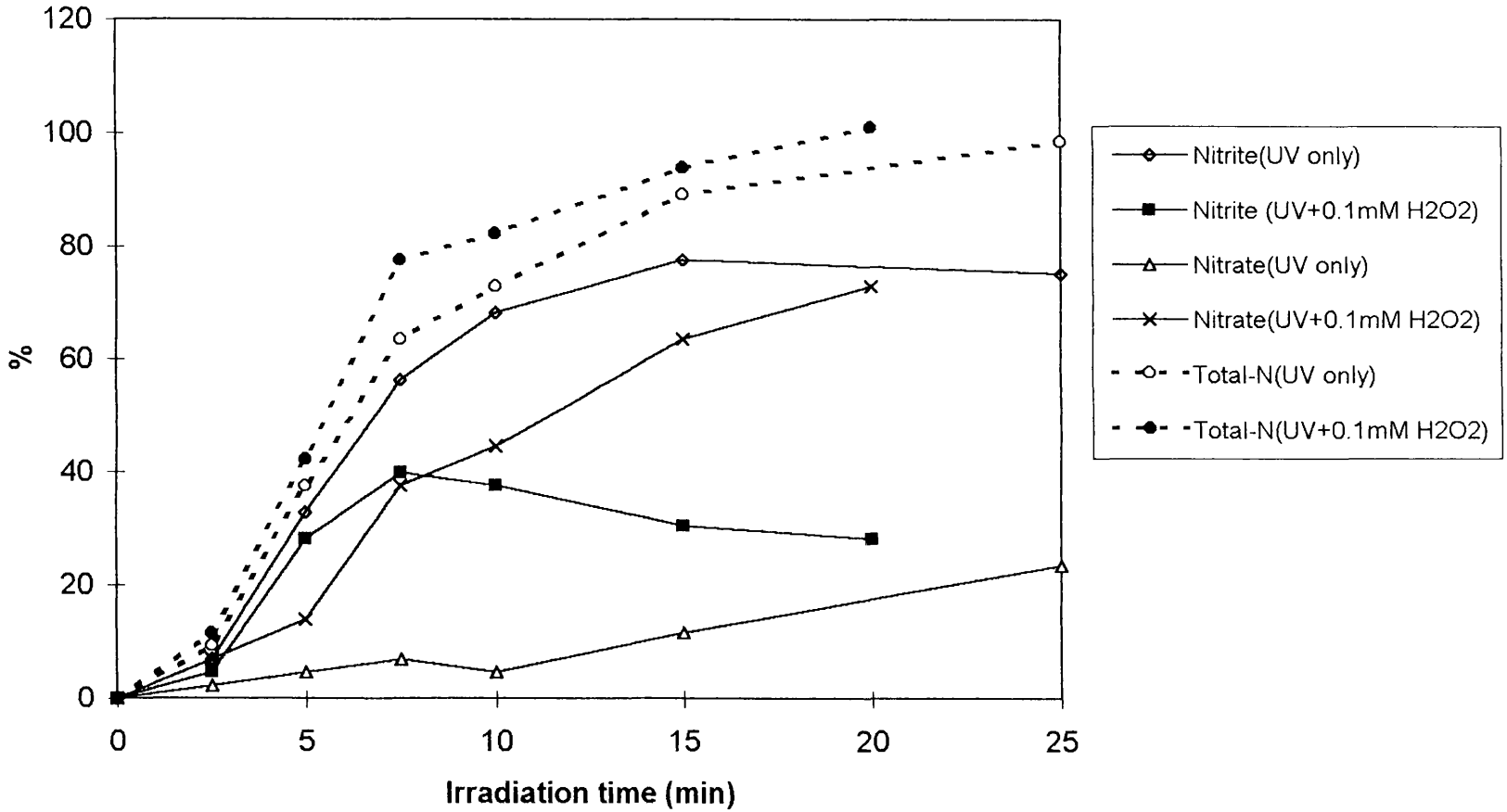


Figure 3.9 Determination of nitrite and nitrate as anionic inorganic photodecomposition products from tecnazene irradiated by UV light in water in the presence and absence of hydrogen peroxide.

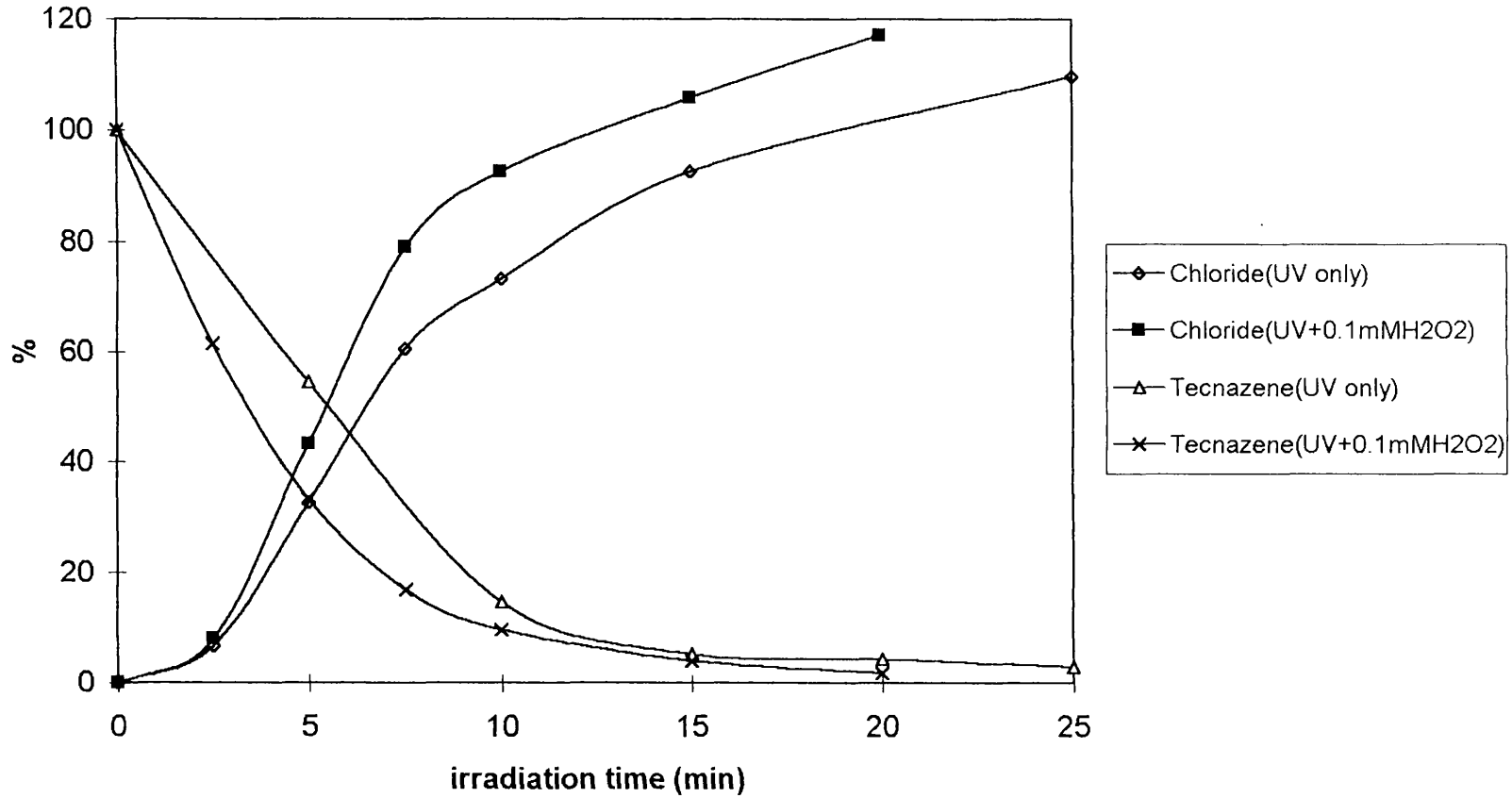


Figure 3.10 Determination of chloride as anionic inorganic photodecomposition products from tecnazene irradiated by UV light in water in the presence and absence of hydrogen peroxide.

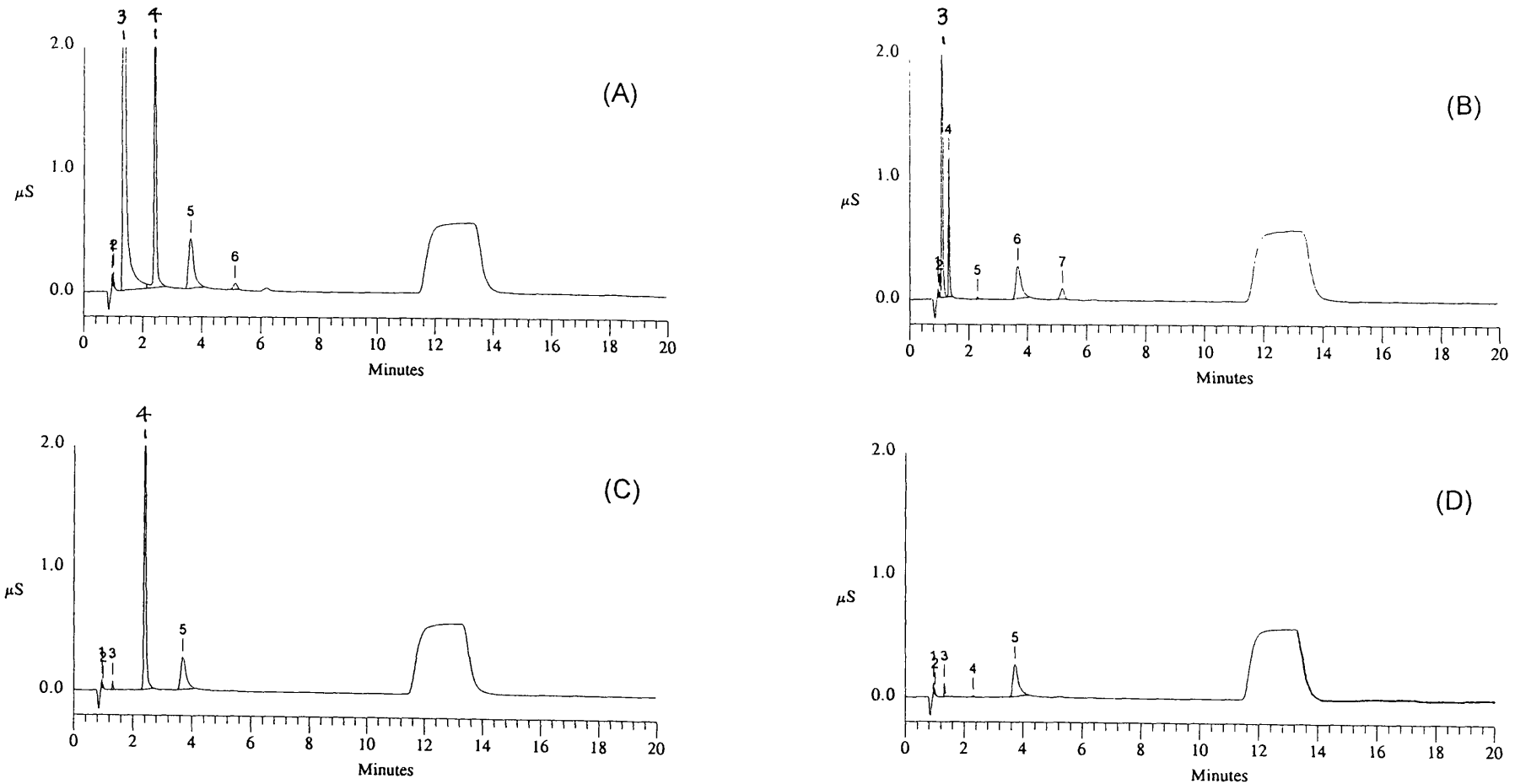


Figure 3.11 Ion exchange chromatograms of standard chlorine oxyanions.
(A) hypochlorite, 10μM; (B) chlorite, 10μM; (C) chlorate, 10μM and (D) perchlorate, 10μM.
Peaks (A) 1 and 2=may be organic anions, 3=chloride, 4=hypochlorite, 5=disolved CO_2, 6=sulphate. (B) 1 and 2=may be organic anions, 3=chlorite, 4=chloride, 5=nitrate, 6=disolved CO_2, 7=sulphate. (C) 1 and 2=may be organic anions, 3=chloride, 4=chlorate, 5=disolved CO_2. (D) 1 and 2=may be organic anions, 3=chloride, 4=nitrate, 5=disolved CO_2.

peak of perchlorate was missing on this occasion because column performance might not be enough for the separation or there might be other reasons. Therefore, further method development would be necessary for their determination if needed. The observed RTs did not correspond to any of the unidentified peak of the sample chromatograms.

In the case of UV irradiation in acetonitrile-water mixtures in the presence and absence of hydrogen peroxide, ion exchange chromatograms at different time points from tecnazene irradiated were given in Figures 3.12 and 3.13, and the results of the determination of chloride, nitrite and nitrate are shown in Figures 3.14 and 3.15. In Figure 3.14 Y axis shows the parentage of nitrogen that became either nitrite or nitrate from the nitrogen of tecnazene, and also shows remaining percentage for tecnazene, while in Figure 3.15 the Y axis shows percentage of chlorine that became chloride ion from the chlorine of tecnazene, and also shows the remaining percentage for tecnazene

As can be seen in Figure 3.14 the levels of nitrite and nitrate were higher than that could be expected from tecnazene (more then 100%). The source of nitrogen was thought to be the acetonitrile used as cosolvent. In fact acetonitrile was found to decomposed by UV light and produced nitrite and nitrate. It can be seen in the control chromatograms (Figure 3.16) for the irradiation in 10% (v/v) acetonitrile-water mixtures. Although the identification was not carried out there is high possibility of production of organic anions especially acetate. The growing peak with time in the early stage of chromatogram is very likely to be an organic anion. The production of organic acids is also structurally possible. Consequently, the determination of nitrate and nitrite release from tecnazene was not possible on this occasion. However, in the case of chloride determination the concentration of chloride increased with decreasing tecnazene concentration during irradiation either in the absence or presence of hydrogen peroxide (Figure 3.15). Although there is no significant difference in the decomposition rate of tecnazene between UV irradiation in the absence and presence of hydrogen peroxide, the rate of release of chlorine produced as chloride is relatively faster in the presence of hydrogen peroxide.

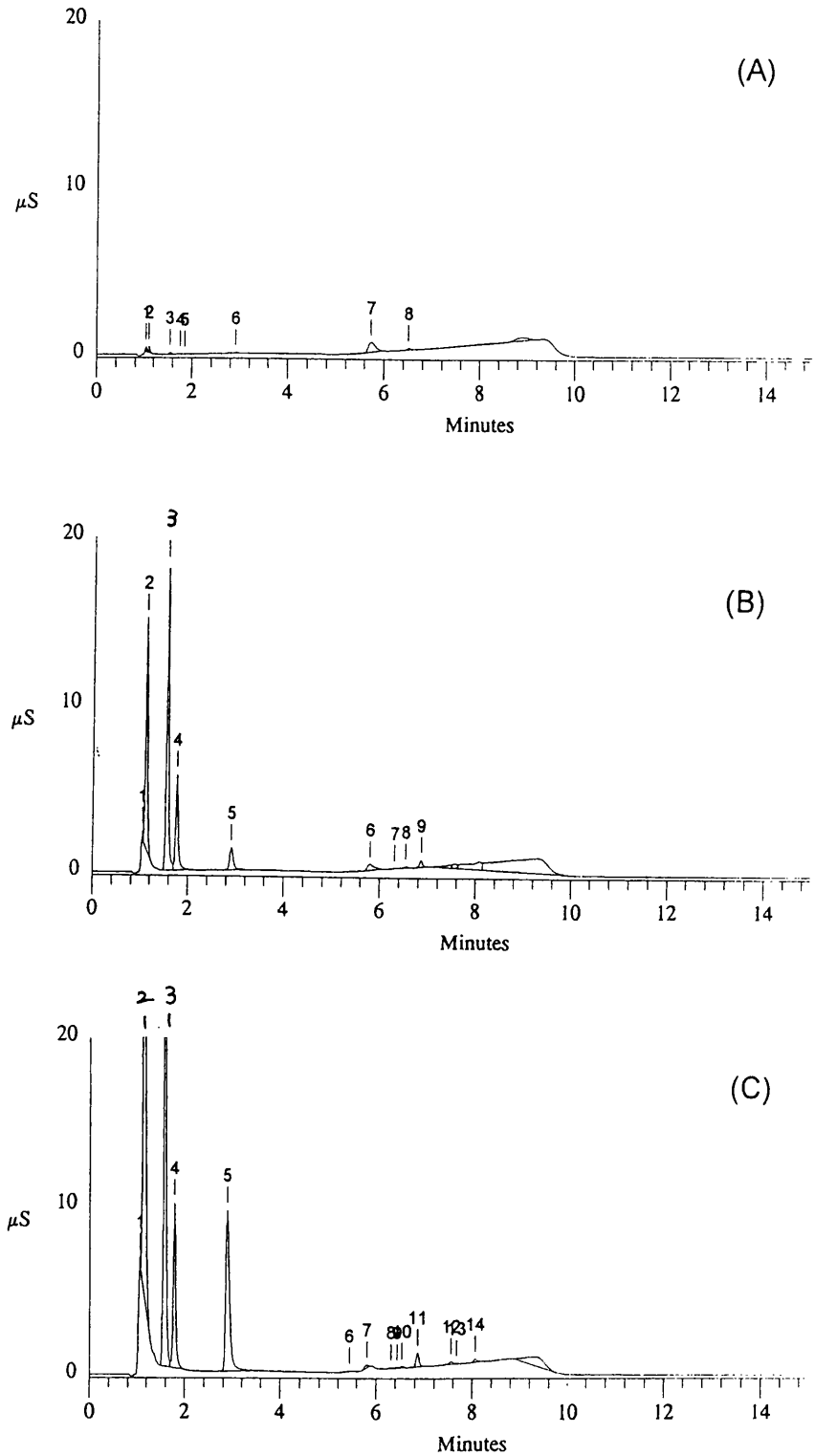


Figure 3.12 Ion exchange chromatograms for inorganic anion analysis from tecnazene irradiated by UV light in 10%(v/v) acetonitrile-water mixtures for (A) 0min, (B) 40min and (C)80min.

Name of anions and peak number

	Unidentified (possibly organic anions)	chloride	nitrite	nitrate	dissolved CO_2	Unidentified (possibly organic anions)
(A) 0min	1,2,	3	-	-	7	-
(B) 10min	1,2	3	4	5	6	7-9
(C) 25min	1,2	3	4	5	6	8-14

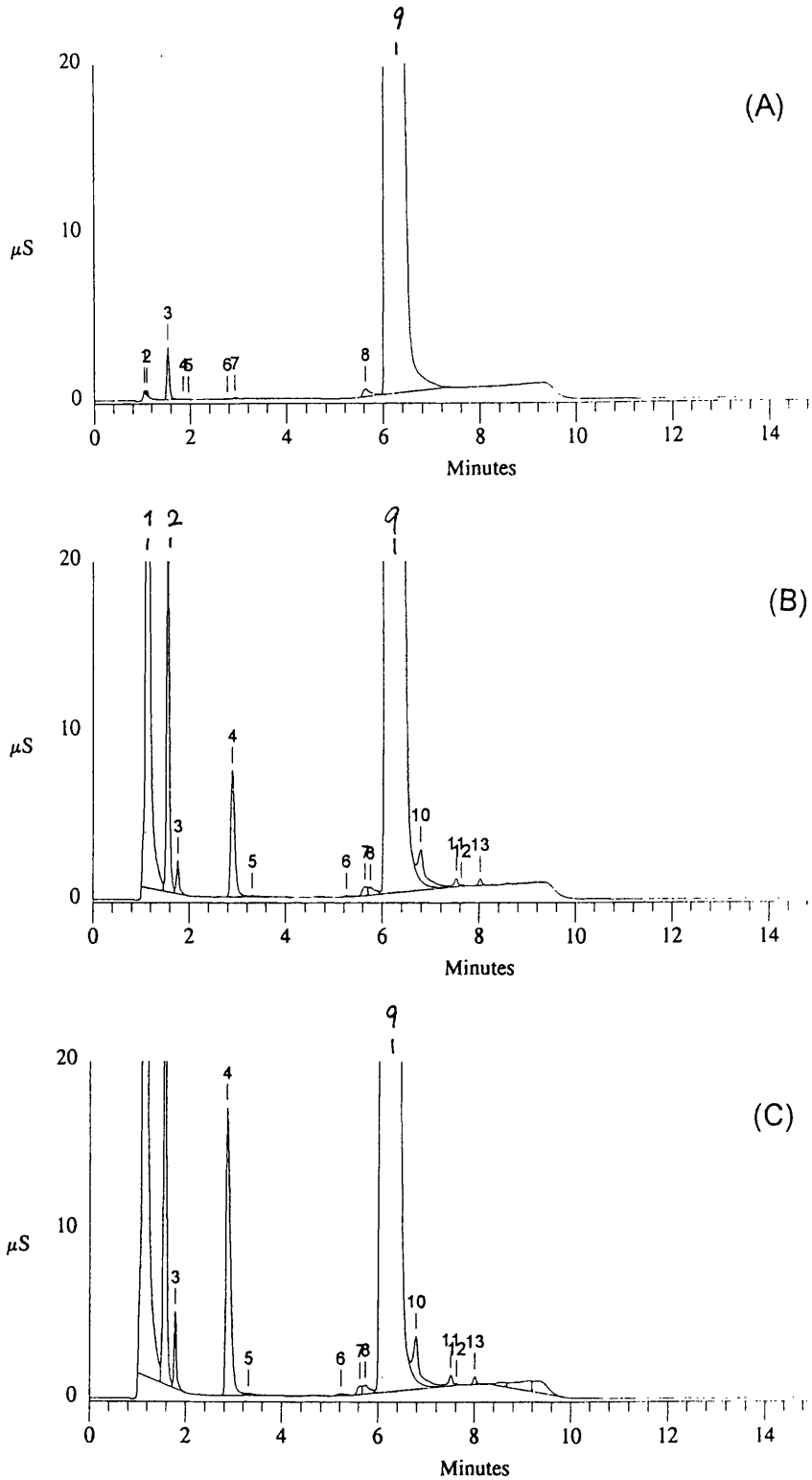


Figure 3.13 Ion exchange chromatograms for inorganic anion analysis from tecnazene irradiated by UV light with 1mM H_2O_2 in 10%(v/v) acetonitrile-water mixtures for (A) 0min, (B) 40min and (C)80min.

Name of anions and peak number

	Unidentified (possibly organic anions)	chloride	nitrite	nitrate	dissolved CO_2	sulphate*)	Unidentified (possibly organic anions)
(A) 0min	1,2	3	-	-	8	9	-
(B) 10min	1	2	3	4	7	9	5,6,8,10-13
(C) 20min	1	2	3	4	7	9	5,6,8,10-13

*) from oxidised disulphite added in order to reduce residual H_2O_2 after collecting samples.

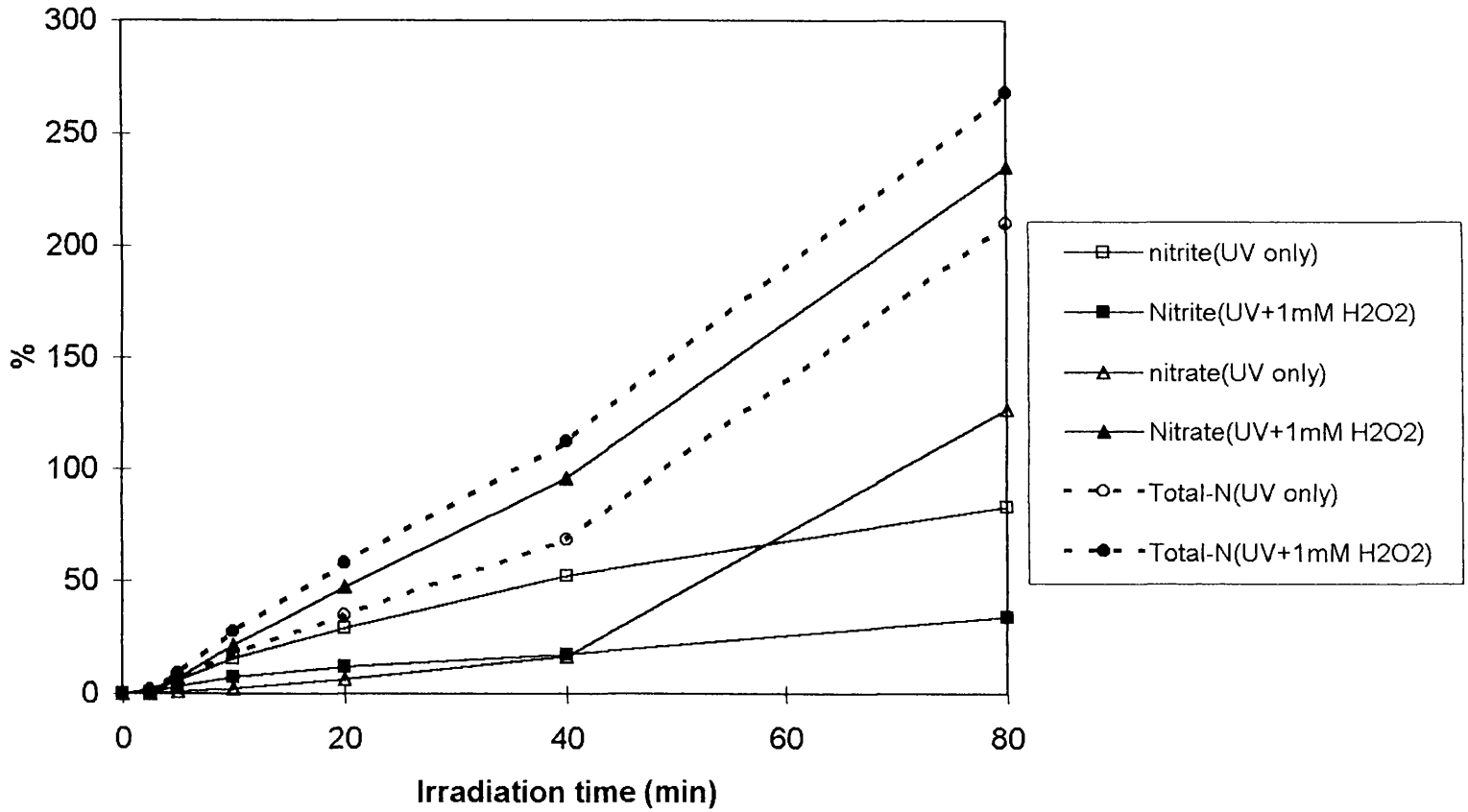


Figure 3.14 Determination of nitrite and nitrate as anionic inorganic photodecomposition products from tecnazene irradiated by UV light in 10%(v/v) acetonitrile-water mixtures in the presence and absence of hydrogen peroxide.

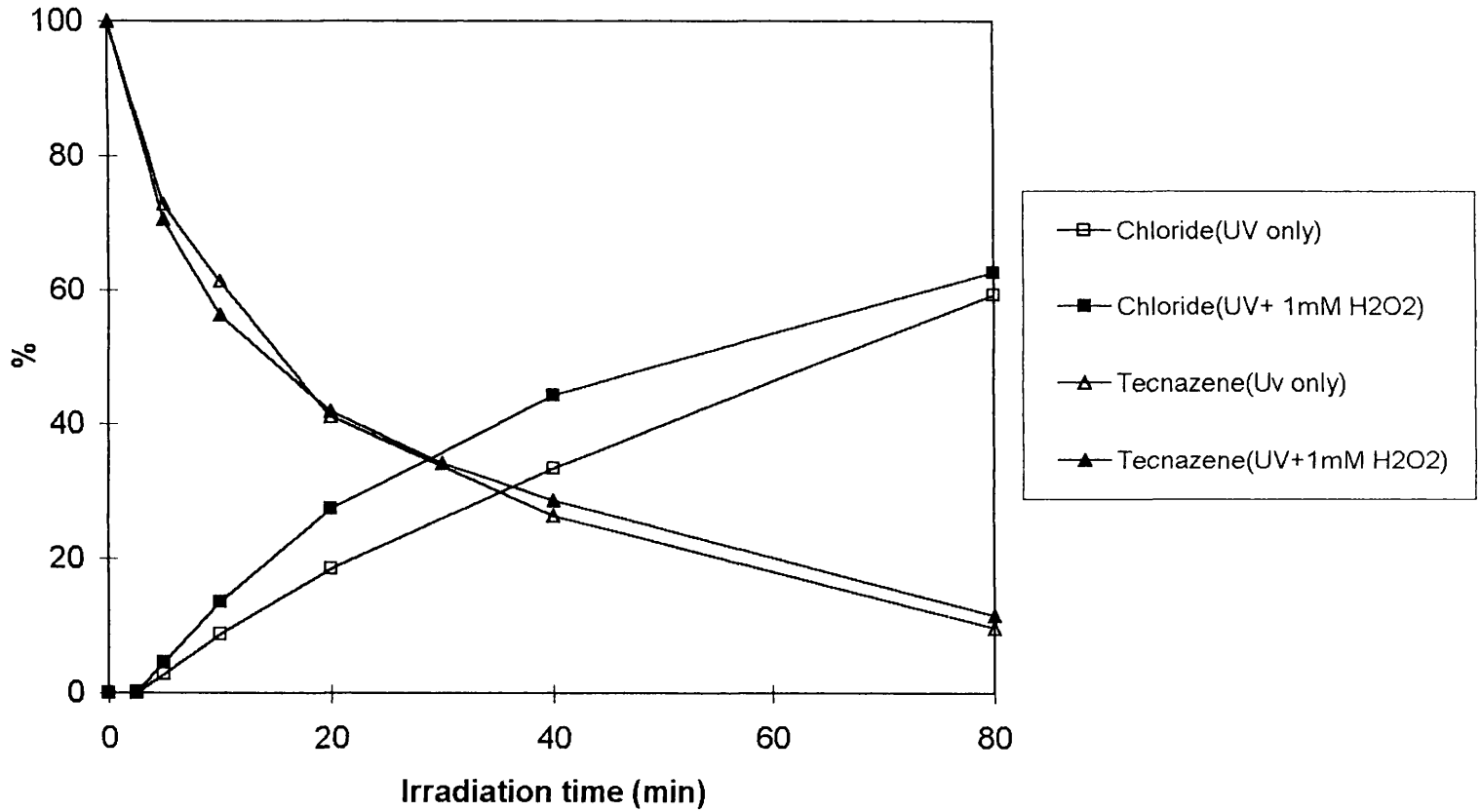


Figure 3.15 Determination of chloride as an anionic inorganic photodecomposition product from tecnazene irradiated by UV light in 10%(v/v) acetonitrile-water mixtures in the presence and absence of hydrogen peroxide.

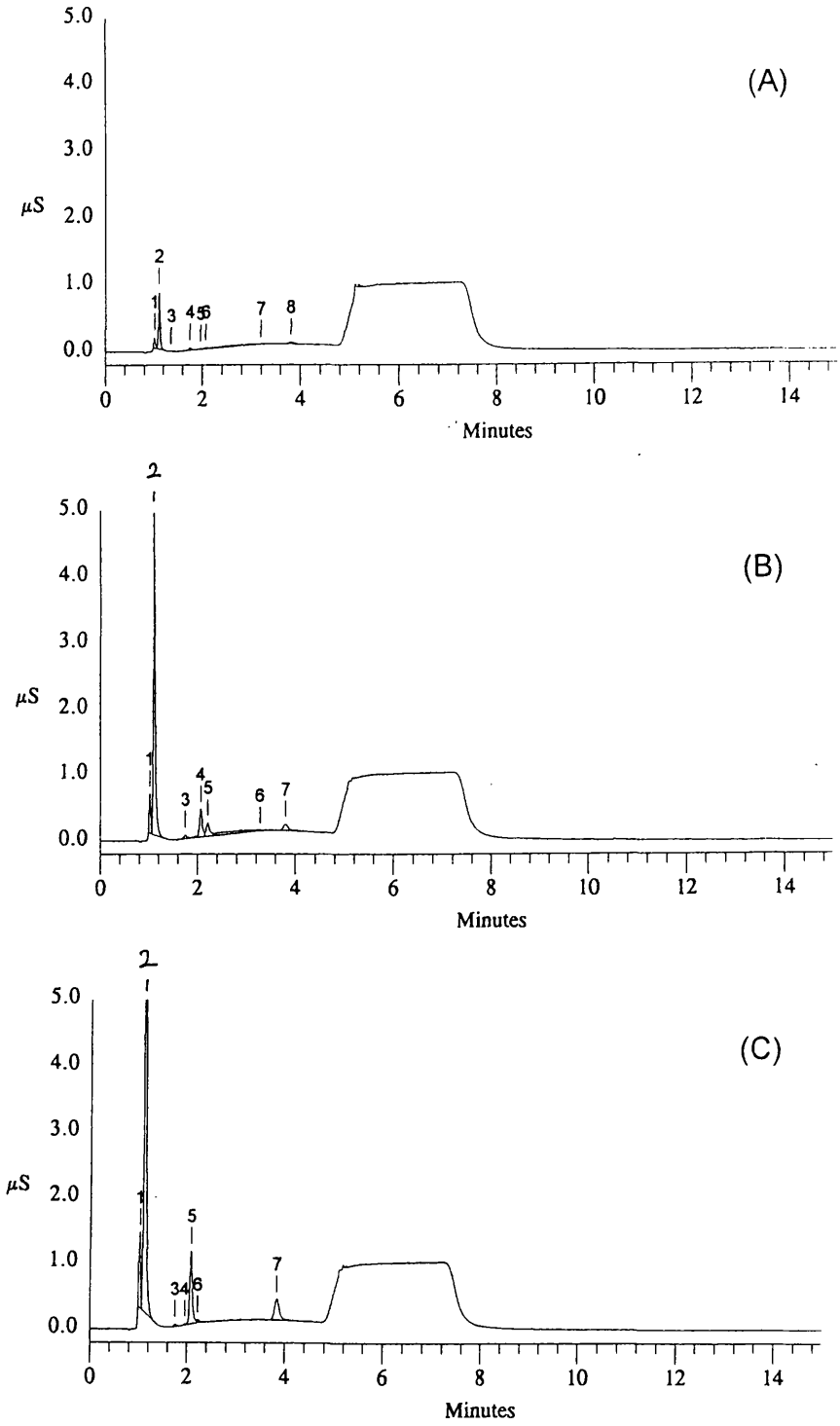


Figure 3.16 Ion exchange chromatograms of control for inorganic anion analysis. 10%(v/v) acetonitrile-water mixtures was irradiated by UV light without tecnazene for (A) 0min, (B) 40min and (C)80min.

Name of anions and peak number

	Unidentified (possibly organic anions)	chloride	nitrite	nitrate
(A) 0min	1,2	-	-	-
(B) 10min	1,2	3	4	7
(C) 25min	1,2	3	5	7

The rate of the release of chloride against the disappearance of tecnazene was relatively slow in acetonitrile-water mixtures compared with water (Figure 3.17). When the remaining tecnazene was reduced to nearly 0%, approximately 100% of the release of chloride was observed in the case of water, while under comparable conditions approximately 60% of chloride was released in 10%(v/v) acetonitrile-water mixtures. These results may indicate that chlorinated decomposition products are more likely to be found in the case of UV irradiation in acetonitrile-water mixtures. Indeed, as described in Section 3.2 the chlorinated decomposition products, trichloronitrobenzene and tetrachlorophenol were identified after most of the tecnazene was decomposed by UV irradiation in acetonitrile-water mixtures. On the other hand, in the case of water it is likely that after tecnazene was decomposed, all the chlorines were released as chloride ions so the formation of chlorinated decomposition products is very unlikely. There is no significant difference in the rate of the release of chloride in the absence and the presence of hydrogen peroxide, especially in acetonitrile-water mixtures.

3.3.3 Determination of phenolic compounds

3.3.3.1 Introduction

Because 2,3,5,6-tetrachlorophenol was found by GC as one of the photodecomposition products, determination of the phenolic compound, 2,3,5,6-TCNB and other possible phenolic compounds were carried out by ion exchange chromatography. The determination of phenolic compounds by ion exchange chromatography has an advantage compared to by GC because further sample preparation, such as extraction by an organic solvent and acetylation, are not necessary. Phenolic compounds are not expected to be detected by a conductivity detector as they would be unionised after passage through the suppressor. Therefore, a UV detector was used for detection of phenolic compounds since phenolic compounds absorb UV light. Two detectors, conductivity detector for inorganic anions and the UV detector for phenolic compounds were used in series. Four

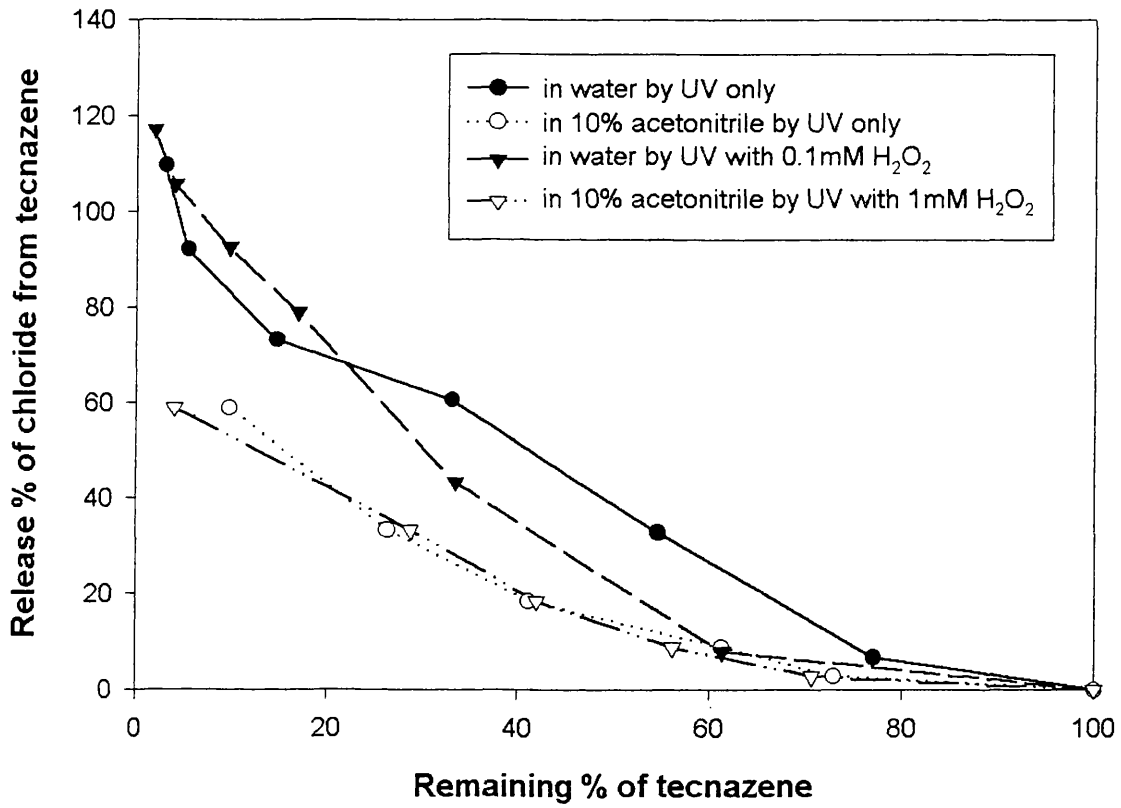


Figure 3.17 Release of chloride and remaining % of tecnazene

available phenolic compounds, phenol, 2,4-dichlorophenol, 2,5-dichlorophenol and 2,3,5,6-tetrachlorophenol were tested.

3.3.3.2 Materials and method

Materials

Analytical grade of phenol (detached crystals) was supplied by Fisher Scientific. 2,4-dichlorophenol of purity and supplier was unknown. 2,5-dichlorophenol (Aldrich Chemical Co.) was >98% pure. 2,3,5,6-tetrachlorophenol (Aldrich Chemical Co.) was recrystallised once previously.

Preparation of standard solution

Except for phenol other three chlorinated phenolic compounds used in this study are practically insoluble in water. Therefore, 10% (v/v) acetonitrile-water mixtures was used instead. 10mg/L phenol aqueous solution, 10mg/l of other three phenolic compounds in 10% (v/v) acetonitrile-water mixtures were prepared.

Absorption spectrum of inorganic anions and phenolic compounds

The absorption spectrum of inorganic anions (chloride, nitrite, nitrate and sulphate) were measured in neutral conditions by ultraviolet spectroscopy. Phenolic compounds (phenol, 2,3,4,5-tetrachlorophenol, 2,4-dichlorophenol and 2,5-dichlorophenol) were measured in both neutral and alkaline conditions.

Ion exchange chromatography

A UV detector (Model 450 variable wavelength detector (Water Associates)) was placed between the separating column, and the suppressor and the conductivity detector. Other conditions for ion exchange chromatography were the same as for the determination of inorganic anions (chloride, nitrite and nitrate) (see Section 3.3.2).

3.3.3.3 Results and discussion

Chloride and sulphate did not absorb UV light, but nitrite and nitrate showed absorption peak at 210nm and 200nm (Figures 3.18 and 3.19). The peak absorption of the phenolic compounds shifted a little bit in alkaline conditions (≅ pH10) compared to in neutral conditions (see Figures 3.20 and 3.21). In the ion exchange chromatography system, the condition is alkaline due to the eluent, sodium hydroxide as the UV detector was placed before the suppressor. Therefore, the wavelength of UV detector were set up according to the UV spectra in alkaline condition. At near 200nm of wavelength, the base line of the UV detector was noisy. Moreover, nitrite and nitrate were detected at this range. Therefore, in order to obtain a better base line and avoid the interfere by nitrite and nitrate, wavelengths around 300nm was used. 285nm for phenol, 310nm for 2,3,5,6-tetrachlorophenol, 305nm for 2,4-tetrachlorophenol, and 300nm for 2,5-tetrachlorophenol were set up. Single component standard solution of 10mg/L were injected. However, only phenol was detected by the UV detector on this occasion. The possible reason that the chlorinated phenolic compounds were not detected is that these compound might have been stuck on the column due to their insolubility in water. Therefore, further method development was not carried out because there was a risk to damage the column.

As mentioned before, determination by ion exchange chromatography is useful because extra sample preparation is not necessary. Although the determination of chlorinated phenolic compounds did not succeed on this occasion, using other types of column or mixing methanol in the eluent may be useful for further method development. A polarographic detector may also be useful to provide greater sensitivity than can be achieved with a UV detector.

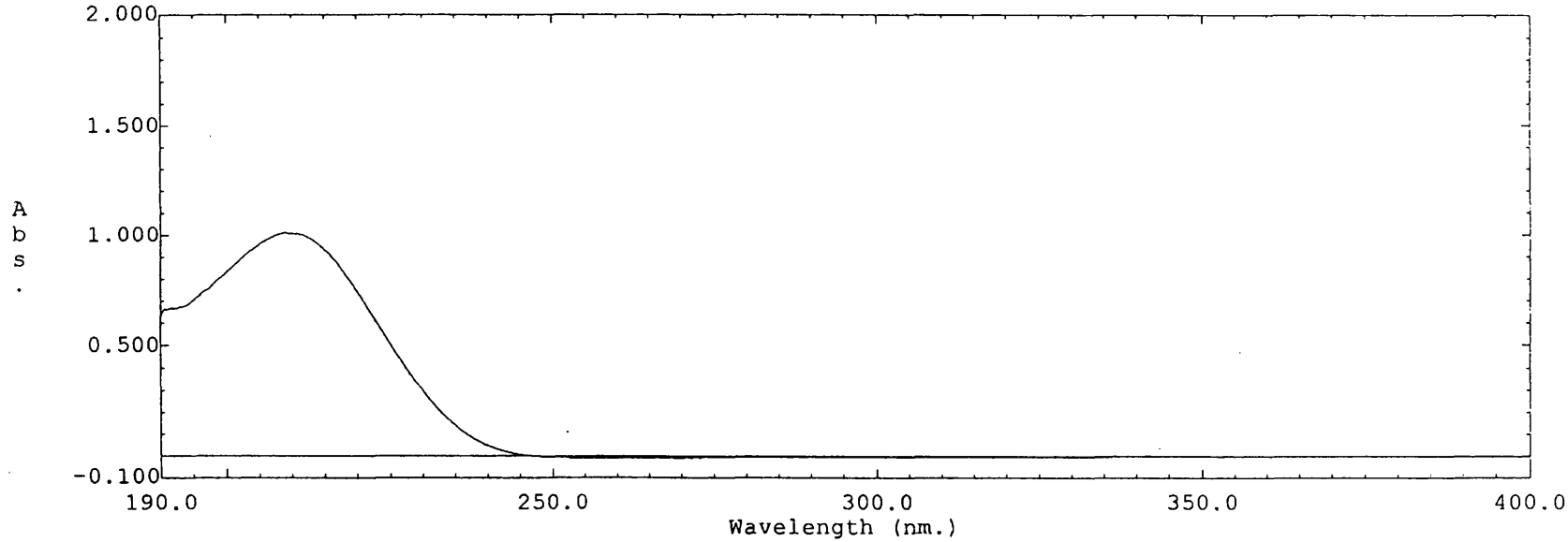


Figure 3.18 The UV absorption spectrum of nitrite in aqueous solution. Nitrite concentration; 2.5mg/L.

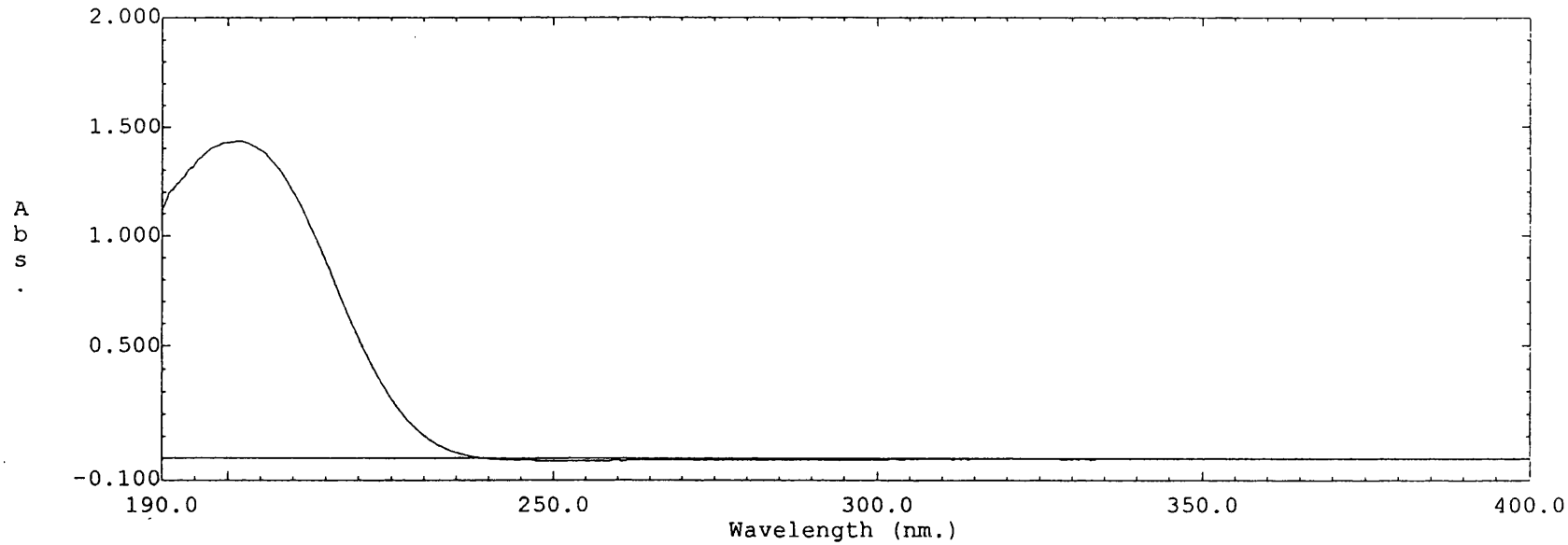


Figure 3.19 The UV absorption spectrum of nitrate in aqueous solution. Nitrate concentration; 2.5mg/L.

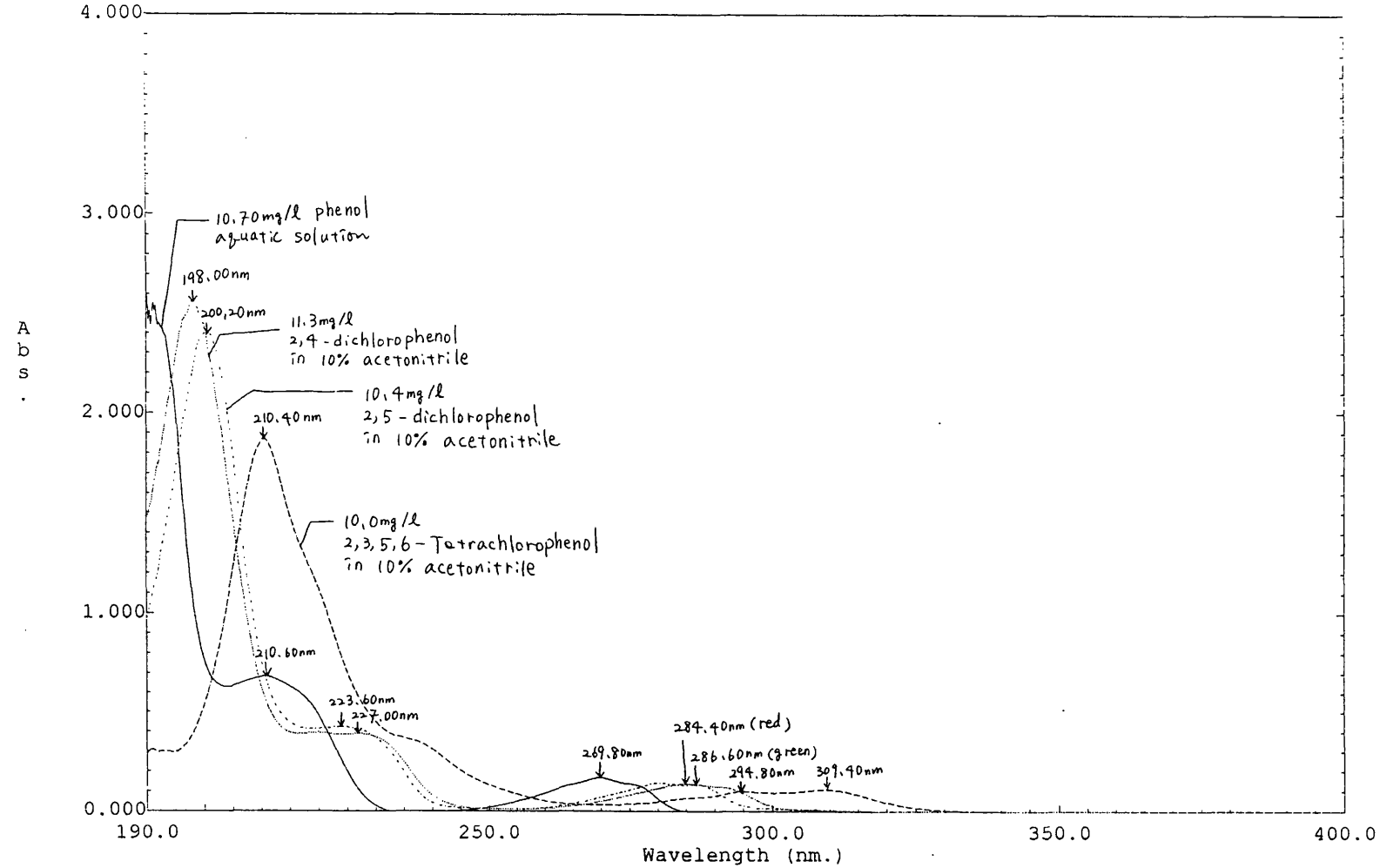


Figure 3.20 The UV absorption spectrum of phenolic compounds in neutral condition.

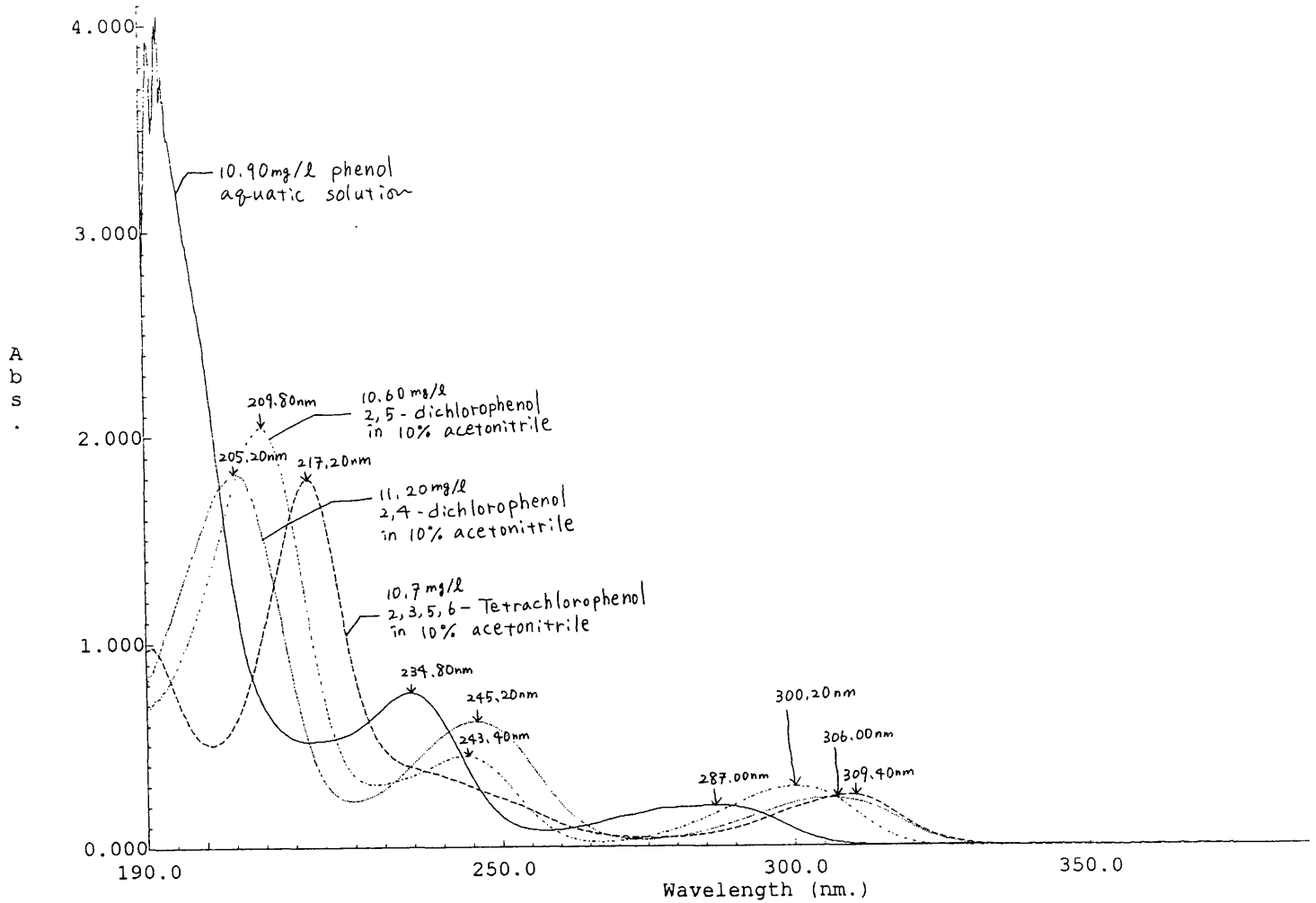


Figure 3.21 The UV absorption spectrum of phenolic compounds in alkaline condition (pH ≅ 10).

3.3.4 Identification and quantification of organic anionic decomposition products

3.3.4.1 Introduction

Scheuer *et al.* (1995) have found that several anions of carboxylic acids such as acetate, glycolate, malonate and oxalate were produced as decomposition products during UV irradiation treatment in the presence of hydrogen peroxide of 2,4-dichlorophenoxyacetic acid (2,4-D), 2-nitrobenzoic acid and nitrobenzene. Tecnazene was also expected to be converted into organic anions due to a ring opening reaction during treatment. Therefore, determination of organic anions was carried out using ion-exchange chromatography. Although acetonitrile was considered as a cosolvent to obtain high starting concentration of tecnazene, it was decided to carry out the reaction in water only since acetonitrile was found to be decomposed by UV light as mentioned in previous section and there is a possibility of the formation of organic anions from acetonitrile.

3.3.4.2 Materials and methods

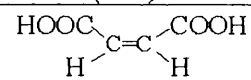
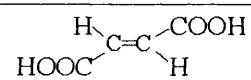
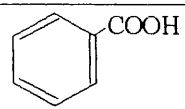
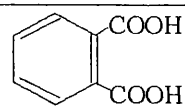
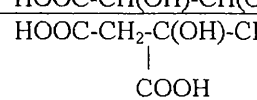
Materials

Methan-sulfonic acid was supplied by Fulka Chemie and was >99% pure. The detail of materials which used for preparation of organic anion standard solutions is given in Table 3.2.

Preparation of standard solutions

Stock solutions (100mM or 10mM for less soluble materials) of organic acids were prepared with the salts of the acids. When a salt was not available, the corresponding organic acid was used instead. Stock solutions were stored at 4°C in order to minimize the micro-organism activity. Therefore these stock solutions could be used for a few months. 10μM or 5μM standard solutions were freshly prepared from the stock solution each time for each analysis. In the case of using the stock solutions prepared with organic acids, the standard solutions were neutralized using 0.1M sodium hydroxide.

Table 3.2 A list of carboxylic acid and their materials for standard

	Common name	Structure	Material	Purity*)	Supply company
Monocarboxylic acid	formic acid	H-COOH	sodium formate	>99%	Koch-light Laboratories Ltd.
	acetic acid	CH3-COOH	sodium acetate	>99.9% (AR)	FSA Laboratory Supplies
	propionic acid	C2H5-COOH	propionic acid	>99% (GPR)	BDH Ltd.
	butyric acid	CH3-(CH2)2-COOH	butylic acid	>99% (GPR)	BDH Ltd.
Dicarboxylic acid	oxalic acid	HOOC-COOH	ammonium oxalate	>99% (AR)	BDH Ltd.
	malonic acid	HOOC-CH2-COOH	malonic acid	>99% (GPR)	Hopkin & Williams
	succinic acid	HOOC-(CH2)2-COOH	succinic acid	>99.5% (AR)	BDH Ltd.
	glutaric acid	HOOC-(CH2)3-COOH	glutaric acid	>99%	Aldrich Chemical Co. Inc.
	maleic acid		maleic acid	99.5-100.5% (LR)	BDH Ltd.
	fumaric acid		fumaric acid	ROS	Hopkin & Williams
Aromatic carboxylic acid	benzoic acid		benzoic acid	>99.9% (AR)	Formachem Ltd.
	phthalic acid		phthalic acid	>99% (LR)	BDH Ltd.
Hydroxylic acid	glycolic acid	CH2(OH)-COOH	glycolic acid	ROS	Hopkin & Williams
	lactic acid	CH3-CH(OH)-COOH	Ammonium lactate	About 60% w/w	BDH Ltd.
	malic acid	HOOC-CH(OH)-CH2-COOH	DL-malic acid	>99% (LR)	BDH Ltd.
	tartaric acid	HOOC-CH(OH)-CH(OH)-COOH	ammonium tartrate	>99% (AR)	Hopkin & Williams
	citric acid		sodium citrate	AR	BDH Ltd.

*) AR (Analytical Reagent); GPR (General Purpose Reagent); LR (Laboratory Reagent); ROS (Reagent for Organic Synthesis).

Ion exchange chromatography

Determination of organic anions was carried out using a Dionex DX-500 Ion Chromatograph equipped with a conductivity detector and Dionex ASRS-1 suppressor using a sodium hydroxide gradient to measure ions. Separation was performed on a Dionex IonPac AS11 column (250×4 mm I.D.) with Dionex AG11 guard column. The injection volume was 100μl using a sample loop. AS40 auto sampler was used when analysing a series of samples.

Preparation of eluents for Dionex

The eluents were prepared under a helium atmosphere using 50% (w/v) of sodium hydroxide solution (Vickers Laboratories Ltd.). The concentrations of sodium hydroxide eluents prepared for Dionex analysis were and 1mM, 20mM and 200mM. Helium atmosphere was constantly applied over the eluents.

Method development

First, a method of Dionex analysis for organic anions was developed. Determination of organic anionic ions was divided into two parts, namely for low-affinity (single charged organics) anions and high-affinity anions (doubly charged organics).

(1) Determination of low affinity organic anion

From the literature review (Chen, 1996; Ammann & Ruttimann, 1995; Scheuer *et al.*, 1995), possible organic anions as decomposition products were selected. Six organic anions, formate, acetate, propionate, butyrate, glycolate and lactate were chosen as low affinity organic anions. Kerr (1996) developed the method for the determination of organic acids, such as acetate and formate using the Dionex system. Therefore, this method was employed as the starting point of further method development. Although several gradient concentrations of sodium hydroxide were tried in order to obtain a better resolution, acetate was co-eluted with glycolate, and a method which can separate these two anions was not established. Acetate has simpler structure than glycolate, and is more likely to be a decomposition product. Therefore, the method

was established with five organic anions except glycolate. The final method that has the most effective resolution and peak conformation compromise for the five organic anions are given in Table 3.3. The flow rate was 1ml/min. A gradient from 0.15mM up to 0.25mM sodium hydroxide followed by a gradient to 20mM sodium hydroxide and re-equlibriation at 0.15mM were used.

Table 3.3 Gradient conditions for the ion exchange chromatographic analysis of low affinity organic anions.

time (min)	Eluent 1, water (%)	Eluent 2, 1mM sodium hydroxide (%)	Eluent 2, 200mM sodium hydroxide (%)	Eluent concentration (mM sodium hydroxide)
0.1	85	15	0	0.15
10	75	25	0	0.25
12	90	0	10	20
20	90	0	10	20
20.1	85	15	0	0.15
30	85	15	0	0.15

Using the method described above a typical chromatogram for the standards is shown in Figure 3.22.

Kerr (1996) mentioned the variation in retention times of organic anions and suggested the use of methane-sulfonic acid as internal standard in order to obtain relative retention times of each anion. There was also variation in retention time on this occasion. Therefore, it was decided to use methane-sulfonic acid as an internal standard. The absolute retention time of standard samples and methane-sulfonic acid, the relative retention time calculated as the fraction (sample RT/MSA RT) are given in Table 3.4. As can be seen the relative retention time is less variable compared to the absolute retention time, and can be reliably used for the identification of unknown peaks.

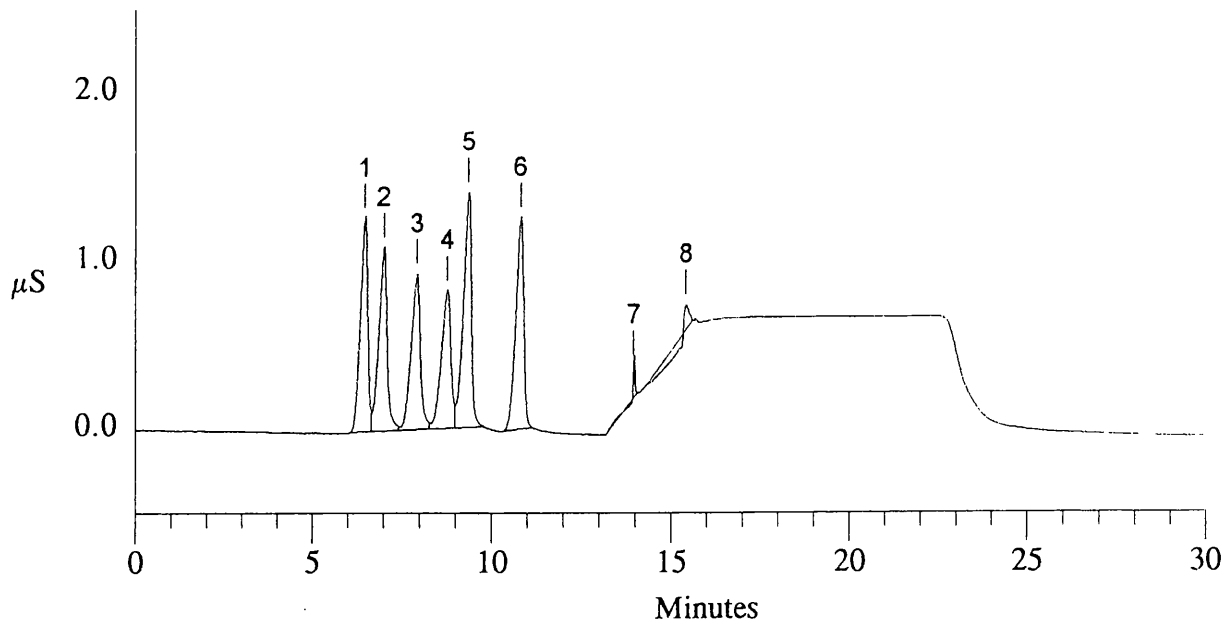


Figure 3.22 Typical ion exchange chromatogram of low affinity organic anions. Mixed standard.

Peaks 1=lactate, 10μM; 2=acetate, 10μM; 3=propionate, 10μM; 4=butyrate, 10μM; 5=formate, 10μM and 6=methane sulphonate, 10μM.

Table 3.4 Absolute retention time of standard samples and methan-sulfonic acid (MSA), and relative retention time of samples for determination of low affinity organic anions by ion exchange chromatography

	MSA	lactate		acetate		propionate		butyrate		formate	
	ART	ART	RRT	ART	RRT	ART	RRT	ART	RRT	ART	RRT
	11.12	6.83	0.614	7.32	0.663	8.33	0.749	9.22	0.829	9.68	0.871
	10.90	6.70	0.615	7.23	0.659	8.33	0.754	9.18	0.829	9.63	0.866
	10.78	6.57	0.609	7.08	0.657	8.00	0.742	8.83	0.819	9.35	0.867
	10.93	6.67	0.610	7.20	0.659	8.13	0.744	8.98	0.822	9.50	0.869
	10.93	6.67	0.610	7.18	0.657	8.13	0.744	8.98	0.822	9.50	0.869
	10.98	6.72	0.612	7.23	0.658	8.18	0.745	9.03	0.822	9.55	0.870
Mean value	10.94	6.69	0.612	7.22	0.659	8.18	0.746	9.04	0.824	9.54	0.869
Stdev	0.11	0.085	0.002	0.094	0.002	0.13	0.004	0.14	0.004	0.12	0.002
RSD%	1.01	1.26	0.36	1.30	0.33	1.57	0.59	1.59	0.50	1.21	0.19

*)ART; Absolute retention time, RRT; Relative retention time (=sample ART/MSA AST)

(2) Determination of high affinity organic anion

In the case of the determination of high affinity organic anions, there are many possible organic anions. Therefore, the method development was established with actual UV irradiated samples rather than all the high affinity organic anion standards in Table in 3.2. Initially, the method for the determination of inorganic ions was considered as the starting point of method development. However, it was found that a gradient gave a noisy baseline for the determination of organic ions because the concentrations of ions in the samples were relatively low. Therefore, an isocratic separation approach was adopted in order to obtain a better base line. The final method that has the most effective resolution and peak conformation compromise is given in Table 3.5. The flow rate was 2ml/min. Isocratic separation in 6.2mM sodium hydroxide followed by gradient to 20mM and re-equilibration at 6.2mM was used.

Table 3.5 Gradient conditions of ion exchange chromatography analysis for high affinity organic anion

time (min)	Eluent 1, water (%)	Eluent 2, 20mM sodium hydroxide (%)	Eluent 2, 200mM sodium hydroxide (%)	Eluent concentration (mM sodium hydroxide)
0.1	69	31	0	6.2
10	69	31	0	6.2
10.1	90	0	10	20
20	90	0	10	20
20.1	69	31	0	6.2
30	69	31	0	6.2

There was also a variation in retention time in this series of analysis. Therefore, it was decided to use sulphate ions as an internal standard on this occasion. The absolute retention time of two representative standards (succinate and oxalate) and sulphate, the relative retention time calculated as the fraction (sample RT/sulphate RT) are given in Table 3.6. As can be seen the relative retention time is less variable compared to the absolute retention time, and can be reliably used for the identification of unknown peaks.

Table 3.6 Absolute retention time of standards and sulphate, and relative retention time of standards for determination of high affinity organic anions by ion exchange chromatography

		succinate		oxalate	
	ART	ART	RRT	ART	RRT
	4.29	2.73	0.636	5.18	1.207
	4.45	2.82	0.634	5.37	1.207
	4.72	2.99	0.633	5.70	1.208
	4.27	2.71	0.635	5.15	1.206
	4.42	2.80	0.633	5.33	1.206
	4.75	3.01	0.634	5.72	1.204
Mean value	4.48	2.84	0.634	5.41	1.206
Stdev	0.21	0.13	0.0011	0.25	0.0012
RSD%	4.6	4.5	0.18	4.6	0.10

*)ART; Absolute retention time, RRT; Relative retention time (=sample ART/MSA AST)

Sample preparation

Two conditions, UV irradiation of tecnazene in presence and absence of hydrogen peroxide in water, were conducted for the determination of organic anions. In the case of the presence of hydrogen peroxide 0.1mM concentration was used. After being withdrawn, in the case of the determination of low affinity organic anions methane sulfonic acid was added to a final concentration of 10μl/L as internal standard in order to obtain relative retention times of organic anions in the samples. In the case of the determination of high affinity organic anions, the sulphate ion that is present in all samples as an impurity was used to obtain relative retention times. All samples were then filtered through a 0.2 micron filter (Millipore) for Dionex analysis.

3.3.4.3 Results and discussion

Identification of the products was established by comparison of the chromatographic properties with authentic standards. In both treatment conditions, UV irradiation in the presence and absence of hydrogen peroxide, three unknown peaks were obtained with the method for low affinity organic anions and then identified as lactate, acetate and formate. Chromatograms at different time points were given in Figures 3.23 and 3.24.

In the case of determination of high affinity organic anions three unknown peaks were also obtained in both treatment conditions. Identification of these peaks were carried out by comparison of possible eleven organic anion standards (see Table 3.2). As a result, two of unknown peaks were identified as succinate and oxalate. Altough oxalate was co-eluted with fummarate, from the literature review (Scheuer *et al.*, 1995; Ammann & Ruttimann, 1995) oxalate is more common anion as decomposition products so one of unknown peaks was identified and quantified as oxalate. The retention time of the other unknown peak did not match any of the possible eleven organic standard anions. Therefore, identification of this peak was not established on this occasion. Among the eleven organic standard anions tartrate and malonate were co-eluted with carbonate. Therefore, further method development is necessary for the confirmation of the presence of these anions as decomposition products. The

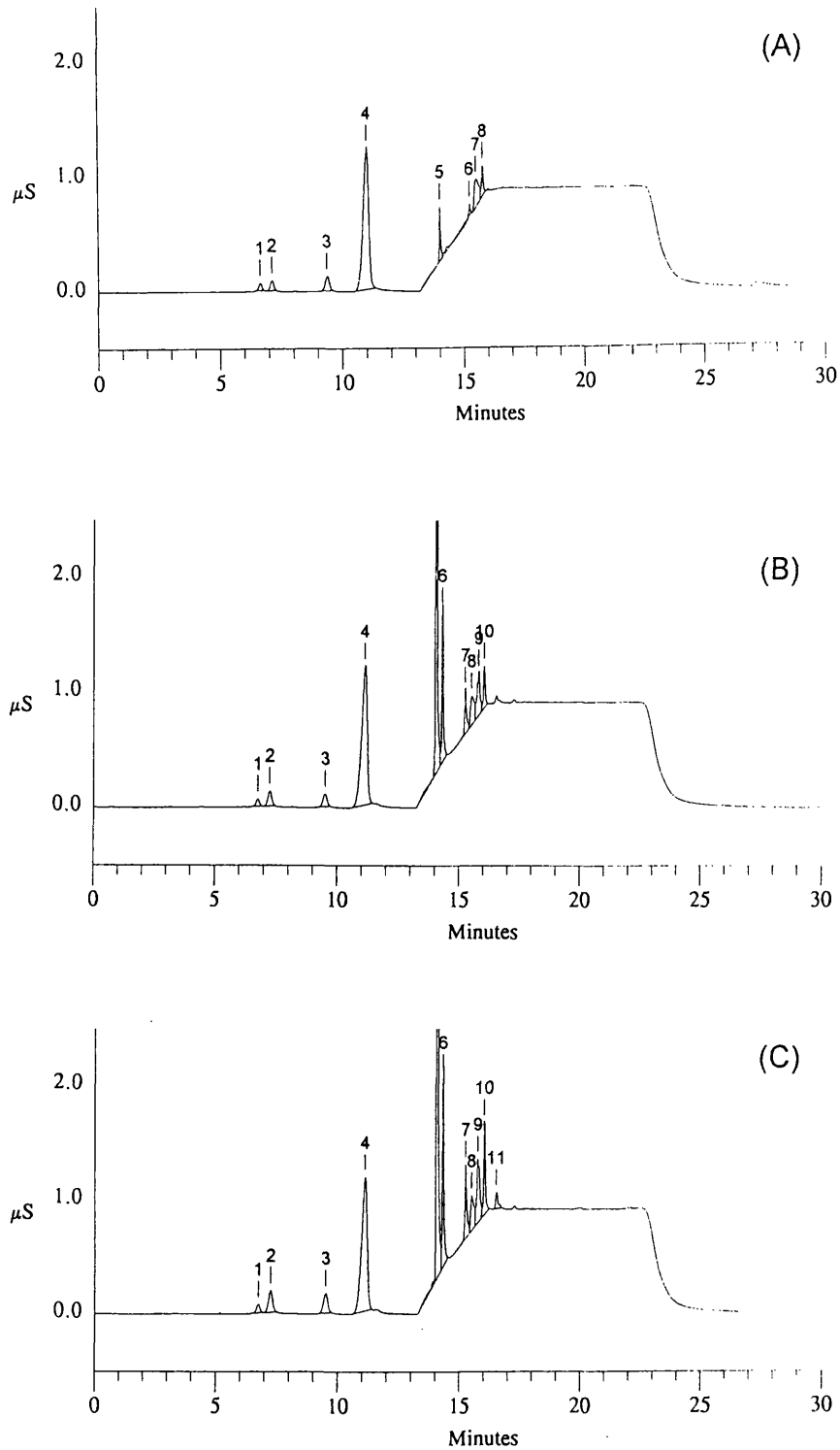


Figure 3.23 Ion exchange chromatograms for analysis of low affinity organic anions from tecnazene irradiated by UV light in water for (A) 0min, (B) 10min and (C) 25min.

Peaks (A): 1=lactate, 2=acetate, 3=formate and 4=methane sulphonate. (B): 1=lactate, 2=acetate, 3=formate and 4=methane sulphonate. (C): 1=lactate, 2=acetate, 3=formate and 4=methane sulphonate.

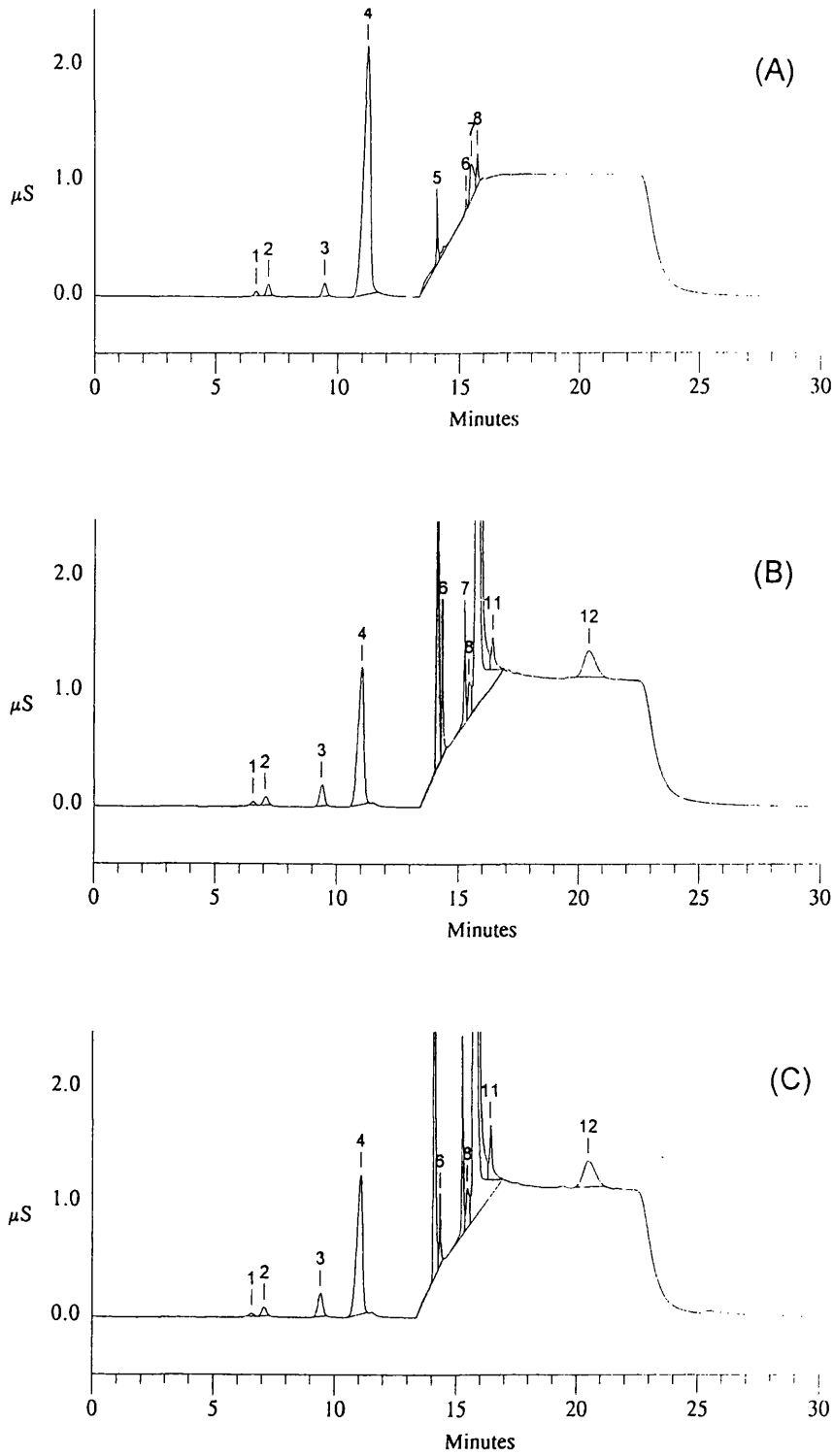


Figure 3.24 Ion exchange chromatograms for analysis of low affinity organic anions from tecnazene irradiated by UV light with 0.1mM H_2O_2 in water for (A) 0min, (B) 10min and (C) 20min.

Peaks (A): 1=lactate, 2=acetate, 3=formate and 4=methane sulphonate. (B): 1=lactate, 2=acetate, 3=formate and 4=methane sulphonate. (C): 1=lactate, 2=acetate, 3=formate and 4=methane sulphonate.

chromatogram of standard succinate and oxalate are given in Figure 3.25. Chromatograms at different points are given in Figures 3.26 and 3.27. Because succinate was detected only at trace levels on both conditions, UV irradiation in absence and presence of hydrogen peroxide, only oxalate was quantified on this occasion.

The results are shown in Figures 3.28 and 3.29. The Y axis shows the percentage of carbon from tecnazene that is used for each organic ion. In the case of decomposition using UV only, formate was detected at only trace levels so lactate, acetate and oxalate were quantified. All three compounds were increasing slightly with time (Figure 3.28).

In the case of UV irradiation in the presence of hydrogen peroxide, acetate was only detected at trace level, and lactate, formate and oxalate were quantified. Oxalate was increasing significantly with time, while lactate was increasing slightly with time and started decreasing after 7.5min and finally was not detectable after 15min (Figure 3.29). Formate was also found to increase slightly with time and started decreasing after 15min.

In comparison between the two treatments, in the case of UV irradiation in the presence of hydrogen peroxide since acetate was not detected at all irradiation times it seems acetate was quickly converted into formate, while transformation of acetate into formate seems to be slow in the case of UV irradiation because formate was not detected with this condition. Furthermore, more oxalate was formed in the presence of hydrogen peroxide than in the absence of hydrogen peroxide, and the major organic anionic decomposition product formed under UV irradiation in the presence of hydrogen peroxide was identified as oxalate. In the case of UV irradiation in the absence of hydrogen peroxide in total 13% of the carbon in tecnazene was transformed into organic anions at 25min, and it seems to be still increasing after that, while in the case of UV irradiation in the presence of hydrogen peroxide, 35% of the carbon in tecnazene was transformed into organic anions at 15min. Furthermore, the total carbon as organic anions started decreasing at 20min in the case of UV

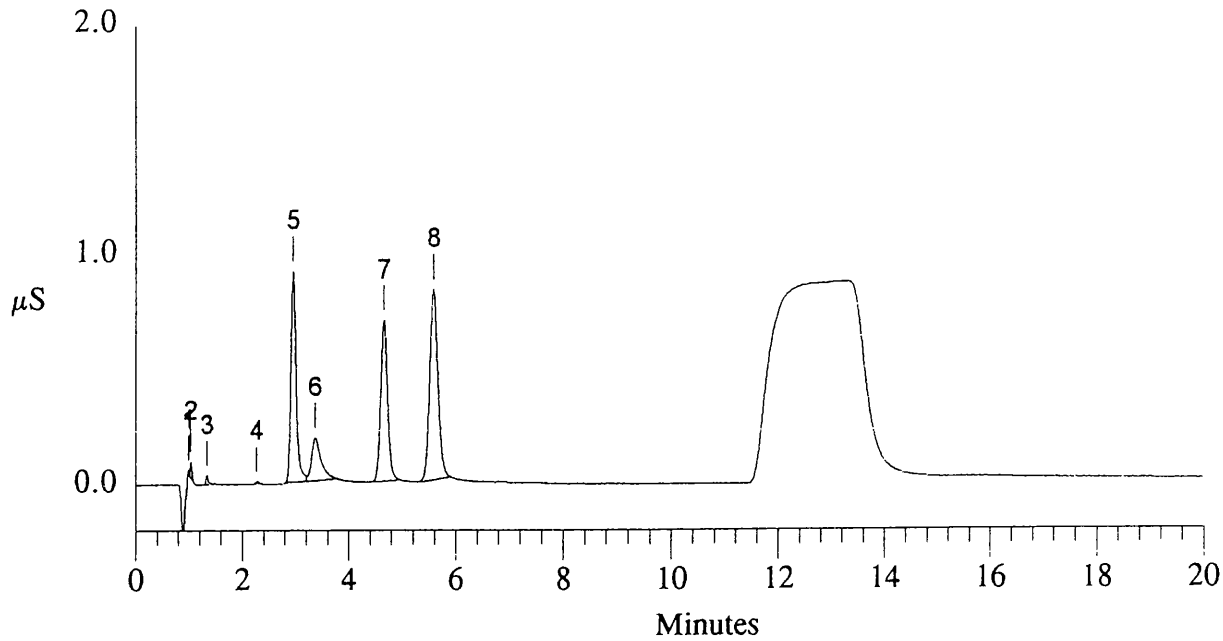


Figure 3.25 Typical ion exchange chromatogram of standard high affinity organic anions.

Peaks 5=succinate, 5μM; 6=disolved CO_2; 7=sulphate, 0.1mg/L and 8=oxalate, 5μM.

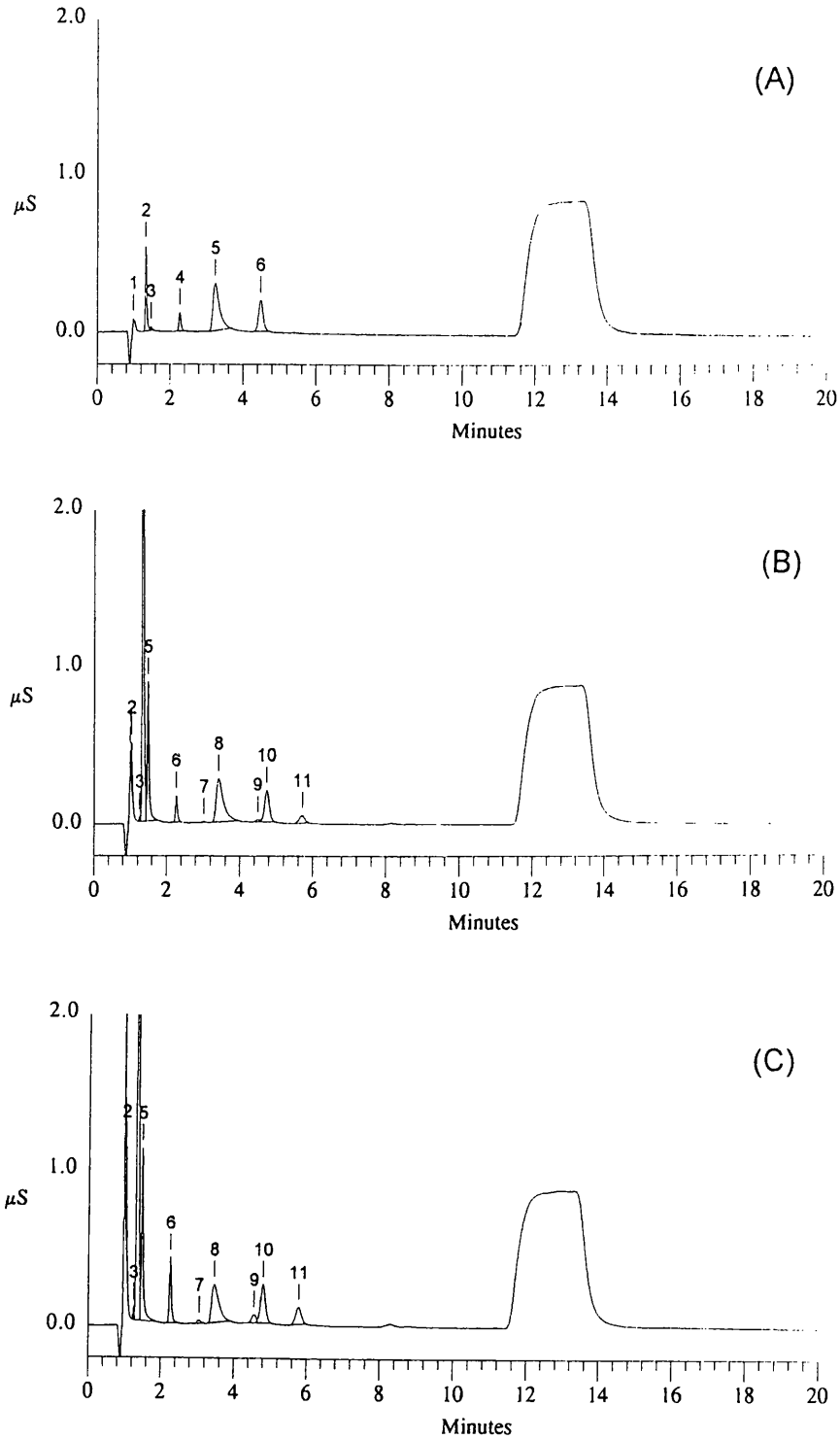


Figure 3.26 Ion exchange chromatograms for analysis of high affinity organic anion from tecnazene irradiated by UV light in water for (A) 0min, (B) 10min and (C)25min.

Name of anions and peak number

	Unidentified (possibly organic anions)	Cl^-	NO_2^-	NO_3^-	succinate	dissolved CO_2	unknown decompositi on products	sulphate	oxalate
(A) 0min	1	2	3	4	-	5	-	6	-
(B) 10min	1,2,3	4	5	6	7	8	9	10	11
(C) 25min	1,2,3	4	5	6	7	8	9	10	11

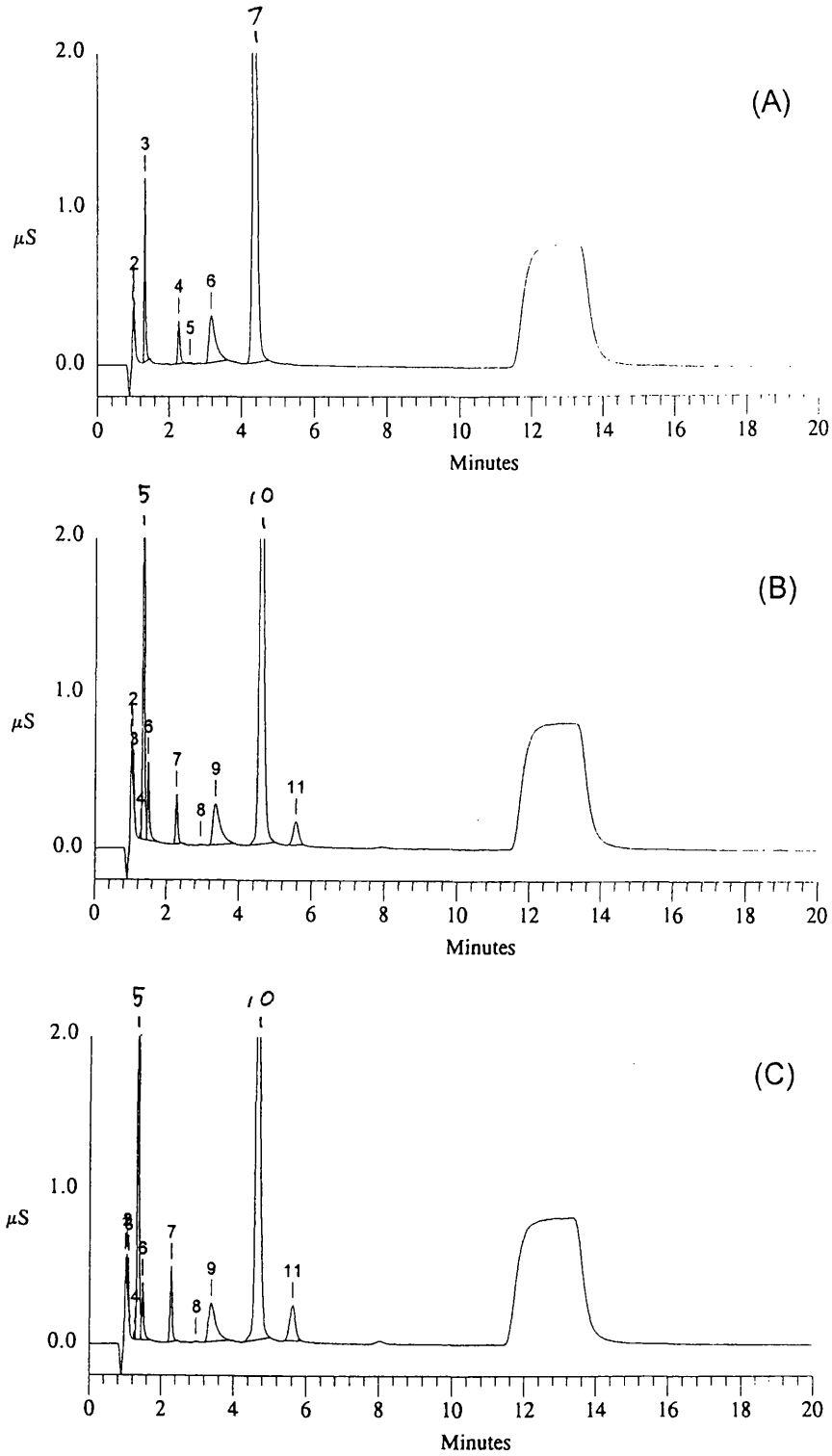


Figure 3.27 Ion exchange chromatograms for analysis of high affinity organic anion from tecnazene irradiated by UV light with 0.1mM H_2O_2 in water for (A) 0min, (B) 10min and (C)25min.

Name of anions and peak number

	Unidentified (possibly organic anions)	Cl^-	NO_2^-	NO_3^-	succinate	dissolved CO_2	sulphate*)	oxalate
(A) 0min	1,2	3	-	4	-	6	7	-
(B) 10min	1,2,3,4	5	6	7	8	9	10	11
(C) 25min	1,2,3,4	5	6	7	8	9	10	11

*) from oxidised disulphite added in order to reduce residual H_2O_2 after collecting samples.

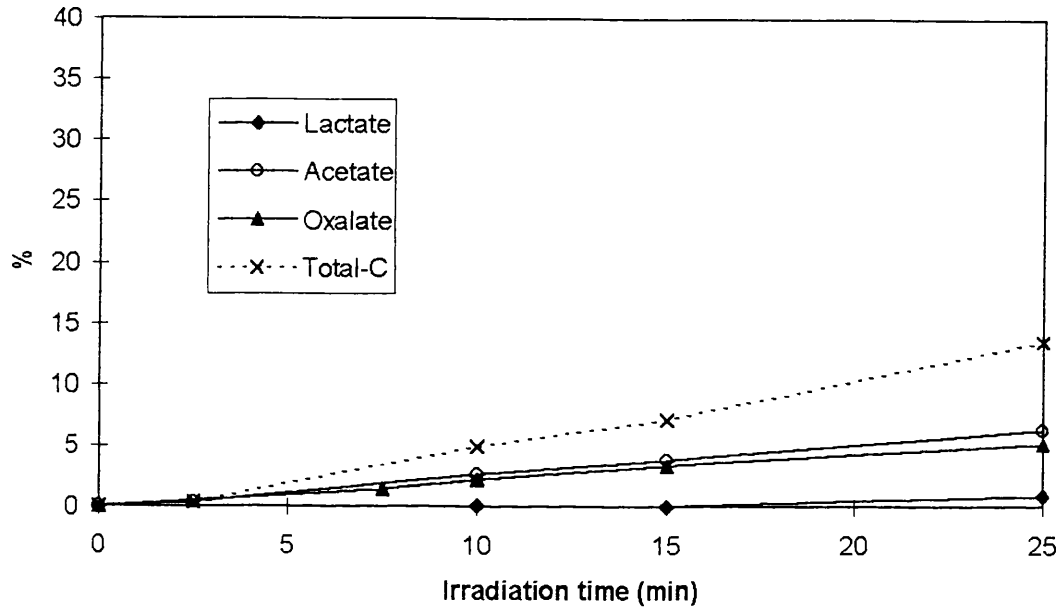


Figure 3.28 Determination of organic anionic decomposition products form tecnazene irradiated by UV light in water.

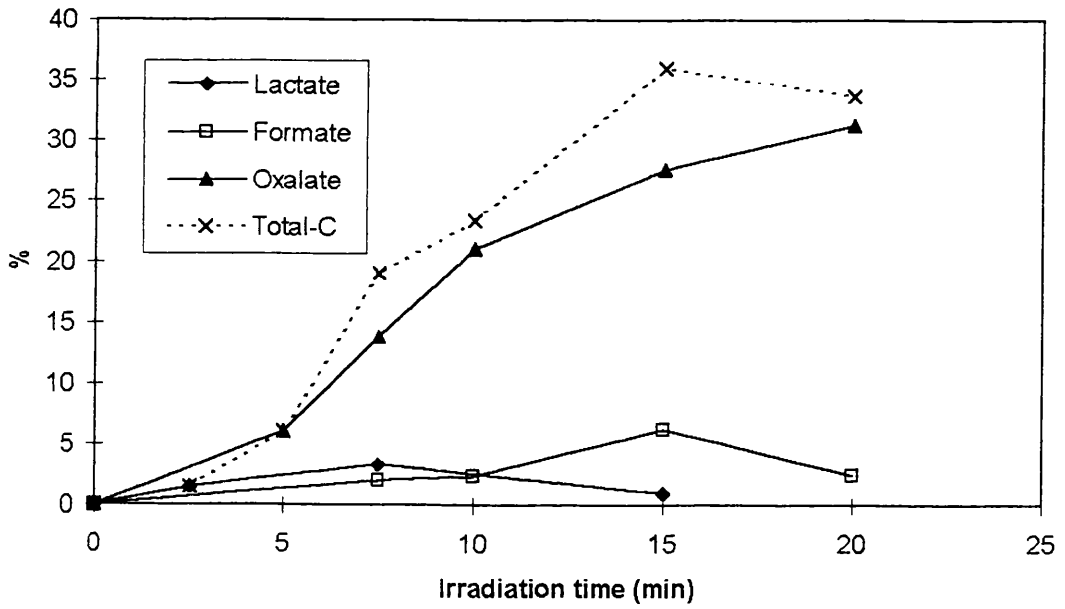


Figure 3.29 Determination of organic anionic decomposition products form tecnazene irradiated by UV light with 0.1mM H_2O_2 in water .

irradiation in the presence of hydrogen peroxide. Therefore, it is likely that these anions undergo further decomposition probably into water and carbon dioxide.

As discussed in Chapter 2 UV irradiation in either the absence or presence of hydrogen peroxide leads almost to complete decomposition of tecnazene within half an hour. However it seems the formation of the decomposition products are different between the treatments. In other words, UV irradiation in the presence of hydrogen peroxide brought about rapid transformation into simple organic compounds which was not the case with UV light only.

Finally, although five organic anions, acetate, formate, lactate, succinate and oxalate were identified as decomposition products, there is still a co-elution problem in the method in the case of determination of acetate and oxalate as mentioned in the method development section. On this occasion, because glycolate, co-eluted with acetate, and fumarate, co-eluted with oxalate were unlikely to be decomposition products than acetate and oxalate, unknown peaks were quantified as acetate and oxalate. However, the final proof of absence of co-eluted anions can be done with analysis which gives structural information, such as mass spectrometry.

3.4 Conclusions

The results of the determination of decomposition products of tecnazene are given in Table 3.7. It was observed that almost all nitrogen and chlorine from decomposed tecnazene were converted to nitrite, nitrate and chloride ions. In terms of the conversion of carbon from tecnazene into smaller fragments, in the case where hydrogen peroxide was absent 13% of carbon of tecnazene was converted into organic anions, while in the case where hydrogen peroxide was present 35% of carbon was converted into organic anions which was approximately 3 times more compared to the case where hydrogen peroxide was absent. The UV treatment in the presence of hydrogen peroxide brought about faster decomposition of tecnazene (see Chapter 2). Therefore, the results may indicate the UV irradiation treatment in the presence of hydrogen peroxide brought about the faster decomposition of tecnazene, and the faster

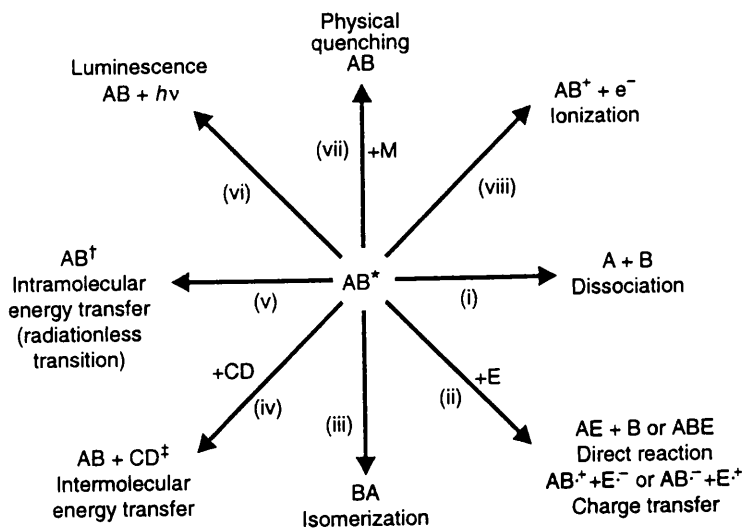
Table 3.7 The determination of decomposition products of tecnazene

Experimental conditions \ Determination method		GC or GC-MS	Ion exchange chromatography		
			Inorganic anions		Organic anions
			Release of chloride	Release of nitrogen	
in water	without H_2O_2	No decomposition product was detected on the GC chromatogram.	Almost all chlorine from decomposed tecnazene released as chloride ion at any UV irradiation time points	Almost all nitrogen from decomposed tecnazene released as nitrite and nitrate at any UV irradiation time points	Lactate, acetate, formate(trace level), succinate(trace level) and oxalate were identified. In total 13% of carbon was converted to organic anions at 25 min.
	with H_2O_2	No decomposition product was detected on the GC chromatogram.	Almost all chlorine from decomposed tecnazene released as chloride ion at any UV irradiation time points	Almost all nitrogen from decomposed tecnazene released as nitrite and nitrate at any UV irradiation time points. Nitrite was quickly converted to nitrate.	Lactate, acetate(trace level), formate, succinate(trace level) and oxalate were identified. In total 35% of carbon was converted to organic anions at 20min.
in 10% (v/v) CH_3CN-water mixtures	without H_2O_2	Trichloronitrobenzene and tetrachlorophenol were identified.	Approx. 60% of Cl was released at 80min when 90% of tecnazene was decomposed.	The determination was not possible on this occasion.	The determination was not possible on this occasion.
	with H_2O_2	Trichloronitrobenzene and tetrachlorophenol were identified.	Approx. 60% of Cl was released at 80min when 90% of tecnazene was decomposed.	The determination was not possible on this occasion.	The determination was not possible on this occasion

conversion of tecnazene into smaller fragments than UV irradiation only. Presumably some of the rest of the carbon of tecnazene may be still presence in the form of organic compounds or other organic anions non of which were detectable on this occasion. Some possible organic compounds which could be present as decomposition products of tecnazene were discussed in the next Section. Some of the carbon were might have been converted into carbon dioxide which could be the final stage of decomposition. Finally, further study is required in order to investigate the fate of the remainder of the carbon of tecnazene.

3.5 Possible decomposition pathway of tecnazene

In a study of photochemistry there are several processes initiated by electronically excited species. Some of the most important routes are given in Figure 3.30.



Note; The use of the symbols *, † and ‡ is only intended to illustrate the presence of electronic excitation and not necessarily differences in states. One or both of the products of processes (i)-(iii) may be excited.

Figure 3.30 The several routes to loss of electronic exitation (Wayne and Wayne, 1996).

In the case of tecnazene an electronically excited tecnazene is delivered by the absorption of UV irradiation. The next step of this excited tecnazene was considered according to the diagram. However, as Wayne and Wayne (1996) wrote the strictly chemical routes are (i)-(iii). Therefore, only these three routes were considered in the case of tecnazene. Presumably the formation of organic ions is as a result of combination of several routes including dissociation of tecnazene (route (i)). This route is often referred to as photolysis (Wayne and Wayne,1996).

From the results discussed in early sections, the release of chloride ions could be evidence of reductive dechlorination pathway of tecnazene, while the release of nitrite and nitrate and the formation of 2,3,5,6-tetrachloronitrophenol could indicate the replacement of the ring nitro group by hydroxyl. The replacement of chloride by hydrogen and nitro groups by hydroxyl can be examples of route (ii). Reductive dechlorinaiton has been reported as one of the most important transformation pathway for polychlorinated aromatic compound (Choudhry *et al.*, 1986). The replacement of chlorine by hydroxyl was also studied in photodecomposition of aromatic chlorinated compounds (Crosby and Tutass, 1966; Moilanen and Crosby, 1972). Therefore, it could be possible further photodecomposition of 2,3,5,6-tetrachlorophenol leads to polymerise the compound.

In the case of structual isomerizations or rearrangements (route (iii)) there are two isomers of tecnazene, namely 2,3,4,5-tetrachloronitrobenzene and 2,3,4,6-tetrachloronitrobenzene but these isomers were not detected on this occasion. However, the isomerization transformation pathway of chlorinated aromatic compounds was reported by Choudhry *et al.* (1986). According to their work the UV irradiation of 1,2,4,5-tetrachlorobenzene in acetonitrile-water mixtures yielded two isomers, 0.45% of 1,2,3,4-tetrachlorobenzene and 1.11% of 1,2,3,5-tetrachlorobenzene. In this respect, isomerization could be one of the important decomposition pathways of tecnazene although it was not observed on this occasion.

Apart from the pathways mentioned above there is a possibility of the formation of azobenzene and azoxybenzene derivatives, which tend to be unpleasant compounds in

the environment, from the photodecomposition of tecnazene. Sullivan *et al.* (1980) identified several azobenzene and azoxybenzene derivatives as decomposition products of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) from UV irradiation in benzene solution. These azo-compounds were thought to be coupled up directly using nitro groups on the benzene ring of trifluralin. Tecnazene also has a nitro group on the benzene ring so it is possible to produce an azo-compound. There may be another route to deliver them through aniline intermediate derivatives although in the available literature the formation of azobenzene from aniline via photochemical pathways has not been demonstrated yet. However biological pathways of several azobenzenes from aniline were reviewed by Corker *et al.* (1979). It is possible that tecnazene yields aniline derivatives, such as 2,3,5,6-tetrachloroaniline which are usually produced via biological pathways, although they were not detected through the study of UV irradiation of tecnazene.

In terms of the role of hydrogen peroxide for the formation of decomposition products, no differences in the formation of decomposition products were observed between UV irradiation treatment in the absence and presence of hydrogen peroxide as discussed in previous sections. However, hydrogen peroxide brought about the rapid oxidative reaction of nitrite and some organic anions which could be an advantage of using hydrogen peroxide for UV irradiation treatment of tecnazene.

In accordance with the above consideration the transformation pathway of tecnazene by UV light was proposed and given in Figure 3.31.

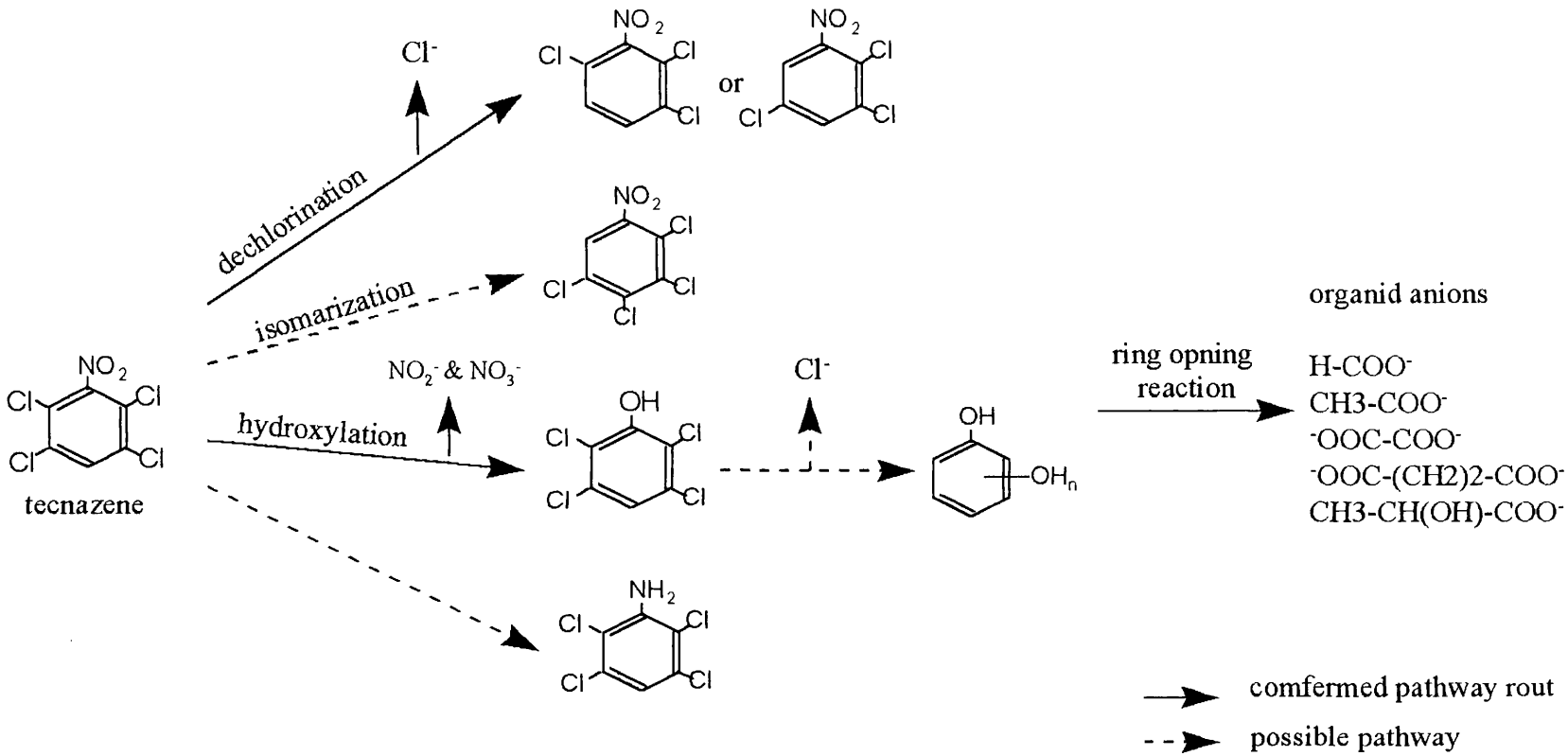


Figure 3.31 Reaction scheme proposed for tecnazene photodecomposition

CHAPTER 4. General conclusions and suggestions for further study

4.1 General conclusions

Tecnazene was expected to be decomposed by UV light because of its high intensity absorption of UV light. UV irradiation treatment of tecnazene in pure water leads to a rapid decomposition, and almost complete decomposition of tecnazene was observed within half an hour. Hydrogen peroxide enhanced the rate of photolysis in the presence of UV light although hydrogen peroxide alone hardly oxidised any tecnazene. This is because of the formation of hydroxyl radicals, which are known to be strong oxidants, as a consequence of photolysis of hydrogen peroxide. The decomposition rate increased with the increase in the concentration of hydrogen peroxide.

One further advantage of UV irradiation treatment in the presence of hydrogen peroxide was that the nitrite was oxidised to nitrate permitting control over the form of N. Moreover, organic anions detected as decomposition products were oxidised quicker than in the absence of hydrogen peroxide. Consequently, although it was not investigated on this occasion, presumably UV irradiation in the presence of hydrogen peroxide could transform tecnazene finally to carbon dioxide, water and inorganic salts faster than UV light alone due to its powerful oxidation reaction toward tecnazene and the decomposition products of tecnazene.

In order to increase the concentration of tecnazene in solution, acetonitrile was employed as a cosolvent for the study of decomposition. However, in the case of UV irradiation of tecnazene in 10% (v/v) acetonitrile-water mixtures, different behaviour was observed compared with the situation which existed in the case of water. It was likely that this was because of mainly the higher initial concentration of tecnazene in the case of UV irradiation alone. However, in the presence of hydrogen peroxide the results seemed to be more complicated. In other words, hydrogen peroxide had no effect on the decomposition rate for 10.0mg/L initial concentration of tecnazene, while hydrogen peroxide slowed down the decomposition rate in the case of 1.0mg/L initial

concentration of tecnazene. On the other hand, as mentioned before in the case of water hydrogen peroxide accelerated the decomposition rate of tecnazene and the decomposition rate increased with the increase in the concentration of hydrogen peroxide.

The reason for this different behaviour of hydrogen peroxide between water and acetonitrile-water mixtures is possibly that acetonitrile may produce substances that cause reduction of decomposition rate as a consequence of the decomposition of acetonitrile by UV light. Indeed, acetonitrile itself was found to be decomposed by UV light.

Therefore, it may be observed that the study of decomposition of tecnazene in acetonitrile-water mixtures is probably not representative of what happens to the UV irradiation treatment in water.

In terms of the study of decomposition products of tecnazene the results obtained indicate several pathways. The release of chloride ions determined by ion-exchange chromatography could be evidence of a reductive dechlorination pathway of tecnazene decomposition. The release of nitrite and nitrate ions determined by ion-exchange chromatography and the formation of 2,3,5,6-tetrachloronitrophenol identified by GC-MS could indicate the replacement of the ring nitro group by hydroxyl. Furthermore, the formation of organic anions are evidence of the ring opening reaction of tecnazene. The effect of the presence of acetonitrile on the formation of decomposition products was obscure on this occasion. However, use of a cosolvent is necessary in this field of study because of low aquatic solubility of tecnazene in order to identify decomposition products as many as possible.

Obviously much more work is required in this area before the full picture of tecnazene breakdown is revealed and the conversion of the tecnazene to innocuous compounds confirmed. However, supporting results do suggest the reductive dechlorination, the ring opening reaction following the formation of organic anions which should not cause undue concern.

4.2 Suggestions for further study

This type of approach (UV light plus hydrogen peroxide) does show promise particularly as a compliment to existing practices such as sedimentation and biodegradation. However, the influence of additional components, e.g. the presence of soil, slurry, organic matter, etc. in these reactions will need further investigation before such an approach could be recommended with confidence for all commercial situations.

Oliver *et al.* (1979) reported the affect of suspended sediment on the photolysis rate of organic pollutants. They pointed out that the photolysis rate may be reduced by suspended sediment due to shielding the organics from the available light or quenching the excited states of the organic molecules before they react to from products. They also pointed out the possibility of enhancement of the photolysis rate by suspended particulates because of the production of excited states or free radicals that can then react with the organics. Like these examples photolysis in the presence of additives is complicated, and photolysis rates are dependent on the nature of the additives.

Although it was not included in these studies, temperature and pH are other important factors that affect photolysis rate. Beltran-Heredia *et al.* (1996) conducted experiments for the study of photolysis of bentazone, a herbicide, at various temperatures and pH values, and they reported that the photolysis rate was faster at higher temperature, and at higher pH conditions. This aspect was also reported by Benitez *et al.* (1994) in experiments of decomposition of organic pollutants using UV light.

In the case of UV irradiation in the presence of hydrogen peroxide the temperature and pH have the same influence on the decomposition rate as in the case of direct photolysis (Beltran-Heredia *et al.*, 1996). In other words, the rate increased with the increase of temperature and pH. In terms of pH Legrini *et al.* (1993) have also reported in their review work that pH is one of the factors for the contribution of

hydroxyl radicals, and in alkaline conditions the rate of hydrogen peroxide photolysis increases. However, the hydroxyl radical is rapidly converted to base $\cdot O^-$(Eqn. 1) in strong alkaline solution, which has a slower reaction rate than hydroxyl radicals (Buxton *et al.*, 1988).

$$\cdot OH + OH^- \leftrightarrow \cdot O^- + H_2O \qquad (1)$$

They also have studied that the reduction potential of hydroxyl radicals is stronger in acidic solution (2.7V) rather than in neutral solution (1.8V). In the presence of hydrogen peroxide the reaction rate towards organic substances depends on the rate of hydroxyl radical generation and competition by other hydroxyl radical scavengers in solution as Haag and Yao (1992) pointed out.

In terms of the study of decomposition products several issues came up. For instance, the influence of the presence of cosolvent which was acetonitrile on this occasion on the formation of decomposition products was obscure on this occasion, while it was found acetonitrile affects the decomposition rate in the presence of hydrogen peroxide. However, use of cosolvent is necessary in this field of study because of low aquatic solubility of tecnazene in order to identify decomposition products as many as possible. Therefore, the study of the influence of cosolvent on the formation of decomposition products should be conducted at the same time as the study of the decomposition products

In terms of the methods for the identification of decomposition products of tecnazene, using GC-MS may have limited the finding to volatile, thermally stable and relatively non-polar compounds due to the extraction by hexane, a non-polar organic solvent, and characterisation by GC analysis. In this regard, other methods, such as LC-MS may be useful for the determination of products which are much more polar and less volatile than tecnazene.

One other issue for the identification of the decomposition products is that most of the standard products are not commercially available. In general, the identification of

decomposition products is established by comparison with the appropriate model compounds. Therefore, availability of these products as standard is important. In this regard, it may be necessary to synthesise authentic model compounds for verification of the structure.

References

Allchin, C. R. and Law, R. J. (1988) The analysis of tecnazene (2,3,5,6-tetrachloronitrobenzene) and its metabolites in water and fish. *Chemistry and Ecology*, **3**, 255-264.

Ammann, A. A. and Ruttimann, T. B. (1995) Simultaneous determination of small organic and inorganic anions in environmental water samples by ion-exchange chromatography. *Journal of Chromatography A*, **706**, 259-269.

Beltran, F. J.; Gonzalez, M.; Rivas, F. J. and Alvarez, P. (1996) Aqueous UV radiation and UV/H_2O_2 oxidation of atrazine first degradation products: Deethlatrazine and deisopropylatrazine. *Environmental Toxicology and Chemistry*, **15**, 868-872.

Beltran-Heredia, J.; Benitez, F. J.; Gonzalez, T.; Acero, J. L. and Rodriguez, B. (1996) Photolytic decomposition of bentazone. *Journal of Chemical Technology and Biotechnology*, **66**, 206-212

Benitez, F. J.; Beltran-Heredia, J.; Gonzalez, T. and Real, F. (1995) Photooxidation of carbofuran by a polychromatic UV irradiation without and with hydrogen peroxide. *Industrial & Engineering Chemistry Research*, **34**, 4099-4105.

Benitez, F. J.; Beltran-Heredia, J.; Gonzalez, T. and Acero, J. L. (1994) Photochemical oxidation of protocatechuic acid. *Water Research*, **28**, 2095-2100.

Beurskens, J. E. M.; Dekker, C. G. C.; Van den Heuvel, H.; Swart M.; De Wolf, J. and Dolfing, J. (1994) Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. *Environmental Science & Technology*, **28**, 701-706.

Buckley, D. C.; Hill, A. R. C.; Sivyer, V. L. and Wilkins, J. P. G. (1994) Residues of tecnazene and its metabolites in potatoes in commercial stores. *Crop Protection*, **13**, 87-92

Bunce, N. J. (1978) Photolysis of 2-chlorobiphenyl in aqueous acetonitrile. *Chemosphere*, **8**, 653-656.

Buxton, G. and Wilmarth, W. K. (1963) Aqueous chemistry of inorganic free radicals. V. The use of carbon monoxide as a scavenger for hydroxyl radicals generated by the photolysis of hydrogen peroxide. *Journal of Physical Chemistry.*, **67**, 2835-2841.

Buxton, G. V.; Greenstock, C. L.; Helman, W. P. and Ross, A. B. (1988) Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (·OH/·O⁻) in aqueous solution. *Journal of Physical and Chemical Reference Date*, **17**, 513-886.

Chen, J. (1996) Determination of organic acids in industrial streams by ion chromatography after solid-phase extraction. *Journal of Chromatography A*, **739**, 273-280.

Choudhry, G. G. and Barrie Webster, G. R. (1985a) Photochemistry of halogenated benzene derivatives. Part V. Photolytic reductive dechlorination and isomerization of tetrachlorobenzenes in acetnitrile-water mixtures. *Toxicological and Environmental Chemistry*, **9**, 291-308.

Choudhry, G. G.; Van den Broecke, J. A. and Hutzinger, O. (1983) Formation of polychlorodibenzofurans (PCDFs) by the photolyses of polychlorobenzenes (PCBzs) in aqueous acetonitrile containing phenols. *Chemosphere*, **12**, 487-492.

Choudhry, G. G.; Van der Wielen, F. W. M.; Barrie Webster, G. R. and Hutzinger, O. (1985b) Photochemistry of halogenated benzene derivatives. Part VI. Photoreactions of tetra- and pentachlorophenols in water-acetonitrile mixtures. *Canadian Journal of Chemistry*, **63**, 469-475.

Choudhry, G. G.; Webster, G. R. B. and Hutzinger, O. (1986) Environmentally significant photochemistry of chlorinated benzenes and their derivatives in aquatic systems. *Toxicological and Environmental Chemistry*, **13**, 27-83.

Corke, C. T.; Bunce, N. J.; Beaumont, A. and Merrick, R. L. (1979) Diazonium cations as intermediates in the microbial transformation of chloroanilines to chlorinated biphenyls, azo compounds, and triazenes. *Journal of Agricultural and Food Chemistry*, **27**, 644-646.

Cox, A. and Kemp, T. J. (1971) *Introductory photochemistry*. McGraw-Hill Book Company ltd., England, UK.

Crosby, D. G. and Tutass, H. O. (1966) Photodecomposition of 2,4-dichlorophenoxyacetic acid. . *Journal of Agricultural and Food Chemistry*, **14**, 596-599.

Dalziel, J.(1978) Potato sprout suppressants with particular reference to tecnazene. Glasgow University Ph.D. Thesis.

Duncan, H. J.; Boyd, I. M. G. and Muir, J. W. (1992) Methods for controlling sprouting in potatoes. *Aspects of Applied Biology*, **33**, 189-195.

Durand, G.; Abad, J. L.; Sanchez-Baeza, F.; Messeguer, A. and Barselo, D. (1994) Unequivocal identification of compounds formed in the photodegradation of fenitrothion in water/methanol and proposal of selected transformation pathways. *Journal of Agricultural and Food Chemistry*, **42**, 814-821.

Glaze, W. H.; Kang, J. and Chapin, D. H. (1987) The chemistry of water treatment processes involving ozone, hydrogen peroxide and ultraviolet radiation. *Ozone Science & Engineering*, **9**, 335-352.

Haag, W. R. and Yao, C. C. D. (1992) Rate constants for reaction of hydroxyl radicals with several drinking water contaminants. *Environmental Science & Technology*, **26**, 1005-1013.

Hamadmad, N. (1967) Photolysis of pentachloronitrobenzene, 2,3,5,6-tetrachloronitrobenzene, and pentachlorophenol. Diss. Abst. B, 1419

Hartley, D. and Kidd, H., Eds. (1983). *The agrochemicals hand book.* Royal Society of Chemistry, England, UK.

Hoigne, J. and Bader, H. (1983) Rate constants of reactions of ozone with organic and inorganic compounds in water–I Non-dissociating organic compounds. *Water Research*, **17**, 173-183.

Jardine, W. G. (1994) A study into the extraction techniques and photodegradation of tetrachloronitrobenzene. Glasgow University 4th year student report.

Kerr, J. (1996) Grass silage effluent project. Glasgow University 4th year student report.

Lappin, G. J.; Pritchard, D.; Moore, R. B. and Laird, W. J. D. (1996) Metabolism of 2,3,5,6-tetrachloronitrobenzene(tecnazene) in rat. *Xenobiotica*, **26**, 65-77

Legrand, M.; De Angelis, M. and Maupetit, F. (1993) Field investigation of major and minor ions along Summit (Central Greenland) ice cores by ion chromatography. *Journal of Chromatography*, **640**, 251-258.

Legrini, O.; Oliveros, E. and Braun, A. M. (1993) Photochemical processes for water treatment. *Chemical Reviews*, **93**, 671-698.

Leifer, A. (1988) *The kinetics of environmental aquatic photochemistry. Theory and practice.* American Chemical Society, New York, NY, U.S.A.

Leonard, M. J. (1988) Studies on the potato sprout suppressant/fungicide tecnazene. Glasgow University Ph.D. Thesis.

Mathew, R. and Khan, S. U. (1996) Photodegradation of metolachlor in water in the presence of soil mineral and organic constituents. *Journal of Agricultural and Food chemistry*, **44**, 3996-4000.

Moilanen, K. W. and Crosby, D. G. (1972) Photodecomposition of 3',4'-dichloropropionanilide (propanil). . *Journal of Agricultural and Food Chemistry*, **20**, 950-953.

Oliver, B. G.; Cosgrove, E. G. and Carey, J. H. (1979) Effect of suspended sediments on the photolysis of organics in water. *Environmental Science & Technology*, **13**, 1075-1077.

Oliver, B. G. and Niimi, A. J. (1985) Bioconcentration factors of some halogenated organics for rainbow trout: Limitations in their use for prediction of environmental residues. *Environmental Science & Technology*, **19**, 842-849.

Peterson, D.; Watson, D. and Winterlin, W. (1990) Destruction of pesticide and their formulations in water using short wavelength UV light. *Bulletin of Environmental Contamination and Toxicology*, **44**, 744-750.

Roche, P. and Prados, M. (1995) Removal of pesticide by use of ozone or hydrogenperoxide/ozone. *Ozone Science & Engineering*, **17** 657-672.

Sanlavilley, Y.; Guittonneau, S.; Mansour, M.; Feicht, E. A.; Meallier, P. and Kettrup, A. (1996) Phtosensitized degradation of terbuthylazine in water. *Chemosphere*, **33**, 353-362.

Scheuer, C.; Wimmer, B.; Bischof, H.; Nguyen, L.; Maguhn, J.; Spitzauer, P.; Kettrup, A. and Wabner, D. (1995) Oxidative decomposition of organic water pollutants with UV-activated hydrogen peroxide-Determination of anionic products by ion chromatography. *Journal of Chromatography A*, **706**, 253-258.

Simmons, M. S. and Zepp, R. G. (1986) Influence of humic substances on photolysis of nitroaromatic compounds in aqueous systems. *Water research*, **20**, 899-904.

Staehelin, J. and Hoigne, J. (1982) Decomposition of ozone in water: Rate of initiation by hydroxide ion and hydrogen peroxide, *Environmental Science & Technology*, **16**, 676-681.

Steinwandter, H. (1987) Resarch in environmental pollution. II. Determination of polychlorinated nitrobenzene (PCB's) in main river fish. *Fresenius Zeitschrift fur Analytische Chemie*, **326**, 139-141.

Sullivan, R. G.; Knoche, H. W. and Markle, J. C. (1980) Photolysis of trifluralin: Characterization of azobenzene and azoxybenzene photodegradation products. *Journal of Agricultural and Food Chemistry*, **28**, 746-755.

Wayne, C. E. and Wayne, R. P. (1996) *Photochemistry*. Oxford University Press, England, UK.

Whale, G.; Sheahan, D. and Matthiessen, P. (1988) The toxicity of tecnazene, a potato sprouting inhibitor, to freshwater fauna. *Chemosphere*, **17**, 1205-1217.

Yurawecz, M. and Puma, B.J. (1983) Identification of chlorinated nitorobenzene residues in Mississippi River fish. *Journal of the Association of Official Analytical Chemists*, **66**, 1345-1352.

Zepp, R. G. and Cline, D. M. (1977) Rate of direct photolysis in aquatic environment. *Environmental Science & Technology*, **11**, 359-366.

Zwiener, C.; Weil, L. and Niessner, R. (1995) Atrazine and parathion methyl removal by UV and UV/O_3 in drinking water treatment. *International Journal Environmental Analytical Chemistry*, **58**, 247-246.